Characterization of the spatial expression of flightin among arthropods: an evolutionary investigation of the specialization of a flight muscle protein

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Within *Drosophila melanogaster,* the ancillary myofibrillar protein flightin is unique to the indirect flight muscles (IFM), where it has been shown to serve an essential function in maintaining sarcomeric integrity and the ability to fly. Recent studies have identified a highly conserved ~55 amino acid region of flightin, denoted as WYR, that is found in the genomes of many other arthropods, although its expression and function in organisms other than Drosophila has yet to be explored. In this study, we characterized the transcriptional expression patterns of WYR within a specific subset of arthropods. Understanding the expression of WYR within basal arthropods may help explain how flightin became IFM-specific in the flight muscles of Diptera and give us insight into what other role(s), if any, flightin fulfills outside the genus Drosophila. Tissue specific RT-PCR was conducted using degenerate primers flanking the WYR region, and DNA sequencing was used to identify any amplified products. Species providing positive matches to WYR were then further examined by means of *in situ* hybridization of cryosections using a riboprobe complementary to WYR. Using RT-PCR, WYR was identified in the thorax of larval Megaloptera (fishfly) and in the head, thorax, abdomen, and legs of larval Odonata (dragonfly). Using the previously amplified *Daphnia magna* (waterflea) WYR sequence as a riboprobe, *in situ* hybridization within this crustacean was conducted. Resulting staining indicated partial specificity of flightin transcription, with signal lacking altogether from particular organ systems. These results indicate that the flightin-like sequences in these basal arthropods are expressed more broadly than in Drosophila. This suggests that the specialization of flightin has changed over time, resulting in distinct differences in the spatial expression of flightin in various arthropods, ranging from partial tissue specialization within Odonata, Megaloptera and Daphnia, to Drosophila, where expression is entirely limited to a specific subset of thoracic musculature.