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THE EVOLUTION OF FIBRILLAR MUSCLE IN INSECTS

By J. W. S. PRINGLE

Department of Zoology, Oxford

Fibrillar muscle is a peculiar type of striated muscle found only in the power-producing flight muscles of certain higher Orders of insects and in the main timbal* muscle of the sound-production system of certain cicadas. It is recognizable both physiologically and histologically and is distinguishable from the tubular and 'close-packed' striated muscles found in nearly all other orders of insects in the flight system (Pringle, 1975). There are good reasons for thinking that tubular and close-packed muscles are the more primitive evolutionarily and that fibrillar muscle has arisen independently many times in the evolution of insects. Since it is inconceivable that, at any stage in these evolutionary developments, there was a loss of the capacity for flight or of sound-production, the problem arises of determining how the change could have come about. This is the subject of this lecture.

I must first describe in some detail the differences between the types of striated muscle in insects. Fibrillar muscle is often called 'asynchronous' muscle because of its most important physiological characteristic, which was, indeed, the means for its discovery (Pringle, 1949). The asynchronous mode of operation is easily demonstrated by an experiment which is now repeated annually by students at Oxford. A living insect is glued to a stiff wire in such a way that it can perform normal wing movements and the wire is, in turn, mounted on an electromechanical transducer such as a gramophone pick-up. Fine wire electrodes are inserted into the thorax so that they record the potentials from the thoracic muscles. The insect is started into tethered flight by removing a pith ball from the grasp of the legs.

If this experiment is repeated with different species of insect (Fig. 1) the difference in the physiology of control of the flight muscles is very obvious. In the first two cases (a cockroach and a moth) the potentials and the contractions of the flight muscles are *synchronous*; that is, they occur at the same frequency. In the last two cases (a fly and a wasp) there is no temporal correlation between the two traces; and control is *asynchronous*.

In order to understand the mechanism of the difference between the two types of muscular control, we must look at the sequence of events when a striated muscle is excited through its motor nerve (Fig. 2). The impulse in the nerve is transmitted across the neuromuscular junction and leads to excitation of the surface membrane of the muscle cells. The processes of intra-muscular excitation-contraction coupling then produce activation of the contractile myofibrils. The excitation-contraction coupling is, in fact, two processes in sequence. The excitation of the membrane of the

* I now feel that this is the correct spelling.

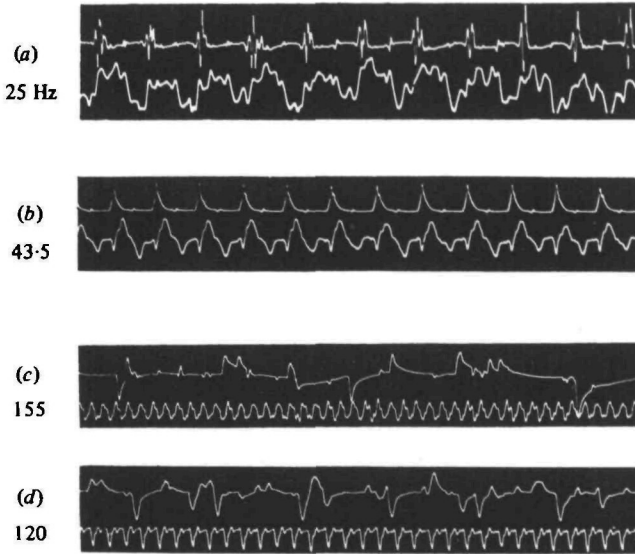


Fig. 1. Electrical (upper trace) and mechanical (lower trace) records from the thorax of tethered flying insects. (a), (b) Synchronous type: (c), (d) asynchronous type. (a) Cockroach: (b) moth: (c) fly: (d) wasp. The wing-beat frequency is shown in each case. (Adapted from Pringle, 1957.)

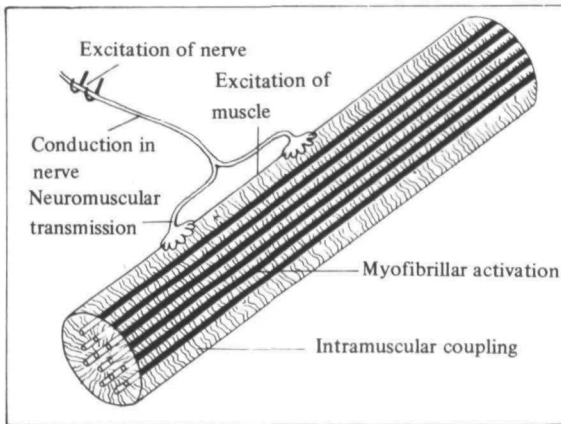


Fig. 2. Diagram showing the sequence of events when a striated muscle is excited by stimulation of its motor nerve. (Pringle, 1975.)

fibre is carried inwards by the tubules of the T-system, which are invaginations of the plasma membrane. The mainly transverse tubules make close contact by diads or triads with the second internal membrane system, the sarcoplasmic reticulum (SR), which consists of the network of closed vesicles containing a relatively high concentration of calcium ions. The excitation leads to the release of Ca^{2+} from the sarcoplasmic reticulum into the general sarcoplasm surrounding the myofibrils and it is this rise of intracellular Ca^{2+} that produces activation of the actomyosin enzyme system of the myofibrils. When excitation stops, the sarcoplasmic reticulum takes up Ca^{2+}

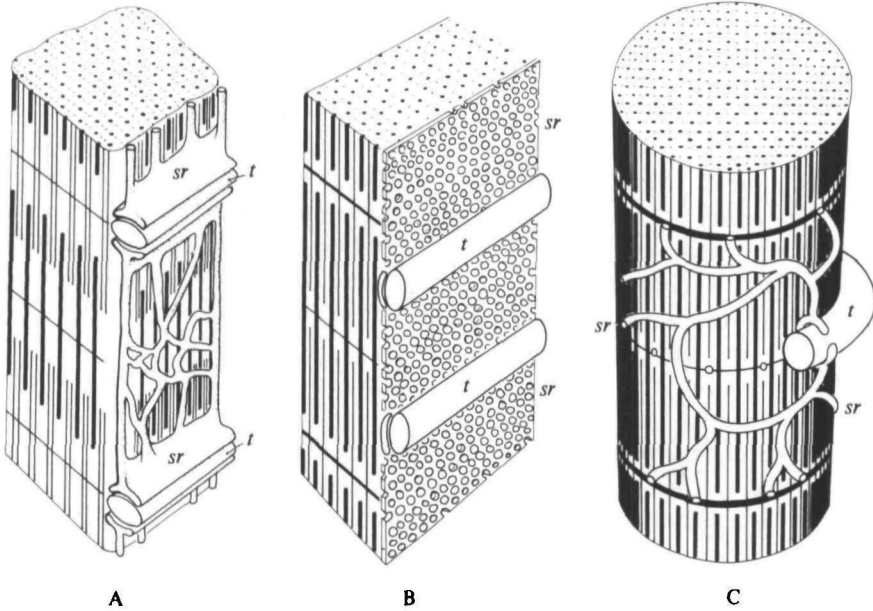


Fig. 3. Reconstructions of the arrangement of sarcoplasmic reticulum (sr) and transverse tubules (t) in (A) vertebrate skeletal muscle; (B) synchronous insect muscle; (C) asynchronous insect muscle. (Information from various sources, drawn by J. Rodford.)

reducing the sarcoplasmic concentration from the 10^{-7} M required for activation to less than 10^{-9} M.

In vertebrate striated muscle and in synchronous insect flight muscle, the surface area of the SR is sufficiently large (Fig. 3) for the rise and fall of sarcoplasmic Ca^{2+} to take place in as little as 10 ms and the muscles can twitch rapidly. This movement of Ca^{2+} takes place at each contraction of the thoracic muscles: that is at each wing beat. In the best-known asynchronous or fibrillar flight muscles the SR is reduced, leading to a much slower rate of change of sarcoplasmic Ca^{2+} . In a fly or a wasp, quite a low frequency of nerve impulses is sufficient to maintain a constant raised level of Ca^{2+} in the environment of the myofibrils and the contractions and relaxations are brought about by a different mechanism, residing entirely in the myofibrils.

To find out what is the property of fibrillar muscle that enables it to generate a series of contractions at constant Ca^{2+} , it is necessary to isolate the myofibrils. The most suitable preparation for this is the dorsal longitudinal muscle of large tropical water bugs of the genus *Lethocerus*. The muscles are treated with 50% glycerol/water in a buffer solution which destroys the continuity of the plasma membrane and then, if necessary, with a non-ionic detergent such as Tween-80 or Triton X. The myofibrils of most fibrillar muscles are distinct and of large diameter (Fig. 4A) and after this treatment a single fibre consists of little more than a bundle of about 200 myofibrils (Fig. 4B). This bundle is mounted on apparatus by means of which controlled length changes can be imposed and the tension measured. The bundle is then immersed in a buffered solution of the right ionic strength to mimic the internal environment of the cell and the Ca^{2+} concentration is controlled with the chelating agent

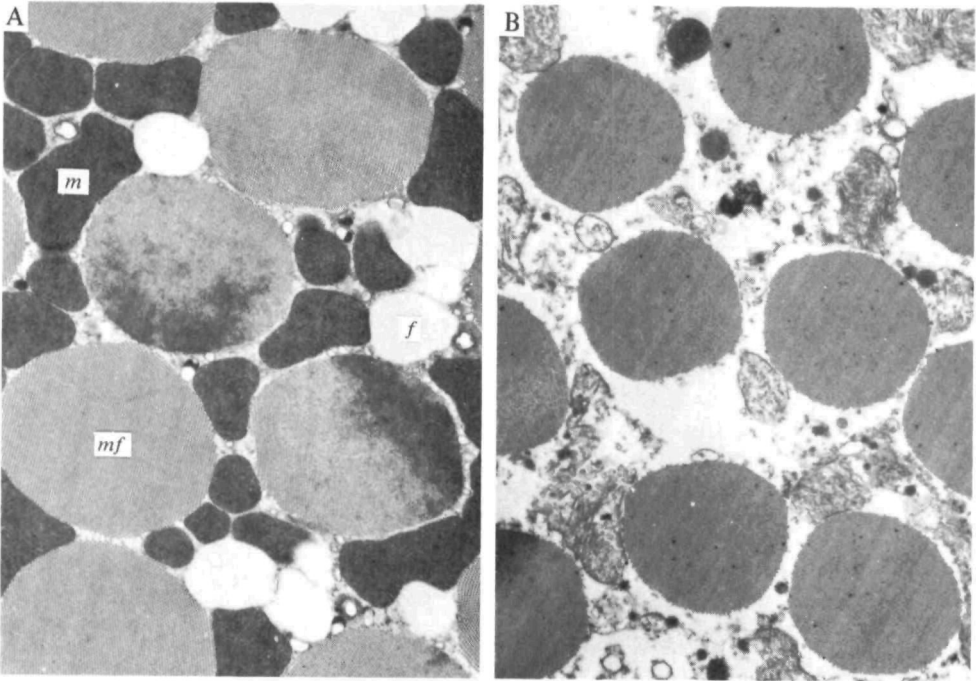
EGTA. With MgATP^{2-} in the solution and a Ca^{2+} concentration of less than 10^{-8} M, the myofibrils are relaxed. When the Ca^{2+} concentration is raised, one has the equivalent of normal activation.

The most easily analysed length changes to impose on the activated myofibrils are square-wave quick stretch and quick release or sinusoidal modulation. Quick stretch or quick release lead to delayed changes of tension (Fig. 5*a*). Low-amplitude sinusoidal modulation of length produces sinusoidal changes of tension that are delayed in phase relative to the length; a simultaneous display of tension and length on *Y* and *X* plates of the oscilloscope shows a loop rotating in an anticlockwise direction (Fig. 5*b*). For length changes of 0.1 % or less, the responses to quick length steps are symmetrical and the loop is an ellipse. At 2.0 % length change, as in Fig. 5*b*, there is some non-linearity, but the loop has a larger area. The area of the loop is a measure of the amount of work being done by the preparation on the apparatus at each cycle of oscillation.

This delayed change of tension in Ca^{2+} -activated fibres is all that is required to enable the muscle to feed energy into the mechanically resonant system of the thorax and wings in the intact insect. Fig. 6 shows an earlier experiment on the living basalar muscle of a rhinoceros beetle, *Oryctes* (Machin & Pringle, 1959). These are tension/length plots, as displayed on the oscilloscope. Graph (*a*) is the curve for the unstimulated muscle; graph (*b*) is the curve for tetanically stimulated muscle under isometric recording conditions, when length changes are prevented. The loop (*c*) is the response when the load consists of a mechanical inertia. This load, coupled with the muscle's own elasticity, generates a mechanical resonance and the stimulated muscle feeds energy into this, maintaining the oscillation for as long as the stimulus persists. At this large amplitude of oscillation, the response is very non-linear.

So the essential property for asynchronous behaviour is the delayed tension response to changes of length. When this result was first described, it was difficult for physiologists to accept it, since the analysis of mechanical properties by A. V. Hill specifically excluded the possibility that changes of length could have a direct effect on the 'active component' of contraction (Pringle, 1960). In fact, there had already been isolated reports of oscillatory behaviour in glycerol-extracted rabbit muscle (Goodall, 1956; Lorand & Moos, 1956). Oscillatory behaviour was later shown to occur in living frog fibres and also delayed tension changes after quick-stretch (Armstrong, Huxley & Julian, 1966) but the amplitude of these phenomena was small and seemed functionally insignificant. Finally, Aidley & White (1969) showed a clear delayed tension in the timbal muscle of a cicada in which the normal song was produced by a synchronous mechanism and Rüegg, Steiger & Schädler (1970) showed marked activation by stretch in mammalian skeletal muscle. The most recent paper is by Kawai & Brandt (1980), in which they identify the property clearly in rabbit psoas, frog semitendinosus and a fast leg flexor muscle of the crayfish leg. It is now clear that the delayed tension of stretch activation is a general property of striated muscles. Does it have any function in normal synchronous muscle?

In vertebrate striated muscle, it seems unlikely that there is any functional advantage. Quick stretches and releases are not normally encountered in life. Periodic activity, even in the rhythmic swimming oscillations of a fish or the beating of a bird's



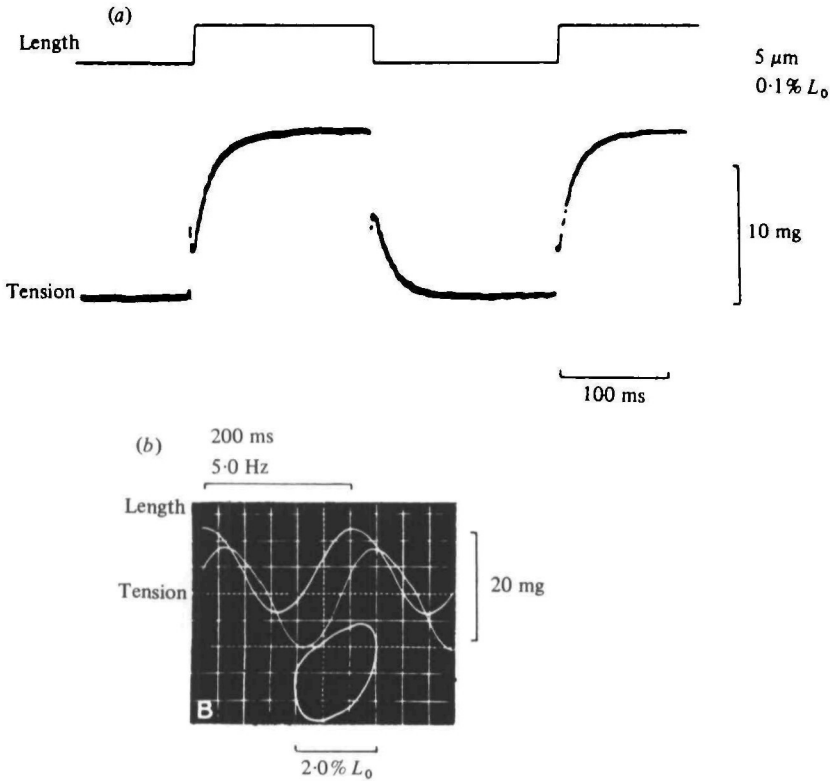


Fig. 5. Tension changes in glycerol-extracted *Lethocerus* muscle with (a) quick stretch and release, (b) sinusoidal modulation of length. The loop in (b) shows the same results as a tension/length plot. L_0 is the mean length. (Pringle, 1979.)

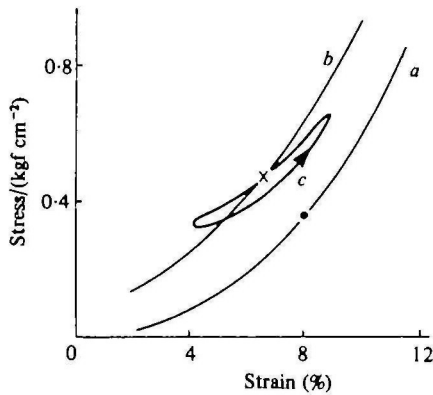


Fig. 6. Stress-strain curves for living beetle flight muscle. (a) Unstimulated; (b) stimulated with isometric or damped isotonic recording; (c) oscillation when the load contains an inertia. $1 \text{ kgf} \sim 9.81 \text{ N}$. (Machin & Pringle, 1959.)

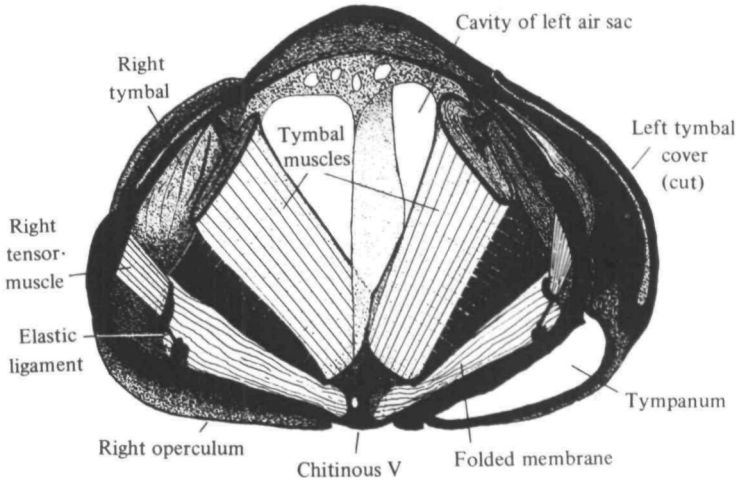


Fig. 7. Rear view of a slab containing the sound-producing apparatus of a male cicada. (Pringle, 1954.)

wings, is produced by synchronous discharge of nerve impulses from the central nervous system and there is an elaborate system of proprioceptive feed-back to regulate the activity. But in insects, it is possible to suggest a functional advantage that may have led to the development of this general property of muscle to the point where it becomes, in asynchronous muscles, the main method of generating the rhythm.

The easiest system to understand is cicada timbal sound production, where there is no antagonist to the timbal muscle and re-extension is achieved by the elastic mechanism of the timbal. The sound-producing timbals are situated on the dorso-lateral part of the 1st abdominal segment. The timbal is an elaborately ribbed cuticular membrane which clicks suddenly like the lid of a tin can when the tension on the muscle apodeme reaches a certain value, producing a pulse of sound. Just as in the flight system some species have a synchronous and some have an asynchronous rhythmic mechanism, so in cicadas some species are synchronous and some are asynchronous in the excitation of their timbal muscles. Josephson & Young (1981) compare the synchronous Australian *Cyclochila australasiae* with the asynchronous *Platypleura capitata* from Sri Lanka (Fig. 8). Simultaneous electrical records from the timbal muscle and sound records were made during the shrieks emitted when a captive cicada is held in the hand. In *C. australasiae* each excitation of the muscle fibres produces an IN and OUT click. In *P. capitata* a volley of nerve impulses leading to a burst of excitatory muscle potentials induces a rhythm of mechanical activity, which dies away slowly when excitation stops.

Fig. 9. shows diagrammatically how the delayed increase in tension on quick-stretch and the delayed decrease in tension on quick-release may generate the mechanical rhythm in asynchronous species. The cytoplasmic concentration of Ca^{2+} controls the rate of redevelopment of tension and the rhythmic IN and OUT clicks stop when the tension fails to reach the critical value for the IN click.

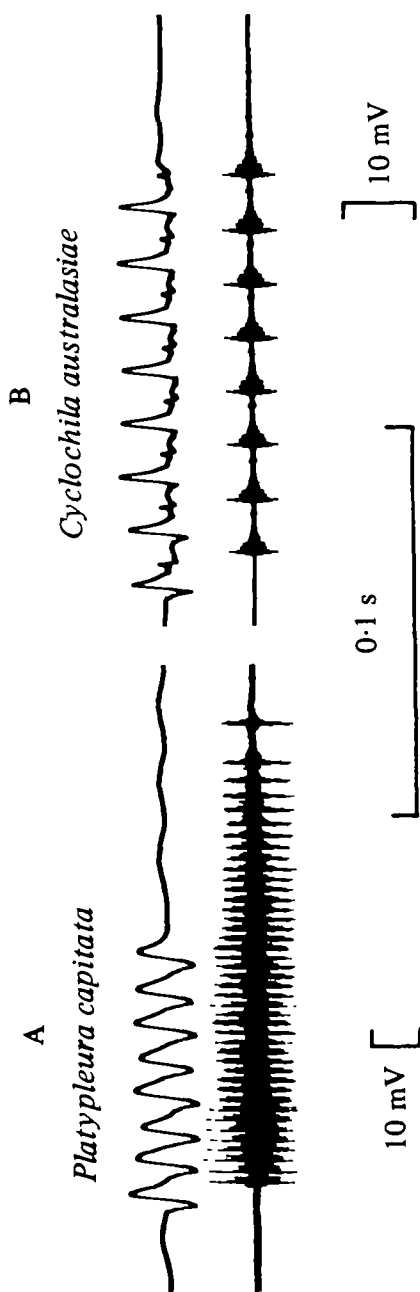


Fig. 8. Timbal muscle potential (upper trace) and sound (lower trace) records during the 'shrieks' of cicadas. (A) Asynchronous: (*Platypleura capitata*). (B) Synchronous: (*Cyclochila australasiae*). (Josephson & Young, 1981.)

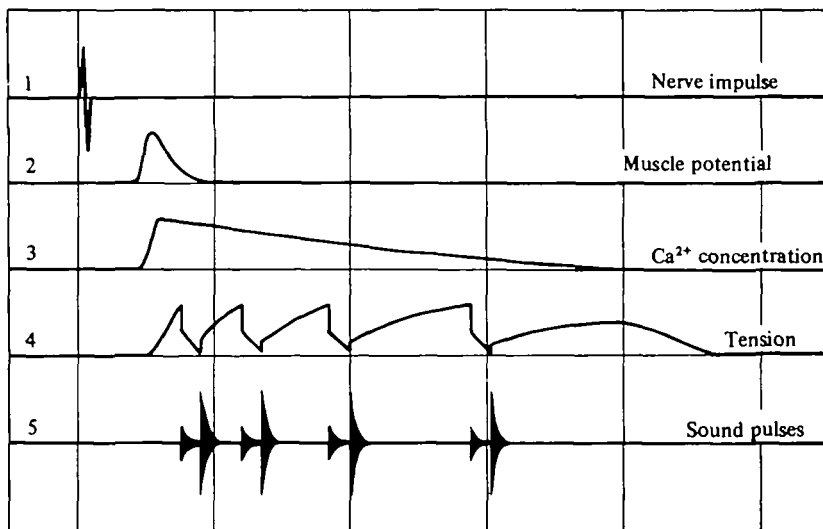


Fig. 9. Diagrammatic representation of the course of events when the timbal muscle of an 'asynchronous' cicada is excited by an impulse in its motor nerve. Lines 1, 2 and 5 can be recorded experimentally; lines 3 and 4 are hypothetical. (Adapted from Pringle, 1957.)

As already mentioned, Aidley & White (1969) showed that delayed changes of tension are present also in a synchronous species of cicada from Brazil. On a recent visit to Australia I was able in collaboration with Dr Gunther Rossmanith to confirm this result in the synchronous species used by Josephson & Young (1981) (*Cyclochila australasiae*). Using sinusoidal length modulation of activated fibres from glycerol-extracted timbal muscle, one can obtain a tension-length loop of area comparable to that obtainable with the flight muscle fibres of the asynchronous water-bug *Lethocerus* on which most of the recent experiments have been done. What then is the function of this property in a synchronous timbal muscle? Aidley & White pointed out that it could shorten the interval between IN and OUT clicks and so permit a higher frequency of twitching (Fig. 10). It appears to be advantageous in cicada song to have a high frequency of sound pulses, since several different ways are found of achieving this in different species. In many synchronous species the timbals on the two sides alternate, doubling the pulse frequency. In some American species the construction of the timbal allows the many ribs to buckle in sequence, so that it produces up to 13 pulses of sound for a single muscular contraction. If a high frequency of twitching is to be achieved in a synchronous muscle, the OUT click must be over before the next twitch occurs, so shortening of the IN-OUT interval would be of functional advantage.

The same type of advantage could arise in the flight system. Synchronous excitation is perfectly satisfactory for the flight muscles provided that the wing-beat frequency is low, leaving time for the muscles to twitch and relax at each beat. The first flying insects were large and will have had a low wing-beat frequency. With diversification of the terrestrial environment and the availability of many new niches with transient food supply, there would be advantage in becoming smaller and this necessarily means a higher wing-beat frequency unless the wing-leading is very low. Delayed tension changes after changes in length could speed up relaxation which is the critica

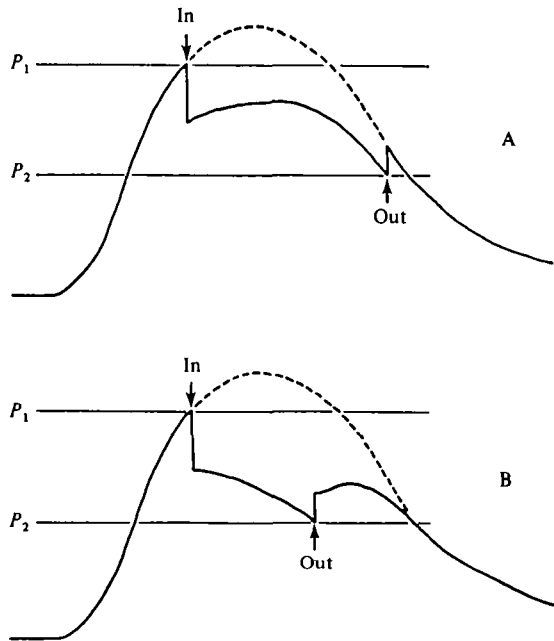


Fig. 10. Diagram to illustrate the hypothesis regarding the functional significance of delayed tension changes in the timbal muscle of a 'synchronous' cicada. Time course of tension in a twitch in the absence (A) and presence (B) of delayed tension changes after quick release and quick stretch produced by clicking of the timbal. (Aidley and White, 1969.)

process in achieving a high frequency of separate twitches without the development of tetanus.

Another consideration is that a harmonic, sinusoidal motion of the wings is not the optimum aerodynamically. More lift is generated if the UP and DOWN strokes occur rapidly with pauses at the top and bottom of the beat. This is easily achieved by the development of a click mechanism in the pterothroax through the action of the pleurosternal muscle, as first described by Boettiger & Furshpan (1952). A well-developed click mechanism is found in many Lepidoptera which always have a synchronous mechanism. The quick-release of length which this produces in the flight muscles will increase the delayed tension fall on sudden shortening.

The picture is then of functional advantage of the delayed tension changes in synchronous muscles in both the flight and cicada sound-production systems, up to the point where this becomes adequate actually to generate the mechanical rhythm and change over to an asynchronous mechanism. The argument would be convincing were it not for a histological difficulty. There is no obvious difference between synchronous and asynchronous muscles in the organisation of the myofibrils. There is, however, a difference in the components of the excitation-contraction coupling system. A synchronous muscle capable of a high frequency of twitching requires a very-well-developed sarcoplasmic reticulum. This is found in the flight muscles of, for example, the dragonflies and in the synchronous timbal muscles of *Cyclochila australasiae* (Fig. 11). By contrast, the best-known asynchronous muscles, like the

Table 1. *The type of control of power-producing flight muscles in insects*

Synchronous	Asynchronous
Odonata	Psocoptera
Orthoptera	<i>Psocus</i>
Ephemeroptera	Heteroptera
Psocoptera	Homoptera
<i>Trogia</i>	Jassidae, Aphidae,
Homoptera	Psyllidae.
Cicadidae, Cercopidae,	Thysanoptera
Membracidae, Aleurodidae.	Diptera
Neuroptera	Hymenoptera
Mecoptera	<i>Xyela</i> , <i>Tenthredo</i> ,
Lepidoptera	Apocrita.
Hymenoptera	Coleoptera
<i>Sirex</i> , <i>Cephus</i>	

flight muscles of a wasp and the timbal muscle of *Platypleura capitata*, have the SR reduced to a loose network of vesicles. If the evolutionary sequence that I have suggested is correct, one would expect that, as size is reduced and the wing-beat frequency increases, a greater and greater development of the SR would be needed. It is then necessary to suppose that in the most advanced asynchronous muscles a secondary reduction of the SR has occurred once rapid twitching is no longer needed.

A further difficulty is that there is a consistent difference in the location in the sarcomere of the transverse tubules of the T-system as between synchronous and asynchronous muscles. In synchronous flight muscles, the tubules are always located at $1/4$ and $3/4$ of the distance between Z-lines, whereas in asynchronous muscles the location is always at the half-way point, opposite the M-line (Figs. 3 and 11). No one has been able to suggest any physiological significance for this. This change of location of the T-tubules must also have occurred secondarily.

There has not yet been an extensive investigation of the extent of the SR and the T-system location in different asynchronous muscles. An alternative is to look at the speed of isometric contraction and relaxation when the mechanical conditions needed for oscillatory behaviour are not present. The asynchronous basalar muscle of the beetle *Oryctes* used by Machin & Pringle (1959) is not capable of twitching and a certain frequency of nervous excitation is needed for it to develop any isometric tension. The asynchronous timbal muscle of *Platypleura capitata* does show a small twitch tension in response to single stimuli, but a smooth tetanus occurs at a stimulus frequency of 46 Hz. The dorsal longitudinal muscle of *Bombus* also shows small isometric twitches. I should not be surprised if someone found an asynchronous muscle which can twitch quite well and has a well-developed SR.

Evidence about the evolution of asynchronous muscle is, in my opinion, likely to come from a study of those insect Orders where both synchronous and asynchronous muscles are to be found in related genera. Table 1 shows the distribution of the two types of power-producing flight muscle in the main insect Orders. The most interesting are the Homoptera and the Hymenoptera. The Homoptera have been extensively examined by Cullen (1971, 1974) using electronmicroscopy; Fig. 12 is his suggested evolutionary tree, showing two separate occurrences of asynchronous muscle in

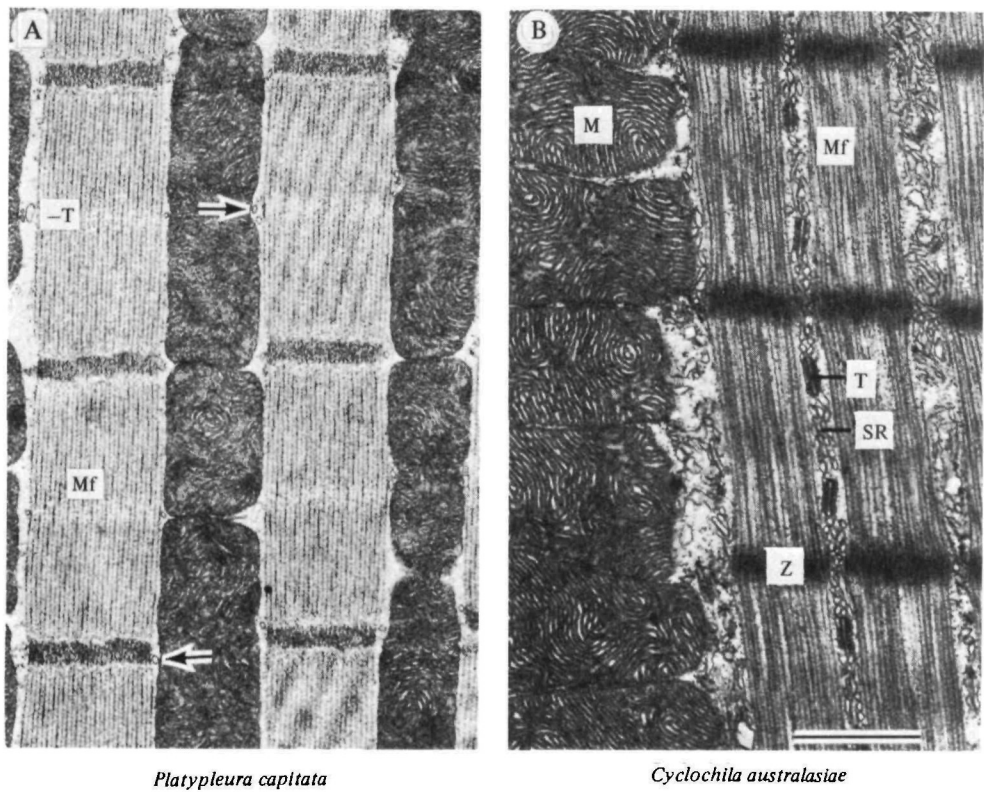


Fig. 11. Longitudinal electronmicrographs of the timbal muscles of (A) asynchronous; (B) synchronous cicadas. Note the different location of transverse tubules (T) and the sparse sarcoplasmic reticulum (SR) in A (arrowed). See also diagrams of Fig. 3. (Josephson & Young, 1981.)

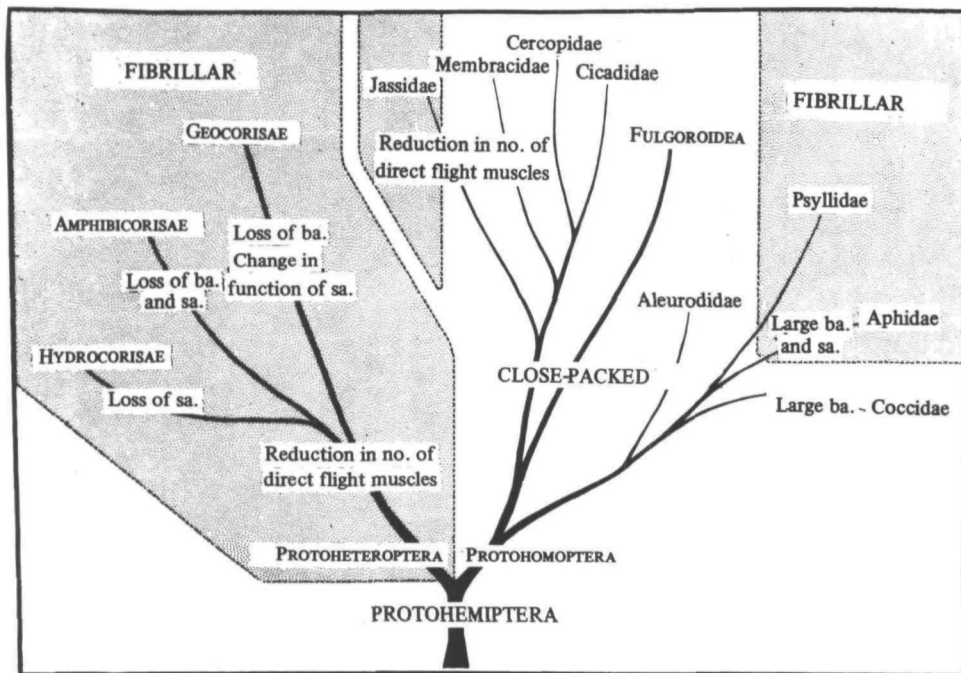


Fig. 12. Evolutionary tree of the Hemiptera, showing the occurrence of close-packed (synchronous) and fibrillar (asynchronous) power-producing flight muscles. (Cullen, 1971).

addition to the original separation of Homoptera and Heteroptera. Just why the Psyllidae and Aphidae have asynchronous muscles is not at all clear, since many of these have lower wing-beat frequencies than, for example, the synchronous Aleurodidae, in which the wing-beat frequency can be as high as 180 Hz (Wootton & Newman, 1979).

In the Hymenoptera the main interest is in the more primitive Symphyta since all the Apocrita seem to be asynchronous. No comparative study by electronmicroscopy or electrophysiology has been done on these insects and we have only a paper using light microscopy (Daly, 1963). According to him, *Tenthredo* is asynchronous, while *Sirex* and *Cephus* are synchronous. The primitive genus *Xyela* has fibrillar muscles in both the meso- and metathorax; if this really means that the muscles function asynchronously, it has evolved further even than the Apocrita, in which the asynchronous muscles are limited to the mesothorax. Electronmicroscope and physiological research is much needed on this Suborder and it will be important to look at as many anatomical muscles as possible. Fig. 13 shows the mesothoracic basalar muscle of *Tenthredo*; the sparse sarcoplasmic reticulum is typical of an asynchronous fibrillar muscle and the T-tubules are located at the M-line; the triadic connection to the SR has not previously been described in an insect. This particular muscle is not asynchronous in the hive bee.

One thing which does seem to be clear is that once the asynchronous mechanism has developed in a group of insects, the reverse evolution never occurs. For example, all Coleoptera are asynchronous, even large Dynastid beetles, where the wing-beat

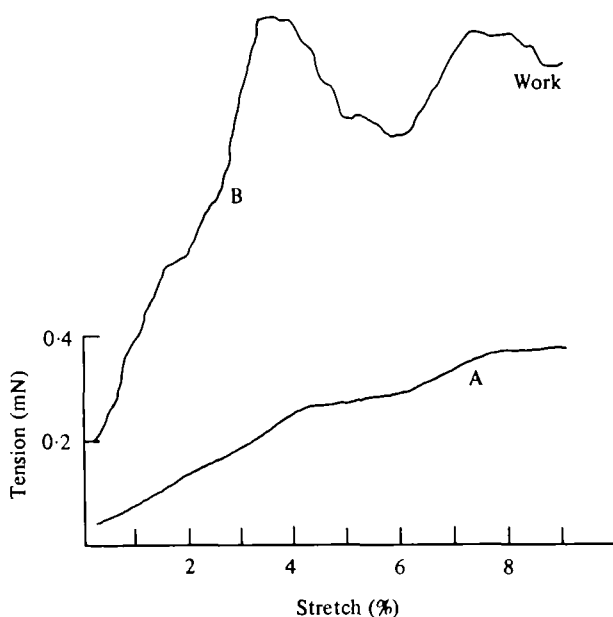


Fig. 14. Periodicity of stretch activation of glycerol-extracted *Lethocerus* flight muscle. A single fibre is extended progressively in length steps of 0.5 % under conditions of partial Ca^{2+} activation. Curve A shows the steady tension at each length step; curve B shows the oscillatory work (arbitrary scale) when sinusoidal length modulation of 0.2 % at 8 Hz is superimposed on the steady extension. (Original, similar to Abbott & Cage, 1979.)

frequency is as low as 25 Hz. Again, all Diptera are asynchronous, even the large Tipulids. If I am right in suggesting that small size and the resulting need for a high wing-beat frequency was the determining factor in the development of the asynchronous mechanism, then these insects must have become large again at a later stage in their evolution.

Finally, let me consider why the asynchronous mechanism has appeared only in insects and not, for example, in humming-birds, where one might have thought it would be advantageous. The argument goes back to physiology. I said earlier that the delayed tension change after quick change in length is found in all striated muscles. This direct influence of change of length on the active tension generation of the myofibrils is evidently a basic property of the molecular mechanism of contraction. There is, however, a difference between vertebrate and insect muscles. In vertebrate muscles, maintained extension of relaxed fibres *reduces* the magnitude of tension generation on subsequent activation, due to the reduction in overlap between the myosin and actin filaments (Gordon, Huxley & Julian, 1966). In asynchronous insect flight muscle, extension up to about 9 % *increases* the active tension. Furthermore, the magnitude both of the active tension and of the oscillatory work done when small sinusoidal modulation of length is superimposed on the steady stretch fluctuate periodically as the fibres are extended over this range (Fig. 14). It has been suggested (Wray, 1979) that this periodic effect on extension arises from the particular packing of myosin molecules into the myosin filament that is found in asynchronous insect flight muscle; this produces a match between the helical periodicities of the heads of the myosin

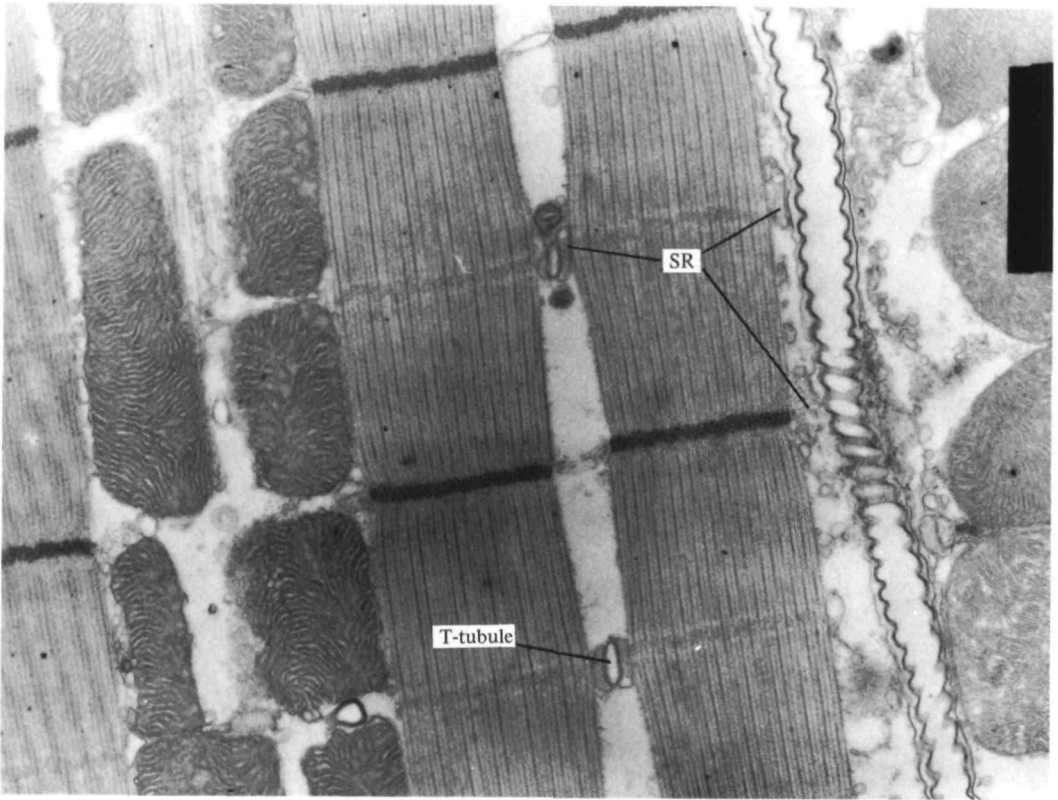


Fig. 13. Longitudinal section of mesothoracic basalar muscle of *Tenthredo* (Hymenoptera, Symphyta), showing sparse sarcoplasmic reticulum with triadic connexion to T-tubules. Scale 1 μ m. (Original by B. M. Luke.)

molecules and the sites on the actin filament with which they react and contrasts with the state of affairs in crustacean and vertebrate striated muscles where there are no such preferred relative positions. We badly need to know if this effect is peculiar to asynchronous muscles or if it is universal in all insect flight muscles, including those that function synchronously. Since its effect will be to increase the magnitude of oscillatory work over the range of lengths found in the intact insect during flight (1-2 %; Boettiger, 1960), it could well be the reason why asynchrony is found only in insects.

Altogether, I hope I have shown that there is a lot more work which can be done on insect flight and cicada tymbal muscles. In this lecture I have stressed particularly those aspects of the study that should be the concern of the experimental zoologist, rather than those that interest people wishing to understand the basic mechanism of contractility (Tregear, 1975). I have argued (Pringle, 1979) that insect fibrillar muscle also provides particularly good material for this latter type of investigation, which is really part of molecular biophysics. The Society for Experimental Biology has always been the main forum for people interested in more extensive rather than intensive researchers. I shall be disappointed if the papers read at some of its next 200 meetings do not include some on this topic.

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