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ULTRASTRUCTURAL PATTERN DIFFERENCES IN SOME MUSCLE FIBRES OF NIMPHAL *EPHEMERA DANICA* (Ephemeroptera)

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INTRODUCTION

In recent years, attention has been paid to the analysis of the ultrastructural organization of muscle fibres in the Insects (Smith, 1966; Hagopian, 1966; Pasquali-Ronchetti, 1970; *et al.*).

It is, in fact, evident that an extremely high specialization exists between the different groups of muscles in the same individual (Saita and Camatini, 1967; Reger and Cooper, 1967; Saita, 1967) and in the different species (Camatini and Saita, 1972).

The muscular fibres of Insects are striated and may be schematically distinguished in various cathegories, according to the differences in the ultrastructural morphology, probably related to functional diversities. We can generally recognize the typical myofilament arrangement of phasic muscles (synchronous and asynchronous, both marked by a low ratio between thin and thick filaments, 3: 1) and of tonic ones (with a high ratio of thin and thick filaments, 6:1). Furthermore, there are fibres with rather short myosin filaments, e.g. in fast acting flight muscles, which present reduced possibilities of filament sliding, and fibres with longer myosin filaments, (e.g. visceral and some skeletal muscles) which have increased possibilities for filament sliding through a longer overlap region. Other differences regard the sarcoplasmic reticulum and dyads distribution (Smith, 1962; Candia Carnevali and Valvassori, 1973).

Although these generalizations are, at the present, generally accepted, some unusual features have been observed in researches made on the ultrastructural organization of muscle fibres of mayfly nymphs of *Ecdyonurus helveticus* and *Baetis rhodani* (Saita, 1969), *Ephemera danica* (present paper), *Cloëon dipterum* and *Epeorus torrentium* (in preparation). In order to study the relationships between the basic structure of the striated myofibrils and their contractile mechanism, electron microscope observations have been carried out together with a high speed cinephotography (800 photograms/sec.) of the movements produced by selected muscular systems in living nymphs.

Particular attention is paid in this work to walking and burrowing muscles (thoracic coxal and femoral muscles) and to swimming or gill moving muscles (abdominal muscular systems) because of their different contraction speed, resulting from cinemaphotography

observations; (quantitative data on film analysis will be published later).

MATERIALS AND METHODS

Nymphs of *Ephemera danica* have been used.
Thorax, abdomen, legs have been injected with 3% glutaraldehide in phosphate buffer, pH 7,2, and postfixed in 1% osmic acid phosphate buffered, pH 7, or according to Karnowsky (1967). Different muscle bundles have been dissected: femoral muscles, coxal (pleural-coxal, coxal-trochanteral) muscles, intrasegmental abdominal and gill direct muscles. During dehydration 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 II ES and HS 8 electron microscopes.

OBSERVATIONS

Femoral muscles. At the electron microscope the fibres show an organization recalling that of other swimming Insects, (Notonecta, Saita, 1967). Nuclei are in peripheral position; the few mitochondria seem to be mainly localized at the I band level, showing rather different shapes and dimensions. The fibres volume is mainly occupied by the contractile material, forming a great number of irregularly shaped myofibrils, which look approximately circular, poligonal or strip-like, (fig. 1), and are outlined by a nearly continuous sarcoplasmic reticulum. The myofibrils, which are well delimited at the periphery of the fibre by deep invaginations of the T-system, tend to fuse together towards the center. Often, tubules of the T-system pair up with reticulum cisternae forming the dyads; these are rather regularly localized at the A band level as results from both cross and longitudinal sections. In the latter ones, (fig. 3), the Z lines appear in register and are emphasized by the presence of mitochondria. The sarcomere lenght varies in contracted or released muscles; anyway, thick filaments are long about 3 μ. The reciprocal arrangement of thick and thin myofilaments follows the pattern already observed in tonic muscles of Arthropoda. There is, in fact, a high ratio (~ 6) between two kinds of myofilaments: thick ones are arranged according to a hexagonal pattern and are surrounded by a crown of about 10-12 thin filaments, (fig. 2).

Thoracic muscles. In the thorax we have analyzed various types of coxal muscles (pleural-coxal, coxal, coxal-trochanteral muscles), not concerning the flight, but related with the motion of the legs (Tsui and Peters, 1972).
The ultrastructural morphology of the examined fibres shows a high ratio of filaments: the fibres are characterized by peripheral nuclei and myofibrils of poligonal shape, somewhat more regular than in the femoral muscles, (fig. 5). Particulary towards the center of the fibre, they may assume a roundish profile, being fairly distinctly separated by the well developed reticulum, and a great number of dyads. The extreme frequency of these structures represents one of the marking characters of these fibres and myofibrils completely outlined by dyads are easily found. In longitudinal section, the dyads show a uniform distribution, as in femoral muscles, at the A band level, but it is difficult to detect more than two dyads in a single sarcomere.
Sarcomeres, in longitudinal section, (fig. 4), appear regularly in register: the lenght of the thick filament is about 3.5 μ. Also in the coxal muscles, mitochondria have an elongated shape and do not occupy a considerable volume within the muscle.
The ratio thin to thick filaments is rather high (~ 5) and also in this case it is found a precise hexagonal pattern of myosin fila-

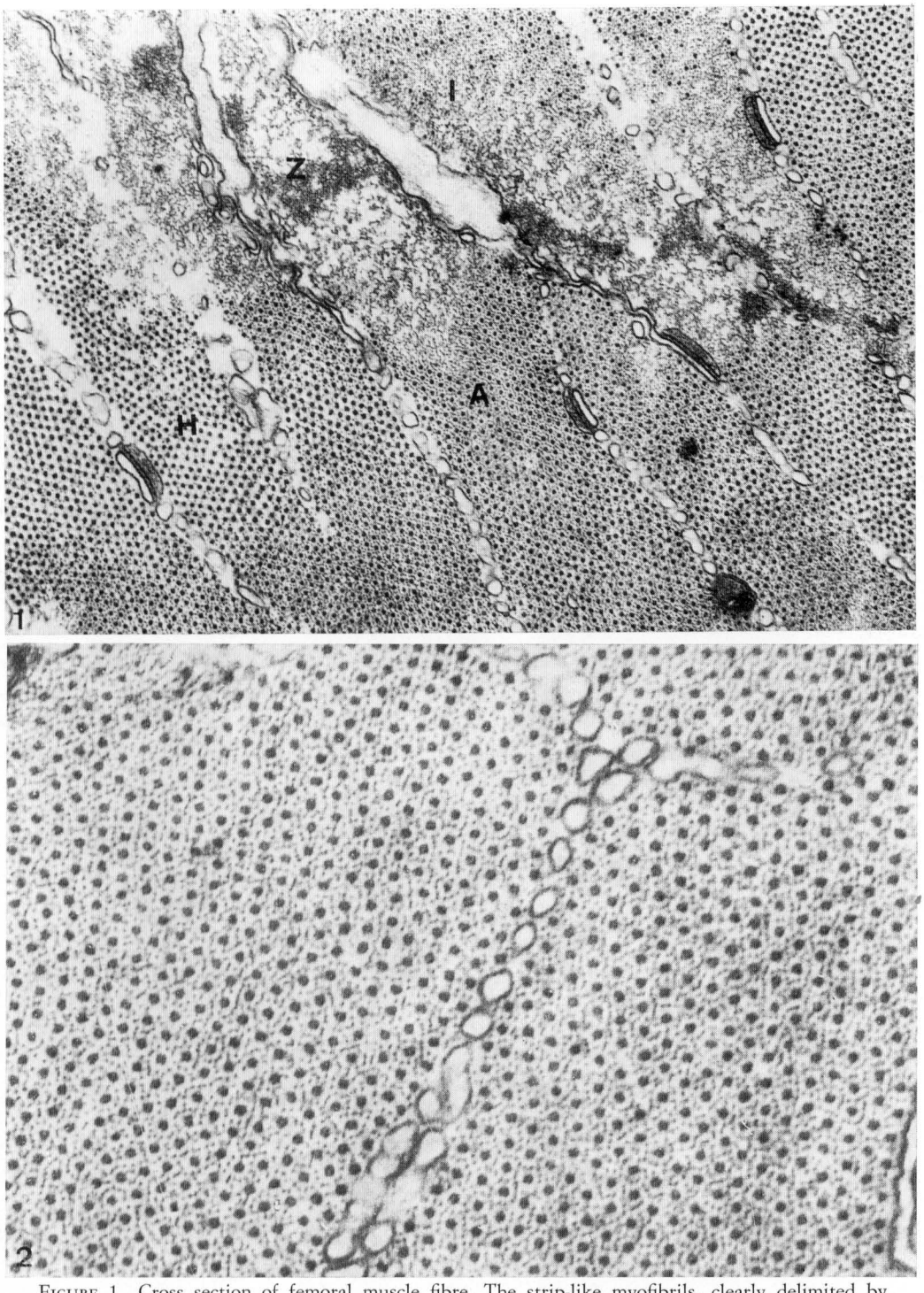


FIGURE 1 Cross section of femoral muscle fibre. The strip-like myofibrils, clearly delimited by sarcoplasmic reticulum, are sectioned at different levels. H band = H; A band = A; I band = I; Z line = Z. × 35,200.

FIGURE 2 Cross section of a femoral muscle fibre at the A band level, to show the high ratio (~ 6) between thin and thick myofilaments. × 95,000.

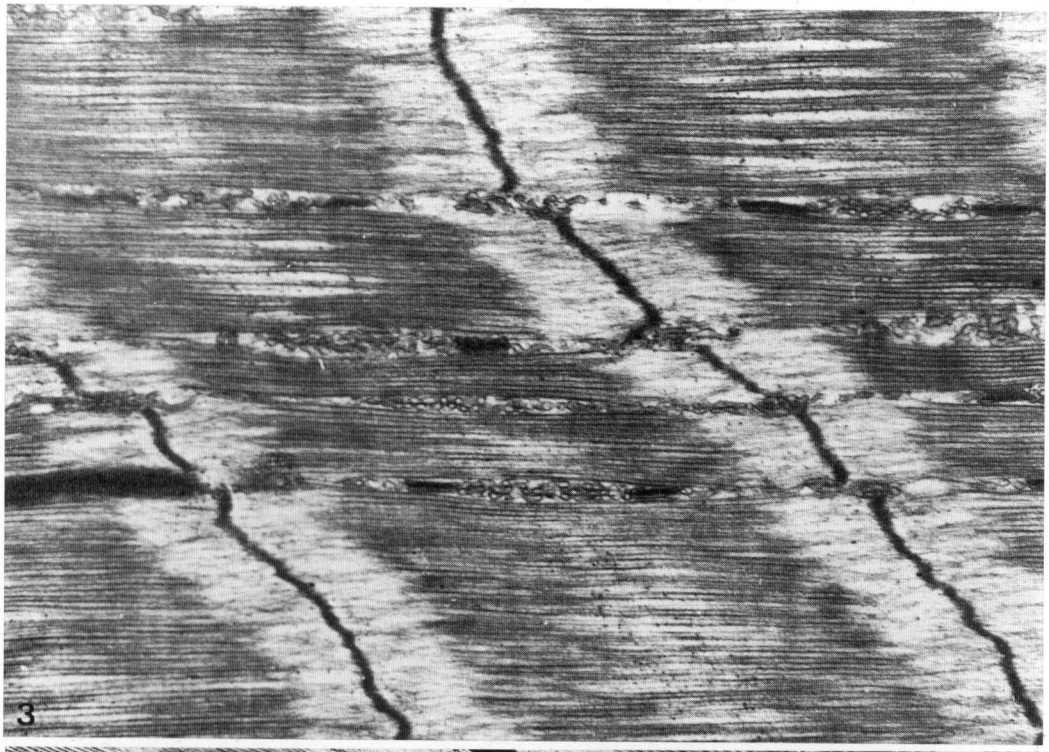


FIGURE 3 Longitudinal section of femoral muscle fibre. Sarcomeres are different in size but regularly packed and aligned; myosin filaments are 3μ long. × 18,200.

FIGURE 4 Longitudinal section of coxal muscle fibres. Sarcomeres are similar in size and regularly aligned; myosin filaments are 3μ long. × 12,000.

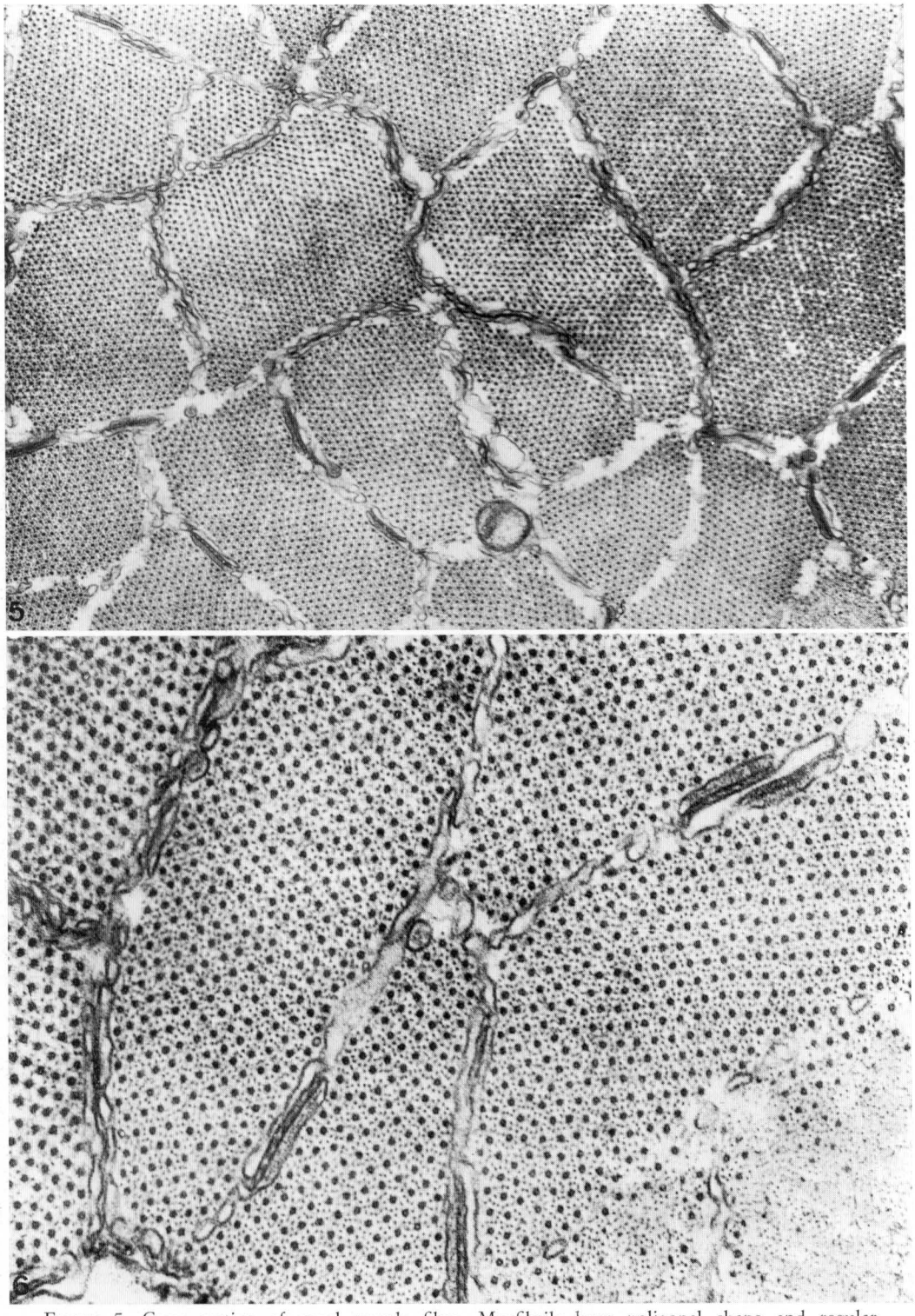


FIGURE 5 Cross section of coxal muscle fibre. Myofibrils have poligonal shape and regular size and are completely outlined by sarcoplasmic reticulum cisternae and T-system tubules with numerous dyads. × 42,000.

FIGURE 6 Coxal muscle fibre; cross section showing the myofilament pattern at the A-band level and the 5:1 ratio between thin and thick filaments. × 63,000.

ments; they appear encircled by a regular crown of 9-10 actin filaments, (fig. 6). The formers are, in some sections, hollow at the center at the A band level, while look rectangular in the H band.

Principal abdominal muscles. Abdominal muscles of Ephemeroptera nymphs may be separated in two main groups: the principal, or medial, muscles are longitudinally arranged along the whole abdomen either dorsally (tergal muscle bundles) or ventrally (sternal muscle bundles); smaller fibres, directly or indirectly related with the gills, are usually indicated as lateral or tergo-sternal muscles (Grandi, 1962-1963).

Both the abdominal muscle types carry out very different functions; the principal ones are responsible for the abdominal movements, particularly during the swimming, while the others are related with the motion of the gills (Eastham, 1937-1939-1958).

In *Ephemera danica,* the principal muscles, both sternal and tergal, show mostly an intrasegmental pattern: this situation allows to each segment to move independently from the adjacents.

From the ultrastructural point of view, the principal abdominal muscles of Ephemera, in contrast with those of Ecdyonurus and Baetis, cannot be regarded as fasic ones (filament ratio $\sim$ 3). Their organization, however, does not even follow the typical pattern of the abdominal fibres of other Insects, belonging to the tonic muscles, characterized by a high ratio of filaments ($\sim$ 6). The poligonal fibres appear subdivided in a great number of myofibrils with roundish profile and more or less constant dimensions, (fig. 7): the boundaries of the various areas are clearly marked by the presence of numerous dyads, by the reticulum cisternae and few mitochondria, scattered without an apparent order. The exagonal arrangement of the thick myofilaments is very regular, and thin myofilaments form crowns of approximately 8 elements, so that the mutual ratio is $\sim$ 4 (fig. 8).

In the longitudinal sections (fig. 9) the lenght of the thick filaments is about 2.5 μ.

Muscles of the gills. The lateral muscles can be distinguished in «direct» and «indirect», with relation to the presence or not of an insertion at the basis of the gills. The direct fibres are composed by three small bundles which, inserting in different positions at the basis of each gill, (fig. 10), cooperate to the fulfilment of the typical elliptic movement. The ultrastructure of these muscles is characterized by a high filament ratio ($\sim$ 6), with crowns of 10-12 thin filaments surrounding each thick filament (fig. 13).

The general organization of these fibres follows the patterns of the femoral muscles: the myofibrils, in fact, present round outlines towards the center of the fibre, becoming irregular at the periphery; reticulum is well developed and dyads are rather frequent. Particularly abundant are the mitochondria at the I band level, where they form fairly consistent clusters near the peripheral area or at the center of the fibre (fig. 12).

In longitudinal sections (fig. 11) the morphology of these fibres does not show particular features, except for the lenght of the thick filament (approximately 3 μ). The myofibrils, regularly aligned, are separated by an abundant reticulum; the nuclei appear laterally arranged as well as thracheae, always set out in peripheral position.

DISCUSSION

Since the modality of contraction is related to the different patterns of contractile fibres (Lanzavecchia, 1967), observations made by means of electron microscopy on striated muscles can supply comparative data, be-

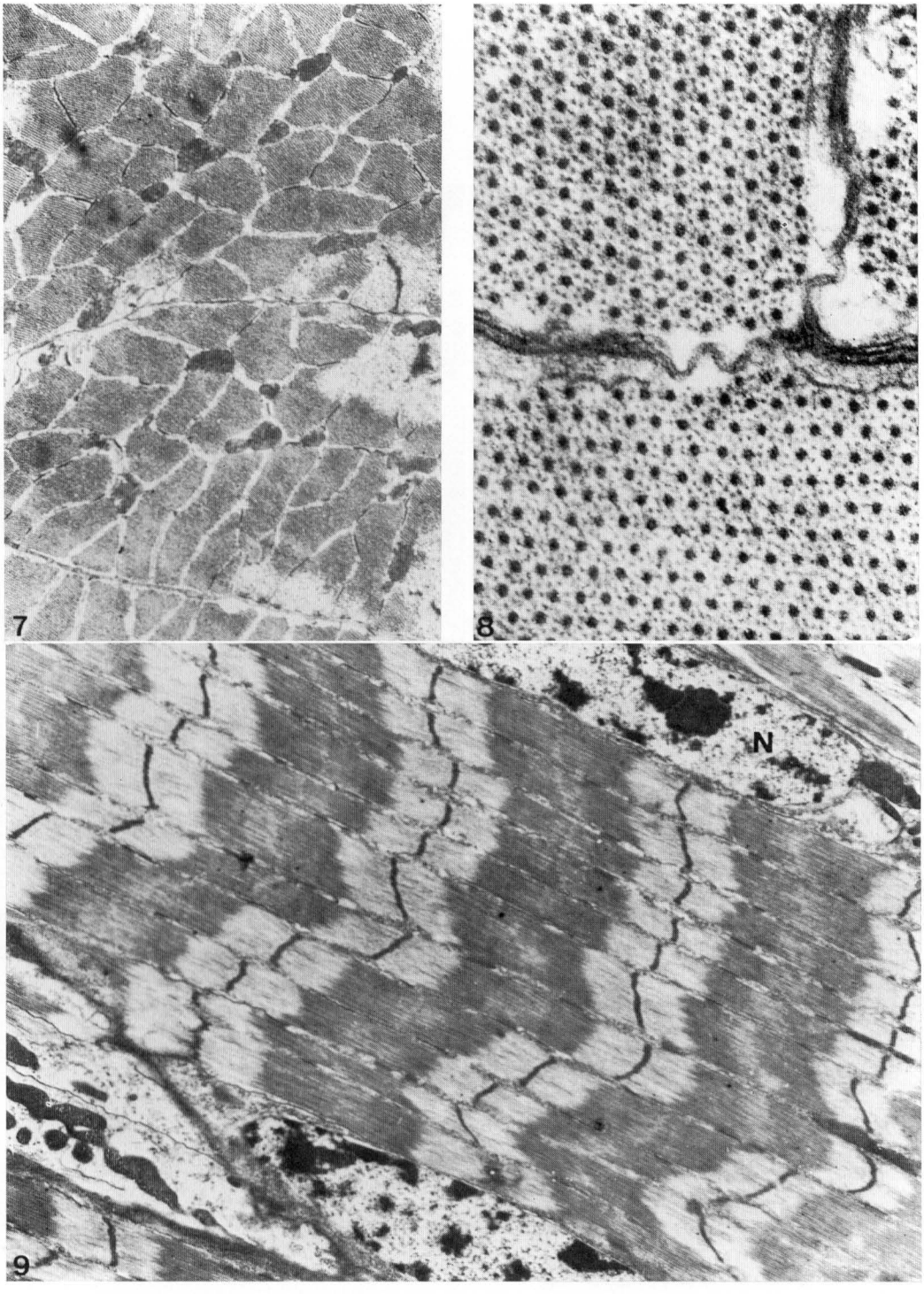


FIGURE 7 Principal abdominal muscle fibres. Numerous and poligonal myofibrils of different size are closely packed with mitochondria irregularly distributed. × 7700.

FIGURE 8 Higher magnified images of principal abdominal muscle myofibrils where a lower actin-myosin ratio (4:1) is observed. × 95,000.

FIGURE 9 Intrasegmental abdominal muscle fibres in longitudinal section. In these muscles the thick filament is 2,5 μ long. N = nucleus. × 7700.

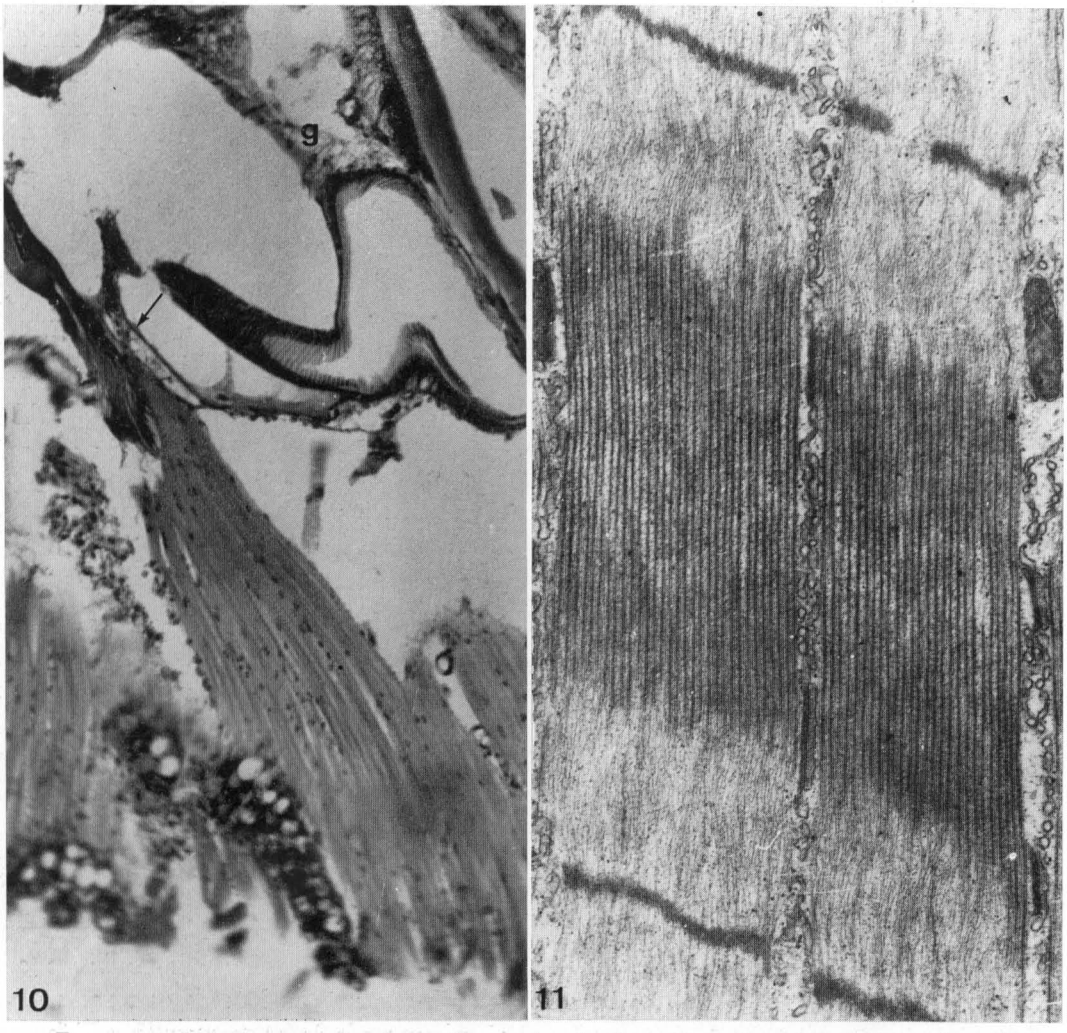
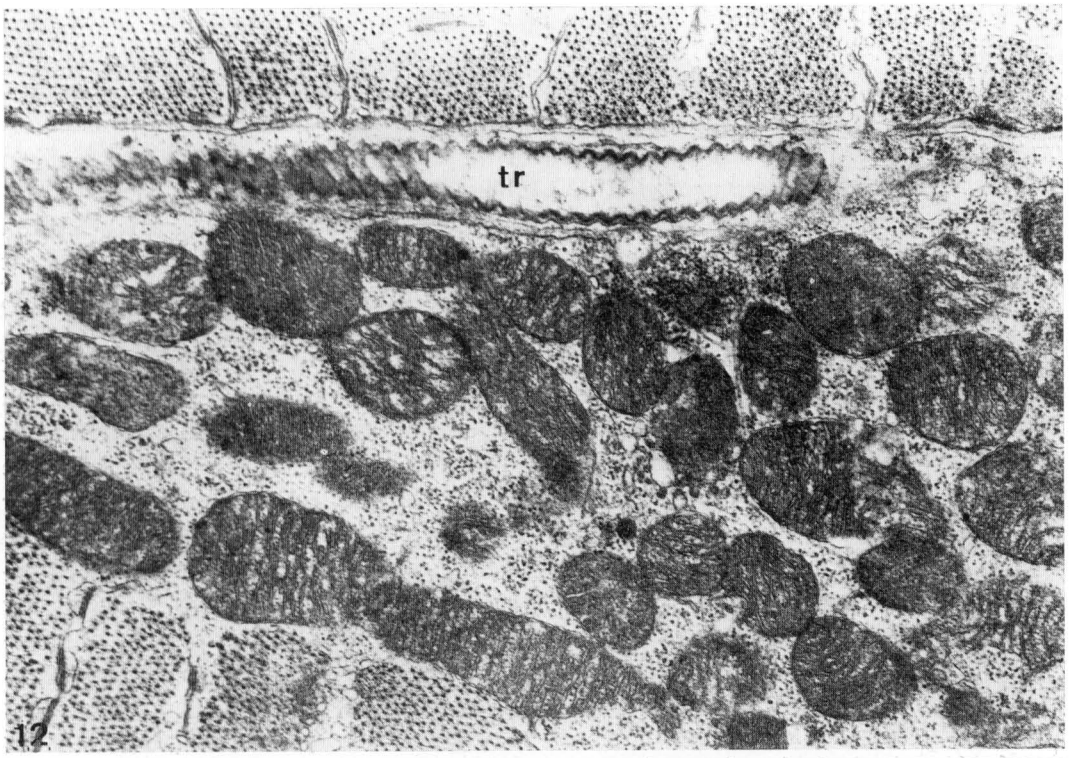


FIGURE 10 Light micrograph of a gill muscle showing direct insertion (↗) at the gill basis. g = gill. × 1000.

FIGURE 11 Longitudinal section of gill muscle fibre sarcomeres. Thick filament is 3μ long × 26,000.



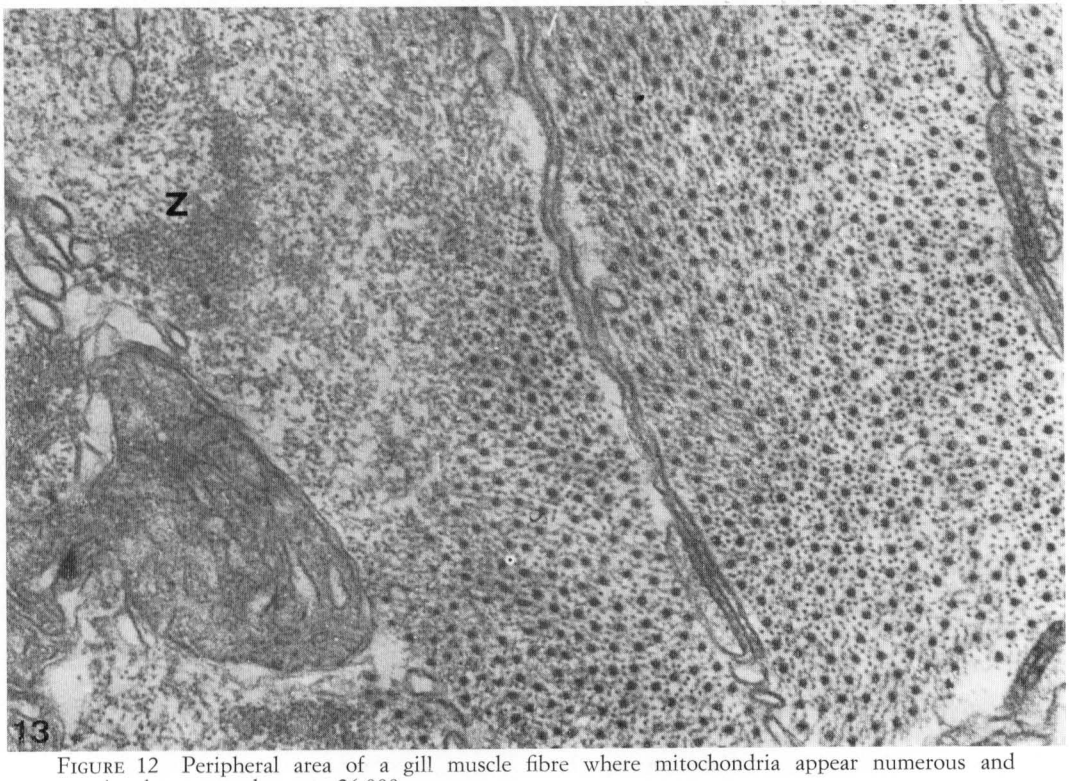


FIGURE 12 Peripheral area of a gill muscle fibre where mitochondria appear numerous and associated. tr = trachea. × 26,000.

FIGURE 13 Gill muscle fibre. Cross section showing an high ratio (6:1) between myofilaments. Z = Z line. × 90,000.

cause cross-striation represents a valuable « reference scale » by which « muscle structure research can be compared with each other and can be characterized by the use of exact quantities, e.g., sarcomere lenght, filament lenght, etc. » (Garamwolgyi, 1971). Among these researches are of particular interest the observations on the ratio between actin and myosin filaments in muscles with different contraction speed; the double exagonal array of the filaments, in fact, and the thin to thick filaments ratio in the overlap zones of the A-band may present remarkable differences in Arthropoda and may be related to different kinds of interaction of myosin and actin when the muscle is active (speed of contraction, oscillatory properties, etc.).

Although the intriguing questions concerning the physiological effects of side-spacing of filaments and regular « side by side » array of the filament lattice have not been completely solved, it has been, however, possible to establish morphofunctional correlations in the observed muscles of *Ephemera*. The legs of these nymphs are engaged sometimes in moving stones and in burrowing in a sandy floor with strong and slow movements; the femoral and coxal muscles show consequently the typical structure of tonic muscles, with long thick filaments and a high thin to thick filaments ratio.

A similar filament pattern and sarcomere lenght is observed in the gill moving muscles; the speed of the gill oscillation is small with respect to that of *Cloëon* and *Ecdyonurus*. The ultrastructure of the muscles is always of the tonic type (high ratio between filaments). As they are, however, muscles with continued activity, it will be necessary a considerable number of mitochondria for a much continuous energetic supply.

The intrasegmental muscles of the abdomen are involved in swimming, which is carried out by means of rapid but unfrequent oscillations. They show, consequently, a reduction in the number of thin filaments, (ratio thin to thick filaments about 4), roundish myofibrils, and short thick filaments. These features have already been found in flight muscles of some Lepidoptera with low contraction frequence (Auber, 1967; Camatini and Saita, 1969) and in the indirect flight muscles of cockroaches (Hagopian and Spiro, 1968).

The observed variability of the thick filament lenght seems to be related to the differences observed in the thin to thick filaments ratio. The myosin filament lenght is 2.5 μ in the abdominal principal muscles, while in other muscles it is 3-3.5 μ.

The distribution of the sarcoplasmic reticulum, T-system and dyads indicate that all observed muscles are synchronous, without possibility of myogenic auto-oscillations.

The morphological modulations pointed out in the muscular systems of *Ephemera danica* concern only the tonic type muscles, excepted the principal muscles of the abdomen. These may be considered as a transition form, in relation to their peculiar myofilament arrangement. These observations will acquire a more precise morpho-functional meaning after further observations on muscular systems in other Ephemeroptera, concerning both the ultrastructural pattern and the physiological properties.

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SUMMARY

Femoral, coxal, intrasegmental, abdominal and gill muscles of nimphal *Ephemera danica* have been examined at the electron microscope.

Differences between myofilament arrangement and thick filament lenght in the various muscles have been described; ultrastructural findings have been analyzed in connection with the physiological behaviour of different muscles.

The structural organization and relationship of T-system and sarcoplasmic reticulum clearly point to a syncronous nature of these fibres, because all muscles observed show an high developed and ordered distribution of T tubules, dyads and sarcoplasmic cisternae. The shape and size of myofibrils, the ratio between thin and thick filaments, the myosin filament lenght change in connection with physiological properties of contractile mechanism.

RIASSUNTO

Sono stati osservati al microscopio elettronico i muscoli femorali, coxali, intrasegmentali addominali e diretti delle tracheobranchie della ninfa di *Ephemera danica.*

Sono state messe in evidenza differenze relative alla distribuzione dei filamenti e alla lunghezza del filamento primario; i dati ultrastrutturali sono stati correlati con le prestazioni funzionali dei diversi muscoli.

Tutti i muscoli osservati, come organizzazione, sono da considerarsi di tipo sincrono, dato il notevole sviluppo del reticolo sarcoplasmatico, l'ordinamento preciso del sistema T e l'abbondanza delle diadi. Per quanto riguarda, invece, la forma e le dimensioni delle miofibrille, il rapporto reciproco tra i miofilamenti, la lunghezza del miofilamento primario, questi variano tra un tipo di muscolo e l'altro in rapporto alle diverse funzioni svolte dai diversi sistemi muscolari.

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