To Sing or To Fly: Role of Muscle Proteins in *Drosophila* Mating and Flight Behaviors

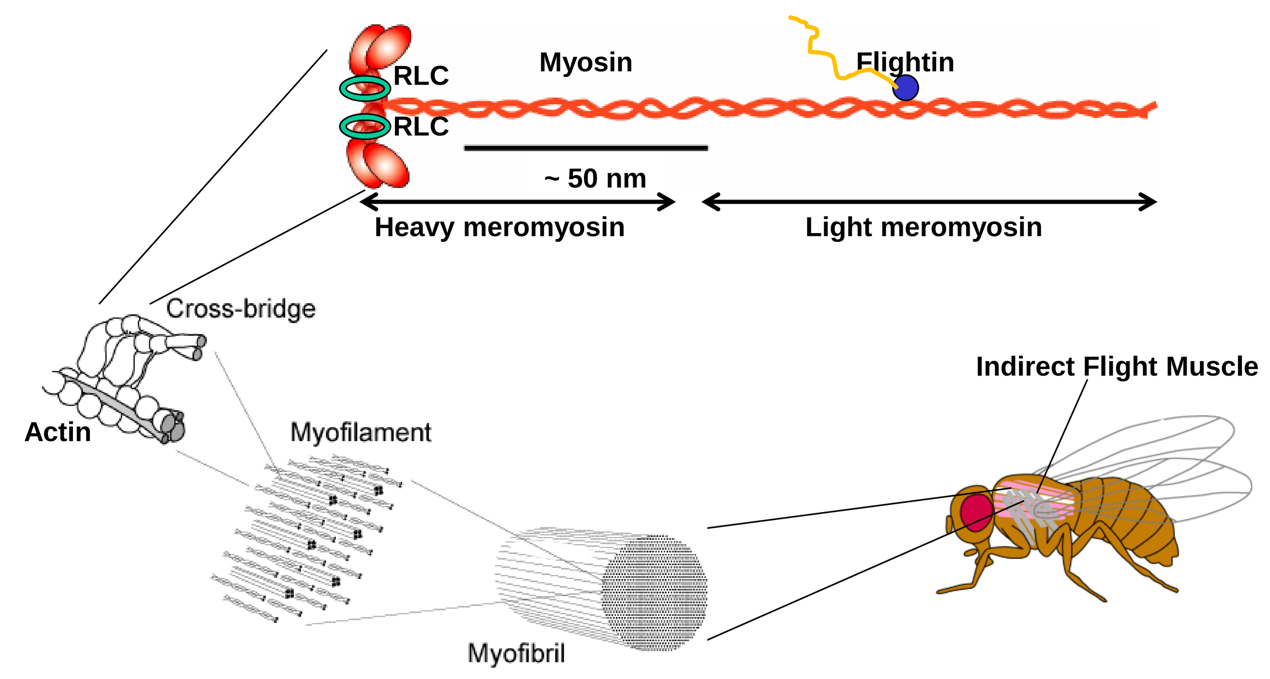
Category: Health and Biological Sciences

Samya Chakravorty, PhD Candidate

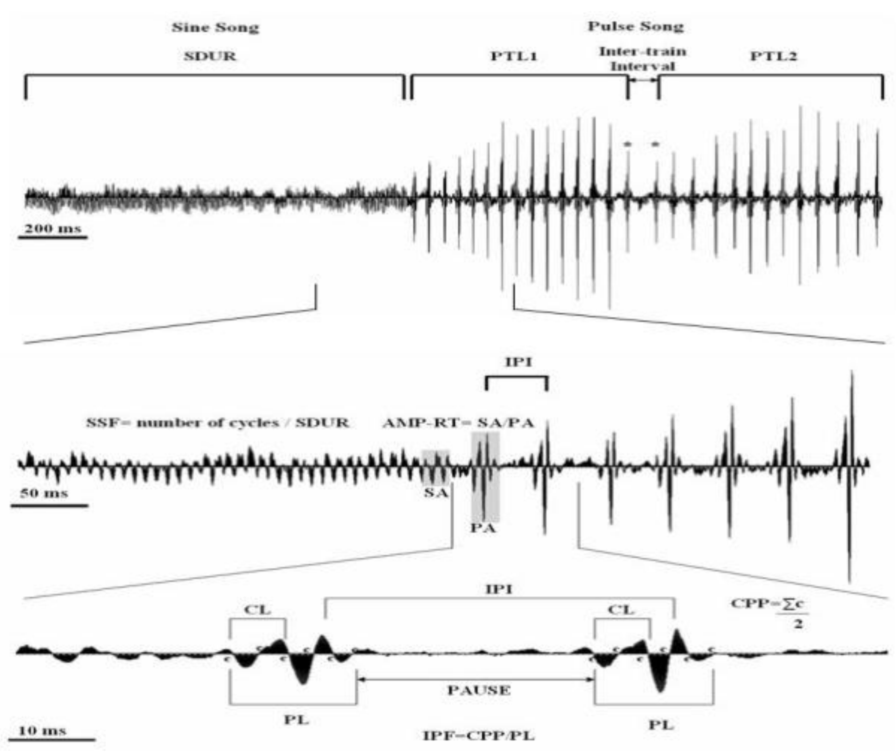
Advisor: Jim O. Vigoreaux, Department of Biology, College of Arts and Sciences

Background

- Indirect flight muscles (IFM) deform the thorax by oscillatory contractions to drive wing motions for flight.
- *Drosophila* males produce species-specific courtship song through wing vibrations, a trait under sexual selection. Electrical recording of *D. melanogaster* IFM, the major power producing muscles for flight, revealed that these muscles are neurally activated during the courtship song.
- Absence of flightin (*fln⁰*), an orphan exclusive to IFM, results in flightlessness and abnormally compliant IFM fibers unable to produce or withstand contractile forces.
- Myosin regulatory light chain (RLC), a conserved myosin-associated protein from worms to humans, is essential for fast wing beat required for flight power. (Miller et al. 2011 Biophys J)
- Male fln^0 flies do not produce courtship song [unpublished data].



Drosophila Male Courtship Song



Chakravorty S et al. 2012

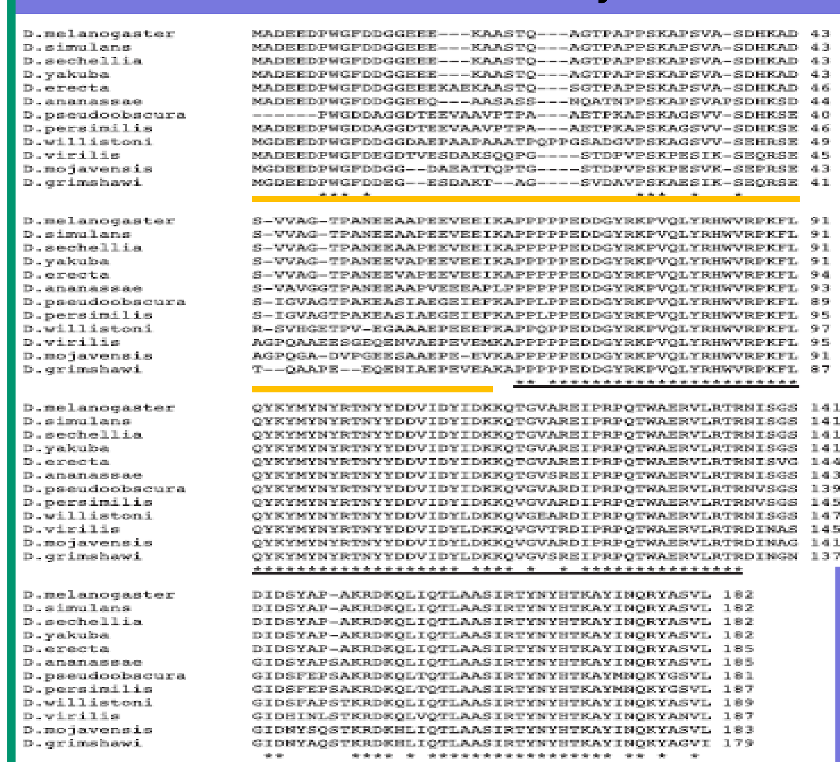
Project Description

How complex behaviors of organisms to survive and reproduce arise from the neuronal sensory perceptions, decision making, to motor neuronal activation and finally muscle action output, is a fundamental biological question. Here we are interested in the receiving end i.e., the role of the muscle proteins. The model organism fruit fly (*Drosophila melanogaster*) uses their flight muscles for fast wing beats of flight to survive and the males produce subtle wing vibrations for sexually selected species-specific courtship singing to attract females. Utilizing varied techniques like creating muscle protein mutations using *Drosophila* genetics, electron microscopy structural study, muscle fiber mechanics, and quantitative behavioral analysis, we unraveled that the flight muscle potentially uses distinct proteins or protein domains for flying and singing purposes, subject to evolutionary natural and sexual selections respectively. This finding brings a step closer to understanding the genetic and physiological basis of muscle-driven complex behaviors in general.

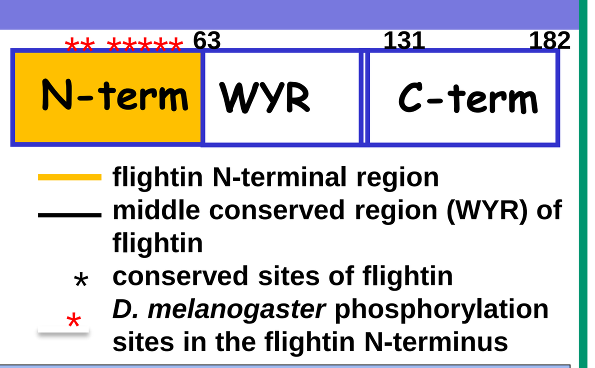
Background

Characteristics of flightin N-terminal region

- Low sequence conservation (~15% identity, compared to >70% identity in the rest of protein) among 12 *Drosophila* species covering about 42 million years of evolutionary time.
- Cluster of 7 phosphorylation sites identified in *D. melanogaster* by LC-MSMS.
- Predicted to be an intrinsically disordered region.



Modified from Tanner et al. 2011



Questions:
- What is the role of flightin N-terminal region in IFM structure and flight mechanics?
- Does the N-terminal region influence courtship song parameters that are under sexual selection?

Results

N-terminal truncated flightin rescues flightlessness and wing beat frequency of fln^0, but impairs sexually selected courtship song parameter, notably Interpulse Interval (IPI) important for female mate choice

Flight Properties

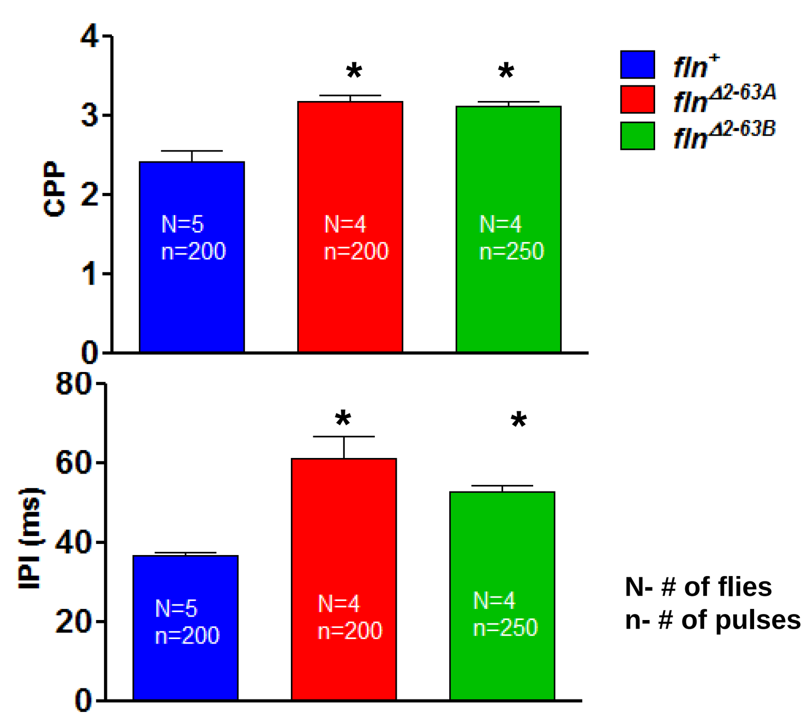
Line	Flight Index (0-6)	Wingbeat Frequency (Hz)
fln^+ (control)	4.2 ± 0.36 (35)	198 ± 2 (25)
$fln^{\Delta 2-63A}$	2.64 ± 0.32 (31) *	198 ± 4 (20)
$fln^{\Delta 2-63B}$	3.12 ± 0.34 (32) *	191 ± 4 (25)

Flight Index : "6" means vertical upward flight towards light source and "0" means no flight, i.e., the fly falls to the ground.

All values mean ± SE. Numbers in parenthesis indicate # of flies tested; * p<0.05 vs fln^+

fln^0 flies have a flight score of 0 and no wingbeat frequency

Courtship Song Properties

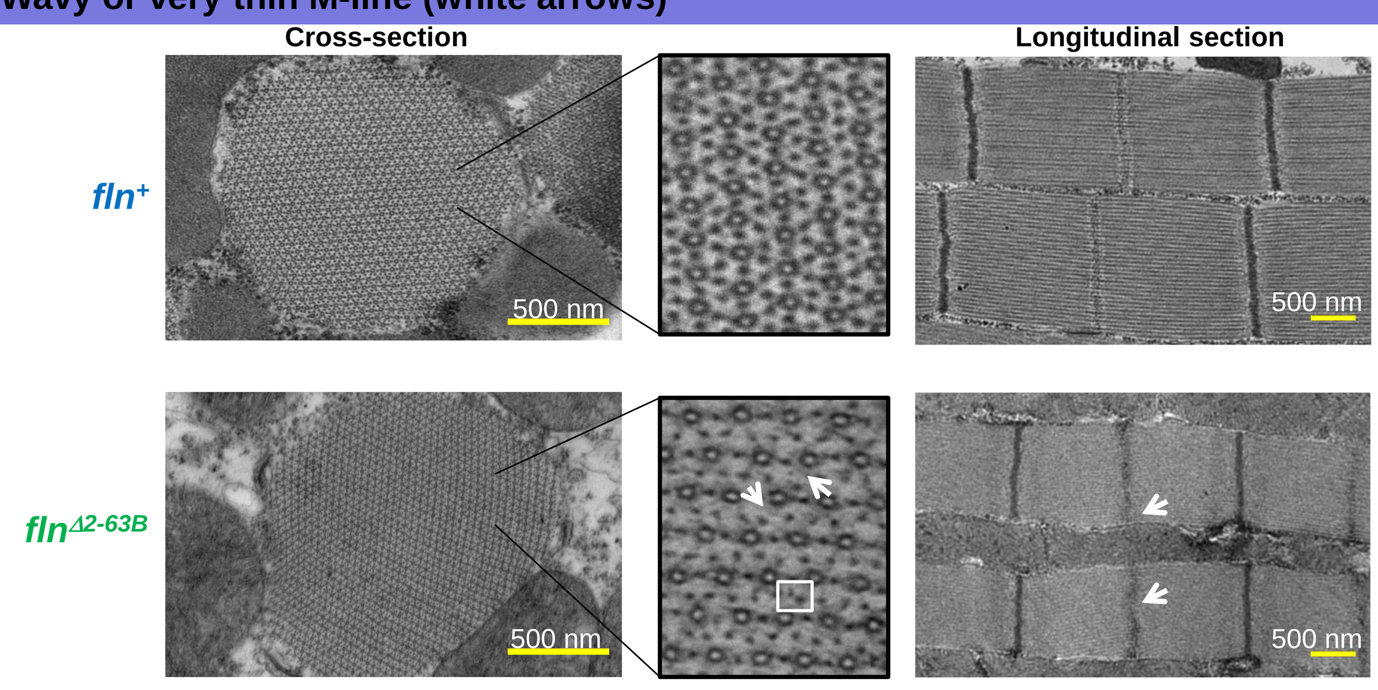


Courtship song parameters:
CPP- Cycles per Pulse
IPI- Interpulse Interval
* p<0.05 vs fln^+

Results

IFM Ultrastructure by Transmission Electron Microscopy

N-terminal truncation of flightin ($fln^{\Delta 2-63}$) results in subtle alterations in sarcomere structure:
- Shorter sarcomeres
- Irregularities in hexagonal lattice evidenced as uneven spacing of thin filaments (white box and arrows in middle panel)
- Wavy or very thin M-line (white arrows)



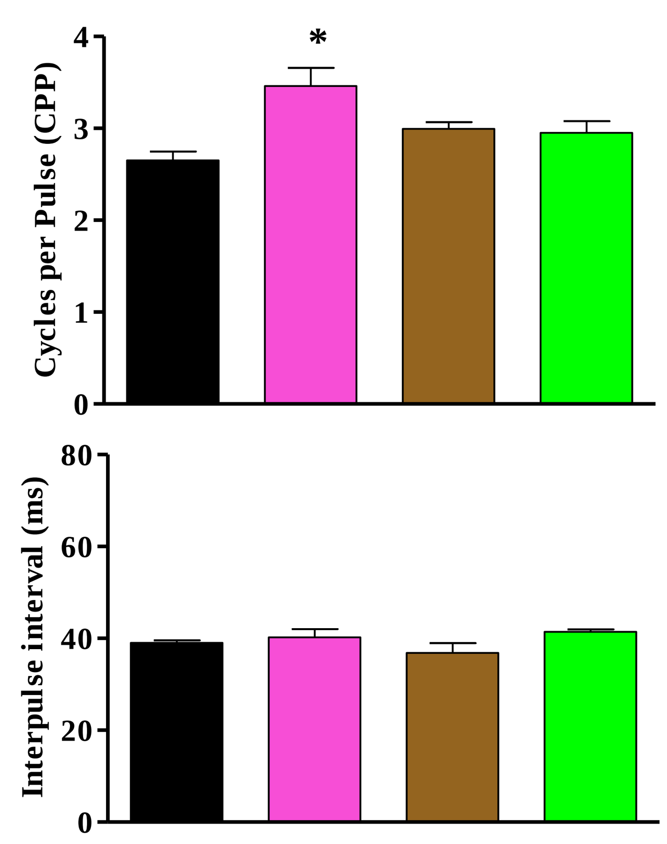
Line	Sarcomere Length (μm)
fln^+	3.30±0.09 (n=316, N=2)
$fln^{\Delta 2-63A}$	2.68±0.01 * (n=213, N=2)
$fln^{\Delta 2-63B}$	2.80±0.005 * (n=658, N=2)

n- # of sarcomeres; N- # of fly thoraces; * p<0.05 vs fln^+

RLC mutation affecting flight ability does not have similar effect on song

D*mlc2*⁺ (control)	5 ± 0.1 (60)	202 ± 3 (52)	Miller et al (2011) Biophysical Journal
$Dmlc2^{\Delta 2-46}$	4.6 ± 0.2 (60)*	165 ± 2 (44)*	*ibid*
$Dmlc2^{S66A,S67A}$	0.1 ± 0.1 (53)*	158 ± 3 (11)*	*ibid*
$Dmlc2^{\Delta 2-46; S66A,S67A}$	0 (53)*	0 (30)*	*ibid*

All values mean±SE. Numbers in parenthesis indicate # of flies tested; * indicate significant difference from control



Summary/Discussion:

- Flightin N-terminus does not affect wing beat frequency for flight, but is required to maintain the behaviorally critical proper timing of pulses (IPI) of the song structure.
- None of the RLC mutations affecting flight ability and wing beat frequency, dictates IPI as well as any other song component.
- IFM potentially uses distinct muscle proteins or protein domains to fulfill its versatile functions of high power flight subject to natural selection pressure, and subtle vibrations for courtship song subject to sexual selection.

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