Eating Cactus Takes Guts

Exploring the contributions of host evolutionary history and diet in shaping the gut microbiota of cactophilic flies

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Background

Gut microbial communities often provide functions that have significant implications for their host's fitness. However, understanding how a host's gut microbiome has been shaped by its evolutionary history can be difficult because the influence of diet can be lesser (1) or greater (2) than host genetic background.

Flies in the genus *Drosophila* have been previously used as model systems for understanding various factors pertaining to gut microbial community shape and function. We propose that the various species of cactophilic *Drosophila* native to North America could be a powerful comparative model system for discerning the contributions of host evolution and diet in shaping host gut microbial communities. In this study, we compare the bacterial gut microbiomes from three obligate cactus feeding *Drosophila* species to two sympatric dietary generalists.

Questions

By comparing cactus specialist and generalist flies, we first asked if dietary generalists differ from specialists in gut bacterial diversity. We initially hypothesized that because they encounter a wider array of foods, generalists would have more diverse gut microbiomes. We then asked if fly diet or relatedness is more predictive of gut microbial composition and structure.

Study Design

Compare the gut bacterial communities of generalist and cactus specialist flies across the *D. repleta* species group

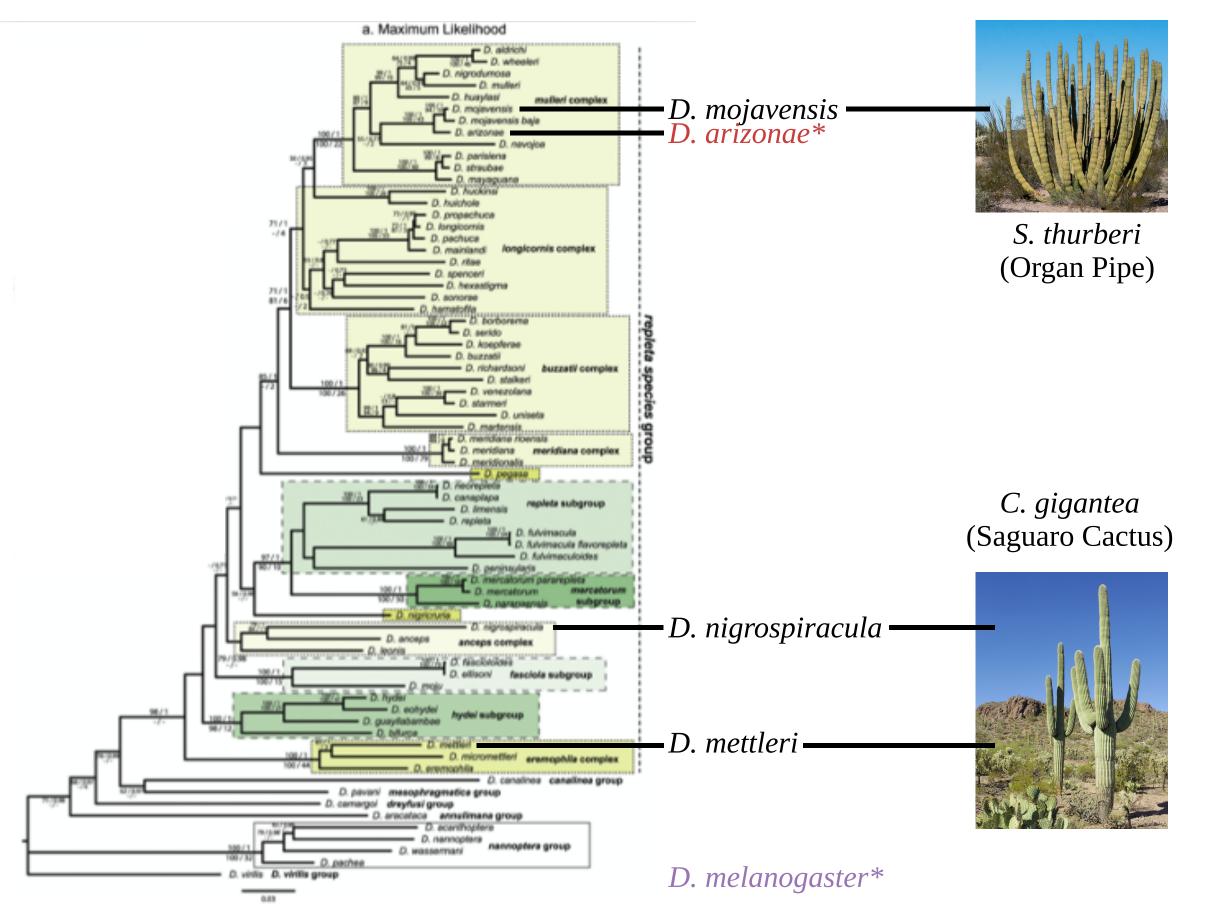


Figure 1: Phylogeny of the *D. repleta* species group (3). Lines indicate the species sampled in this study, as well as the cactus they specialize on. Species marked with an * indicate generalist species.

Alpha-Diversity Comparisons

Generalist flies do not have more diverse bacterial microbiomes

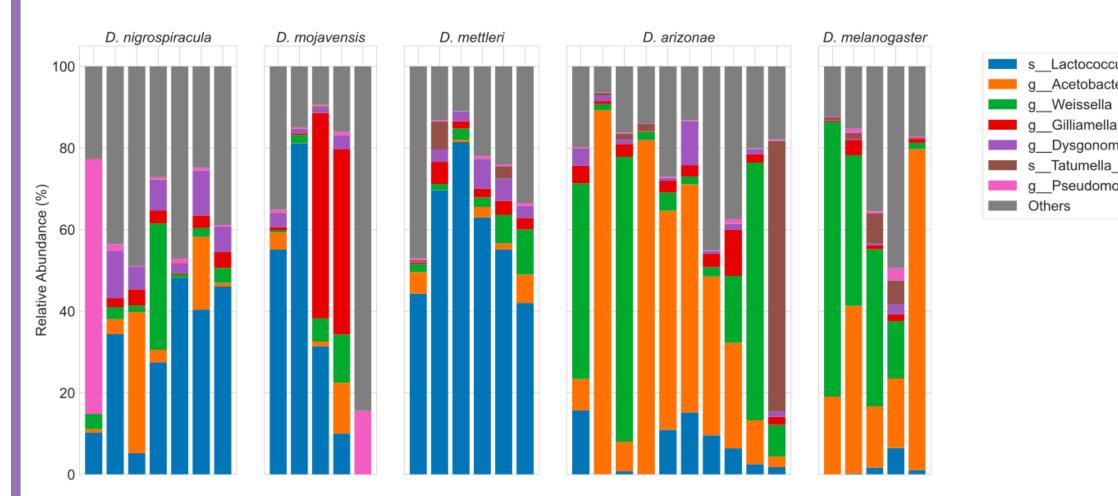


Figure 2: Taxonomy barplot showing the bacterial taxa and their relative abundances in each fly gut microbiome.

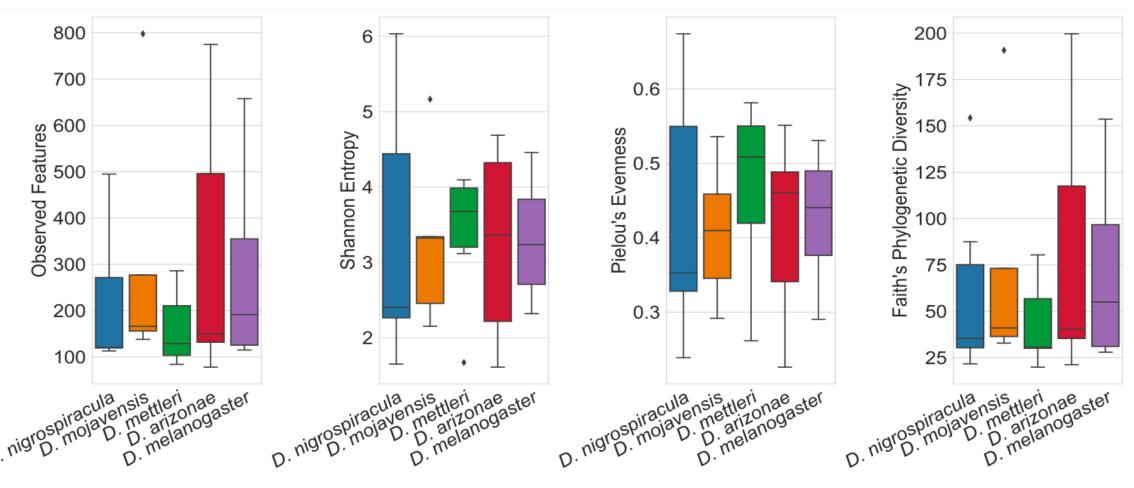


Figure 3: Boxplots showing various alpha-diversity metrics of each fly species. No statistically significant differences were observed (KW: $p \ge 0.13$).

Beta-Diversity: Compositional and Structural Comparisons

Generalist and specialist fly microbiomes are compositionally similar but structurally distinct

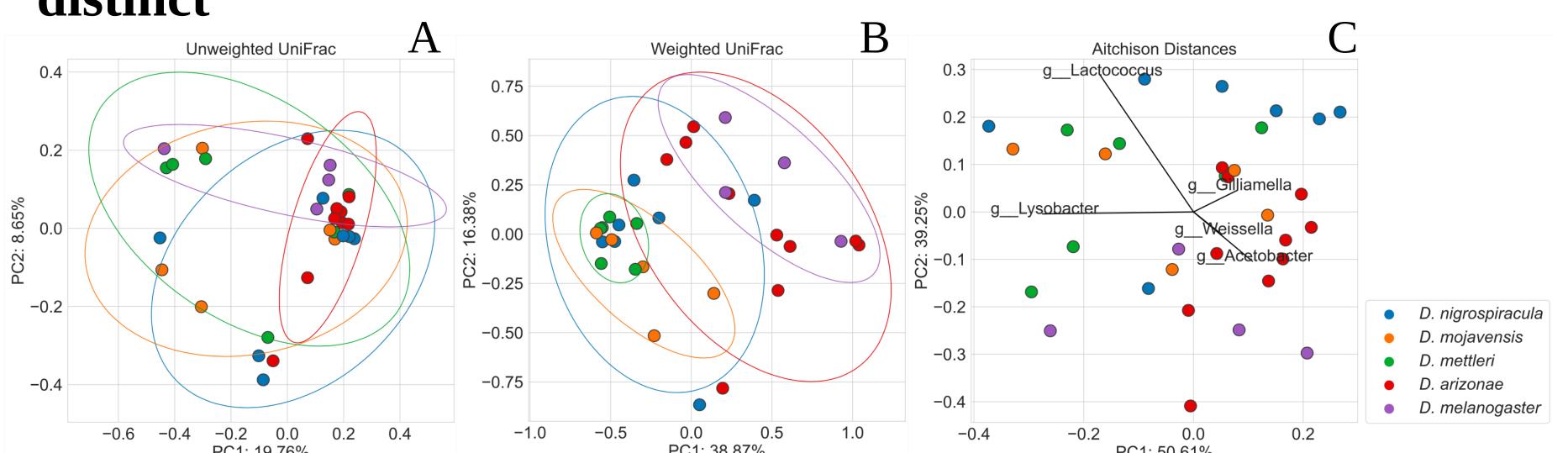


Figure 4: Ordination plots showing A) Fly gut bacterial microbiome compositional dissimilarities evaluated using Unweighted UniFrac distances. No significant differences between any fly species were found (PERMANOVA: p=0.116) B) Fly gut bacterial microbiome structural dissimilarities using weighted UniFrac distances. Significant differences between generalist and specialist flies were found (PERMANOVA: p=0.001, PERMDISP: p=0.086), and pairwise differences are highlighted in figure 5. C) Fly gut bacterial microbiome structural dissimilarities using Aitchison distances. The same significant results were found as described in B (PERMANOVA: p=0.001, PERMDISP, p=0.379). Vectors indicate bacterial taxa that had significant roles in sample clustering.

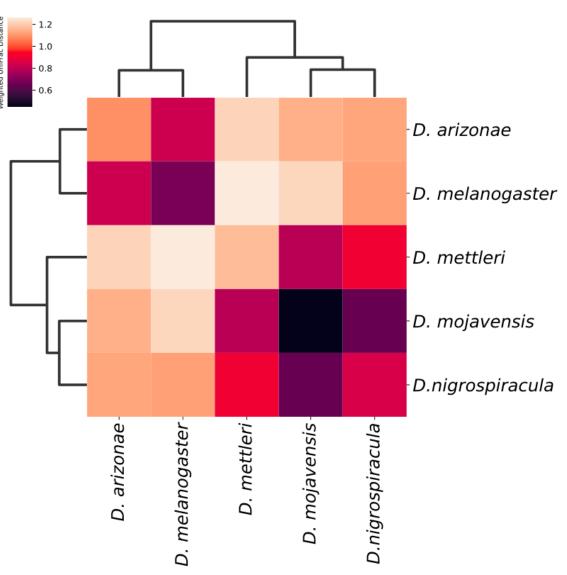


Figure 5: Heatmap showing mean pariwise weighted UniFrac distances between each fly species gut microbial communities. Clustering patterns show differences between the generalist flies and specialist flies, and mirror statistically significant pairwise comparisons.

- Compositional comparisons showed no differences between any fly species
- Strucural comparisons showed differences between generalist and specialist flies
- Lactococcus lactis and Acetobacter persici are significant contributors to sample clustering

Discussion

- The lack of difference in bacterial diversity between generalist and specialist flies could indicate that more general diets select for generalist bacteria, not more diverse microbiomes (4, 5)
- Compositional similarities but structural differences could indicate that certain microbes have evolved to proficiently colonize host flies (6), but diet then shapes community dynamics

Future Directions

- Identify if and how fly gut microbes are functionally relevant
- Quantify bacterial abundances to better compare gut microbiome structure

References

- 1) Ley et. al. 2008, Science, 320:1647-1651
- 2) Delsuc *et. al.* 2014, Mol Ecol, 23:1301-1317
- 3) Deodoro *et. al.* 2012, Mol Phyl and Evol, 64:533-544 4) Chen *et. al.* 2022, Microbiol Spect, 10:4
- 4) Chen *et. al.* 2022, Microbiol Spect, 10:4 5) Blankenchip *et. al.* 2018, PeerJ, 6:e4793
- 6) Martinson et. al. 2017, Appl Env Microbiol, 83:e01551-17

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