EVOLUTION OF FLORAL MICROBES AND THE RESULTING EFFECTS ON POLLINATOR PREFERENCES

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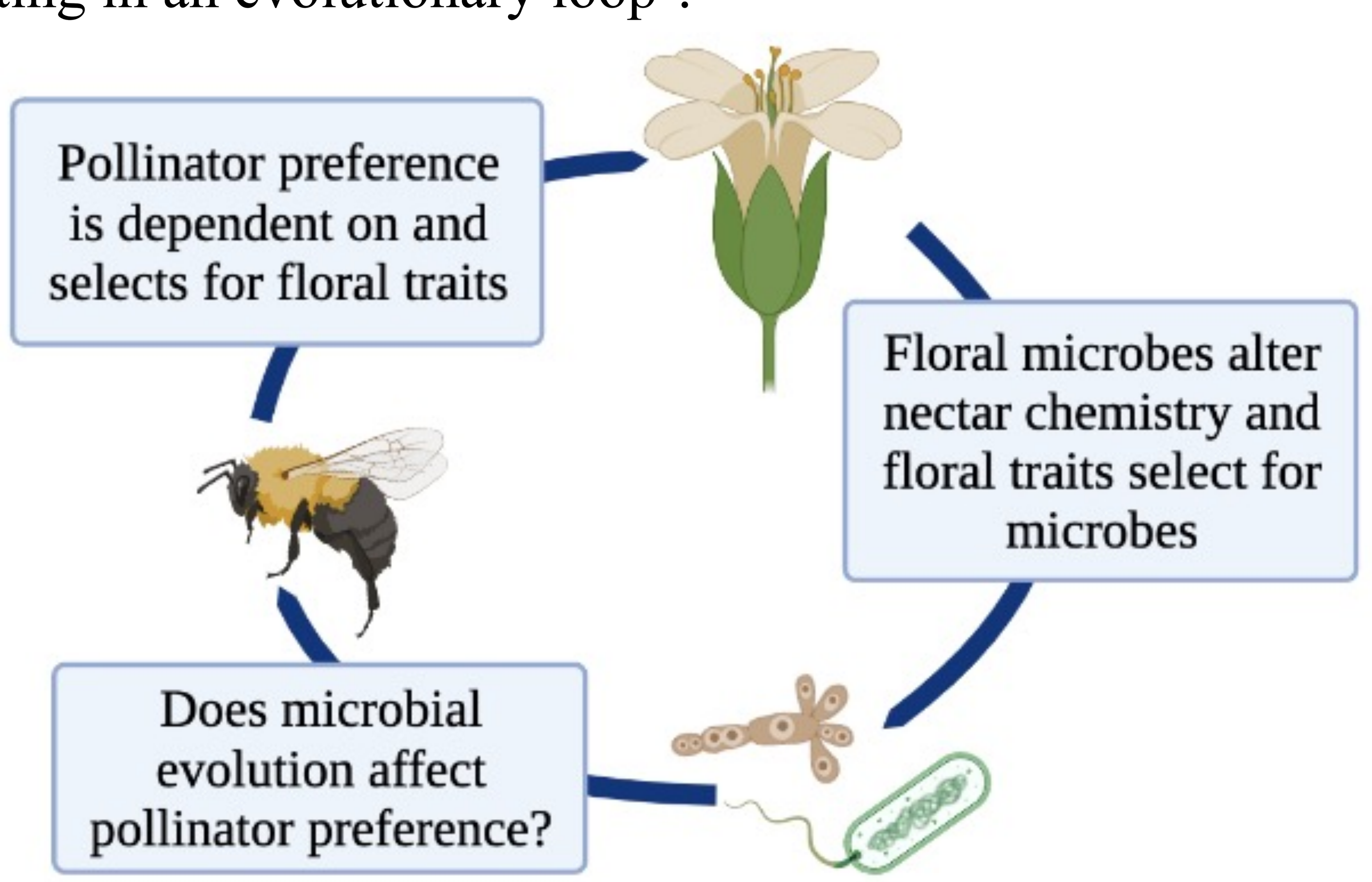
INTRODUCTION

Flowers offer a unique system to study phenotypic evolution. Floral microbes impact the floral phenotype via their metabolism of nectar sugars and other nectar compounds.[1] Pollinator choice is largely determined by floral morphology and olfactory traits.[2] Because microbes play a role in altering floral chemistry,[3] they also influence pollinator preference.[4,5,6] As a result, microbe-induced changes in floral nectar may impact pollinator selection of floral traits.[1,7] While little is known about how floral microbes affect plant fitness directly, if floral microbes can affect floral traits, pollinator choice in turn may select on floral microbe traits, resulting in an evolutionary loop[1].



METHODS

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

- Generated nectar mimics of the model plant *Brassica rapa*, including nectar secondary metabolite sinigrin, a glucosinolate
- Exposed floral microbes to each nectar background for 8 generations
- Monitored microbial resource use via sugar consumption (BRIX) and microbial abundance via plating (CFU)

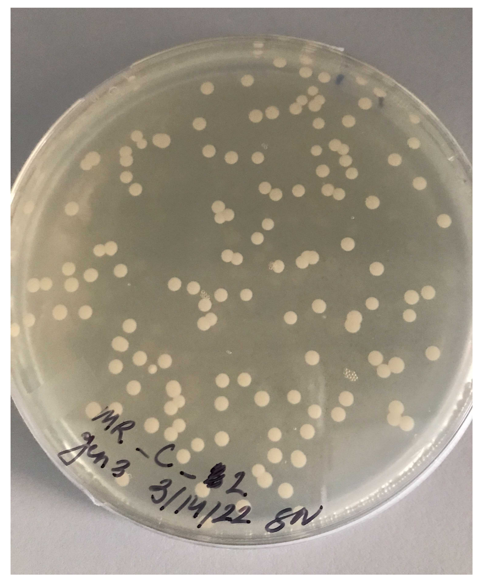
How do the resulting phenotypic changes alter pollinator preference?

- Bumble-bee foragers were released in a flight cage to choose among artificial flowers with nectar containing isolates from each generation and nectar background
- Recorded total choices, time spent at each flower, and calculated a weighted preference score

RESULTS

Table 1: ANOVAs for microbe performance via CFU

Effect	DFn	DFd	F	p
B. subtilis				
Generation	3	27	1.04	0.388
Nectar	1	9	0.798	0.395
Generation:Nectar	1.42	12.76	1.37	0.278
M. reukaufii (CO)				
Generation	3	27	1.08	0.349
Nectar	1	9	0.999	0.595
Generation:Nectar	1.42	12.76	2.45	0.356



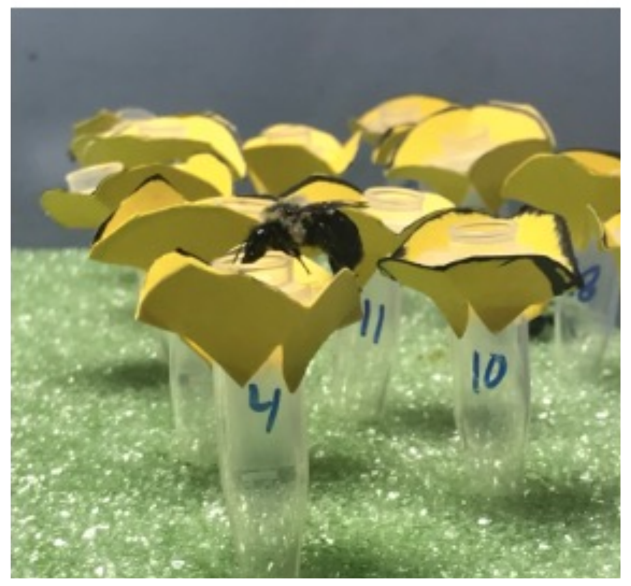


Fig 1. (left) *M. reukaufii* plated for CFU counts

Fig 2. (right) *B. impatiens* drinks nectar from an artificial flower

Table 2. ANOVA for 3 preference indicators comparing *B. impatiens* microbial preference

Effect	DFn	Sum Sq	Mean Sq	F	Pr(>F)
Total visits					
Microbe	5	5.741	1.148	1.216	0.311
Trial	2	14.130	7.065	7.480	0.001*
Nectar	1	0.148	0.148	0.157	0.693
Microbe:Trial	10	12.093	1.209	1.280	0.258
Microbe:Nectar	5	6.963	1.393	1.475	0.209
Trial:Nectar	2	0.574	0.287	0.304	0.739
Microbe:Trial:Nectar	10	6.981	0.698	0.739	0.695
Time per flower					
Microbe	5	8015	1603.1	0.592	0.706
Trial	1	9203	9202.7	3.397	0.069*
Nectar	1	164	163.8	0.061	0.806
Microbe:Trial	5	11956	2391.3	0.883	0.496
Microbe:Nectar	5	10763	2152.7	0.795	0.557
Trial:Nectar	1	3016	3016.1	1.113	0.294
Microbe:Trial:Nectar	5	7213	1442.6	0.533	0.751
Weighted preference score					
Microbe	5	11.958	2.392	1.033	0.404
Trial	1	2.302	2.302	0.995	0.322
Nectar	1	1.445	1.445	0.624	0.432
Microbe:Trial	5	12.115	2.423	1.047	0.396
Microbe:Nectar	5	9.434	1.887	0.815	0.542
Trial:Nectar	1	0.453	0.453	0.196	0.660
Microbe:Trial:Nectar	5	7.254	1.451	0.627	0.680

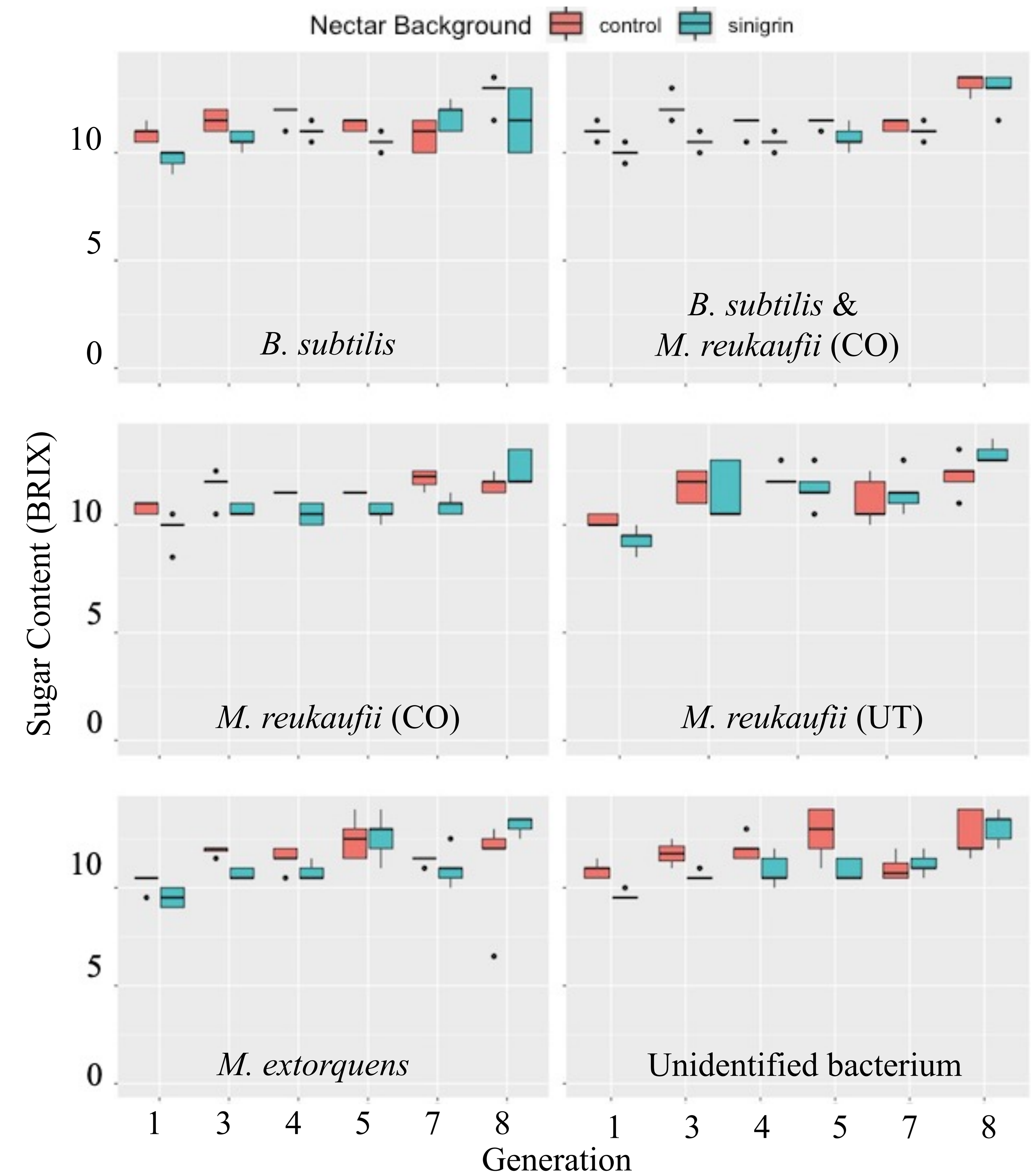


Figure 3. Box plots of sugar content in artificial flowers after multiple generational transfers of floral microbial species

DISCUSSION

- Nectar consumption decreasing over time due to decreased need for sugar. Additionally, they could have adapted to be more efficient at resource utilization resulting in lower metabolism
- Higher sugar consumption in the presence of sinigrin to compensate for environmental stress
- *B. impatiens* has shown preference for yeasts such as *M. reukaufii*.[8] However, nectar was inoculated immediately before presentation to the foragers, leaving limited time for microbes to alter nectar quality enough to be detected

Literature Cited

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