

# VARIABILITY IN THE SIZE, COMPOSITION, AND FUNCTION OF INSECT FLIGHT MUSCLES

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■ **Abstract** In order to fly, insects require flight muscles that constitute at least 12 to 16% of their total mass, and flight performance increases as this percentage increases. However, flight muscles are energetically and materially expensive to build and maintain, and investment in flight muscles constrains other aspects of function, particularly female fecundity. This review examines ways in which insects vary the size of their flight muscles, and how variation in the relative size and composition of flight muscles affects flight performance. Sources of variability in flight muscle size and composition include genetic differences within and between species, individual phenotypic responses to environmental stimuli, and maturational changes that occur before and during the adult stage. Insects have evolved a wide variety of ways to adjust flight muscle size and contractile performance in order to meet demands imposed by variation in life history and ecology.

## INTRODUCTION

Insect flight muscles operate at high contraction rates and produce the highest known rates of mechanical power output and metabolic power input. These characteristics, along with the activation of certain insect flight muscles by mechanical rather than neural stimuli (stretch activation), have long attracted the attention of physiologists interested in the mechanical and biochemical bases of flight muscle contraction and metabolism (1-4). The same characteristics have also attracted the attention of comparative physiologists interested in determining the causes and consequences of variation in muscle function within this exceedingly diverse group of organisms. Among the topics examined by comparative physiologists, interspecific variation in flight-related thermoregulatory mechanisms and thermal ecology has been fairly well explored and summarized (5). Other aspects of variability in insect flight muscle function have been more sparsely studied and less frequently reviewed. Here I present an overview of what is known about variation in the size, composition, and contractile physiology of insect flight muscles, focusing particularly on how that variation affects whole-organism function.

## VARIATION IN OVERALL SIZE OF THE FLIGHT MUSCULATURE

The flight muscles of insects represent a major allocation of energy and material. Flight muscles constitute as much as 55 to 65% of body mass (6–8), with the percentage dropping to nearly zero in species that temporarily or permanently break down their flight muscles and lose the ability to fly. The variation is not continuous, since total flight muscle mass must be at least 12 to 16% of body mass for weight-supported flight in still air (7). Thus insects are forced to either make a substantial investment in flight muscle or forego the benefits of aerial travel.

Nearly every order of insects contains species that are either flightless or polymorphic in the extent of flight muscle development (9–15). In some cases polymorphisms are caused by simple Mendelian genetic differences. For example, flight muscle development in female *Heptophylla picea* beetles (Scarabaeidae) is a homozygous recessive trait (16). Muscle suppression occurs in heterozygous as well as homozygous dominant females, and the frequency of flight-capable females varies from 0 to 100% at different geographic locations (17). Males in this species are always flight capable. These data suggest that a single copy of an uncharacterized flightless allele gives rise to a threshold level of a flight muscle repressor in females, but two copies of this allele fail to produce a threshold level of the repressor in males.

In a wide variety of insects, flight muscle size responds in a flexible manner to environmental factors such as local population density and food availability (9–15). For example, both sexes of bark beetles (*Ips pini*; Scolytidae) undergo degeneration of their flight muscles within five days of entering a tree that is a suitable breeding site (18). Males mate with females and remain paired in order to assist in initiating the construction of a tunnel system in which the young will develop. Within about one month, males gradually regenerate their flight muscles and become capable of emigrating to another breeding site. Males that have located a tree and undergone muscle degeneration, but are unable to locate a mate, regenerate their flight muscles to emigration-phase levels within only five days.

Many insect taxa undergo fairly predictable changes in flight muscle size and ultrastructure during the course of adult maturation. The flight muscles of tsetse flies (*Glossina* spp., Diptera) are not fully mature until after they have consumed a number of blood meals, undergoing a total increase in mass of about 75% (19). Nearly all taxa of dragonflies (Odonata) undergo substantial growth of their flight muscles during adult maturation (20), with dragonflies of the genus *Libellula* showing as much as a doubling or tripling of muscle size (8). The exoskeletal size of insects is fixed shortly after adult eclosion when the cuticle hardens, so maturational increases in muscle size occur by displacement of air sacs and perhaps by some compression of the expandable volume of the foregut. Because of

this, maturational and other changes in flight muscle size are difficult or impossible to detect externally.

It has long been assumed that material and energy costs of building and maintaining flight muscles limit reproductive output, and indeed, it is generally true in polymorphic species that flightless morphs reach sexual maturity more rapidly and attain higher fecundity (9–15). This is particularly true for females, which in many taxa display the well-characterized oogenesis-flight syndrome, which involves a seasonal or ontogenetic transition between high flight capability and ovarian development (9). The costs of developing a flight motor are less clear for males, but some evidence exists that male reproduction is also enhanced in flightless morphs that lack or have histolyzed their flight muscles (21, 22).

## EFFECTS OF RELATIVE FLIGHT MUSCLE SIZE ON AERIAL PERFORMANCE

Effects of flight muscle development on aerial performance have frequently been assessed by examining flight endurance, which is typically measured as the number of revolutions accomplished while an insect is tethered to a rotary flight mill (23–28). Flight endurance tends to show a strong positive correlation with relative muscle size and a negative correlation with female ovarian development, although in some species there are seasons when females show high flight capacity even when their ovaries are highly developed (23, 24, 29). More fully developed flight muscles tend to possess higher levels of aerobic enzyme activity, mitochondrial density, tracheation, energy stores, and proteins involved in the translocation of fatty acids from storage depots (30–36). For example, a fatty acid binding protein that is undetectable in migratory locusts at adult emergence becomes the most abundant soluble protein in flight muscle by 10 days of age, comprising 18% of total soluble protein in the sarcoplasm (31). During adult maturation in *Libellula* dragonflies, the fractional cross-sectional area of mitochondria increases from 0.15 to 0.46 (8) over a period when the percentage of time spent flying by free-living dragonflies increases from 2 to 32% (38). These observations indicate that many of the gross changes in composition that occur during flight muscle hypertrophy involve an up-scaling of aerobic metabolic capacity, which allows greater endurance.

In addition to the need to periodically remain airborne for long periods of time, insects frequently need to achieve high levels of aerial performance in order to evade predators, compete for mates, lift loads, or overcome low muscle temperature. Thus it is also important to consider how variation in flight muscle development affects maximal short-burst flight performance. Load-lifting experiments using a wide variety of taxa (7) indicate that insects at warm muscle temperatures achieve a fairly consistent amount of short-burst lift per unit muscle mass (60–80 N/kg). The relationship between lift force and flight muscle mass scales iso-

metrically and is not affected by variation in the relative size or shape of the wings, within the naturally occurring extremes exhibited by flight-capable insects. The minimum amount of flight muscle required for weight support is 12 to 16% of total body mass, and increases in the flight muscle ratio (the ratio of flight muscle mass to total body mass) above the marginal level bring about a linearly increasing ability to lift loads and to accelerate.

Consequences of the relationship between flight muscle ratio (FMR) and aerial performance have been explored by comparing FMRs of butterflies that vary in their susceptibility to aerial predation by birds. Due to variability in the chemical composition of host plants eaten during the caterpillar stage, butterflies vary in a highly dichotomous fashion in the degree to which they are preyed upon by birds (39, 40). This stimulated the prediction that palatable species should be more reliant on short bursts of high-performance flight to evade birds and thus they should have higher FMRs than do unpalatable species. In a comparison of 122 species of neotropical butterflies, FMRs of females of unpalatable, mimetic species averaged 0.24, whereas females of palatable, non-mimetic species averaged 0.35 (41). Unpalatable female butterflies have significantly larger abdomens (42) and ovaries (41), thus demonstrating that unpalatability not only reduces predation but also allows greater reproductive effort. Phylogenetically based statistical analyses show that these associations between FMR and palatability have evolved independently in numerous lineages.

Male butterflies also show differences in mean FMR between unpalatable (0.31) and palatable species (0.42), although less of the variation in male FMR is explained by palatability status (41). This suggests that other aspects of male function, such as aerial pursuit of mates, might affect the evolution of FMR in males. This prediction was tested in a study that compared mean FMRs of a broad taxonomic selection of male butterflies that employ different mating tactics (43). Species in which males fly continuously in search of females showed lower FMRs and different wing shapes than did males that perch and use short-burst flight to rapidly overtake passing females. The importance of burst performance for aerially mating male insects has also been shown by experiments that reduced the FMR of territorial male dragonflies. Small weight loads (6–13 % of body mass) attached to the bodies of territorial males reduced their territorial and mating success (8).

An alternative hypothesis for the observed variability in butterfly body design is that a smaller abdomen places the center of rotation closer to the thorax and wingbase, thereby allowing more rapid body rotation and greater aerial maneuverability (44). According to this hypothesis, differences in FMR are a secondary result of selection acting on the relative size of the abdomen. There is statistical evidence against this (41), as the relative mass of flight muscle among species that differ in palatability and mimicry status varies independently of relative abdomen mass, but not vice versa. This pattern argues for a primary effect of FMR rather than abdomen size. Experiments presently underway (45) are further exploring this issue by attaching weight loads to butterflies at different distances

from the body center of rotation, then releasing the marked butterflies and determining mortality rates in nature. Results of this experiment will show whether the effect of weight alone or both weight and location are important.

A high FMR is also beneficial in ecological contexts that require lifting large external loads. For example, male Empidid flies (Diptera, Empididae) capture small insects and present them to females as “nuptial gifts.” Females permit males to copulate while they consume these prey items, and while doing so they are carried in flight by the male. In order to mate, a male Empidid fly must support his own weight along with that of the female and the prey item. The overall size and FMR of males is positively related to the total load that they can support during aerial copulation (46). Female cicada killer wasps (*Sphex speciosus*) are another example of extraordinary load lifting. Female cicada killers are unusual in that their FMR is as high as that of conspecific males. A relatively high FMR allows them to carry prey that average 88% heavier than their own weight (47). Even so, carrying such heavy prey causes a threefold reduction in FMR compared with the unloaded state, which drops them below the marginal flight muscle ratio. Female cicada killers lack sufficient force production to take off with an average-sized cicada, but they compensate behaviorally by repeatedly climbing trees and descending under power in the direction of their burrows.

In ectothermic insects, variation in the relative size of flight muscles also affects the thermal breadth for flight (i.e. the range of ambient and muscle temperature over which flight is possible). Insects with higher FMRs should be capable of maintaining flight at lower thoracic temperatures, and this appears to be part of the suite of adaptations that allow male winter-flying Geometrid moths (*Operophtera bruceata*) to fly at ambient and thoracic temperatures ranging from approximately 0 to 28°C (48). *Operophtera* females are flightless and their thoracic cavity is filled with eggs rather than muscle. Based on the thermal sensitivity of power output in male flight muscles, *Operophtera* females would require a 17% reduction in egg number to fly over a fairly narrow range of temperatures (13 to 22°C), whereas an 82% reduction in egg number would be required to fly over the broad temperature range accomplished by males.

## CONTROL OF FLIGHT MUSCLE HYPERTROPHY

Hypertrophy of the flight muscles of insects begins during the nymph or pupal stages prior to adult emergence and in some taxa continues during adult maturation. This hypertrophy is controlled by the same hormones that orchestrate the insect molting process, juvenile hormone (JH), and ecdysteroids. The time course of the relative concentration of these hormones regulates muscle and wing development, including morph determination in species that are polymorphic for flight capability (14).

A long-standing hypothesis is that an elevated JH titer at a critical stage of development suppresses morphogenesis of flight muscles and wings. Experi-

mental evaluation of this hypothesis has progressed the farthest in studies of Orthopterans (crickets and locusts). Treatment of final instar nymphs of the monomorphic, flight-capable cricket *Teleogryllus oceanicus* with the JH-analog methoprene slows the 30-fold increase in muscle mass that normally occurs during the first few days of adult life and blocks the growth of mitochondria and tracheoblasts (49). The flight motor neural pattern in *Teleogryllus* crickets is detectable well before the final nymphal instar and therefore might also play a role in stimulating muscle growth and differentiation. Denervated muscles of *T. oceanicus* show a reduced muscle growth rate, but no change in mitochondrial or tracheoblast proliferation. These results indicate that both neural and endocrine factors contribute to flight muscle hypertrophy in *T. oceanicus* but that endocrine factors alone appear to determine ultrastructural differentiation of mitochondria and tracheoblasts. In *Schistocerca gregaria* locusts, methoprene treatment has a curiously different effect on muscle development (50). Methoprene-treated final instar nymphs molt to a supernumerary nymph stage rather than to the adult stage. Thoracic muscles within these supernumerary nymphs show a fairly normal increase in the size and distribution of mitochondria, but little growth of myofibrils, i.e. a pattern that is opposite that produced by JH treatment in *T. oceanicus* crickets. These contrasting effects in two Orthopterans indicate that there is likely to be wide diversity in the details of JH inhibition of muscle hypertrophy among species.

The most detailed studies of endocrine effects on flight muscle development have been performed using the cricket *Gryllus rubens*. In this species, experimental application of JH during the penultimate or ultimate nymph stage redirects the development of a flight-destined morph to a flight-incapable morph (51). Native levels of JH are higher in final instar nymphs of the flight-incapable morph, and newly emerged adults of the flight-capable morph have higher activity of the degradative enzyme, JH esterase (52). Regulation of JH and its degradative enzymes may not be the only endocrine mechanism at work in *G. rubens*, for there are also differences in the level and timing of the ecdysteroid peak during the last stages of nymphal development and early adult maturation (53). Thus morph determination in this species is likely to be regulated by covariation in both JH and ecdysteroid titers (15, 53).

To date there is little understanding of the molecular details of regulatory processes controlling flight muscle hypertrophy, but evidence obtained from other types of insect muscles suggests an important role for ecdysteroids and ecdysteroid receptors. In an abdominal body wall muscle present in pupae of *Manduca sexta*, temporal and spatial patterning of different isoforms of the ecdysone receptor (EcR) match the developmental response of the muscle to changing steroid titers and to the pattern of innervation (54). Only one fiber of this muscle participates in the regrowth of the muscle during the adult stage, and only this fiber shows an upregulation of a particular EcR isoform (EcR-B1). Denervation of the muscle prevents both the upregulation of EcR-B1 and myoblast proliferation. Thus growth of this muscle appears to be regulated by the pattern of expression

of hormone receptor isoforms, rising and falling ecdysteroid titers, and local interactions with nerve cells (54).

## CONTROL OF FLIGHT MUSCLE DEGENERATION

Certain insects are capable of degenerating their flight muscles in response to environmental stimuli that signal a temporary or permanent end to the need to fly. Numerous aspects of the degeneration process indicate that it is not a passive process resulting from muscle disuse, but rather an active process of programmed cell death, triggered by specific environmental and social signals (55). Post-migratory muscle degeneration is blocked by environmental stimuli that signal habitat unsuitability (i.e. food shortage) (9, 56), by antibiotics that inhibit RNA and protein synthesis (56), and by chemicals that inhibit secretion of JH from the corpus allatum (56). Muscle degeneration is stimulated by treatment with a JH analog (55, 57) and by transplantation of brain tissue from reproductive females into pre-reproductive females (58).

One of the species in which the degeneration process has been best characterized is the fire ant, *Solenopsis* spp. (Hymenoptera). After performing a mating flight and initiating a terrestrial search for a colony founding site, fire ant queens shed their wings and undergo flight muscle histolysis. When hemolymph from mated females is injected into virgin females, within 24 h there is a marked breakdown of the flight musculature, but no such breakdown occurs in control females injected with hemolymph from virgin females, or those injected with male seminal fluids or heat-degraded (70°C) hemolymph from mated females (59). Lysis of the flight muscles begins within 2 h after mating, appearing first as lesions in the membranes of the sarcotubular system and mitochondria, followed by breakage and dissolution of the myofilaments, and lastly by the disappearance of sarcomeric Z-lines (60–62). The initial membrane disruption is thought to cause mitochondria and SR to release calcium into the sarcoplasm (62), thereby activating the calcium-dependent proteases that are constitutively present in striated muscle (63). As this is occurring, there is also a sharp rise in ubiquitination of the myofilaments, Z-lines, and mitochondria (64), thus indicating that muscle proteins are tagged for ultimate degradation by the ATP/ubiquitin-dependent proteolytic pathway (65). In fire ants, this process is apparently all-or-none; once triggered, the flight muscles are rapidly and completely degraded.

It is interesting to note that a similar process of muscle degeneration, featuring both calcium-dependent proteases and the ubiquitin pathway, occurs in crustaceans. Lobsters undergo a specific regional atrophy of the distal portion of their claw muscles that allows withdrawal of the large distal portion of the muscle through narrow proximal joints during molting (66). The ubiquitin pathway has also been shown to function during a muscle-specific and hormonally regulated adult-stage atrophy of abdominal intersegmental muscles in *Manduca* moths (67–

69). These muscles are used to free the nascent adult from the pupal exoskeleton during molting and then disappear during early adult life. The process is hormonally regulated by falling ecdysteroid titers, and features selective repression of actin and myosin heavy chain genes (70). Flight muscle histolysis in insects is likely to be derived evolutionarily from these processes that arthropods use to remodel muscle before, during, and after molting.

Flight muscle degeneration is thought to allow a large re-allocation of energy and protein, which females may subsequently use for ovarian development and oocyte provision (14, 15). Direct evidence for this is rare, although protein transfer from degenerating flight muscles to developing oocytes has been demonstrated in *Dystdercus cingulatus* bugs (Heteroptera) (71). Surgical removal of the wings of female *Velarifictorus parvus* crickets (Orthoptera) stimulates flight muscle histolysis and ovarian development (72), and when denied access to food, wing-intact females produce no eggs during the first 5 days after adult emergence, whereas de-alated females under the same conditions break down their flight muscles and produce an average of 23 eggs.

Recent studies of *Gryllus* crickets have shown that simply maintaining the flight muscles has negative impacts on female fecundity (73–75). A flight-capable morph in *G. firmus* has pink-colored thoracic muscles that contain higher lipid and triglyceride energy reserves, three- to sevenfold higher mass-specific metabolic enzyme activities, and a higher resting metabolic rate than white-colored thoracic muscles from a flight-incapable morph. The mass-specific resting metabolic rate of pink-colored flight muscles is ninefold higher than that of ovarian tissue (75). Flight-capable and flight-incapable crickets do not differ in the amount of food consumed or assimilated, but the high maintenance costs and disproportionate energy consumption by their flight muscles causes flight-capable crickets to have lower efficiencies of energy assimilation, which is manifested ultimately as a decrease in fecundity. These patterns of muscle physiology, energetics, and fecundity were first observed in the naturally polymorphic species *G. firmus* and *G. rubens* (73, 74) and subsequently reproduced experimentally by hormonally manipulating the monomorphic species *G. assimilis* by using a JH analog (methoprene) to stimulate flight muscle histolysis and increased ovarian development (75).

## VARIABILITY IN METABOLIC ENZYMES AND THEIR EFFECTS ON FLIGHT

In addition to varying in size, insect flight muscles show functionally important variation in their molecular composition, ultrastructure, and biochemistry. One such category of variability involves the effects of polymorphism at gene loci that encode enzymes which participate in energy metabolism. A detailed review of older literature is available (76), and here I discuss primarily the few recent studies



that have specifically addressed the effects of allozyme variation on flight performance.

One of the main motivations for studying allozymes is to help distinguish between neutrality versus selection as causative factors for the unexpectedly high levels of heterozygosity found in most populations. The predominant approach in this field has been to analyze gene sequence data for patterns of nucleotide diversity indicative of selection, and this approach has been highly successful in providing evidence for natural selection acting on polymorphic loci (77). However, statistical analyses of nucleotide data reveal little about organismal physiology and function. In this regard, studies of the effects of polymorphic loci on insect flight metabolism and performance have the potential to provide particularly clear insights. Flying insects possess the highest known mass-specific metabolic rates, and therefore they may be particularly sensitive to the effects of allozymes on metabolic performance.

A prominent set of studies in this area has been the work of Watt and colleagues (78–85), who examined the impact of different phosphoglucose isomerase (PGI) allozymes on *Colias* butterflies. Purified PGI allozymes differ in reaction kinetics and thermostability, and genotypes differ in the flux rate of radiolabeled carbon in muscle. Field studies show temperature-dependent variation among genotypes in survivorship, flight activity, male mating success, and female fecundity. These results have been interpreted as an indication that kinetic differences among PGI allozymes affect glycolytic metabolite flux, thereby limiting ATP production rate and ultimately the power output of the flight muscles. The specific effects of PGI genotype on flight physiology remain to be determined, as there are no data showing genotype or genotype  $\times$  temperature effects on muscle contraction, wingbeat kinematics, energetics, or other such measures of flight motor function.

An interesting recent development in the biology of PGI polymorphism in insects comes from a study of two species of crickets (*Gryllus veletis*, *G. pennsylvanicus*) that each show clinal variation in PGI allozyme frequency (86). Each species possesses six distinct PGI bands on starch electrophoresis gels; however, nucleotide sequence data show that amino acid substitutions have occurred at different sites in the two species, thus indicating independent evolution of a similar level of allozyme diversity. Moreover, the variable sites in both species have undergone predominately radical, as opposed to conservative, amino acid substitutions (i.e. substitutions that change the size, charge, or hydrophobicity at a site). The authors of the study suggest that there may be balancing selection occurring on gross physical characteristics such as overall charge of the PGI molecule. Rapid accumulation of radical amino acid substitutions in a metabolic enzyme is an unusual observation and warrants an examination of physiological effects.

In *Drosophila melanogaster* (Diptera), there is clinal and seasonal variation in alleles at the glycerol-3-phosphate dehydrogenase (GPDH) locus that has been shown to have subtle effects on flight performance. GPDH enzyme activity is highest for the SS genotype, intermediate for FS, and lowest for FF (87). The SS

genotype shows an approximately 2 to 4% greater aerodynamic power output during tethered flight than the FF genotype among flies raised at 15°C and flown at 15°C, whereas the reverse is true for flies raised at 30°C and flown at 30°C. These temperature effects on power output are consistent with the geographical and seasonal variation observed at the GPDH locus in nature. Larval development rate also varies subtly among GPDH genotypes (88), and here again the S allele slightly outperforms the F allele at cooler temperatures, in a manner consistent with clinal variation in allele frequency.

In the moth *Epiphyas postvittana* (Lepidoptera), polymorphism at the phosphoglucomutase (PGM) locus is associated with significant variation in tethered flight duration (89). Artificial selection on flight capacity resulted in significant genotypic differentiation.

Polymorphism at the malate dehydrogenase allele (MDH-1) in honeybees (*Apis mellifera*, Hymenoptera) shows clinal variation on three continents and has been shown to affect the metabolic rate of honeybees during free flight (90, 91). A 20% difference between FF and SS MDH-1 genotypes was found in the original study (90), but since each genotype came from a different colony, it could not be determined what portion of this variation was attributable to genotypic as opposed to colony-level effects. A subsequent study (91) solved this problem by using sister queens (MF heterozygotes) to establish two adjacently located colonies. Both queens were artificially inseminated with sperm from a single S genotype drone (male Hymenoptera are haploid). This established replicate colonies of highly related worker bees having either the SM or the SF MDH-1 genotype. Flight metabolic rates of SF genotype worker bees significantly exceeded those of SM genotype bees by an average of 3%, with a significant colony effect of about the same magnitude. This effect of malate dehydrogenase genotype on honeybee flight metabolism is puzzling because the malate-aspartate shuttle, which is the main cytosol-to-mitochondria redox shuttle in mammalian muscles, is not known to operate in insect flight muscles. There may be some as-yet undetected activity of this shuttle in bee flight muscle, or perhaps the known gluconeogenic role of the malate-aspartate shuttle in the fat body affects the rate of metabolite supply to the working muscles during prolonged flights (91).

It was recently shown that a flight muscle-specific isoform of GPDH in *Drosophila* is located at specific locations on the sarcomere (the Z-lines and M-line), and imposes spatial organization on the two enzymes (GAPDH and aldolase) that catalyze adjacent reactions in glycolysis (92). The flight muscle-specific isoform contains three C-terminal amino acids not present in other GPDH isoforms. A mutant line that expresses a non-muscle isoform in flight muscle shows no spatial organization of GPDH or adjacent glycolytic enzymes, and the flies are flightless. This finding indicates that spatial organization of glycolytic enzymes has powerful functional consequences for insect flight muscles. No studies have yet examined the effects of naturally occurring allozyme variation on spatial organization of glycolytic enzymes in flight muscle or the age- or morph-related differences in GPDH isoform expression within individuals, but this may be an important source

of functional differences. Furthermore, these results indicate that catalytic properties measured from purified enzymes or tissue homogenates may not always be an accurate indication of catalytic properties that exist in the spatially defined setting of the intact flight muscle sarcomere, thus reiterating the need for detailed *in vivo* studies.

## VARIABILITY IN FLIGHT MUSCLE CONTRACTION

Insect flight muscles operate over a broad and unusually high range of contraction frequencies, from a low of about 5 Hz, up to values in excess of 500 Hz. Individual insects vary their muscle power output by changing both the frequency and amplitude of muscle contraction, and we have some knowledge of how variability in the composition of insect flight muscles affects these processes. As a starting point to understanding variability in the contractile function of insect flight muscles, it is instructive to consider how vertebrate striated muscles adjust to different contraction frequencies. A general trend in vertebrates is that striated muscles operating at higher contraction frequencies have faster shortening velocities and higher myosin ATPase rates (93, 94). Vertebrate muscles adjust to changes in contractile regimes that arise from growth, training, or environmental temperature variation (ectotherms) by qualitative and quantitative changes in expression of myosin heavy chain genes (95, 96), thereby changing the composition and contractile characteristics of their myosin cross bridges.

Insects also vary myosin expression, but not within their flight muscles. Insects use alternative splicing to generate myosin heavy chain isoforms in an age- and tissue-specific manner (97–99). These different myosins are likely to have functional differences, but flight muscles appear to express only one isoform. Furthermore, insect flight muscles show fairly uniform unloaded shortening velocities ( $V_{\max}$ ) across species that vary widely in wingbeat frequency.  $V_{\max}$  measurements from katydids (100), sphinx moths (48), and dragonflies (101) show values ranging from 10 to 16 muscle lengths per s, with no correlation between  $V_{\max}$  and contraction frequency (range = 20–200 Hz). Tetanic tension per muscle cross-sectional area also shows little variation among species (generally about 10–13 N/cm<sup>2</sup>). Thus the common vertebrate mechanism of adjusting myosin heavy chain composition and cross-bridge cycling rates in order to accommodate different contraction frequencies does not appear to be used by insect flight muscles.

Rather than adapt to different contractile regimes by varying the nature of the molecular motor itself, insect flight muscles vary the regulatory processes that turn muscle contraction on and off. The most radical such change is that between synchronous and asynchronous muscle activation. Synchronous muscles have a 1:1 relationship between neural stimuli and contractions, with contraction initiated by intracellular calcium release and terminated by calcium uptake by the SR. This is the typical regulatory mechanism for striated muscle. Asynchronous muscles

are divergent; they show an approximately 1:10 ratio of neural stimuli to contractions. Neural stimulation in asynchronous muscles releases intracellular calcium that removes thin filament inhibition, but the cross bridges themselves are activated by stretch and deactivated by sarcomere shortening. Asynchronous flight muscles are stretched by thoracic deformation caused by contraction of antagonistic muscles, and this mechanical feedback keeps asynchronous muscles contracting over many cycles (1, 2). The large power-producing asynchronous muscles are controlled by a set of small synchronous muscles that produce little power (some in fact absorb power) but are capable of rapid and finely graded responses to neural stimuli (4, 102). This dichotomy of muscle size and function has led to the colorful characterization of “big dumb power-producing muscles” versus “small smart steering muscles.”

In synchronous insect flight muscles, as well as in the synchronous sound-producing tymbal muscles of cicadas (Homoptera), there is a strong positive relationship between the relative density of SR and twitch contraction kinetics and operating frequency of the muscles (103). Similarly, there is an inverse relationship between the diameter of myofibrils and operating frequency. Having a higher density of SR and more narrow myofibrils reduces diffusion distances and increases calcium pumping capacity, which presumably results in more rapid calcium cycling within the sarcoplasm, and thus shorter response times for both activation and deactivation of the cross bridges. The drawback of adapting synchronous muscles to function at high frequencies is that elaboration of SR can occur only at the cost of reductions in the relative volume of the force-generating myofibrils, and/or the energy-supplying mitochondria. An extreme example of this tradeoff is a sound-producing muscle in the cicada, *Okanagana vanduzeei*, which has the highest known contraction frequency of any synchronous muscle (500 Hz). SR comprises nearly a third of this muscle's cross-sectional area, whereas myofibrils constitute only 22% (104). Flight muscles are more constrained than sound-producing muscles because they have an absolute requirement to generate sufficient force to counteract body weight, and there are no such extreme examples of ultrastructural specializations in synchronous insect flight muscles. The highest contraction frequencies of any synchronous flight muscles are probably those of sphingid moths, of which the smallest species have wingbeat frequencies in the range of 50–100 Hz (105).

The relationship described above between ultrastructure and contraction kinetics also occurs between muscles that perform different functions within an individual insect (100, 106). In a katydid that uses its metathoracic muscles strictly for flying (20 Hz) and its mesothoracic muscles for sound production (200 Hz) in addition to flying, the metathoracic muscles have a much briefer twitch duration (6–8 versus 12–15 ms from onset to 50% relaxation), narrower myofibrils, and a smaller ratio of myofibril volume to SR volume. One curious result is that the increase in SR and reduction of myofibrillar diameter, thought to allow faster twitch kinetics, develop prior to the attainment of faster twitch kinetics, thus suggesting that additional factors with a different developmental time course must

also be affecting twitch kinetics. Densities and/or activities of  $\text{Ca}^{2+}$  ATPase pumps and  $\text{Ca}^{2+}$  gates in the SR are likely candidates, but this possibility remains unexplored.

In addition to ultrastructural changes that affect the rate of calcium diffusion, recent studies indicate that variability in calcium regulatory processes also affects cross-bridge recruitment and force production. In flight muscles of the dragonfly *Libellula pulchella*, the mixture of isoforms of the alternatively spliced calcium regulatory protein troponin-t changes during adult maturation, with correlated changes in calcium sensitivity of muscle activation and twitch force (101). The same muscles show no maturational changes in  $V_{\max}$  or tetanic tension, thus indicating that variation in twitch tension is the result of changes in the way the muscle responds to a transient pulse of calcium rather than a difference in cross-bridge kinetics. The mechanisms by which increased calcium sensitivity affect twitch force remain to be determined, although it is quite likely that higher calcium sensitivity increases the probability or duration of activation of any given cross-bridge binding site during a single calcium transient, particularly at myofibril locations that are relatively distant from the SR membrane. A spatially explicit model for calcium movement and troponin binding during single SR release events in frog muscle (107) indicates that there is spatial variation in the rate and extent of force development and that differences in calcium sensitivity of troponin units can alter these effects.

It is interesting to consider why Libellulid dragonflies, which have the highest known investment in flight muscle (FMR reaches as high as 0.63 at maturity), have a mechanism that allows them to modulate the force and power production of those muscles. Mark-recapture studies of newly emerged free-living *Libellula* dragonflies indicate that a large proportion of individuals fail to gain mass and apparently starve during the first few days of adult maturation (108). It has been suggested that power output, and therefore energy consumption during flight, is downregulated during the early adult stage when the only function of flight in these dragonflies is for capturing small, non-evasive prey. Muscle power output and energy consumption are then upregulated at maturity when high-performance flight is used during intense aerial competition for mating territories. Interestingly, the protein whose isoform expression appears to mediate this functional switch, troponin-t, has recently been suggested to perform a similar role in modulating power output and energy consumption in mammalian heart muscle during severe stress and impending failure (109). Thus troponin-t isoform variation may be generally involved in modulating performance versus economy in striated muscles.

In addition to regulation by troponin-tropomyosin, contraction of insect flight muscles is also regulated, or at least modulated, by phosphorylation of myosin light chains (MLCs). In *D. melanogaster* flight muscles, myosin light chain kinase (MLCK) and other phosphorylases appear to become active during the first few hours following adult emergence, since only dephosphorylated MLC is present in late pupae, and phosphorylated MLC accumulates in the hours following adult

emergence (110, 111). MLC phosphorylation increases the ATPase activity of purified *D. melanogaster* myosin (110, 112). These observations, along with the similar time course of MLC phosphorylation and flight acquisition in newly emerged adults, suggest that MLC phosphorylation upregulates muscle activation.

Genetic manipulations of *D. melanogaster* have been used to characterize the in vivo functional effects of variability in MLC phosphorylation (113, 114). Flightless heterozygotes of homozygous-lethal MLC null mutants have been rescued to normal muscle ultrastructure and flight ability by P-element transformation with the wild-type allele. Site-directed mutagenesis was subsequently used to create cDNA constructs in which two serine residues, the sites of MLC2 phosphorylation by MLCK, were replaced by unphosphorylatable alanines. These constructs were transposed into MLC2 null mutants, resulting in lines of flies in which the only full-length, functional copy of MLC2 lacked either one or both of the sites that can be phosphorylated by MLCK. The resulting flies were examined for flight muscle ultrastructure, skinned fiber mechanical characteristics, and aerodynamic power output and metabolic power input during tethered flight (114). Muscles from the flies transformed with MLC2 lacking one or both MLCK phosphorylation sites showed no apparent changes in myofibrillar ultrastructure during rest, maximal activation, or rigor, nor did they show significantly altered calcium sensitivity, cross-bridge kinetics, or maximum steady state isometric tension. Mutant muscles did show mechanical features indicative of a reduced recruitment of force-producing cross bridges during stretch activation. Mechanical power output of mutant lines during tethered flight was reduced by 19 to 28% compared with wild-type transformants, along with a similar decrease in metabolic power input, with no change in efficiency. Mutant flies could generally produce sufficient vertical net aerodynamic force to support their body weight, but significantly less than the 1.35 force/weight ratio produced by wild-type rescued and unmanipulated control flies.

These results demonstrate that phosphorylation of MLC2 has a modulatory effect on stretch-activated flight muscle force and power production in *Drosophila*, although the significance of the naturally occurring changes in phosphorylation that occur during maturation remains to be determined. One possibility is that the delay in MLC2 phosphorylation during the first few hours following adult emergence allows the thoracic exoskeleton and wings to harden prior to full force and power production. This would be analogous to the neural suppression of muscle tension that prevents damage to the legs of grasshoppers during the period following molting, when the cuticle is not yet structurally mature (115).

Flightin, a novel protein found to date only in insect flight muscle, also shows a change in phosphorylation during adult maturation in *Drosophila* (116). Flies that are heterozygous for a chromosomal mutation that deletes the flightin gene are flightless, and their isolated muscles show patterns of stretch activation that suggest altered cross-bridge kinetics (117). How phosphorylation of flightin affects cross bridges and the functional significance of maturational changes in flightin phosphorylation have yet to be determined.

Different strains of *D. melanogaster* are polymorphic for a flight muscle-specific form of tropomyosin (the misnamed troponin-H), but different forms of this protein show no qualitative effects on flight performance (118). Like many other studies examining *Drosophila* flight ability, this study used a fairly crude and qualitative performance assay (94–99.5% of the flies are reported to have gone “up”), so subtle differences would not have been revealed. Recent development of methods for obtaining quantitative assays of *Drosophila* performance during tethered (114, 119) and free flight (120) will allow in future studies a more sensitive resolution of in vivo consequences of variability in motor performance.

A large number of studies have examined the effects on flight and/or muscle contractile function of mutant muscle contractile proteins or altered tissue specificity of protein isoform expression in *Drosophila*. Because these alterations have no known role in natural variation, I forgo reviewing them here.

## SUMMARY

Like other forms of striated muscle, insect flight muscles are highly labile. In order to provide sufficient aerodynamic power output for weight-supported flight, insect flight muscles must be very large and metabolically active, and thus they constitute a considerable expense to synthesize and maintain. Materials and energy consumed by flight muscle growth and maintenance have a large negative impact on reproductive output. However, in ecological settings where flight is important, relatively larger flight muscles are clearly beneficial. Thus relative size of the flight musculature presumably evolves to a level that balances selective forces favoring different and often conflicting aspects of organismal performance. Because the balance of selective forces shifts in different habitats, at different life stages, and in specific ecological and social contexts, many insects have evolved the ability to rapidly build up, break down, or alter the composition of their flight muscles.

Our current understanding of the physiological details underlying these processes are, in general, fairly rudimentary. Endocrine studies have revealed the gross patterns of hormonal regulation of flight muscle size and composition, but the molecular details are poorly known. Metabolic biochemistry in insects shows ample intraspecific variation, but there is little understanding of how allozymes affect specific metabolic pathways or whole-organism performance. Ultrastructural variation that affects twitch kinetics and contraction frequency in synchronous flight muscles is well documented, but recent studies show that contractile mechanics are also strongly affected by molecular variation in regulatory proteins associated with both the thin and thick filaments. Naturally occurring molecular variation affecting contractile regulation has been examined in only two taxa, so it is impossible to make generalizations regarding the prevalence of these mechanisms. The emergence of *Drosophila* as a genetically malleable model organism has allowed powerful manipulative experiments, but the predominant focus has

been on determining the effects of experimentally induced mutations rather than naturally occurring variation. Variability in insect flight muscle size and function has been much more extensively studied by researchers interested in ecology, evolution, and biomechanics than by comparative physiologists whose main interests lie in determining the mechanistic bases and root causes of naturally occurring variability. It is abundantly clear that insect flight physiology constitutes a relatively untapped source for future exploration and discovery by comparative physiologists, and a fertile ground for integrative studies that combine mechanistic approaches with ecology, evolution, and behavior.

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