# Histological and Ultrastructural Study on the Atrophied Flight Muscle of the Female Usherhopper, *Poekilocerus bufonius* (Klug)

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ABSTRACT. The present study was undertaken to describe the histology and fine structure of the longitudinal flight muscle of female *P. bufonius* at different ages. The appearance of female flight muscle remain translucent in all ages studied of the adult insect. Histological and ultrastructural study showed that the muscle fibers are reduced to thread-like structures and the mitochondria are scarce and abnormal in shape. The tracheoles are scarce or absent from female flight muscle fibers of all ages studied.

Signs of muscle fibers degeneration was noted at the periphery of some muscle fibers at various ages studied. Disintegration and loss of myofibril striations are coupled with the proliferation of sarcoplasmic reticulum. It was concluded that the atrophy of adult female flight muscle is developmental and due to incomplete development (retardation) and degeneration in old insects. Possible causes for incomplete muscle development is outlined in the discussion.

### Introduction

A previous comparative study on the fine structure of flight muscle of mature male and female *Poekilocerus bufonius*<sup>[1]</sup> indicate that the flight muscle fibers are reduced in size to slender thread-like structures. The nuclei are located peripherally and are surrounded by undifferentiated sarcoplasm, suggesting nuclear pyknosis. In addition the myofibrils are small, cross-striations are poorly defined and Z-bands are irregularly arranged or absent in some cases. Furthermore, the mitochondria are scarce and the sarcoplasmic reticulum is abundant with clear indication of dilation.

The mature adult female of *P. bufonius*, is incapable of flight or wing fluttering, whereas the male of this species can fly for a very short distance. Degeneration and atrophy of muscle have been observed in a variety of experimental and pathological diseases<sup>[2-4]</sup>. This indicates that whatever the causes of muscle degeneration and atrophy are, the process is similar. Edwards<sup>[5]</sup> reported that degeneration of the indirect flight muscle in adult female of *Dysdercus intermedius* coincides with oocyte growth and is under endocrine control. However, Scudder<sup>[6]</sup> discussed the degeneration of flight muscle in detail and threw doubt about the possible physiological link between flight muscle and egg production. He demonstrated that the smaller flight muscle in flightless stages of *Cenocorixa bifida* are a consequence of retarded growth, not degeneration.

*Poekilocerus bufonius* in nature probably feeds almost exclusively on asclepiad plants<sup>[7]</sup> one of which is *Calotropis procera*, which contains toxic cardiac glycosides (CGs). The latter are specific inhibitors to Na<sup>+</sup>/K<sup>+</sup> – transporting ATPase activity and minute amount of CG is said to cause death to most vertebrates and invertebrates<sup>[8-10]</sup>.

*Poekilocerus bufonius* ingest and sequester CGs in to their tissues and bilobed gland and use them for protection from predators<sup>[7,11,12]</sup>. It is possible that the species utilizing a toxic chemical may suffer some kinds of physiological cost or place stress on their bearers<sup>[13]</sup>.

It is remains to be established whether the atrophy of flight muscle of mature adult female of *P. bufonius* coincides with oocyte growth or whether the muscle never develops (developmental atrophy). The latter may due to a physiological cause affected by the toxic CGs through interaction between CGs and endocrine factors<sup>[14,15]</sup>.

In order to suggest one or refute the other of the above assumptions, the present study was carried out to investigate to what extent the flight muscle component varies during developmental stages of *P. bufonius*. For comparative purposes some results of studies on flight muscles of male *P. bufonius* and *Locusta migratoria* are included.

#### **Material and Methods**

#### Sampling of the Insect Population

*Poekilocerus bufonius* at various stages of development, were collected from Wadi Asail and Gizan area. Insects (males and females) were kept in large cages and supplied daily with fresh branches of *Calotropis procera*.

The method of sampling used in the present study is the same as was already used by Al-Robai<sup>[14]</sup> for *Locusta migratoria*. Using this method of sampling, it was possible to obtain the required stages (first day adult after final ecdysis, 3, 10, 20 days, during mating and egg deposition stages).

#### **Flight Muscle Preparation**

Adult females and males, *P. bufonius*, of the required stage were killed as reported previously<sup>[1]</sup>.</sup>

#### **Light Microscope Preparation**

For the light microscope preparations, the female and male flight muscles were fixed in Bouin's solution. Within 20 minutes after fixative application, the tissues were dissected out and placed in fresh Bouin's solution. After the normal routine processes, sections of approximately 5 mm were stained by haematoxylin and eosin.

### **Transmission Electron Microscope Preparation**

For electron microscope study, the muscle fixation, dehydration, embedding and staining were carried out as was already reported<sup>[1]</sup>.

# Results

#### **General Observations**

Body measurements showed that the mature adult female (gravid) of *P. bufonius* is 5.5-6.5 cm long and 3.5-6.5 gm in weight. The wings (fore and hind) are 3.2-3.5 cm and the distance between the tips of the hind wings is 5.8 cm in the average.

Anatomical observations indicate that the fibers of flight muscles of all adult female stages are very small and reduced to thread-like structures. They are characteristically pale in appearance.

In contrast, the mature adult male is approximately 4 cm long and about one gram in weight. The lengths of fore and hind wings (the distance between the wing tips) are 6.5 cm (3.1 cm each), and 6 cm (2.8 cm each), respectively. The male flight muscles are relatively large and are reddish-brown in colour.

#### **Histological Studies of Flight Muscle**

Examination of longitudinal sections of the flight muscle of female *P. bufonius*, at various stages, shows a reduction in the width of muscle fibers compared to that of male (see latter) (Figs. 1-5); the flight muscle being very thin and tend to be thread-like structure. The nuclei are more or less centrally placed and their number per muscle fiber is high at all ages studied (Figs. 2 and 4-7). The transverse sections (Figs. 8-10) show that the sarcoplasm of some muscle fibers contains an amorphous material and the nuclei are more centrally placed, and tend to occupy larger volumes of the sarcoplasm (Figs. 8 and 11). It appears that in later stages (15-20 days after final ecdysis) the muscle fibers tend to be fragmented (Fig. 10).

The cross-striations of the female flight muscle are poorly defined (Figs. 1,2,6 and 12) and this was a general characteristic of all the stages studied. However, some regions of the flight muscle fibers retain the more or less normal cross-striation and appearance of muscle fibers (Figs. 3 and 13) especially in those of the last nymphal instar (Fig. 1) and the first few days of adult life (Figs. 2 and 3).

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FIG Photomicrograph of longitudinal section (PLS) of flight muscle of the last nymphal instar of female *P. bufonius*. Muscle fibers are reduced to thread-like structure and the cross-striations are absent. N: Nucleus; MF: Muscle fiber. (× 1280).



FIG. 2. PLS of reduced flight muscle of third day old adult female *P. bufonius.* Note the high number of nuclei (N) and the absence of the cross-striation. (× 1280).

Developmental stages of female flight muscle fibers of the last nymphal instar and adult are clearly seen namely: Primary mature muscle fiber, immature muscle fiber (Fig. 8) and satellite cells (Figs. 4 and 8). Multinucleated muscle fibers are observed in the flight muscle of the last nymphal stage (Fig. 1) and various ages after final ecdysis (Figs. 6 and 12).

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FIG. 3. PLS of flight muscle of the fifth day old adult female *P. bufonius*. The muscle fibers are further apart and cross-striations (arrow) are evident. N: Nucleus (× 1280).



IG. 4. PLS of flight muscle of 20 days old adult female P. bufonius. The number of nuclei are high and focal degeneration of muscle is shown (arrow head). A satellite cell is seen on the upper part (arrow). (× 1280).

Focal regions of the flight muscle fibers (Figs. 5, 7 and 14) undergo cytolytic alteration as witnessed by disappearance of myofibrils and the aggregation of nuclei, suggesting very severe degeneration of flight muscle.

Tracheal branches are frequently seen in close proximity to or penetrating among muscle fibers (Figs. 15 and 16, respectively).



FIG. 5. PLS of flight muscle fibers of female *P. bufonius* at the stage of eggs deposition. Focal region undergoes cytolytic alteration (arrows) and aggregation of nuclei (N) is demonstrated. (× 510).

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FIG. 6. PLS of female flight muscle fiber of gravid female *P. bufonius*. A high number of nuclei (N) are seen in the more or less central part of the fibers (lower fiber). Note the absence of the cross-striations. (× 1280). A.A. Al-Robai & H.S. Al-Ghamdi

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In contrast to the above abnormal flight muscle fibers of female *P. bufonius*, examination of transverse (Fig. 17) and longitudinal (Fig. 18) sections of male flight muscle shows normal flight muscle fibers. There is a considerable variation in the shape and size of each individual fiber profile. The peripheral position of the nuclei as well as transverse striations are evident.

## **Ultrastructural Studies of Flight Muscle**

The tracheoles are very scarce or absent from female flight muscle fibers of all ages studied (Fig. 19). However, tracheae are frequently found among muscle fibers of female (Fig. 19). In contrast, very well developed tracheoles are encountered in the flight muscle fibers of the male (Figs. 20 and 21).

Mitochondria are very scarce and, occasionally, small or vacuolated mitochondria are evident (Fig. 19) in female. In some peripheral regions of the muscle fiber, where degeneration has commenced, flattened mitochondria are seen (Figs. 22 and 23).



FIG. 7. PLS of flight muscle fibers of female *P. bufonius* (10 days old). Degenerated muscle fiber is seen in the middle. N: Nucleus (× 1280).



FIG. 8. Photomicrograph of transverse section (PTS) of female flight muscle (last nymphal instar). The nuclei (N) are large in size and centrally placed in the amorphous region of muscle fibers. Primary (arrow), immature (arrow head) and satellite muscle fiber(s) are shown (× 1280).



FIG. 9. PTS of flight muscle of 10 days old adult female P. bufonius. Amorphous muscle fibers (arrow) are shown with large size nuclei (N). ( $\times$  1280).



FIG. 10. PTS of flight muscle of 15 days old adult female P. bufonius. Note the amorphous fibers (arrow head) with centrally placed nuclei (N). Some muscle fibers are reduced to small ones (arrow). (× 510).



FIG. 11. PTS of abnormal muscle fibers (compare with Fig. 17) of 10 days old adult female *P. bufonius*. Large volume of muscle sarcoplasm is occupied by nucleus (N) and sign of degeneration is also shown (arrows). (× 1720).

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FIG 2. PLS of flight muscle of 10 days old adult female P. bufonius. Cross-striations are absent. (× 1280).



FIG. 13. PLS of flight muscle of three days old adult female *P. bufonius*. Although the muscle fibers are further apart (arrow heads), cross-striations are evident (arrows). (× 510).

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IG. 14. PLS of flight muscle of 10 days old adult female *P. bufonius*. Focal degeneration is clearly shown (arrows). N: nucleus (× 800).



FIG. 15. PTS of flight muscle of female *P. bufonius* (5 days old adult). Tracheal branch is seen in close proximity to muscle fiber. (× 510).



16. 16. PLS of flight muscle (6 days old adult) of female *P. bufonius*. Tracheal branch is seen penetrating among muscle fibers (arrows). ( $\times$  510).



FIG. 17. PTS of flight muscle of 5 days old adult male *P. bufonius*. Muscle fibers of normal appearance with small nuclei (N) in the peripheral region are evident. Stripe of myofibrils can be recognized in the muscle fibers (arrow heads). (× 640).



FIG. 18. PLS of flight muscle fibers of 5 days old adult male *P. bufonius*. Normal appearance muscle fibers with distinct cross-striation and peripheral nuclei (arrow heads) are demonstrated. (× 1280).

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FIG. 19. Transmission electron micrograph (TEM) of transverse section through flight muscle of 10 days old adult female *P. bufonius.* showing the absence of tracheole. A considerable variation of the shapes of myofibrils and scarcity of mitochondria. Sign of degeneration is seen in the peripheral region (arrows) and sarcoplasmic reticulum (SR) is developed. However, prominent dilation is evident (see inset). T: Trachea (× 9000).

The more or less normal mitochondria of the female flight muscle contain very dense cristae (Fig. 23). There is no much difference in size, abundance and fine structure of female flight muscle mitochondria of all ages studied. The mitochondria are abundant in muscle fibers of male and are packed between myofibrils (Fig. 20). The mitochondrial arrangement is clearly shown in longitudinal sections where they appear as columns of different sizes (Figs. 21 and 27). Similar arrangement is also shown in the flight muscle of male *Locusta migratoria* (Fig. 24).

The cross-striations of the female flight muscle fibers are poorly defined and the Zbands are irregularly arranged (Figs. 25 and 26). Sarcomeres of the peripheral myofibrils are replaced by patchy electron-dense material, presumably, derived from disintegrated Z-band and myofibrils. The degenerated myofilaments and indistinct sarcomere bands are also demonstrated (Fig. 26). The flight muscle of all ages studied of female *P. bufonius* showed the same patterns of cross-striations. This is in contrast to the general characteristic pattern of the flight muscle fibers of male *P. bufonius* (Fig. 27) and male *L. migratoria* (Fig. 24). The myofibrils of the later insects are in almost perfect transverse register, as indicated by the relatively straight rows of Z-bands.



FIG. 20. TEM of transverse section through flight muscle fiber of mature adult male *P. bufonius*. Very well developed tracheole (t) is seen in between myofibrils. Mitochondria (M) are abundant and packed between myofibrils. (SR: Sarcoplasmic reticulum: D: Dyad). (× 16000).

The myofibrils represent the bulk of the muscle fibers with the nuclei situated in a peripheral position of female flight muscle of *P. bufonius* (Fig. 23). There are considerable variation in the shape of the myofibrillar profiles (Figs. 19 and 23). The peripheral myofibrils tend to be somewhat larger in size than the more centrally placed ones, which tend to be polygonal (Fig. 28). The arrangement of the myofibrils and mitochondria of the flight muscle fibers of male *P. bufonius* is irregular and described as closed-packed (Fig. 20).

The nuclear chromatin of the female muscle fiber is aggregated or clumped into numerous masses adjacent to nuclear membrane (Fig. 22). It appears that the nucleus is abnormal in size and shape and contrast sharply with the normal ones of the flight muscle fibers of male (Fig. 29). Occasionally, an abnormal nucleus of female flight muscle fibers surrounded with undifferentiated sarcoplasm is in the peripheral region (Fig. 30).

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FIG. 21. TEM of longitudinal section through flight muscle fiber of male *P. bufonius*. Tracheole (t) is seen and columns of mitochondria (M) between myofibrils are shown. Normal appearance of sarcomeres bonds are evident. Note also the normal distribution of sarcoplasmic reticulum (SR) and its association with T-system to form dyads (D). (Z:Z-band). (× 12500).

Well developed sarcoplasmic reticulum and dyads are present in the flight muscle fibers of female (Fig. 19) and male (Figs. 20 and 21) *P. bufonius*. However, prominent dilation is a general characteristic of female muscle fibers (Fig. 28).

The peripheral myofibrils sometime, especially in gravid stages of female flight muscle fibers, appears abruptly interrupted by muscle degeneration (Figs. 19 and 31). The inner myofibrils of the same muscle fibers, on the other hand, reflect ordinary structures, although the inter myofibrillar sarcoplasmic reticulum spaces are slightly enlarged (Fig. 31).

# Discussion

The morphometric pattern and anatomical observation for male and female of all collections are similar for all populations collected from various sites. Field observations showed that the population of *P. bufonius* generally move, let alone travel, little during the course of their lives. Eggs are laid in the ground, usually below, or in close proximity to their host plant, *Calotropis procera*, so that there is normally very little displacement between the food/shelter and oviposition habitat. There is thus a



FIG. 22. TEM of transverse section through flight muscle fiber of 20 days old adult female *P. bufonius*. Commenced muscle degeneration (arrows) is shown in the peripheral region of muscle fiber (see inset). Note also the presence of flattened mitochondria (WM). Abnormal nucleus with undifferentiated sarcoplasm is shown (N). SR: Sarcoplasmic reticulum. (× 10000).

tendency for any *Poekilocerus* individual to pass its entire live within the confines of a single habitat. The tendency to shorter-winged condition in female, would seem to rule out any major displacement of the populations as a whole, though evidently the males are capable of flying, which probably facilitating encounters between sexes.

The appearance of female flight muscle remain translucent in all ages studied of adult insect. This is supported by the histological and ultrastructural studies in which the muscle fibers are reduced to thread-like structure and the mitochondria are scarce. The above mentioned finding is in sharp contrast to the situation reported for male L. migratoria<sup>[14]</sup>; during the first week of mature adult life, muscle colour changed from white-translucent to reddish-brown.

The results presented in this study indicate that the severe atrophy of adult female flight muscle is due largely to retrenchment rather than degeneration, which commenced in advanced age. Similar observations have been reported for the flightless grasshopper *Romalea microptera*<sup>[16]</sup>, *Leptinotarsa decemlineata*<sup>[17]</sup> and *Coenocorixa bifida*<sup>[6]</sup>. Scudder<sup>[6]</sup> discussed the degeneration and atrophy of insect flight muscles in

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FIG. 23. TEM of transverse section of flight muscle fiber of 20 days old adult female *P. bufonius* showing the peripheral degeneration. M: Mitochondria. (× 16000).

detail and threw doubts of any possible physiological link between flight muscle degeneration and egg production, which was suggested by Edwards<sup>[5]</sup> for *Dysdecus intermedius*.

The flight muscle of female *P. bufonius* is incompletely developed at emergence and does not grow after the final ecdysis as indicated by the histological and fine structure of the present study. The appearance and components of the flight muscle are more or less similar at all ages studied. Signs of degeneration or autolysis<sup>[17]</sup> appear at the periphery of the muscle fibers where disintegration and loss of myofibrils is coupled with proliferation of sarcoplasmic reticulum. It is tempting to suggest that the atrophy of adult female flight muscle is developmental, namely due to incomplete development. A similar suggestion has been made for *Leptinotarsa decemlineata*<sup>[17]</sup>. Normally, the degeneration of the flight muscles of insects leads to atrophy and occurs after sexual maturity in many species<sup>[18]</sup> or setting on their hostplant after migratory flight<sup>[19-21]</sup>.

Locomotion and flight are processes animals develop for the purpose of mating, seeking food, escaping from predators and hostile environment conditions<sup>[22]</sup>. The male and female *P. bufonius*, in nature feed almost exclusively on asclepiad plants,

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FIG. 24. TEM of transverse section through flight muscle fiber of adult male *Locusta migratoria* (A). Muscle fiber components are similar to that of Fig. 21. Normal arrangement, which is characteristic to synchronous insect flight muscle. Note also the arrangements in longitudinal section (24 B). (M: Mitochondria, N: Nucleus; t: Tracheole). (× 6000).

one of which is *Calotropis procera*<sup>[7,12]</sup>, which contains toxic CGs. These toxic compounds are ingested and sequestered into bilobed gland and utilized for defense against predators<sup>[12,23]</sup>. It is well established that a minute amount of CGs, which are known as specific inhibitors of Na<sup>+</sup>/K<sup>+</sup> transporting ATPase activity, is toxic to most vertebrates and invertebrates<sup>[10,24]</sup>. The dose causing 50% mortality (LD<sub>50</sub>) in vertebrates is in the range of 0.1-1.5 mg/kg body weight<sup>[18]</sup>. However, the LD<sub>50</sub> which has been determined for *P. bufonius* by ouabain injection was 2 g/kg body weight (quoted from Euw *et al.*<sup>[7]</sup>. Pharmacologically active sugar derivatives of sterols, which are known collectively as CGs, are found in the haemolymphs of *P. bufonius*. Their most important action is to improve the contractility of the failing heart and to produce partial heart block in arterial fibrillation<sup>[8,9,24]</sup>.

It has been reported that the chemical deterrents in insects are definitely toxic and may place stress upon their bearers<sup>[13]</sup>. There is some evidence that the *P. bufonius* (male and female) suffers some kind of physiological cost as a result of feeding on *C. procera* and sequestering CGs. For example, Al-Fifi<sup>[25]</sup> observed that the nymphal development period of *P. bufonius* is very long (about four months) compared with that of *Schistocera gregaria* (about one and half month). In addition, the female has lost the ability to fly whereas the male retains the ability to fly for short distance. Furthermore, it is possible that the autotoxicity may also present a serious and unappreciated cost for those species utilizing a toxic deterrent. It is worth recalling that

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FIG. 25 & 26. TEM of longitudinal section through flight muscle fiber of female *P. bufonius* during mating (25) and eggs deposition (26). Poorly defined cross-striation and irregular Z-bands is shown (arrows). (SR: Sarcoplasmic reticulum). (25. × 6000, 26. × 17500).



FIG. 27. TEM of longitudinal section through flight muscle fiber of mature adult male *P. bufonius*. Normal muscle fiber (compare with that of Figures 25 and 26). (SR: Sarcoplasmic reticulum; Z:Z-band; D:Dyad; M: Mitochondria). (× 12500).

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FIG. 28. TEM of transverse section through flight muscle fiber of mature adult female P. bufonius (15 days old adult) showing the arrangement of myofibrils. (SR: Sarcoplasmic reticulum). (× 16000).

the *P. bufonius* has developed some tolerance to toxic cardiac glycosides as indicated by the presence of ouabain-resistant Na<sup>+</sup>/K<sup>+</sup>-transporting ATPase (PI<sub>50</sub> = 3.7) in the membrane microsomal preparation from Malpighian tubules and hind-gut<sup>[26]</sup>.

Atrophy of muscle has been observed in a variety of pathological and experimental studies (see introduction). Duncan and Jackson<sup>[27]</sup> have studied the skeletal muscle damage induced by either calcium ionophore (A23187) or 2, 4-dinitrophenol in incubated mouse soleus muscle. They concluded that there are at least two separate pathways in cell damage: (1) phospholipase A activation and lipoxygenase activity culminating in sarcolemma damage and (2) a system that produced characteristic destruction of the myofilament apparatus.

There are two possible explanations for the factor or factors, which may cause the flight muscle atrophy. One is that the CGs ingested with the food, may impose an alteration of ionic transport through sarcolemma of flight muscle and may render the flight muscle irresponsive to nervous stimulation. It has long been recognized that denervation of muscle leads to atrophy and eventually the degeneration of the muscle fibers. Goldspink<sup>[28]</sup> reported that it was not clear whether the atrophy and degeneration.

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FIG. 29. TEM of transverse section of flight muscle fiber of adult male *P. bufonius*. Note the arrangement of myofibrils and the normal appearance of the nucleus (N). (× 10000).

eration was due to changes in activity of the muscle or whether the nerve produces substances or in some other way imports stability to muscle fibers. Finlayson<sup>[29]</sup> indicated that the nervous system do exert some kind of trophic influence on musculature, both during development, and in maintaining those already differentiated. It has been reported that the innervation is required for normal muscle growth in the cockroach<sup>[30]</sup>. However, the feeding behaviour of male *P. bufonius*, which retain ability for short distance flight, is similar to that of female. This may refute the above possible explanation, unless other study prove that the male of P. bufonius consume little amount of C. procera than that of female. The other possible explanation is that the feeding and ingestion of C. procera, which contains CGs may act on the endocrine system so as to alter the juvenile hormone (JH) titre and thereby determine the flight muscle development. JH titre reach a minimum during the last instar in hemimetabolous insects<sup>[31]</sup>. Poels and Beenakkers<sup>[32]</sup> have shown that flight muscle growth is greatly inhibited if corpora allata (CA) that are producing juvenile hormone are transplanted from adult into last instar nymphs of L. migratoria. This result suggests that flight muscle growth may be controlled by changing JH titre during the last instar. Implantation of either adult CA or four pairs of larval CA gives rise to an extra larval stage and consequently an extra (sixth) ecdysis<sup>[33]</sup>. During this extra lar-



FIG. 30. TEM of longitudinal section of flight muscle of gravid female *P. bufonius* showing abnormal nucleus surrounded with undifferentiated sarcoplasm (arrows). ( $\times$  40000).

val period, flight muscle development appeared to be retarded in comparison with control insect, as reflected by lower specific activities of enzymes indicative of aerobic metabolic pathways. Ready and Josephson<sup>[15]</sup> reported that the control mechanism for flight muscle growth may be complex, and it is possible that the effect of JH is not directly on muscle but rather is mediated through the nervous system.

The atrophy and degeneration of flight muscle of female *P. bufonius* are difficult to assign to one of the above mention explanations. Further studies are needed about the physiological effect of the interaction between cardiac glycosides and endocrine system, which may affect flight muscle development.

It appears that the development of flight muscle fibers are retarded after final ecdysis and does not attain the well developed flight muscle fibers of male *P. bufonius*. It may be safe enough, however, to suggest that atrophy of flight muscles of female *P. bufonius* is developmental rather than pathological, and may due to a complex physiological interaction between the endocrine and nervous systems, and the influence of the feeding habit of this insect.

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FIG. 31. TEM of transverse section through flight muscle fiber of gravid female *P. bufonius* showing the start of degeneration at the peripheral region. (× 15500).

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#### Reference

- Al-Robai, A.A., Assagaf, A. and El-Gohary, M., Comparative study on the fine structure of the flight muscles of mature male and female *Poekilocerus bufonius* Klug (Orthoptera, Pyrogomorphidae), *J.K.A.U.: Sci.* 5: 47-64 (1993).
- [2] Neville, H.E., Ultrastructural changes in muscle diseases. In: Dubowitz, V. and Brooke, M.H. (eds.) Muscle biopsy: A modern approach, W.B. Saunders Company Ltd., London, Philadelphia and Toronto, pp. 383-470 (1973).
- [3] Adams, R.D., Diseases of muscle: A study in pathology, (3rd edition), Medical Department, Harper and Row, Hagerstown, Maryland, New York, 388 p. (1975).
- [4] Ghadially, F., Ultrastructural pathology of the cell and matrix, (2nd edition). Butterworths, London, 947 p. (1982).
- [5] Edwards, F.J., Endocrine control of flight muscle histolysis in Dysdercus intermedius, J. Insect Physiol. 16: 2027-2033 (1970).

- [6] Scudder, G.G.E., The post-embryonic development of the indirect flight muscles in Cenocorixa bifida (Hung.) (Hem., Corixdae), Can. J. Zool., 49: 1387-1398 (1971).
- [7] Euw, J.V., Fischelson, L., Parsons, J.A., Reichstein, T. and Rothschild, M., Cardenolides (Heart poisons) in a grasshopper feeding on milkweeds, *Nature* 214: 35-39 (1967).
- [8] Lewis, R.J. Sr. and Tatken, R.L. (eds.) Registration of toxic effects of chemical substances, U.S. Dept. of Health and Human Services (NIOSH) Publication No. 80-111 (1979).
- [9] Stekhoven, F.S. and Bonting, S.L., Sodium potassium activated adenosine triphosphatase. In: Bonting, S.L. and DePont, J.J. H.H.M. (eds.) Membrane Transport, Vol. 2: 159-182 (1981).
- [10] Moore, L.V. and Scudder, G.G.E., Ouabain-resistant Na<sup>+</sup>, K<sup>+</sup>-ATPases and cardenolide tolerance in the large milkweed bug, Oncopellus fasciatus, J. Insect. Physiol. 32: 27-33 (1986).
- [11] Abushama, F.T., The repugnatorial gland of the grasshopper Poekilocerus hieroglyphicus (Klug), J. Ent., Lond. (A) 47: 95-100 (1972).
- [12] Duffrey, S.C., Sequestration of plant materials products by insects, Ann. Rev. Ent. 25: 447-477 (1980).
- [13] Huheey, J.E., Warning coloration and mimicry. In: Bell, W.J. and Card, R.T. (eds.) Chemical Ecology of Insects. pp. 257-297, Chapman and Hull Ltd., New York (1984).
- [14] Al-Robai, A.A., Mitochondrial and myofibrillar development in the flight muscle of Locusta migratoria L. (Orthoptera, Acrididae), J.K.A.U.: Sci. 3: 113-153 (1991).
- [15] Ready, N.E. and Josephson, R.K., Flight muscle development in a hemimetabolous insect, J. Exp. Zool. 220: 49-56 (1982).
- [16] Wilson, D.M., Bifunctional muscles in the thorax of grasshoppers, J. Exp. Biol. 39: 669-677 (1962).
- [17] De Kort, C.A.D., Hormones and the structural and biochemical properties of the flight muscles in the Colorado beetle, *Meded. lanbouwhogeschool Wageningen*, 69(2): 1-63 (1969).
- [18] Richards, O.W. and Davies, R.G., Imm's Outlines of Entomology, (6th ed.) Chapman and Hall, London, p. 254 (1976).
- [19] Johnson, B., Studies on the degeneration of the flight muscle of alate aphids II, J. Insect Physiol. 3: 567-377 (1959).
- [20] Atkins, M.D. and Farris, S.H., A contribution to the knowledge of flight muscle changes in scolytidae (Coleoptera), Can. Ent. 94: 25-32 (1962).
- [21] Bhakthan, N.M.G., Borden, J.H. and Nair, K.K., Fine structure of degenerating and regenerating flight muscle in a bark beetle, *Ips confusus* (Co., Scolytidae), *J. Cell Sci.* 6: 807-819 (1970).
- [22] Chesky, J., Comparative aspects of declining muscle function, Aging 6: 179-210 (1978).
- [23] Blum, M.S., Chemical Defenses of Arthropods, Academic Press, New York, 562 p. (1981).
- [24] Detweiler, D.K., Comparative pharmacology of cardiac glycosides, Fedn. Proc. 26: 119-124 (1967).
- [25] AI-Fifi, Z.I., Studies on the activities of Na<sup>+</sup>/K<sup>+</sup> ATPase and phosphofructokinase from the alimentary canal of Poekilocerus bufonius (Klug.) M.Sc. Thesis, Department of Biological Sciences, King Abdulaziz University, Jeddah, S.A. 131 p. (1990).
- [26] Al-Robai, A.A., Khoja, S.M. and Al-Fifi, Z.I., Properties of ouabain-resistant Na<sup>+</sup>/K<sup>+</sup>-transporting ATPase from the excretory system of *Poekilocerus bufonius*, *Insect Biochem.* 20: 701-707 (1990).
- [27] Duncan, C.J. and Jackson, M.J., Different mechanisms mediate structural changes and intracellular enzyme afflux following damage to skeletal muscle, J. Cell Sci. 87: 183-188 (1987).
- [28] Goldspink, G., Development of muscle. In: Goldspink, G. (ed.) Differentiation and growth of cell in vertebrate tissues, pp. 69-99. Chapman and Hall, London (1974).
- [29]Finlayson, L.H., Development and degeneration. In: Usherwood, P.N.R. (ed.) Insect Muscle, Academic Press, New York, pp. 75-149 (1975).
- [30] Nuesch, H., The role of the nervous system in insect morphogenesis and regeneration, Ann. Rev. Ent. 13: 27-44 (1968).
- [31] Highnam, K.C., Insect hormones, J. Endocranial 39: 123-159 (1967).
- [32] Poels, C.L.M. and Beenakkers, A.M. Th., The effect of Corpus allatum implantation on the development of flight muscles and fat body in *Locusta migratoria*, *Entomol. Exp. Appl.* 12: 312-324 (1969).
- [33] Van Den Hondel-Franken, M.A.M., Van Den Broek, A. Th. M. and Beenakkers, A.M. Th., Flight muscle development in *Locusta migratoria*: Effect of implantation of Corpora allata on the attainment of metabolic enzyme activities, *Gen. Comp. Endocranial.* 41: 477-486 (1980).

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دراسة نسيجية وتركيبية دقيقة على عضلات الطيران الضامرة في أنثى نطاط العشر بوكيلوسيرس بوفونيوس (كلوج)

> علي بن أحمد الرباعي و حامد بن صالح الغامدي قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز جــــدة ، المملكة العربية السعودية

المستخلص . أجريت هذه الدراسة على عضلات الطيران الطولية لأنثى نطاط العشر بوكيلوسيرس بوفونيوس وذلك بهدف وصفها نسيجيًا والتعرف على تركيبها الدقيق في أعمار مختلفة . وقد تبين أن المظهر العام لعضلات طيران الأنثى شفاف "Translucent" في جميع الأعرار التي تمت دراستها . كما أوضحت الدراسة النسيجية والتركيب الدقيق أن عضلات الطيران تشبه التراكيب الخيطية "Thread-like structure" والميتوكوندريا بها قليلة جدًّا وأشكالها غير عادية ، والقصيبات الهوائية نادرة أو غير موجودة داخل الألياف العضلية للأنثى .

هذا وقد تبين حدوث التفسخ في الأجزاء المحيطية للألياف العضلية في عضلات طيران الأنثى وخاصة في الأعمار المتقدمة ، ويتجلى هذا التفسخ في تحطم أو تكسر الليبفات العضلية وفقدها لتخططها الطبيعي الذي يتزامن مع انتفاخ الشبكة البلازمية اللحمية . وقد استنتج من هذه الدراسة أن ضمور العضلات "Muscle atrophy" في الأنثى هو ضمور تكويني "Developmental atrophy" ويعزي إلى عدم اكتال النمو وحدوث التفسخ في عضلات الحشرات المتقدمة في السن ، وتمت مناقشة الأسباب المحتملة لعدم اكترال تكوين العضلات وبالتالي ضمورها .