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Zoology

On Peculiarities of Insect Functionally Distinguished Flight Muscles Structure

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ABSTRACT. In this paper the ultrastructure, morphometry, histochemistry of locust (*Locusta migratoria migratorioides* R.F.) functionally different monofunctional (MOF) and bifunctional (BIF) flight muscles are discussed. Electron-microscopic, histochemical and morphometric investigations have revealed ultrastructure and metabolic peculiarities of monofunctional (muscle-113) and bifunctional (muscles-119,120) muscles. The structure of sarcomeres, their sizes, the number of actin filaments on orbit of myosin filaments, mitochondrial number and their layout, the high activity of succinatedehydrogenaze confirm the homogeneity and phase nature of muscle-113. The fibers of muscles-119, 120 involved in flight and extremities movement differ in size of sarcomeres and variability of number of actin filaments around myosin filaments. The results of morphological investigations allow to conclude that bifunctional muscles may be composed of heterogeneous fibers that functionally are divided into phase and tonic fibers. Thus, the existence of different parameters characteristic for mono- and bifunctional flight muscles are due to their functional specialization. *© 2015 Bull. Georg. Natl. Acad. Sci.*

***Key words**: flight muscles, monofunctional muscle, bifunctional muscle, ultrastructure, morphometry, histochemistry, locust.*

Introduction

Driving system of insects during the evolution process, which is 400 million years old, reached extraordinarily high level of functional specialization [1], the development of flight and other locomotor activities facilitated to the distribution of this kind of wildlife in our planet. The small sizes of insects correspond to perfectness of behavioral activity that causes researchers interest in studying of muscle tissue structural and functional peculiarities. Although the functionally distinguished flight muscles are being studied for many decades, the theme still has not lost its actuality.

In fulfillment of flight act the main role belongs to muscles acting indirectly, these muscles in their turn are divided into dorsal longitudinalis and dorsoven-

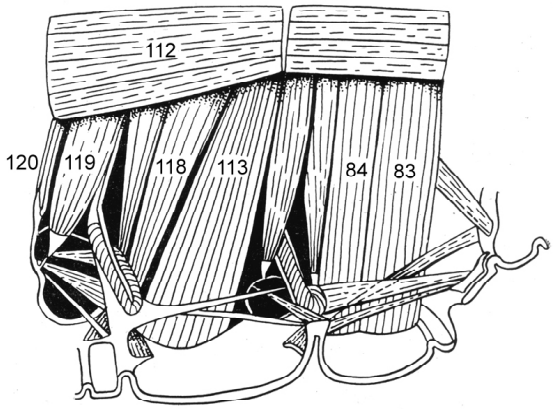

Fig. 1. Layout of locust flight muscles in meso- and metathorax.
83, 84, 113 – tergo-sternal muscles (monofunctional)
118, 119, 120 – tergo-coxal muscles (bifunctional)
112 – dorsal longitudinal muscle

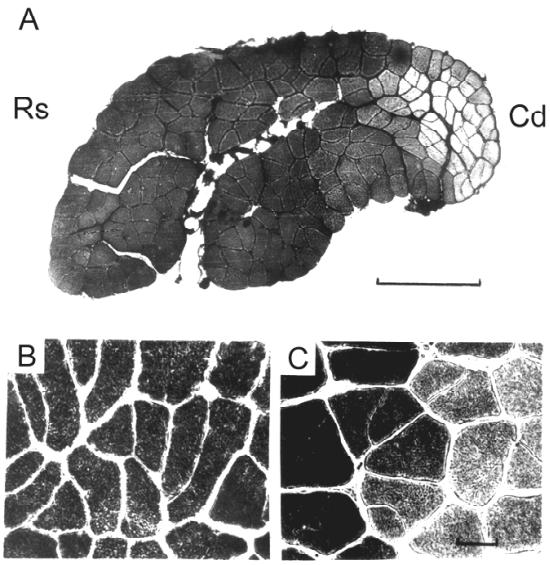

Fig. 2. Localization of lipids and succinatedehydrogenaze activity in locust bifunctional muscle (muscle -120). Microphotography. A – cross- section of the whole muscle. Staining with sudan black. Fibers of rostral (Rs) and caudal (Cd) parts differ by lipid content. B, C – cross-sections of fibers of rostral and caudal parts after the revealing of sdh localization. Caudal fibers are detected to have the lowest sdh activity Scale: A – 200 μm.; B, C – 15 μm.

tral muscles. There are tergo-sternal and tergo-coxal muscles in locust dorsoventral muscles. The contraction of tergo-sternal muscles causes only the wing rising, it means that they are monofunctional. The contraction of tergo-coxal muscles, in contrast to the tergo-sternal muscles, along with the rise of the wings (when the insect flies) participates in the movement of methatoracal extremities (when walking), so they perform two functions, and hence, are bifunctional [2, 3].

The major part of the research of functionally different muscles was conducted on monofunctional (MOF) muscle-113 and bifunctional (BIF) muscles - 119, 120 of locust (*Locusta migratoria migratorioides* R.F.) mature imago (Fig. 1) [4]. The figural marking of muscles is given according to anatomical nomenclature of Snodgrass [5].

The Morphology of 113, 119 and 120 Muscles

The muscle -113 is attached to tergite and sternite and is monofunctional. It is the thickest in dorsoventral muscles and consists of 400-500 fibers. The equal staining with sudan black of muscle cross section indicates to their structural homogeneity. The difference in succinatedehydrogenaze (sdh) activity was not observed either [6]. The use of the above-mentioned histochemical methods showed that muscle - 113 consists of fibers that are homogeneous according to the content and distribution of mitochondria. Further, these data were confirmed by electron microscopy studies [7]. The diameter of muscle-113 fibers is 20-48 μm. The average diameter is 30.11 ± 1.25 μm [8].

The muscle -119 is attached to tergite and methatoracal extremity coke. It is bifunctional and consists of 200-300 fibers. While staining with sudan black all fibers are equally stained alike the muscle 113. Sdh activity coincides with the results obtained by sudan black. The diameter of muscle 119 fibers is 20-48 μm. The average diameter is 35.24 ± 2.74 μm [8].

Thus, MOF muscle 113 and BIF muscles 119 consist of fibers that do not distinguish by distribution of mitochondria . These muscles do not differ in size of their consisting fibers either.

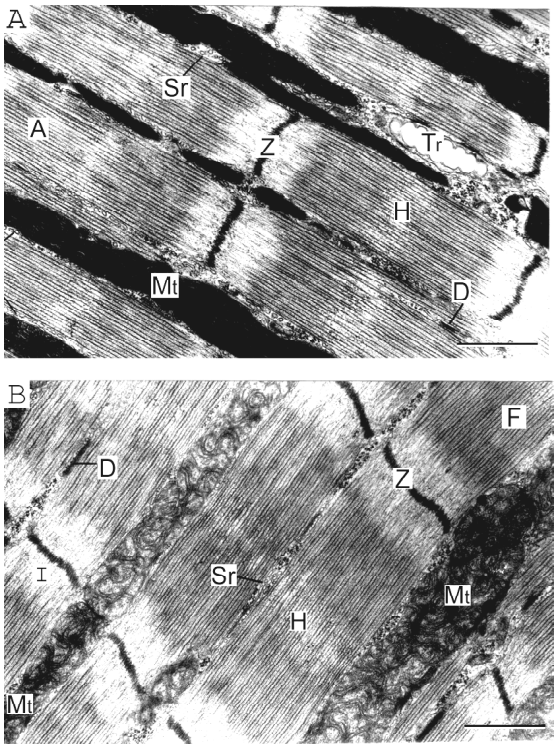

Fig. 3. The ultrastructure of locus thorax mono- and bifunctional muscle sarcomeres. A – muscle 113, B – muscle 119. The muscles are characterized by existence of H-zone and different sizes of A-discs. Tr-tracheola Scale: 1 μm.

The muscle-120 is of particular interest as earlier it was shown to contain fibers with different lipid composition [9] and having unequal ATP-aze activity [10]. The muscle is bind to tergite and methatoracal extremity coke and is bifunctional. Kutsch and Usherwood were first who described in detail the anatomy of the muscle [11]. According to their data the muscle is composed of bunches of rostral and caudal fibers. The first bunch is innervated by fast, the second - by fast, slow and restrictive axons. The muscle contains 170 -190 fibers. While staining with sudan black 75% of fibers are intensely, 13% – on average and 12% slightly stained. According to sdh activity, the proportion of different types of fibers is similar. The part of muscle rostral fibers in contrast to caudal ones is characterized by high lipid content and high activity of the sdh (Fig. 2) [7, 12]. Studies focused on only two types of fibers – intensely and slightly stained. Based on physiological and histochemical studies conducted in the past [10, 13] and our histochemical data, these fibers were classified as phase and tonic. The diameter of phase fibers is in the range of 16-52 μm, the maximum is expressed slightly and ranges in 36 - 40 μm interval. The diameter of tonic fibers is less and ranges in 16 - 40 μm interval with the maximum between 24-28 μm. The average diameter of the fibers was 27.90 ± 0.19 μm [14].

The Ultrastructure of 113, 119 and 120 Muscles Fibers

The participation of these muscles in different locomotor activities flight and walking and as the result, their functional distinction creates the bases for studying the organization of their contractile mechanism. Myofibrils (F) of -113 and 119 muscles are characterized by all items of sarcomeres (A and I discs, Z-line, H -zone etc.) The sizes of sarcomeres and A-discs (A) of -113 and -119 muscles are different. The lengths of sarcomeres and A-discs are 3.6 ± 0.05 and 2.7± 0.08 μm in muscle -113, 4.0 ± 0.07 and 3.0 ± 0.09 μm in muscle -119 correspondingly. Z-line (Z) in both muscle sarcomeres is straight, but its width in muscle -119 is somewhat more -111.0 ± 1.0 nm, than in muscle 113 – 102.0 ± 3.6nm [7].

A-discs of both muscle sarcomeres are characterized by well-pronounced H-zone (H) (Fig. 3). On the both sides of sarcomere at the border of A and I discs there are located dyads (D). The myofibrils of 113 and 119 muscle fibers (in both muscles they occupy 58% of area) have polygonal form at cross-section (Fig. 4, A, B). The important part of fibrils cross-section area is occupied by mitochondria (Mt), in muscle-113 – 24.6% ± 2.4 and in muscle-119 – 23,3 ± 2.1%. The areas occupied by the membrane of sarcoplasmic reticulum (Sr) and T-system tubes are 17.3 ± 1.2% and 18.5±0.4 % correspondingly [15]. The comparison of percentage data of areas occupied by mitochondria allows to assume that fibers of 113 and 119 muscles slightly differ by the intensity of metabolic processes. In MOF muscle 113 myofibrils actin filaments form the straight (double) hexagonal structure (Fig. 4, C). Such layout of myofilaments is

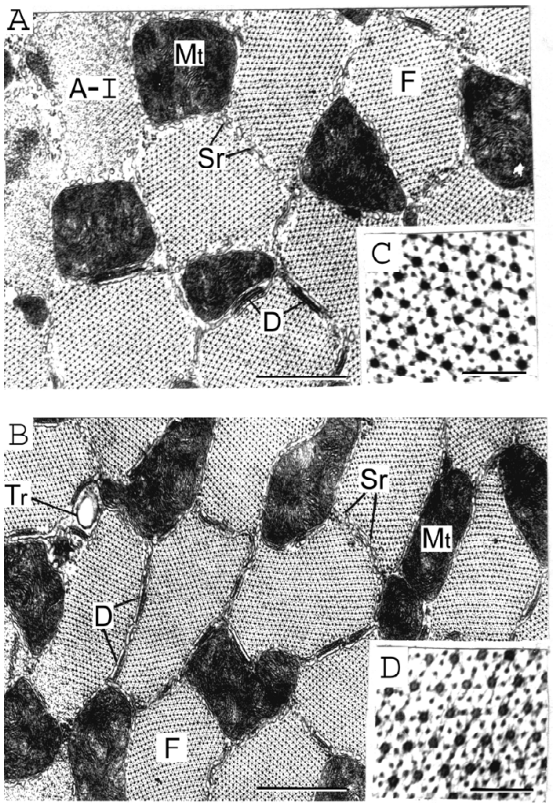

Fig. 4. Ultrastructure of myofibrils and spatial disposition of actin and myosin threads in mono- and bifunctional muscles. A, C – muscle-113, B, D – muscle-119. Thin – actin thread, thick – myosin thread. Tr-tracheola Scale: A, B – 1 μm. C, D – 0.1 μm.

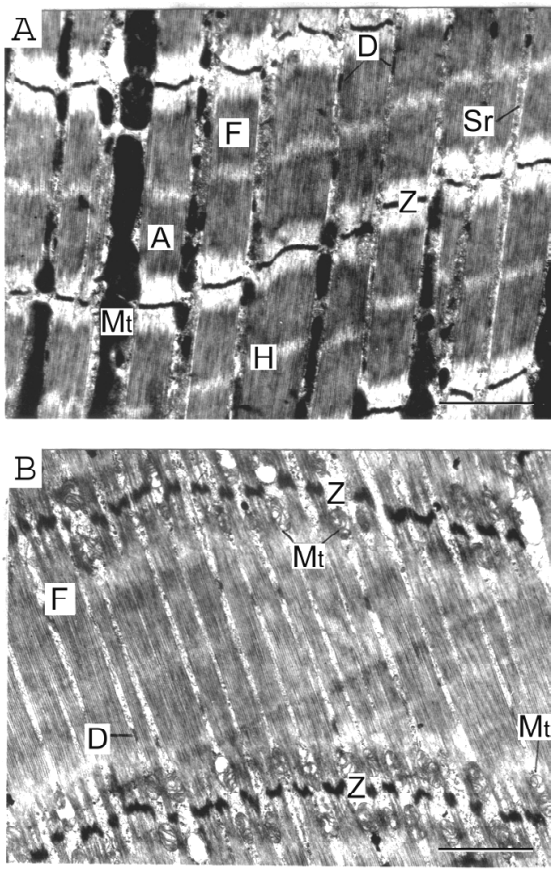

Fig. 5. Ultrastructure of rostral (A) and caudal (B) bunches fibers sarcomeres of muscle-120. The fibers of caudal bunch are characterized by large size sarcomeres, mitochondria arranged in pairs near Z-line and absence of H-zone. Scale: 2 μm.

typical for dorsal flying muscles of locusts [16], bumblebees [17], dragonflies, butterflies and other insects [18]. Between two myosin threads there is always located one actin filament. The average diameter of myosin filament is 16nm. The ratio of actin and myosin filaments is 3:1 (Fig. 6, A). The sizes of BIF muscle 119 threads are the same as those of muscle 113, but actin filaments do not form hexagonal lattice. Around the myosin filaments there are located 7-8 actin filaments (Fig. 4, D). Due to such topography of contractile fibrils the ratio of actin and myosin filaments is more than 3:1 (Fig. 6, B).

Thus, electron-microscopic investigations have revealed a number of differences in the structure of the MOF and BIF flying muscles contractile apparatus. The same number and layout of mitochondria in fibers of these muscles are in good accordance with the results obtained by light microscope, which showed that the fibers of investigated muscle do not differ by lipid composition, sdh activity, the existence of H-zone, relatively small sizes of sarcomeres and Z-line structure. These data allow to conclude that fibers of MOF muscle-113 and BIF muscle-119 are phase by their nature [15].

The fibers of rostral and caudal parts of muscle-120 differ in phospholipid content and sdh activity (Fig. 2). This difference is primarily due to uneven content of mitochondria in fibers. In mitochondria of rostral part interfibrilar space is evenly filled by mitochondria, myofibrils are closely located with each other, which create a typical picture of closely disposed muscles at cross-sections [19]. The measurements showed that the mitochondria accounts for 19.0± 0.3% of the fiber cross-sectional area, myofibrils

-66.4 ± 2.9%, interfibrilar space -14.6 ± 0.1%.

It is necessary to specially emphasize the fact that the number of actin filaments around the myosin thread, unlike 113 and 119 muscles, is more and within the range of 9-10. The ratio of actin and myosin filament in rostral part of fibers is more than 4:1 (Fig. 6, C). The sarcomeres of these fibers have the structure of phase-type fibers. The sizes of sarcomeres and A-disc are 4.0 ± 0.04 and 3.1 ± 0.04 ìm correspondingly. In the central part of the A-disc the H-zone is well seen, Z-line is straight and its width is equal to 130.0 ± 5.0 nm [12]. Mitochondria with dense matrix, which are distributed between the fibrils, are quite big in their size. One mitochondria can take whole length of the sarcomere. Dyads are located at the border of A and I discs (Fig. 5, A).

The fibers of caudal part of muscle-120 significantly differ from previously discussed muscles. In these fibers mitochondria are smaller, they have light matrix and less crista. They account for about 6.1 ± 0.27% of the area of the fibers. Mitochondria are distributed unevenly and are located in the I-disc region near Z-line (Fig. 5, B). Interfibrilar space occupies an area of 13.3 ± 0.16% [7]. It is interesting, that the diameter of myosin threads in these fibers is the longest (23.0 ± 0.6nm) in studied muscles (see Table). Around one myosin thread there are 10-12 actin threads and hence the ratio is always more than 5:1 (Fig. 6, D).

The ratio of actin and myosin threads in insects' sarcomeres, unlike that of vertebrate (2:1), is more and depends on muscles functional peculiarity – the intensity of their contraction [1]. For example, in fast flying muscles this ratio is 3:1, in relatively slow – 3:1 – 4:1, in fast muscles of extremities 5:1 – 6:1 and sometimes (in visceral muscles) 7:1 [20].

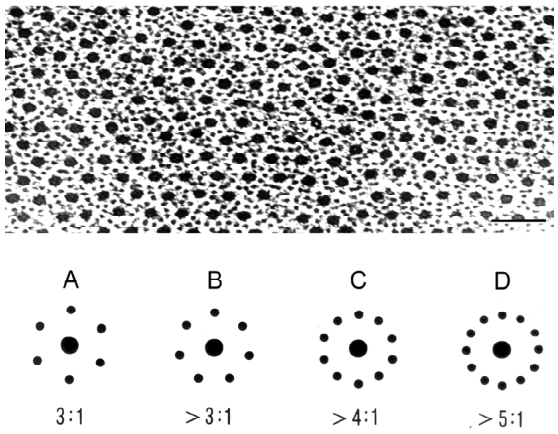

Fig. 6. The ultrastructure of cross-section of muscle-120 caudal bunch fibers myofibril. Myofibril contains large amount of actin threads (thin threads) Scale: 0.1 μm. Actin filaments layout (disposition) around myosin threads in myofibrils of mono- and bifunctional muscles. (Diagram) A, B, C, D – phasic fibers of 113, 119, 120 muscles and tonic fibers of muscle 120. Under the scheme numerical ratios of actin (thin) and myosin (thick) threads are indicated.

The fibers of caudal part of muscle-120 also differ by variability of sarcomere sizes of muscle 120 rostral part (Fig. 5, A, B. Table). The biggest sarcomeres characterize the fibers with lowest sdh-activity and faintly staining by sudan black. The sizes of their sarcomeres and A-discs are 6.3 ± 0.09 μm and 4.1 ± 0.12 μm correspondingly [12,21]. Z- line is significantly wide and has a toothed profile (Table, Fig. 5, B). In the center of A-disc the fibers do not have H-zone (Fig. 5, B). The structure of Z-line, big sizes of sarcomeres and

Table. The structural differences of locust tergo-sternal and tergo-koxal muscles

Muscle	Sarcomere size (m)	Size of A-disk (m)	The width of Z-line (nm)	The diameter of myosin treads (nm)	Mitochondria (%)	Myofibrils (%)	Interfibrillar space (%)
Muscle-113	3,6±0,05	2.7±0.08	102.0±3.6	15.9±4.08	24.6±2.40	58.0±2,71	17.3±1.23
Muscle-119	4,0±0,07	3.0±0.09	111.0±1.0	15.6±1.35	23.3±2.11	58.0±2,08	18.5±0.42
Phazic fibers (muscle-120)	4.0±0.04	3.1±0.04	130.0±5.0	19.1±0.5	19.0±0.3	66.4±2,91	14.6±0.12
Tonik fibers (muscle-120)	6.3±0.09	4.1±0.12	245.0±16.5	23.0±0.6	6.1±0.27	80.8±3,18	13.1±0.16

Notice: percentage of mitochondria, myofibrils and interfibrillar space were calculated from total area of fiber cross- section.

ratio of myofilaments are the typical morphological signs of tonic fibers contractile apparatus of locomotor muscles of arthropoda [22-24].

Thus, basing on the above studies, we are the first who morphologically confirm the existence of tonic fibers in flying muscles of insects. This fact is new and has principal significance for understanding the peculiarities of functioning of insect flying muscles.

The morphology of the muscle fibers and the number of mitochondria are linked to functional specialization of specific muscle. The summary results of the morphometry of discussed muscles are given in Table. The relationship between muscle functional specialization and the sarcomere sizes of muscle fibers of individual groups is particularly evident. MOF muscle -113 has the shortest sarcomeres and the tonic fibers of caudal bunch of muscle 120 have the longest ones. The number of actin filaments located around myosin threads also correlates with functional specialization of fibers. The lowest number of actin filaments is in fast phase MOF muscle-113, the highest – in caudal part of muscle-120 (Fig. 4, C, 6. Diagram). Thus, not only the sarcomere structure, but also the ratio of contractile filaments confirms the tonic nature of fibers of caudal part of locust bifunctional muscle-120 [12, 20].

While studying the percentage content of myofibril proteins (actin, myosin) in MOF and BIF muscles the difference was detected only in the case of actin. In particular, MOF muscle -113 contains 18.21% of actin, BIF muscles 119 and 120 include 21.12% and 26.18% correspondingly [25]. These data correlate with the ratio of actin and myosin filaments (Fig. 6, Diagram).

Comparative investigation of intra cellural space volume and Na^+, K^+, Mg^{2+}, Ca^{2+} content in locust locomotor muscles shows that the difference between water, Na^+, Mg^{2+}, Ca^{2+} content in locust flight and leg muscles was insignificant. As to K^+ content, it varies in the range of 88.4-104.5 mM/kg (wet weight) in flight muscles and 115.5-129.1 mM/kg (wet weight) in leg muscles. In the mixed muscles K^+ content is high. Intra cellural space volume is maximum in locust flight muscles and minimum –in leg muscles, which must be connected with their functions [26]. Recent studies demonstrated that MOF and BIF muscles do not significantly differ by intensity of DNA synthesis [27].

Thus, the existence of different parameters characteristic for mono- and bifunctional flight muscles are due to their functional specialization.

ზოოლოგია

მწერების ფუნქციურად განსხვავებული საფრენი კუნთების აგებულების თავისებურებათა შესახებ

გ. პაპიძე*, ნ. გაჩეჩილაძე**, ო. ახმეტელაშვილი*, თ. ერისთავი**

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შრომაში განხილულია კალიის (***Locusta migratoria migratorioides*** **R.F.**) ფუნქციურად განსხვავებული – მონოფუნქციური (მოფ) და ბიფუნქციური (ბიფ) საფრენი კუნთების ულტრასტრუქტურა, მორფომეტრია, ჰისტოქიმია და სხვ. ელექტრონულ-მიკროსკოპული, ჰისტოქიმიური და მორფომეტრული გამოკვლევებით დადგენილია მონოფუნქციური (კუნთი-113) და ბიფუნქციური (კუნთები-119, 120) კუნთების ულტრასტრუქტურული და მეტაბოლური თავისებურებანი. სარკომერების აგებულება, მათი ზომები, მიოზინის ძაფების ორბიტაზე აქტინის ძაფების რიცხვი, მიტოქონდრიების რაოდენობა და მათი განლაგება, სუქცინატდეჰიდროგენაზის მაღალი აქტივობა ადასტურებს 113 კუნთის ერთგვაროვნებას და მის ფაზურ ბუნებას. 119, 120 კუნთების ბოჭკოები, რომლებიც მონაწილეობენ ფრენასა და კიდურების მოძრაობაში, განსხვავდება სარკომერის ზომებით და მიოზინის ძაფის ირგვლივ აქტინის ძაფების რიცხვის ვარიაბელობით. მორფოლოგიური კვლევის შედეგები უფლებას გვაძლევს დავასკვნათ, რომ ბიფუნქციური კუნთები ბოჭკოების შემადგენლობით შეიძლება იყოს ჰეტეროგენური, რომლებიც ფუნქციურად იყოფა ფაზურ და ტონურ ბოჭკოებად. ამრიგად, მონო- და ბიფუნქციური საფრენი კუნთებისათვის დამახასიათებელი განსხვავებული პარამეტრების არსებობას განაპირობებს მათი ფუნქციური სპეციალიზაცია.

REFERENCES

1. *Svidersky V.L.* (1988) Lokomotsiia nasekomykh, Leningrad, 259 p. (in Russian).
2. *Wilson D.M.* (1962) J. Exp. Biol., **39**: 669-677.
3. *Wilson, D.M. Weis-Fogh T.* (1962) J. Exp. Biol., **39**: 643-667.
4. *Albrecht F.D.* (1953) The Anatomy of the migratory Locust. London, Atione press 118 p.
5. *Snodgrass R.F.* (1935) Principles of insect morphology. N.Y., 667 p.
6. *Pirs E.* (1962) Teoreticheskaia i prikladnaia gistokhimia. M., 890 p. (In Russian)
7. *Papidze G.P., Mandelshtam Yu.E.* (1987) Bull. Georg. SSR Acad. Sci. 126: 169-172 (in Russian).
8. *Papidze G.P.* (2003) Proc. Georgian Acad. Sci. Biol. Ser. A. **29**: 705-711, (in Russian).
9. *Shumova I.A.* (1973) Zh. Evol. Biokhimii Fiziol., **9**: 306, (in Russian).
10. *Shumova I.A., Mandelshtam Yu.E., Grigorev V.V.* (1982). Tsitologia, **24**: 647-651 (in Russian).
11. *Kutsch W., Usherwood P.N.R.* (1970) J. Exp. Biol., **52**: 299-312.
12. *Mandelshtam Yu.E., Mashanskii V.F., Papidze G.P.* (1986) Zh. Evol. Biokhimii Fiziol., **22**: 211-213 (in Russian).
13. *Grigorev V.V., Mandelshtam Yu.E.* (1981) Neurophysiology, **13**: 98-103 (in Russian).
14. *Papidze G.P.* (2004) Proc.Georgian Acad. Sci. Biol. ser. A, **30**: 257-262, (in Russian).
15. *Mandelshtam Yu.E., Papidze G.P.* (1987) Zh. Evol. Biokhimii Fiziol., **23**: 780-785 (in Russian).
16. *Mandelshtam Yu.E.* (1983) Neiron i myshtsa nasekomogo, Leningrad, 168 p. (in Russian).
17. *Mandelshtam Yu.E.* (1968) Fiziologia i Biokhimia bespozvonochnykh, Leningrad, p. 196-205 (in Russian).
18. *Elder H.Y.* (1975) Muscle structure (Insect muscle). London, Acad. Press, 1-74 p.
19. *Tiegs O.W.* (1955) Phil. Trans. Roy. Soc.(B) 238: 221-359.
20. *Hoyle G.* (1983) Muscle and their neural control. N-Y., John Willeg Sons, 689 p.
21. *Papidze G.P.* (1988) Abstracts of IV International Congress of Cell. Biology. Montreal, Canada, 120.
22. *Rhuben M.B., Kammer A.E.* (1980) J. Exp. Biol., 84: 103-118.
23. *Cochrane D.G., Elder H.Y., Usherwood P.N.R.* (1972) J. Cell. Sci., **10**: 419-441.
24. *Atwood H.L.* (1973) Amer. Zool. **13**: 357-358.
25. *Papidze G.P. et al.* (2006) Proc. Georgian Acad. Sci., Biol. ser. B, 4: 19-23.
26. *Papidze G.P. et al.* (2010) Bull. Georg. Natl. Acad. Sci., **4**: 125-129.
27. *Papidze G.P. et al.* (2014) Bull. Georg. Natl. Acad. Sci., **8**: 118-121.

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