Harnessing single-molecule sequencing to characterize the fast-evolving Drosophila subtelomere

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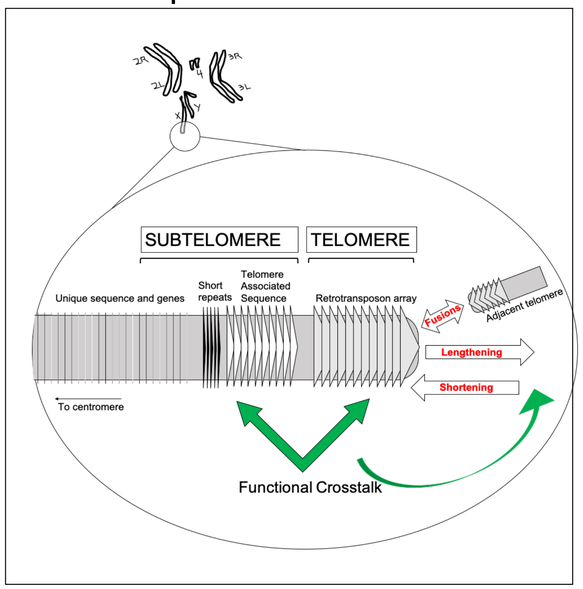
Abstract

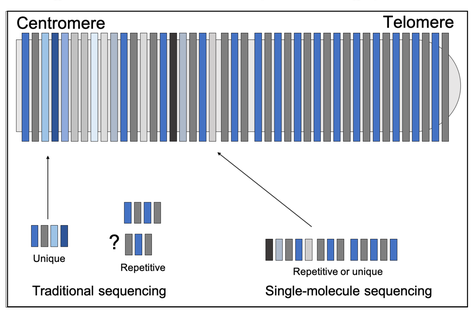
The telomere and subtelomere are repetitive sequences at the ends of chromosomes required for chromosome length preservation. In Drosophila, telomere and subtelomere are highly plastic; each of them varies in copy number and sequence both within and across species. In addition, there is evidence of functional crosstalk between telomere and subtelomere, suggesting that the two regions may co-evolve to maintain system fidelity. However, without characterizing the sequence of the subtelomere, we cannot investigate whether subtelomere evolution affects telomere function. This characterization has recently been made possible due to the advent of single-molecule sequencing, which can be used to assemble repetitive regions using long, 100 kilobase reads. Here, we begin to characterize the composition and variability of subtelomeric genes, focusing on exon duplications, intergenic distance variability, and functional open reading frame polymorphism.

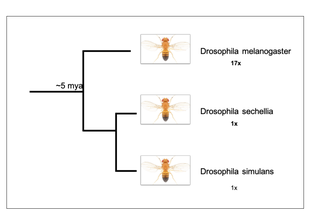
The Drosophila Subtelomere



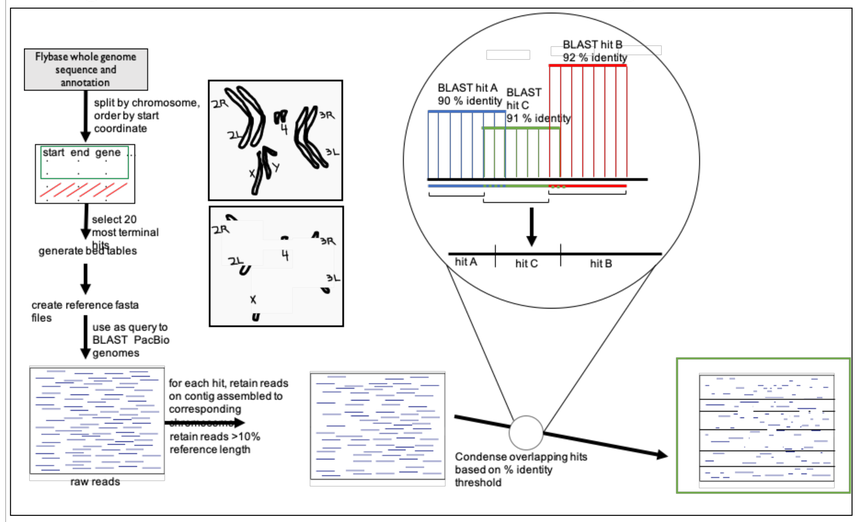
- Highly variable in copy number and sequence within species
- Rapidly evolving across species
- Pervasive terminal deletions
- Functional crosstalk with telomere has implications for genome integrity

Single-molecule sequencing permits subtelomere assembly

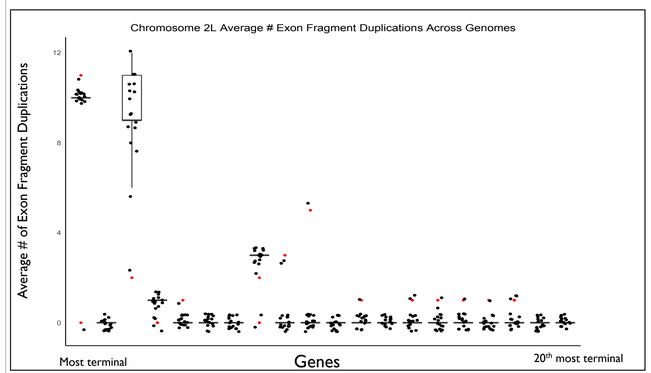


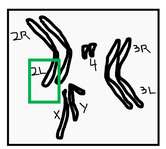


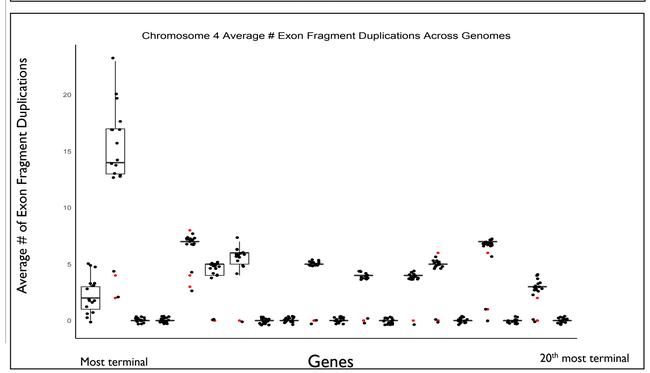
Use genome BLAST to find subtelomeric genes

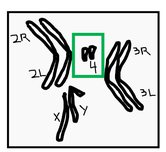


Exon fragment duplications are more common closer to the telomere

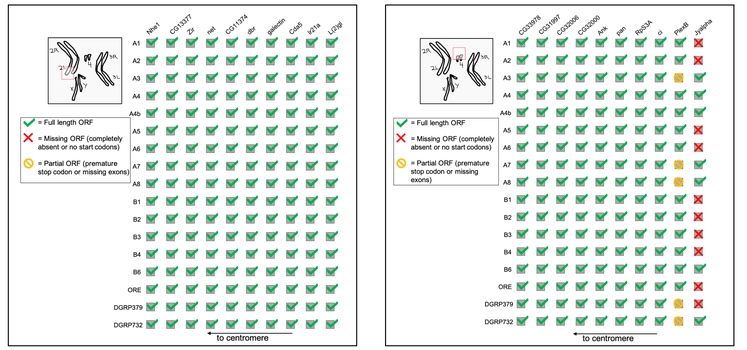


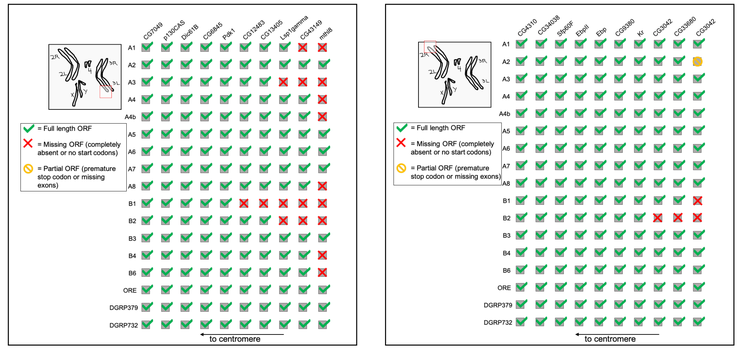






ORF polymorphism is concentrated closer to telomere





Experimental Validation:

- PCR: primers to absent genes, primers to unorthodox break points, primers across gaps
- Cell biology: DNA FISH to gene sequences, IF to proteins RNAseq to dysfunctional ORFs

References:

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