Analyzing Host-Microbe Relationships in Experimentally Evolved *Drosophila melanogaster* Populations

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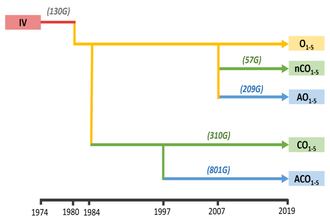
Abstract

The microbiome, or community of microbes, found within an organism affects its fitness, influencing traits such as development, immune defense, and lifespan. We studied how experimental evolution for longevity divergence in *Drosophila melanogaster* populations affects the associated microbiome. Moreover, we tested how flies from short- and long-lived populations are affected by manipulations to the microbiome. Quantitative analysis of bacterial abundance and composition was done by homogenizing and plating whole fly bodies. From this we found that the associated microbiome of populations that have been evolved for prolonged lifespan largely consisted of bacteria from the phylum Proteobacteria. In contrast, the associated microbiome of populations evolved to be shorter-lived were dominated by bacteria from the phylum Firmicutes. To manipulate the microbiome of short- and long- lived flies, axenic flies were created by dechorionating *D. melanogaster* eggs. These eggs were subsequently inoculated with symbiotic bacterial species and abundance was surveyed. Preliminary results show that long-lived flies were more extensively colonized when inoculated with *A. pomorum* and *A. tropicalis*. Short lived populations had overall higher microbial abundance, particularly when inoculated with *L. brevis* and *L. plantarum*. Continued experimentation is necessary to further elucidate the interactions between the *Drosophila melanogaster* microbiome and longevity.

Introduction

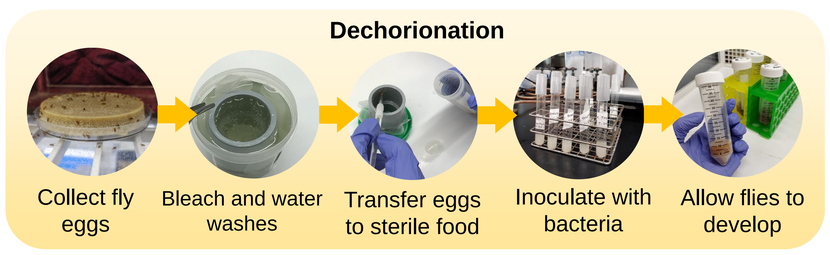
- ACO_{1-5} have been selected for accelerated development and have shorter lifespans as a result.
- CO_{1-5} have been selected for prolonged development and have longer lifespans as a result.
- Existing studies show that *Drosophila* populations experiencing different selection pressures differ in bacterial load and composition.
  - Proteobacteria accelerated development and negatively impacted longevity.
  - Firmicutes encouraged prolonged development and increased overall longevity.
- We hypothesize that *Drosophila* populations undergoing selection pressure for accelerated development will have microbiomes dominated by bacteria from the Proteobacteria phylum, greater microbial abundance, and be more readily colonized by Proteobacteria.

***Experimentally Evolved Drosophila* Populations**



Generation Cycle
14 days 28 days
70 days 9 days

Methods



Results

Phylum	ACO1	ACO2	ACO3	ACO4	ACO5	AO1	AO2	AO3	AO4	AO5
Firmicutes	85	88.24	100	9.09	54.3	99.42	77.19	56.95	67.47	83.59
Proteobacteria	15	11.77	0	25.96	45.7	0.58	22.81	43.04	32.52	16.41
Wolbachia	0	0	0	64.95	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0
Phylum	**CO1**	**CO2**	**CO3**	**CO4**	**CO5**	**nCO1**	**nCO2**	**nCO3**	**nCO4**	**nCO5**
Firmicutes	8.21	0.68	19.67	2.57	3.08	1.11	8.71	0.04	1.18	1.55
Proteobacteria	12.46	2.74	18.51	18.54	7.41	2.79	24.66	0.05	4.42	4.86
Wolbachia	78.89	96.52	61.66	78.87	89.51	96.07	66.49	99.91	94.39	93.58
Other	0.44	0.06	0.16	0.02	0	0.04	0.13	0	0.01	0.01

Table 1 — Genomic frequencies of microbes in 10 short-lived and 10 long-lived fly populations. 120 female flies were collected from each population replicate. They were whole body homogenized, before DNA was extracted, amplified and DNA libraries were created. We then analyzed the bacterial genome using MetaPhlAn 2.0. Values shown are the frequency of Firmicutes, Proteobacteria and *Wolbachia* DNA found in the metagenome of all 20 populations. Short-lived flies have a higher Firmicutes:Proteobacteria ratio while long-lived flies have proportionally more Proteobacteria:Firmicutes when *Wolbachia* is considered in this analysis. It is important to note that *Wolbachia* is in the Proteobacteria phyla but does not colonize the gut of the fly, and is instead found in the reproductive tissues. Data labelled Proteobacteria is excluding *Wolbachia*.

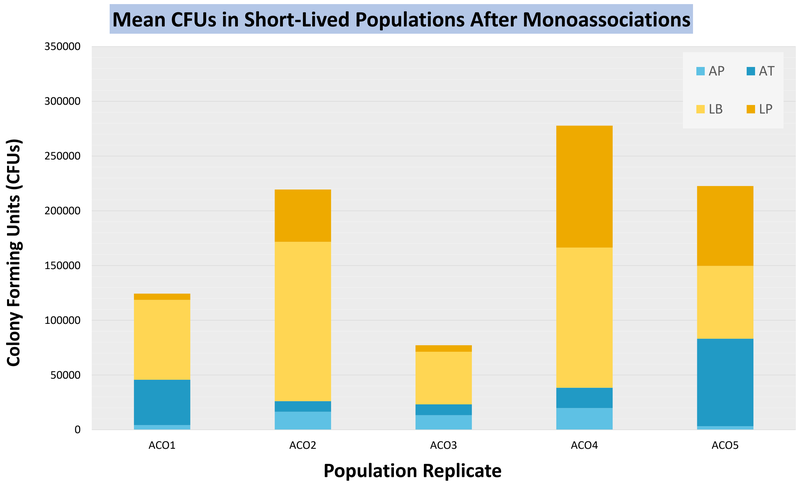


Figure 2 — Mean CFU counts of short- and long-lived fly populations that have been dechorionated and monoinoculated with *Acetobacter pomorum, Acetobacter tropicalis, Lactobacillus brevis, or Lactobacillus plantarum.* Short-lived flies (ACO_{1-5}) show increased bacterial abundance when inoculated with bacteria from the phylum Firmicutes. Long-lived flies (CO_{1-5}) show increased bacterial abundance when monoinoculated with bacteria from the phyla Proteobacteria.

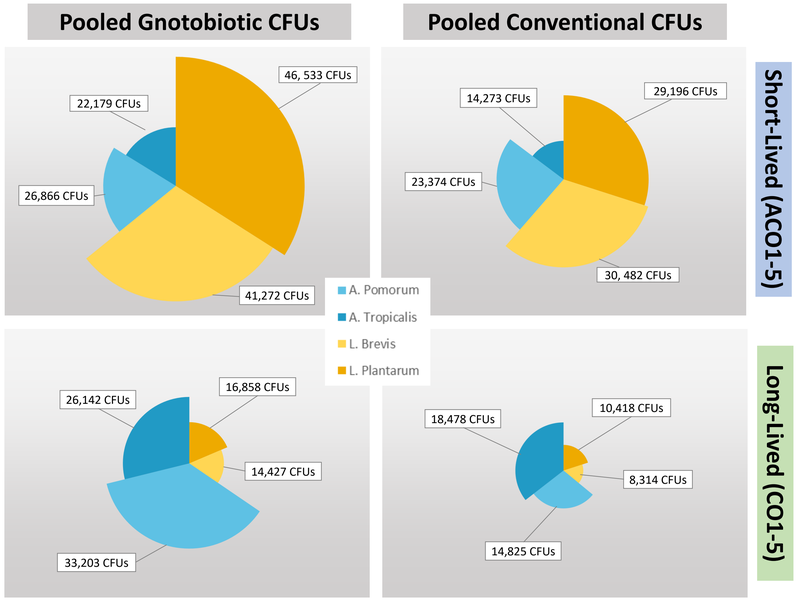


Figure 3 — Pooled mean CFUs of Gnotobiotic and Conventional treatments across all population replicates (ACO_{1-5} and CO_{1-5}). Gnotobiotic flies were dechorionated and inoculated with an equal mixture of all four bacteria. Both Gnotobiotic and Conventional short-lived flies show increased colonization by *L. brevis* and *L. plantarum.* Long-lived populations show the reverse outcome and overall lower bacterial abundance. Amongst both short- and long-lived populations, Gnotobiots exhibit greater bacterial quantities.

Discussion

Prior research suggests that commensal bacteria of the phylum Proteobacteria promotes acceleration of development in *Drosophila melanogaster*. Preliminary results from our experiments show a reversed outcome; short-lived flies that require accelerated development have higher abundance of bacteria from the phylum Firmicutes. Following manipulation of the microbiome of long-lived flies we saw a distinctly higher abundance of Proteobacteria. Furthermore, bacterial colonization was greatest when short-lived flies were monoinoculated with *Lactobacillus Brevis.* In contrast, long-lived flies had increased bacterial colonization by *Acetobacter tropicalis* and *Acetobacter pomorum.* Further experimentation is needed to explore the significance of microbial composition and abundance on development time and longevity in these populations. We will deepen our study of the host-microbe relationship in experimentally evolved populations by continuing experiments with the ACO and CO populations. Longevity, development, and other assays will be employed to further analyze the specific effects of microbiome manipulation.

References

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