

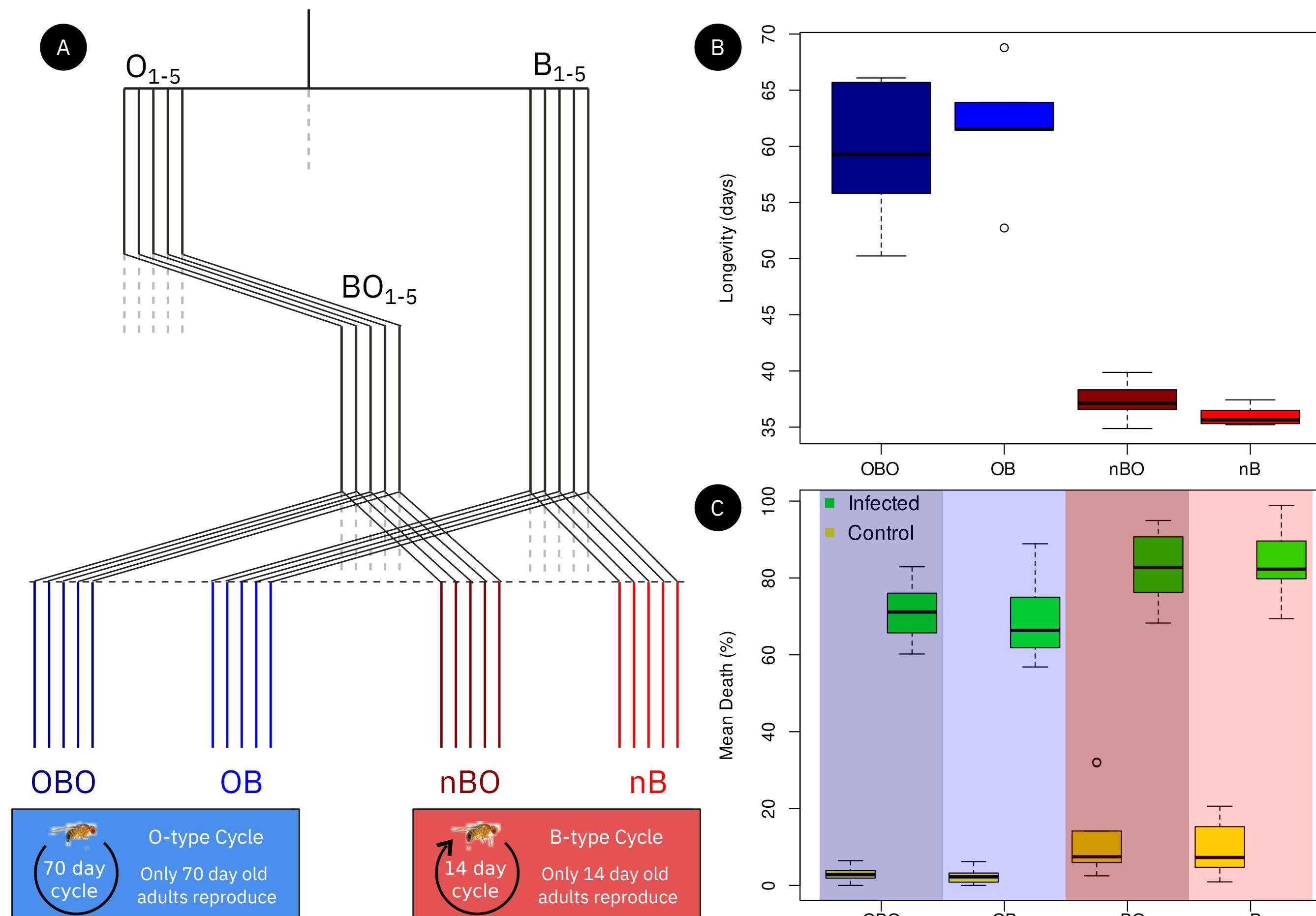
# Genomics of experimentally-evolved postponed reproduction in *Drosophila melanogaster*

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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 ( $N = 560$  flies/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 ( $N \sim 200$  flies/cage). Only female data are shown.

## Questions

- What genomic changes can we associate with experimental evolution for postponed reproduction?
- How much parallelism do we observe across independent replicate populations?
- Does recent evolutionary history affect populations adapting to a novel selection regimen?

## Methods

Gen. 1 (O) Gen. 20 (O)  
Gen. 1 (B) Gen. 56 (B)

100 females/pop;  
Nextera genomic  
library prep;  
Pool-SEQ

Illumina  
NextSeq  
PE 150

fastqs GATK 4 R

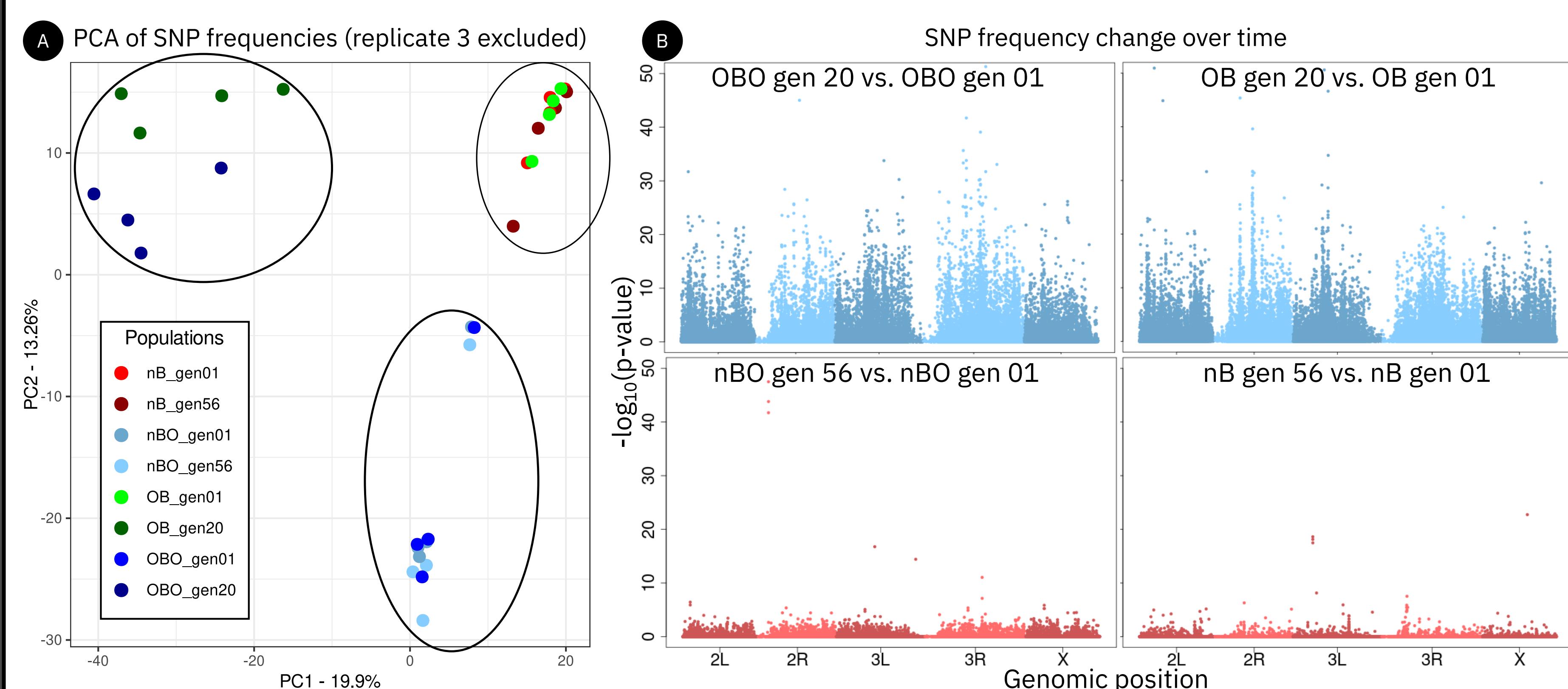
### Analysis Parameters

- Replicate 3 dropped due to low coverage
- MAF cutoff:** 0.025
- Genome-wide SNP coverage filter:** 5 to 300
- R Packages:** poolSEQ [1], ACER [2]
- SNP analysis:** Principal Components Analysis (PCA); Cochran-Mantel-Haenszel (CMH) test.

### Outcomes

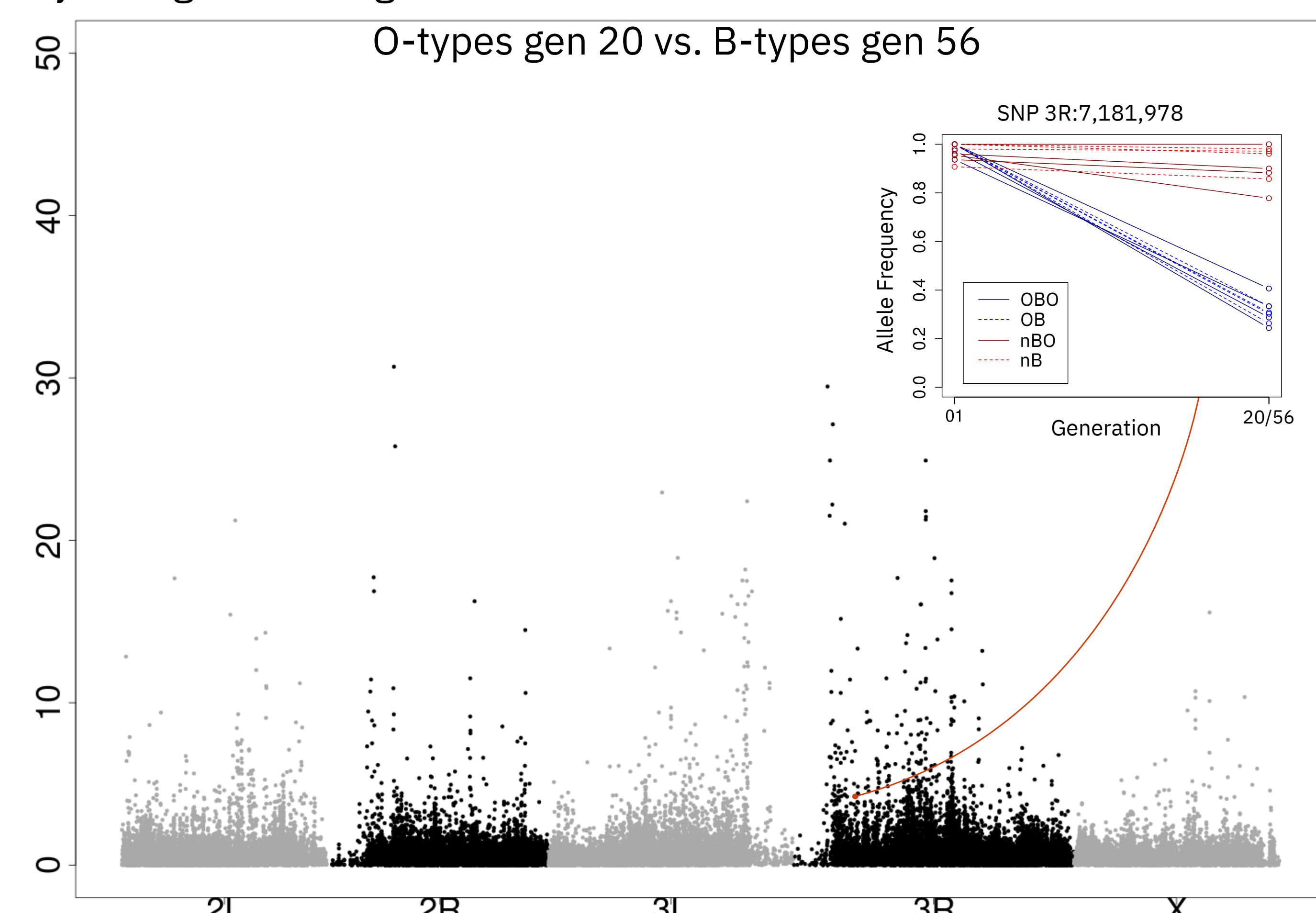
- 53,290,208 Reads/pop (mean)
- 35X genome-wide coverage/pop (mean)
- 67,781 SNPs (post filtering)

## Results & Discussion



**Fig 2 | A) PCA of SNP frequencies.** A total of 67,781 frequencies were used to assess genomic similarity across all populations at each timepoint. Bottom oval: nBO generations 1 and 56 & OBO generation 1. Top right oval: nB generations 1 and 56 & OB generation 1. Top left oval: OBO & OB at generation 20. O-type populations cluster together at generation 20, regardless of ancestry.

**B) CMH tests of frequencies for each treatment.** Results from CMH tests comparing replicates from each selection treatment. Top: results for O-type treatments indicate dramatic SNP frequency change over 20 generations. Bottom: results for B-type treatments indicate negligible SNP frequency change over 56 generations.



**Fig 3 | Combined O-type populations vs. combined B-type populations.** Results from CMH tests comparing all O-type populations at 20 generations with all B-type populations at the corresponding generation (56). Clustered "peaks" of data points indicate potential candidate regions associated with selection for postponed reproduction. Inset: SNP frequencies from a SNP in a peak region on chromosome 3R showing the reference genome allele decreasing in the O-type populations, but not in the B-type populations.

## Conclusion

- Initial results suggest dozens of genomic regions associated with selection for postponed reproduction, evidenced by consistent SNP frequency changes across all O-type populations.
- We observe a high degree of within-treatment parallelism, evidenced by the PCA of SNP frequencies.
- We observe rapid change in SNP frequencies in all O-type populations, suggesting that evolutionary history matters little to future adaptation.

## Acknowledgments and References

We thank Dr. Michael Rose (UC Irvine) for generously providing the ancestral populations that were used to find this experiment.

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[1] Taus, Futschik, and Schlötterer 2017, Mol. Bio. Evol.

[2] Spitzer, Pelizzolla, Futschik 2020, Ann. Appl. Stat.