



## Structure, function and evolution of insect flight muscle

Hiroyuki Iwamoto<sup>1</sup>

<sup>1</sup>Research and Utilization Division, SPring-8, Japan Synchrotron Radiation Research Institute, Hyogo 679-5198, Japan

Received January 21, 2011; accepted February 2, 2011

**Insects, the largest group of animals on the earth, owe their prosperity to their ability of flight and small body sizes. The ability of flight provided means for rapid translocation. The small body size allowed access to unutilized niches. By acquiring both features, however, insects faced a new problem: They were forced to beat their wings at enormous frequencies. Insects have overcome this problem by inventing asynchronous flight muscle, a highly specialized form of striated muscle capable of oscillating at >1,000 Hz. This article reviews the structure, mechanism, and molecular evolution of this unique invention of nature.**

**Key words:** insect flight muscle, asynchronous operation, stretch activation, thin-filament regulatory system

Insects are deeply implicated in human life by doing both good and harm; many are integrated members of the ecosystem and support production in forest and farmland, while some others are notorious pests that transmit infectious diseases and compromise agricultural production. More than a million species of insects live on earth, but they often evade our attention because the majority of them are only millimeters to 1–2 centimeters long. Tiny as they are, one may be surprised to know how elaborately built each individual are. So elaborate that they deserve the name of ultimate micro-machines, filled with mechanisms for survival and prosperity. A honeybee has a microbrain<sup>1</sup> that is probably 6 orders of magnitude smaller than a human brain, but it can remember

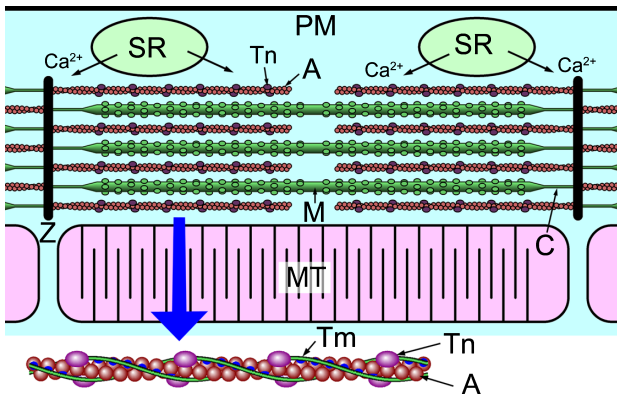
the location of food, and can communicate the information to its hivemates by means of a sophisticated dance language. A human brain is apparently no match for a bee brain in terms of performance per unit volume. This example tells that honeybees and other insects have evolved in such a way to maximize the efficiency of individual's function within a severely limited space of the body. This review focuses on another insect organ that has evolved to pursue maximal efficiency and has gained a symbolic status in insect evolution: the flight muscle. Here we briefly overview the current understanding about its structure, function and its molecular mechanism and its evolutionary origin, and try to provide a bridge to future studies.

### In order to fly

It is believed that insects owe their prosperity largely to the acquisition of flight ability and body miniaturization. Body miniaturization has made it possible to utilize new niches unexploited by larger animals. For example, the smallest insect ever known is a species of parasitic Trichogrammatid wasps (Hymenoptera; body length, 0.2 mm), which parasitize the eggs of thrips (Thysanoptera), another representative group of minuscule insects (body length, 1–2 mm). To have the ability of flight and miniaturized bodies at the same time, however, insect faced a major problem to overcome: they must beat their wings at higher frequencies as they downsize their bodies. This is because of aerodynamic reasons: the lift is proportional to the 4th power of body length, while the body weight is proportional to the 3rd<sup>2</sup>. In fact, mosquitoes beat their wings at 500 Hz, and smaller midges can beat at 1,000 Hz<sup>3</sup>. It is difficult to attain these frequencies by accelerating the ordinary cycles of contraction and relaxation.

Like vertebrate skeletal muscle, insect flight muscle (IFM) is a cross-striated muscle, and the regulatory mechanism for

Corresponding author: Hiroyuki Iwamoto, Research and Utilization Division, SPring-8, Japan Synchrotron Radiation Research Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-6198, Japan.  
e-mail: iwamoto@spring8.or.jp



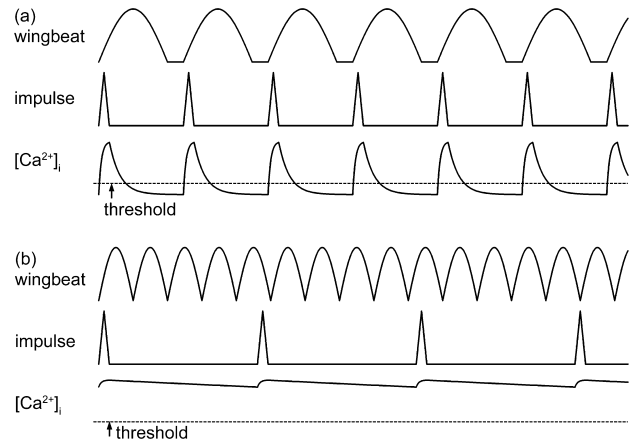
**Figure 1** Basic structure of insect flight muscle (IFM) and control of contraction and relaxation. Structure of a single sarcomere, the minimum unit of muscle function, is shown. A, thin filament; C, C-filament; M, thick filament; MT, mitochondrion; PM, plasma membrane; SR, sarcoplasmic reticulum; Tm, tropomyosin; Tn, troponin; Z, Z-line. In actual muscle, there is no one-to-one correspondence between mitochondria and SR.

its contraction and relaxation is basically identical to that of vertebrate skeletal muscle (Fig. 1): The excitation of the plasma membrane of muscle cell causes the sarcoplasmic reticulum (SR) to release calcium ions, which binds to troponin, a regulatory protein on the thin filament. This in turn causes tropomyosin (another regulatory protein) to move from its inhibitory position and initiate contraction. On the other hand, relaxation occurs as the calcium pump on the SR membrane transports the calcium ions back to the SR lumen. This transport is an energy-consuming active process done against the concentration gradient. If an IFM is to attain a high wing-beat frequency by accelerating this ordinary contraction-relaxation cycle, the volumes of both SR and mitochondria (which supply ATP as energy) must increase to keep up with an increased speed of calcium release and reuptake. This volume increase can only be done at the expense of the space for myofibrils. For this reason, an upper limit is believed to exist for the wing-beat frequencies, which is around 100 Hz. The IFMs of lower insects, such as locusts (Orthoptera), operate under this limit. They contract and relax each time when an impulse arrives each time from the motor nerve. This mode of IFM operation is called “synchronous” (Fig. 2a).

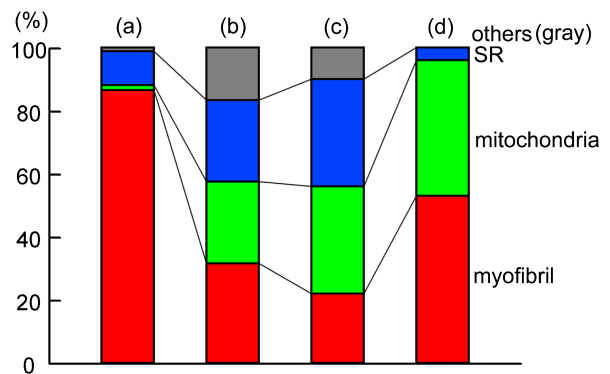
Some 30 years ago, a debate was triggered regarding the upper limit of frequencies when a species of whitefly (a minute Hemipteran) was reported to beat at frequencies well above 100 Hz. The debate ended when its IFM turned out to be “asynchronous”<sup>4</sup> (as will be explained below). In those good old days, breaking the 100-Hz limit was a big news to take the pages of Nature journal.

### Croaking fish and rattlesnake

Besides IFM, muscles that repeat high-frequency contraction-relaxation cycles are found among sound-



**Figure 2** Schematic diagram of action of flight muscles. (a), synchronous IFM; (b), asynchronous IFM. Upper trace, wing-beat; middle trace, impulses from motor nerve; bottom trace, intracellular calcium level. The broken line indicates the threshold calcium level above which contraction is initiated. Wing-beat frequencies vary greatly, depending on insect species.



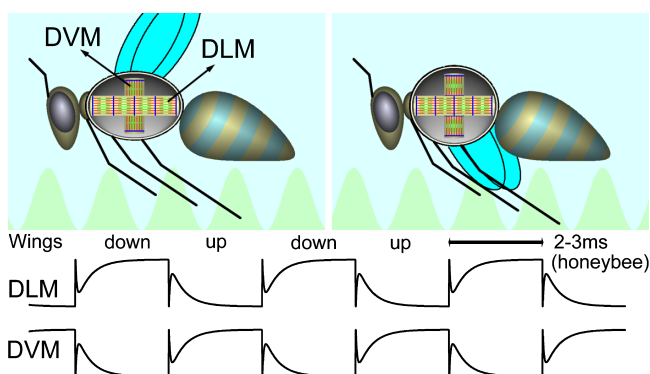
**Figure 3** Relative occupancy of various structure in the volume of muscle cell. (a), ordinary skeletal muscle of rattlesnake; (b) sound-producing tail muscle of rattlesnake; (c) timbal muscle of cicada; (d) flight muscle of bee. Others refers to the volume not occupied by any of these organelles (myofibril, SR, mitochondria). Modified from ref. 6.

producing animals. In this case, some muscles oscillate at above 100 Hz even if they are “synchronous”. In these muscles, contractile force is sacrificed as a price for speed. A toadfish, for example, produces sound by oscillating its swimbladder at 200 Hz. The fast contraction-relaxation cycles are made possible by reducing the time for actin-myosin binding at the expense of contractile force<sup>5</sup>. In the case of a rattlesnake, whose sound frequency goes up to 100 Hz, myofibrils occupy only 32% of the volume of the sound-producing muscle, while mitochondria and SR occupy 26% each<sup>6</sup> (Fig. 3). In a more extreme case of a cicada (Hemiptera) that sings at 550 Hz, myofibrils occupy only 22% of the volume of the timbal muscle, while mitochondria and SR occupy 33% each<sup>6</sup>. Speed at the expense of force may be good for producing sound, but it is not a right strategy for IFMs that require large power output.

## Asynchronous flight muscle

How do IFMs of those small insects meet the conflicting demands for high wing-beat frequencies and necessary power output at the same time? The answer is the development of the “asynchronous” mode of flight muscle operation. In asynchronous IFM, the intracellular calcium ion is maintained at an activating level by low-frequency nerve impulses, while the myofibrils oscillate autonomously (Fig. 2b). Because the calcium level is held constant, the amount of SR and calcium pumps can be minimized, and the energy produced by mitochondria can be effectively utilized by myofibrils. The upper limit of wing-beat frequencies is also eliminated. In the case of bees (Hymenoptera), myofibrils occupy 53% of the muscle cell volume, while mitochondria and SR occupy 43% and only 4%, respectively<sup>6</sup>. The relatively large volume of mitochondria is apparently due the high energy consumption rate of myofibrils, as will be explained later. In addition to bees, all advanced orders of insects like Diptera (flies, mosquitoes and midges) and Coleoptera (beetles) have asynchronous IFMs.

If it is not the frequency of nerve impulses, what determines the wingbeat frequencies in these insects? In many insects, IFMs do not directly drive the wings, but do so indirectly by deforming the thoracic exoskeleton (indirect flight muscle, Fig. 4). There are two sets of major flight muscles in the thorax: the dorsal longitudinal muscle (DLM) that runs along the anterior-posterior axis and the dorsoventral muscle (DVM) that runs along the dorsal-ventral axis. These muscles work antagonistically, i.e., when one shortens, the other is stretched. Because asynchronous IFMs have the ability of stretch activation (SA), they are activated when they are stretched by the antagonistic muscle, and produce large force and stretch back the opponent. By repeat-



**Figure 4** Schematic diagram showing the action on indirect flight muscle and stretch activation. Upper left, phase in which dorsoventral muscle (DVM) shortens; upper right, phase in which dorsal longitudinal muscle (DLM) shortens. Note the relation between wing position and the deformation of thoracic exoskeleton. Lower panel, relation between the forces of DLM and DVM. Stretch activation (SA) refers to the delayed rise of force after stretch. The forces of DLM and DVM are complementary to each other. The diagram shows the responses to step stretches, but in live insects, the length change is sinusoidal.

ing this process, insects keep beating their wings even if the calcium level does not fluctuate. Here the primary factor to determine the wing-beat frequency is the mechanical resonant frequency of the thorax and the wings. However, insects can modulate the wing-beat frequency by varying the frequency of nerve impulses, which results in a change of intracellular calcium level. In fruitfly (*Drosophila*), it is reported that the wing-beat frequency is increased from 185 to 195 Hz when the frequency of nerve impulses is increased from 3 to 5.5 Hz<sup>7</sup>. In any event, by developing asynchronous IFM, insects have introduced a system of “distributed information processing”, which relieves the central nervous system from the burden of controlling each wing beat. This might have contributed to the realization of “microbrain” as explained earlier.

## Structural features of asynchronous flight muscle

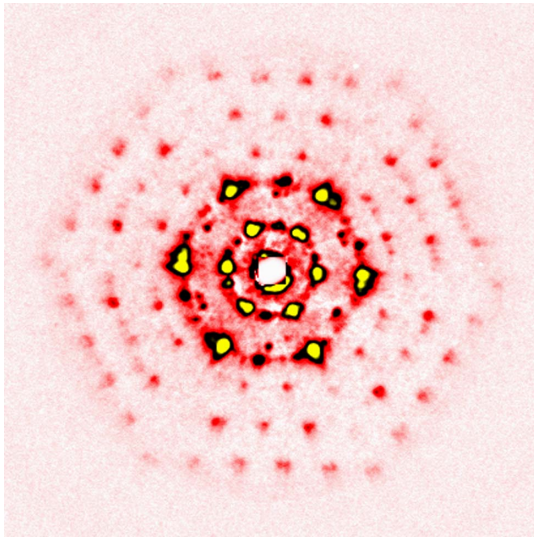
Asynchronous IFMs are found in various insect orders, but they have many structural features in common. First, both ends of the myosin filaments are connected to the Z-lines (a structure that separates neighboring sarcomeres) with short, stout filaments (C-filaments) made of elastic proteins. Because of these connections, IFM fibers are highly resistant to stretch, and unlike vertebrate skeletal muscle fibers, their length cannot be changed much.

Second, the constituent proteins of sarcomere, such as actin and myosin, are very regularly arranged so that the whole sarcomere may be regarded as a protein crystal. Because of this regular arrangement, asynchronous IFMs give rise to a number of isolated reflection spots when irradiated with X-ray, just as artificially grown protein crystals.

Furthermore, this regularity is not confined within a sarcomere but extends over a long distance: It is shown that the lattice plane orientation of myofilaments (thick and thin filaments) is strictly preserved along the entire length of a myofibril, although the myofilaments themselves are disrupted at the Z-lines<sup>8,9</sup>. In other words, the entire myofibril may be regarded as a single, millimeters-long giant protein crystal. Because of this structure, the myofibril gives rise to a number of reflections indexable to a single hexagonal lattice of myofilaments, when irradiated along its long axis by an X-ray microbeam (diameter, 2  $\mu\text{m}$  — the same size as a single myofibril) (Fig. 5). The features as described above are observed in all insects with asynchronous IFM examined to date.

## Molecular mechanism of stretch activation

The ability of SA, as mentioned above, is considered essential for the asynchronous operation of IFM, and many investigators have pursued its molecular mechanism. The characteristically high regularity of protein arrangement led to the proposal of the “match-mismatch hypothesis<sup>10</sup>”, which assumes that a stretch brings actin and myosin into



**Figure 5** Example of end-on X-ray diffraction pattern from a myofibril of asynchronous IFM, originating from a single hexagonal lattice of myofilaments.

right geometry for interaction. In general, only a limited number of actin monomers on a thin filament are suitably oriented for interaction with myosin heads. These monomers are clustered in small areas called “target zones”. The target zones on the 6 thin filaments surrounding a thick filament are helically arranged (Fig. 6). At a certain sarcomere length, none of the myosin heads are close to the target zones. However, a 19-nm stretch of the sarcomere will bring the myosin heads close enough to interact with actins in the target zones, causing SA.

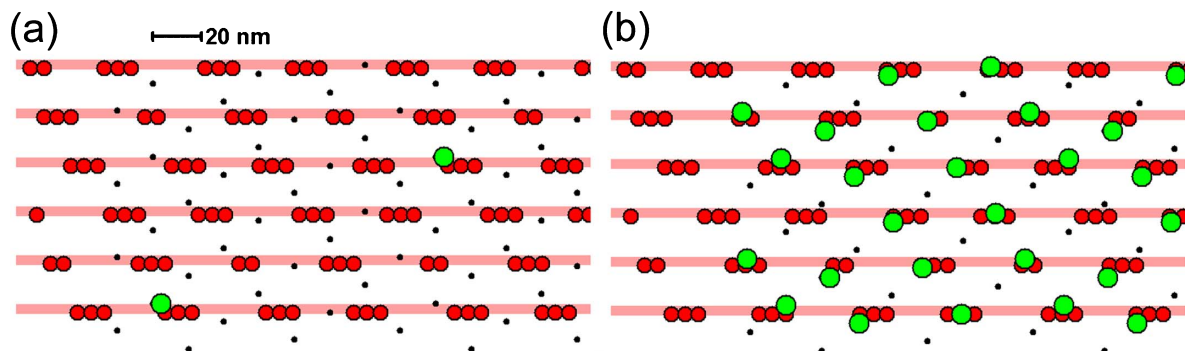
However, there are several lines of evidence or arguments against this match-mismatch hypothesis. For example, it has been argued that the matches and mismatches occur out-of-phase in different thick filaments in a sarcomere, and that the peaks and bottoms of force in each thick filament would be averaged out<sup>11</sup>. It is also known that the amplitude of

oscillation of IFM in living insects is  $\sim 3\%$  of its length<sup>12</sup>. This corresponds to a  $\sim 45$ -nm stretch, far greater than theoretically expected from the match-mismatch mechanism (19 nm, see above).

### Role of regulatory proteins on stretch activation

Turning eyes to the biochemical side of the matters, there is increasing evidence that the regulatory proteins on the thin filament (troponin and tropomyosin) plays a role in SA.

The troponin complex, the calcium sensor on the thin filament, consists of three components: Troponin-C (TnC) which binds calcium ions, troponin-I (TnI) which inhibits actin-myosin interaction, and troponin-T (TnT) which anchors the whole complex to tropomyosin. TnC is a calmodulin-like dumbbell-shaped molecule, and it typically has four binding sites for divalent cations (two in the N-terminal end and two in the C-terminal end). In the vertebrate skeletal muscle isoform, the 1st and 2nd binding sites from the N-terminus play a regulatory role, while the 3rd and 4th binding sites bind magnesium ions rather than calcium, and are considered to play a structural role. The IFM of the giant waterbug (*Lethocerus*, Hemiptera) is known to have two TnC isoforms (F1 and F2). F1 has only one functional calcium binding site at the 4th position, and F2 has two calcium binding sites at the 2nd and 4th positions. F1 and F2 are expressed in *Lethocerus* IFM with a molar ratio of 7:1–10:1<sup>13</sup>. From the result of TnC-exchange experiments, Bullard and colleagues<sup>13</sup> postulate that F1 causes SA by sensing stretch rather than calcium binding, while F2 is responsible for eliciting steady-state isometric force as the vertebrate skeletal muscle isoform. The 3-D structure of the F1 molecules is almost identical to that of other TnC isoforms, so that it is unlikely that F1 directly senses stretch. In this respect, Bullard and colleagues propose that the stretch sensor resides in TnI<sup>14</sup>. The TnI of IFM has a long Pro-Ala-rich extension, and is called TnH (H is for heavy) because of its heavier molecular weight. The extension is postulated



**Figure 6** Match-mismatch theory of stretch activation. The diagram shows the relations among 6 thin filaments (pink lines) surrounding a thick filament, the positions of target zones on the thin filament (red circles) and the positions of myosin heads (dots). Myosin heads bound to actin are represented as green circles. In (a) most of myosin heads are unable to bind to actin, but after a  $\sim 20$ -nm stretch, many myosin heads can bind to actin.

to reach the thick filament, and to detect the relative sliding between the thick and thin filaments. However, the extension is also found in insects with synchronous IFM (which hardly exhibits SA), and the reduced expression of this extension in mutant fruitflies has been reported to have unexpectedly light effects<sup>15</sup>. Therefore, one should carefully draw conclusions about the role of the Pro-Ala-rich extension.

In a very recently published paper, Reedy and colleagues<sup>16</sup> propose that it is myosin heads themselves that transmit the information of stretch to the thin filament. Electron-microscopic evidence shows that some weak-binding myosin heads are found in the troponin region of the thin filament, whereas strong-binding (force-producing) heads are exclusively found in the target zones located midway between two neighboring troponin complexes<sup>17</sup>. These weak-binding heads are found also in relaxed IFM, and make direct contacts with tropomyosin rather than actin. These heads are assumed to move tropomyosin molecules away from their inhibitory position<sup>16</sup>.

In any event, it is likely that the thin filament regulatory proteins play an important role in SA, and X-ray diffraction studies also support this. The 2nd actin layer-line reflection (2nd ALL) reports the azimuthal movement of tropomyosin molecules on the thin filament. Its intensity increases as tropomyosin moves away from its inhibitory position. In IFM, its intensity is known to increase not only upon calcium-activation but also upon SA<sup>16,18,19</sup>, and in bumblebee IFM, the intensity change has been reported to occur in a millisecond time scale<sup>19</sup>. Even after invention of asynchronous IFM, insects seem to keep oscillating their tropomyosin synchronously with the wing-beat, as in their ancestors with synchronous IFM.

### Constituent proteins of asynchronous flight muscle

As has been described, asynchronous IFM is a kind of cross-striated muscle just as vertebrate skeletal muscle, but a highly specialized variety of it. Both structure and function of the asynchronous IFM have maximally adapted for small-amplitude, high-frequency vibrations. Unexpectedly, there are few novel proteins specific to asynchronous IFM, and most of its constituent proteins are isoforms or homologs of proteins already known to occur in vertebrate skeletal muscle. However, many of the constituent proteins are expressed as IFM-specific isoforms (non-specialized isoforms are found in non-IFM muscles and non-muscle cells of the same insect). In other words, insects have created a highly specialized muscle by modifying preexisting raw materials and by modifying the way in which they are integrated into a system. The following is a brief description of each constituent protein of asynchronous IFM.

### Myosin

X-ray diffraction studies show that myosin heads attach to and detach from actin in each SA event (i.e., in each wing-beat)<sup>19</sup>. This implies that the higher the wing-beat frequency, the faster is the attachment/detachment. Currently no experimental evidence has been reported for multiple attachment/detachment events within a single ATP hydrolysis cycle, and actually IFM myosins from faster-beating insects are shown to exhibit higher ATPase activities<sup>20</sup>. A detailed kinetic study has been made for myosin isoforms in *Drosophila*, which beat at 200 Hz<sup>21</sup>. There is only a single gene for muscle myosin (myosin II) in *Drosophila*, and all isoforms, including the IFM-specific one, are expressed from this gene through alternative splicing. In the case of the IFM-specific isoform, the rate constant for dissociation from actin (usually regarded as an index for the 'speed' of myosin) is unusually high (3,698 s<sup>-1</sup> as opposed to 200–500 s<sup>-1</sup> in vertebrate skeletal muscle). Thus, the IFM myosin from *Drosophila* is called the fastest myosin II, but the rate constants may be greater in faster-beating insects. It detaches from actin quickly because the step of ADP release (usually the rate-limiting step for actin-activated ATPase reaction) is accelerated. As a result of this acceleration, the affinity for ATP is also reduced ( $K = 0.2 \text{ mM}^{-1}$  as opposed to 0.8–9 mM<sup>-1</sup> in vertebrate). The authors of the paper expect that *Drosophila* IFM may operate at a high intracellular ATP level, but the results in the literature or our own measurement show that the intracellular ATP levels in asynchronous IFM are not much greater than in other muscles. It is possible that, unlike in vertebrate muscle, IFM myosin may operate at substantially sub-saturating levels of ATP.

### Actin

It is well known that *Drosophila* has an IFM-specific actin gene (Act88F). Actin is a conservative protein, and there are 27 differences in amino-acid (a.a.) residues between Act88F and other actin isoforms. The function of IFM is little affected after replacement of a few of these 27 residues by those of other isoforms, but the flight ability is lost after replacement of 18 residues<sup>22</sup>. Therefore, Act88F may have acquired specialized functions by the replacement of these residues.

It is unknown whether other insects express IFM-specific actin isoforms as well. However, an X-ray diffraction study on various insect species has shown that calcium activation of IFM causes actin to change its structure in a manner different from that in vertebrate skeletal muscle<sup>23</sup>. This structural change is also observed in synchronous IFM of dragonflies (Odonata), and is therefore not restricted to asynchronous IFM.

## Troponin and Tropomyosin

The most peculiar of IFM proteins are troponin and tropomyosin. Among the 3 components of troponin, TnI(TnH) has the most striking feature because of the Pro-Ala-rich 200 a.a. residues-long extension at its C-terminus as stated earlier<sup>24</sup>. Because of its size, the troponin complex of IFM can be clearly recognized in electron micrographs. Initially, TnH was expected to explain the SA mechanism of asynchronous IFM, but it is now clear that TnH is ubiquitously distributed among all winged insect orders (although it is still IFM-specific). Interestingly, TnH does not exist in Diptera. In the IFM of these insects, TnI has ordinary molecular weights. Instead, the Pro-Ala-rich extension is associated with tropomyosin. In *Drosophila*, two high-molecular-weight tropomyosin isoforms (~80 kDa) are expressed besides the ordinary isoform (35 kDa). These high-molecular-weight isoforms were first described by Mogami et al.<sup>25</sup> as IFM-specific proteins 33 and 34 (note that these isoforms are called TnH in some literature). Diptera is a monophyletic group of insects, and is considered to have arisen from a common ancestor. Probably the high-molecular-weight tropomyosin isoforms were created by gene transfer in an early stage of evolution. In any event, the fact that the long extension is preserved in all winged insects implies that it has some functional significance. For example, the extension is known to bind glutathione-S-transferase (GST)<sup>26</sup>. GST is an enzyme that detoxifies various noxious substances, and is known to render pesticide-resistance to malaria-transmitting tropical mosquitoes<sup>27</sup>. In human bodies the liver is the organ for detoxification, but in insects, the IFM is the most voluminous organ, and it is not surprising if the IFM takes the role for the liver.

As for TnC, the F1 isoform of *Lethocerus* IFM has only one functional calcium-binding site at the 4th position, and it is important for SA according to Bullard's hypothesis, as described earlier. However, according to Marco et al., who examined in detail the evolution of the TnC gene groups, the F1 type is ubiquitously expressed in the body and it is the F2 type, which has two binding sites at the 2nd and 4th positions, that is the IFM-specific species that emerged with the development of flight muscle functions<sup>28</sup>. According to them, the F2 type further generated its subtypes by gene duplication. The number of its copies is one in *Lethocerus* that beats at 30 Hz, two in *Drosophila* and *Apis* (honeybee) that beat at 200 Hz, and four in *Anopheles* (mosquito) that beat at 500 Hz. The F2 types of *Apis* IFM have evolved from an ancestral gene different from that of Dipterans, and therefore the similarities of the F2 type molecules in these insects are the result of convergence<sup>28</sup>.

As we have reviewed, the current understanding of the TnC isoforms in IFM is still in its early stage. The largest problem would be the difficulty in identifying which of the TnC isoforms of those holometabolous insects are the true

homologs of the F1 or F2 isoform of *Lethocerus*. The difficulty is simply because hemimetabolous *Lethocerus* and holometabolous insects are phylogenetically remote to each other. The functionality of each divalent cation binding site has not been determined experimentally, but is only inferred from its a.a. sequence. Clearly more studies are needed to clarify the identity and the role of each TnC isoform.

## Projectin

Projectin is one of so-called modular proteins, consisting of many immunoglobulin- and fibronectin-like domains connected in series. Projectin is a homolog of connectin (titin) in vertebrate, and is expressed by sls gene through alternative splicing<sup>29</sup>. In asynchronous IFM, it is a component of the C-filament as described earlier, and anchors the thick filament to the Z-line. Projectin is also found in non-IFM (leg) muscles, but its intracellular localization is different, and it seems to run along the thick filament as connectin in vertebrate skeletal muscle<sup>29</sup>. Projectin is probably an elastic protein commonly distributed among protostomes, and may have been diverted for specialized purposes with the development of asynchronous IFM, and localized around the Z-line.

The features of the proteins mentioned above are summarized in Table 1 as well as other properties of IFM and non-IFM muscles in insects.

## Molecular evolution

To overview the proteins described above in the light of molecular evolution, the specialization of the constituent proteins of IFM seems to have occurred in three steps. The first occurred when IFM emerged, and the second occurred when asynchronous IFM emerged. The third one is the fine-tuning of the proteins for the sizes and other factors of individual insect species. The third one applies to the modulation of the enzymatic properties of myosin according to body size. As is evident from the fact that a group of closely related insects contains large and small species, the change in body size and concomitant changes in enzymatic properties seem to belong to minor evolution, which can occur in relatively short time. Small changes in a.a. sequences of specific loops of myosin may account for such modulations of enzymatic properties.

On the other hand, the first and the second steps belong to major evolution, which occurs in a longer period of time. To know specifically what changes occurred in the insect genes, genome information from a vast number of insects will be required, including that of primitive species. Currently the whole genome information is available for only a limited number of insect species, and most of them are those with asynchronous IFM. Therefore, one should be careful in evaluating generalized conclusion drawn from these few species.

**Table 1** Summary of structure, function and constituent proteins in insect muscle

	muscle type			
	asynchronous IFM	asynchronous IFM (Diptera)	synchronous IFM	non-IFM muscles
beat frequency	>>100 Hz in small species	>>100 Hz in small species <sup>3</sup>	<100 Hz even in small species <sup>4</sup>	—
sarcomere	near-crystalline <sup>17,18</sup>	near-crystalline <sup>23</sup>	non-crystalline <sup>23</sup>	non-crystalline
myofibril	single giant protein crystal <sup>9</sup>	single giant protein crystal <sup>9</sup>	non-crystalline <sup>9</sup>	non-crystalline <sup>9</sup>
myosin	faster kinetics in faster-beating species <sup>20</sup>	faster kinetics in faster-beating species <sup>20,21</sup>	not studied	not studied
actin	unique structural change <sup>23</sup>	unique structural change <sup>23</sup> , IFM-specific (Act88F in <i>Drosophila</i> ) <sup>22</sup>	unique structural change <sup>23</sup>	various isoforms ( <i>Drosophila</i> ) <sup>22</sup>
tropomyosin	normal MW	heavy MW with Pro-Ala-rich extension (TnH) <sup>25</sup>	normal MW	normal MW
troponin C	IFM-specific, 2 or more isoforms <sup>13,28</sup>	IFM-specific, 2 or more isoforms <sup>28</sup>	single isoform?	general-expression isoforms <sup>28</sup>
troponin I	heavy MW with Pro-Ala-rich extension (TnH) <sup>24</sup>	normal MW	heavy MW with Pro-Ala-rich extension (TnH)	normal MW
projectin	connects thick filament and Z-line <sup>29</sup>	connects thick filament and Z-line <sup>29</sup>	localized in A-band	localized in A-band <sup>29</sup>

References are limited to those quoted in the text. MW, molecular weight.

## Summary

As we have overviewed, the asynchronous flight muscle of insects is a highly specialized form of cross-striated muscle. As a means to achieve such specialization, insects did not opt the method to add novel proteins, but the method to tune existing proteins for high-frequency oscillations. Asynchronous IFMs created in this way are very similar to each other among different species in all aspects, including actions, molecular architecture and appearance. Very similar as they are, asynchronous IFMs are known to have independently emerged in various insect groups which are not phylogenetically close to each other. In other words, those insect groups invented asynchronous IFMs independently, without knowing what others were doing, and the resulting inventions are almost identical to each other. This phenomenon is called convergence, and it implies that there is functional necessity to take such a specific form. Classical examples of convergence include the triangular face of carnivorous animals such as fox and mantis, and such cases would be easily understood. However, the case of asynchronous IFM is much more complex, and defies understanding by investigators. Even today, we encounter more fresh surprises as we study further, and each effort of study makes us recall the profoundness of IFM research. Those tiny insects keep challenging our will and ability to clarify the whole picture of insect evolution, and we hope that more of young investigators will join the force to accept the challenge.

## Acknowledgements

This article is the English translation of a review originally published in Japanese<sup>30</sup>. Some latest information has

been incorporated since the publication of the original review.

## References

- Mizunami, M. *Insects — amazing microbrain* (in Japanese). (Chuo-Kouron-Sha, Tokyo, 2006).
- Dickinson, M. Insect flight. *Curr. Biol.* **16**, R309–R314 (2006).
- Sotavalta, O. Recordings of high wing-stroke and thoracic vibration frequency in some midges. *Biol. Bull.* **104**, 439–444 (1953).
- Smith, D. S. 100 Hz remains upper limit of synchronous muscle contraction—an anomaly resolved. *Nature* **303**, 539–540 (1983).
- Rome, L. C., Cook, C., Syme, D. A., Connaughton, M. A., Ashley-Ross, M., Klimov, A., Tikunov, B. & Goldman, Y. E. Trading force for speed: Why superfast crossbridge kinetics leads to superlow forces. *Proc. Natl. Acad. Sci. USA* **96**, 5826–5831 (1999).
- Conley, K. E. & Lindstedt, S. L. Energy-saving mechanisms in muscle: the minimization strategy. *J. Exp. Biol.* **205**, 2175–2181 (2002).
- Gordon, S. & Dickinson, M. H. Role of calcium in the regulation of mechanical power in insect flight. *Proc. Natl. Acad. Sci. USA*, **103**, 4311–4315 (2006).
- Iwamoto, H., Nishikawa, Y., Wakayama, J. & Fujisawa, T. Direct X-ray observation of a single hexagonal myofilament lattice in native myofibrils of striated muscle. *Biophys. J.* **83**, 1074–1081 (2002).
- Iwamoto, H., Inoue, K. & Yagi, N. Evolution of long-range myofibrillar crystallinity in insect flight muscle as examined by X-ray cryomicrodiffraction. *Proc. R. Soc. Lond. B* **273**, 677–685 (2006).
- Wray, J. S. Filament geometry and the activation of insect flight muscles. *Nature* **280**, 325–326 (1979).
- Squire, J. M. Muscle filament lattices and stretch-activation: the match-mismatch model reassessed. *J. Muscle Res. Cell Motil.* **13**, 183–189 (1992).

12. Chan W. P. & Dickinson, M. H. In vivo length oscillations of indirect flight muscles in the fruit fly *Drosophila virilis*. *J. Exp. Biol.* **199**, 2767–2774 (1996).
13. Agianian, B., Kržič, U., Qiu, F., Linke, W. A., Leonard, K. & Bullard, B. A troponin switch that regulates muscle contraction by stretch instead of calcium. *EMBO J.* **23**, 772–779 (2004).
14. Kržič, U., Rybin, V., Leonard, K. R., Linke, W. A. & Bullard, B. Regulation of oscillatory contraction in insect flight muscle by troponin. *J. Mol. Biol.* **397**, 110–118 (2010).
15. Kreuz, A. J., Simcox, A. & Maughan, D. Alterations in flight muscle ultrastructure and function in *Drosophila* tropomyosin mutants. *J. Cell Biol.* **135**, 673–687 (1996).
16. Perz-Edwards, R. J., Irving, T. C., Baumann, B. A. J., Gore, D., Hutchinson, D. C., Kržič, U., Porter, R. L., Ward, A. B. & Reedy, M. K. X-ray diffraction evidence for myosin-troponin connections and tropomyosin movement during stretch activation of insect flight muscle. *Proc. Natl. Acad. Sci. USA* **108**, 120–125 (2011).
17. Wu, S., Liu, J., Reedy, M. C., Tregear, R. T., Winkler, H., Franzini-Armstrong, C., Sasaki, H., Lucaveche, C., Goldman, Y. E., Reedy, M. K. & Taylor, K. A. Electron tomography of cryofixed, isometrically contracting insect flight muscle reveals novel actin-myosin interactions. *PLoS One* **5**, e12643 (2010).
18. Bekyarova, T. I., Reedy, M. C., Baumann, B. A. J., Tregear, R. T., Ward, A., Kržič, U., Prince, K. M., Perz-Edwards, R. J., Reconditi, M., Gore, D., Irving, T. C. & Reedy, M. K. Reverse actin sliding triggers strong myosin binding that moves tropomyosin. *Proc. Natl. Acad. Sci. USA* **105**, 10372–10377 (2008).
19. Iwamoto, H., Inoue, K. & Yagi, N. Fast X-ray recordings reveal dynamic action of contractile and regulatory proteins in stretch-activated insect flight muscle. *Biophys. J.* **99**, 184–192 (2010).
20. Molloy, J. E., Kyrtatas, V., Sparrow, J. C. & White, D. C. S. Kinetics of flight muscles from insects with different wing-beat frequencies. *Nature* **328**, 449–451 (1987).
21. Swank, D. M., Vishnudas, V. K. & Maughan, D. M. An exceptionally fast actomyosin reaction powers insect flight muscle. *Proc. Natl. Acad. Sci. USA* **103**, 17543–17547 (2006).
22. Fyrberg, E. A., Fyrberg, C. C., Biggs, J. R., Saviile, D., Beall, C. J. & Ketchum, A. Functional nonequivalence of *Drosophila* actin isoforms. *Biochem. Genet.* **36**, 271–287 (1998).
23. Iwamoto, H. Evidence for unique structural change of the thin filaments upon calcium-activation of insect flight muscle. *J. Mol. Biol.* **390**, 99–111 (2009).
24. Bullard, B., Leonard, K., Larkins, A., Butcher, G., Karlik, C. & Fyrberg, E. Troponin of asynchronous flight muscle. *J. Mol. Biol.* **204**, 621–637 (1988).
25. Mogami, K., Fujita, S. C. & Hotta, Y. Identification of *Drosophila* indirect flight muscle myofibrillar proteins by means of two-dimensional electrophoresis. *J. Biochem.* **91**, 643–650 (1982).
26. Clayton, J. D., Cripps, R. M., Sparrow, J. C. & Bullard, B. Interaction of troponin-H and glutathione S-transferase-2 in the indirect flight muscles of *Drosophila melanogaster*. *J. Muscle Res. Cell Motil.* **19**, 117–127 (1998).
27. Ranson, H., Cornel, A. J., Fournier, D., Vaughan, A., Collins, F. H. & Hemingway, J. Cloning and localization of a glutathione S-transferase class I Gene from *Anopheles gambiae*. *J. Biol. Chem.* **272**, 5464–5468 (1997).
28. Herranz, R., Mateos, J. & Marco, R. Diversification and independent evolution of troponin C genes in insects. *J. Mol. Evol.* **60**, 31–44 (2005).
29. Bullard, B., Burkart, C., Labeit, S. & Leonard, K. The function of elastic proteins in the oscillatory contraction of insect flight muscle. *J. Muscle Res. Cell Motil.* **26**, 479–485 (2005).
30. Iwamoto, H. Structure, function and evolution of insect flight muscle (review in Japanese). *Seibutsu Butsuri* **50**, 168–173 (2010).