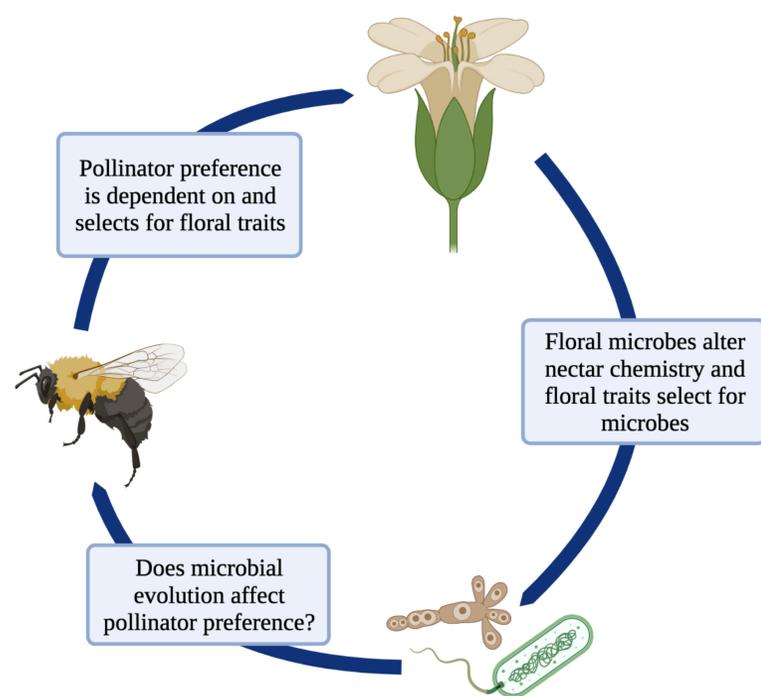


## INTRODUCTION

Understanding how natural selection acts on phenotype is complex because phenotypic variation results from the interaction between genotype and environmental response. Flowers are the key to angiosperm evolution because of their role in reproduction<sup>1</sup>. In turn, pollinators are a major selective pressure in plants because they select for different favorable traits, including colors, shapes, and scents that we observe among angiosperm flowers.

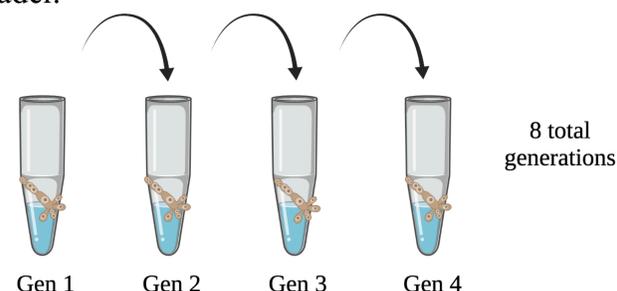
The floral microbiome is an important and understudied component of the extended floral phenotype. Floral microbes impact the floral phenotype via their metabolism of nectar sugars, amino acids, and proteins found in nectar and pollen<sup>1</sup>. They also contribute their own volatiles that impact floral scent. Pollinator preference is largely determined by floral morphology and olfactory traits<sup>2</sup> including, nectar sugar content, scent, color, and additional nectar volatiles.<sup>3,4,5</sup> Because microbes play a role in altering floral chemistry,<sup>6</sup> they also influence pollinator preference.<sup>7,8,9</sup> As a result, microbe-induced changes in the floral phenotype may impact affect floral selection and consequent floral trait evolution.<sup>1,10</sup> While little is known about how floral microbes affect plant fitness directly, if floral microbes can affect floral selection, floral traits, in turn may select on floral microbe traits, resulting in an evolutionary feedback loop<sup>1</sup>.



## METHODS

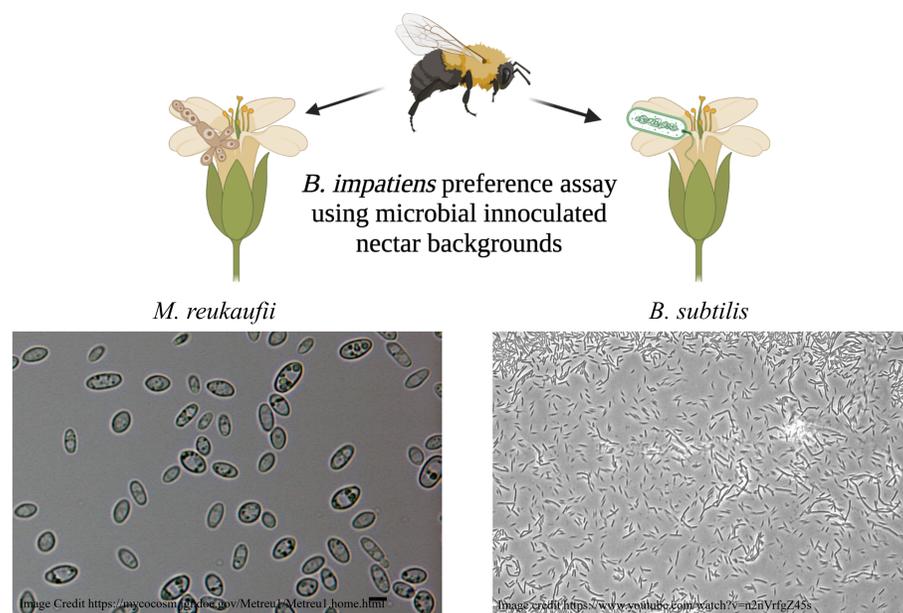
### How do floral nectar traits and microbe competition affect microbial evolution?

- Generated nectar mimics of the model plant *Brassica rapa* utilizing volatile compounds
- Included *B. subtilis* and *M. reukaufii* and exposed them to each background for 8 consecutive generations
- Monitored microbial resource use via shifts in pH and sugar consumption (BRIX)
- Recorded microbial abundance via plating and recording colony-forming units (CFU) and logged growth rates with a plate reader.

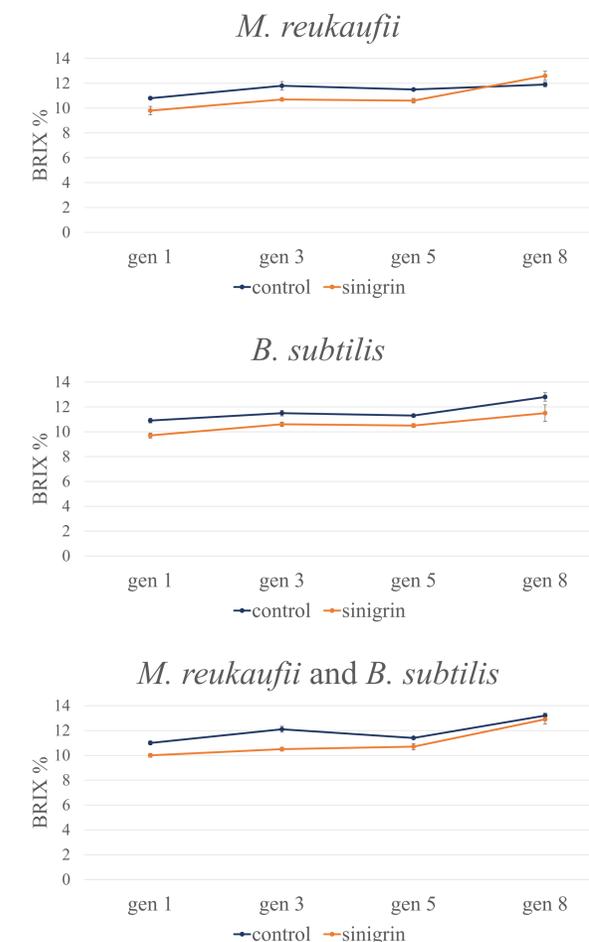


### How do the resulting phenotypic changes alter pollinator preference (and ultimately floral trait selection)?

- A single bumble-bee forager will be starved for 3 hours and released in a flight cage to choose among artificial flowers with isolates from each generation
- Recording total choices, nectar consumed, and nectar quality



## CURRENT RESULTS



## FUTURE DIRECTIONS

Preliminary results show that microbes react differently to a nectar background containing sinigrin. We will also conduct the same generational tests using nectar containing AITC.

Because the extended floral phenotype is altered by floral microbes, we predict that *B. subtilis* and *M. reukaufii* will impact bumblebee preferences. *Bombus impatiens* preference assays will be conducted to test this prediction.

## Literature Cited

1. Rebolledo-Gomez, M., Forrester, N.J., Russell, A.L., Wei, N., Fetter, A.M., Stephens, J.D., & Ashman, T. 2019. Gazing into the anthosphere: considering how microbes influence floral evolution. *New Phytologist*, 224(3), 1012-1020.
2. Zu, P., Schiestl, F.P., Gervasi, D., Li, X., Runcie, D., & Guillaume, F. 2020. Floral signals evolve in a predictable way under artificial and pollinator selection in *Brassica rapa*. *BMC evolutionary biology*, 20(1), 127.
3. Cai, J., Zu, P., & Schiestl, F.P. 2016. The molecular bases of floral scent evolution under artificial selection: Insights from a transcriptome analysis in *Brassica rapa*. *Scientific Reports*, 6, 4.
4. Gervasi, D.D.L. & F.P. Schiestl. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, 8, 5.
5. Ramos, S.E. & Schiestl, F.P. 2019. Rapid plant evolution driven by the interaction of pollination and herbivory. *Science*, 364(6436), 193-196.
6. Rering, C.C., Beck, J.J., Hall, G.W., McCartney, M.M., & Vannette, R.L. 2018. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytologist*, 220(3), 750-759.
7. Álvarez-Pérez, S., Herrera, C.M., & de Vega, C. 2012. Zooming-in on floral nectar: a first exploration of nectar-associated bacteria in wild plant communities. *FEEMS Microbiology Ecology*, 80(3), 591-602.
8. Vannette, R.L., Gauthier, M.P.L., & Fukami, T. 2013. Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism. *Proceedings of the Royal Society B-Biological Sciences*, 280(1752), 9.
9. Vannette, R.L. & Fukami, T. 2016. Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. *Ecology*, 97(6), 1410-1419.
10. Vannette, R.L. 2020. The floral microbiome: Plant, pollinator, and microbial perspectives. *Annual Review of Ecology, Evolution, and Systematics*, 51, 363-386.