

REVIEW

Mechanics of the thorax in flies

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ABSTRACT

Insects represent more than 60% of all multicellular life forms, and are easily among the most diverse and abundant organisms on earth. They evolved functional wings and the ability to fly, which enabled them to occupy diverse niches. Insects of the hyper-diverse orders show extreme miniaturization of their body size. The reduced body size, however, imposes steep constraints on flight ability, as their wings must flap faster to generate sufficient forces to stay aloft. Here, we discuss the various physiological and biomechanical adaptations of the thorax in flies which enabled them to overcome the myriad constraints of small body size, while ensuring very precise control of their wing motion. One such adaptation is the evolution of specialized myogenic or asynchronous muscles that power the high-frequency wing motion, in combination with neurogenic or synchronous steering muscles that control higher-order wing kinematic patterns. Additionally, passive cuticular linkages within the thorax coordinate fast and yet precise bilateral wing movement, in combination with an actively controlled clutch and gear system that enables flexible flight patterns. Thus, the study of thoracic biomechanics, along with the underlying sensory-motor processing, is central in understanding how the insect body form is adapted for flight.

KEY WORDS: Miniaturization, Biomechanics, Asynchronous muscles, Insect flight, Gear box, Clutch mechanism, Mechanical linkages

Introduction

Insects are by far the most successful metazoan taxa and represent over 60% of all animals on earth (Zhang, 2013a). With nearly a million species already described and putative estimates saturating at 8 million, insects exceed by two orders of magnitude the next most species-rich group, arachnids (Mora et al., 2011). They occupy varied niches across habitats ranging from domestic kitchen corners to inhospitable Antarctica, which – despite temperatures as low as -89°C – is home to the flightless midge *Belgica antarctica*. Insects are commonly categorized into 29 orders, of which 27 are pterygotes, or winged insects (Misof, 2014). Of these, five prominent orders – Diptera (flies), Hymenoptera (bees and wasps), Coleoptera (beetles), Lepidoptera (moths and butterflies) and sometimes Hemiptera (true bugs) – are classified as ‘major’ or ‘hyper-diverse’ because they account for nearly 80–90% of all extant insect species (Dudley, 2000; Zhang, 2013b). In addition to the evolution of holometaboly (i.e. complete metamorphosis; e.g. Nicholson et al., 2014) in the first four of these orders, the evolutionary success of hyper-diverse orders is thought to predicate on the combination of two major factors: one, the evolution of small

body size, which greatly facilitated adaptability by increasing their ecological range; and two, the evolution of flight, which enabled dispersal, migration, predation or rapid escape from predator attacks.

Although miniature body forms are a common evolutionary trend among other animals, including birds and mammals (e.g. Hanken and Wake, 1993), miniaturization takes on a rather extreme form in insects. For example, the size of adult parasitic chalcid wasps such as *Kikiki huna* ($\sim 150\ \mu\text{m}$) or the trichogrammatid wasp *Megaphragma mymaripenne* ($\sim 170\ \mu\text{m}$) is comparable to that of some unicellular protozoan organisms (Polilov, 2012, 2015); these wasps are among the smallest metazoans ever described. Such extreme miniaturization is especially common among parasitoid insects belonging to three of the five insect groups – Diptera (flies), Hymenoptera (wasps) and some families of Coleoptera (beetles) – which have evolved a lifestyle that, for the most part, is contained within another organism, or even the eggs of other insects (Sane, 2016). Whereas *Megaphragma* are among the smallest winged insects, the wingless males of the egg parasitoid wasp *Dicopomorpha* (body length of $140\ \mu\text{m}$; Mockford, 1997) are even smaller. The smallest free-living non-parasitic insects, such as the Ptiliidae beetles (body length of $300\text{--}400\ \mu\text{m}$), with feathery hind-wings (Sorensson, 1997), are only slightly larger. At the other end of the size spectrum are certain species of Lepidoptera, which are among the largest extant insect species (e.g. the Queen Alexandra’s birdwing or saturniid Atlas moths, with wing spans of $25\text{--}30\ \text{cm}$), although the maximum diversity is skewed towards the micro-lepidopteran body size. In general, the selective evolutionary pressures that impinge on insect size are the subject of much speculation, with some recent results arguing that insect diversification may be independent of body size (Rainford et al., 2016).

Gigantism and miniaturization in insects presents a curious scientific problem: what limits insect size at both ends of the size spectrum? Moreover, how do insects maintain aerial flight ability at length scales ranging over a staggering three orders of magnitude? Insect gigantism during the Carboniferous and Permian periods is often attributed to the peak in oxygen levels (Graham et al., 1995; Harrison et al., 2010), which coincides with fossil records of giant dragonflies (order Protoodonata; wingspan $\sim 70\ \text{cm}$), mayflies (order Ephemeroptera; wingspan $\sim 20\ \text{cm}$) and some paleodicypteran insects (wingspan $\sim 55\ \text{cm}$) (Carpenter, 1953). A subsequent drop in atmospheric oxygen levels during the late Permian coincided with extinction of these giant insects (Graham et al., 1995). The physiological basis of the oxygen–size correlation is unclear (Klok et al., 2009; Clapham and Karr, 2012). One hypothesis invokes the passive gas transport across the highly branched tracheal respiration system of insects as a limiting factor for body size (Dudley, 2000), although this view has been challenged by studies that show active transport within trachea (Lehmann, 2001; Westneat et al., 2003). Alternatively, the upper and lower bounds on insect sizes may be set by biomechanical, rather than physiological, limitations. In large flying insects, these would be manifest due to limits imposed by the flexural stiffness of

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Glossary**Aerodynamic force coefficient**

A non-dimensional or scale-free measure of the aerodynamic effectiveness of an airfoil of specific geometry in generating forces.

Angle of attack

The angle at which the inclined airfoil experiences the incident ambient fluid.

Apophysis

An elongate projection of the fly cuticle, often as invaginations into the thorax serving as attachment points for muscles.

Asynchronous (or myogenic) muscle

A special class of muscle found in certain insect species where the muscle contraction rate is decoupled (or asynchronous) from the neural input rate, resulting in multiple muscle contractions for each neural input.

Bistable click mechanism

A wing motion mechanism suggested by Boettiger and Furshpan (1952) based on two stable wing positions – extreme dorsal and extreme ventral in CCl_4 -anesthetized flies. They proposed that wing motion was a result of clicking between these two stable positions. This hypothesis was later rejected by Miyan and Ewing (1985a,b) based on high-speed videos and study of the wing hinge morphology of liquid nitrogen frozen flies in mid-flight.

Campaniform sensillae

Dome-shaped mechanosensillae found on the legs and wings of insects (and halteres in flies) that detect strains in the cuticle.

Clap-and-fling

An aerodynamic mechanism in which the two flapping wings dorsally meet (clap) and come apart (fling), thereby enhancing aerodynamic force production.

Coriolis force

A force that acts to restore the plane of rotation of a rotating (or vibrating) object when there is an externally imposed change in its plane of rotation. It is a consequence of the law of conservation of angular momentum.

Delayed stretch activation

A property of myogenic muscles where strain continues to increase even as it is held after being stretched during stretch–hold–release–hold cycles. This is unlike neurogenic muscles, where the strain rises as long as it is stretched and plateaus as it is held.

Sarcoplasm reticulum

A cellular compartment in a muscle cell specialized for storing and recycling calcium.

Sclerite

The insect body is covered with cuticle which is often heavily sclerotized or hardened, forming body wall plates or the sclerites. These sclerites intercalate together to form the exoskeleton of the insect.

Synchronous (or neurogenic) muscle

Muscles like those found in the vertebrate skeletal system, where the muscle contraction rate is synchronous to the neural input rate, resulting in a single muscle contraction for each neural input.

membranous wings, affecting their ability to withstand the aerodynamic as well as inertial forces of flight. At the lower size limit, the larger surface area-to-body mass ratio in smaller insects would increase viscous interactions (drag) relative to wing inertia, meaning that these insects must use smaller muscles and yet expend more energy in flight despite lesser energy storage in their small bodies. This might severely compromise their ability to sustain flight, although such insects may rely on passive dispersal or phoretic (i.e. mediated by a host species) means of transport (e.g. Sane, 2016). Myriad other factors may also contribute to size diversity in insects, including minimum cell number, reproduction times, heat exchange, habitat usage, limitations on cellular size, etc.

From the flight perspective, the phenomenon of miniaturization is particularly interesting because it touches upon many facets of flight, ranging from the diverse physical and physiological challenges for generating forces and energetics, to the substantial ecological impact of small-sized insects (e.g. Sane, 2016). It also points to neat evolutionary tricks that may serve as inspiration for engineers aiming to build micro-robotic flappers. In this Review, we specifically discuss the thoracic adaptations that enable small insects to generate powerful, high-frequency wing strokes while precisely coordinating their rapid flight movements.

Aerodynamic constraints of miniaturization

Flapping insect wings are subject to unsteady aerodynamic forces that have been the topic of intense study in the past two decades (e.g. Sane, 2003; Chin and Lentink, 2016). These forces primarily result from aerodynamic mechanisms owing to leading edge vortices produced during wing translation (Ellington et al., 1996), rotational mechanisms during pronation and supination of the wing (Dickinson et al., 1999), and other mechanisms such as ‘clap-and-fling’ (see Glossary) which are observed more commonly in miniature insects (Weis-Fogh, 1973). Miniaturization of the insect body means a smaller wing span, and hence decreased aerodynamic forces. The aerodynamic force generated by a wing may be given by:

$$F = \frac{1}{2} C_F(\alpha) \rho V^2 S, \quad (1)$$

where the aerodynamic force coefficient [$C_F(\alpha)$; see Glossary] depends on the wing shape and its angle of attack (α ; see Glossary). Force also depends on the density of the air medium (ρ), the wing velocity (V) and the projected area of the wing (S). For an angular amplitude of the wing stroke (ϕ), the total angular excursion of the wing in each stroke is given by 2ϕ . If n is the wingbeat frequency, R the wing length and \bar{c} the average chord length, then the average wing velocity V and projected area S may be further written as:

$$V = 2\phi n R, \quad (2)$$

$$S = \bar{c} R, \quad (3)$$

and Eqn 1 can now be re-written as:

$$F = 2C_F(\alpha) \rho \phi^2 n^2 R^3 \bar{c}. \quad (4)$$

In this equation, the aerodynamic forces depend on the fourth power of wing length (assuming that average chord length also scales with wing length). Thus, miniaturization of the body means that insects lose mass (or volume) as $\sim L^3$, but they lose aerodynamic forces as $\sim L^4$, where L represents the length dimension. The dependence of $C_F(\alpha)$ on Reynolds number (the ratio of inertial to viscous forces) is roughly conserved from medium to high Reynolds numbers (10^1 – 10^3), and its dependence on the wing shape is rather moderate and fixed for any specific insect (Usherwood and Ellington, 2002). Thus, the modulation of wing kinematics plays a major role in overcoming the effects of scaling with size. The dependence of $C_F(\alpha)$ on the angle of attack, α , is a crucial determinant of force modulation during flapping, in addition to the two major kinematic parameters, ϕ and n , which can modulate aerodynamic forces.

To compensate for the lower aerodynamic forces relative to body mass, miniature insects fly with greater stroke amplitudes and higher wingbeat frequency. Higher wingbeat amplitudes may cause the wings of a large number of miniature insects to display the dorsal clap-and-fling (Dudley, 2000), which further aids in force generation. Additionally, miniature insects also flap their wings at

high frequency, which makes a large difference in their aerodynamic force output because of the n^2 dependence (see Eqn 4). Not surprisingly, enhanced wingbeat frequencies are a common feature of miniaturization throughout insects (Fig. 1). This is achieved through a combination of biomechanical and musculo-skeletal adaptations, as discussed in the following sections.

Asynchronous flight muscles enhance wingbeat frequency in insects

Although the thoracic volumes are much reduced in miniature insects, this smaller muscle mass must generate sufficient power to move the wings against the fluid medium at frequencies that often exceed 100 Hz. In insects of the orders Diptera, Hymenoptera, Coleoptera and some Hemiptera, the gross wing motion is powered by specialized flight muscles called the ‘asynchronous’ or ‘myogenic’ muscles (see Glossary). Asynchronous muscles are also reported in other insect orders such as Thysanoptera, Psocodea and Strepsiptera (Cullen, 1974; Smith, 1984). Unlike the synchronous (or neurogenic) muscles (see Glossary; Fig. 2A, left panel), asynchronous muscles do not require a neural impulse to generate every muscle twitch. Instead, they go through multiple cycles of contraction for each nerve impulse (Roeder, 1951) (Fig. 2A, right panel). We must note here that the nervous system is, in principle, capable of generating neural output at >100 Hz. Indeed, the fastest known synchronous (or neurogenic) muscle is the tymbal muscle, which can contract at 500 Hz to produce the distinctive chirps of cicadas. However, there is a trade-off between the high-frequency stimulation and the power generated by the muscle, which means that faster synchronous muscles cannot move large loads (Josephson and Young, 1985). How is this power–frequency trade-off resolved in insects, especially those with a smaller body size, which require high wingbeat frequencies for flight?

Pringle (1949) showed that this problem is solved by a combination of the asynchronous muscles and the resonant mechanics in the insect thorax, which is detailed in later sections. The motor neurons innervating the dipteran flight muscle fire at frequencies that are approximately an order of magnitude lower (i.e. on the order of 10 Hz) than the muscle and wing oscillations in insects with asynchronous muscles (on the order of 100 Hz). In Diptera, the asynchronous muscles power the oscillations of both the forewing and the modified hind-wings, which have evolved into gyro-sensory halteres that provide rapid mechanosensory feedback about aerial maneuvers (Pringle, 1948). Although the asynchronous muscles do need periodic nervous input to remain in an excited state, their stroke-to-stroke contractions depend on a property called ‘delayed stretch activation’ (see Glossary; Pringle, 1949, 1978), which is also found in human cardiac muscles (Tu and Daniel, 2004).

Delayed stretch activation drives the contraction of asynchronous muscles

Upon the arrival of a nerve impulse, the sarcoplasmic reticulum (SR; see Glossary) releases calcium ions (Ca^{2+}) into the cytosol of the muscle fibers. To contract at the same rate as the incoming nerve stimulus, the Ca^{2+} levels in muscle fibers must return to the resting level before the next nerve impulse arrives. Faster synchronous muscles require rapid Ca^{2+} cycling, which is ensured by the relatively large volume of the SR. They also require more mitochondrial volume to furnish the ATP required by Ca^{2+} pumps to rapidly sequester the Ca^{2+} from the muscle into the SR. In contrast, the asynchronous flight muscles contract rapidly but have both sparse SR and reduced mitochondrial volume as compared with other fast muscles (Josephson et al., 2000), and they are packed

with micro-fibrils. The lack of SR enveloping the asynchronous muscle fibers gives them their typical fibrillar appearance under a light microscope (hence the term ‘fibrillar muscles’; Pringle, 1978; Josephson et al., 2000). The densely packed micro-fibrils ensure that asynchronous muscles generate greater force per unit volume, but the reduced SR volume means slower cycling of Ca^{2+} and hence reduced responsiveness to neural stimulation.

How do asynchronous muscles generate power at high frequencies? In asynchronous muscles, delayed stretch activation makes high-frequency contractions possible despite low-frequency neural input (Pringle, 1978; Josephson et al., 2000). In isolated muscle preparations, the force generated by the muscles can be measured using a force transducer hooked at one end, while the muscle length is oscillated in a controlled manner (e.g. Tu and Dickinson, 1994). The measured force and length can be used to derive the stress (force per unit cross sectional area) and strain (the non-dimensional ratio of extension to actual muscle length), respectively. In isolated synchronous muscles of insects such as locusts, stress rises as the muscle is extended but it falls after the value of strain reaches a plateau (left panel, Fig. 2B) (Josephson et al., 2000). In contrast, force generation by asynchronous muscles shows a delayed rise, and stays at a high value even after the strain has plateaued (right panel, Fig. 2B). This property has therefore been termed ‘delayed stretch activation’ (Pringle, 1978). Delayed stretch activation enables the asynchronous muscles to perform work when subjected to stretch–hold–release–hold cycles (Fig. 2Ci,ii), in contrast to synchronous muscles that absorb work when continuously stretched and released. The increased number of myofibrils per volume of the asynchronous flight muscles allows these muscles to generate the high power required for flight, and delayed stretch activation ensures that the muscles beat the wings at rapid rates despite low, steady Ca^{2+} levels.

Molecular basis of delayed stretch activation

Although the phenomenon of delayed stretch activation has been extensively studied since Pringle’s initial discovery in 1949, its molecular mechanism in muscle fibers remains quite unclear. The sliding filament model describes the molecular events underlying muscle contraction in synchronous muscle fibers, which undergo contraction–relaxation cycles as Ca^{2+} cycles with each nerve impulse (Fig. 3) (e.g. Aidley and Ashley, 1998). However, in stretch-activated fibers, muscle contraction results from the muscle deformation that is due to stretch. Both *in vivo* and *in vitro* studies reveal specific differences in the regulatory proteins, troponin C and troponin I, of asynchronous and synchronous muscles (reviewed in Bullard and Pastore, 2011). One isoform of troponin C, called F1 troponin C, is necessary for stretch activation in isolated muscle fibers. It has only one Ca^{2+} -binding site, unlike troponin C in vertebrate muscles, which has four sites (Agianian et al., 2004). The binding of Ca^{2+} induces troponin C to transition from a closed to a partially open state, even in the presence of low levels of Ca^{2+} ; this may be essential to prime the muscles for stretch activation. An isoform of troponin I, called troponin H, has a long tail that has been implicated in stretch activation. *In vivo* X-ray diffraction studies indicate that the troponins form a cross-bridge, called the troponin bridge, which directly connects them to myosin and is instrumental in releasing or blocking active sites on actin during stretch and release cycles (Perz-Edwards et al., 2011). A recent X-ray study on live flapping insects confirmed that contraction in stretch-activated muscles is mediated by actin–myosin cross-bridge formations, without requirement of a novel mechanism (Iwamoto and Yagi, 2013).

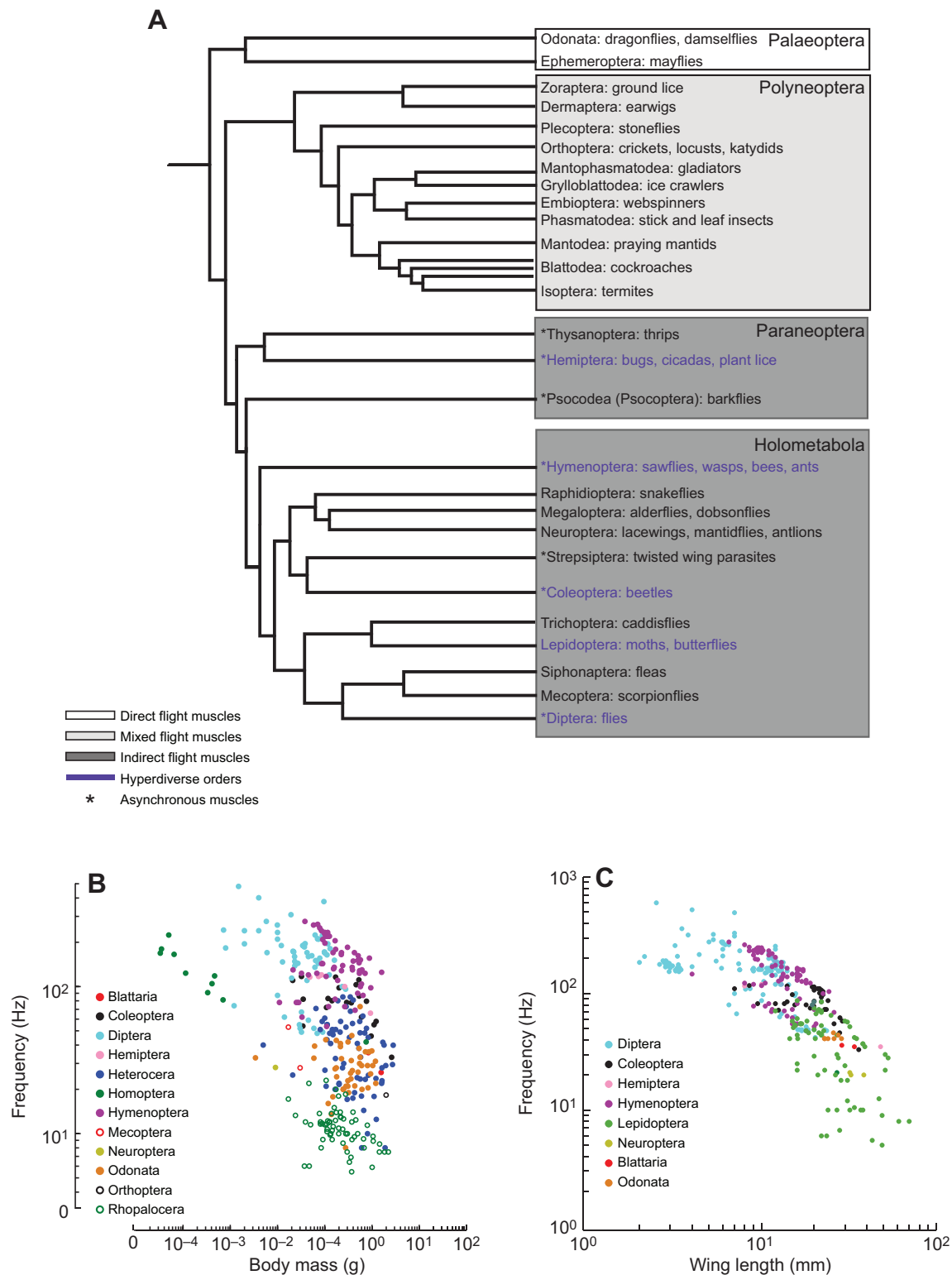


Fig. 1. Flight muscle organization in insects. (A) The direct (white box), mixed (light grey box) or indirect (dark grey boxes) arrangement of flight muscles in the insect thorax shows a strong phylogenetic basis (insect phylogeny adapted from Misof, 2014). Although the indirect and direct flight muscle arrangements are typically reported as morphologically distinct, the transition between these muscles is best viewed as a continuum because of the presence of intermediate forms in certain basal insects such as Ephemeroptera and Plecoptera. In a subset of the insects with indirect flight musculature, the muscles are also asynchronous or myogenic (*). In this figure, Paraneoptera does not include Phthiraptera because they are flightless. All hyperdiverse orders (blue), with the possible exception of Lepidoptera, share the presence of indirect flight muscles combined with asynchronous muscle type. There is also a strong overlap between hyperdiverse taxa (blue) and species with miniature (<1 mm) size (Diptera, Hymenoptera, Coleoptera). Greater wingbeat frequency is correlated with smaller size both in terms of body mass (B) and wing length (C). B and C are redrawn from data in Dudley, 2000 and Greenewalt (1962), respectively.

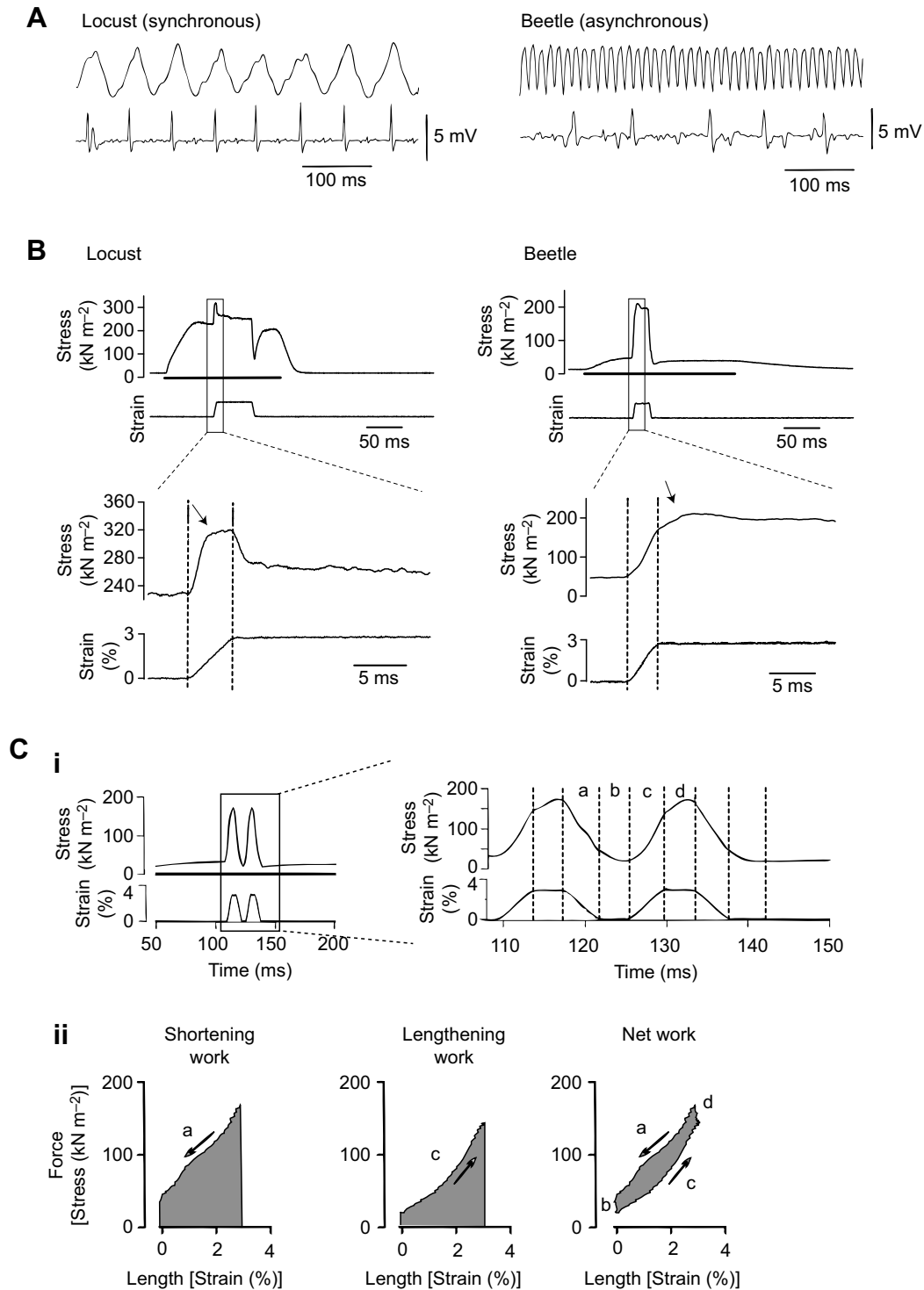


Fig. 2. Synchronous versus asynchronous muscle activation powers wing flapping. (A) Wing oscillations (upper trace) generated during tethered flight and the corresponding muscle potentials (lower trace) in the synchronous muscles of locusts (left) and asynchronous muscles of beetles (right) (adapted from Syme and Josephson, 2002). (B) Tetanically stimulated flight muscles of locust (left) and beetle (right) generate stress in response to an imposed stretch in the muscle. The period of stimulation is shown as a thick horizontal line in both cases. For a step increase in strain, stress in the synchronous flight muscle of a locust increases as the muscle is stretched but sharply declines to a plateau value smaller than the peak stress after strain has reached its maximum value. In contrast, the stress in the asynchronous flight muscle of a beetle continues to rise even after strain has reached its maximum value, and plateaus at a peak value after some delay. The arrows mark the point at which the stress yields (from Josephson et al., 2000). (C) Work loop technique to determine power output of muscles. (i) Stress generated by tetanically stimulated asynchronous flight muscle of a beetle in response to stretch–hold–release–hold cycles. Areas marked a, b, c and d correspond to the different parts of the work loop cycle illustrated in ii. (ii) As the muscle is lengthened, the work done on the muscle can be calculated as the area under the stress–strain curve. As the muscle shortens, the force decreases (leftmost panel). As the muscle lengthens, the force increases (center panel). The net work done by/on the system can be calculated by subtracting the area from the shortening cycle and lengthening cycle (shown in the rightmost panel). An anticlockwise loop indicates work done by the muscle, whereas a clockwise loop indicates that work is done on the muscle (adapted from Josephson et al., 2000).

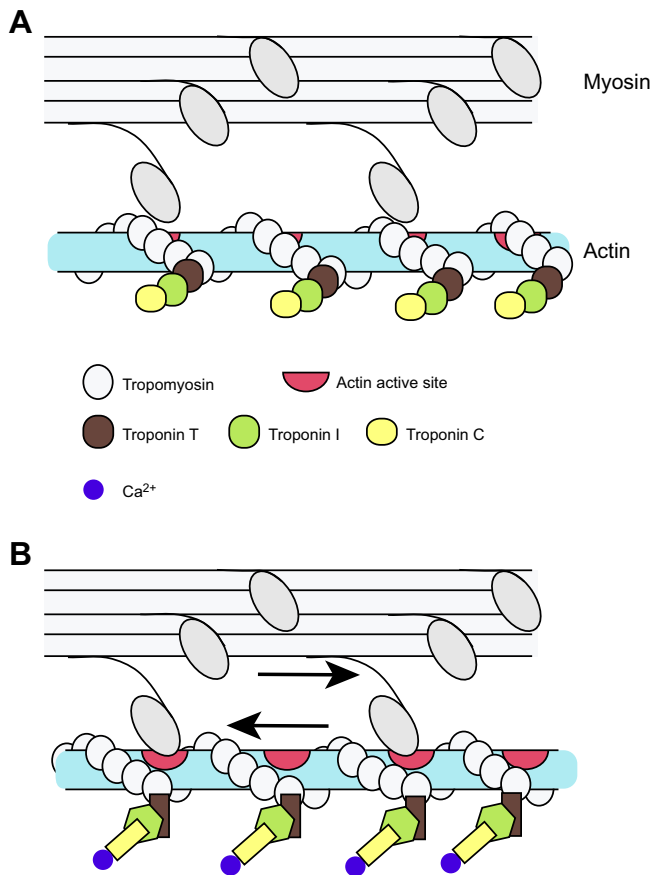


Fig. 3. Actin–myosin binding in muscles. (A) Myosin heads (grey) projecting from the myosin fibers are unable to bind to the active sites on actin (pink) because of steric blockage by tropomyosin (light grey circles). This blocking is regulated by the troponin complex: troponin T (brown circle), troponin I (green) and troponin C (yellow). (B) On binding to Ca²⁺, the troponin complex goes through a conformational shift inducing tropomyosin to release the block on the active site. Myosin heads then bind to these active sites and induce actin–myosin filaments to slide over one another, causing muscle contraction.

Antagonistic asynchronous muscles set up resonance oscillations of the thorax

The asynchronous flight muscles of Diptera, Hymenoptera and Coleoptera are arranged in the so-called ‘indirect muscle architecture’, which appears to be a feature of all paraneopteran and holometabolous insects that can fly (Fig. 1). Unlike palaeopteran and polyneopteran insects in which all or only a subset of flight muscles directly connect to the wing hinge, the indirect flight muscles are arranged as two antagonistic sets called the dorso-longitudinal and the dorso-ventral muscles (Fig. 4A,B). When a fly takes off, the dorso-ventral muscles contract and deform its thorax, causing the dorso-longitudinal muscles to extend and undergo delayed stretch activation. The consequent delayed force generation by the dorso-longitudinal muscles causes them to contract and the dorso-ventral muscles to extend; this establishes the cycle of stretching and contracting and repeated oscillations of the thorax for each nerve impulse (Fig. 4C).

Thus, the asynchronous indirect flight muscles power the back-and-forth movement of the wings at substantially enhanced frequencies. The subtler stroke-to-stroke alterations of wing kinematics are carried out by a separate set of muscles called synchronous steering muscles, which directly or indirectly actuate subtler wing movements and respond to rapid neural feedback

during flight (Wisser and Nachtigall, 1984; Dickinson and Tu, 1997). To gain insight into their role, it is first necessary to understand how the wing hinge is configured from its component parts, as discussed below.

Passive cuticular linkages and direct steering muscles

Wing hinge components and their actuation via steering muscles

How do the thoracic deformations outlined above transform into complex wing kinematics in flying insects? Although this question has intrigued insect flight researchers for several decades, it remains largely unresolved because of our inability to image and quantify the mechanics of the wing hinge, which acts as the transmission between thoracic oscillations and the consequent wing movement (Nachtigall et al., 1998). The dorsal side of the thorax is divided into the anterior scutum and posterior scutellum (Fig. 5A). Lateral projections of the scutellum, called the ‘scutellar lever arm’, extend close to the wing base. During oscillations of indirect flight muscles, the entire thorax, including the scutellum and scutellar lever arm, vibrates and actuates the wing strokes via a series of small, sclerotized cuticular structures called ‘axillary sclerites’ (for sclerite, see Glossary; Fig. 5B,C, Movie 1), which form the wing hinge. The synchronous, direct steering muscles connect to a subset of these sclerites and other cuticular projections to finely control wing motion (Wisser and Nachtigall, 1984; Miyan and Ewing, 1985a,b; Dickinson and Tu, 1997). In the flesh fly *Parasarcophaga dux*, wing kinematics are controlled by approximately 19 neurogenic steering muscles, of which 14 attach directly to the wing base, whereas the remaining five muscles exert an indirect influence on wing motion. The arrangement of the thoracic sclerites and the underlying steering muscles is similar, but not identical, in various Diptera. The wing hinge structure has also been described in the four-winged Lepidoptera (various moths and butterflies; Sharplin, 1963a,b, 1964; *Manduca sexta*, Rheuben and Kammer, 1987) and Hymenoptera (*Apis mellifera*; Nachtigall et al., 1998; also see Snodgrass, 1993), but the description below is based primarily on our investigations of X-ray tomography images of the steering muscles and wing hinge sclerites in *P. dux*.

Axillary sclerite 1 and associated muscles

The end of the scutellar lever arm (called the post-medial notal process or the posterior notal wing process) connects to the posterior ventral lobe of a four-lobed sclerite called ‘axillary sclerite 1’ (or pterale I or Ax1; Fig. 5C, dark blue; Movie 1). At the anterior dorsal end, the motion of Ax1 is constrained by a lateral projection of the scutum called the ‘parascutal shelf’ (Fig. 5C, purple). The region of parascutal shelf that attaches to Ax1 forms the anterior notal process. Attached to the anterior ventral lobe of axillary sclerite 1 are two muscles – I1 and I2 (Dickinson and Tu, 1997; Miyan and Ewing, 1985a; Nachtigall and Wilson, 1967; Wisser and Nachtigall, 1984) – both aligned in slightly different directions such that their contraction causes an effective rotation of Ax1 (Fig. 6A). The base of the I1 muscle is attached to the anepisternal ridge, whereas I2 is more internal and sits on the pleural apophysis (for apophysis, see Glossary) (Figs 5B, 6A, Movie 2). A recent X-ray tomography study (Walker et al., 2014) showed that the tendon connecting the I1 muscle to Ax1 is in a buckled state at the onset of downstroke, when the wing is above the wing hinge. It transitions from a buckled to a taut state as it approaches the end of the downstroke. This suggests that high-amplitude wing motion is not achieved by the pulling of Ax1 because of contraction of I1 at the end of upstroke. Rather, I1 contraction acts to restrict amplitude at the end of downstroke (Walker et al., 2014); these results explain why I1 is active when stroke amplitude decreases (Heide, 1975; Balint and Dickinson,

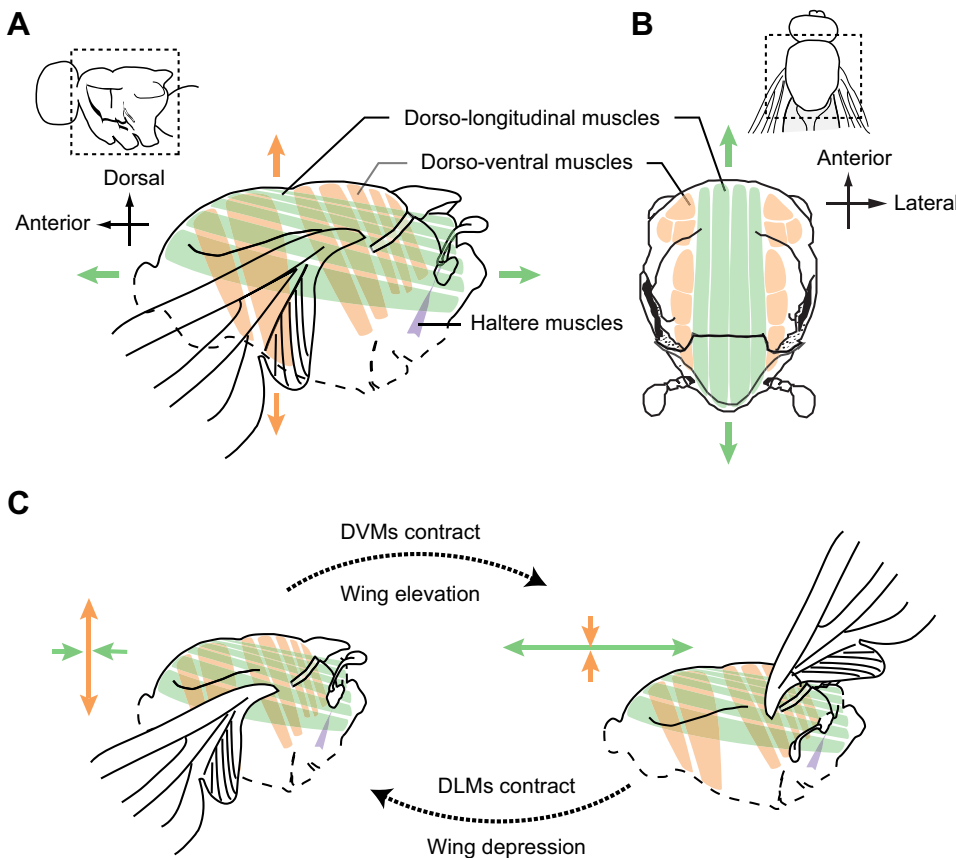


Fig. 4. Alternating contraction of antagonistic indirect flight muscles causes flapping wing movements in insects. The indirect, asynchronous flight muscles in the lateral (A) and dorsal (B) views. Dorso-longitudinal muscles (DLMs) are shown in green, and dorso-ventral muscles (DVMs) are shown in orange. (C) The resonant cycle of contraction and extension of the antagonistic pairs of DLMs and DVMs, and the corresponding wing movements.

2001, 2004). The II muscle is also reported to be active when the ipsilateral wing is folded (Miyan and Ewing, 1985a).

Axillary sclerite 2

Axillary sclerite 2 (or pterale II or Ax2; Fig. 5C, yellow; Movie 1), located between two dorsal lobes of Ax1, connects to the pleural wing process (green) on the lateral wall of the thorax through a hook articulation. Ax2 is also continuous with the ventral end of the wing and, hence, may directly actuate the radial wing vein and a projection on the radial vein called the radial stop (red). Ax2 is not directly actuated by any muscles.

Axillary sclerite 3 and associated muscles

Axillary sclerite 3 (or pterale III or Ax3; Fig. 5C, deep pink; Movie 1) lies on the posterior end of the wing. Four muscles, referred to as the III1–4 muscles (Fig. 6B) (Dickinson and Tu, 1997; Miyan and Ewing, 1985a; Nachtigall and Wilson, 1967; Wisser and Nachtigall, 1984), attach to Ax3. The III1 muscle has its base on the anepisternal ridge that is posterior to the muscle II. III2, however, is more internal on the pleural apophysis (Fig. 6B, Movie 2). These two muscles form a single tendon and together actuate Ax3. The two smaller muscles – III3 and 4 – also fuse and exert force via the common tendon. These muscles are thought to assist in wing retraction. They are active when the ipsilateral wing is folded (Miyan and Ewing, 1985a; Wisser and Nachtigall, 1984). However, when both wings are flapping, the muscle on the side of the wing with greater amplitude is active.

Axillary sclerite 4 and associated muscles

Axillary sclerite 4 (or pterale IV or Ax4; Fig. 5C, green; Movie 1) is a sclerotized region under the scutellar lever arm. In some flies, such

as *Drosophila*, the two structures are fused and are hence referred to as the posterior notal wing process. In other insects, such as flesh flies, the posterior notal wing process appears as a distinct structure. Five muscles, hg1–5, that attach to Ax4 (Fig. 6C) (Dickinson and Tu, 1997; Miyan and Ewing, 1985a; Nachtigall and Wilson, 1967; Wisser and Nachtigall, 1984) lie atop the pleural apophysis (Fig. 6C, Movie 2). Hg1 is the anterior-most muscle with a long tendon attached to the posterior end of Ax4. In the flesh fly, the other four muscles, hg2–5, are posterior to hg1 but their fused tendon crosses the hg1 tendon and attaches to the anterior end of Ax4. To the best of our knowledge, the tiny hg5 has not been described before although it is possible that different fly species have variable number of hg muscles (Fig. 6C). Co-activation of hg1 and hg3 with II is correlated with a decrease in wingbeat amplitude (Dickinson and Tu, 1997). Hg1–4 fire when the ipsilateral wing is folded but are silent when it is flapping (Nachtigall and Wilson, 1967). In addition to their role in flight, the hg muscles have also been implicated in other natural history contexts. For example, in an elegant set of experiments, Shirangi et al. (2013) demonstrated that the hg1 muscle is essential for producing the sine component of courtship song in *Drosophila*.

Basalar muscles

Three basalar muscles – b1, b2 and b3 – attach to the basalar apophysis (Fig. 5B) at a location anterior to the wing (Fig. 6D, Movie 2) (Dickinson and Tu, 1997; Miyan and Ewing, 1985a; Nachtigall and Wilson, 1967; Wisser and Nachtigall, 1984). These muscles are accessible for electrophysiology recordings because they lie right under the cuticle, and hence have been extensively studied (Heide and Götz, 1996; Tu and Dickinson, 1994, 1996). The b1 motor neurons fire once every wingbeat. Both b1 and b2 muscles are arranged such that their contraction moves the apophysis in the

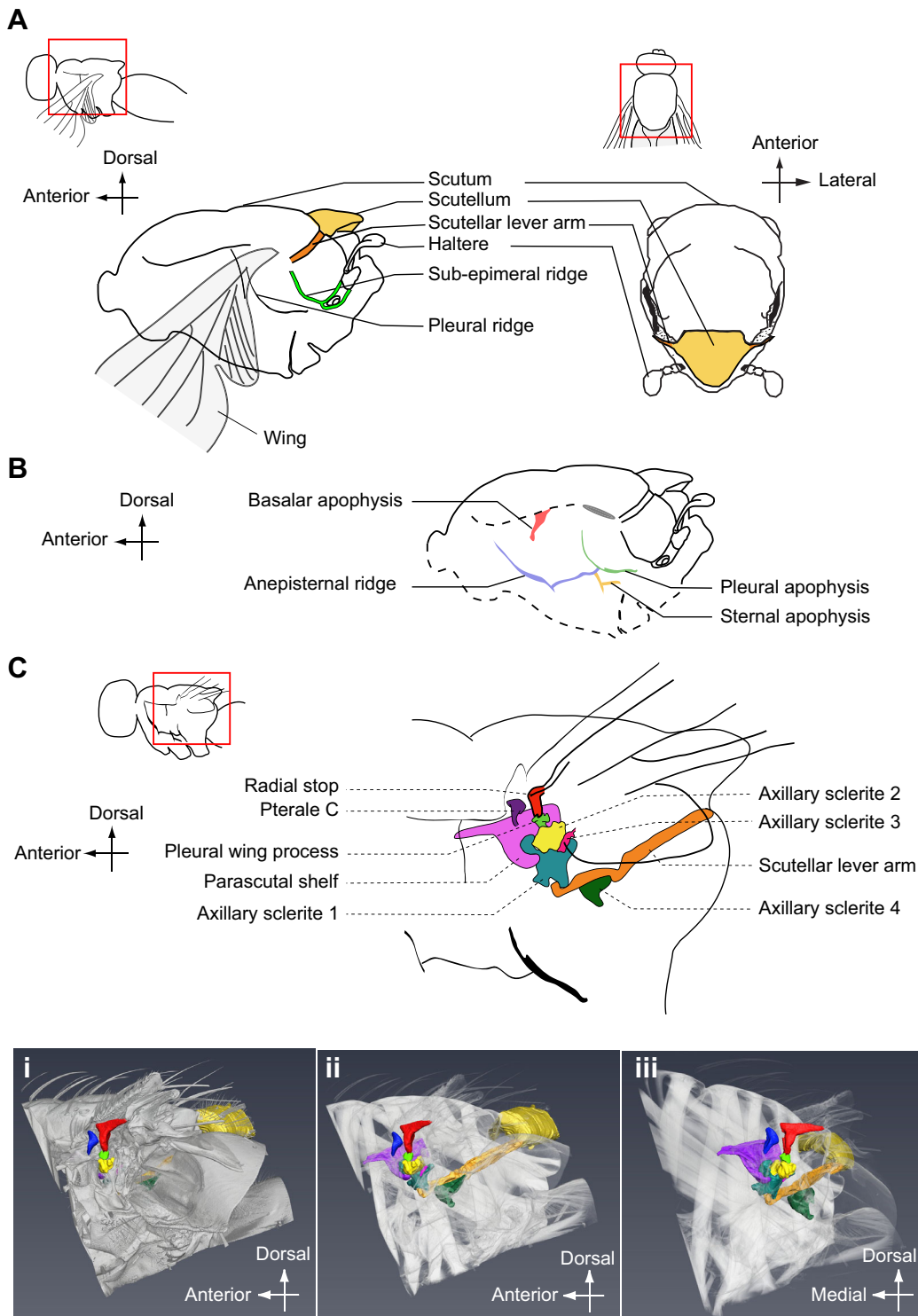


Fig. 5. X-ray microtomographic reconstruction of external and internal thoracic anatomy of the flesh fly, *Sarcophaga dux*. (A) Diagram of the external anatomy of the thorax in the lateral (left) and dorsal (right) views (adapted from Deora et al., 2015). (B) Location of internal apophyses that form the attachment sites for steering muscles. (C) Lateral view of the various axillary sclerites and other cuticular structures involved in wing actuation. Also shown are X-ray microtomographic projections of the external structure alone (i), and external as well as internal structures in a lateral (ii) and anterior view (iii). See Movies 1 and 2 for additional detail. Note that these images were obtained from a fixed preparation, which may be different in some respects as compared with live preparation owing to fixation-related artifacts.

anterior direction, thereby pulling the wing forward for wing amplitude modulation (Nachtigall and Wilson, 1967). The b3 is a smaller muscle with a long tendon attached close to the parascutal shelf, with its fibers aligned antagonistically to b1 and b2 (Fig. 6D,

Movie 2). During yaw maneuvers, the firing of b2 muscles together with III1 muscles is correlated with an increase in wing amplitude (Dickinson and Tu, 1997), but b2 fires only when the ipsilateral wing is flapping.

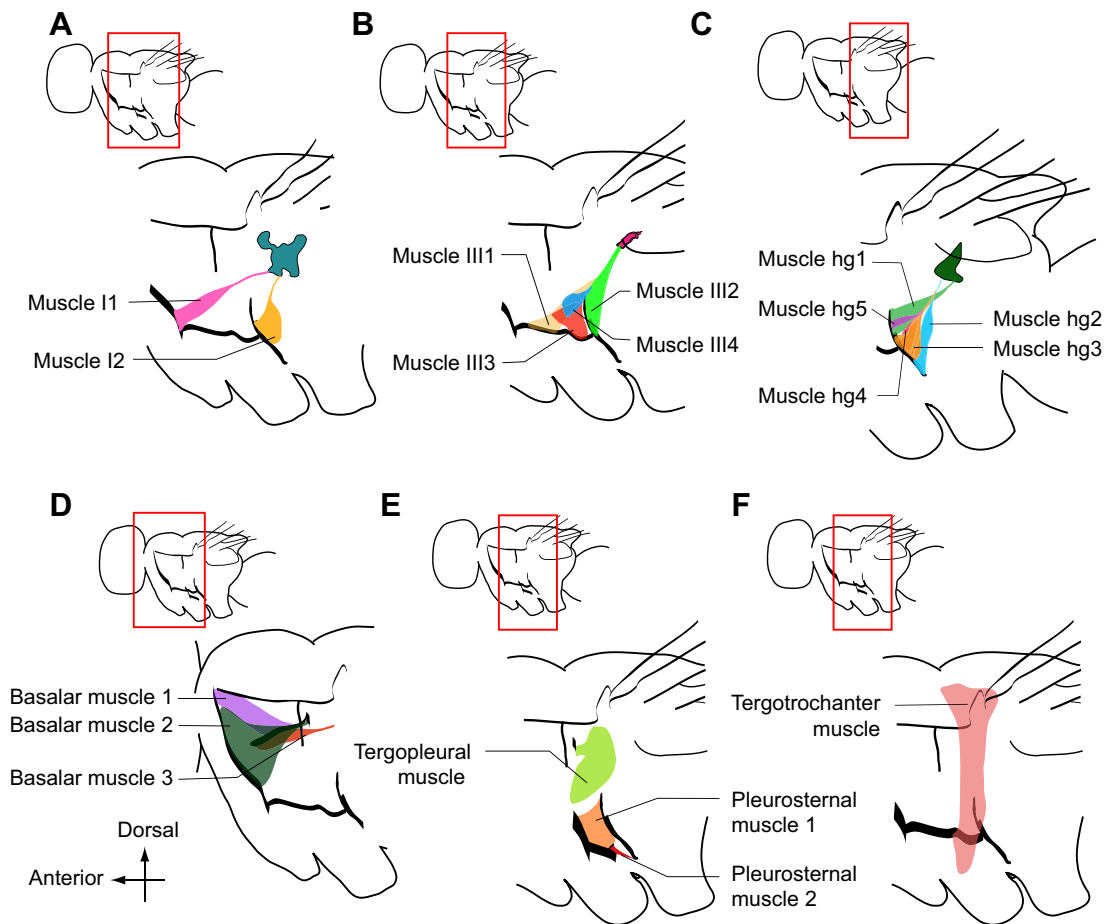


Fig. 6. The steering muscles in the lateral view based on X-ray microtomography data from *Sarcophaga*. Insets show different sets of muscles in an enlarged view. (A) Ax1 (blue) with muscles I1 (pink) and I2 (yellow). (B) Ax3 (deep pink) with muscles III1 (light brown), III2 (green), III3 (red) and III4 (blue). (C) Ax4 muscles – hg1 (green), hg2 (blue), hg3 (brown), hg4 (pink) and hg5 (purple). hg5 has not been described previously. (D) Basalar muscles – b1 (purple), b2 (green) and b3 (red). (E) Tergopleural muscle (green) and the pleurosternal muscles psm1 (buff) and psm2 (red). (F) Tergotrochanter muscle (pink).

Indirect control muscles

The remaining five indirect control flight muscles do not directly attach to any apophyses that influence the wing motion. Pleurosternal muscles 1 and 2 connect the pleural and sternal apophyses (Fig. 6E) (Dickinson and Tu, 1997; Miyan and Ewing, 1985a; Nachtigall and Wilson, 1967; Wisser and Nachtigall, 1984). Of these, pleurosternal muscle 1 has recently been implicated in generating specific features of pulse song during courtship in *Drosophila* (Shirangi et al., 2013). The fan-shaped tergopleural muscles 1 and 2 are situated at the base of the wing. The tergopleural muscle in *Parasarcophaga* is a single muscle, unlike in insects such as *Drosophila*, in which it is present as two separate muscles (Fig. 6E). The function of pleurosternal and tergopleural muscles is not well understood. Because firing in these muscles is correlated with wingbeat frequency they are thought to control thoracic geometry and stiffness (Miyan and Ewing, 1985a), thereby causing changes in the resonant tuning and the wingbeat frequency (Nachtigall and Wilson, 1967). The tergotrochanter muscle (or tergal depressor of the trochanter) is a large muscle that aligns almost parallel to the dorso-ventral muscles (Fig. 6F). Its activation initiates the jump during take-off, thereby triggering the resonant contraction cycles of indirect flight muscles. Activation of the tergotrochanter muscle may not, however, be a necessary condition for jumping, because flies in which this muscle is surgically removed can also jump or initiate flight (Nachtigall and Wilson, 1967).

The wing hinge translates muscle contraction into wing motion

How does the combinatorial activity of these 18–19 pairs of steering muscles control wing kinematics? This remains one of the most difficult open questions in insect flight. Previous approaches to inferring the function of steering muscles involved independently actuating each muscle in fresh dissections and noting their effect on the wing. However, this approach has severe limitations, as was evident from various studies which used work loop techniques as well as simultaneous recordings from multiple muscles. These studies showed that the control of wing kinematics results from the combined activation of two or more muscles (Tu and Dickinson, 1994, 1996; Balint and Dickinson, 2001). For instance, activity in neighboring muscles can dramatically alter the work output of a particular muscle (Tu and Dickinson, 1994; Dickinson and Tu, 1997). Recent work on *Drosophila* using genetically encoded calcium reporters (GCaMP6f) in active muscles has revealed some very interesting aspects about the organization of steering muscles at the base of the wing hinge sclerites. The steering muscles in *Drosophila* are organized into two groups that are anatomically and functionally separated into the larger phasically activated muscles that control the major alteration in wing motion during distinct maneuvers, and the smaller tonic muscles that control the subtler modulations of wing motion (Lindsay et al., 2017).

The transmission of strain from the indirect flight muscles, via hinge sclerites to the wings, may be described using a four-bar linkage model which includes the parascutal shelf, Ax1, Ax2 and

the wing vein (e.g. Wootton, 2009). In this model, the movements of the scutellar lever arm directly actuate Ax1, causing wing oscillations. To infer the exact mechanism of strain transfer, previous researchers largely resorted to a functional morphological approach by meticulously drawing carefully performed dissections and directly observing wing kinematics using high-speed videography (Wisser and Nachtigall, 1984; Nalbach, 1989). Following the observation that CCl₄-anesthetized flies held their wings in either extreme upstroke or downstroke positions, Boettiger and Furshpan (1952) argued for a bistable ‘click’ mechanism (see Glossary) of wing motion. Their model emphasized the rotation of Ax1 on the scutellar lever arm as an important component in generating this bistability. However, this idea was rejected by Miyan and Ewing (1985a,b) based on the more continuous modulation of wing kinematics in live tethered flies. They argued that the bistable wing positions in flies were most likely an artifact of CCl₄ anesthesia, and instead proposed that Ax1 locks onto the parascutal shelf and drives the wing motion. The two other axillary sclerites – Ax3 and Ax4 – may be involved in wing protraction, extension and changing stroke planes. Similarly, Ennos (1987) argued for the scutum as the driving agent for the back-and-forth motion of the wings, via the axillary sclerites. Later experiments by Wisser (1988) invoked a ‘gear change’ mechanism that emphasized a wing ‘stop’ during downstroke as an essential feature of wing oscillations. Because these structures were mostly internal and not amenable to ready observations during flight, direct evidence for the precise mechanism of wing actuation has been difficult to obtain.

This scenario is rapidly changing, however, in large part owing to modern techniques such as synchrotron-coupled X-ray imaging in live insects and genetic manipulations in *Drosophila* to better understand the role of direct steering muscles in various behaviors. The synchrotron-coupled X-ray imaging of live flapping flies has been used to visualize the small motion of these tiny structures during active wing motion. In addition to the motion of internal sclerites, these techniques also allow visualization of the direct steering muscles during flight (Walker et al., 2014). In parallel, recent breakthroughs in *Drosophila* genetics also show great promise in revealing the role of specific direct steering muscles both individually and in combination with other muscles in different behaviors (Shirangi et al., 2013; Lindsay et al., 2017).

Coordination of wings and halteres involves passive linkages and an active clutch

Rapid mechanosensory control of wing kinematics during flight maneuvers

The physiological and anatomical adaptations described above enable small insects to generate sufficient forces at rapid rates during flight. Unlike most insects that use two pairs of wings to generate lift, some orders – such as Coleoptera, Strepsiptera and Diptera – use only one wing pair; the other pair adopts auxiliary protective or sensory functions (Chapman, 1982). In Coleoptera, the forewing has evolved to form protective elytra which cover the hind wings when the insect is not flying. During flight, the elytra lift up and the hind wings unfold to flap and generate the necessary flight forces. In Diptera, the hind wings have evolved into specialized mechanosensory organs called halteres, which act as gyroscopic sensors that detect body rotations at rapid rates (Pringle, 1948; reviewed in Yarger and Fox, 2016) (Fig. 7Ai–iii). Although a more detailed discussion of haltere function is beyond the scope of this Review, we offer here a brief summary of the existing ideas.

Halteres are club-shaped organs with most of the mass concentrated at the end knob and multiple fields of

mechanosensory campaniform sensillae (see Glossary) at the base (Fig. 7Aii,iii) (Agrawal et al., 2017). Halteres oscillate in a tight plane synchronously with the wings. During aerial maneuvers, the law of conservation of angular momentum imposes an inertial resistance to externally imposed changes in their plane of rotation. This inertial force is manifest as Coriolis forces (see Glossary) that cause the haltere stalk to bend in directions orthogonal to the rotation plane, thereby stimulating the mechanosensory fields located at the base (Pringle, 1948; Nalbach, 1993, 1994). The mechanosensory feedback, encoding information about pitch, roll and yaw turns, thus rapidly informs the fly about its own body rotations (Nalbach and Hengstenberg, 1994; Fox and Daniel, 2008; Fox et al., 2010, Yarger and Fox, 2016). Although insects also combine visual and olfactory information with mechanosensory feedback to stabilize flight and steer towards a target (Götz, 1968; Heide and Götz, 1996; Willis and Arbas, 1998; Egelhaaf and Kern, 2002), the mechanosensory feedback acts on a much shorter time scale than vision or olfaction (Hengstenberg et al., 1986; Hengstenberg, 1988; Trimarchi and Schneiderman, 1995a,b; Sherman and Dickinson, 2003, 2004; Bender and Dickinson, 2006). In insects of the order Strepsiptera, the forewings, rather than the hindwings, have evolved into halteres. The strepsipteran haltere is relatively understudied, but may function in a manner similar to that of dipterans (Pix et al., 1993).

Halteres provide wingbeat-synchronous feedback to the wing steering muscles

Wing steering muscles receive rapid, monosynaptic feedback from both the wing and haltere mechanosensors (Fayyazuddin and Dickinson, 1999), and this feedback is phase-locked to wing oscillations. In most Diptera, the wings and halteres beat precisely antiphase (i.e. 180 deg) relative to each other, whereas in some others with higher wingbeat frequencies, the phase relationship may not be 180 deg (Hall et al., 2015). From the perspective of flies, the phase difference is extremely important because any change in the relative phase between wings and halteres is indicative of an aerial maneuver that must be corrected if unintentional; alternatively, the tendency to correct such deviations should be overridden if the maneuver is intentional. Because even small deviations in wing kinematics can lead to large changes in body kinematics (Fry et al., 2003), the left and right wing motion must also be precisely coordinated. In all cases, the relative phase difference between the wings and halteres is exactly maintained despite oscillating at frequencies >100 Hz. Absence of this coordination causes severe impairment of flight control (Deora et al., 2015).

Passive mechanisms coordinate wing and haltere motion

Unlike Diptera or Strepsiptera and some wingless insects, most insects have two pairs of wings, which are either independently controlled or externally coupled. For instance, dragonflies (order Odonata) can independently control each wing pair, and likely use their relative motion to maneuver (Usherwood and Lehmann, 2008). In other insects, specialized anatomical structures on both pairs of wings ensure that the two ipsilateral wings remain coupled and flap as a single unit (Chapman, 1982). For example, the hind wings of some lepidopterans have a bristle-like structure called the ‘frenulum’ that latches onto a hook-like structure called the ‘retinaculum’ on the forewing to ensure synchronous flapping. In Hymenoptera, the hindwings have a row of tiny hooks called the ‘hamuli’, which latch onto a fold on the forewing and help keep both ipsilateral wings together. Although the wings and halteres of Diptera are not externally coupled, they too oscillate with precise

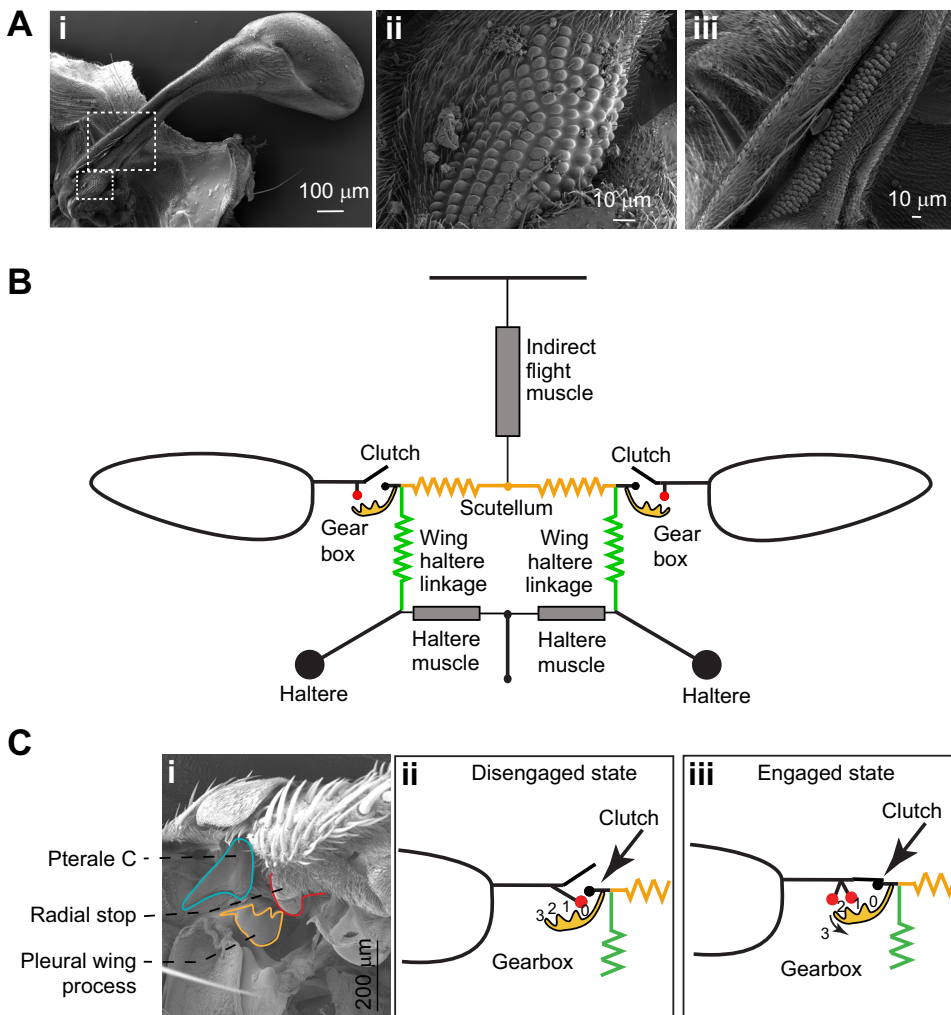


Fig. 7. Wing–wing and wing–haltere coordination is passively mediated by cuticular linkages. (A) Dorsal view of the haltere of a soldier fly, *Hermetia illucens*. (i) Regions on the haltere base marked in small (enlarged in ii) and large (enlarged in iii) boxes highlight the mechanosensory fields of campaniform sensillae. (B) Mechanical model of the dipteran thorax showing wings and halteres coupled by passive cuticular linkages with the clutch and gear box (adapted from Deora et al., 2015). (C) SEM image of the wing base (i) with the pleural wing process (yellow), radial stop (red) and pterale C (blue) outlined, and illustration of the gear box in the disengaged (ii) and engaged (iii) forms.

phase relationships relative to each other. How do wings and halteres maintain such precise phase at high frequencies?

Passive mechanisms of wing–haltere coordination

In insect orders that contain miniature members, rapid yet precise coordination is mediated by passive cuticular linkages embedded within the thorax, which mechanically couple the wings and halteres and constrain their motion to maintain the same relative phase despite changes in wing kinematics (Deora et al., 2015). This coupling ensures that haltere timing relative to wing movement remains fixed, and the wing muscles receive unambiguous information about the timing of haltere movement.

The two contralateral wings are coupled by the scutellum, whereas each haltere is coupled to the ipsilateral wing by the sub-epimeral ridge on the lateral side of the thorax (Deora et al., 2015) (Figs 5A, 7B). The two halteres are independent of each other and are only indirectly coupled via the wings. There is mounting evidence that the wings and halteres, connected by the thoracic linkages, act as coupled oscillators. If the wing frequency increases (caused for instance, by clipping the wing), the haltere frequency concomitantly increases (Deora et al., 2015). Beyond an increase of ~150%, however, haltere frequency becomes decoupled from the wingbeat frequency, and reverts to its original frequency. By contrast, if the frequency of haltere oscillations is altered, the wing continues to oscillate at the original wingbeat frequency, suggesting that the wing–haltere coupling is unidirectional. This ensures that

halteres maintain phase and frequency synchrony with the wings, irrespective of wing damage owing to predator attacks or natural age-related wear and tear.

Active engagement and control of the bilateral wings

The mechanical coupling described above ensures precise and rapid wing coordination, but it also imposes constraints on the independent movement of the wing–wing or wing–haltere pair. We know, however, that flies are capable of such independent movement (e.g. during courtship songs in *Drosophila*; Bennet-Clark and Ewing, 1968). They must, therefore, possess a ‘clutch’ mechanism that can decouple their wings from the vibrating thorax and enable independent control of each wing (Deora et al., 2015). Unlike passive mechanical control, which provides rapid and precise coordination, active neural control of the clutch provides variability and flexibility in the face of environmental perturbations. The clutch at the base of each wing must be actively synchronized by the nervous system, for instance, during flight onset when both wings begin flapping at the same time. In *Drosophila*, the ventral unpaired medial dopaminergic interneuron coordinates wing engagement at flight initiation (Sadaf et al., 2015). Inactivating this neuron in mutant flies causes disruption of the synchronous wing engagement at flight onset. However, even though the wings do not simultaneously engage at flight onset in these mutants, the mechanical linkages ensure perfectly synchronous flapping throughout a flight bout. Inactivating the ventral unpaired medial

neuron does not lead to defects in courtship song production, suggesting that single wing engagement may be independent of the flight pathway (Sadaf et al., 2015).

Active neural control induces conduction delays. During take-off, flies use two different pathways to initiate flight (Trimarchi and Schneiderman, 1995a; von Reyn et al., 2014). During slow, voluntary take-off, the two wings lift together and start flapping just as the fly jumps off. Clutches on both sides engage synchronously during such voluntary movements. However, during fast or involuntary take-off during escape responses, the fly jumps but the wings fail to engage synchronously (Trimarchi and Schneiderman, 1995b; Card and Dickinson, 2008). After a few wingbeats though, both wings oscillate in phase as a result of the scutellar linkage.

In addition to the active clutch, a ‘gearbox’ under each wing is proposed to allow flies to asymmetrically alter their wing kinematics during aerial maneuvers (Nalbach, 1989; Balint and Dickinson, 2001). The gearbox is a multi-grooved structure on the lateral thorax composed of the pleural wing process in combination with a protrusion at the base of the radial vein of the wing, the radial stop. It enables the subtle and unilateral amplitude modulation of each wing. During downstroke, the radial stop briefly contacts one of the grooves of the pleural wing process, which acts as a stop to restrict the wing amplitude to one of four modes (Fig. 7B,C). Of these, mode 0 corresponds to the disengaged state, in which the wing remains isolated from the thoracic vibration. In modes 1–3, the wing clutch is engaged. Different modes correspond to different wingbeat amplitudes (Nalbach, 1989). In modes 1 and 2, the radial stop makes contact with the pleural wing process grooves 1 and 2, respectively, whereas in mode 3 the radial stop moves anteriorly to the pleural wing process without any contact with it (Fig. 7C). Another structure, pterale C, appears to act as a shock absorber or a mechanosensory structure during the downstroke (Miyan and Ewing, 1984), but its precise function remains to be determined.

The proposed gear change mechanism, as described above, leaves several unanswered questions. Wingbeat amplitude ranges from being the lowest in mode 1 to intermediate in mode 2 and the highest in mode 3. In mode 3, the radial stop does not engage with the pleural wing process, thereby not necessitating fine control. However, the hypothesized gear shift between modes 1 and 2 requires actuation to be finely controlled within length scales of 10 μm at wingbeat frequencies in excess of 100 Hz. This seems implausible, because such fine control requires rapid sensory feedback, the source of which is not apparent. Moreover, the number of grooves in the pleural wing process varies greatly across different fly species which flap with flight frequencies greater than 100 Hz, yet all are capable of continuous amplitude modulation. We propose the alternative hypothesis that, rather than representing gears, the pleural wing process grooves act as a rough surface upon which the radial stop can rest without slipping. The presence of specific grooves in the pleural wing process of certain flies could then greatly enhance the frictional coefficient; such surface roughness further minimizes the chances of slippage between the radial stop and the pleural wing process. This means that modes 1 and 2 are not separate states, and the amplitude modulation in the mode 1 and 2 states is driven by differential activity in the underlying steering muscles. Thus, the gearbox may be coarser than previously argued. Careful tests of these hypotheses will offer new and exciting avenues for work on the wing hinge mechanism.

Is an active clutch mechanism common to all flying insects?

Hymenopteran and coleopteran insects, which also contain asynchronous indirect flight muscles, show wing-related behaviors

that are also suggestive of an actively controlled clutch. Bees thermoregulate by vibrating their thorax without moving their wings (Hrcir et al., 2008), and some beetles are capable of producing sound by vibrating their thorax with their wings folded (Leston et al., 1965). Both behaviors require insects to decouple their wings from the thorax, and are indicative of the presence of a clutch mechanism. Lepidopteran insects, specifically sphingid or saturniid moths, possess indirect but synchronous muscles. During pre-flight warm-up, they vibrate their wings with low amplitudes, suggesting constant engagement with the thorax. During warm-up, contractions of antagonist flight muscles overlap, thereby preventing larger vibrations of the thorax and the wings (Kammer, 1968). As the thorax heats up, the twitch duration of the muscle shortens, decreasing the overlap of the antagonistic muscle contraction phases. At some point, the antagonistic flight muscles begin contracting out of phase with each other and produce full-amplitude wing motion and abrupt flight. The wing vibration during warm-up suggests that these moths lack the clutch mechanism. However, we know very little about the other members of Lepidoptera, specifically Microlepidoptera. These present a unique comparative study system from which to gain insights into the evolutionary pressures of miniaturization on muscles and thoracic architecture, and on the flight system.

Perspectives and conclusions

Mechanical linkages across insects

One conclusion from the data reviewed above is that miniaturization has driven the evolution of passive mechanical structures that link and coordinate the motion of various body parts at high frequencies. This enables speed and precision, which may not be possible for the nervous system to maintain when frequencies are high. Passive mechanical linkages are not unique to Diptera, but also occur in other invertebrates. In froghoppers, which jump up to 100 times their own body length (Burrows, 2010), even a small delay between the extension of the bilateral legs can cause the jump to be unstable and disoriented. However, this is prevented by mechanical coupling between the trochanters of both legs, which ensures that both legs exert equal force on the substrate by moving simultaneously during a jump. Similarly, in male cicadas, which produce repetitive, high-frequency song to attract mates, a single muscle powers the movements of a cuticular ‘tymbal’ to produce this song (Young and Bennet-Clark, 1995). The mechanical structure of the tymbal amplifies the muscle contractions to produce highly efficient, rhythmic movements that we perceive as the high-frequency calls of cicadas.

There are numerous examples of similar mechanical structures that ensure precise coordination in insects or other arthropods (Patek et al., 2011). Because such structures are typically involved in fast and repetitive movements, they are compelling systems in which to explore adaptations for energy efficiency, such as elastic storage or amplification. For example, the rubber protein resilin, one of the most efficient rubber proteins known, was first discovered in the tendons of flight muscles of dragonflies (Weis-Fogh, 1961; Bennet-Clark, 2007). It has since been documented in the wing hinge of insects with low wingbeat frequencies such as locusts and dragonflies (Andersen and Weis-Fogh, 1964), in addition to wing folding margins (Haas et al., 2000), the legs of fleas (Bennet-Clark and Lucey, 1967), cicada tymbals (Young and Bennet-Clark, 1995) and cockroach leg joints (Neff et al., 2000). However, the mechanical resilience of resilin is high only at low frequencies. Hence, it is unlikely to confer elastic advantage at high frequencies (Gosline et al., 2002; Vincent and Wegst, 2004). For instance, the cuticular links of the dipteran thorax contain no resilin, but are primarily composed of rigid, chitinous material.

General conclusions

As described above, the neuromuscular adaptations in the form of indirect flight muscle architecture and asynchronous (myogenic) muscle type combine with the linkage-driven thoracic mechanics to generate fast resonant oscillations of the thorax in flying insects. These ensure a wingbeat frequency well in excess of what may be possible purely through neural activation of each flight muscle. The thoracic oscillations are transduced into powerful wing strokes by a complex wing hinge, which is configured by 18–19 pairs of steering muscles that control the finer aspects of wing motion in combinatorial ways that remain to be fully understood. A clutch-like mechanism engages or isolates the wing from the thoracic oscillations, and a multi-grooved gearbox helps control stroke amplitude during flight. These observations indicate that, under evolutionary pressure to increase wingbeat frequency, the flight system has evolved biomechanical adaptations for rapid coordination, combined with slower neural modulation of diverse stroke parameters. This ensures both high-frequency wing motion as well as perfect coordination of wings and other flight-related appendages (such as halteres) in the smaller insects, making possible the spectacular diversity of flight behaviors in insects.

Acknowledgements

We wish to thank Mehrab Modi, Anupratap Tomar, Anand Krishnan, Robert Dudley and members of the Insect Flight lab for feedback; Shilpa Naik, Nehal Johri, Akash Vardhan and Abin Ghosh for various discussions and dissections; Taruni Roy for help with plots; and the AFMM facility staff at the Indian Institute of Science for help with X-ray microtomography. Akash Vardhan and Shilpa Naik contributed to the preparation of the supplementary movies.

Competing interests

The authors declare no competing or financial interests.

Funding

Funds were provided by the US Air Force Office of Scientific Research, Asian Office of Aerospace Research and Development (AOARD 114057); the International Technology Center–Pacific (ITC-PAC); and Ramanujan Fellowships from the Science and Engineering Research Board, Department of Science and Technology, Government of India (to S.P.S. and N.G.).

Supplementary information

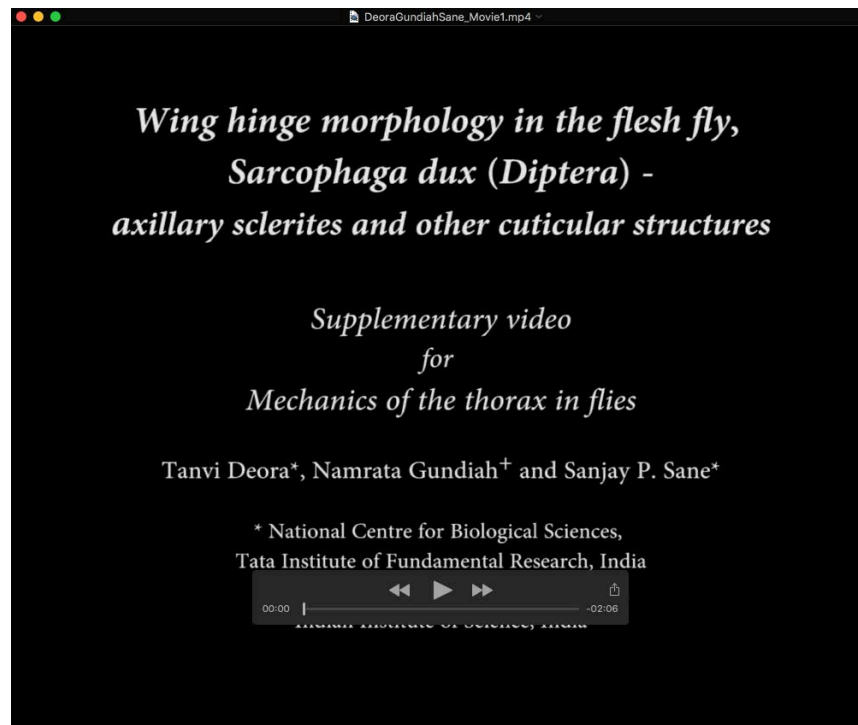
Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.128363.supplemental>

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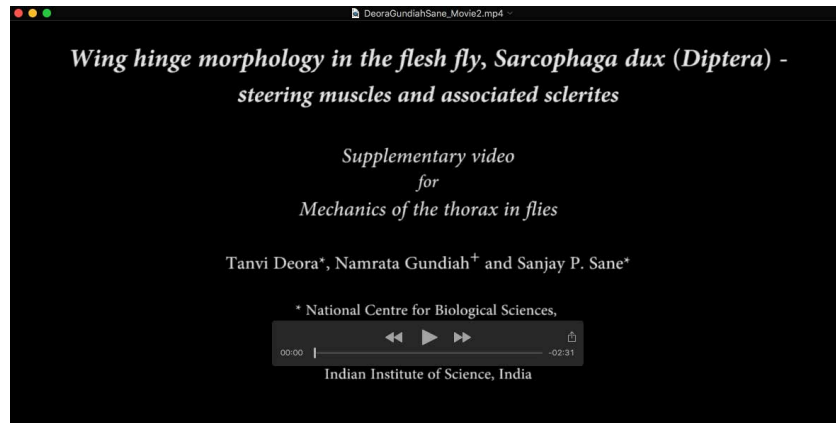
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Supplementary Videos



Supplementary Movie 1: Wing hinge morphology in the flesh fly, *Sarcophaga dux* (Diptera) – axillary sclerites and other cuticular structures. This video shows the structures involved in transmitting forces from the power muscles to the wings using contrast-enhanced X-ray microtomography of a flesh fly thorax.



Supplementary Movie 2: Wing hinge morphology in the flesh fly, *Sarcophaga dux* (Diptera) – steering muscles and associated sclerites. This video shows all the steering muscles responsible for fine kinematic control of wing strokes using contrast enhanced X-ray microtomography of a flesh fly thorax.