

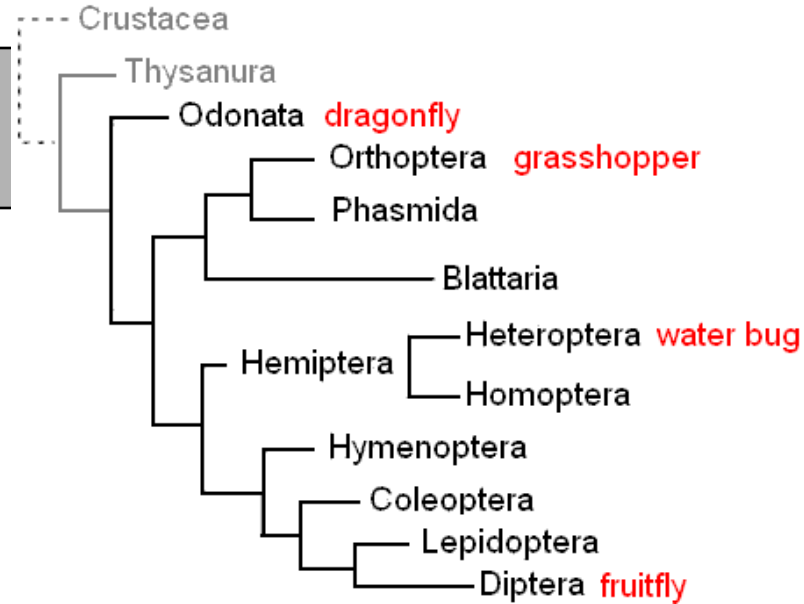
# Comparative Genomics and Structure-Function of Projectin in Relation to the Elasticity of Insect Flight muscles.



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# Insect flight muscles



❖ structure

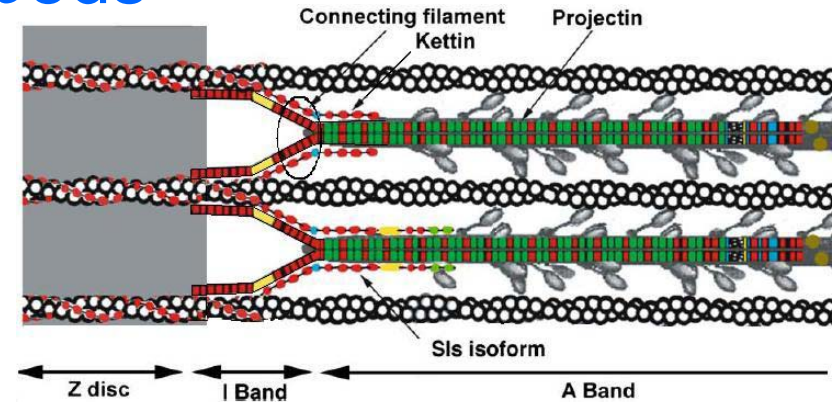
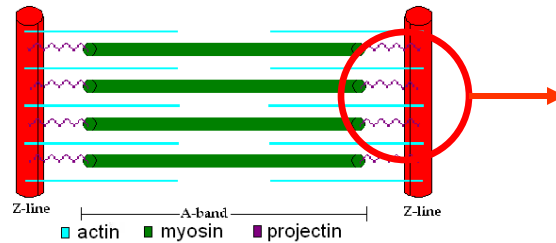
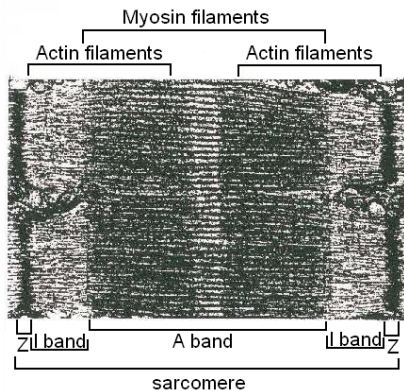
❖ physiology

	Rabbit psoas	<i>Libellula</i>	<i>Lethocerus</i>	<i>Apis</i>	<i>Drosophila</i>
Relaxed stiffness	3.5	22	98-120	82	35
Active stiffness	329	213	147-177	233	53
Rigor stiffness	318	230	581	NA	NA

**Muscle stiffness in various insects.** Stiffness was measured as pN/ A filament/ % length change under various conditions

# Myofibril stiffness and C-filaments

- 3<sup>rd</sup> elastic filament in all muscles, named C-filament in insects
- Titin in vertebrates
- Kettin and projectin in arthropods

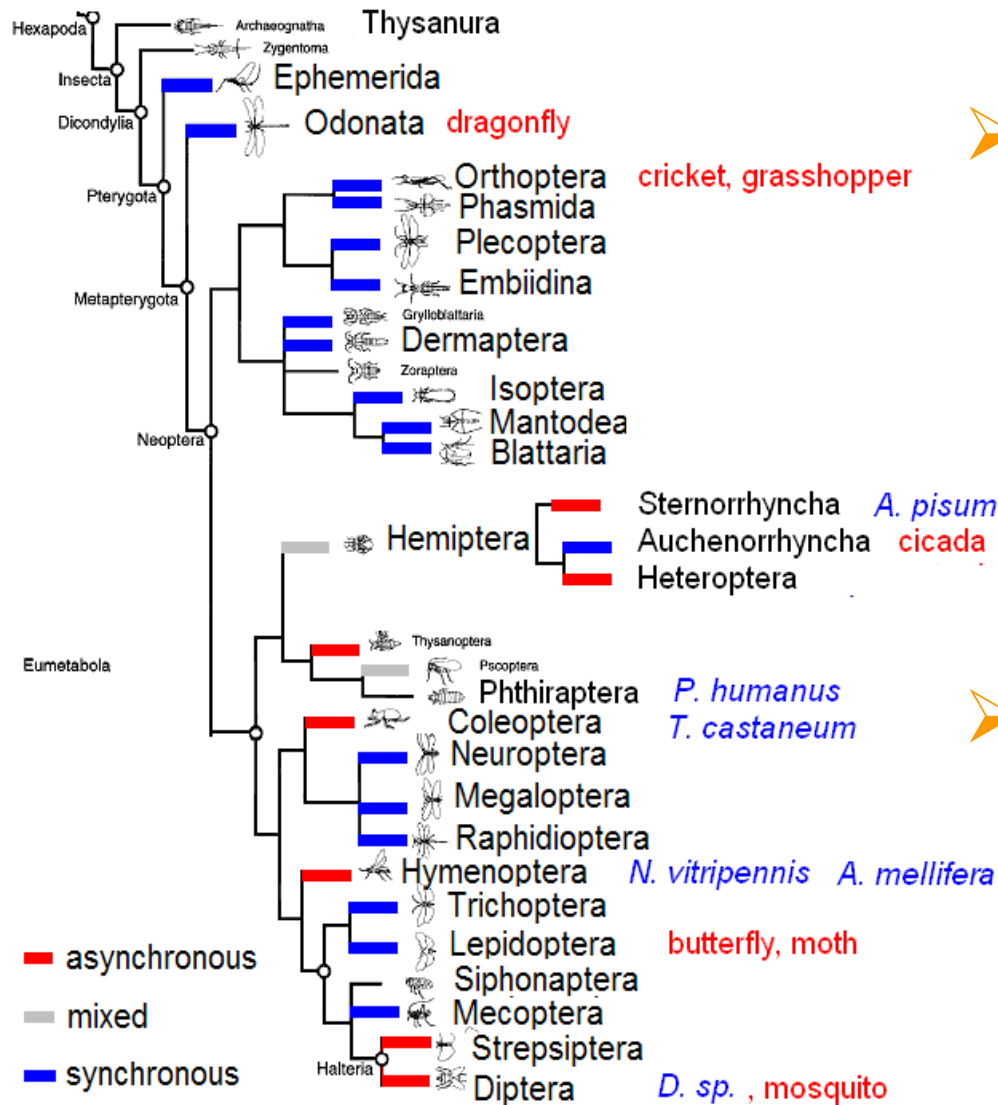


*From Bullard et al 2005*

## QUESTION:

Is there a correlation between variation in the structure of projectin and variation in muscle stiffness?

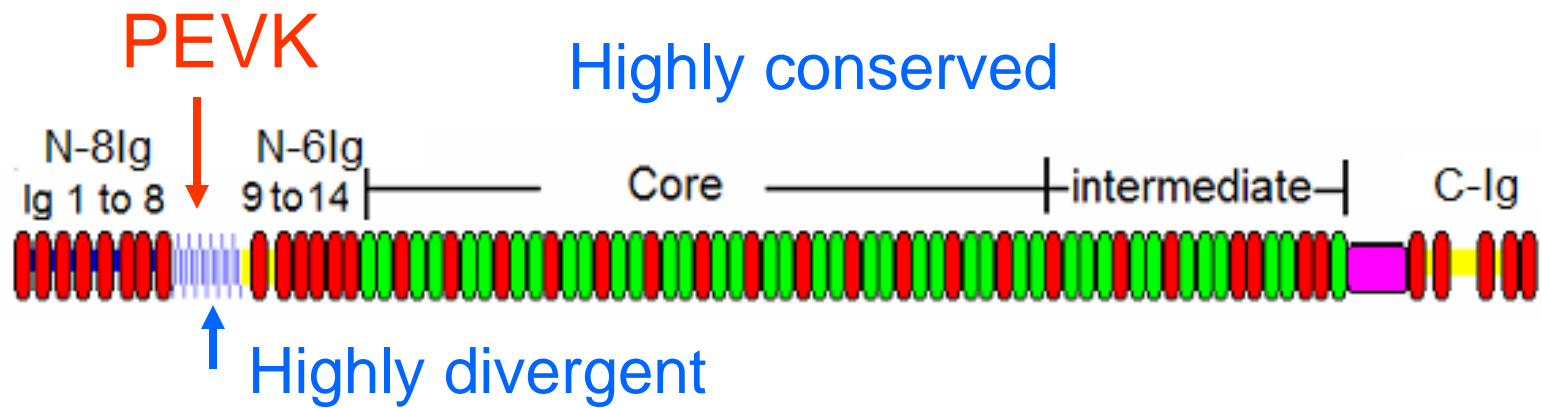
# Sequence retrieval and annotation



➤ Mining genome projects to retrieve either:

- annotated genes
- assembled genomic sequences
- traces

➤ Cloning and sequencing cDNA



	Dmelano	Agam	Nvit	Amel	Tcas	Api
gene size (bp)	51,073	>31,000	48,634	66,952	37,040	>70,000
cDNA size (bp)	27,154	23,809	26,547	26,012	26,184	26,132
# exons	46	>27	71	97	64	144
largest exon	9462	10046	4371	2325	2670	1054
smallest exon	22		22	15	22	22
average exon size (bp)	590	na	373	271	409	181
Amino acids #	8,923	na	8,912	8,730	8,379	8,607
Calculated MW	998292	na	995868	995346	933354	960331

# PEVK is divergent in its sequence

*Api/1-448* --- EKMEVDEKDEEVKETKEKTVKRKSSITTKKTTETETENAPP--EVKRRKSSILKTTNESEKQEDGQPEVKKRRSSILKSSSESTTTT-----TVEEQKDKQEKRR----  
*Lpul/1-313* -----EVKVKVEKKKE-----EKKK-----EPEKK-----EPKKEAE-----EKIQEP-----  
*Amel/1-482* ---KKEEKKKEEKEEKKKVE---KKEEKK--EKKKKEEKKKEEKKVEKKEEKKKEE---KLEVIQKKPEEK-IEE-----KKE---VEKKIAKKEE---K  
*Nvi/1-629* ---KKEKKKKKKDEEEKKEKKKVEEKKKKEEKKKVEEKKKKEEKKLEEKKKIEEEKIKVEKKEEKEEIKIEQKKIEERKKIEEKKIKEPESKPNGIDKPTSKKDKKEEVEKRRSIIKKEPEAK  
*Tca/1-466* EKKEEKKRKEEAEEIEEEEEEEEEEEEDTDKQITRKKDDVVKKEEEEIKKKEEKKVEKKEEKKVEKKEEAKKVEKKEAKKVN-----DVGKNAVEKVEET--  
*Dmel/1-655* -KVAKKKEEKPKKEKTKNEKEVEQKEMEEDKNESGQSVAQTEGRINIEQISEGDPKEELTVKEEILDKRDTEVKESSVELQDSAGHEVPEPKKATSDSKLDQSNQKNLKKHTDQSESKTNK  
*Dvir/1-656* EKDKKKKDEKS-EQDKKEEAEKRRKVIETKTKTKKEISQQEDLAEREQQTN---QISLKVQNNQIVNQIISVTSYEQAYIQEQSTLIVQQTQTEQQQVSETHVSYSSVQKKAHQELLVQE

*Api/1-448* -----SSILKNSSNEKTEVKDEIKKPLIKKTLDEKVEVKDEVK-----EKIKKP-----  
*Lpul/1-313* -----NEVKAEEKVEAKAPKKKEEPKKEPAKKEEP-----EKIQEP-----  
*Amel/1-482* KEE-----ALLKAEKTVSRKQSIKVEELRTESRRSSLIATDEKIEEVKT-----ESRRASIEKK-----VITEKKD  
*Nvi/1-629* KPES-----PREQTPEKVVGRKSSVSRVEELKTESRRSSISRTEETIHEDGTGTPQRRVSIQSSQEEESTMESRRSSIMSQKEEKISKPKSKLIEDKETTERRSSIKRKE  
*Tca/1-466* -----KVAAKKAERKASVQKIEEFKSESRRSSVVEKQIVQGD-----KVSRR-----SVSTQV  
*Dmel/1-655* NVS---LAEPKISNKQESSEEQQATEQIGLKKVDRKASIVSVKEEISSDVRKSTIKAKEEITVDKKAASSRR---SSLAVEESNTESRRSSIIDKKPLEQVDNKPIDANKNPQLKEEIPRLKPA  
*Dvir/1-656* HSSPKVQAKVSSSTCTIETQPTPEAEQIGLKKVERRASIVSVKESKSETRRRSIIKSHQTSSEEDTKTVSRR---SSFAVEENVSESRSSVVDKKALEQVESKKEIANNNPQAIKEEIPKPKPA

*Api/1-448* SVKNLSDEKTEVKNNEET-----KKTLLKTSVUKDEE-----KKPLIKKSTIEKTEVKDEGIIKKP----  
*Lpul/1-313* --KKLEPGK--KPEPA-----KKLVDNKAPEEK-----KGPCLKKVEEMNDVQPKKATKEP----  
*Amel/1-482* --EVVAKAKTVGKEEK-----KKEEPAPK-----PKVTLTKPEEAKKEEP-----AQKPKTLGKPEEAKKEEAPK----  
*Nvi/1-629* SAEVISDTKKYVDEEKNQSVKRPSVVKRDSVKKDSITPKDETPEEKNGSVKRPSVVKRDSVKKDITIKKNEVTEEKNGTVDRLSVVKRDSVKKDITIKKVEEKVPVKTDKVEDKPKETPSLKPTLKTS  
*Tca/1-466* ELETRSQSRKSSIADEK-----KVVQTKKLETTAEALTP-----TNNIEEKFDTKKEPKLAPTKEP----  
*Dmel/1-655* EKRRTSKVIIEPKPDEG-----LPKLRKASIAQVKEEAKPAAPKLKAK--AKAKPKYEELPEIPDYERPQLEKYEKSDFTPSDFARDLEIPNKMEKPIIDSGKKEPAVLADKNGIPK  
*Dvir/1-656* DKRRIPKAVEEQKPAEE-----LPKLRKTSIAKPKKEEIPPEAAKPKAKGKLKAKPKYEELPEIPDYERPALAEKYEKSDFTPSDFARELEIPNKMEKPALETAKKPAEIVPQKNGLPKK

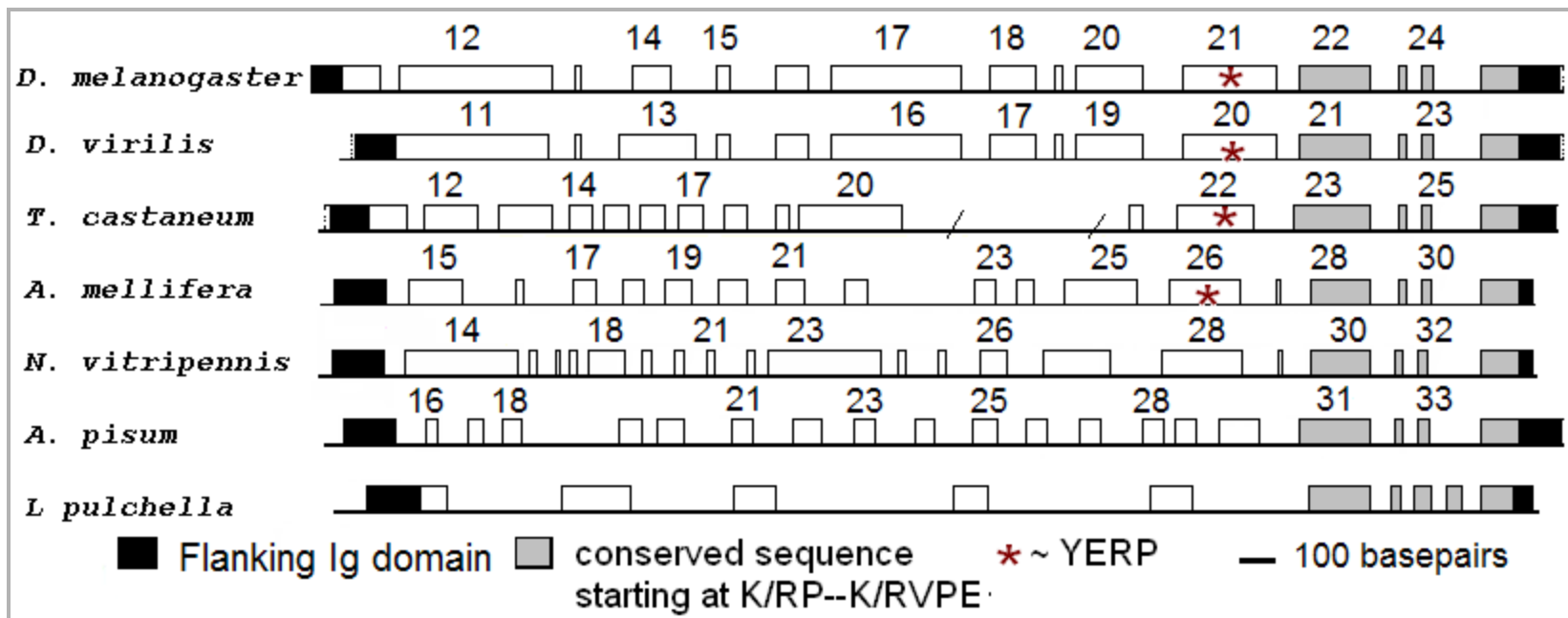
*Api/1-448* -----LLKKAPIEKPAVKAMDFTKVLKLPVTRTE-----GKTPTPEEKPAANKRLSVKKKKDTIEAEHPEKMRPTISNGTKVDNSQDSFDDKSIDERTPETSVDYSSMMSED  
*Lpul/1-313* -----ALAKKPVFA-----KKPD-KPKPRYNMQFQLPSDDEESEE-----EHDTYDINDSRLGTRKE-----  
*Amel/1-482* -KEEE-----QTRKLSLKPGAKIEEEAKTLNKVELKPVAAEMNEKKSGLRKPPLYEIKADSFQSIQLKPVSRDPKT--PQKAENGGIELKTEKTEKVEAKMKIGKAGKEKSYELPEIPDY  
*Nvi/1-629* GKPEDSKLKPGEPESSRASLKPQVGRGDERKDSLKVELKPVGSPRSSGK--WRRPEKQEVVIEF AEIQLKPVPKTVTKQPDKAENGLVELKKTDRMDKLEVKKVSKKADKEKSYELPEIPDY  
*Tca/1-466* -----EKLDTKSKLAPAKEPPKEKVDTKPQLKTEPEK-----KPKLKKPEPEPEKPELKTADLTKK--NEAKANKEVELKKTGEKEKSKTVAKKSTAKKDEIEAWQNIIPDY  
*Dmel/1-655* TDIIIEQYADEPKGLKVGGKLLPDEGDDGRDGAFLKPMIEPEKEILDGKNNKHADKPTVLDIIKQRRSSIRNLMKPEIQNESFLGVLKPVIKDTRQEAAPQQAIALTKANATEQFSPTKA  
*Dvir/1-656* TEVVEQPAEPEPTIKLGGKLLPDEDTRDGAATLKPVIIEPEK--DKQNTDKSEAEKPSLMDIIKQRRSSVVRNLMKPEIQNESFLGVLKPVVVDKAEQVAPQAKQLHKANITENFNPTKS

*Api/1-448* ENSIQEPSIK-----LDSSDEKAPT-----GRPSRVPEIKAP-----EMPKIEVIRETTFGDSRKNLSGSPNSGQSSRRGSLIPPEDNKGRRPSLIIISDEE  
*Lpul/1-313* -----IKVPEIKAP-----EAPKIEVIREKTPQDSSRKGLAPG--SGPSSRRGSLIPP--EELQRRPSLIIISDEE  
*Amel/1-482* ERPVLEKPPQE-----FDFGEHVARDKTKLERPATQKFEKPKVPEIKAP-----EAPKIEVIREKTPAG-SRKSLSLIPG--SGTSSRRGSLIPP--EELQRRPSLIIISDEE  
*Nvi/1-629* ERPVLEKPPQE-----FDFGEHVARDKTKLERPATQKFEKPKVPEIKAPA-----EAPIKIEIKDVTPIG-SRRGSLVPG--SGTSSRRGSLIPP--EELQRRPSLIIISDEE  
*Tca/1-466* ERPVLEKPEP-----GEHPEYAGREKTKLTK-----DKPSLPIKTP-----EAPKIAVVEKTEPEP--RKQSLPES--GPPSSRRGSLIPP--EAMTRRASLIIISDEE  
*Dmel/1-655* VKAQVADLKKPETLATLEDNYERPVLKEDPFSIDKTKSEKSTPSIITPDIRGPEVVKLEQETKE-KQVKMQPPAPGDFPKIEVIREKRPSSLAPEPPSSRRGSLIPP--ADTGRRPSLIIINDEK  
*Dvir/1-656* SKAQISDMKKPEALATIVDDYERPKELEKYEPIEFKTKSEKPTPVVKSPPDQNTPEKLEPVEDSKEEKPKVPMQPPAPGDFPKIEVIREKRPSSLAPEPPSSRRGSLIPP--ADTGRRPSLIIINDEK

*Api/1-448* NRKLRPGEVLEEKK-----VGKLRPGQVIDAK---RRRPSDEMRRPSIAELEEKIDKPSLPLKDFGAP  
*Lpul/1-313* G-KLRPGEVLDISKRLARPGELIEEKNKQENRKLKRPGEVLEEKK--RRRPSDMRRPSVAELEEKIDKPSLPLKPIGSP  
*Amel/1-482* NRKLRPGEVVDERKLG-----KLRPGEVLDTK---RRRPSDVRPPSVADLENKINQPSLPLKDVGGP  
*Nvi/1-629* NRKLRPGEVLEEKKR-----FRPGELLDQ---RRRPSDARRLSVADIE--NKPSTPLKDVGGP  
*Tca/1-466* G-KLRPGEVLDTKPKS-----KRPQQLQDPNEKQRRRPSDVRPPSIADMEMDINKPSLPLKPIGPA  
*Dmel/1-655* K-LRPGEVMDTRLLR-----PGEVGEQ---RRRPSIDVRRPSVQDLEDLIDKPSLPLKDVGGP  
*Dvir/1-656* K-LRPGEVMDTRLLR-----PGEVGEQ---RRRPSIDVRRPSVQDLEDLIDKPSLPLKDVGGP

Except for last ~  
100 amino acids

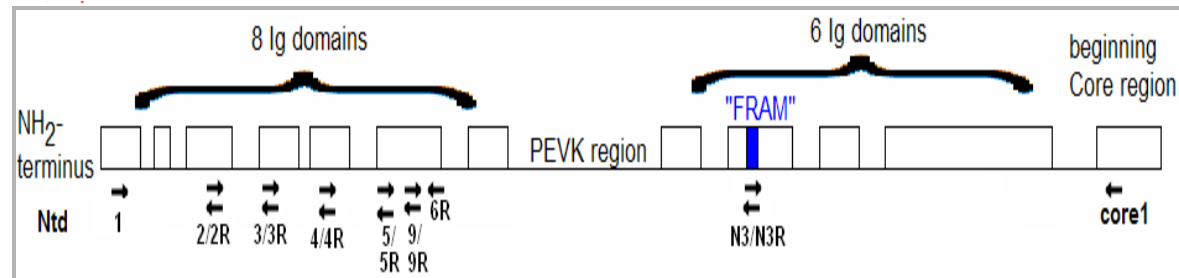
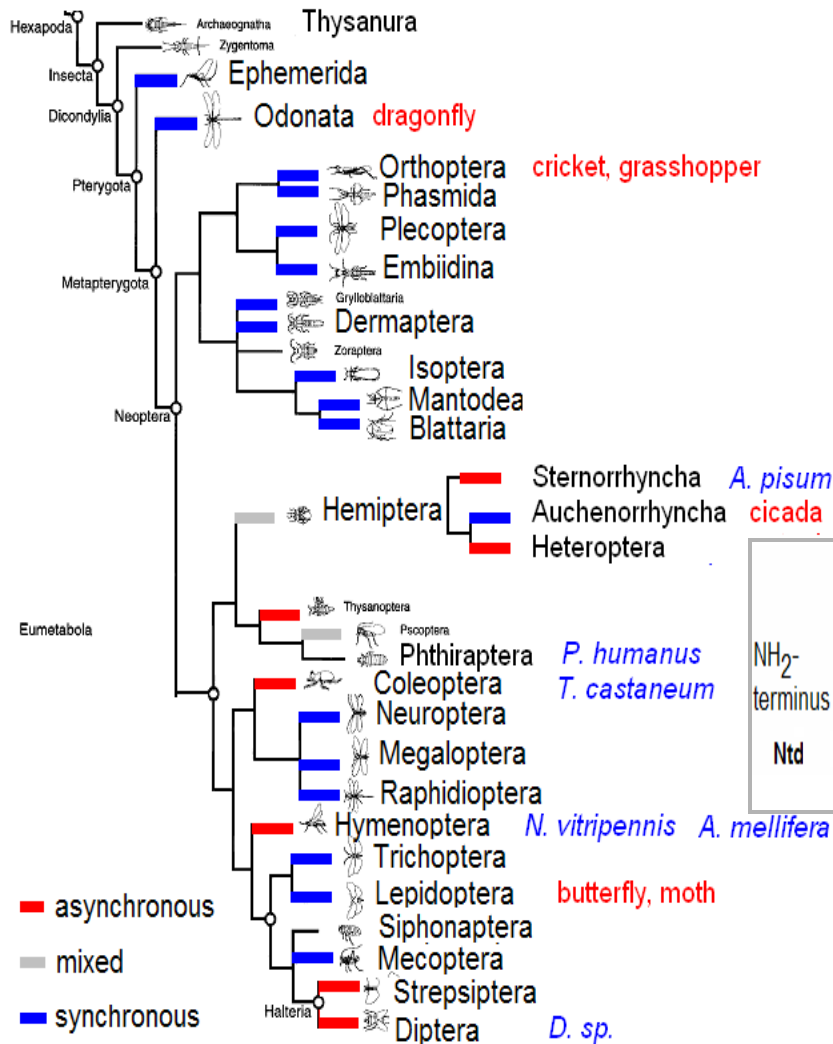
# Alternative splicing in the PEVK region



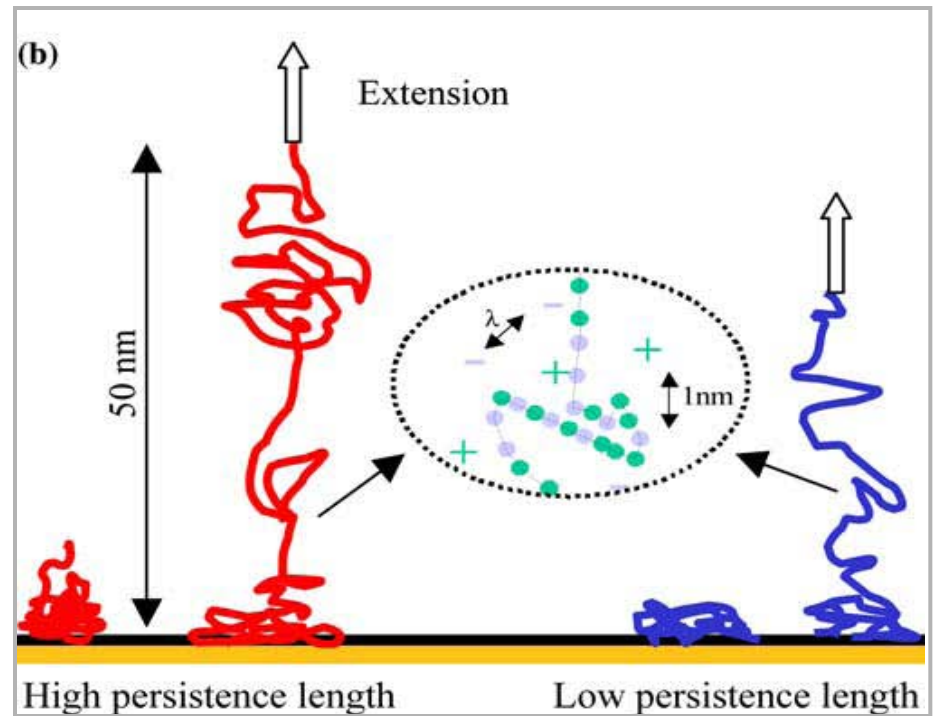
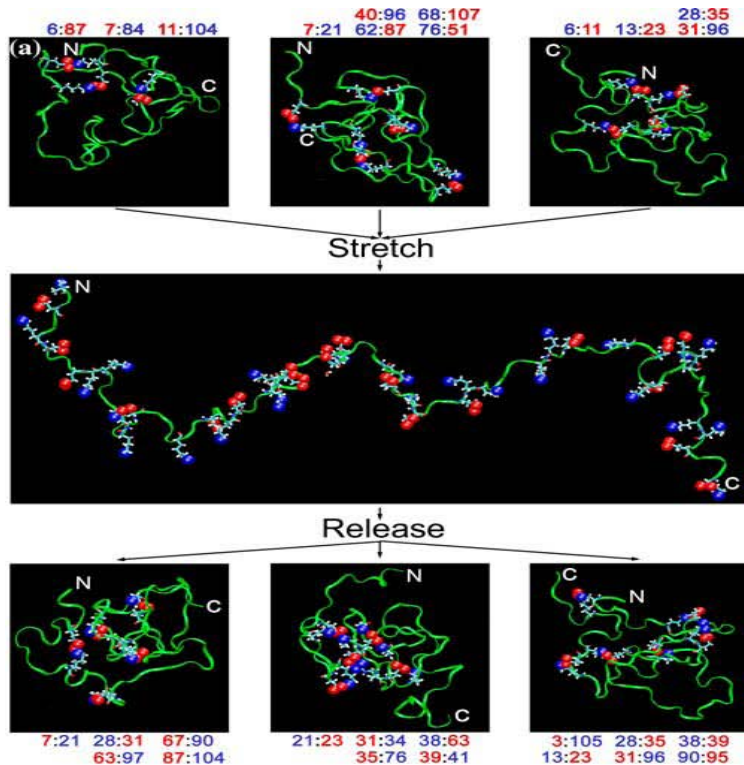
	alternative splice	exon #	longest variant aa	%PEVK	shortest variant aa	%PEVK
<i>D. melanogaster</i>	yes, flight shortest	<b>15</b>	<b>655</b>	<b>44.5</b>	<b>131</b>	<b>46.5</b>
<i>T. castaneum</i>	yes, flight shortest	<b>16</b>	<b>464</b>	<b>59.6</b>	<b>93</b>	<b>63.4</b>
<i>A. mellifera</i>	yes, flight shortest	<b>16</b>	<b>482</b>	<b>62.8</b>	<b>39</b>	<b>32</b>
<i>L. pulchella</i>	yes, flight shortest	at least <b>10</b>	<b>&gt; 313</b>	<b>56</b>	<b>129</b>	<b>54</b>

# Molecular identification in insects with no genome data

- Design battery of degenerated primers from conserved sequences
- cDNA sequencing, assembly, and annotation.



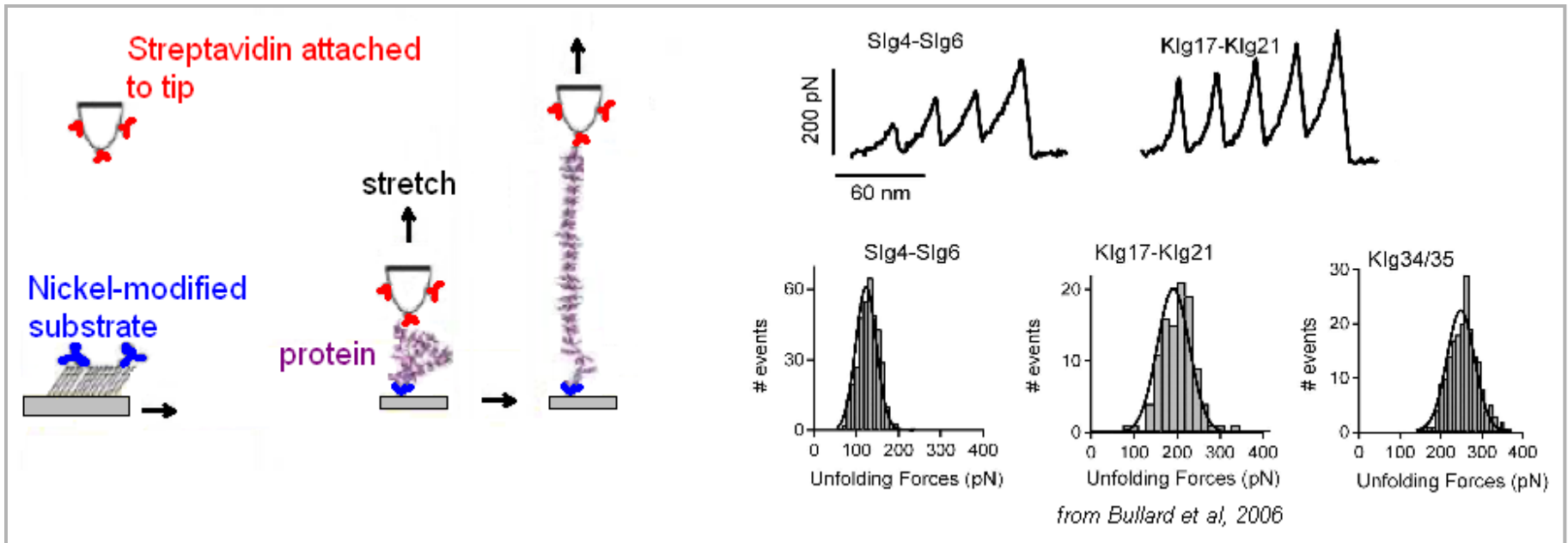
# PEVK and resting stiffness: can PEVK be a multi-conformational domain responding to stretch-release?



*Forbes et al, 2005*

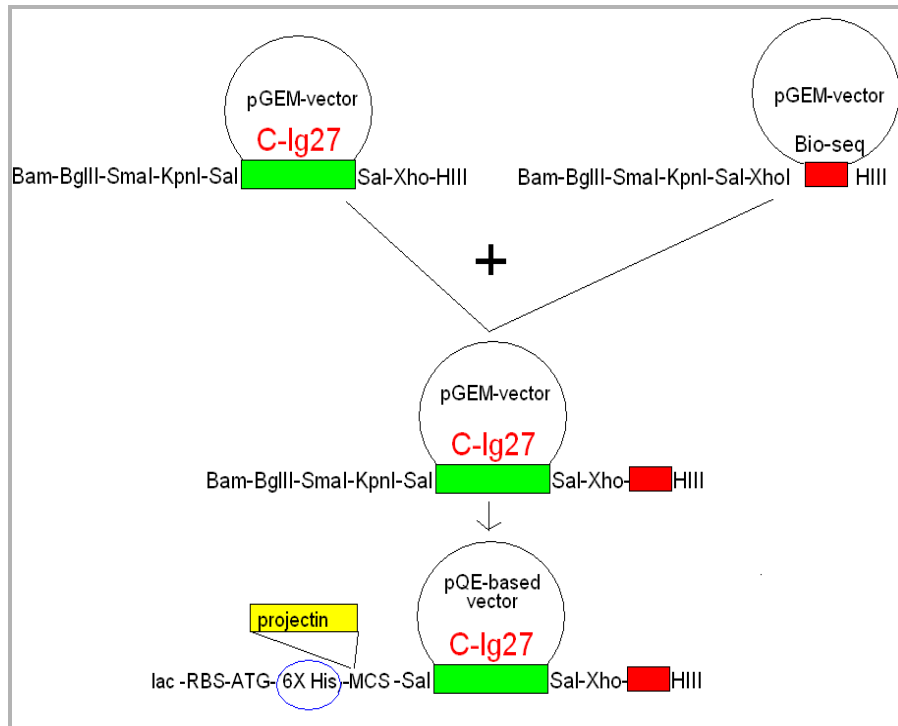
Titin PEVK under stretch and release regimen adopts multiple, alternative, equivalent conformations.

# PEVK mechanical behavior under stretch and release test (AFM)

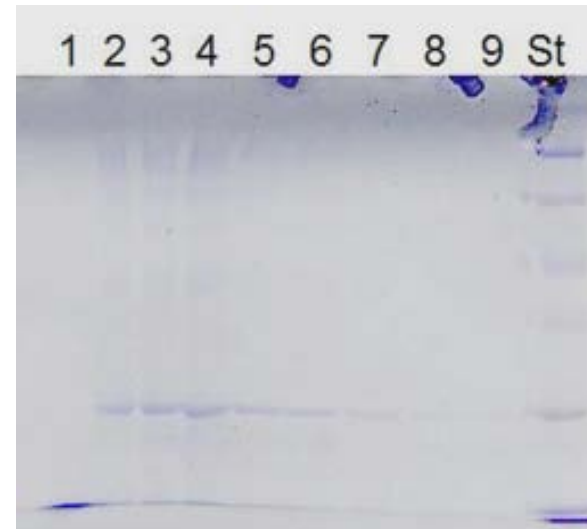


**ATG** ● Bam ————— Sal-Xho ————— HIII = 0 Ig27 handle  
**ATG** ● Bam ————— Sal ————— Sal-Xho ————— HIII = 1 Ig27 handle  
 ● 6X His tag    ■ projectin domain    ■ C-terminal titin Ig 27    ■ biotinylation seq.

# Cloning and expression of PEVK fusion peptides



species		
<i>Drosophila</i>	Short ~ 300 bp	Long ~1, 200 bp
<i>Libellula</i>	Short ~350 bp	Long ~ 700 bp



# PEVK splice variants and flight muscle output

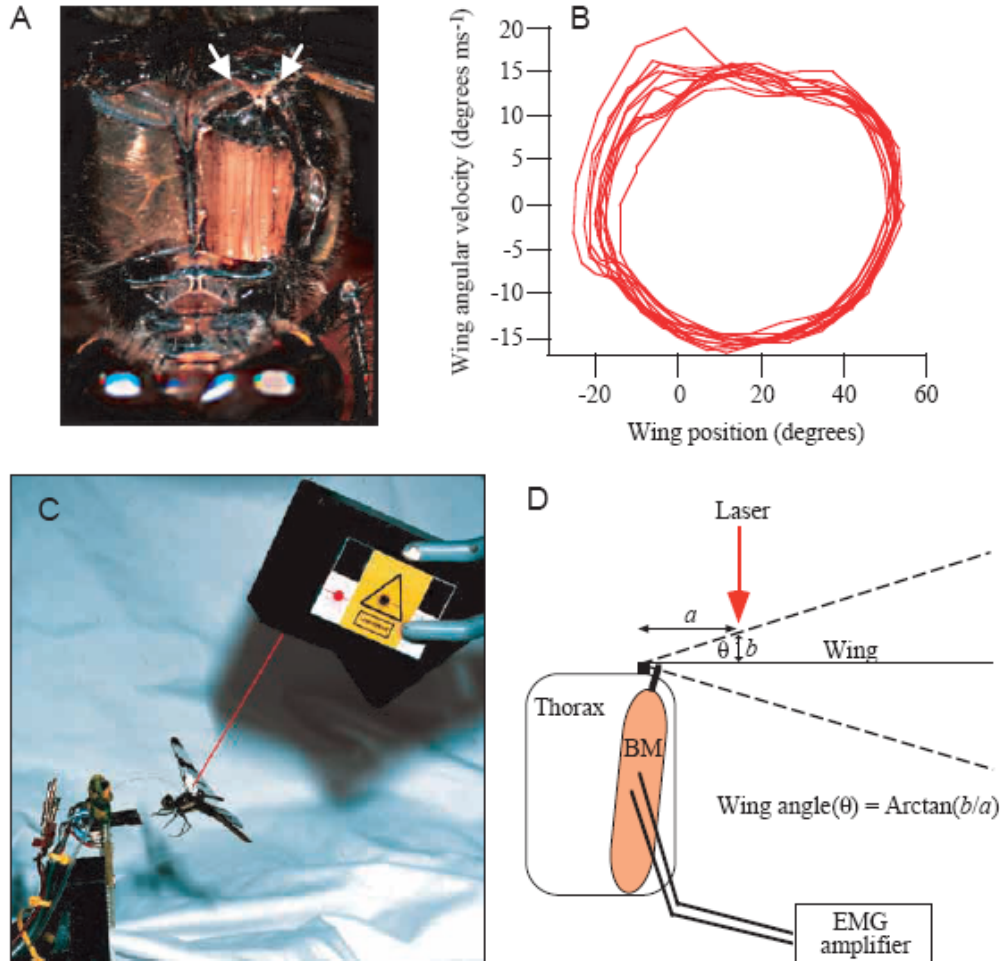
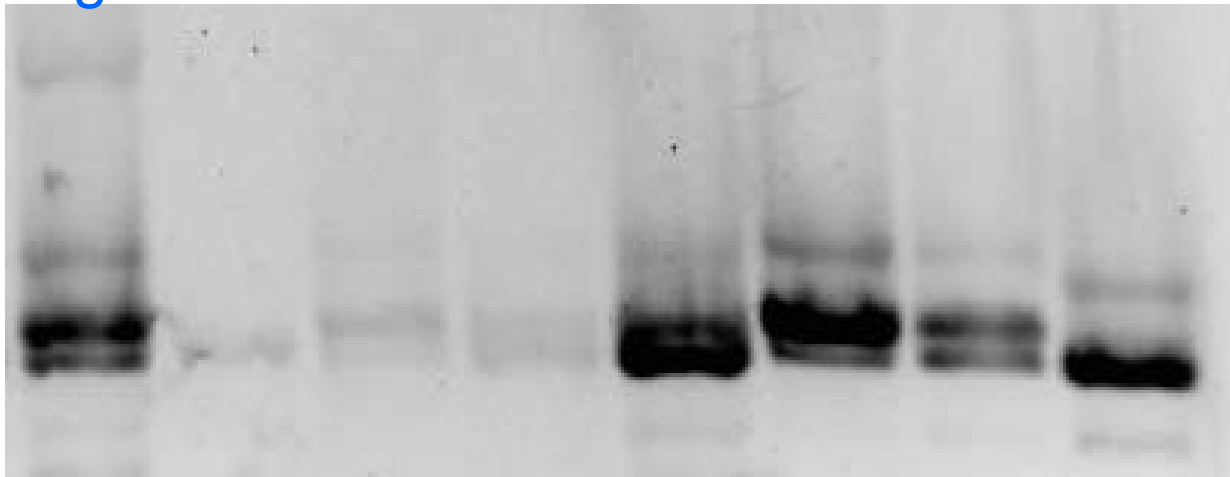


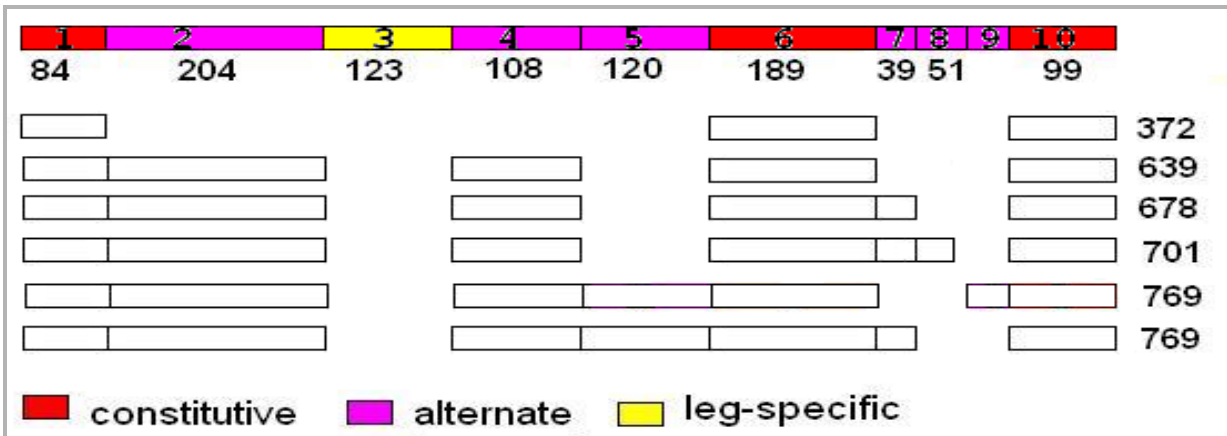
Fig. 1. (A) The basalar muscle (BM) of the mesothorax exposed by removal of a portion of the cuticle and underlying air sacs. The viewpoint is looking posteriorly from the rear margin of the head and slightly above the dragonfly; the base of the left forewing is visible near the upper right. White arrows show the lateral edges of the humeral plate, around which incisions were made to isolate the basalar muscle mechanically for studies of contractile performance. (B) Sample trace of wing position and velocity from a recording made using a laser distance sensor on the base of the forewing. (C) Photograph showing a dragonfly attached by its ventral thorax to a narrow glass beam (not visible) that extends from the base of the forewing. A fine-gauge thermocouple and EMG electrodes are inserted into the thorax. The laser distance sensor is shown at the upper right. We have drawn a red line to depict the laser beam striking a white spot painted at the base of the right forewing. (D) Diagram showing the geometry of the basalar muscle attachment to the base of the forewing, an approximation of the EMG recording electrodes (these are differential electrodes referenced to a third electrode in the abdomen that is not shown) and the method used to calculate wing position from the laser distance sensor.

# PEVK splice variants and flight muscle output

RT-PCR analysis of individual *L. pulchella* flight muscles

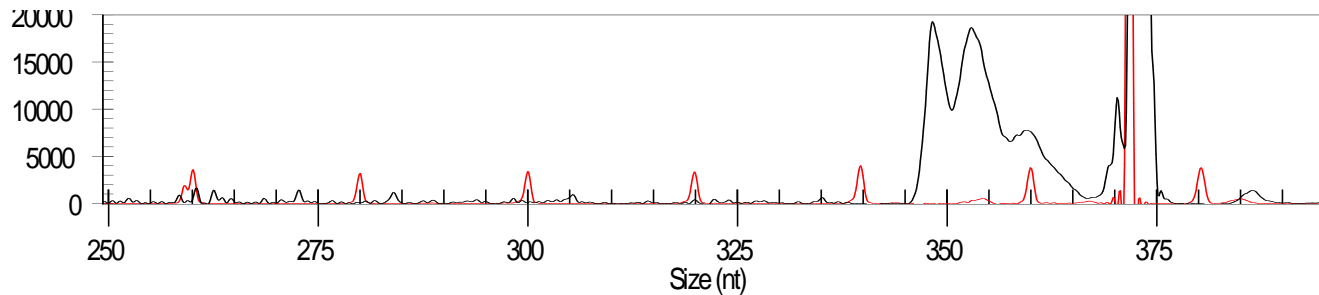


Each individual *L. pulchella* has a specific pattern of PEVK isoforms.

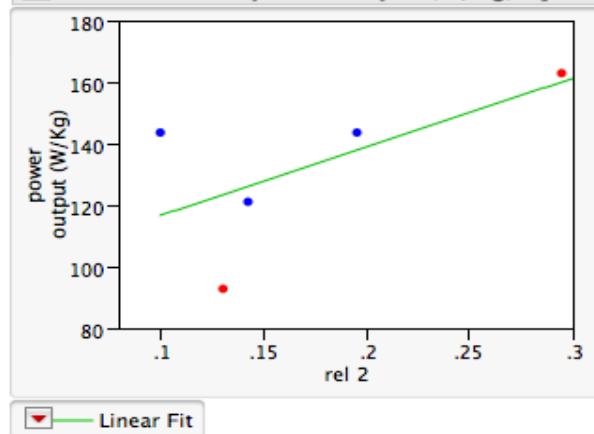


# PEVK splice variants and flight muscle output

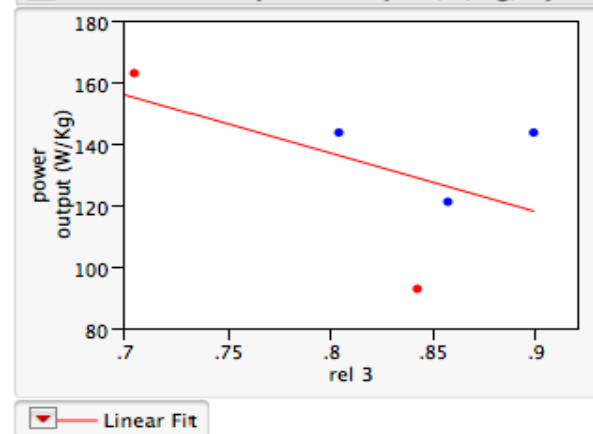
Is there a linear relation between the ratio of certain PEVK isoforms and the power output of individual flight muscles? Alternative Fragment Length Analysis and Bivariate fit.



▼ Bivariate Fit of power output (W/Kg) By rel 2



▼ Bivariate Fit of power output (W/Kg) By rel 3



# Acknowledgements

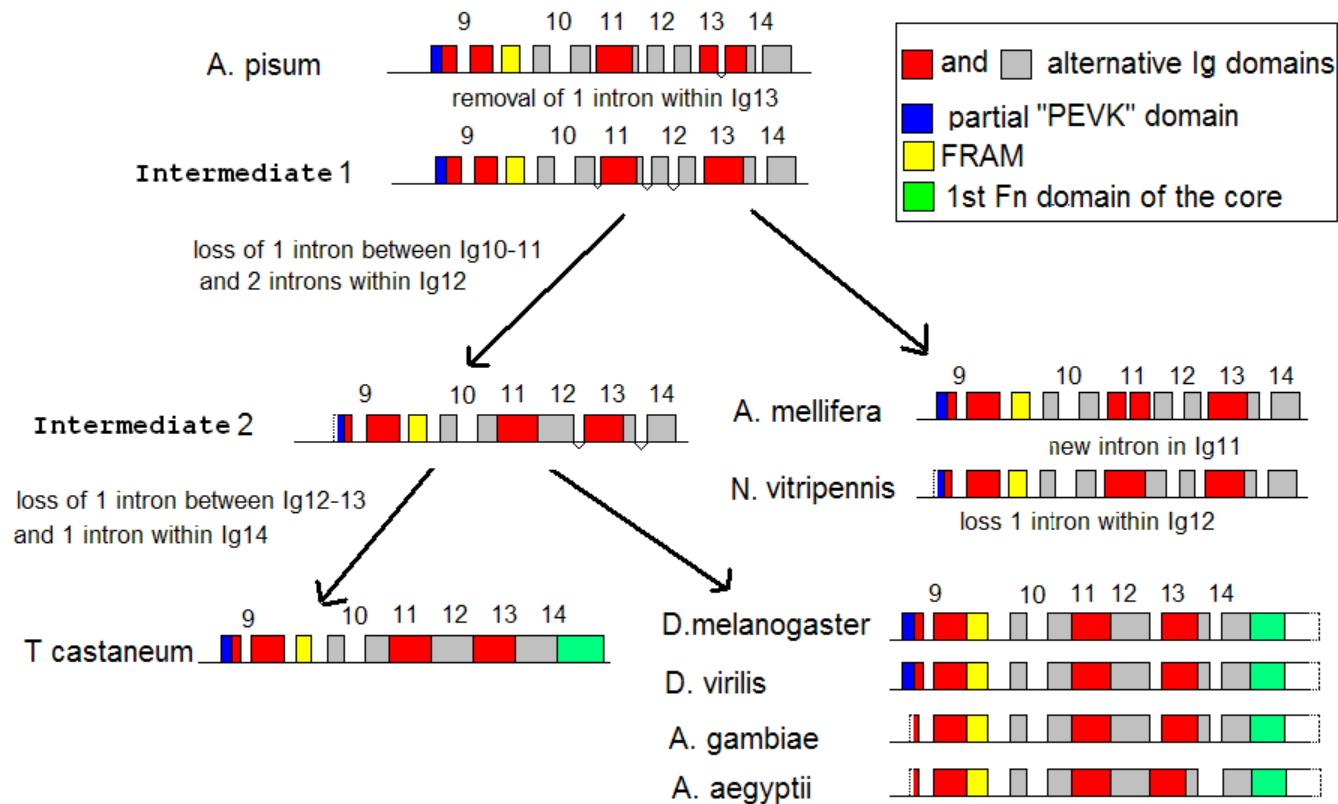
Collaborators: Richard Southgate (CofC), Kuan Wang (NIH/NIAMS), and Jim Marden (PennState)

Current students: Sean Bear, Jamie Rodriguez, and Larchinee Turner.

Past students: Drew Philipp and Catherine Gumps

- SUPPORT: South Carolina INBRE program NIH Grant P20 RR-016461 from the National Center for Research Resources (P.I. L. Pirisi-Creek).

# Evolution of intron position: example of the NH<sub>2</sub>-terminal Ig region



The number and position of the splice sites indicate that Hymenoptera might be more basal than in current phylogenies

# Revised phylogeny for insect orders based on projectin data

