Experimental evolution for postponed reproduction improves longevity and immune defense in *Drosophila melanogaster*

Giovanni Crestani*, Karen Walsh#, Alejandro Moran#, Hannah S. Dugo*, Parvin Shahrestani#, and Molly K. Burke*

* Department of Integrative Biology, Oregon State University.
# Department of Biological Science, California State University, Fullerton.

Oregon State University

Introduction

Research with *Drosophila melanogaster* and other species shows that a population's mean longevity responds to experimental evolution on generation time [1]. Other life-history traits, including those associated with stress resistance, also evolve concomitantly [2]. Here, we compare four selection treatments, each with 5-fold replication. The goals of this novel selection experiment are to: a) determine the phenotypic correlations between experimentally-evolved postponed longevity and immune defense; and b) to determine how rapidly populations that share a selection treatment converge phenotypically and genomically, regardless of their ancestral origin.

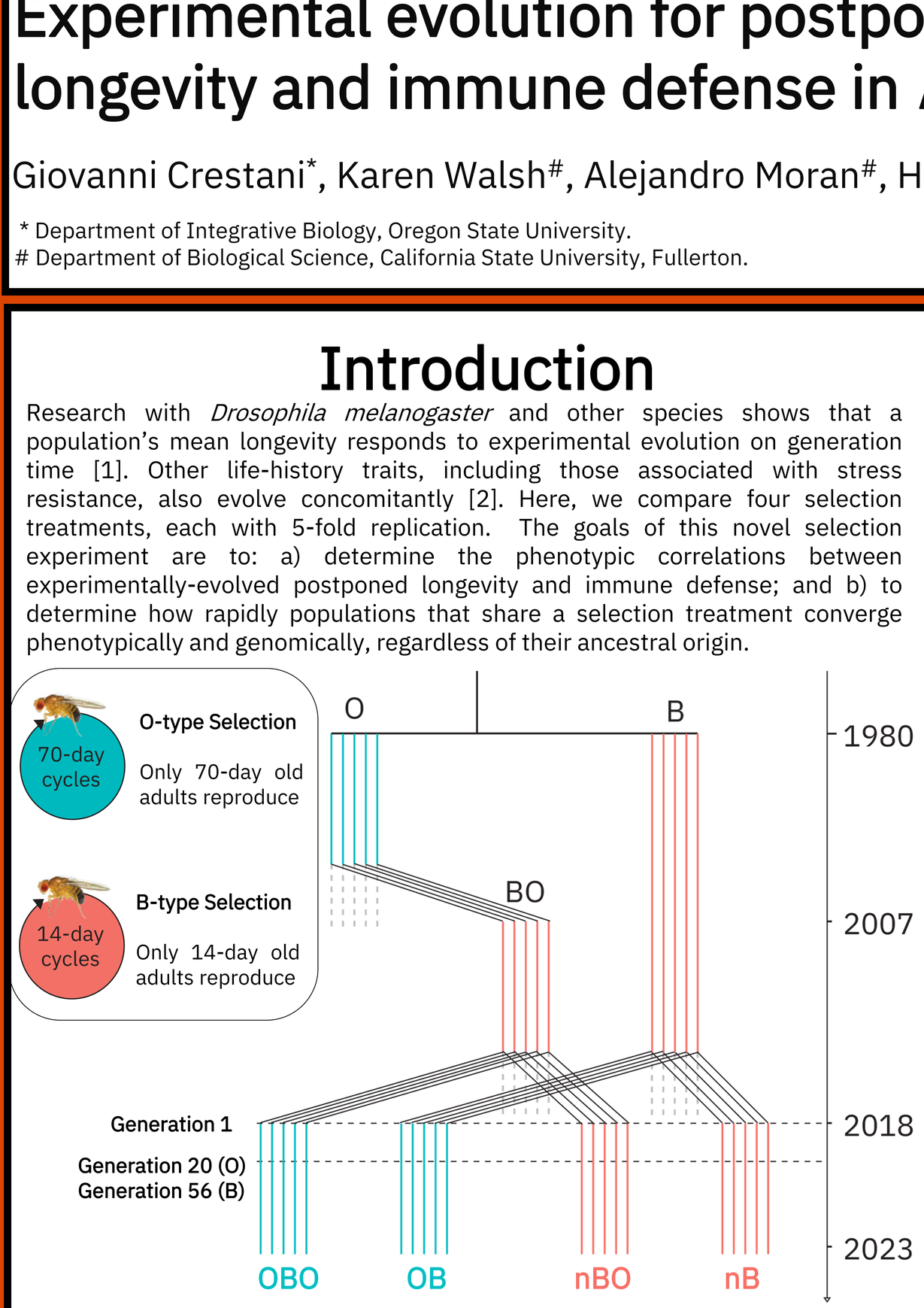


Fig 1 | Experimental evolution study system. Colors indicate the two selection treatments: O-type (nB & nBO) for 70-day generations, and B-type (OB & OBO) for 14-day generations. Populations nB and OB share recent ancestry, as do populations nBO and OBO.

Objectives & Hypotheses

Our objectives with this project are to evaluate innate immune defense in laboratory populations with and without extended longevity, and to characterize the genomic basis of this phenotype.

Hypothesis 1: Innate immune defense will be higher in the O-type populations, compared to the B-type populations, when surveyed at the same age.

Hypothesis 2: We will observe convergence across all the O-type populations, at both the phenotypic and genomic level, within 20 generations.

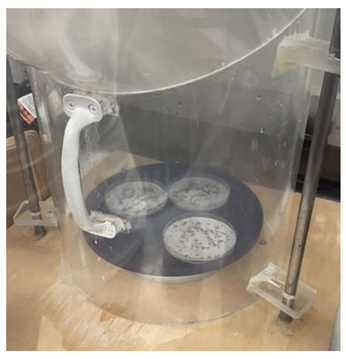
Methods

Phenotype assays

- Longevity assayed after 20 gens of O-type selection (56 gens of B-type selection)
- Immune defense, as measured by survival post-infection with an entomopathogenic fungus [3], assayed after 22 gens of O-type selection (65 gens of B-type selection)

Genomic analysis

- O-type flies sequenced at gen 1 and gen 20; B-type flies sequenced at gen 1 and gen 56
- 100 females from each replicate population were processed for Pool-SEQ genomic analysis.
- Sequencing libraries prepared with Illumina Nextera reagents
- For the initial library, all 40 samples multiplexed into a pool sequenced on a NextSeq P2 (PE150)



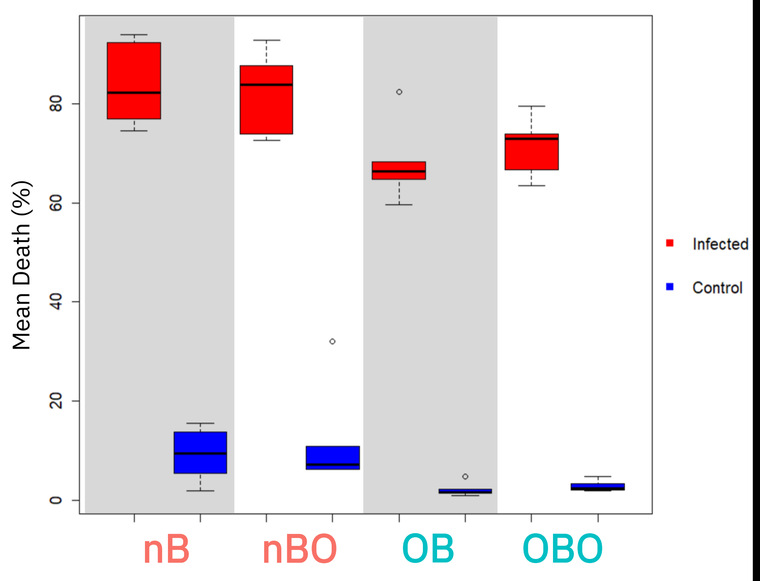
*Sharestani's lab Spray Tower spraying spores of **Beauveria bassiana** on adult flies.*

Results

Phenotypes

Fig 2 | O-type populations survive infection at higher rates than B-type populations (ANOVA; $p<10^{-5}$). This result provides support for Hypothesis 1. We did not observe a significant effect of ancestry (ANOVA; p=0.094), providing support for Hypothesis 2.

Methods: Each population was split into 2 "infection" cages sprayed with spores of *Beauveria bassiana* GHA strain, and 2 uninfected control cages. Dead flies were sexed, counted, and removed from cages over 14 days (N~200 flies/cage). Only data from females shown.



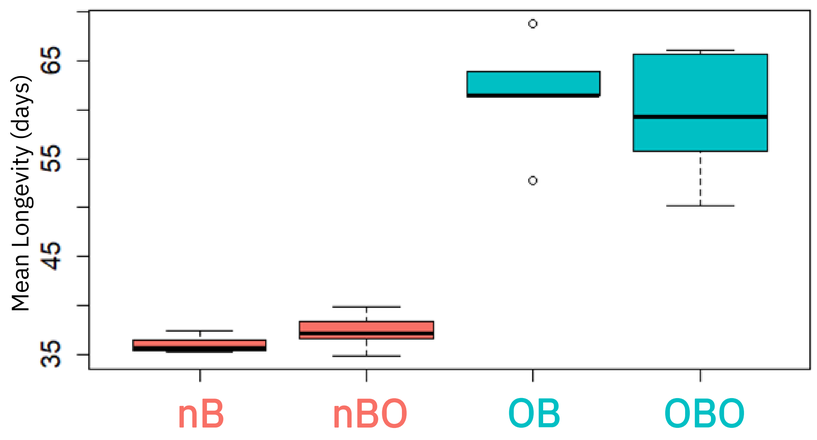


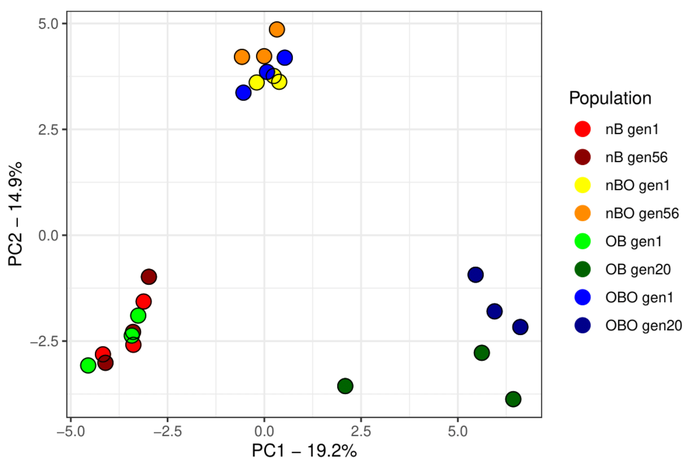
Fig 3 | O-type populations live longer than B-type populations (ANOVA; $p<10^{-16}$), though we also observed a significant effect of ancestry (ANOVA; p=0.00137). This result does not provide strong support for Hypothesis 2.

Methods: 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 data from females shown.

Preliminary genomics

Fig 4 | O-type populations cluster together at gen 20, regardless of ancestry. This result provides support for Hypothesis 2.

Methods: Genome-wide allele frequencies were estimated as the counts of non-reference alleles over total coverage at every bi-allelic SNP. Implementing a minimum coverage filter of N=6 severely reduced the number of SNPs to 32,690 and resulted in dropping replicate populations 3 and 5 due to low coverage. The first two principal components of a standard PCA of these allele frequencies are plotted here.



Discussion

- Longevity and immune defense phenotypes have both increased in the O-type populations after 20 generations, compared to B-types.
- Immune defense phenotypes in all O-type populations have converged, though for longevity we still observe small phenotypic differences due to recent evolutionary history.
- Preliminary genomic analysis suggests that O-type populations (OB & OBO) have converged by generation 20.
- Preliminary genomic analysis suggests little change in B-type populations (nB & nBO) within 56 generations.

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

Next steps

- Re-sequence populations to achieve deeper genomic coverage, enabling additional statistical testing.
- Sequence additional timepoints to resolve allele frequency trajectories at a finer scale.

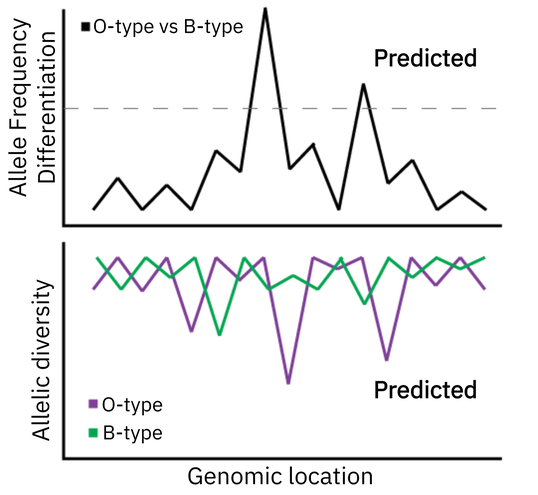


Fig 5 | Predicted patterns in the genomic data. We expect high levels of divergence when comparing O and B-type treatments. We expect more regions with reduced diversity in the O-type populations as a result of strong selection.

References [1] Rose 1984, *Evolution*. [2] Burke et al. 2010, *Nature*. [3] Sharestani et al. 2021. *G3*.