Genetic Modifiers Associated with Fly *ND2 M*odels of Complex-I Mitochondrial Diseases

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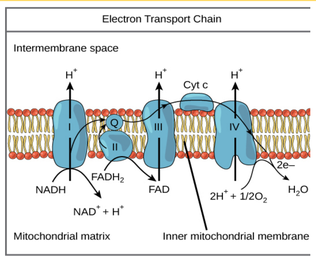
Introduction

- Complex-I mitochondrial diseases are associated with mitochondrial myopathy, progressive neurodegeneration, and shortened lifespan.
- The *ND2 (NADH d*ehydrogenase 2) gene encodes the ND2 protein subunit of complex-I.
- *Drosophila melanogaster ND2^{del1}* mutants exhibit behaviors such as paralysis that are similar to the effects of mitochondrial complex-I mutations in humans.
- Interactions between proteins encoded by mitochondrial and nuclear genomes are necessary for overall fitness and mitochondrial function.

Mitochondrial diseases exhibit phenotypic variation in human populations. There is a lack of studies aimed at understanding the causes for this variation. Genetic and metabolic variation present in a population might explain this observed disease phenotype variation.

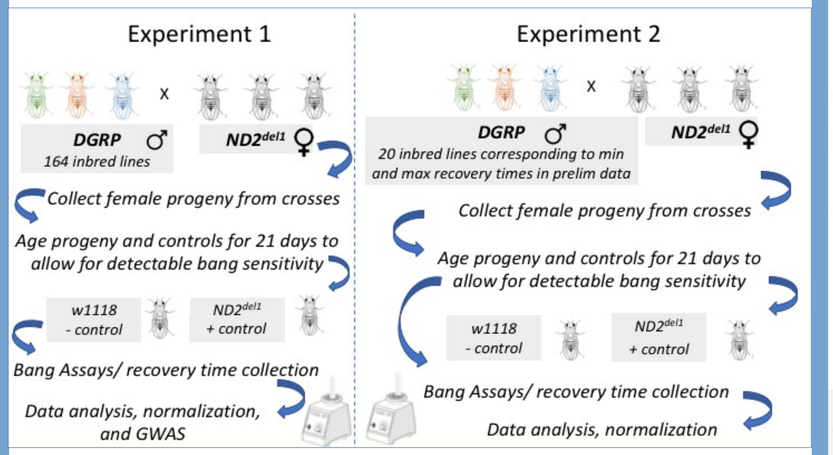
Objective



To determine how nuclear genetic variation present in a population can exacerbate or ameliorate the effects of the *ND2* gene mutation.

Methods

- ***ND2^{del1}*** flies were backcrossed to ***w1118*** flies
- ***Bang Assay:*** Flies are paralyzed using a vortexer
- ***Recovery Time:*** Required duration of time to recover from paralysis, measured in seconds.



- Performing experiment 2 allowed us to test for reproducibility of the *ND2^{del1}* phenotype.

Acknowledgements

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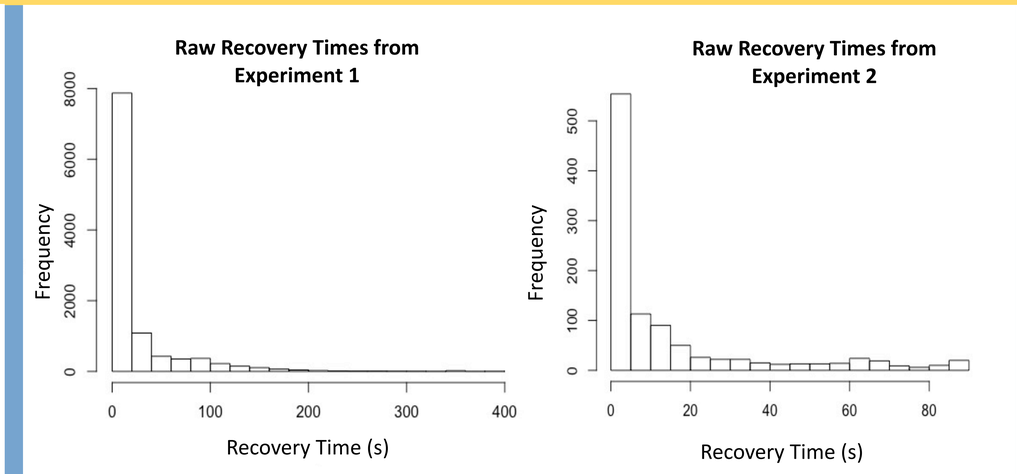
Results:



Figs. 1, 2: Recorded recovery times for both experiments 1 and 2 following bang assays. Flies that recovered from mechanical paralysis immediately were assigned a value of zero seconds. "Recovery" was defined as a fly's ability to completely stand up. Previous tests on the experiment 1 dataset demonstrated no background effect of the DGRP on bang sensitivity. Both datasets demonstrated significant variance in recovery times between different DGRP and control lines (Kruskal Wallis: $\chi 2$= 259.02, df = 21, $P < 2.2 \times 10^{-16}$ for the experiment 2 dataset and $\chi 2$= 2357.8, df=166, $P < 2.2 \times 10^{-16}$ for the experiment 1 dataset). This indicates significant variance in mutant *ND2* phenotypes between different genotypes.

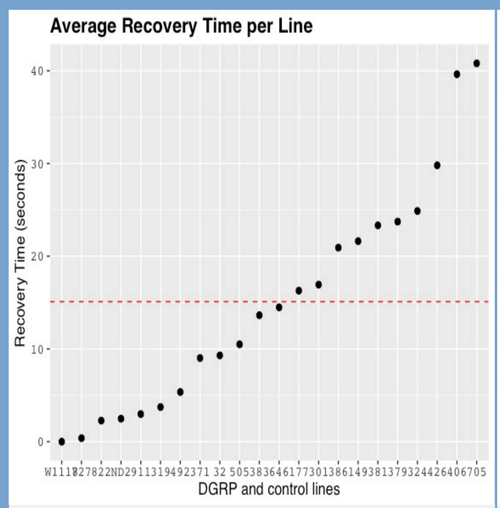


Fig. 3: Average Recovery Time Values for each DGRP and control line from experiment 2. The average recovery time across all lines (dashed red line) is equal to 15.097.

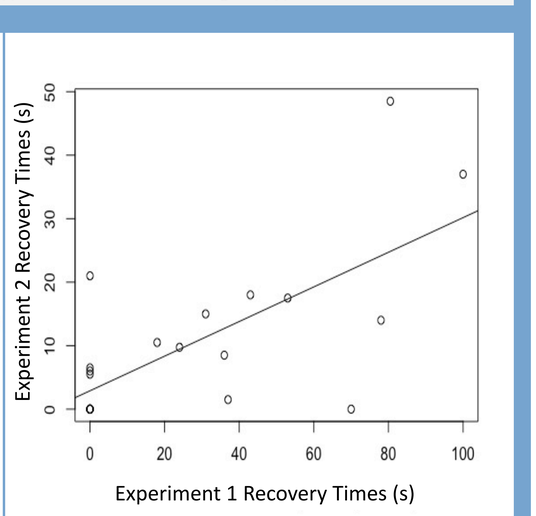


Fig. 4: Correlation between median recovery time values from both experimental datasets. The two datasets demonstrated mild correlation in median recovery times between corresponding different DGRP and control lines (Pearson's r= 0.69, df = 20, *P < 0.0004).*

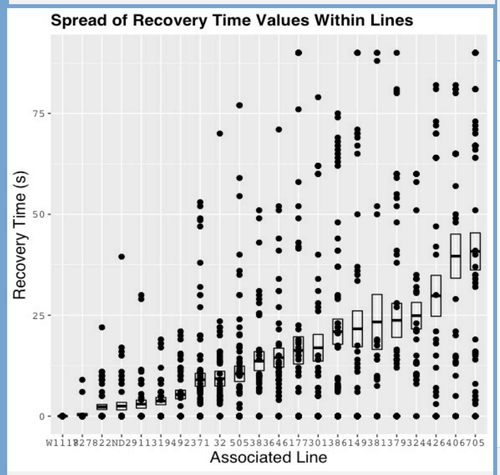


Fig. 5: Visualizing the spread of recovery time values within lines from the experiment 2 dataset. Bars represent standard errors within each line.

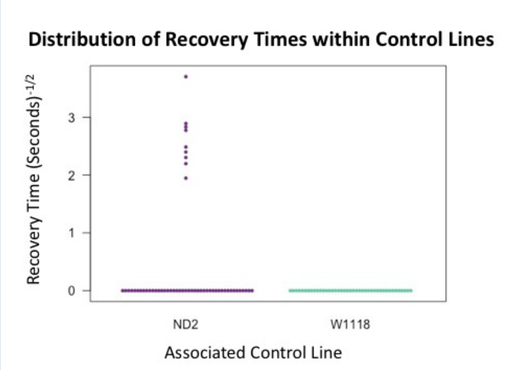
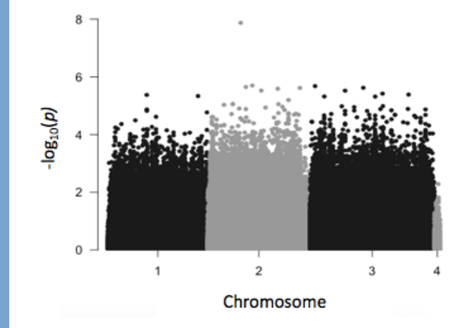


Fig. 6: Testing for ND2 effect in comparison to wildtype *w1118* from the experiment 2 dataset. Average recovery time of *ND2* is 2.48 seconds and average recovery time of *w1118* is 0 seconds among 48 samples per line.

Fig. 7 (right): Attempt at conducting GWAS on experiment 1 dataset to identify possible SNPs associated with mutant *ND2* phenotype variation. Dataset consisted of 164 distinct DGRP lines as well as *ND2* and w*1118* controls from 23 groups. No significant SNPs were identified.



Discussion

Significance:

- Our results demonstrate significant evidence for genetic variation for mitochondrial variation (Figs. 1-3, 5).
- Our results were significantly correlated between both datasets (Fig. 4), indicating that the *ND2^{del1}* phenotype is reproducible.
- Our results confirm and expand upon the conclusions of previous studies that have indicated links between mitochondrial haplotypes and nuclear genomes.
- ***Collectively,*** these results suggest a possible explanation for variable mitochondrial complex-I disease phenotypes within populations.

Future Work:

- Test for heteroplasmy between lines
  - ***Why:*** As a possible explanation for why *ND2^{del1}* did not demonstrate significant bang sensitivity in comparison to wild-type *w1118* (Fig. 5).
- Conduct targeted metabolomics for metabolites such as NAD^+.
  - ***Why:*** Previous studies demonstrated links between variable NAD^+ concentrations and complex-I dysfunction as well as links between the *Drosophila* metabolome and age-related disease phenotypes.

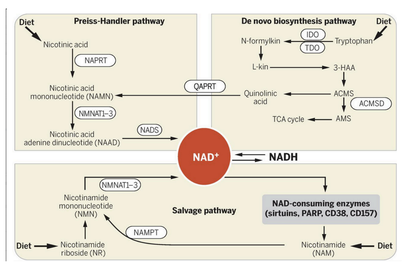


Fig. 2 from Verdin, E. "NAD+ in Aging, Metabolism, and Neurodegeneration." *Science* 350, (2015): 1208-1213. Web.

Conclusion

Our results indicate significant epistatic interactions between nuclear and mitochondrially encoded mutation associated with neurodegeneration.

References

Burman, J. L., Itsara, L. S., Kayser, E.B., Suthammarak, W., Wang, A.M., Kaeberlin, M., Sedensky, M. M., Morgan, P. G., and Pallanck, L.J. "A *Drosophila* model of mitochondrial disease caused by a complex I mutation that uncouples proton pumping from electron transfer." *Disease models & mechanisms,* vol. 7,(2014): 1165- 74. Web.

Ederer, K. A., Jin, K., Bouslog, S., Wang, L., Gregory, G.S., Glenn, R.C., Abadir, P., Raftery, D., Moellering, D., Promislow, D., Jumbo-Lucioni, P., and De Luca, M. "Age- and Genotype-Specific Effects of the Angiotensin-Converting Enzyme Inhibitor Lisinopril on Mitochondrial and Metabolic Parameters in *Drosophila melanogaster*." *International Journal of Molecular Sciences,* vol. 19, (2018): 1-17. Web.

Katsyuba, E. et al. "De novo NAD+ synthesis enhances mitochondrial function and improves health." *Nature,* vol. 563, (2018): 354-359. Web.

Mackay, T. F. C., et al. "The *Drosophila melanogaster* Genetic Reference Panel." *Nature,* vol. 482, (2012), 173–178. Web.

Montooth, Kristi L et al. "Mitochondrial-nuclear epistasis affects fitness within species but does not contribute to fixed incompatibilities between species of Drosophila." *Evolution; international journal of organic evolution* vol. 64,12 (2010): 3364-79. Web.

Mossman, J.A., Ge, J.Y., Navarro, F., and Rand, D.R., "*Mitochondrial DNA Fitness Depends on Nuclear Genetic Background in Drosophila." G3: Genes | Genomes | Genetics*, G3: Genes, Genomes, Genetics, vol. 9, (2019): 1175-1188. Web.

Reynolds, E. R. "Shortened Lifespan and Other Age-Related Defects in Bang Sensitive Mutants of Drosophila Melanogaster." *G3: Genes | Genomes | Genetics*, G3: Genes, Genomes, Genetics, vol. 8, (2018): 1-8. Web.

Ugalde, C., et al. "Mutated ND2 Impairs Mitochondrial Complex I Assembly and Leads to Leigh Syndrome." *Molecular Genetics and Metabolism*, vol. 90, (2007): 10–14. Web.

Verdin, E. "NAD+ in Aging, Metabolism, and Neurodegeneration." *Science, American Association for the Advancement of Science,* vol. 350, (2015): 1208-1213. Web.