Genomics of experimentally-evolved postponed reproduction in *Drosophila melanogaster*

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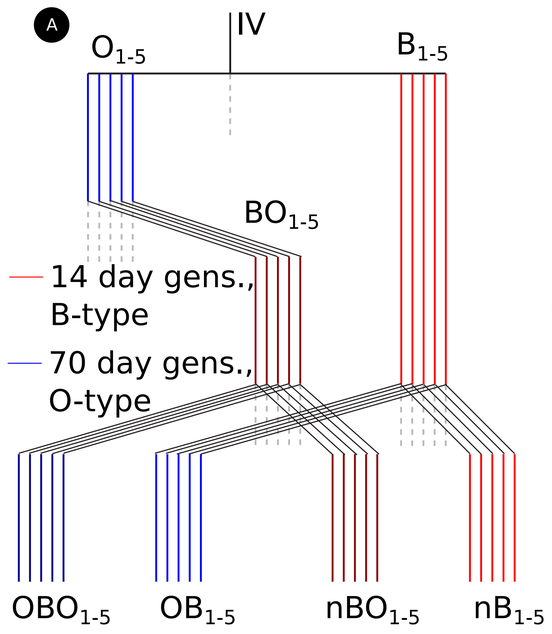
Introduction



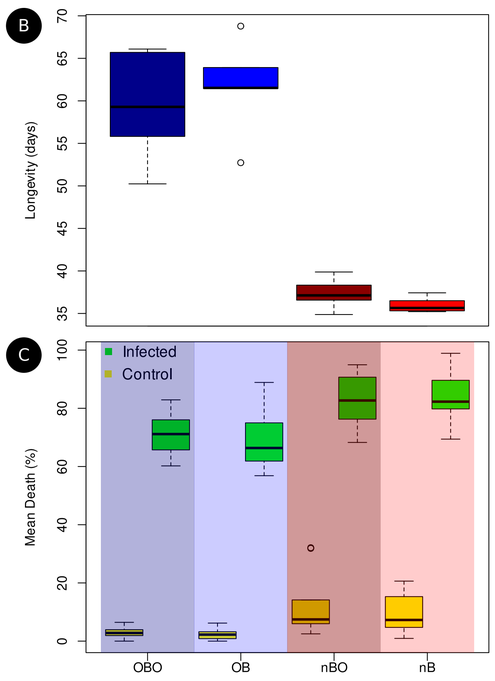


Fig 1 | A) Experimental System. Blue and red colors indicate the O-type and B-type selection regimes, respectively. Treatments OBO and nBO share recent ancestry, as do treatments OB and nB. All treatments include 5-fold replication, and replicates have continuous ancestry. Populations were assessed initially, and after ~20 generations of O-type selection.

B) Longevity. O-type populations live longer than B-type populations (ANOVA; $p<10^{-16}$).
Methods: At O-type generation 20, dead flies were sexed, counted, and removed from cages 4x/week until all flies died, in 3 replicate cages per population (N=560 files/cage). Only female data are shown.

C) Immune defense. O-type populations survive infection at higher rates than B-type populations (ANOVA; $p<10^{-5}$).
Methods: At O-type generation 22, two infection cages were sprayed with spores of Beauveria bassiana *GHA strain, and two control cages were handled in parallel. Dead flies were sexed, counted, and removed from cages over 14 days (N ~ 200 flies/cage). Only female data are shown.*

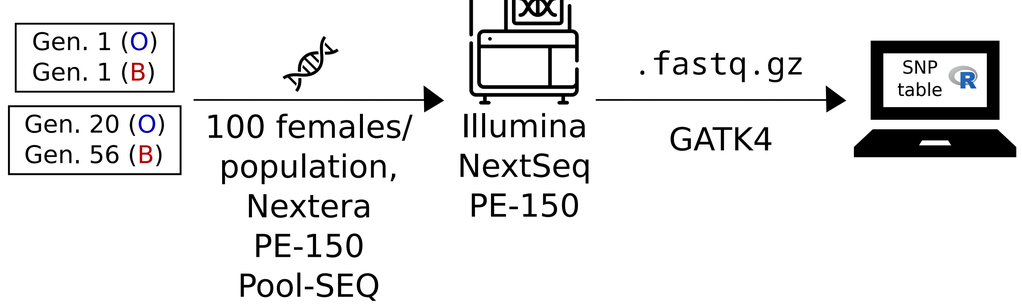
Questions

1. What genomic regions can we associate with adaptation to experimental postponed reproduction?

2. How much parallelism do we observe across independent replicate populations?

3. Does recent evolutionary history affect populations adapting to a novel selection regimen?

Methods



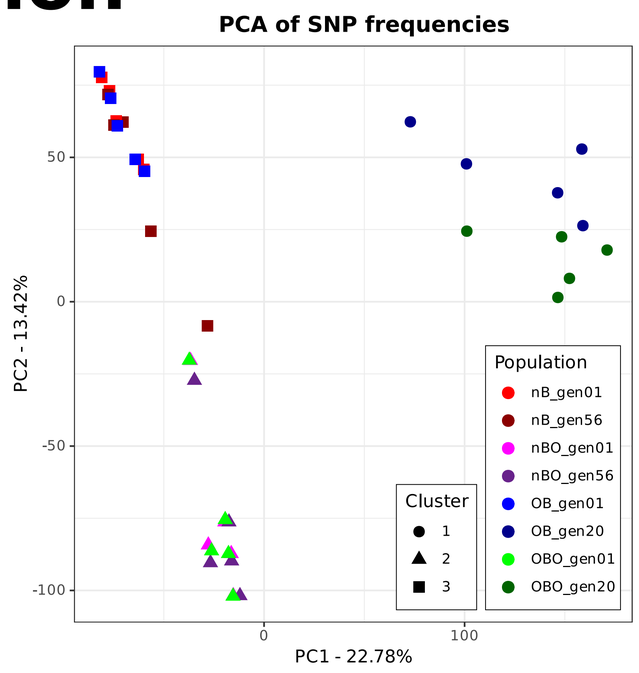
Outcomes
171,539,930 reads/pop (mean)
106X genome-wide coverage/pop (mean)
1,086,149 SNPs (post filtering)

SNP analysis: Principal Components Analysis (PCA), Adapted CMH test [1] [2].
Gene Ontology (GO) Enrichment Analysis: biomaRt [3], clusterProfiler [4].

Results & Discussion

Fig 2 | PCA of SNP frequencies.
A total of 1,086,149 frequencies were used to assess genomic similarity across all populations at the two timepoints. A k-means cluster algorithm grouped populations according to selection treatment: nBO generations 1 and 56 & OBO generation 1, nB generations 1 and 56 & OB generation 1, and OBO & OB at generation 20. Evolved O-type populations cluster together, regardless of ancestry.

Methods: Principal components and k-mean clusters calculated using the stats package in R.



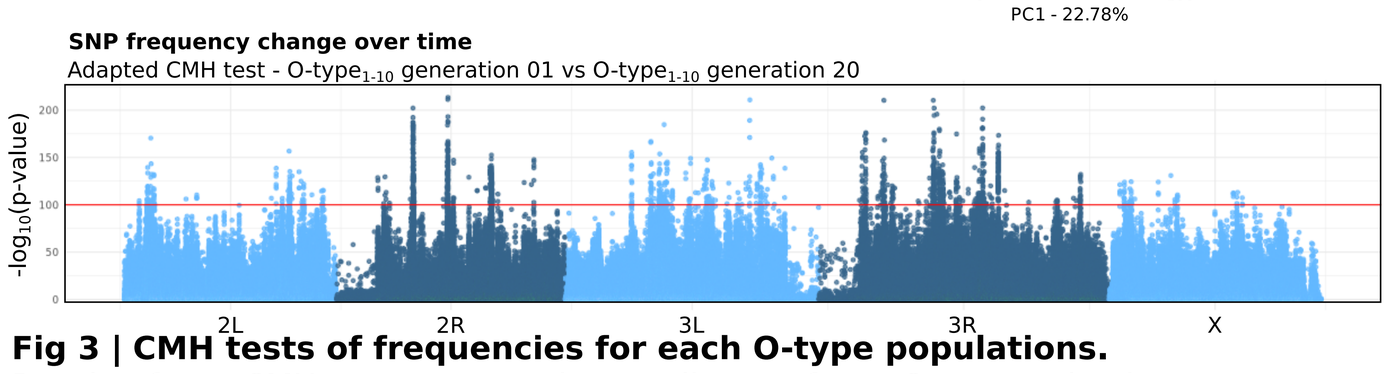


Fig 3 | CMH tests of frequencies for each O-type populations.
Results from CMH tests comparing replicates from O-type selection treatments. Points show significance of SNP frequency change over time. "Peak" regions above the threshold are potentially associated with selection for postponed reproduction.
Methods: P-values calculated using the adapted.cmh.test [1]. Horizontal red line represents an empirical threshold of statistical significance. Data were scaled to normalize coverage. P-values are FDR-corrected.

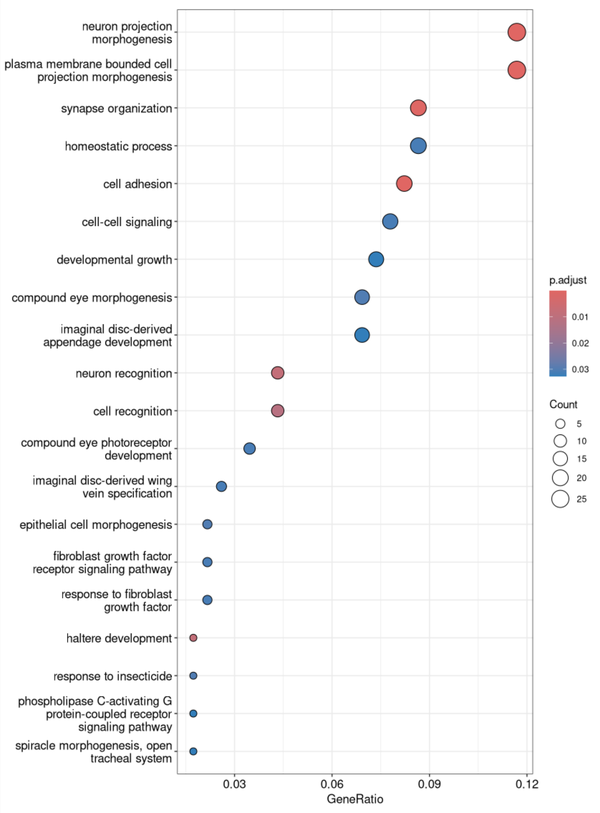


Fig 3 | Gene Ontology (GO) Enrichment Analysis of significant genes.
Dotplot showing top enriched Biological Process GO terms. Datapoints represent GO terms, and x-axis indicates Gene Ratio (ratio of genes associated with the term relative to all genes). Color represents FDR-adjusted p-value, and size corresponds to number of genes associated with each term. We observe 1,284 significant SNPs in 300 genes. Most significant GO terms include **neuron projection morphogenesis**, **plasma membrane bounded cell projection morphogenesis**, **synapse organization**, and **cell adhesion**.

Methods: Using the biomaRt R package, we obtained a list of genes that contain a statistical significant SNP. clusterProfiler R package was used for GO enrichment analysis and simplification.

Conclusion

1. Major changes in allele frequency are present in regions potentially associated with postponed reproduction. These regions are enriched for the general terms **cell morphogenesis** and **nervous system development** [5].

2. We observe a high degree of within-treatment parallelism, evidenced by the PCA of SNP frequencies.

3. We observe rapid change in SNP frequencies in all O-type populations, suggesting that evolutionary history matters little to future adaptation

Acknowledgments and References

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[1] Taus, Futschik, and Schlötterer 2017, *Mol. Bio. Evol.*
[2] Spitzer, Pelizzola, Futschik 2020, *Ann. Appl. Stat.*
[3] Durinck et al. 2009, *Nature Protocols*.
[4] Yu et al. 2012, *The Innovation*.
[5] PANTHER.db, 10.18129/B9.bioc.PANTHER.db