ATYPICAL MYOFILAMENT ARRANGEMENT IN SOME ABDOMINAL MUSCLES OF MAYFLY NYMPHS

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Introduction

The various types of muscle fibres found in Insects differ vastly both in the same animal and between animals from different species as regards the ultrastructural morphology and contraction speed. They thus offer material for morphological and functional research on striated muscle.

Analysis of the ultrastructure of *Ephemeroptera* muscle has already produced some important findings [28, 30, 10] which do not fall completely into the generally accepted picture for other Insects. In particular, it has proved difficult to interpret the findings referring to the muscle systems of the nymphs analysed to date; these systems are responsible for highly complex movements. With the aim, therefore, of clarifying the morphological and functional relationships at this level, a comparative study has been conducted on the abdominal muscles of *Ecdyonurus helveticus*, *Cloeon dipterum* and *Ephemera danica* nymphs. The muscle bundles used for swimming, and the lateral abdominal fasciae, which govern the rhythmic gill movements [12, 13, 14], were subjected to close scrutiny.

In addition, since the literature provides no data on the speed and rate of contraction of these muscles, parallel to the electron microscope studies, high-speed and ultra-high-speed cinemaphotography was made of the movements of living nymphs, in order to clarify their mechanisms. These observations have been reported at this Congress by SAITA [29].

Materials and Methods

Nymphs of *Ecdyonurus helveticus*, *Cloeon dipterum* and *Ephemera danica* have been used.

The abdomens have been injected with 3% glutaraldehide in phosphate buffer, pH 7,2, and postfixed in 1% osmic acid phosphate buffered, pH 7. Different boundles have been dissected: principal abdominal internal and external muscles and various gill direct muscles. During dehidration the specimens were prestained "en bloc" in uranyl acetate 1% in 90% ethanol; then embedded in Araldite-Epon 812. The sections, cut with Ultrotome I and III, and stained with lead citrate, were observed with Hitachi HU 11 ES and HS 8 electron microscopes.

Observations

The abdominal muscles of *Ephemeroptera* nymphs can be divided into two groups [18, 19]. One is the group of "medial, or principal" muscles which are used for swimming; the other is the "lateral" group, inserted differently, which move the tracheal gills (SAITA, in preparation).

The medial, or principal muscles run in broad longitudinal bundles dorsally (the tergal musculature) and ventrally (sternal musculature) in the abdomen; in turn they can be divided into the "external" bundles, closer to the exoskeleton, and the "internal" ones, lying deeper in the abdomen.

In *Ecdyonurus helveticus* almost all the principal abdominal muscles lie in two robust longitudinal bands, arranged intersegmentally; the external tergal muscles, on the contrary, are intrasegmental. The fibres are oval or rounded in section, with peripheral nuclei; they appear to consist of numerous myofibrils, with fairly regular shapes (Fig. 1), with a few, small mitochondria. Myofilament distribution is similar to that in the flight muscles; the thick filaments are laid out hexagonally, each surrounded by 6 actin myofilaments. The thin to thick filament ratio is therefore 3:1 (Fig. 2).

Where the Z line is evident in the section, a typical "perforated plate" structure can be seen (Fig. 1); the distance between one "hole" and the next is the same as between primary filaments in band A.

In longitudinal section the sarcomeres appear fairly short, and the thick myofilaments are about 2 μm long. The I-band is always very limited, because the two types of filaments, as in flight muscles, are almost completely interdigitated. Fenestrated cisternae of sarcoplasmic reticulum surround each myofibril, frequently pairing up with the *T*-system to form dyads.

The lateral muscles are intrasegmental, and are all involved in gill movement. Only two small bundles, almost parallel and lying anteroposteriorly and sterno-tergally, insert directly at the base of each gill. The others all act indirectly. The tracheal gill muscles in *Ecdyonurus* are similar in ultrastructural morphology to the principal abdominal muscles; the myofibrils are polygonal in section, there are few mitochondria, numerous dyads and the thin to thick filament ratio is 3:1. The myosin filaments are all fairly short, about 2.2. μm long.

In *Cloeon dipterum* the principal, tergal and internal sternal muscles all lie in equal broad bands which form a "cross-over" arrangement on each segment [14]; the tergal bundles are independent of the sternal ones. These muscles are not completely intersegmental. The individual fibres are fairly coarse, with ultrastructural features very similar to the corresponding muscles in *Ecdyonurus*. The myofibrils are surrounded by a well-developed reticulum, and the ratio of thin to thick filaments is 3:1. The Z-line has the same "perforated" structure (Fig. 3). In transverse section, between the I-band and the Z-line, a C filament can be seen, connecting the thick filament with the Z-line (Fig. 3). The myosin filament is very short, only 1.5 μm (Fig. 4).

The principal external muscles, adhering more closely to the chitin, show a different ultrastructure. They are typical tonic muscles, with the same features as the abdominal muscles of all the other known Insects. The myofibrils are extremely irregular in shape and size (Fig. 5), long T-tubules invaginate from the periphery, and the reticulum is poorly developed. The thick myofilaments are still hexagonally arranged, surrounded by crowns of 10–12 thin filaments (Fig. 6). The ratio of thin to thick filaments is 6:1. The myosin filaments are nevertheless fairly short (2.5 μm).

In *Cloeon* the lateral muscles also form two small direct bundles, crossing slightly over each other, inserted at the base of each gill. These muscles have the same ultrastructure as the principal internal abdominal muscles (Fig. 7 and 8); the actin to myosin filament ratio is 3:1.

In *Ephemera danica* the picture differs: the principal tergal and sternal muscle fibres are almost all intrasegmental, allowing each segment to move independently of the others. The internal tergal muscles, covering most of the inner surface of each segment are highly developed. The fibres of all these muscles are divided into small, regular polygonal myofibrils (Fig. 10), outlined by a well developed reticulum, forming numerous dyads with the T-tubules. The Z-line structure does not show the perforated pattern as in the other muscles studied; the myofilament is often surrounded by a crown of 8 thin filaments, with a ratio of 4 actin to 1 myosin filaments (Fig. 11). Viewing the relaxed fibres in longitudinal section (Fig. 9), the I-band appears fairly extensive, and the myosin filaments are 2.5 μm long.

The lateral musculature consists of both direct and indirect bundles, the latter divided into three smaller bands which insert at three different positions at the base of the gills. Very probably these are the muscles which govern the typical elliptical pattern of the gill movements.

The ultrastructure of these muscles is almost the same as that of the external abdominal muscles in *Cloeon*. The myofibrils have irregular outlines, and a poorly developed reticulum. The ratio of thin to thick filaments is 6:1, each myosin filament being crowned with 12 actin filaments (Fig. 12). Mitochondria are numerous, most of them clumped peripherally in the I-band. In longitudinal section (Fig. 13) the I-bands are clearly visible and in the A-band the myosin filaments are 3 μm long.

Discussion

Ultrastructural observations on the muscles of Ephemeroptera nymphs have revealed that certain characteristics of the various types of fibres differ from the normal abdominal musculature of other Insects [23, 27, 35].

Among *Ephemeroptera* a ratio of 3 : 1 has been found between thin and thick myofilaments (in the principal and lateral abdominal muscles of *Ecdyonurus* and *Cloeon*), 4 : 1 in the principal abdominal muscles of *Ephemera*, and 6 : 1 in the principal external abdominal muscles of *Cloeon* and the lateral muscles of *Ephemera*. These ratios are normally considered typical respectively of very fast-moving muscles such as those of flight [31, 32, 33], or slow-contracting muscles [11, 34]. This range of variation in the arrangement and ratio of myofilaments may be related to the function and morphology of the muscles concerned [22], but it is nevertheless not easy to provide and interpretation in the light of the paucity of physiological data available to date. Study of the abdominal movements of the nymph while swimming, by means of high-speed cinemaphotography, is now in progress, and should prove of great assistance in finding a functional explanation of the ultrastructural features found.

In any event, the principal abdominal muscles of *Ephemera*, which show an atypical ratio of myofilaments, 4 : 1, (more often found in the flight muscles of some Lepidoptera, with a low contraction rate [8, 9, 20]), suggest they may involve some transitional form between tonic muscles, such as the external abdominal muscles on *Cloeon*, and phasic muscles, e.g. *Ecdyonurus* and *Cloeon*, internal abdominal muscles.

Our data on gill speed and rate of movement in individual animals [29] appears to agree encouragingly with the ultrastructural findings. In *Cloeon* and *Ecdyonurus* the gills beat at a very high rate, as sustained by the quantitative data obtained by high-speed and very high-speed film; their ultrastructure is typical of phasic muscle. In *Ephemera*, whose gills move much more slowly, the gill muscle ultrastructure morphology is of the tonic fibre type.

The data on the lengths and degree of interdigitation between filaments in the various muscles also correlates well with the findings concerning myofilament distribution and ratios, and agrees with the functional features of the various fibres both as regards contractile speed and — in particular — as regards the maximum tension developed by the sarcomere [21]. The longer the myosin filament, in fact, the more numerous bridges can be made to the actin filament [6, 24, 25].

As regards reticulum distribution and the T-system in the muscles studied [15, 16, 36], the broad cisternae development and the frequent dyads in the abdominal muscles of *Cloeon* and *Ecdyonurus* — morphologically similar to synchronous flight muscle fibres — would appear to exclude the possibility of self-oscillating or myogenic contraction [1].

This problem does not even arise for the muscles with a 6 : 1 thin to thick filament ratio, such as the gill muscles of *Ephemera* and the external abdominal muscles of *Cloeon*, since these are obviously tonic.

In this case, the perforated-pattern of Z-line in the muscles of *Ecdyonurus* and *Cloeon*, similar to that of the asynchronous flight muscles of other Insects [7], and the probable existence of a fine extension filament (filament C) connecting the myosin filament to the Z-plate, brings up the question of what exactly filament C exists for [4, 5]. In known contractile systems, it appears to be connected with the elasticity of the sarcomere, which is intrinsic not only to asynchronous muscles [1, 2], but also to all synchronous systems [26, 3]. In *Ephemera* muscle fibres, where the Z-line does not show this "perforated" pattern, filament C has never been observed.

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SUMMARY

Atypical myofilament arrangement in some abdominal muscles of mayfly nymphs

The electron microscopic study of Insect striated muscle has always been useful to clearify the relationships between sarcomere ultrastructural and muscle contraction. Among these researches, the ultrastructural analysis of *Ephemeroptera* muscles revealed some peculiarities uncomparable with the results obtained in the other Insects. We have examined some muscles, related to particular movements, suitable for a comparative study of contractile apparatus fine structure and physiological behaviours. Our research has been carried on thoracic and abdominal muscles of three different mayfly nymphs, *Ecdyonurus helveticus* (*Heptageniidae*), *Cloeon dipterum* (*Baetidae*), *Ephemera danica* (*Ephemeridae*): particular attention has been paid to the abdominal longitudinal muscles, performing the swimming, and to the abdominal lateral muscular boundles, in relation with the gill movements.

The three species show some ultrastructural characteristics: in *Ecdyonurus* and in *Cloeon* all muscles examined present a myofilament arrangement with 3 : 1 thin to thick myofilament ratio. This is an atypical condition because 3 : 1 ratio has been commonly found, in Insects only in muscles which contract speedly (v.g. synchronous and asynchronous flight muscles or tymbal muscles of *Cicadae*). The length of the thick filament is different in the various muscles. In *Ephemera*, however, we have found different features: the myofilament ratio changes from 4 : 1 to 6 : 1 and another ultrastructural difference is the filament lenght.

At the present we cannot establish precise conclusions, because these are preliminar data: some results, however, may be interpreted by studying with high speed cinemaphotography the movements produced by selected muscular systems in living nymphs.

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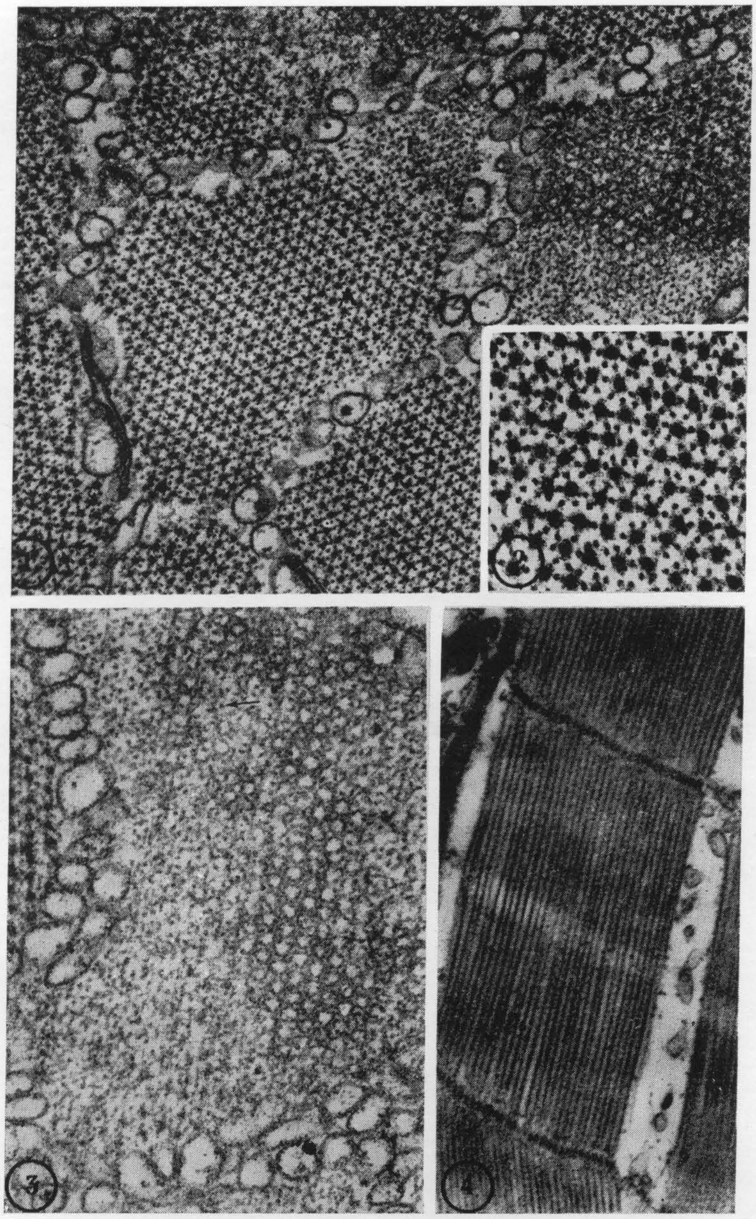
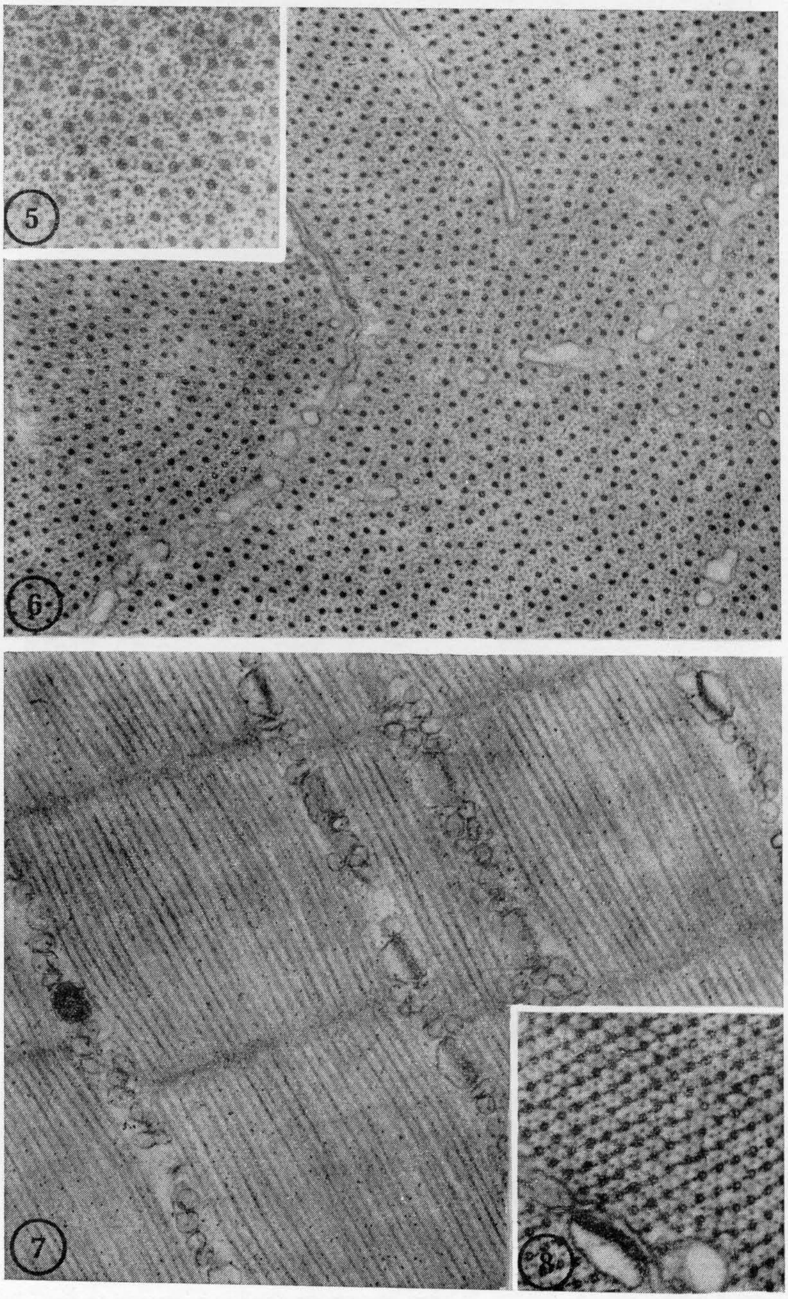
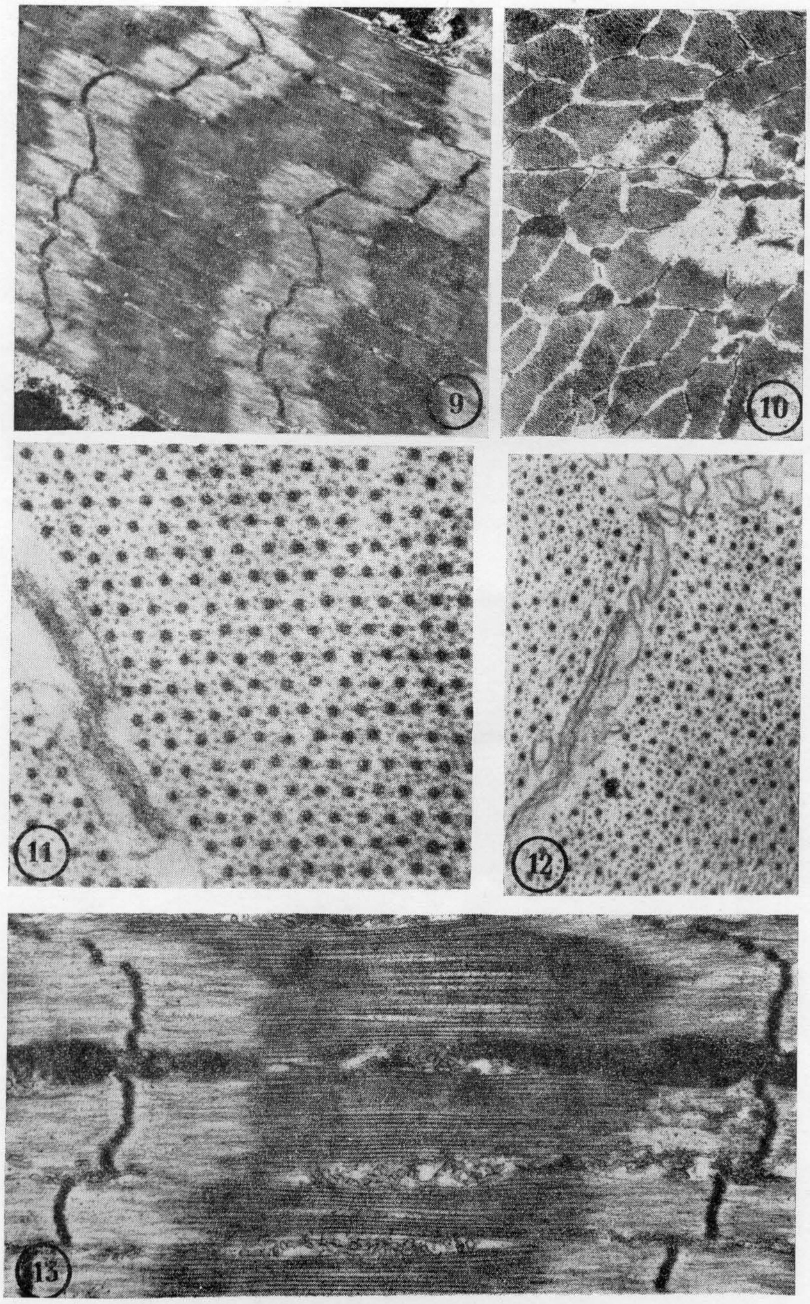


Figs. 1–4. *Ecdyonurus helveticus* (1, 2) and *Cloeon dipterum* (3, 4). **1**: transverse section of principal abdominal muscle fibre, showing the regular-outlined myofibrils, surrounded by sarcoplasmic reticulum and numerous dyads. The myofilament regular distribution is clearly visible. A-band (A) can be seen gradually giving way to the I-band (I), and to the "perforated" pattern of Z-line (Z). X 40.000; **2**: detail of a myofibril from the principal abdominal musculature. 6 actin filaments surround each myosin one, with a ratio of 3 : 1. X 150.000; **3**: transverse section of the principal internal abdominal muscle. Detail of the Z-line, with perforated pattern. Filament C is visible at some points arrow. X 65.000. **4**: longitudinal section of the principal internal abdominal muscle. The sarcomere is relaxed, but the I-band is still very milited. The myosin filament is about 1.5 μm long. X 32.000



Figs. 5–8. *Cloeon dipterum*. **5**: transverse section of the principal external abdominal muscle, showing the irregular morphology of the myofibrils nad poorly developed reticulum. Myosin filaments have a crown of 10–12 actin filaments. × 60.000. **6**: enlarged detail of the previous figure, showing the arrangement of thick and thin filaments. × 90.000. **7**: longitudinal section of the gill muscle. The sarcomeres are short, and the myosin myofilaments are about 1.8 μm long. × 25.000. **8**: detail of a gill muscle fibre in cross section. The ratio of actin to myosin myofilaments is 3 : 1. The myosin filament appears hollow in the middle. × 65.000



Figs. 9–13. *Ephemera danica*. **9**: longitudinal section of the principal abdominal muscle, showing fairly extensive I-bands. The A-band is about 2.5 μm long. $\times$ 8.000. **10**: transverse section of the principal abdominal muscle. The polygonal myofibrils are visible, separated by sarcoplasmic reticulum. $\times$8.000. **11**: enlargement of the previous section. Eight thin filaments crown each thick one (ratio of 4 : 1). $\times$ 60.000. **12**: transverse section of the gill muscle, with the thick filaments crowned by 12 thin ones, giving a 6 : 1, thin to thick filament ratio. $\times$ 63.000. **13**: longitudinal section of the gill muscle. The sarcomeres are fairly extensive, with primary filaments 3 μm long. $\times$ 20.000

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