Insect flight muscles are extraordinary in their diversity at the anatomical, physiological and molecular levels; in particular muscle stiffness can be very different across insect orders. In derived insects a large part of the elasticity of flight muscles is attributed to the protein, PEVK sequence, whereas insect muscles with low stiffness present longer isoforms. Variation in the relative concentration of various PEVK isoforms is correlated with differences in the mechanical properties of flight muscles.

**ABSTRACT**

**HYPOTHESIS**

Insect flight muscles with high resting stiffness contain predominantly but not exclusively a short PEVK sequence, whereas insect muscles with low stiffness present longer isoforms. Variation in the relative concentration of various PEVK isoforms is correlated with differences in the mechanical properties of flight muscles.

**BACKGROUND**

Every striated muscle in vertebrates and invertebrates is composed of bundles of muscle fibres containing myofibrils. The sarcomeres are the repeated unit of myofibrils. They are bound on each side by the Z band and contain regular arrangements of actin thin filaments and myosin thick filaments. Figure 1

Mechanical properties of muscles such as maximum work output are often associated with properties of the myofibrils, in particular passive and active stiffness. Many insect flight muscles are relatively stiff from being fused, flails, and dragonflies. The elasticity and high resting stiffness of muscles has been associated in derived insects with a third elastic filament. (Figure 1) In addition, the PEVK domain contains a unique sequence known as the PEVK domain. The PEVK domain is characterized by an unusually elevated percentage of four specific amino acids: Proline (P), Glutamic acid (E), Valine (V), and Lysine (K). This region of the gene undergoes complex alternative splicing events, generating PEVK domains of different lengths. In derived insects, the indirect flight muscles contain predominantly the shorter form. The longer form is present in the direct flight muscles.

**GENERAL PROTOCOLS**

- RNA is extracted from the basalar muscles of individual Lilbellula pulchella using TRIzol (Invitrogen). The samples were provided by Dr. Jim Marden who had previously obtained the mechanical flight data for each insect (Figure 6, right).
- RNA is used in Reverse Transcriptase-PCR reactions (RT-PCR; SuperScript Platinum II, Invitrogen) with different combinations of primers. We used a duplex approach, i.e. each RT-PCR reaction is done with 2 sets of primers, one for the PEVK and one for the kinase domain as the internal control. Two different primer sets selectively amplify different PEVK isoforms (Figure 9, below). Each forward primer (Lp6 or Lp7) has a fluorescent dye attached to it (blue in Figure 8, right). The kinase forward primer (Lp8) has a different dye attached so we can distinguish it from the PEVK (green in Figure 8, right).
- RT-PCR products are separated according to size by agarose gel electrophoresis to test the validity of the reaction (Figure 7, right).
- The reaction products are sent to the GENEWIZ company for AFLP analysis using capillary electrophoresis and fluorescence measurement of peak intensity. AFLP (Amplified Fragment Length Polymorphism) is widely used to distinguish DNA size variants due to mutation, allelism, or in our case, alternate splicing.
- Dr. Marden’s lab performed the bivariate statistical analysis to test the correlation between their data on flight mechanics and our molecular evaluation of PEVK splice variants. (Figure 9, right)

Dr. Marden’s lab performed the bivariate statistical analysis to test the correlation between their data on flight mechanics and our molecular evaluation of PEVK splice variants. The longer isoform contains the PEVK sequences. The kinase fragment as an internal control represents 100% of the projectin transcripts. Information regarding peak intensity can be interpreted to provide data regarding the relative amount of PEVK variants in each individual.

**REFERENCES**

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**FIGURES**

- Figure 1: Schematic of the PEVK mechanical behavior and muscle stiffness
- Figure 2: Schematic of the PEVK mechanical behavior and muscle stiffness
- Figure 3: PEVK mechanical behavior and muscle stiffness
- Figure 4: Schematic of three types of insect flight muscles based on attachment of the muscles to the wing base: direct, indirect or mixed
- Figure 5: L. pulchella PEVK region. This schematic indicates the known exons of the PEVK segment and the position of the primers used. Region coded red indicate constitutive exons and regions coded pink indicate facultative exons. Constitutive exons are always present in the transcript, there is some variability regarding facultative exons which are only present in some individuals. The yellow “leg” region indicates an exon that has only been amplified in the leg. The two highlighted fragments are the most abundant products, which were further quantified by AFLP.
- Figure 6: Analysis of PEVK isoforms. (A) The basalar muscle of Lilbellula pulchella (Lp) is depicted at a portion of the outside and the underlying wing base. (B) Shows the PEVK region and position from the laser distance sensor on the base of the wings. (C) Photograph shows a dragonfly attached by its ventral thorax to a narrow glass beam (not visible) that extends from a strain gauge setup showing the geometry of the basalar muscle attachment to the base of the forewing, an approximation of the high recording electrodes and the measurement of the relative position from the laser distance sensor. From Marden et al. 2001

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