Identification of the projectin gene in *Nasonia vitripennis* and analysis of the elastic domains.

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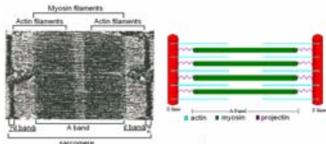
ABSTRACT

The sequencing of the two Hymenopteran genomes (*Apis mellifera* and *Nasonia vitripennis*) has now been completed. Using projectin from *Drosophila melanogaster* as a query sequence we isolated a complete projectin gene from the genome of both species using BLAST and multiple alignment softwares. The gene is highly conserved between *Drosophila* and the two hymenoptera, except for the sequence of the putative elastic domain or PEVK domain. We have verified the predicted exon-intron patterns obtained from the genome annotations and identified several new exons encompassing the PEVK domain. We used standard molecular biology techniques to clone and sequence exons and intron boundaries. Data will be presented describing the gene annotation process as well as the phylogenetic analysis between diptera and hymenoptera in the context of the evolution of insect flight.

BACKGROUND

All striated muscles in vertebrates and invertebrates contain a third elastic filament (Figure 1). In *Drosophila* Indirect Flight Muscles (IFM), these filaments are called the connecting or C-filaments.

FIGURE 1: filament systems in myofibrils



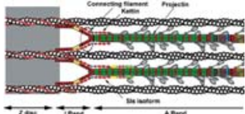
This filament is responsible for the elasticity and resting stiffness, which is associated with the physiological process of stretch activation. In return this process enables the rapid contraction of flight muscles leading to the high wing beat of some insects.

FIGURE 2: table with insect flight details

Insect	beats/second	flight speed km/hour
dragonfly	20-28	25
beetles	46-90	5
butterflies	9-12	9
mosquito	300-550	32
Honey bee	200	22
wasp	110	9

The major component of C-filaments is the protein projectin. It is an extremely large protein at ~1 MgDa. Projectin has been found in all tested invertebrate muscles, and antibodies often show cross-reactivity, yet the physiology of these muscles can be quite different. *Drosophila* Projectin is composed of 39 repeating Ig and FnIII domains, and contains two unique sequences: a kinase domain at the COOH-terminus and a PEVK-like sequence at he NH_2-terminus (Figure 3). Analysis of the gene encoding projectin from *Nasonia vitripennis* indicates a domain organization very similar to the *Drosophila* protein, except possibly for the PEVK domain.

FIGURE 3: projectin orientation within the sarcomere



MATERIALS and METHODS

BLAST *Nasonia* genomic data using *Apis* core cDNA sequences. Retrieve contig containg projectin gene. Using translation and alignment tools identify exon intron pattern.

RNA extraction: In order to extract the RNA from the *Nasonia*, the *Nasonia* are homogenized with Trizol™ and chloroform. Then the mixture is centrifuged and the aqueous phase is transferred to a fresh tube. Isopropyl alcohol is added to the extract to precipitate the RNA. This is centrifuged and the pellet is washed and dried and resuspended into water.

Exon-Intron splicing: Reverse Transcription-PCR (RT-PCR) is done by adding RNA, primers, buffer, and enzymes to a PCR tube with mineral oil on top to prevent evaporation. Then the mixture is placed in the PCR machine and run through one cycle of reverse transcription and 30 cycles of PCR.

The samples are then run on an agarose gel. The gel is prepared by using agarose powder, TAE and water. Ethidium bromide is added to stain the DNA samples. The agarose gel is placed in a gel apparatus filled with TAE as a buffer. The PCR reactions are prepared for the gel by adding a loading dye. Then they are loaded into the wells of the gel. The gel is then run for about an hour. Using UV light the gel is looked at using gel imager. Pictures are taken and the band is then cut out using a razor blade and UV light to visualize the band

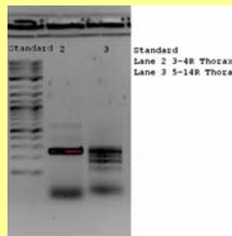
DNA gel extraction: To extract the DNA from the band it is dissolved affinity beads (Qiagen™). The DNA is resuspended in water.

Cloning: The DNA is then inserted into a vector, pGEM-T by adding the DNA, buffer, vector, and T4 DNA ligase to a tube and incubating it at room temperature for 1-2 hours. To transform the DNA, the previous mixture is added to competent cells JM109 and then heat shocked. LB is added and the transformation reaction is incubated at 37° C. X-gal dissolved in DMSO and IPTG are added and the mixture is plated on the LB/Amp plate and the plates are incubated in an incubator overnight. The presence of white colonies indicates the PCR fragment has been inserted into the vector.

Plasmid DNA extraction: In order to isolate the DNA from the bacteria, a single white colony is transferred form the plate into a tube with LB + Amp and incubated overnight at 37° C. The DNA is isolated using standard miniprep procedure. The DNA pellet is resuspended in TE and RNAase is added.

Restriction enzyme analysis: To confirm the cloning of the insert into the vector, the plasmid DNA along with buffer and enzymes are added to a tube and incubated at 37° C. Restriction digests are analyzed by gel electrophoresis as described above.

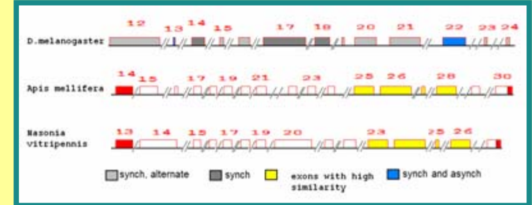
FIGURE 5: RT-PCR analysis of *Apis* projectin's transcript in the thorax.



RESULTS

Table 1: Projectin Gene Statistics

Projectin In *Nasonia*		Projectin in *Apis*	
cDNA Size	26547	cDNA Size	25708
Number of Exons	73	Number of Exons	96
Size of PEVK	1560	Size of PEVK	1309
Number of Exons in PEVK	14	Number of Exons in PEVK	16



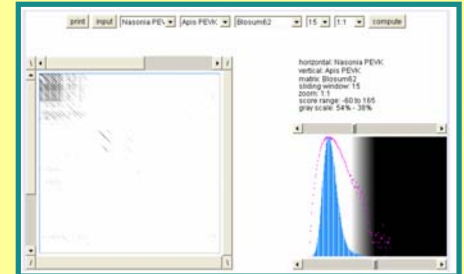


Figure Legend

Figure 6: PEVK domain Map of *Drosophila, Apis,* and *Nasonia*

Figure 7: Dotlet of *Apis* PEVK and *Nasonia* PEVK

Figure 8: Alignment of amino acids in PEVK of *Apis* and *Nasonia*

CONCLUSION

Exons were the same size in Fn and Ig domains for *Apis* and *Nasonia.* Where there are discrepancies the *Nasonia* exon has been split but *Apis* into multiple exons that still when added together are the same size as the original exon. The biggest difference in the gene between the two species is in the PEVK domain. In this domain, *Nasonia* has several much larger exons than *Apis* resulting in a larger domain and gene. While we do not yet no of any alternative splicing in either species, it is still something that the lab is working on.