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SYNCHRONOUS AND ASYNCHRONOUS MUSCLES IN CICADAS*

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SUMMARY

1. The tymbal muscles of the cicada *Cyclochila australasiae* Donovan are synchronous, those of *Platypleura capitata* (Oliv.) asynchronous. The tymbal structure and the individual sound pulses are similar in the two species. The sound pulse frequency during calling song is higher in *P. capitata* (389 Hz) than in *C. australasiae* (234 Hz).

2. Muscle action potentials during protest songs are simultaneous in the two tymbal muscles of *P. capitata*, alternating in *C. australasiae*. Each action potential, or direct electrical stimulus to the muscle, is followed by a single, sometimes bimodal, sound pulse in *C. australasiae*, and by a burst of sound pulses in *P. capitata*.

3. The isometric twitch duration (30 °C) is much longer in *P. capitata* (107 ms) than in *C. australasiae* (18 ms) and the tetanic fusion frequency correspondingly lower.

4. Myofibrils are slightly wider and sarcoplasmic reticulum much sparser in tymbal muscles of *P. capitata* than in those of *C. australasiae*; otherwise the muscles are structurally similar.

5. Flight muscles in both species are synchronous with an action potential for each contraction.

6. Tymbal muscles of the two species are used to illustrate the diagnostic features of synchronous and asynchronous muscles.

INTRODUCTION

Insect muscles can be divided into synchronous and asynchronous types. In synchronous muscle there is a 1:1 correspondence between muscle fibre depolarization and contraction; each contraction is initiated by a fibre depolarization or burst of depolarizations due to antecedent activity in the motor neurone or motor neurones to the fibre. In asynchronous muscle there is no such direct correspondence between electrical and mechanical activity under normal operating conditions. The contraction frequency of an asynchronous muscle can be much higher than that of motor neurone

* This paper is dedicated to the late Professor K. D. Roeder who contributed importantly to the development of concepts about asynchronous muscle.

impulses to the muscle. For example, in a housefly the action potential frequency the flight muscles may be about 10 Hz, although the wings are beating, and therefore the flight muscles contracting, at nearly 150 Hz (Roeder, 1951). This comes about because a neurally-excited asynchronous muscle, when attached to a resonant load, can oscillate at the resonant frequency of the load. The ability of asynchronous muscle to give oscillatory contractions is a result of a property termed stretch activation (Pringle, 1978) whereby changes in muscle length lead to delayed changes in active tension.

Most insect asynchronous muscles are flight muscles, but the tymbal muscles of some cicadas are also asynchronous. Tymbal muscles are the sound-producing muscles. They are found as a pair of large muscles in the first abdominal segment of male cicadas, having their origin ventrally upon a complex infolding of the cuticle, the chitinous V. Each tymbal muscle inserts dorso-laterally on a tymbal, which is a ribbed, cuticular membrane in the first abdominal tergum. Contraction of the tymbal muscle buckles the tymbal, producing sound pulses. Hagiwara, Uchiyama & Watanabe (1954) and Pringle (1954*a*,*b*) first reported the occurrence of asynchronous tymbal muscles in the genus *Platypleura* and possibly also in *Meimuna*. However, members of nine other cicada genera examined at that time and subsequently have proven to have synchronous tymbal muscles (Hagiwara & Ogura, 1960; Aidley, 1969; Reid, 1971; Young, 1972*a*). Further, in some species it has been shown that the tymbal bears a series of ribs which buckle sequentially during contraction of the tymbal muscle (Reid, 1971; Young, 1972*a*). In these species a single motor neurone impulse produces many sound pulses, thereby mimicking the asynchronous mechanism. This has raised the possibility (discussed in conversation, if not in print) that the report of asynchronous muscle in *Platypleura* might be a misinterpretation. Accordingly, we have re-examined the same species upon which Pringle (1954 *a*, *b*) worked and have been able to confirm his interpretation and extend the study to the ultrastructure of the muscle.

The confirmed occurrence of both types of muscle within the same family makes it possible to examine the characteristics of homologous synchronous and asynchronous muscles in closely related animals. Since tymbal muscles serve precisely the same function in all cicadas, the differences between synchronous and asynchronous tymbal muscles should represent differences between the two control modes relatively uncontaminated by adaptation to different performance requirements or by different evolutionary origins. The following report compares sound production, tymbal muscle physiology and tymbal muscle ultrastructure in two species of cicada, one of which has synchronous and the other asynchronous tymbal muscles. Our goal in this comparison is to illustrate the basic features of synchronous and asynchronous muscles and to identify more clearly those characteristics which can be used to distinguish between the two muscle types.

MATERIALS AND METHODS

Animals

The two species examined were *Platypleura capitata* from Sri Lanka, the species previously studied by Pringle (1954 *a*, *b*) and *Cyclochila australasiae* from south-eastern Australia, upon which the latest detailed report on sound production is that

[illegible]as (1887). Animals were captured with long-handled nets while they were singing and used for experiments on the same or on the following day. *Platypleura capitata* were obtained from parks and gardens around Colombo in May and June; *Cyclochila australasiae* were obtained from similar situations in Melbourne (Victoria) or Port Macquarie (New South Wales) from November until January.

Sound recordings

The calling songs of animals singing in the field were recorded with a Sennheiser MKH 815 directional microphone and a Nagra IVS tape recorder at a tape speed of 7·5 in/s (*Cyclochila australasiae*) or with a Sony F27 microphone and Sony TC 1585D cassette tape recorder at a tape speed of 1·88 in/s (*Platypleura capitata*). Laboratory recordings of protest songs (the song produced by a cicada when handled or otherwise disturbed) were made with the same equipment.

Electromyogram recording

Electrical activity in a tymbal muscle was recorded with a silver wire, 50 μm in diameter and insulated to the tip, inserted into the muscle through a small hole made in its ventral attachment, the chitinous V. In order to do this, it was necessary to remove the operculum, which covers the chitinous V and tympanum ventrally. The indifferent electrode was a bare silver wire, 0·2 mm in diameter, inserted into the ventral abdomen. The electrodes were held in place by insect wax melted onto the cuticle over the wires at their insertion. Conventional capacitor-coupled amplifiers were used and signals were displayed on an oscilloscope and recorded on tape.

The following sequence was employed in recording protest songs and muscle action potentials.

1. The animal was fixed in place by waxing a rod mounted in a holder to the dorsal prothorax (see Fig. 1 of Josephson & Young, 1979). The mounted animal was allowed to walk on a styrofoam ball. Protest songs initiated by prodding the intact animal were recorded.

2. The opercula were removed, electrodes were placed in the left and right tymbal muscles, and an indifferent electrode was inserted into the abdomen.

3. Muscle action potentials were recorded from both tymbal muscles during protest songs.

4. Muscle action potentials were recorded from one tymbal muscle together with the sound produced by both tymbals during protest songs.

5. The apodeme connecting one tymbal muscle to its tymbal was cut, thus silencing the tymbal apparatus on that side. The unilateral sound and muscle action potentials from the intact side were then recorded during protest song.

6. The posterior nerves leaving the thoracic ganglion were cut with scissors inserted ventrally between the mesothoracic and metathoracic segments, thus denervating the tymbal muscles. The electrode in the muscle on the intact side and the indifferent electrode were attached to an electrical stimulator rather than an amplifier. Sounds produced by direct stimulation of the tymbal muscle were then recorded.

Action potentials from wing muscles of flying insects were recorded with a wire inserted into the dorsal longitudinal muscle of the thorax. Again a bare silver wire inserted into the abdomen served as an indifferent electrode. Wing movements were [illegible]nitored by holding the animal in place with a force transducer waxed to the

prothorax. The transducer recorded the up and down thrust of the thorax created the wing movements. Flight was initiated by raising the animal so its tarsi did not touch the ground and blowing air posteriorly across the animal's head.

Tension during muscle contraction

The animal was beheaded, its legs and wings removed, and the abdomen cut off posterior to the tymbal segment. The posterior nerves from the thoracic ganglion were cut with scissors inserted ventrally between the mesothoracic and metathoracic segments. The abdominal tergum was removed between the tymbals. The animal was then pinned, ventral side down, to a dish lined with resin (Sylgard, Dow Corning Corp., Midland, Michigan) with large pins through the mesothorax, small pins through the midline of the chitinous V, and small pins through the ventral portion of the right tymbal muscle. The left tymbal was cut in a circle centred on the insertion of the muscle apodeme and cuticular elements surrounding the dorsal portion of the left tymbal muscle were removed. This left the dorsal portion of the muscle isolated but still attached to its apodeme and a small piece of the tymbal. Tension was recorded with a strain gauge made from a pair of semiconductor transducer elements (Pixie strain gauges, Endevco, San Juan Capistrano, California). A short segment of an insect pin, bent to a hook at one end, was fixed to the strain gauge. This hook was slipped around the exposed apodeme to connect the transducer to the muscle. The resonant frequency of the transducer and hook was 600–700 Hz. The transducer was mounted on a manipulator which allowed the muscle length to be varied. Tension measurements were made with the muscle stretched to 6% above slack length, slack length being the length adopted by the unloaded muscle. Slack length plus 6% is approximately the length of the muscle in an intact animal. The compliance of the transducer and the pins holding the animal to the resin in the preparation dish was estimated by attaching the transducer hook to a metal ring pinned to the resin and measuring the force generated by a known displacement of the manipulator holding the transducer. The measured compliance was 1·12 μm/mN. The maximum tetanic tension generated by the muscles resulted in a shortening, computed from the estimated compliance, which averaged 1·9% of the muscle length in *C. australasiae* and 2·4% in *P. capitata*. Thus the tension recordings were nearly, but not completely, isometric.

The muscles were stimulated through a pair of silver wires, 50 μm in diameter, inserted into the muscle near its ventral attachment. A thermistor probe inserted into the base of the non-stimulated muscle monitored muscle temperature, and muscle temperature was controlled by varying the intensity of a microscope light directed equally at both tymbal muscles. The tymbal muscles are totally surrounded by a tracheal air sac which prevents drying, and preparations would remain responsive for hours with no further care.

The muscle length was measured with a graduated scale held against the muscle. After completion of tension measurements the preparation was preserved in 70% alcohol. Alcohol fixation makes it possible to cleanly separate the muscle from its attachment. After a few days in alcohol the muscle was removed, rehydrated in locust saline (Usherwood & Grundfest, 1965), and weighed. The average cross-sectional area of the muscle in cm^2 was determined as the ratio of its weight in grams to its length in cm (see Josephson, 1975).

Microscopy

The following solutions were used in preparing tissue for microscopical examination:

1. Primary fixative. 8 ml 25% gluteraldehyde, 5 ml 1 M-sodium cacodylate buffer, 50 ml locust saline (Usherwood & Grundfest, 1965), distilled water to 110 ml, pH adjusted to 6·8 with HCl.

2. Buffer wash. 100 ml locust saline, 25 ml 1 M-sodium cacodylate, distilled water to 220 ml, pH adjusted to 6·8.

3. Post-fixative. 8 ml 4% osmium tetroxide, 3·2 ml 1 M-sodium cacodylate, 10 ml locust saline, distilled water to 35 ml, pH adjusted to 6·8.

The segment containing the tymbal muscle was quickly isolated by cutting off the thorax in front and the remaining abdomen behind, leaving a ring of cuticle supporting the two tymbal muscles. This ring was plunged into gluteraldehyde fixative and the tracheal air sac surrounding the muscle picked away in several places to facilitate penetration of the fixative. After several minutes in fixative, the muscles were freed from the cuticular ring and teased into small bundles. After a total of 1 h in the primary fixative the fibre bundles were washed twice for 30 min each in buffer, post-fixed in cold osmium fixative for 1 h, washed again with buffer, dehydrated in alcohol and propylene oxide and embedded in Araldite. Thick sections (1 μm) stained with toluidine blue were used for light microscopy, thin sections for electron microscopy.

Stereological analysis was done from electron microscope prints of muscle sections taken slightly oblique to the transverse fibre axis. With *Platypleura capitata*, in which the sarcoplasmic reticulum is very regional in distribution, only prints were used which included portions of both the Z line and the M region, thus insuring that all parts of the sarcomere were represented. The prints were at a total magnification of 35,000 and each represented approximately 35 μm^2 of tissue. A grid ruled at 1 cm intervals (corresponding distance on original section = 0·29 μm) was laid over the prints and the number of grid intersections lying over each of the major muscle components was counted. The categories for the intersection counts were: (1) myofibril, (2) mitochondrion, (3) sarcoplasmic reticulum or T-tubule, and (4) 'other', 'other' including undifferentiated sarcoplasm, fields of glycogen granules, cell nuclei, and intracellular tracheoles. The 'other' category underestimates the relative volume occupied by this group of components in the whole muscle since fields dominated by nuclei and large tracheoles tended to be avoided when the original electron microscope photographs were taken. Approximately 450 grid intersection points were counted for each print, the number varying somewhat because of differing registration between the grid and the prints, and because some fields near the edge of a fibre contained extracellular regions which were excluded from the counts. Ten prints were analysed for each muscle, and the relative volume of each component determined as the ratio of the number of intersections lying over that component to the total number of intersections counted. Three different muscles were analysed for each of the two cicada species.

Myofibrillar diameter and cross-sectional area were determined from electron micrographs of transverse sections. The area was measured with a planimeter. Five adjacent myofibrils were measured in two micrographs from each muscle and the ten

numbers averaged to obtain a single value for that muscle. Three muscles were analysed for each species. Sarcomere lengths from thick longitudinal sections were measured with an ocular micrometer. The sarcomere length was determined from the total length of 18-20 adjacent sarcomeres. Five replicate measurements from different fields were made for each muscle and the numbers averaged to obtain a single value for that muscle. Again three muscles were analysed for each species.

RESULTS

Our observations on the gross anatomy, the song, and the muscle action potentials of *P. capitata* are generally in good agreement with those published earlier by Pringle (1954*a*, *b*).

The sound-producing structures

The two species compared in this study differ considerably in size (Fig. 1 *A*) but the arrangement of their sound-producing structures is very similar. This arrangement is illustrated for *P. capitata* in Pringle (1954*b*, Fig. 2) and for *C. australasiae* in Young (1975, Fig. 1). In both species, each tymbal is concealed from view by the tymbal cover, a flap of cuticle projecting forward from the second abdominal segment. This was not removed during the experiments, except when rendering an animal unilateral by sectioning the tymbal apodeme on one side. Ventrally, the chitinous V and auditory tympanum are covered by the operculum, a flap of cuticle projecting backward from the metathorax.

The tymbals are of similar construction in the two species (Fig. 1*B*, *C*), each consisting of a thin, cuticular membrane bearing a number of long, stiffening ribs anteriorly. The long ribs are sclerotized to a moderate degree but are not raised greatly above the surrounding tymbal membrane. They are arranged approximately but not closely parallel to one another. Interspersed between the long ribs are short ribs, which lie along the line of buckling of the tymbal. In *P. capitata* there are 3 long ribs and 2 short ribs, while in *C. australasiae* there are 4 long ribs and 4 short ones. Posteriorly, each tymbal bears an irregularly shaped plate of relatively stiff cuticle, the tymbal plate (Simmons & Young, 1978). The apodeme from the tymbal muscle inserts dorsally on the tymbal plate, the point of insertion being visible externally (Fig. 1*B*, *C* arrows).

The song patterns

Males of both species produce their calling songs from elevated positions, usually a few metres above the ground, in trees and tall shrubs. Both sing continuously for long periods on hot days and *C. australasiae* also sings briefly at dusk. The calling songs of both *P. capitata* and *C. australasiae* are loud, continuous trains of sound pulses (Fig. 2). The songs are of relatively constant amplitude, unlike those of many cicadas in which there are major low frequency amplitude modulations often associated with abdominal movements (e.g. Pringle, 1954*b*; Hagiwara & Ogura, 1960; Young, 1972*a*, *b*). The pulse repetition frequency in the recorded calling songs averaged 389 Hz (S.D. = 31·9; $n = 4$) for *P. capitata* and 234 Hz (S.D. = 5·3; $n = 3$) for *C. australasiae*. The individual sound pulses (Fig. 2*B*) of both species consist of sinusoidal oscillations with relatively low harmonic content. The modulation envelope of the

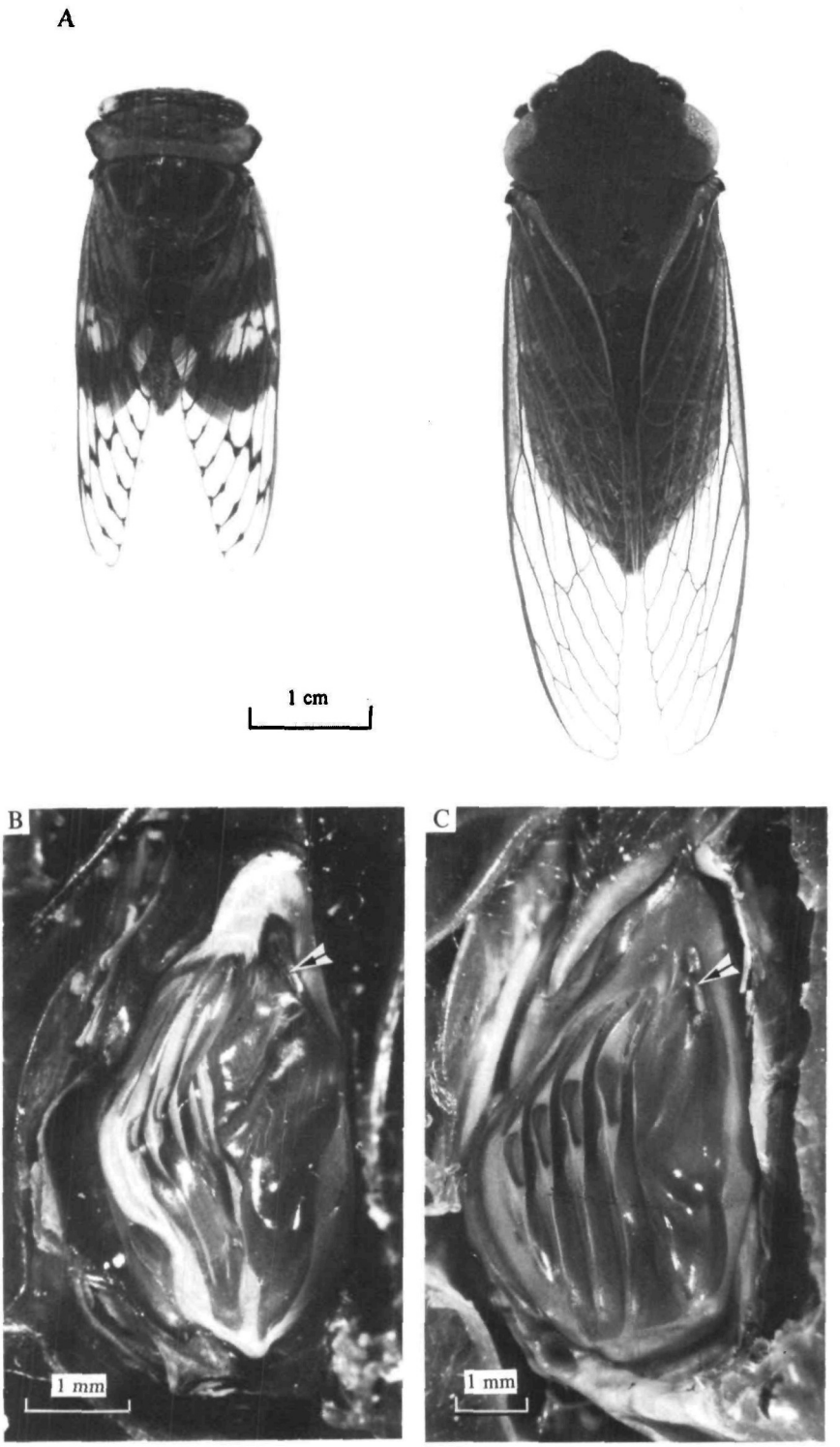

Fig. 1. (A) Preserved specimens of male cicadas: left, *Platypleura capitata* and right, *Cyclochila australasiae*. (B) The tymbal of *P. capitata*. (C) The tymbal of *C. australasiae*. The arrows in B and C indicate the point of insertion of the tymbal muscle. The wings, tymbal covers and part of the 2nd abdominal segment immediately posterior to the tymbal were removed in order to take the photographs in (B) and (C).

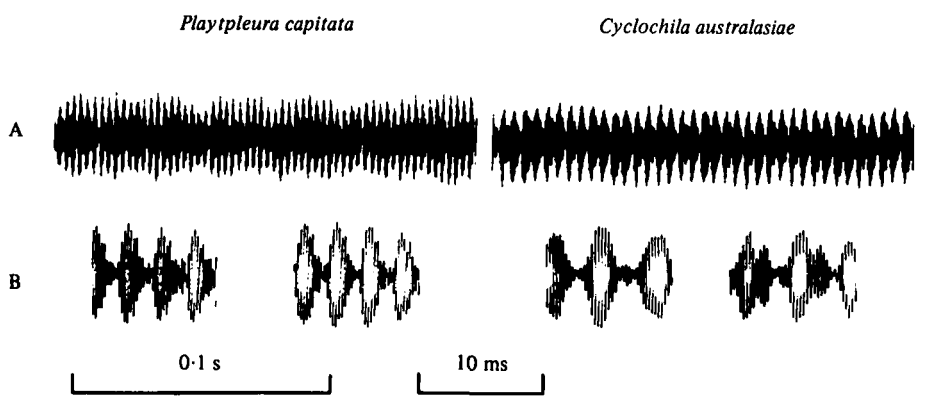

Fig. 2. Oscillograms of the calling songs. (A) Samples from continuous film records showing the train of sound pulses (time-scale: 0·1 s). (B) Single sweep records (two for each species) showing the structure of the individual sound pulses (time-scale: 10 ms).

sound pulses is usually unimodal in *P. capitata* although there are sometimes indications of a second peak. In *C. australasiae* the pulses vary between unimodal and bimodal in shape within individual songs (Fig. 2B). The fundamental frequency of the sound pulses, estimated from oscilloscope traces as in Fig. 2B, is somewhat over 4 kHz for *P. capitata* and close to 4 kHz for *C. australasiae*.

The protest songs, elicited by prodding captive animals, resemble the calling songs in both species, which is by no means always the case in cicadas. The protest song of *P. capitata* is like a short, more irregular segment of the calling song, while in *C. australasiae* the protest song has a distinctly lower pulse repetition frequency than the calling song (Fig. 3B, C). The damping of the sound pulses is greater in protest songs than in calling songs. In *P. capitata* this often renders the pulses clearly bimodal in shape, an effect which is seen even more strongly in pulses following electrical stimulation (Fig. 4). In *C. australasiae* the pulses in protest songs vary between unimodal and bimodal as in calling songs. Pringle termed such bimodal pulses 'double pulses' and provided evidence that they may occur when both the inward and outward movement of the tymbal each generate a pulse. Where the damping is high these appear as double pulses and where damping is less the two pulses merge to become single, i.e. unimodal, as in the calling song of *P. capitata*. Another possibility is that the tymbal may buckle inward in two stages as in *Cystosoma saundersii* (Simmons & Young, 1978). This may well be the case in *Cyclochila australasiae*, where a much quieter sound follows each pulse of the protest song even when these are bimodal. This quieter sound is probably due to the outward movement of the tymbal and is completely obscured in the more powerful calling song. The essential point for the present study is that scrutiny of the protest songs of the two species suggests, in the light of earlier studies, that each tymbal cycle of movement produces either a single pulse or two very close together (= a double pulse). From the resemblance between the calling and protest songs in the two species, we infer that interpretations obtained from experiments with the protest songs may be safely extended to the calling songs.

Silencing one tymbal in *C. australasiae* reduces the pulse repetition frequency of the protest song by one half (Fig. 3B, C), indicating that in the normal protest song

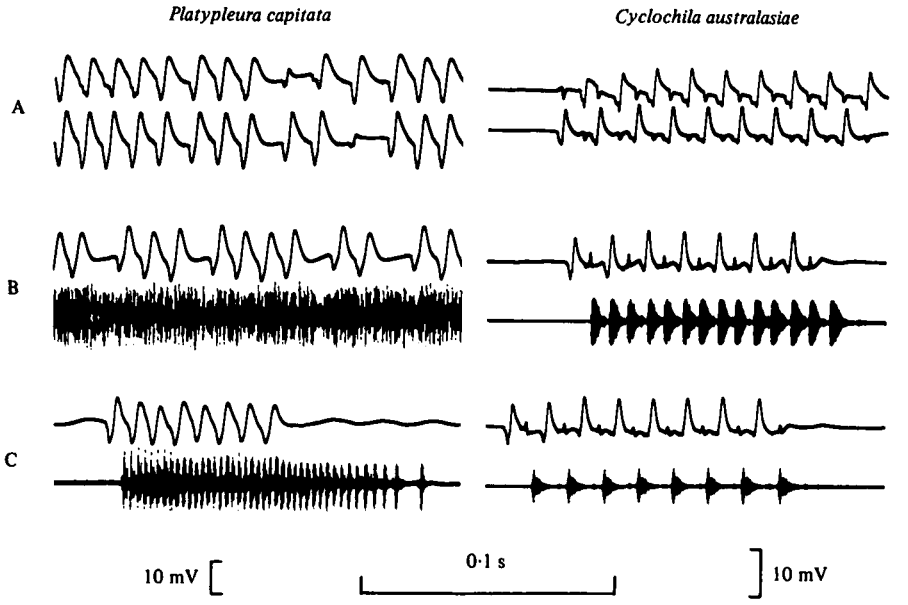

Fig. 3. Protest songs of tethered cicadas. (A) Myograms recorded simultaneously from the two tymbal muscles. (B) Myograms from one tymbal muscle (top trace) and sound output from both tymbals (bottom trace). (C) Myogram and sound record from one side only after destruction of the contralateral tymbal.

the two tymbals buckle in antiphase. In contrast, silencing one tymbal in *P. capitata* does not alter the pulse repetition frequency (Fig. 3B, C), confirming Pringle's (1954*b*) suggestion that in this species the two tymbal muscles contract simultaneously.

Muscle action potentials and sound pulses

In *P. capitata* muscle action potentials in the two tymbal muscles occur simultaneously except for occasional instances in which one muscle produces an action potential but the other does not (Fig. 3A). The pattern of action potentials during protest song is somewhat irregular but the average frequency is of the order of 90 Hz. Irregularities in action potential pattern are poorly reflected in the sound output. Sound pulses continue regularly during short gaps and frequency fluctuations in the action potential train, but decline in frequency during long pauses in the action potentials and stop after 40–60 ms (Fig. 3B). A train of sound pulses lasting beyond the cessation of muscle action potentials is to be expected for asynchronous tymbal muscles because of the slow decay of oscillatory activity following each muscle activation (Pringle, 1954*a*).

Action potentials in the two tymbal muscles of *C. australasiae* are in antiphase with one another (Fig. 3A). Each action potential is followed by a single sound pulse (Fig. 3B). This is seen most clearly in the unilateral preparations (Fig. 3C). Alternating action potentials in the two tymbal muscles seem to be characteristic of cicadas with synchronous tymbal muscles. Alternate firing has been reported in seven genera of cicadas with synchronous muscles (Hagiwara, 1955; Hagiwara & Ogura, 1960; Aidley, 1969; Reid, 1971; Young, 1972*a*). We have found alternate firing of tymbal muscles in nine species of Australian cicadas with synchronous muscles which

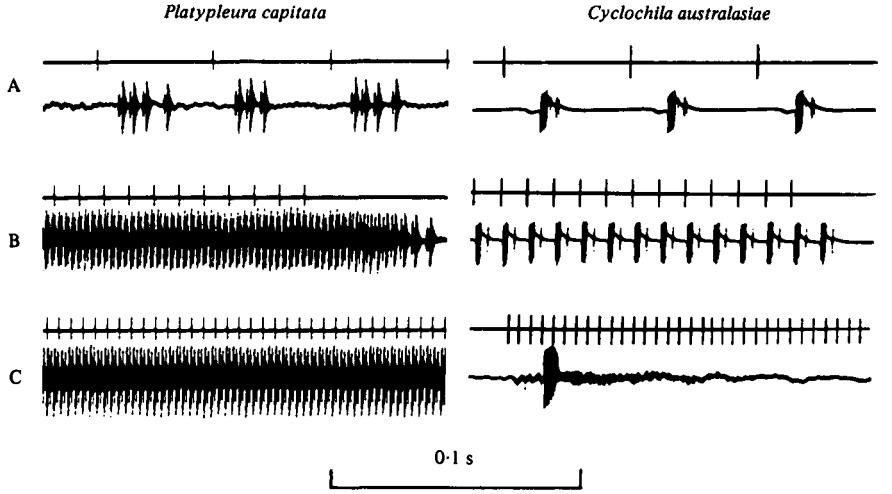

Fig. 4. Sound evoked by electrical stimulation of a single tymbal muscle. (A) 20 Hz; (B) 100 Hz; (C) 200 Hz. In each record, the top trace bears markers of the electrical stimuli and the bottom trace shows the sound output of the tymbal recorded with a microphone. In *C. australasiae* the stimulus markers have been retouched. The small deflection after each sound pulse in *C. australasiae* is probably a click due to the outward movement of the tymbal. Note that it is absent in (C).

we examined, including two (*Arunta perulata, Psaltoda harrisii*) previously reported to contract the two tymbal muscles simultaneously (Young, 1972*a*). Electrical cross-talk between the two recording channels apparently led to the erroneous impression of simultaneous activity in the earlier study. Another species, *Cystosoma saundersii*, is unusual in having tymbal muscles which contract 25% out of phase (Simmons & Young, 1978).

Sound during direct muscle stimulation

Single shocks to the tymbal muscle of *P. capitata* each evoke a short burst of sound pulses, as do individual stimuli of a low frequency train (Fig. 4A). The number of sound pulses per stimulus tends to increase with stimulus frequency up to stimulus frequencies of about 50 Hz when the sound pulse train becomes continuous (cf. Pringle, 1954*a*). At a stimulus frequency of 100 Hz the sound output closely resembles the normal calling song (compare Figs. 2A and 4B), with no modulation at the stimulus frequency. Further increasing the stimulus frequency does not change the sound output pattern, even to stimulus frequencies of 200 to 400 Hz which fully tetanize the isolated tymbal muscle (see below). At the cessation of high frequency stimulation the sound pulse frequency gradually declines to zero (Fig. 4B).

The sound output pattern to direct muscle stimulation is much simpler in *C. australasiae*. At all stimulus frequencies of 100 Hz or less, each stimulus initiates a single sound pulse (Fig. 4). With 200 Hz stimuli, only a single sound pulse is produced: this occurs at the beginning of the stimulus burst and presumably corresponds to the initial shortening of the muscle as it goes into a tetanic contraction (Fig. 4C).

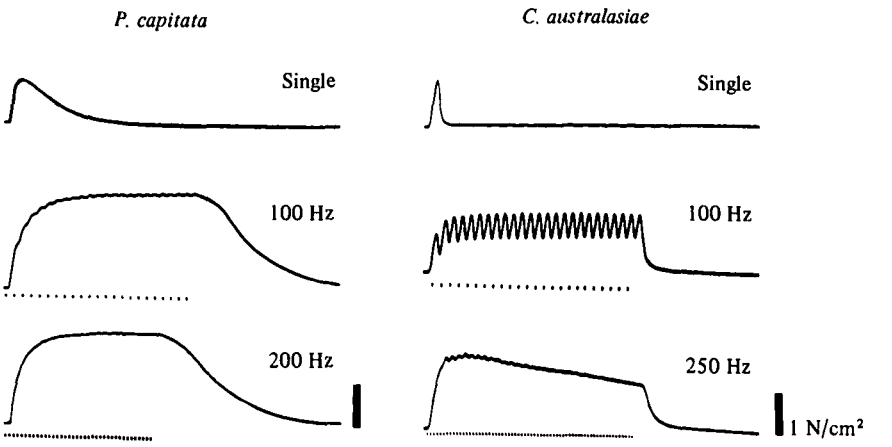

Fig. 5. Isometric tension evoked by direct stimulation of a tymbal muscle. The dots below the lower records mark the stimuli and provide a time calibration for the traces which are all at the same sweep speed. T = 30 °C.

Contraction kinetics

Pringle (1954*a*) and Hagiwara *et al.* (1954) report that each tymbal muscle of cicadas is innervated by a single, rather large motor axon. Recently Wohlers *et al.* (1979) found several small axon profiles in addition to that of the large tymbal motor axon in the branch of the auditory nerve which goes to the tymbal muscle of *Magicicada*. These authors suggest that the small axons are efferent neurones which innervate the tymbal muscle. In our tension measurements from several species, there have been no hints of multiple innervation of the tymbal muscle. The muscles examined gave all-or-nothing twitches with a single sharp threshold whether the stimuli were applied to the tymbal nerve or directly to the tymbal muscle through implanted electrodes. The responses to direct and nerve stimulation are essentially identical, indicating that even with direct stimulation the muscle fibres are being activated through branches of the motor axon which course through the muscle.

Isometrically-contracting tymbal muscles from *P. capitata* are much slower than those from *C. australasiae* (Fig. 5, Table 1). The twitch rise time in *P. capitata* is about twice and the total twitch duration about six times that of *C. australasiae*. Area-specific tetanic tension, however, is about twice as great in *P. capitata* as in *C. australasiae*. Because of the longer twitch duration, the tetanic fusion frequency in *P. capitata* is much lower than in *C. australasiae*. It should be noted that the tymbal muscle action potential frequency during protest calls of *P. capitata* (approximately 90 Hz) is adequate to give nearly smoothly-fused tetanic contractions whereas in *C. australasiae* the action potential frequency during protest songs (about 70 Hz, Fig. 3) is well below the tetanic fusion frequency (Fig. 5). Assuming that the tymbal muscles of *C. australasiae* contract in antiphase during calling songs as they clearly do during protest calls, the contraction frequency during calling songs may be inferred to be one-half the sound pulse frequency or about 120 Hz. In Fig. 5 there was considerable fusion of tension when the tymbal muscle of *C. australasiae* was stimulated at 100 Hz and the muscle was able to relax only partially between tensions

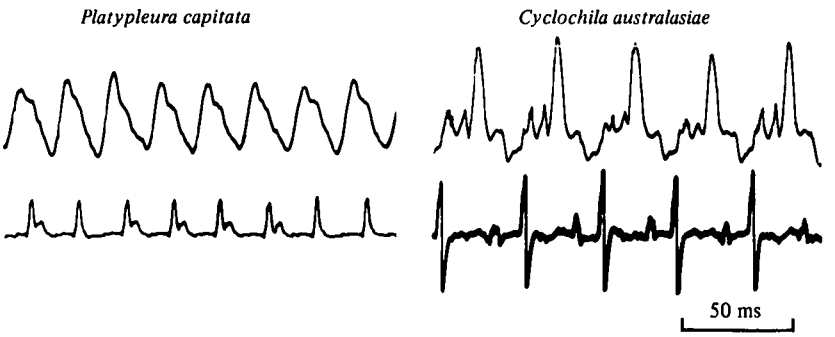

Fig. 6. Thrust generated by wing strokes (upper traces) and action potentials from the dorsal longitudinal flight muscles (lower traces) during flight.

peaks. At 120 Hz stimulation there would be even more fusion and less tension fluctuation. The record in Fig. 5 was taken at 30 °C. *C. australasiae*, like many other cicadas, sings principally in the sun; its body temperature may be well above 30 °C when singing. Further, the temperature of the tymbal muscle almost certainly rises during singing activity (cf. Josephson & Young, 1979). Warming the muscle decreases the twitch duration (Josephson, 1979) so the actual fusion of tension during singing may be considerably less than might be anticipated from Fig. 5.

Table 1. *Some parameters of isometric contractions*

(Mean ± S.E., 30 °C.)

	P. capitata ($n = 8$)	*C. australasiae* ($n = 7$)
Twitch rise time (ms)	15·9 ± 1·3	8·5 ± 0·4
Twitch duration, onset to 90% recovery (ms)	106·8 ± 5·8	18·3 ± 1·5
Tetanic tension (Ncm^{-2})	4·32 ± 0·47	2·27 ± 0·17

Wing movements and muscle action potentials in flight muscles

The wing stroke frequency during tethered flight is about 50 Hz in *P. capitata*, 30 Hz in *C. australasiae* (Fig. 6). In both species there is a 1:1 correspondence between wing movements and muscle action potentials. The flight muscles are synchronous, even in *P. capitata* in which the tymbal muscles are asynchronous. It was earlier concluded from ultrastructural evidence that the flight muscles of cicadas are synchronous (Smith, 1966; Cullen, 1974). The physiological evidence confirms this conclusion.

Tymbal muscle structure

The ultrastructure of tymbal muscles from the two species is compared in Fig. 7 and some quantitative features of muscle morphology are summarized in Table 2. The most obvious structural difference between the two muscles is in the development of the sarcoplasmic reticulum (SR) which is sparse in the muscle from *P. capitata* and well developed in that from *C. australasiae*. In *P. capitata* the SR occurs as single tubules encircling the middle of each sarcomere which are joined to occasional longitudinal tubules. The longitudinal tubules, in turn, connect with scattered SR

Table 2. *Structural features of the tymbal muscles*

(Mean ± S.E. $n = 3$)

	P. capitata	*C. australasiae*
Sarcomere length (μm)	2·35 ± 0·05	2·64 ± 0·08
Maximum myofibril diameter (μm)	1·31 ± 0·05	0·99 ± 0·07
Myofibril cross-sectional area (μm²)	1·12 ± 0·07	0·56 ± 0·08
% muscle fibre as		
Myofibril	49·2 ± 0·4	38·0 ± 0·7
Mitochondria	39·2 ± 0·6	41·9 ± 1·1
SR and T system	2·7 ± 0·3	14·9 ± 0·7

elements at the level of the Z lines. Diadic contacts of conventional appearance (Smith, 1966; Elder, 1975) occur between the SR and transverse tubules, usually on or near the SR circlet surrounding the fibril. In *C. australasiae* the SR forms layers several tubules thick entirely surrounding the myofibrils. The transverse tubules of *C. australasiae* ramify through the fibres and form triadic contacts with the SR near the middle of each half sarcomere. The myofibrils of *P. capitata* are somewhat larger than those of *C. australasiae* and are circular in section, while in *C. australasiae* the myofibrils are polygonal, often nearly square. Other than the considerably greater development of SR in *C. australasiae* and the larger, more circular fibrils of *P. capitata*, the muscles are quite similar. Sarcomeres are nearly the same length in each muscle and in each about 40% of the muscle fibre volume is occupied by large mitochondria. M lines, which are generally prominent in asynchronous flight muscle (reviewed by Elder, 1975), are not obvious in the tymbal muscles of either *P. capitata* or *C. australasiae*. And, as is general in asynchronous muscles and common in fast synchronous muscles of insects (Elder, 1975), thin myofilaments lie midway between each thick filament giving a thin : thick ratio of 3 : 1 in the lattice.

DISCUSSION

The identification of asynchronous muscle

Asynchronous muscle has arisen at least nine to ten times in different insect groups (Cullen, 1974). Despite their diverse origins, asynchronous muscles in all insects share several basic structural and physiological features and form a rather homogeneous set. The difference between synchronous and asynchronous muscles are aptly illustrated by the two tymbal muscles considered here. The features which mark the tymbal muscle of *P. capitata* as asynchronous, and which can be used as criteria for the identification of asynchronous muscles in general, are the following:

1. There is not a 1 : 1 correspondence between electrical activity of the muscle membrane and contraction. This is, of course, the definition of asynchronous muscle. With flight muscle it can be assumed that there is a 1 : 1 relation between wing movement and muscle contraction. Therefore the demonstration that muscle action potentials are not 1 : 1 with wing movement during flight is definitive evidence that the wing muscles are asynchronous. In our study, tymbal muscle contraction during singing was not measured directly but rather inferred from the sound produced. However, the relationship between tymbal muscle contraction and sound pulses depends on the construction of the tymbal, which varies in different species. In

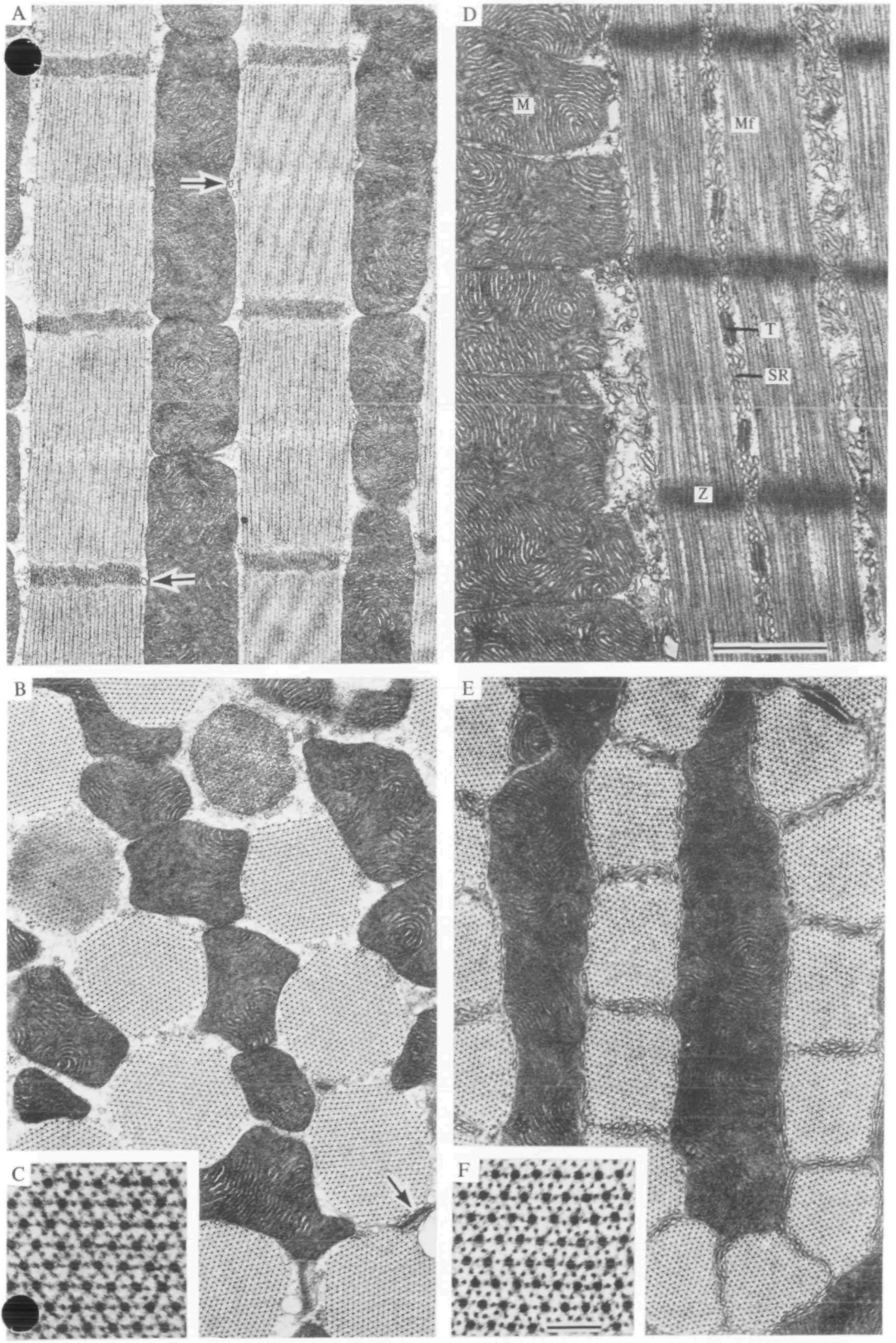

Fig. 7. Ultrastructure of tymbal muscles from *P. capitata* (left) and *C. australasiae* (right). (A), (D) Longitudinal sections. B–F transverse sections. The scale in (D) is 1 μm and applies also to (A), (B) and (E). The scale in F is 0·1 μm and applies to (C) and (F). The arrows in (A) and (B) indicate SR tubules. Abbreviations: M, mitochondrion; Mf, myofibril; SR, sarcoplasmic reticulum; T, transverse tubule; Z, Z-line.

particular, the tymbals of cicadas in the genera *Magicicada* and *Abricta* are able to produce 8 or 9 sound pulses for each muscle contraction. Thus in these cicadas, each tymbal muscle action potential is followed by a short burst of sound pulses even though the muscles are clearly synchronous (Reid, 1971; Young, 1972). The tymbals of *Abricta* and *Magicicada* have a distinctive configuration with about 12 long ribs, which are closely parallel to each other and raised well above the tymbal membrane. The tymbal of *P. capitata* does not resemble this configuration but rather is similar to that of *C. australasiae* (Fig. 1*B*, *C*) which, judging from Figs. 3 and 4, produces only one double pulse per muscle contraction. This makes it probable that each double pulse in *P. capitata* is produced by a separate muscle contraction. Therefore, the lack of correspondence between sound pulses and muscle action potentials (Fig. 3) is good evidence that the muscle is asynchronous.

2. The isometric twitch duration is long compared to the cycle length during presumed oscillatory activity. The sound pulse frequency in a freely-singing *P. capitata* is 389 Hz, indicating that the tymbal muscle can contract and relax partially in 1/389 sec (= 2·6 ms) if the tymbal muscles contract simultaneously as they do during protest song, or 1/195 s (= 5·1 ms) if the tymbal muscles contract alternately during calling song. Either time is a small fraction of the isometric twitch rise time (= 15·9 ms). The tymbal muscle cannot be turned on and off rapidly enough by its neural input to account for its high contraction frequency, and therefore the muscle cannot be synchronous.

3. The muscle does not produce a fused tetanic contraction to high frequency stimulation if it is attached to an appropriate, resonant load or to a click mechanism such as that of a tymbal which is an alternative to a resonant load in creating a phase difference between length and tension changes. Stimulating the *in situ* muscle of *P. capitata* at 200 Hz produces a train of sound pulses, even though this frequency is much greater than that required to give a smoothly fused tetanus when the muscle contracts isometrically (compare Figs. 4 and 5). It must be inherently oscillatory properties of the fibres which allow the muscle to contract and relax repeatedly, even when the stimulus input is adequate to maintain the muscle in a continuously activated state. In contrast to the behaviour of the *P. capitata* muscles, stimulating the synchronous tymbal muscle of *C. australasiae* at 200 Hz produces a single sound pulse as the muscle tetanically contracts and remains contracted until the termination of the stimuli. Similarly, stimulating wing muscles at tetanizing frequencies does not block rhythmic wing movements in insects with asynchronous flight muscles (Boettiger, 1951; Roeder, 1951), while it causes sustained tetanic contraction and cessation of wing beating in insects with synchronous muscles (Heidermanns, 1931).

4. SR is sparse although the contraction frequency is high. This is, in a sense, an alternative way of expressing number 2 above. In striated muscle in general there is an inverse relation between the development of the SR and twitch duration (reviewed by Elder, 1975; Josephson, 1975). Sparse development of the SR indicates long twitch duration which, in a synchronous muscle, is incompatible with high-frequency contraction. Therefore the combination of high frequency performance and little SR is prima facie evidence for asynchronous muscle.

Cullen (1974) reviewed ultrastructural differences between synchronous and asynchronous flight muscles of insects and concluded that abundance of SR, myofibril

diameter, and distribution of diads were reliable criteria for distinguishing asynchronous from synchronous muscle. Myofibrils are larger in asynchronous than in synchronous muscles, SR and T systems are less well developed, and diads are more irregular in position. We would like to emphasize, however, that myofibril diameter is not a reliable indicator of asynchronous muscle, and neither SR abundance nor T system distribution by themselves can be used to uniquely identify asynchronous muscle.

In general the myofibrils of asynchronous muscles are unusually large, typically 1·5–4 μm in diameter (Cullen, 1974; Elder, 1975). In *P. capitata*, however, the maximum diameter of myofibrils averages 1·3 μm and the average diameter is about 1·2 μm. This is within the range of flight muscles identified as synchronous in Homoptera and less than that of muscles thought to be asynchronous in Homoptera and Heteroptera (Cullen, 1974). On the basis of myofibrillar diameter, the tymbal muscles of *P. capitata* would be classified as synchronous even though on physiological grounds they are clearly asynchronous. SR is poorly developed in asynchronous muscles, but also in slowly-contracting synchronous muscles. For example, in the tonic portion of the extensor tibia muscle of the locust *Schistocerca gregaria*, a slow, synchronous muscle, the SR forms only 1·1 % of the fibre volume (Cochrane, Elder & Usherwood, 1972), even less than the relative SR volume in the tymbal muscle of *P. capitata*. The abundance of SR, or lack thereof, is a useful criterion only if combined with information on the contraction frequency. The T system and diads are irregularly dispersed in asynchronous muscles but this is also true in visceral and cardiac striated muscles of insects (Smith, 1966; Smith, Gupta & Smith, 1966; Sanger & McCann, 1968), so irregular diad distribution by itself is also not diagnostic of asynchronous muscle.

Asynchronous or fibrillar or myogenic?

Three different terms have been used to identify the potentially oscillatory muscles of insects: asynchronous, referring to the lack of 1 : 1 correspondence between muscle electrical activity and rhythmic contraction (Roeder, 1951); fibrillar, based on the ease with which myofibrils can be discerned in teased material (Kölliker, 1888); and myogenic, indicating that the frequency of oscillatory contraction is determined by the properties of the muscle and its resonant load and is relatively independent of the pattern of activity in motor neurones from the central nervous system (the dichotomous partner of 'myogenic' is 'neurogenic'). 'Muscle with myogenic rhythmicity' is a more appropriate phrase than 'myogenic muscle', but the latter has been used (e.g. Wilson, 1968; Elder, 1975). This multiplicity of labels for the self-oscillatory muscles of insects is bound to be confusing to the non-specialist, and each of the terms is misleading to some extent. On balance 'asynchronous' seems the preferable label.

The myofibrils can be readily demonstrated in teased preparations of 'fibrillar' muscle because the myofibrils of these muscles are typically unusually large, and because the myofibrils are readily separable, presumably because they are not bound together by sheaths of SR (Cullen, 1974). Teasing apart muscle fibres is today a little practiced skill. With the widespread use of standard histological techniques and especially the advent of electron microscopy, the emphasis in identifying 'fibrillar' muscle has centred on fibril size rather than separability. But, as shown above, fibril

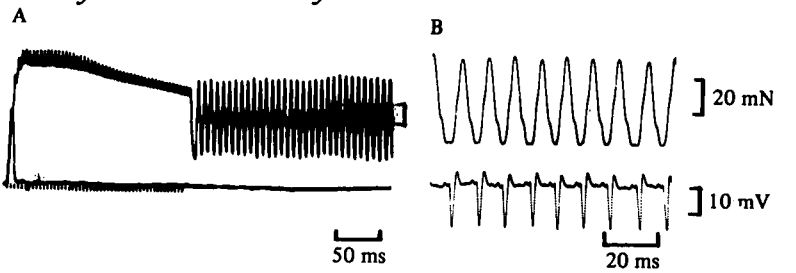

Fig. 8. Myogenic oscillation in isometric tension by a synchronous tymbal muscle. The species is *Psaltoda argentata*. (A) The muscle was first given a single shock (twitch to left) and several seconds later a burst of stimuli at 250 Hz (stimuli marked by dots below trace). Following the stimulus burst the muscle began contracting rhythmically at 160 Hz. T = 35 °C. Scale bar = 20 mN. (B) Isometric tension (upper trace) and muscle action potentials (lower trace) from another preparation during rhythmic contraction like that following the stimulus burst in (A) T = 28 °C.

from the tymbal muscle of *P. capitata* have diameters within the range of those from many synchronous muscles, even though this tymbal muscle is clearly asynchronous and physiologically like 'fibrillar' flight muscles. Further, the contractile filaments of most striated muscles are grouped in bundles termed myofibrils. Because most striated muscles are fibrillar in organization, and because asynchronous muscle need not have unusually large myofibrils, 'fibrillar' seems a poor descriptor of the class of potentially oscillatory muscles.

The term myogenic was applied to smooth and cardiac muscles with rhythmic contraction that is essentially independent of neuronal input long before the term was used for the oscillatory flight and tymbal muscles of insects. The rhythmic activity of myogenic smooth and cardiac muscle is fundamentally different from that of 'myogenic' insect muscle. In myogenic smooth and cardiac muscles, the contraction is associated with rhythmic changes in membrane potential. Thus a myogenic heart is composed of synchronous muscle, muscle in which there is a 1:1 correspondence between electrical and mechanical activity. The term myogenic, then, does not distinguish between asynchronous insect muscles and the larger set of muscles whose rhythmic activity is directly controlled through membrane potential change.

Another complication to the use of 'myogenic' turns up in some cicada muscles. Members of the genus *Psaltoda* have fast, synchronous tymbal muscles. In isometric tension measurements which we have made from three *Psaltoda* species (*P. argentata*, *P. claripennis*, *P. harrisii*), on occasion, especially following a burst of stimuli to the muscle, the muscle suddenly began contracting rhythmically in the absence of additional stimuli (Fig. 8A). The rhythmic contractions could continue for tens of seconds. The tymbal nerves had been cut in these experiments so the oscillatory activity was generated by the muscle fibres or possibly nerve terminals in the muscle, and was not due to rhythmic output along motor neurones from the central ganglion. The amplitude of the rhythmic contractions was similar to that of twitches evoked by electrical stimulation (Fig. 8A), indicating that most or all of the fibres in the muscle were contracting synchronously. During autogenous activity there is an action potential associated with each contraction (Fig. 8B). Similar rhythmic electrical and mechanical activity has been recorded from other cicada muscles (Wakabayashi & Ikeda, 1961) and may be related to the miniature electrical oscillations recordable with intracellular or extracellular electrodes from these muscles (e.g. Wakabayashi & Hagiwara,

1953; Hagiwara, 1953; Ikeda, 1959). The origin of the autogenous activity is obscure as is the mechanism coordinating the many individual fibres of the muscle so that they contract synchronously. The point to be made is that under some, as yet undefined, conditions the tymbal muscles of some cicadas can contract rhythmically without neural input yet with an action potential associated with each contraction. Thus the muscles can be simultaneously myogenically rhythmic and synchronous, emphasizing that myogenic and asynchronous are not synonomous concepts even in tymbal muscles.

There is also a problem associated with the term asynchronous. During oscillatory contraction against a resonant load there is asynchrony between electrical and mechanical events of some insect flight and tymbal muscles, yet during isometric contraction of the same muscles there is a 1:1 correspondence between electrical and mechanical activity. The same muscle can be synchronous or asynchronous depending on its mode of operation. Nonetheless, of the three terms used to identify the potentially oscillatory muscles of insects, asynchronous seems the least misleading and we urge its general adoption.

The evolution of asynchronous muscle

The flight muscles of primitive insects are synchronous while those of many advanced insects are asynchronous, which suggests that asynchronous muscles evolved from synchronous ones (Pringle, 1957*a*; Cullen, 1974). The tymbal muscles of cicadas probably evolved from synchronous, segmental, abdominal muscles (Pringle, 1957*b*); here too asynchronous muscles are apparently derived from synchronous muscles. Pringle (1957*c*) argues that high pulse frequency is advantageous for sound communication in cicadas, and that asynchronous operation is an adaptation for high-frequency contraction. If this line of argument is correct, the path of evolution was from fast synchronous muscles, operating near limiting frequencies, to asynchronous ones capable of even higher frequencies. There are two problems in the conversion of synchronous into asynchronous muscles without disruption of functional continuity: (1) alteration of myofibrillar properties, allowing oscillating contraction against resonant loads, and (2) alteration of the contractile control systems setting a permissive background for oscillatory activity.

The myofibrillar property which allows oscillation is delayed activation of the contractile apparatus following stretch and delayed inactivation upon release (Pringle, 1978). Stretch activation and inactivation by release seem to be properties of many muscles (Aidley & White, 1969; Steiger, 1977) but ones which are especially emphasized in asynchronous flight and tymbal muscles. Pringle (1957*c*) proposed that, in the course of evolution, augmentation of stretch activation and inactivation upon release in fast, synchronous muscles of insects accelerated tension rise and decay, thus decreasing twitch fusion in muscles operating at high frequency. This permitted an increase in the frequency of contraction beyond the limit previously set by tetanic fusion. It is only a small step from this to asynchronous operation.

The conversion of the control systems is, perhaps, a more difficult evolutionary task. Contractile activity in striated muscles, both synchronous and asynchronous, is controlled by the cytoplasmic calcium concentration. Membrane depolarization causes relase of calcium from the SR to initiate contraction and calcium resequestr

on by the SR terminates contraction. In fast, synchronous muscles, such as the tymbal muscle of *C. australasiae*, the SR is well developed, which both decreases calcium diffusion distance (hence diffusion time) and increases the surface area of SR available for calcium transport. This makes possible the rapid fluctuations in calcium levels which are required to turn on and off each cycle. In those asynchronous muscles which have been examined, the time-course of calcium movement is slow, as indicated by a long twitch duration (Fig. 5; Boettiger, 1957; Pringle, 1954*a*) and the low frequency of neural input required to keep the muscle activated. Asynchronous muscle is turned on by elevated cytoplasmic calcium concentration which activates the myofibrils, thereby allowing oscillation. Continued oscillatory activity requires maintained calcium levels without major fluctuation, which might, if the calcium concentration dropped sufficiently, lead to biochemical inactivation and the termination of oscillatory activity. Thus, in terms of muscle activation, the requirements for fast synchronous muscle and for asynchronous muscle are antithetical. The rapid release and resequestration of calcium in fast synchronous muscles would seem to prevent asynchronous activity, while the slow movement of calcium in asynchronous muscles would not allow rapid synchronous activity.

One possibility is that the evolutionary transition from synchronous to asynchronous operation was abrupt and involved an increase in the frequency of neural input to the muscle to tetanizing levels. This would produce sustained, high calcium concentration in the muscle fibres. If stretch activation were a well developed property of the muscle, and if the muscle were connected to a resonant load such as that provided by a tymbal, then tetanically activating the muscle would result in oscillatory contraction with asynchronous control. The immediate advantages of this change are not obvious. The potential advantages which might accrue, once asynchronous control is accomplished, are significant. The contraction frequency, no longer limited by calcium cycling time, could be increased considerably through evolutionary time. The frequency of motor neurone impulses to the muscle could be reduced if this reduction were accompanied by a reduction in the rate of calcium movement so that full muscle activation was still maintained. The benefits, then, are a reduction in the metabolic costs associated with calcium transport, and a concomitant decrease in the necessary investment in SR and hence an increase in space available for myofibrils or mitochondria. In all known asynchronous muscles, the neural activation frequency is quite low during oscillatory activity. We suggest that this condition was reached through a transitional stage in which the activation frequency was very high, high enough to tetanize a fast, basically synchronous muscle. This transitional stage may no longer be represented in any living insects.

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