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Flight and Mating Behavior of a Dual Heterozygote *Drosophila* expressing Flightin NH2-Terminal and COOH-Terminal Truncated Proteins

Flightin is a thick filament associated, myosin-rod binding protein that is essential for the functional and structural integrity of the indirect flight muscles (IFM) of *Drosophila melanogaster*. Flightin consists of an N terminal ~65 amino acid region whose sequence is poorly conserved among *Drosophila* species, a highly conserved ~58 amino acid middle region, and a semi-conserved ~44 amino acid C terminal region. The differences in sequence conservation raise the questions: what evolutionary selection processes are acting on flightin and to what avail? To address this, we created transgenic mutant *Drosophila* expressing an N-terminal truncated flightin missing 62 amino acids ($fln^{\Delta N62/\Delta N62}$), a C-terminal truncated flightin missing 44 amino acids ($fln^{\Delta C44/\Delta C44}$), and a double heterozygous line with both N and C terminal truncated contrasting ($fln^{\Delta N62/\Delta C44}$). To test the effect of these mutations on IFM function, we conducted flight test, wing beat frequency, and courtship assays. Flies that do not express flightin ($fln^{0/0}$) are completely flightless. We found that a single copy of the $\Delta N62$ gene ($fln^{\Delta N62/0}$) is able to partially rescue the $fln^{0/0}$ flightlessness and restore normal wing beat frequency (flight score*: 2.95 ± 0.2 vs. 4.2 ± 0.36 for fln^+ ; $p < 0.05$), indicating that the N-terminal region is not essential for optimal flight. In contrast, flies lacking the C-terminal region ($fln^{\Delta C44/\Delta C44}$ and $fln^{\Delta C44/0}$) are completely flightless, indicating that the flightin C-terminal region is essential for flight. Full flight ability was restored only in flies with the full complement of the C-terminal region ($fln^{\Delta N62/+}$), providing an explanation for the region's higher sequence conservation than the N-terminal region (3.66 ± 0.1). The dual heterozygote line ($fln^{\Delta N62/\Delta C44}$) was only partially able to rescue flight abilities, detailing that the two truncated flightin genes do not genetically complement each other ($2.89 \pm 0.13^*$ vs. 4.2 ± 0.36 for fln^+ ; $p < 0.05$). Current studies are testing the effect of these mutations on courtship song and courtship behavioral outcomes.