

Flightin is a myosin binding protein that in *Drosophila melanogaster* is expressed exclusively in the indirect flight muscles (IFM). Flightin increases thick filament stiffness and is essential for sarcomere stability and flight. In addition to flight, the IFM is activated during male courtship song, but its precise contribution has not been investigated. Here we show that courtship song is abolished in *fln*⁰, a null mutation in flightin that also abolishes flight. Among Drosophilids, the N-terminal flightin sequence is poorly conserved (15% identity) compared to the rest of the protein (>70% identity). Given the role of the courtship song in mate selection and speciation, and the observation that many genes involved in sexual selection are positively selected, we hypothesize that the hypervariable N-terminal flightin region influences species-specific mating song parameters. To test this, we created transgenic *D. melanogaster* strains expressing a truncated flightin missing 63 N-terminal amino acids (*fln*^{A2-63}) and compared its flight and song properties to wild-type rescued null control (*fln*⁺). *fln*^{A2-63} is slightly flight compromised compared to *fln*⁺ (flight index: 3.12±0.34 vs. 4.2±0.36, respectively), but has similar tethered wingbeat frequency (~ 200 Hz) and normal myofibrillar structure as determined by electron microscopy. Courtship song analysis showed that, compared to *fln*⁺ males, *fln*^{A2-63} males produce songs with longer interpulse intervals (IPI, 60±6 ms vs. 40±1.7 ms) and higher sine song frequencies (220±3.2 Hz vs. 149±10.2 Hz). Other studies have shown IPI contributes to species recognition and con-specific mating. Our data shows that flightin N-terminal sequence has a stronger effect in song production than in flight ability. We propose that flightin fulfills dual function, enhancing flight power output for survival and influencing song parameters important for pre-mating isolation, through separate protein domains that are under distinct evolutionary constraints. Positive or sexual selection acting on N-terminal flightin sequences may explain its hypervariability.