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## Z-line in insect muscles: structural and functional diversities

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Morphological observations on striated muscles are normally centred on the parameters traditionally believed to condition the fibre's performance: mainly, the sarcomere length and hence the length of its filaments, and, secondarily, the arrangement and abundance of, for example, the membrane systems and the mitochondrial volume.

Although the Z-line has often been described in detail and its fine organization has been the subject of controversy, yet it has never been interpreted as an element strongly influencing contractile structure performance.

Other than representing a contact point or a connection between the actin filament of two adjacent sarcomeres, the Z-line serves as anchorage for an entire series of filaments, namely the C-filament (Pringle, 1967; Garamvolgji, 1969; Saide & Ullrick, 1973; Trombitas & Tigy-Sebes, 1974, 1975, 1977) and the T-filament (Hoyle, 1967; Peachey, 1968, Goll *et al.*, 1977). The chemical composition of these filaments has not yet been defined and the meaning of the mechanical connection between different sarcomere elements has only been hypothesized.

The main role of the Z in conditioning sarcomere activity can be revealed through a comparative study of muscles with different functional performances in a homogeneous group of animals such as insects. In spite of their being provided only with striated muscles, insects can perform highly diverse activities which would require helical or smooth muscles in other animals. The Z-line is not uniform in different fibres, but is rather a heterogeneous structure both in its general architecture and in its internal fine organization.

The basic structure always consists of a filamentous component (actin and tropomyosin) and an amorphous component (mainly  $\alpha$ -actinin) (Bullard & Sainsbury, 1977). The diverse organizations of the filamentous component and the different quantity and arrangement of the superim-

### ABSTRACT

The Z-line of insect muscle shows different organizations in fibres with diverse performances. The Z-line fine structure and arrangement correlated with the functions of the muscle allow the different cases to be classified in three schematic groups: *unstructured Z*, typical of muscles not particularly specialized either in quick movements or in developing or maintaining strong tensions; *structured Z*, typical of quick synchronous or asynchronous muscles; *fragmented Z*, typical of the supercontracting muscles. The suggested models are briefly discussed on the basis of their functional and evolutionary meanings.

posed amorphous material determine the variability in the appearance of the Z-line, passing from an irregular structure to a rigidly geometric one. Data so far point out three different levels of organization: unstructured Z, structured Z and fragmented or discontinuous Z.

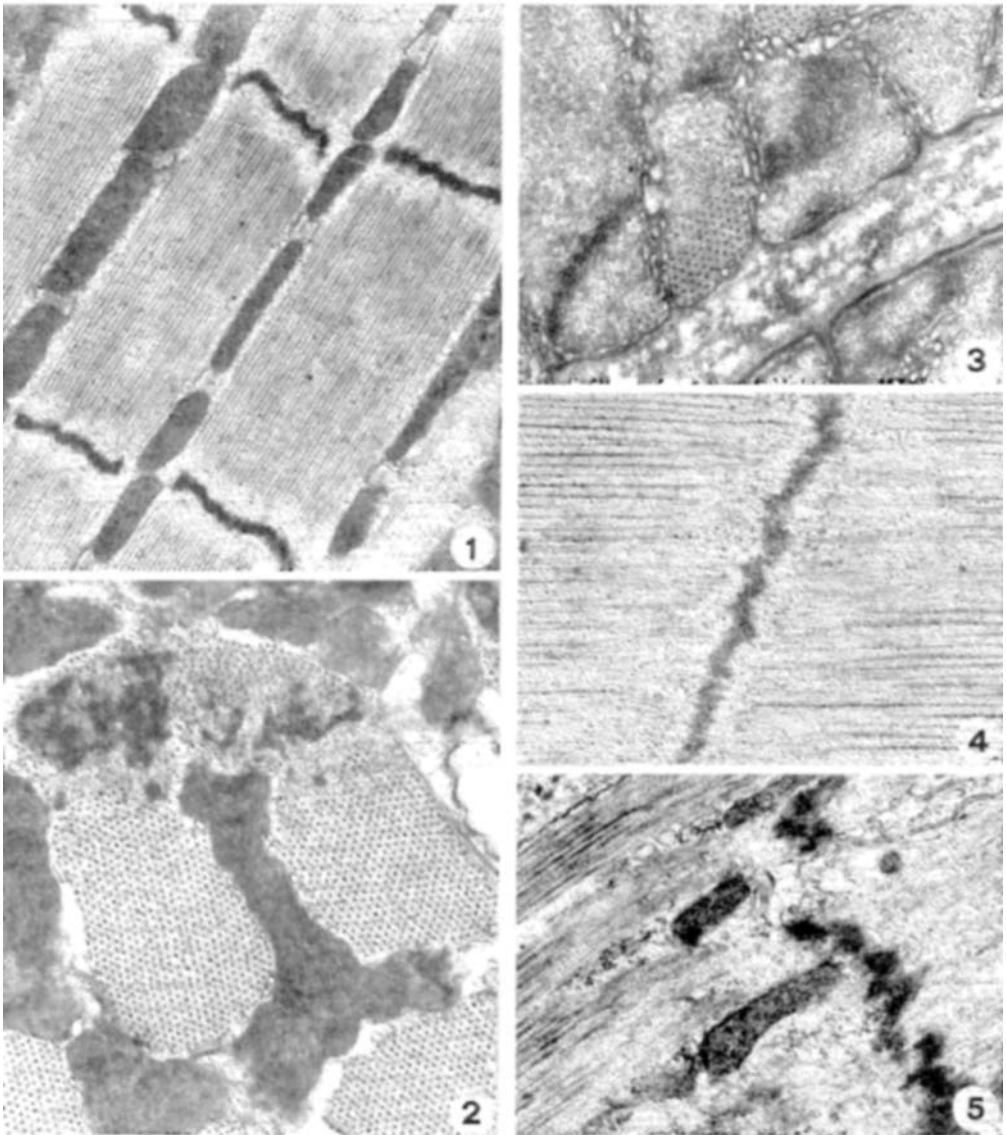
*Unstructured Z* is typical of muscles not particularly specialized either in quick movements or in developing and maintaining strong tensions. This is the case of the classic tonic skeletal muscles, of some flight muscles with low contraction frequencies, and of almost all visceral muscles characterized by long sarcomeres (exceeding  $4\ \mu\text{m}$ ) and thin to thick filament ratios ranging from 4:1 to 6:1 (Toselli, 1965; Hagopian, 1966; Smith, 1966c; Auber, 1967a, b; Hagopian & Spiro, 1968; Camatini & Saita, 1969; Camatini, 1971; Pasquali-Ronchetti, 1971). The Z-line is a structure continuous through the myofibril. In both longitudinal and cross sections, it appears as a heavily electron-dense area with no recognizable arrangement of the filamentous component (Figs. 1-5).

*Structured Z* is typical of muscles with quick movements, whether synchronous or asynchronous flight muscles or synchronous non-flight muscles (Smith, 1961, 1966a, b; 1972; Auber & Coteaux, 1962, 1963; Shafiq, 1964; Ashhurst, 1967, 1971, 1977; Reger, 1967; Reger & Cooper, 1967; Elder, 1971, 1975; Saide & Ullrick, 1973; Cullen, 1974; Trombitas & Tigyi-Sebes, 1974, 1975, 1977). These muscles are characterized by short sarcomeres (less than  $3.5\ \mu\text{m}$ ) and a thin to thick filament ratio of 3:1. Through the sarcomere they have a continuous Z-line which shows a precise geometrical pattern in longitudinal section and, more evident, in cross section. In cross section the Z-line looks like a plate perforated by equidistant holes, with variable outlines in different animals (Figs. 6, 7).

However, these holes form a hexagonal pattern in which the more or less dense

amorphous material fills the spaces in between them. Following lengthy discussions by various authors (Auber & Coteaux, 1963; Saide & Ullrick, 1973; Ashhurst, 1977), a sufficiently precise architectonic model was established but only for asynchronous flight muscles. These have a Z-line uniform in various species which can be schematically defined as a perforated plate in triangled form (or, better yet, in hexagonal form with a triangularly distorted profile, as indicated by Sainsbury & Hulmes, 1977). This architecture is determined by the contribution of all these components: the amorphous matrix, the terminal parts of the thin filaments (arranged according to hexagonal schemes regularly staggered in the two adjacent sarcomeres) and the C-filaments connecting the thick filaments to the Z-line. Such a model is in complete agreement with cross and longitudinal sections of *Notonecta* flight muscles and fundamentally upholds Ashhurst's view (1977), which is the best interpretation of the structural lattice of asynchronous flight muscle (Fig. 9). Among the most authoritative researchers in this field are Auber & Coteaux (1963) and Saide & Ullrick (1973). Their models, however, can be criticized mainly because they tend to consider the actin filaments continuous from one sarcomere to another. At the moment a basic agreement seems to exist on the C-filament connecting the myosin filament to the Z-line. Today there is no doubt of the existence of this filament or of the C-material also identifiable in sections (Pringle, 1977). A generally very similar morphology is found in the Z-line of synchronous flight muscles (Fig. 8) and in the particular non-flight muscles with fast contraction, such as the tymbal muscles of *Cicada* (Aidley & White, 1968, 1969) and the abdominal gill muscles of some Ephemeroptera (Candia Carnevali & Saita, 1975, 1979) (Fig. 7).

The rigid structure of the perforated plates is maintained although it allows



*Unstructured Z-band.* The Z-line looks like a dense, continuous structure through the sarcomere in the longitudinal sections and like a very dense area with no recognizable fine structure in the cross sections.

Fig. 1. — *Philosamia cynthia*: flight muscle fibre in longitudinal section. ( $\times 12\ 000$ ).

Fig. 2. — *Philosamia cynthia*: flight muscle fibre in cross section. ( $\times 24\ 000$ ).

Fig. 3. — *Notonecta* sp.: cross section of coxal muscle. ( $\times 23\ 000$ ).

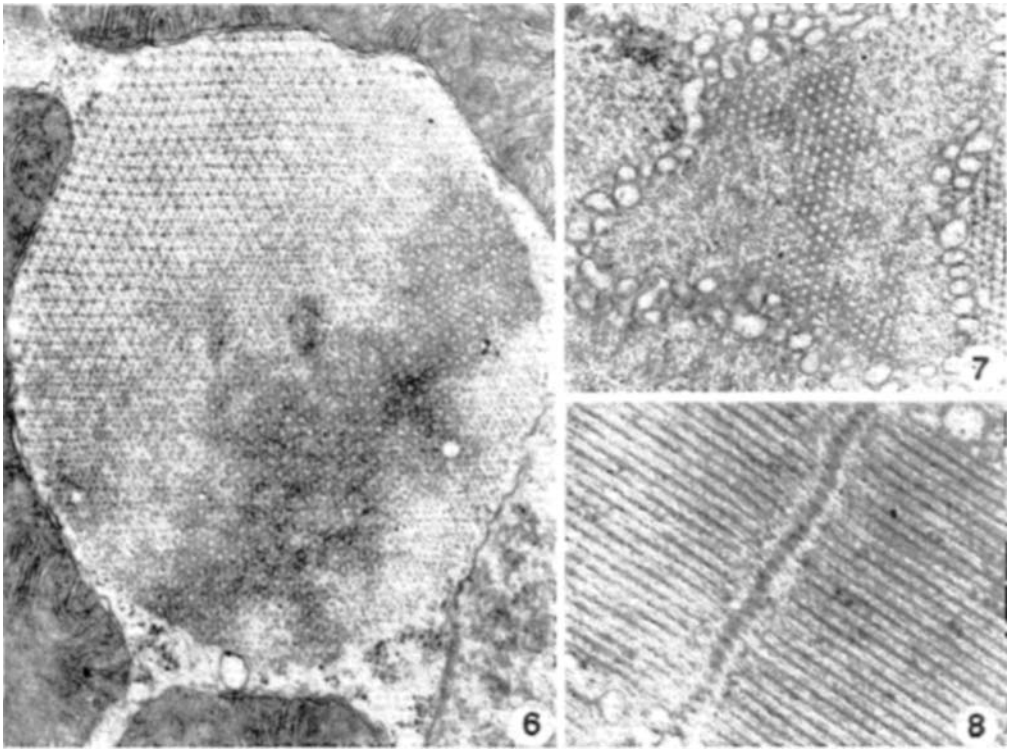
Fig. 4. — *Calopteryx splendens*: longitudinal section of intersegmental abdominal muscle. ( $\times 30\ 000$ ).

Fig. 5. — *Schistocerca gregaria*: visceral muscle (gut longitudinal fibre) in longitudinal section. ( $\times 25\ 000$ ).

some minor differentiations, mainly due to the different combining and packing of the amorphous Z-material on the filamentous component. For this reason the perforation can be seen even in the longitudinal sections at times (Fig. 8), while at

that these two factors are closely related and typical of fast contracting fibres.

*Fragmented Z* appears in all those situations (in both somatic and visceral muscles) in which the capacity of shortening or of maintaining tension required by the



*Structured Z-band.* The Z-line has a typical, perforated-disk structure. The regular distribution of the holes is also identifiable in the longitudinal sections.

Fig. 6. — *Notonecta* sp.: flight muscle fibre in cross section. ( $\times 31\ 000$ ).

Fig. 7. — *Ecdyonurus helveticus* (larva): cross section of abdominal (gill muscle) fibre. ( $\times 30\ 000$ ).

Fig. 8. — *Cloeon dipterum* (larva): flight muscle fibre in longitudinal section. ( $\times 35\ 000$ ).

others the geometrical pattern seems a bit fainter. The connecting C-filament can also be identified in this group of muscles. The presence of an identical connecting C-filament and of a more or less similarly perforated-plate Z-line in all of these phasic muscles, both flight and non flight, shows

system exceeds the effective possibilities of the normal tonic muscle. This is the case in the hydraulic muscle systems of the bodywall of many insect larvae (Osborne, 1967; Crossley, 1968; Candia Carnevali, 1978) and of numerous visceral muscles (Rice, 1970; Nagay & Graham, 1974;

Myklebust, 1975) able to supercontract (shortening by more than 60% their resting length). These so-called supercontracting muscles constitute an extremely atypical example of striated muscle, in which the ultrastructure of the sarcomere yields to functional needs much as does that of helical and smooth muscles. They modify their own morphology and geometry to realize very quick shortenings allowing for a longer maintenance of tension. In these fibres, the Z-line generally looks like a series of dense bodies unaligned and disconnected through the sarcomere. This discontinuity is visible in cross sections, where the dense bodies appear sinuous or globular (Figs. 10, 11), and in longitudinal sections, where the various tapered Z-elements, staggered with respect to each other (Figs. 12, 13), outline the sarcomere in highly irregular fashion. Since they are lacking in cross connections, they can slide against each other causing wide deformations in the muscle fibre. Not only the myosin filaments but also the actin filaments of the two non-adjacent half-sarcomeres can cross the interrupted Z-line. In this way they are further superimposed and interact reciprocally (Fig. 16) as a result of the numeric count of the filaments in cross sections at different sarcomere levels (Fig. 14). Only the actin filaments can directly attach to the various Z-elements. It would be absurd to talk about any connection between the myosin filament and the Z-line in this particular kind of fibre. In fact, in the first place the possibilities of reciprocal sliding of filament which pass beyond the Z-line are highly accentuated, while in the second place the Z-line is discontinuous, with no alignment between the Z-bodies and the myosin filaments. The structure of the Z-line in these muscles is always discontinuous, both in decontracted fibres and in contracted and supercontracted fibres (Figs. 12, 15), contrary to what is verified in the depressor muscle of barnacles (Hoyle

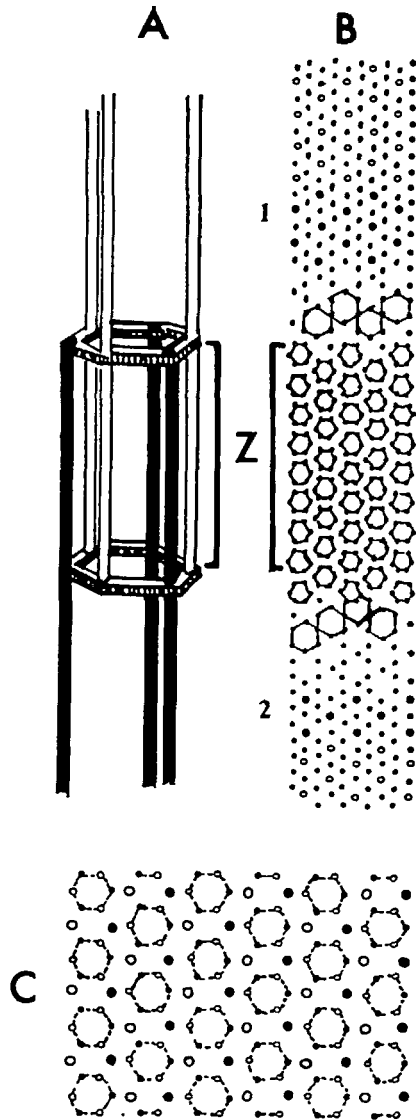
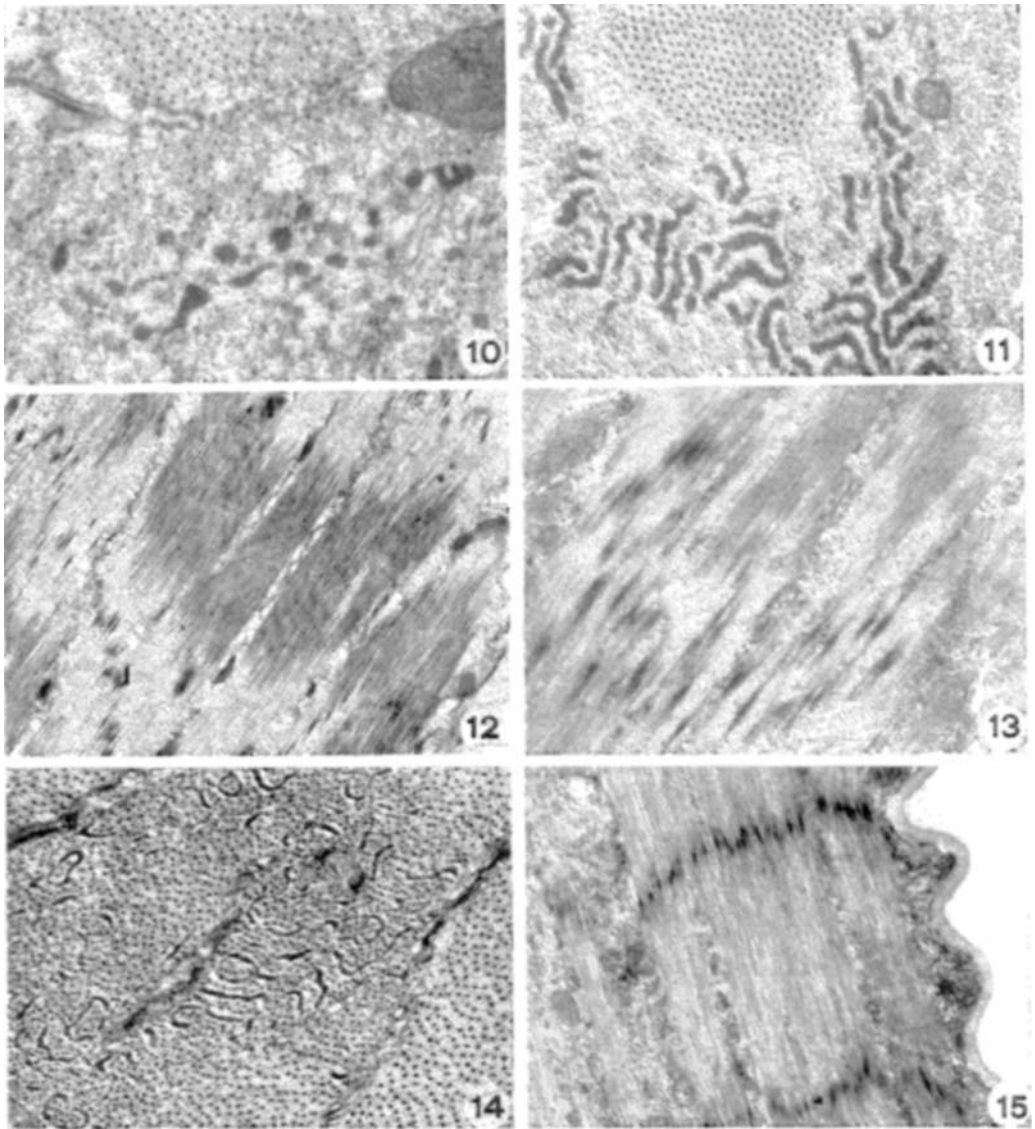


Fig. 9. — Diagram redrawn from Ashhurst (1967) showing the filament arrangement at the Z-line level, in *Letobocerus* flight muscles. A, Thin filament lattice around the Z-holes. B, Superimposition and combining of the hexagonal pattern of the thin filament in two adjacent sarcomeres: a new hexagonal pattern results at Z-level. C, Superimposed diagrams of the complete filament arrangement of two adjacent sarcomeres. In particular the characteristic Z-hexagonal organization arises from the combination of the thin filament patterns.



*Fragmented Z-band.*

Fig. 10. — *Philosamia cynthia* (larva): cross section of decontracted body wall muscles. The globular Z-elements are clearly disjoined. ( $\times 56\ 000$ ).

Fig. 11. — *Noctua pronuba* (larva): cross section of decontracted body wall fibre. The worm-like Z-elements are separated from each other. ( $\times 36\ 000$ ).

Fig. 12. — *Tipula tipula* (larva): decontracted sarcomeres from body wall muscle in longitudinal section. ( $\times 8\ 300$ ).

Fig. 13. — *Philosamia cynthia* (larva): decontracted body wall muscle in longitudinal section. Detail of staggered Z-elements. ( $\times 21\ 000$ ).

Fig. 14. — *Tipula tipula* (larva): cross section of supercontracted body wall fibre. Thick and thin filaments of adjacent sarcomeres can be found between the Z-elements. ( $\times 27\ 000$ ).

Fig. 15. — *Philosamia cynthia* (larva): longitudinal section of supercontracted body wall fibre. The sarcomere is shortened by more than 60% of its resting length. Both thick and thin filaments pass through the interrupted Z-line. ( $\times 9\ 300$ ).

*et al.*, 1965) or in the rolling-up muscles of the milliped *Glomeris* (Candia Carnevali & Vavassori, in preparation) which can even supercontract. In the latter, the supercontraction is correlated to the Z-line's capacity to change its structure according to the fibre's contraction rate. This means that there is an active dilatation process of the Z-line which passes, according to necessity, from an apparently compact and continuous structure to a net-like structure, thereby allowing the passage of thick filaments to the adjacent sarcomeres through its openings. Therefore in this case, the Z-line does not seem like a passive structure inside the contractile system but rather it is actively engaged in the shortening process. In both skeletal and visceral insect muscles nothing similar has yet been described. It does not seem credible to talk about an active process of fenestration in the supercontracting muscles because the various Z-elements appear well-differentiated and independent from each other both anatomically and functionally. This condition is the one most readily adaptable to hydraulic-type muscle systems (a definition also applicable to supercontracting visceral muscles) which physiologically function more like smooth muscles than like traditional striated muscles.

Both the active and passive tensions in the tension-length curves calculated for these fibres are closer to those of the *Mytilus* anterior byssal retractor muscle and the taenia coli in the Guinea-pig than to the diagrams obtained for the classic striated muscles (insect flight or frog skeletal muscles). The tension-length curves for leech helical muscles have already been referred to in this symposium by Lanzavecchia.

If we try to trace the evolutionary pathway of striated muscles on the basis of their Z-line morphology, the resulting interpretation could easily be erroneous. The opinion that the fragmented and irregular Z-line can be the primitive model preceding the achievement of a rigidly

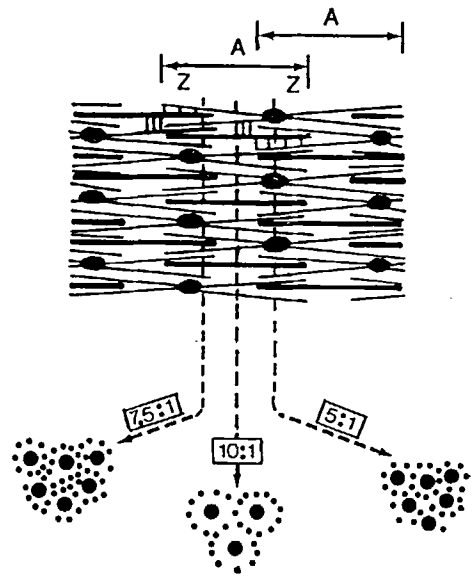


Fig. 16. — Schematic model showing the sarcomere organization in the supercontracting muscles at the supercontracted state: thick and thin filaments pass through the Z-line, overlapping those of the adjacent sarcomere. The thin to thick filament ratios vary at different sarcomere levels.

geometric perforated-plate structure seems hasty. This is contrasted by a series of arguments. In the first place, the fragmented Z (associated with supercontraction) is found in highly evolved animals such as Diptera and Lepidoptera among the Insecta, Diplopoda among the Myriapoda, and Cyrripedia among the Crustaceans. In the second place, the Z-bodies of the supercontracting muscles have a three-dimensional relationship with the two kinds of filaments which allows their passage into adjacent sarcomeres. On the contrary, in unquestionably primitive striated muscle models such as the fibres of *Peripatus* (Lanzavecchia & Camatini, 1978) which have a fragmented Z, but different from that of Insects, there is an arrangement of the single Z-elements and filaments, making a sliding of the filaments beyond the Z-elements impossible



for purely geometric and spatial reasons. The presence of different structural organizations for realizing functionally diverse supercontractions can indicate that these muscular structures originate as secondary modifications of a classic model of cross-striated fibres.

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