Molecular Evolution of Drosophila Flightin: Role of the amino terminal region in species-specific male courtship song.

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Fligthin is a hyperphosphorylated myosin-binding protein that in *Drosophila melanogaster* is expressed exclusively in the asynchronous indirect flight muscle (IFM). In this species, flightin contributes to myosin filament stiffness and is essential for the stretch activation response that drives fast wingbeats required for flight. Analysis of sequences indicate that the central region of flightin, approximately one third of the protein (~ 52 amino acids), is conserved among insects and crustaceans. In contrast, the N-terminal (~ 80 amino acids) and the C-terminal region (~ 51 amino acids) of flightin are not well conserved, with the N-terminal showing the most variability. Preliminary analyses of the flightin sequences from sixteen Drosophila species using Selecton Server for detecting positive selection show that the N-terminus of flightin is evolving at a rate faster than the rest of the protein and the whole-length flightin evolving faster than the rate for other muscle proteins like myosin regulatory light chain. Additionally, we have identified seven phosphorylation sites that reside within the variable N-terminus of *D. melanogaster*, several of which are not conserved among Drosophila. We hypothesize that the variability in N-terminal sequence and phosphorylation potential may influence myofilament stiffness, which in turn dictates wing beat frequency during flight and species-specific male courtship song production using flight musculature during mating ritual. To test this hypothesis, we are creating transgenic *D. melanogaster* expressing a truncated flightin missing the N-terminal region and will test if they can produce wild-type wing-beat frequency for flight and courtship song (sine and pulse song) parameters. While we show here that mutant male flies lacking flightin (fln^0) cannot produce either courtship song at all, we predict that flies expressing the N-terminal truncated flightin will not be able to make song with wild-type parameters. This will show flightin N-terminus has been sexually selected for species-specific courtship song.