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

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#### **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.

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D.simulans	MADEEDPWGFDDGGEEEKAASTQAGTFAPPSKAPSVA-SDHKAD 4 MADEEDPWGFDDGGEEEKAASTQAGTFAPPSKAPSVA-SDHKAD 4	43	
D.sechellia	MADEEDPHGFDDGGEEEKAASTOAGTPAPPSKAPSVA-SDHKAD 4	43	
D. vakuba	MADEEDPWGFDDGGEEEKAASTOAGTPAPPSKAPSVA-SDHKAD 4	43	
D.erecta	MADEEDPWGFDDGGEEEKAEKAASTOSGTPAPPSKAPSVA-SDHKAD 4	4.6	
D.ananassae	MADEEDPWGFDDGGEECAASASSNOATNPPSKAPSVAPSDHKSD 4	44	
D.pseudoobscura	PWGDDAGGDTEEVAAVPTPAAETPKAPSKAGSVV-SDHKSE 4	40	
D.persimilis	MADEEDPWGDDAGGDTEEVAAVPTPAAETPKAPSKAGSVV-SDHKSE 4	46	
D.willistoni	MGDEEDPWGFDDGGDAEPAAPAAATPOPPGSADGVPSKAGSVV-SEHRSE 4	49	
D.virilis	MADEEDPWGFDEGDTVESDAKSOOPGSTDPVPSKPESIK-SEORSE 4	45	flightin N terminal region
D.mojavensis	MGDEEDPWGFDDGGDAEATTOPTGSTDPVPSKPESVK-SEPRSE 4	4.3	Ingritin N-terminal region
D.grimshawi	MGDEEDPWGFDDEGESDARTAGSVDAVPSKAESIK-SEQRSE 4	41	
			middle conserved region (V
D.melanogaster	S-VVAG-TPANEEAAPEEVEEIKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	91	flightin
D.simulans	S-VVAG-TPANEEAAPEEVEEIKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	91	ingitin
D.sechellia	S-VVAG-TPANEEAAPEEVEEIKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	91	
D.yakuba	S-VVAG-TPANEEVAPEEVEEIKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	91	* conserved sites of fildhtin
D.erecta	S-VVAG-TPANEEVAPEEVEEIKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	94	
D.ananassae	S-VAVGGTPANEEAAPVEEEAPLPPPPPPEDDGYRKPVQLYRHWVRPKFL 9	93	D melanogaster phosphory
D.pseudoobscura	S-IGVAGTPAKEASIAEGEIEFKAPPLPPEDDGYRKPVQLYRHWVRPKFL 8	89	
D.persimilis	S-IGVAGTPAKEASIAEGEIEFKAPPLPPEDDGYRKPVQLYRHWVRPKFL 9	95	aitaa in tha flightin N tagmain
D.willistoni	R-SVHGETPV-EGAAAEPEEEFKAPPQPPEDDGYRKPVQLYRHWVRPKFL 9	97	sites in the flightin N-termin
D.virilis	AGPQAAEESGEQENVAEPEVEMKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	95	5
D.mojavensis	AGPOGA-DVPGEESAAEPE-EVKAPPPPPEDDGYRKPVQLYRHWVRPKFL 9	91	NDwo LELN
D.grimshawi	TQAAPEEQENIAEPEVEAKAPPPPPEDDGYRKPVQLYRHWVRPKFL 8	87	7 Dilett EN
			1.0
D.melanogaster	QYKYMYNYRTNYYDDVIDYIDKKQTGVAREIPRPQTWAERVLRTRNISGS 1	141	
D.simulans	QYKYMYNYRTNYYDDVIDYIDKKQTGVAREIPRPQTWAERVLRTRNISGS 1	141	
D.sechellia	QYKYMYNYRTNYYDDVIDYIDKKQTGVAREIPRPQTWAERVLRTRNISGS 1	141	
D.yakuba	QYKYMYNYRTNYYDDVIDYIDKKQTGVAREIPRPQTWAERVLRTRNISGS 1	141	
D.erecta	QYKYMYNYKTNYYDDVIDYIDKKQTGVAREIPRPQTWAERVLKYKNISVG I	144	
D.ananassae	QINIMINIKINI IDDVIDI IDAKQIGVSREI PRPQIMAERVLAI KNISGS I	143	
D.pseudoobscura	QYKYMYNYRTNYYDDVIDYIDKKQVGVARDIPRPQTWAERVLETRNVSGS I	139	
D.persimilis	QIAIMINIKINI IDDVIDI IDAAQAGAADIPAPQIWAEKVLAIKAVSOS I	145	
D.WIIIIstoni	QINIMINIKINI IDDVIDILDAKQVGEARDIPRPQIMAEKVLKIRNISGS I	147	
D. VIFILIS	QIKININIKINI IDDVIDILDKKOVGVINDIPHPOTWARKVLKIKDINAS I	145	0.0
D.Bojavensis	QINIMINIKINI IDDVIDILDAKQVGVAKDIPKPQIWAEKVLKIKDINAG I	132	
p.grimshawi	QINININININIDOVIDILDANOVGVSREIPROTWARKVLRINGN I	137	Residu
		1	
D.melapogaster	DIDSYAP-AKEDKOLIOTLAASIRTYNYHTKAYINORYASVI, 182		Questions
D.simulans	DIDSYAP-AKRDKOLIOTLAASIRTYNYHTKAYINORYASVL 182		
D.sechellia	DIDSYAP-AKEDKOLIOTLAASIRTYNYHTKAYINORYASVL 182	1	
D.yakuba	DIDSYAP-AKRDKOLICTLAASIRTYNYHTKAYINORYASVL 182	- 1	N/hat is the rale of flightin N tory
D.erecta	DIDSYAP-AKRDKOLIOTLAASIRTYNYHTKAYINORYASVL 185		<b>W</b> what is the role of muntin in-terr
D.ananassae	GIDSYAPSAKRDKOLIOTLAASIRTYNYHTKAYINORYASVL 185		· · · · · · · · · · · · · · · · · · ·
D.pseudoobscura	GIDSFEPSAKRDKOLTOTLAASIRTYNYHTKAYNNOKYGSVL 181		realized in ICM structure and
D.persimilis	GIDSFEPSAKRDKOLTOTLAASIRTYNYHTKAYMNOKYGSVL 187		region in IFIVI structure and "
D.willistoni	GIDSFAPSTKRDKOLIOTLAASIRTYNYHTKAYINOKYASVL 189	- 17	
D.virilis	GIDEINLSTKRDROLVOTLAASIRTYNYHTKAYINOKYANVL 187	1	
D.mojavensis	GIDNYSOSTKRDKHLIOTLAASIRTYNYHTKAYINOKYASVL 183		mechanics?
D.grimshawi	GIDNYAOSTKRDKHLIOTLAASIRTYNYHTKAYINOKYAGVI 179		
-	** **** * *********************		
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moune			countship song parameters that

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	N-term	WYR	C-term	
	flightin middle flightin * conser <u>*</u> <i>D. mela</i> sites in	N-terminal r conserved r ved sites of anogaster ph the flightin	region region (WYR) of flightin losphorylation N-terminus	F
>Dme1FLN 1.0 0.8 0.6 0.4 0.2 0.2 0.0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			
uestio What gion echan	ons: is the role of in IFM states	of flightin tructure	N-terminal and flight	

ence are under sexual selection?

# Results

N-terminal truncated flightin rescues flightlessness and wing beat frequency of *fln*<sup>0</sup>, 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)	
<i>fIn</i> ⁺ (control )	4.2 ± 0.36 (35)	198 ± 2 (25)	
fln <sup>∆2-63A</sup>	2.64 ± 0.32 (31) *	198 ± 4 (20)	
fln <sup>42-63B</sup>	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  $\pm$  SE. Numbers in parenthesis indicate # of flies tested; \* p<0.05 vs fln+

fln<sup>0</sup> flies have a flight score of 0 and no wingbeat frequency





## 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)

**Cross-section** Longitudinal section

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

Dmlc2+ (control)	5 ± 0.1 (60)	202 ± 3 (52)	Miller et al (2011) Biophysical Journal
<b>D</b> $mlc2^{\Delta 2-46}$	4.6 ± 0.2 (60)*	165 ± 2 (44)*	ibid
<b>Dmlc2</b> <sup>S66A,S67</sup>	0.1 ±	158 ±	ibid
A	0.1 (53)*	3 (11)*	
<b>Dmlc2</b> <sup>∆2-46</sup> ;	0	0	ibid
S66A,S67A	(53)*	(30)*	





All values mean  $\pm$  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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