



Functional study of the testis-specific nuclear transport retrogenes, *Ntf-2r* and *Ran-like*, in *Drosophila melanogaster*

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Background

Ntf-2r and *Ran-like* are two young RNA-mediated gene duplications in *Drosophila* (Figure 1). Despite being derived from housekeeping nuclear transport parental genes (Figure 2), they are present only in a few *Drosophila* species including *Drosophila melanogaster*. Both *Ntf-2r* and *Ran-like* are testis specific in expression (Mirsalehi, 2021).

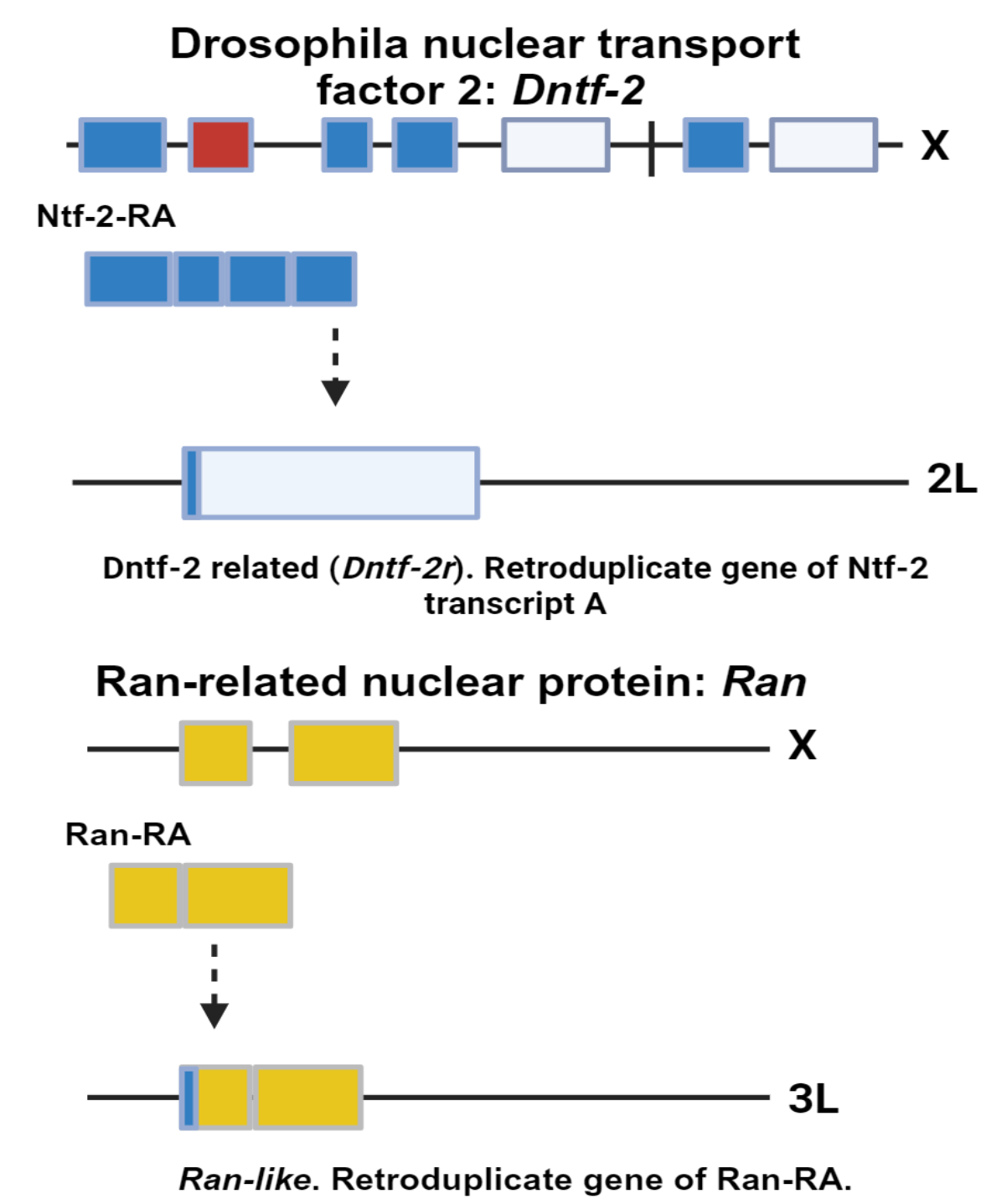


Figure 1. *Ntf-2* & *Ran* retroduplications named *Ntf-2r* & *Ran-like*

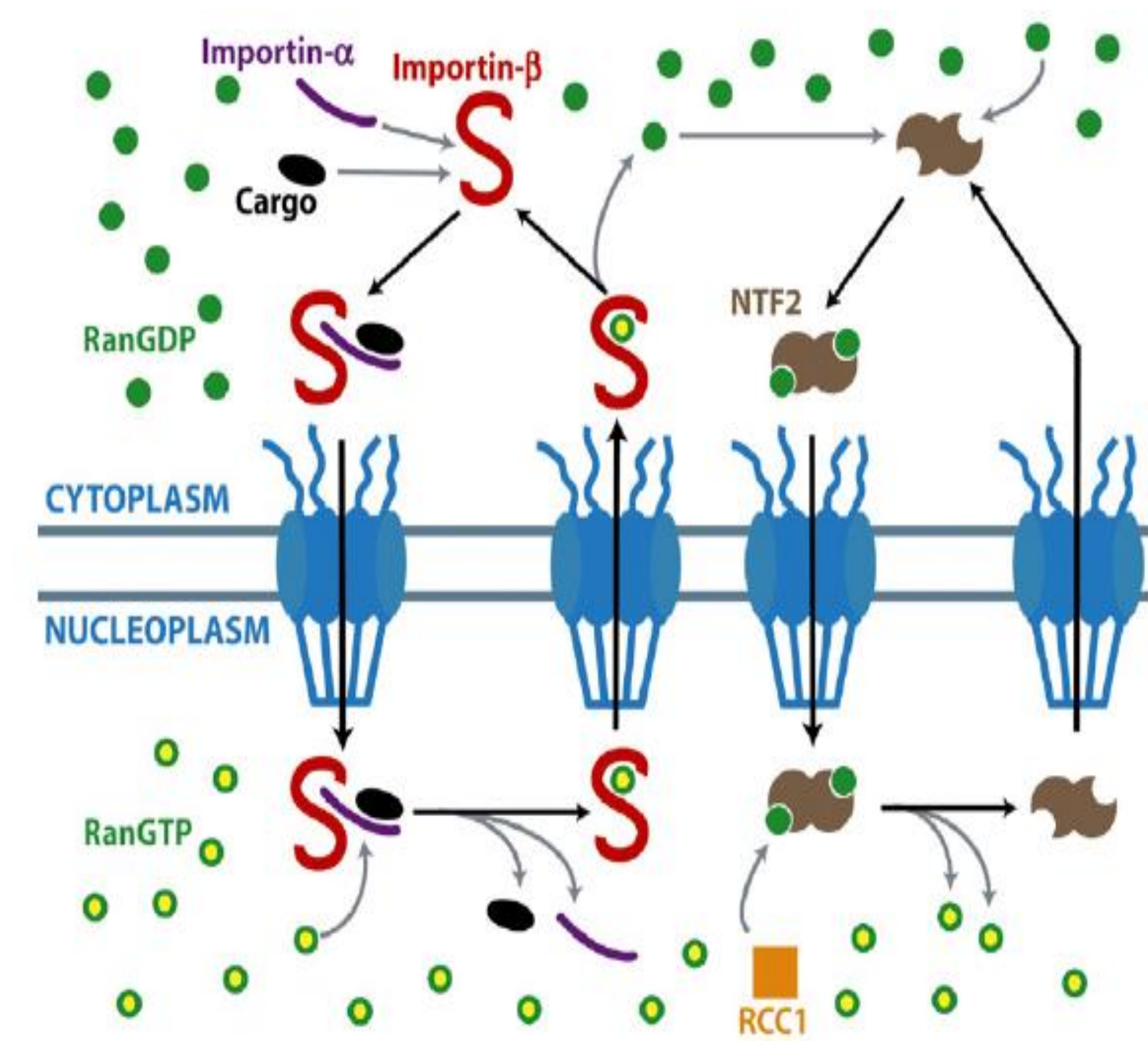


Figure 2. Conventional nuclear transport

Their relocation has facilitated the testis-specific expression and evolution under positive selection (Mirsalehi, 2021). So, they might have a new spermatogenesis function. Transgenes of *Ran-like* and *Ntf-2r* fused to red and green fluorescence protein, respectively, were made and their localization studied during spermatogenesis (Figure 3).

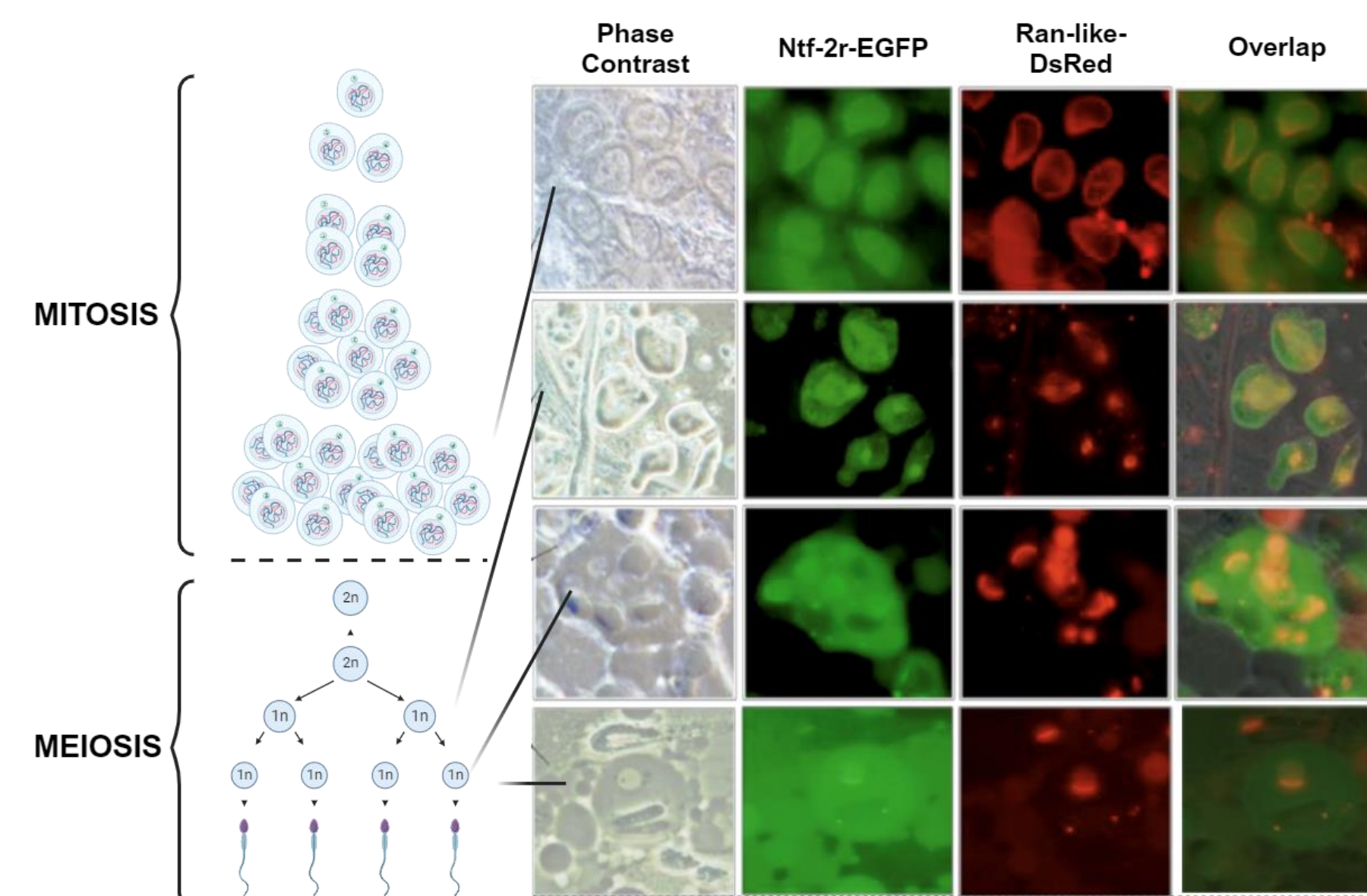
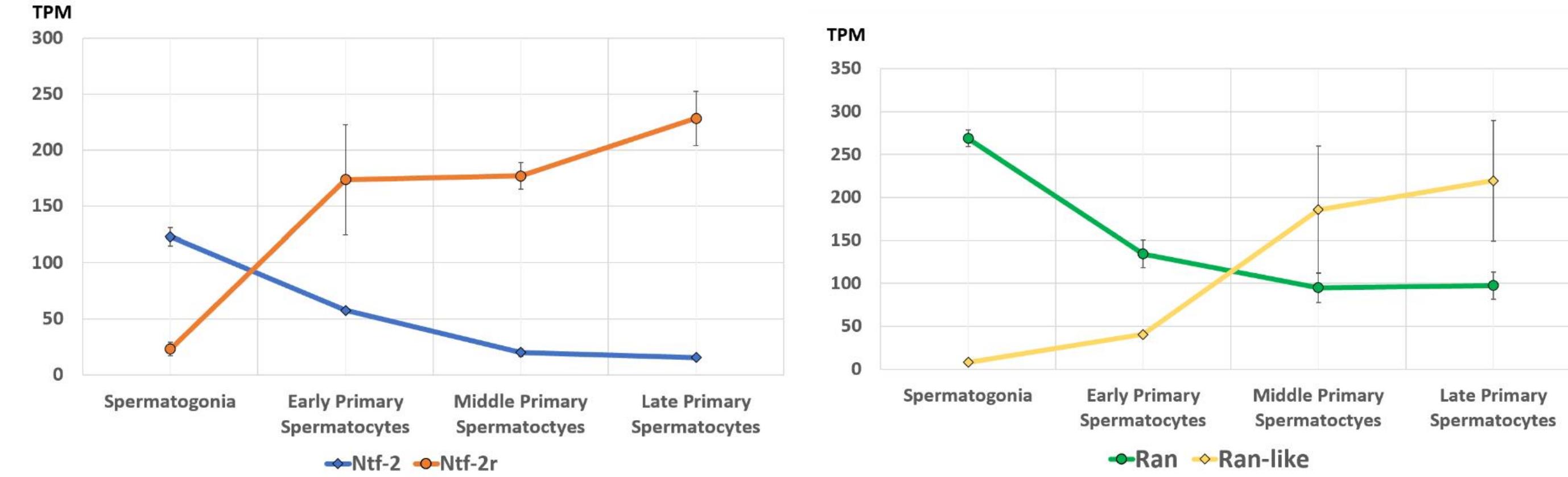


Figure 3. Spermatogenesis phases with expression of *Ran-like-DsRed.T4* column 3 and 4 (red) and *Dntf-2r-EGFP* column 2 and 4 (green). Fluorescence was observed starting at the 16-cell stage (Fabian and Brill 2012) and scRNA-seq data for *Ran*, *Ran-like*, *Ntf-2*, and *Ntf-2r* (Witt, et al.2019)

Materials and Methods

CRISPR-Cas9 technology was used to produce loss-of-function mutants, *Ran-like*-KO and *Ntf-2r*-KO. No male sterility was observed in those lines. So, we set up a long-term cage experiment to detect even small effects.

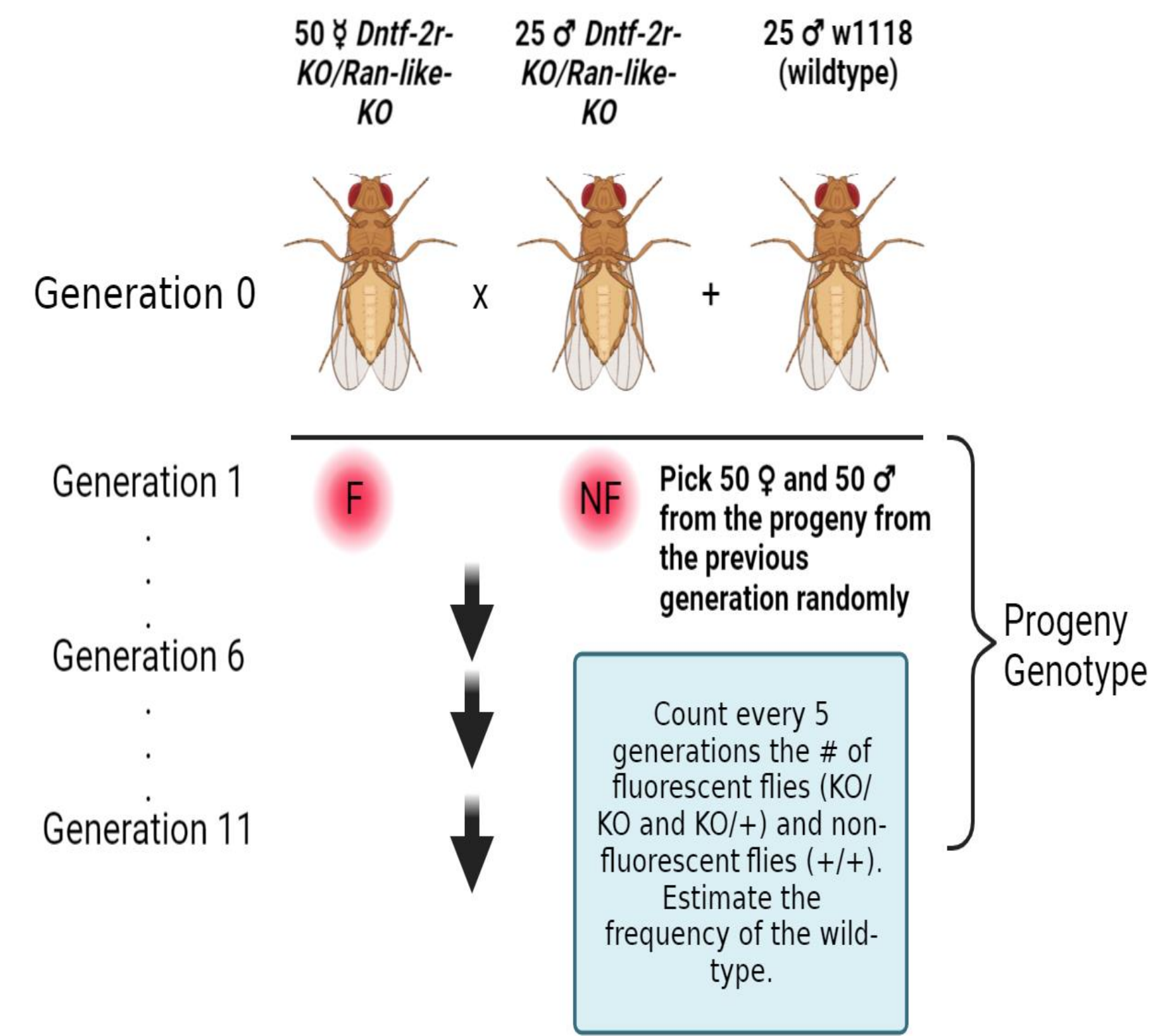


Figure 4. Cage experiment methodology.



Figure 5. Flies with fluorescent eyes due to Ds-Red protein replacing the gene of interest in the KO.

Results

I have been monitoring the change in frequency of the mutant allele, red fluorescence in the eye, every five generations. An increase in the average frequency of the wildtype allele was observed out of five replicates, supporting the idea that these genes have a significant role in spermatogenesis.

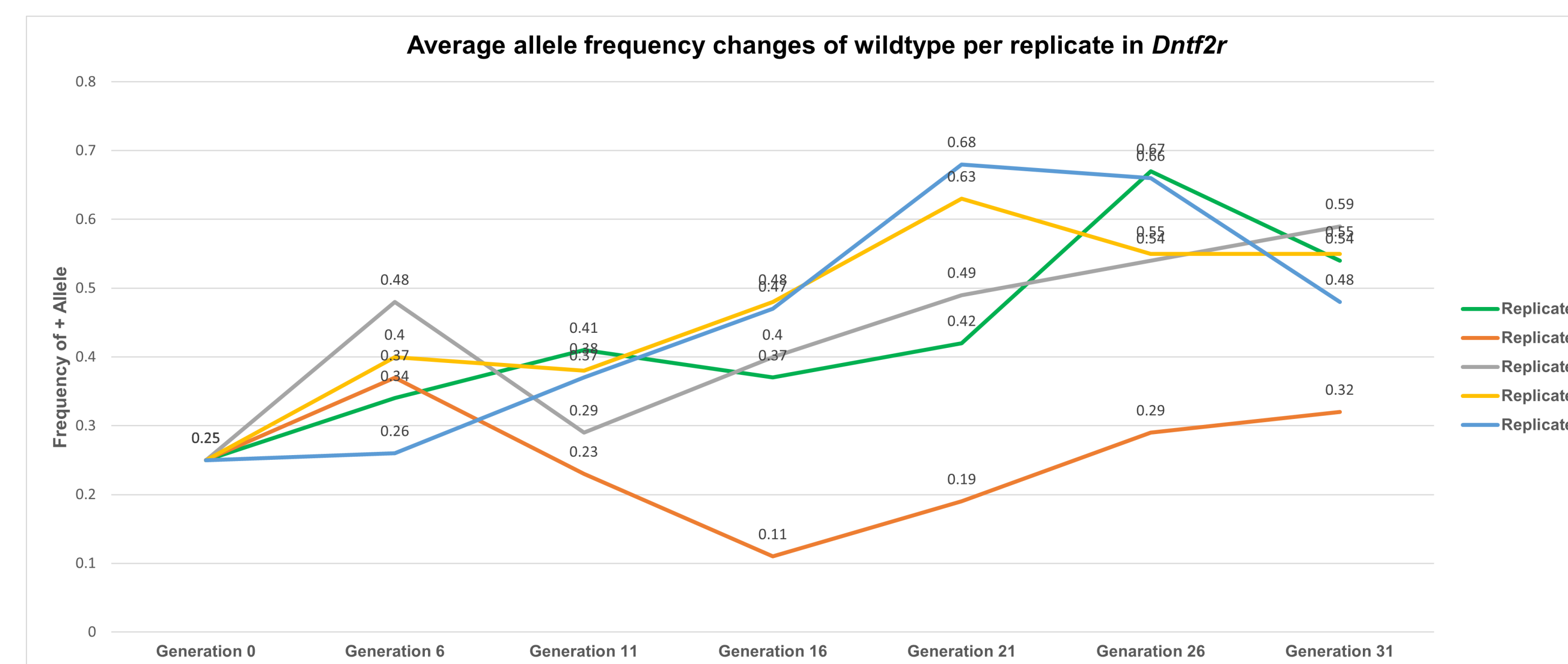


Figure 6. Allele frequency changes of the wildtype per replicate (q) in *Dntf2r*

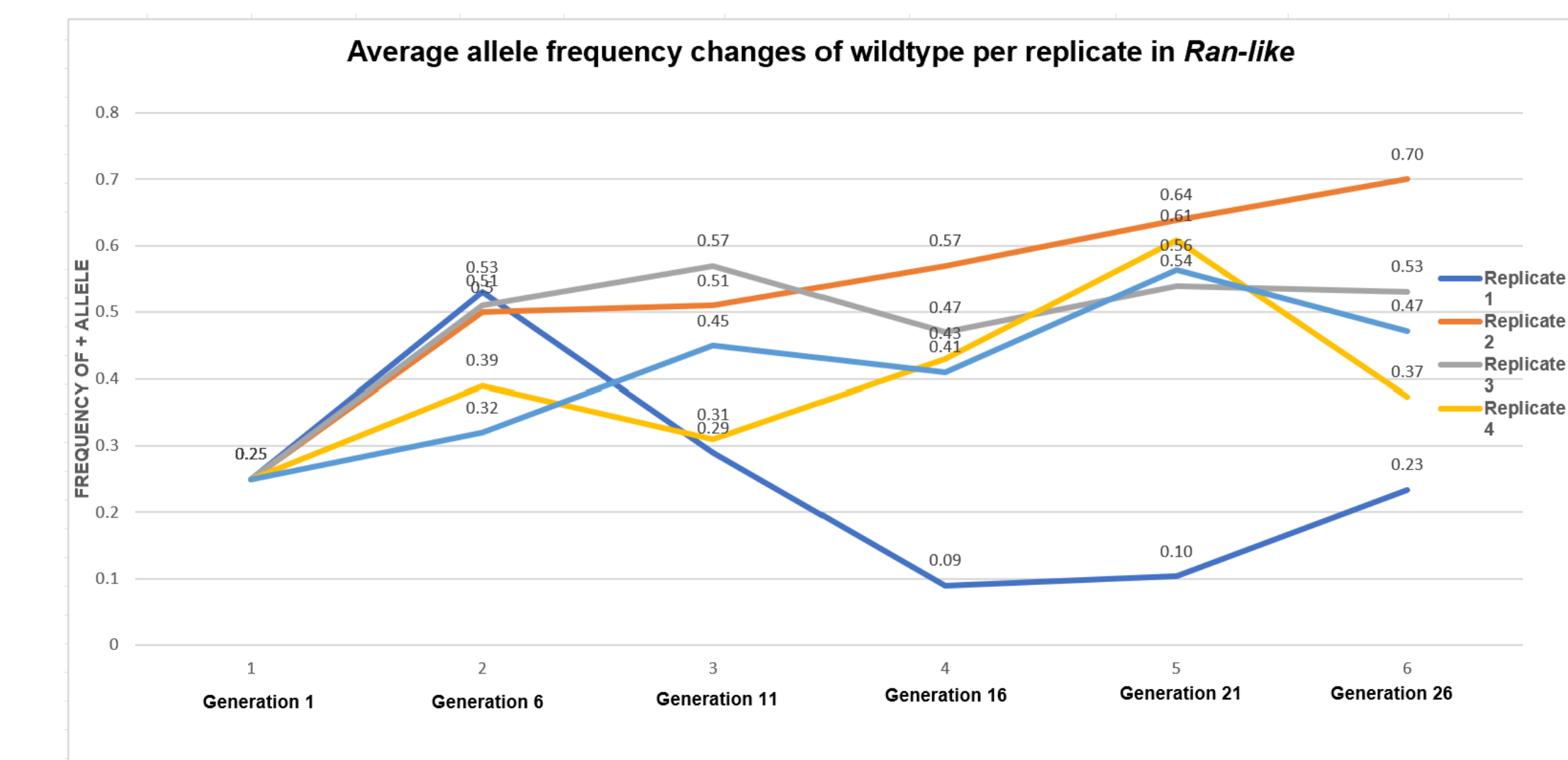


Figure 7. Allele frequency changes of the wildtype per replicate (q) in *Ran-like*

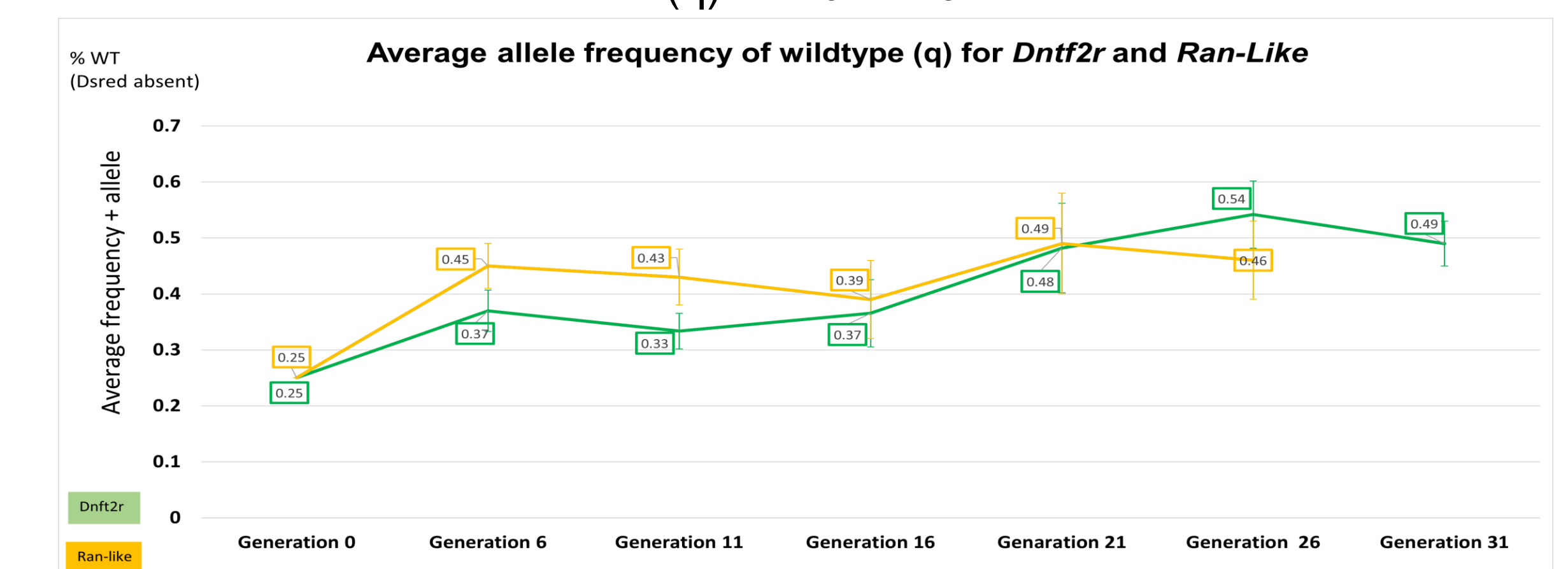


Figure 8. Allele frequency of the wildtype for *Dntf2r* and *Ran-like*

Conclusions

- In this long-term experiment, the frequency of the wildtype allele for both *Ntf-2r* and *Ran-like* has been steadily increasing, which differ from previous short-term experiments where the retrogenes showed no fertility effects in standard laboratory conditions. This demonstrates that the genes have a spermatogenesis function, but these effects are only strong enough to be observed long term
- In future experiments, we can study the reason why there is an effect in spermatogenesis only seen long-term

References

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