

Flightin is a hyperphosphorylated, myosin-binding protein that in *Drosophila melanogaster* is expressed exclusively in the asynchronous indirect flight muscle (IFM). Flightin contributes to myosin filament stiffness and is essential for the stretch activation response that drives the fast wing beats required for flight. Previous studies have shown that IFM, the major power producing muscles for flight, are neurally activated for production of the male courtship song; however, a direct role for the IFM in song production has not been investigated. Here we show that a deletion mutation in flightin affects the courtship song, as well as flight ability. An analysis of flightin sequences from twelve *Drosophila* species revealed that the amino (N) terminal region (~63 amino acids) of flightin is poorly conserved (~15% identity) compared to the rest of the protein (>70% identity). We hypothesize that variability in N-terminal flightin sequences arise from positive selection acting on mating song components that contribute to mate selection and speciation. To test this hypothesis, we have created transgenic *D. melanogaster* (NDL-Fln) strains that express a truncated flightin missing the hypervariable N-terminal region. These mutant strains are flight compromised compared to the control flightin rescued null strain (flight index: 2.209 ± 0.84 vs 4.55 ± 0.39 , respectively). Preliminary analysis of the courtship song showed that NDL-Fln males produce pulse songs with longer interpulse intervals (IPI) than control males (60.2 ± 16.3 ms vs 40.75 ± 1.76 ms, respectively). Other studies have shown that IPI contributes to species recognition and con-specific mating. In summary, our data suggest that the N-terminal region of flightin is necessary for flight and for courtship song production. The hypervariability of the flightin N-terminal region may result from sexual selection, suggesting that flightin fulfills an essential role in defining species-specific mating song parameters.