Determining the Causal Link of Honey Bee Gut Microbial Composition on Behavioral Maturation

Anh Nguyen,[1] Cassondra L. Vernier,[2] and Gene E. Robinson[2]

Franciscan Missionary of Our Lady University, Banton Rouge, Loiusiana[1]

Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign[2]

PRECS Phenotypic Plasticity Research Experience for Community College Students

Background

Introduction

- Emerging studies have supported the association between gut microbiome and host behaviors [1]. However, it is unclear whether changes in the gut microbiome cause changes in host behaviors or vice versa.
- The European honey bee, *Apis mellifera*, is an excellent animal model for identifying the causal link between microbiome and behavioral changes over the lifetime of the host as the honey bee gut contains a simple microbiome composed of only nine bacterial taxa clusters [2].
- In honey bees, division of labor occurs through behavioral maturation where age determines what task a bee does [3]. For example, older bees forage while younger bees perform brood care (nursing) and other in-hive tasks.
- Single cohort colonies (SCCs), or colonies composed of individuals of the same age, uncouple chronological age effects on honeybee behavioral maturation (nursing → foraging). SCCs results from our previous experiment (Figure 1) reveal a highly significant difference in the gut microbiota between nurses and foragers, independent of age, specifically in the abundance of *Lactobacillus mellis* and *Bifidobacterium asteroides.*

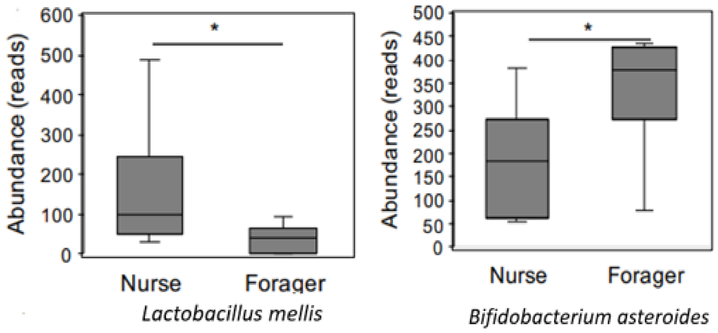
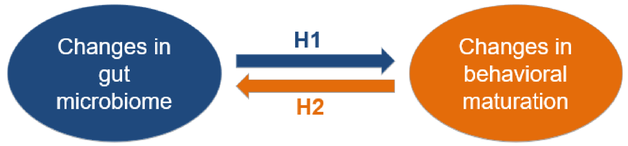


Figure 1: (Left) *L.mellis* Mann-Whitney U, p = 0.03, n = 10. (Right) *B. asteroides*, Mann-Whitney U, p = 0.01, n = 10. Vernier & Robinson, unpublished.

Question: Is behavioral maturation associated with changes in the gut microbiome foraging-expectant or foraging-dependent?

→ We manipulate foraging experience by placing plastic tags on a subset of SCC bees ("big-backs"). In combination with a modified hive entrance, these tags prevent "big-back" bees from leaving the hive and gaining flight experience (Figure 5). We then compare gut microbiomes between experienced foragers and "big-backs" who are attempting to leave the hive.

Hypotheses



Results

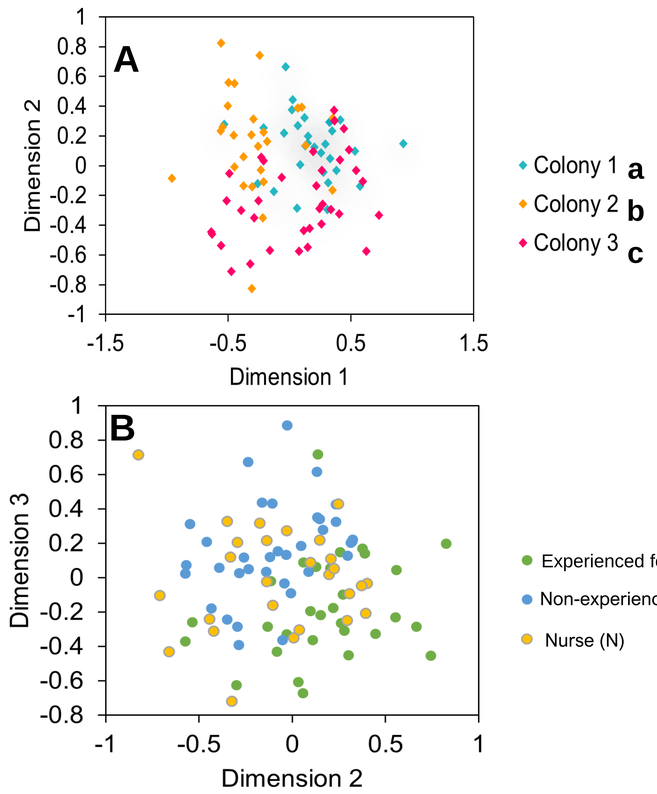


Figure 2: **(A)** Gut microbial community differs between colonies. **(B)** Gut microbial community is different between NFs and EFs but is similar between NFs and Ns. Two-way Permutation MANOVA: Colony, $F(2,91) = 7.21$, $R^2 = 0.13$, $p = 0.001$; Treatment, $F(2,91) = 4.27$, $R2 = 0.08$, $p = 0.001$; Colony*Treatment $F(4,91) = 1.11$, $R2 = 0.04$, $p = 0.30$. Depicted as non-metric multidimensional scaling plot (k =4, stress = 0.14). Lowercase letters denote statistically significantly different groups.

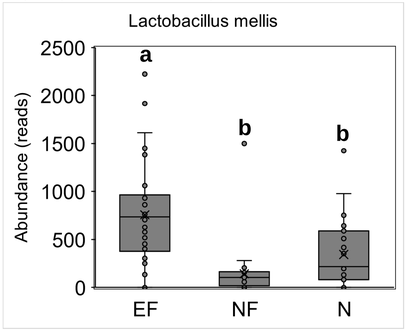


Figure 3: *L. mellis* is similar in abundance between NFs and Ns but differ between NFs and EFs (Linear mixed-effects model with Colony as a random factor, $t = 10.58$, $p < 0.001$).

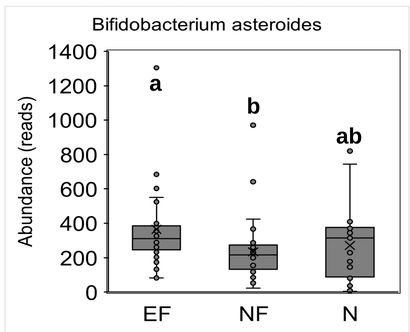


Figure 4: *B. asteroides* is different in abundance between NFs and EFs (Linear mixed-effects model with Colony as a random factor, $t = 7.50$, $p < 0.001$).

Methods

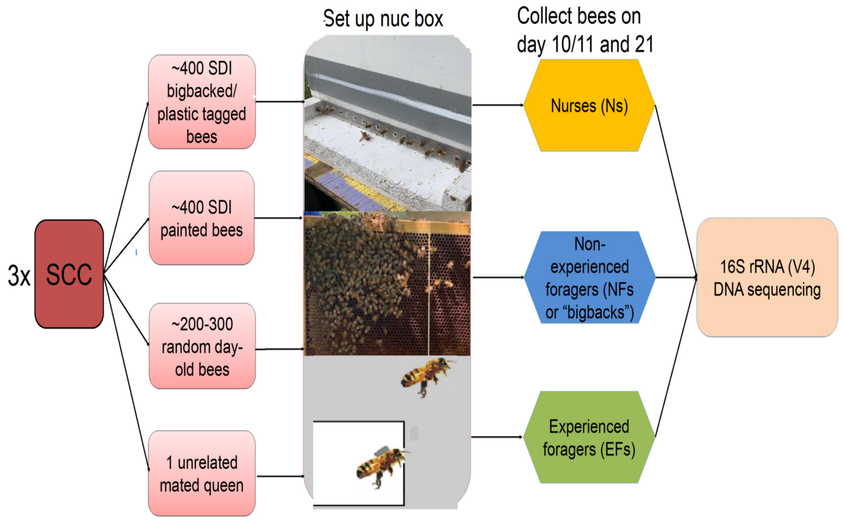


Figure 5 [4]: Restricted bees could not pass through the modified entrance. Single drone inseminated (SDI).

Conclusions

- **Changes in behavioral maturation (nursing → foraging) lead to changes in the bee gut microbiome, specifically in the abundance of *L. mellis* (H2).**
- In contrast to Vernier's previous experimental data, although there is a significant difference in *L. mellis* abundance between experienced foragers and nurses, *L. mellis* is more abundant in experienced foragers than in nurses. Likewise, there is no significant difference in *B.asteroides* abundance between experienced foragers and nurses; level of foraging experience in association with age maybe be accountable for this inconsistency. Future work will address this discrepancy.

Future Work

- Inoculate microbiome-free SDI bees with bacteria previously identified as robustly linked with behavioral maturation → behavioral assays.
- Knock out certain bacterial genes found to be causally linked to host behaviors → determine mechanisms of how individual microbes influence honey bee behavioral maturation.

Acknowledgments

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