

How do organisms acquire their gut microbiomes?

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with David Sivak,
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Carnegie Institution for Science

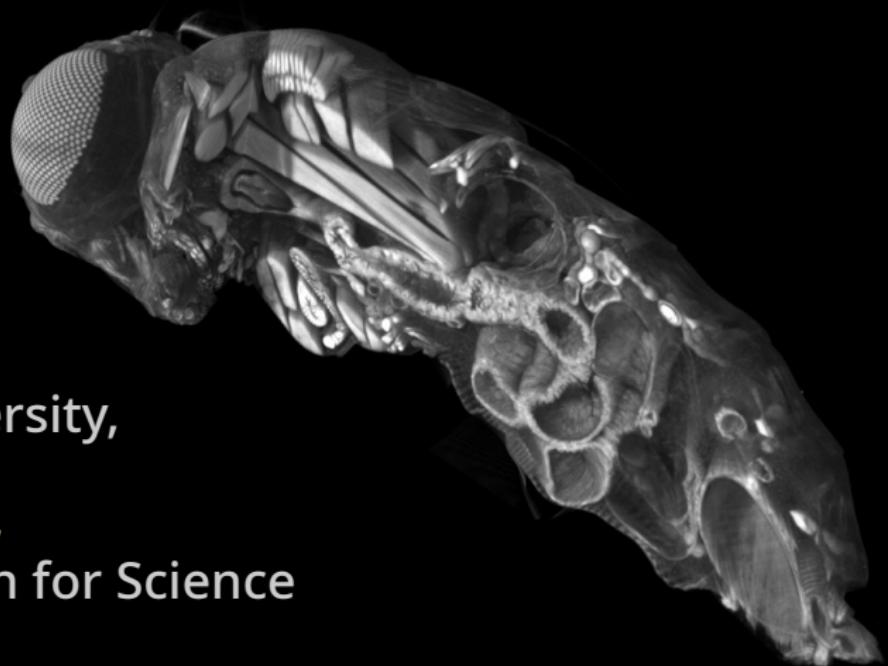
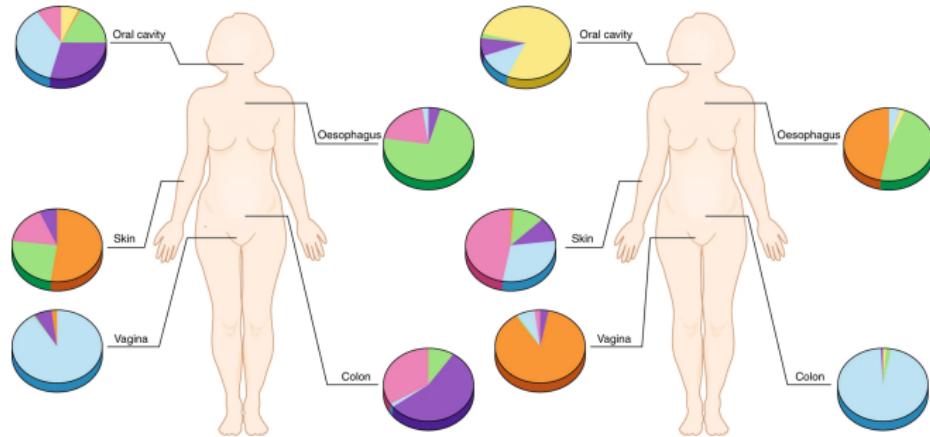
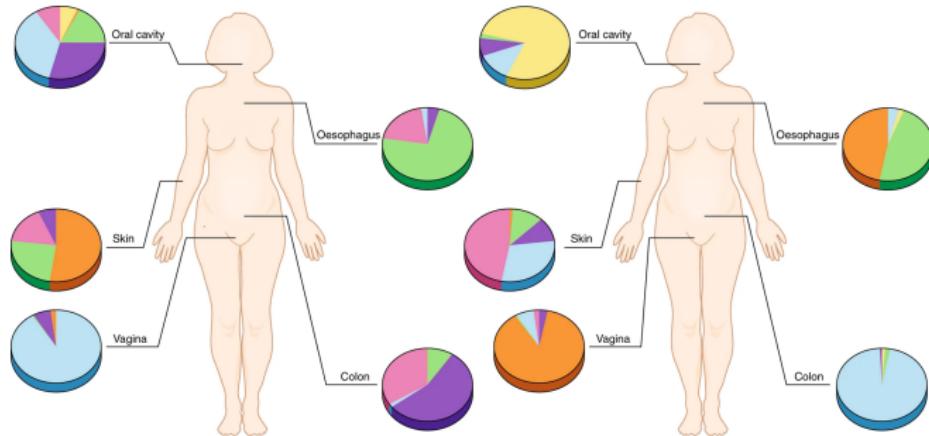


image credit: Marco Voltolini

The human microbiome is as unique as a fingerprint



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and it plays a vital role in human health:

- ▶ some microbes mediate disease (e.g. *C. difficile* infection)
- ▶ “healthy” commensal microbes metabolize, train immune system
- ▶ “unhealthy” dysbiotic microbiome associated w/ obesity, Crohn’s disease, type-2 diabetes, and more

The fruit fly is a model system for microbiome complexity

core set of 5 bacterial species

- ▶ *Lactobacillus sp (yogurt)*
- ▶ *Acetobacter sp (vinegar)*



flies can be reared germ-free and associated with any combination of bacteria

measuring host traits (e.g. fecundity, lifespan) reveals a relationship between microbiome composition and fly physiology

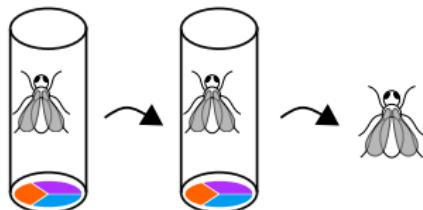
Identical replicates but variable colonization outcomes



germ-free fruit flies
fed bacterial combination



48x replicates per combination



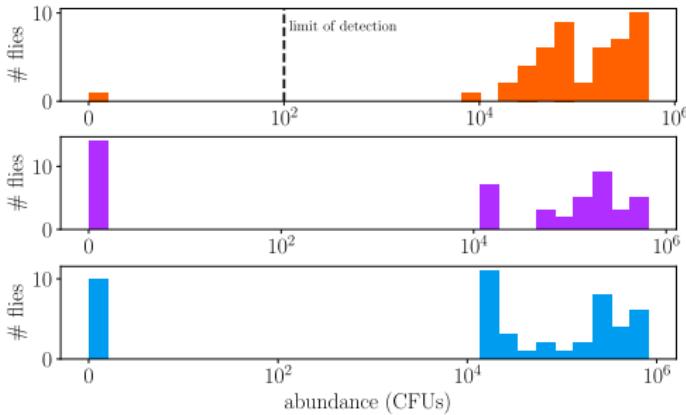
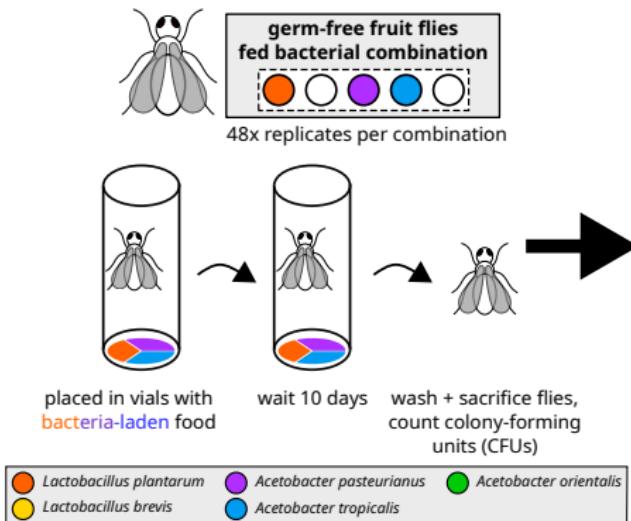
placed in vials with
bacteria-laden food

wait 10 days

wash + sacrifice flies,
count colony-forming
units (CFUs)

| | | |
|--|---|---|
|  <i>Lactobacillus plantarum</i> |  <i>Acetobacter pasteurianus</i> |  <i>Acetobacter orientalis</i> |
|  <i>Lactobacillus brevis</i> |  <i>Acetobacter tropicalis</i> | |

Identical replicates but variable colonization outcomes

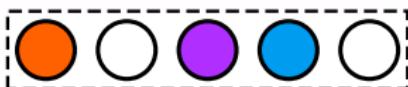


A combinatorial approach to dissect microbiome complexity

a) experimental setup

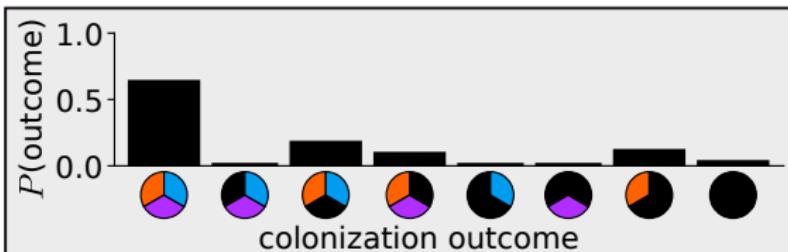


fed bacterial combination



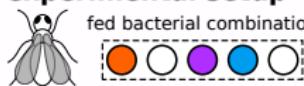
↓ 10 days later (w/ 48 fly replicates)

multinomial distribution of colonization outcomes (based on presence/absence data):



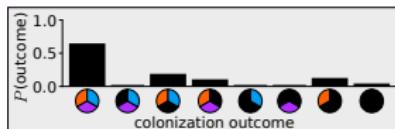
A combinatorial approach to dissect microbiome complexity

a) experimental setup



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multinomial distribution of colonization outcomes (based on presence/absence data):



b) combinatorial dissection:

experimental setup repeated for all 31 combinations

diversity-1
combinations



diversity-2
combinations



diversity-3
combinations



diversity-4
combinations



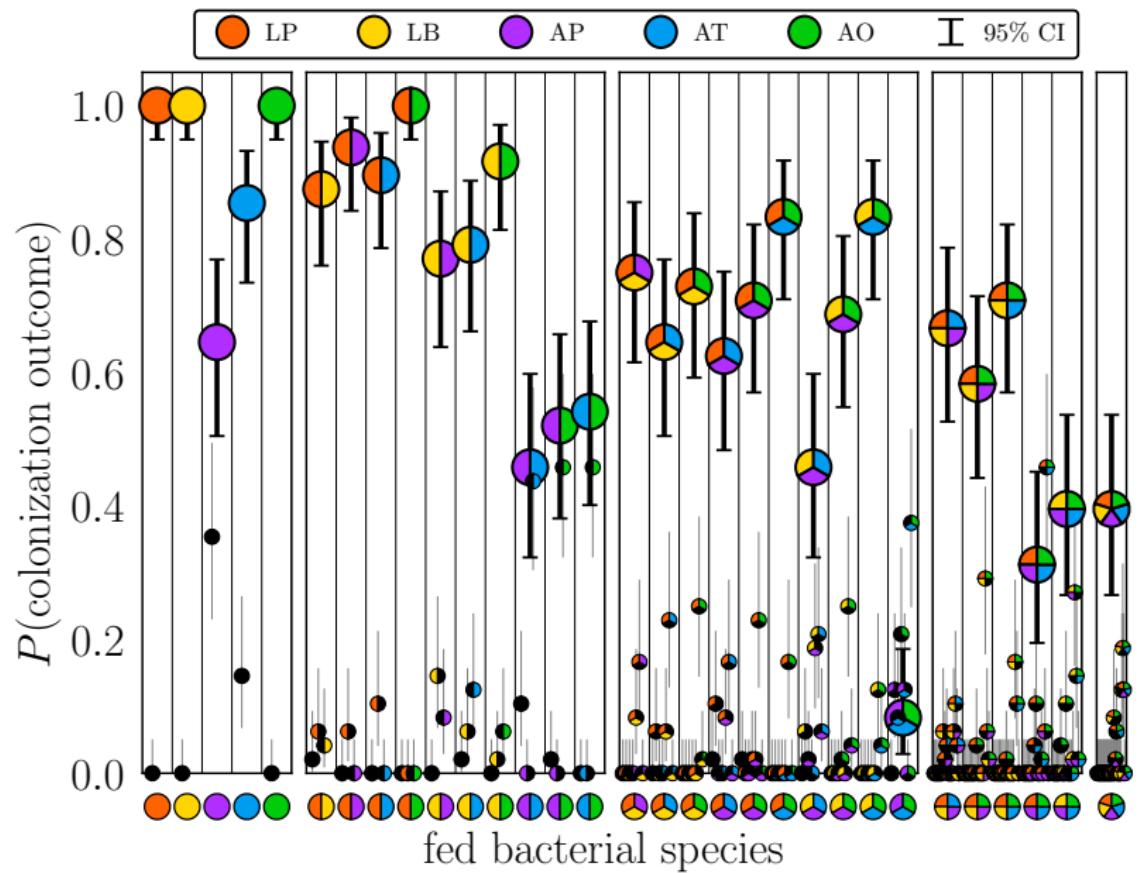
diversity-5
combination



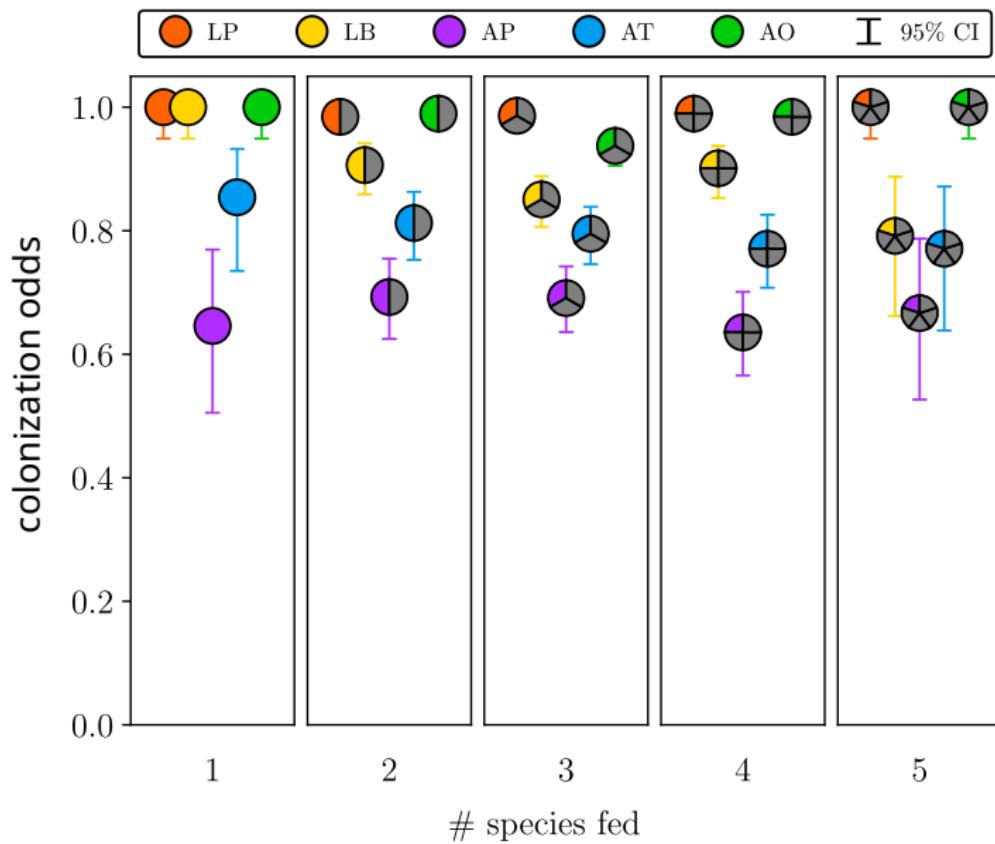
LP LB AP AT AO



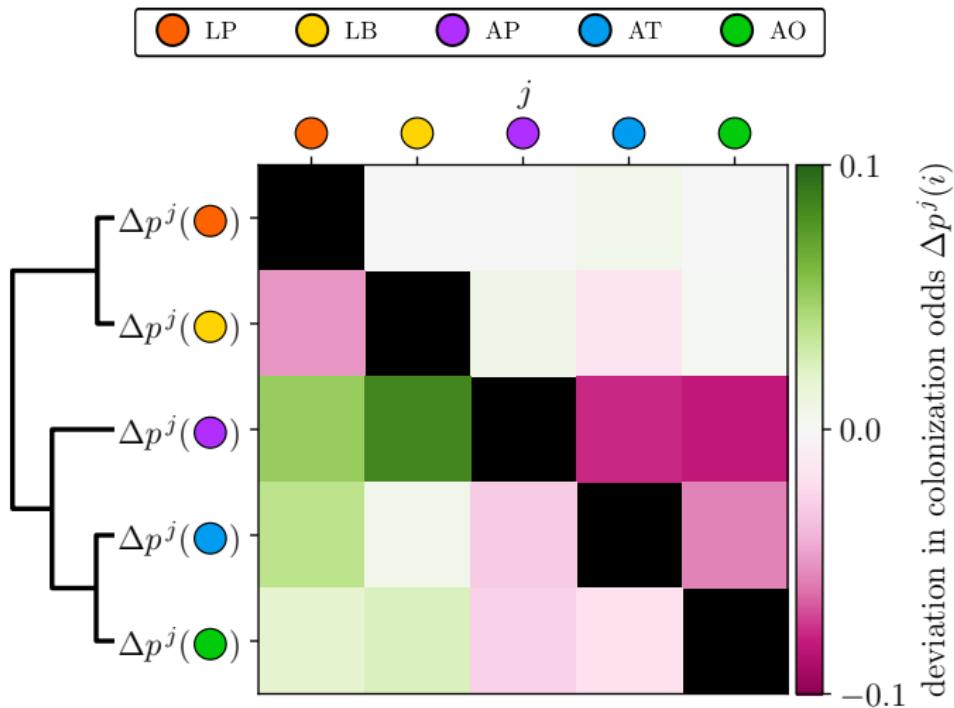
Stochastic colonization of the fly microbiome



Colonization odds are species- and diversity-dependent



Colonization odds are context-specific



$\Delta p^j(i)$... change in colonization odds of species i when fed w/ species j
green ... species j increases colonization odds of species i
pink ... species j decreases colonization odds of species i

Implications for “medicine for the microbiome”

Probiotic therapies and fecal microbiota transplantation (FMT) try to improve a person's health by engrafting a set of healthy microbes into their gut.

- ▶ **Stochastic microbiome assembly underpins probiotic and FMT treatments:** bacterial colonization is not guaranteed even for large doses, and is species-, diversity-, and context-dependent.

Design principles for microbiome therapies:

- ▶ For synthetic transplants, prioritize microbes that are strong colonizers, and include multiple microbes that can perform important functions (functional redundancy)
- ▶ Calibrate dosing regimes (with multiple probiotic or FMT treatments) based on colonization odds of pertinent species

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Modeling stochastic colonization

Which microbes in a probiotic/FMT treatment will actually stick?

c) model the probability
of each colonization outcome
w/ independent marginal probs

e.g.



fed bacterial combination



$$P(\text{circle}) = p(\text{orange}) * p(\text{purple}) * [1 - p(\text{blue})]$$

in general for $R \subseteq S$,

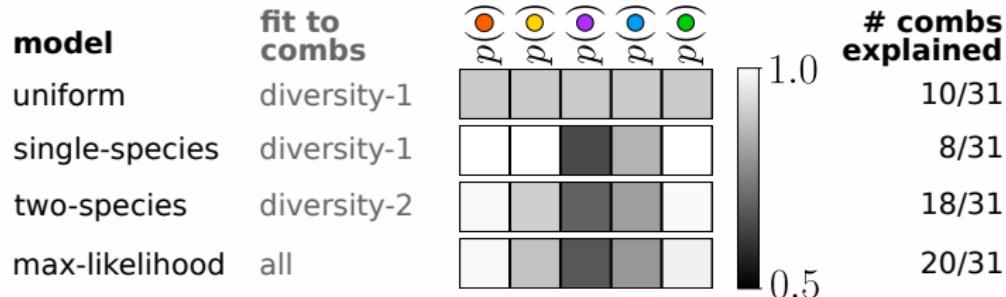
$$P(i \in R \text{ colonize}) = \prod_{i \in R} p_i \prod_{j \in S \setminus R} (1 - p_j)$$

for marginal probabilities p_i

Modeling stochastic colonization



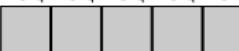
d) models w/ fixed marginal probabilities



Modeling stochastic colonization



d) models w/ fixed marginal probabilities

| model | fit to combs |  | # combs explained |
|----------------|--------------|---|-------------------|
| uniform | diversity-1 |  | 10/31 |
| single-species | diversity-1 |  | 8/31 |
| two-species | diversity-2 |  | 18/31 |
| max-likelihood | all |  | 20/31 |

e) model w/ context-specific marginal probabilities

| | | | | |
|-------------|-----|---|--|-------|
| interaction | all |    | for combination  for combination  | 24/31 |
|-------------|-----|---|--|-------|

Thank you!

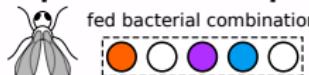
Funding



- **Will Ludington**, Carnegie Institute for Science
- Jean Carlson, UC Santa Barbara

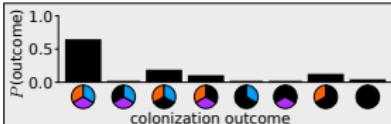
Questions?

a) experimental setup

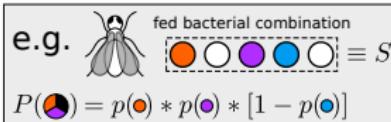


↓ 10 days later (w/ 48 fly replicates)

probability distribution of colonization outcomes (based on presence/absence data):



c) model the probability of each colonization outcome w/ independent marginal probs



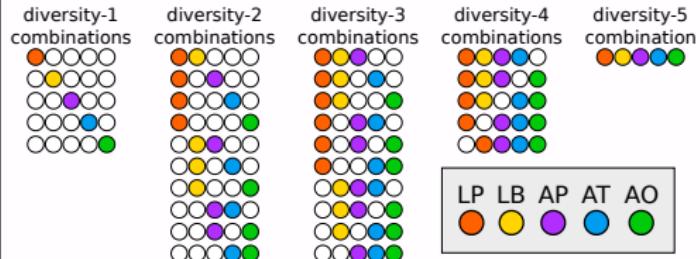
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d) models w/ fixed marginal probabilities

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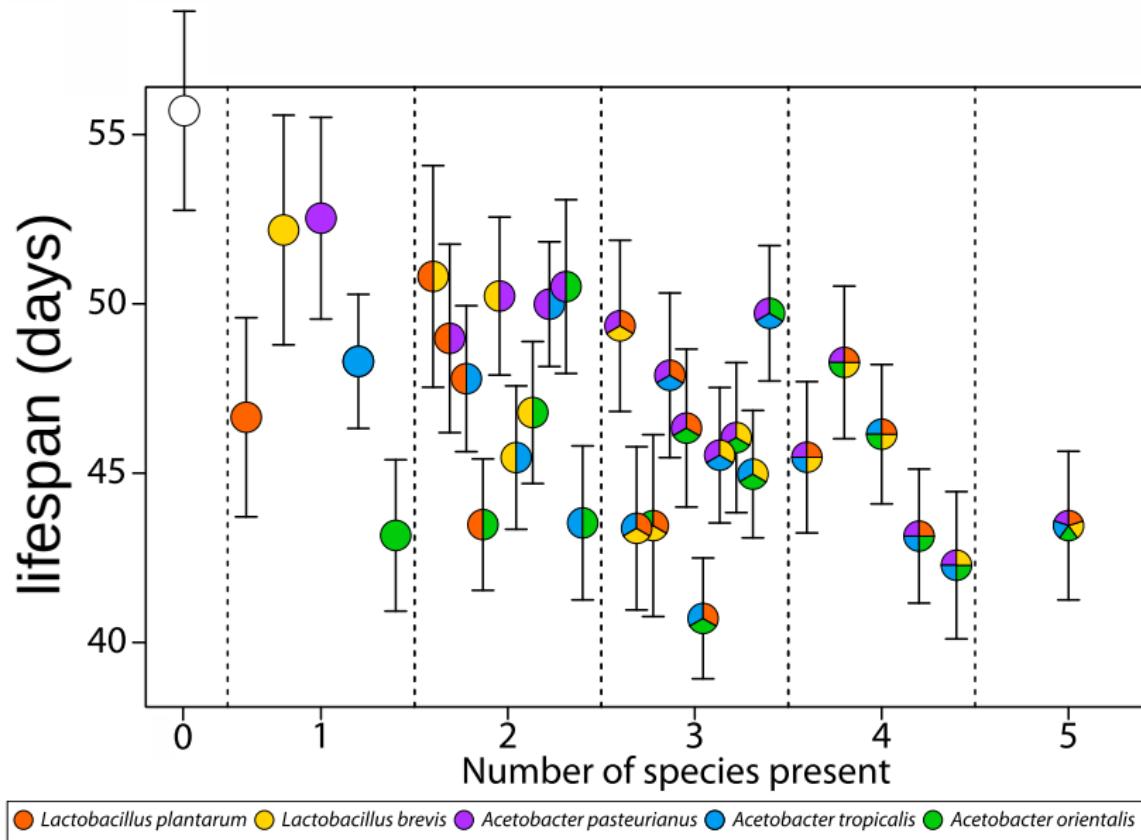
Learn more: "Stochastic microbiome assembly depends on context,"

Jones, Carlson, Sivak, and Ludington, *PNAS* 2022

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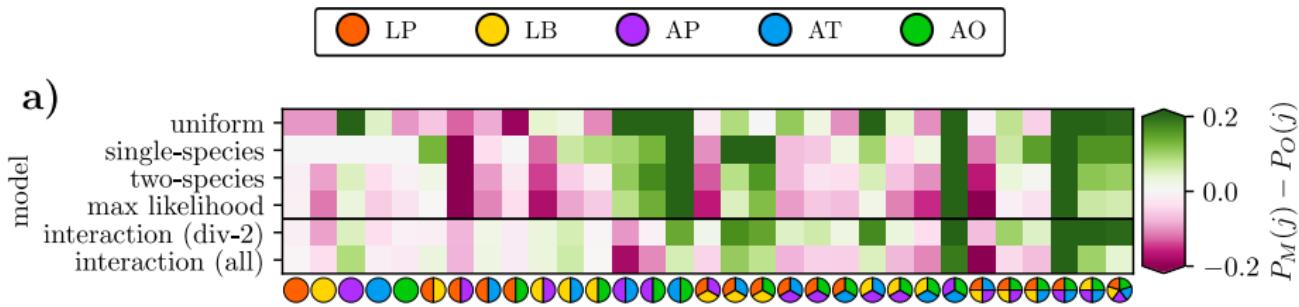
Host phenotypes are a function of microbiome composition



from A. Gould, V. Zhang, L. Lamberti, E. Jones, ..., and W. Ludington, PNAS 2018



How well do models predict the colonization outcome that all fed species colonize?



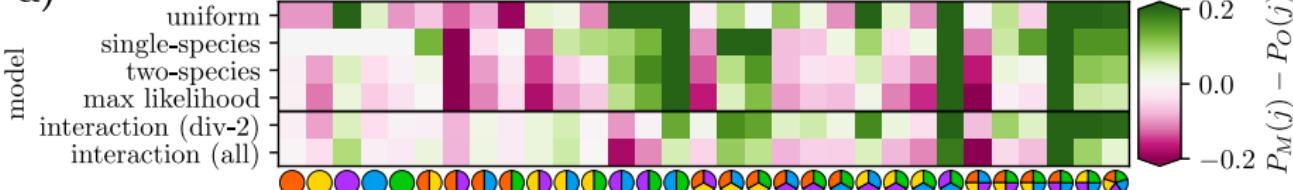
$P_M(j)$... modeled probability that all fed species colonize
 $P_O(j)$... empirical probability that all fed species colonize

green ... model overestimated the odds that all fed species colonized
pink ... model underestimated the odds that all fed species colonized

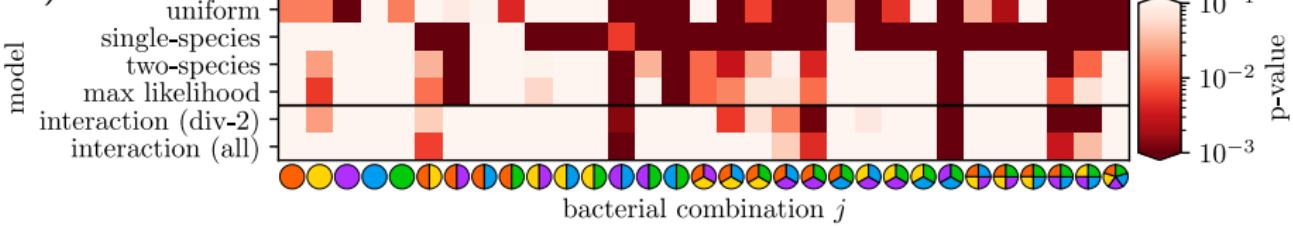
How well do models reproduce colonization outcomes?



a)



b)



p-values computed with multinomial tests (exact or Monte Carlo)

dark red ... model failed to reproduce empirical colonization outcomes
white ... model reproduced empirical colonization outcomes

Comparing observed and predicted colonization outcomes with an exact multinomial test

