Microbial community assembly and competition in boom-and-bust environments

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Z Wang, A Goyal, S Maslov, bioRxiv, July 26, 2023

Astrology → **Astronomy**

Nostradamus → Brahe → **Kepler, Newton**

Biology → **Bionomy**

Terrence Hwa©

Ecology → **Economy**

Physiology → **Physiognomy**

Slow progress in models predicting microbial community assembly

- I started modeling microbial communities 8 years ago, hoping to make predictions
- Growth is described by Monod equation:

$$g(R) = \frac{g_{max} R}{R + K}$$

- For steady state in a chemostat $R \ll K$ so

$$g(R) = \frac{g_{max}}{K} R$$

chemostats are to study oligotrophs not copiotrophs

- But K **varies a lot** between strains**, depends on growth history,** and is **hard to measure**!

TABLE 2. Kinetic constants and their temperature dependencies for *E. coli* grown with glucose as the sole source of carbon and energy

E. coli strain	*T* (°C)	K_s (μg liter^{-1})	μ_{max} (h^{-1})	Cultivation method	Reference
ML 30	40	34[a]	0.75	Chemostat	135
H	37	4,000	0.94	Batch	166
B/r Thy$^-$	37	180	1.04	Batch	260
ML 308	37	3,400	0.75	Batch	125
B/r CM6	37	540	NR[c]	Batch	19
K-12	37	7,160	0.76	Batch	52
ML 308	37	107	0.54	Chemostat	129
		2,340	1.23	Batch	
ML 30	37	53	0.80	Chemostat	224
		72	0.92		
ML 30	37	33[a]	0.76	Chemostat	34
B/r Thy$^-$	30	180	NR	Batch	260
NR[c]	30	77,000–99,000	0.92–1.05	Chemostat	222
ML 30G	30	68[b]	0.78	Batch	226
		12,600			
ML 30	28.4	33[a]	0.54	Chemostat	34
O-124	26	2,400	0.55	Batch	49
OUMI7020	20	8,460[b]	0.55	Batch	109
		46,800			
NR[c]	20	8,000	0.65	Chemostat	111
ML 30	17.4	33[a]	0.19	Chemostat	34

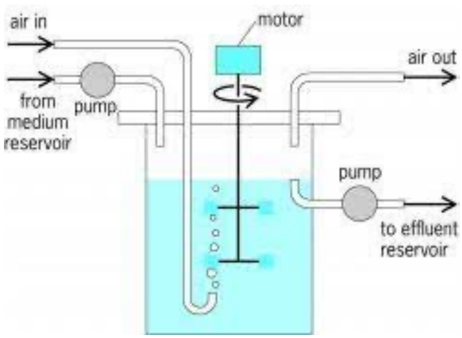
Karin Kovárová-Kovar
Thomas Egli

Growth Kinetics of Suspended Microbial Cells: From Single-Substrate-Controlled Growth to Mixed-Substrate Kinetics.

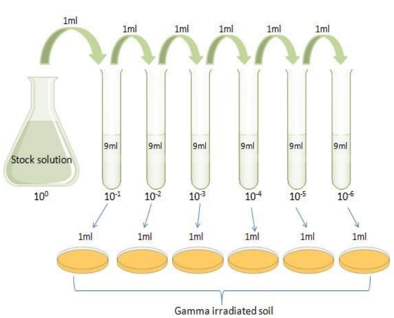
Microbiol Mol Biol Rev 62, 646–666 (1998)

Microbial community dynamics is often not in steady state but boom-and-bust

We model this (chemostat)



But in the lab we do this (**serial dilution/passage = = batch growth**)



Many real ecosystems are dominated by **copiotrophs** not **oligotrophs** (e.g., algal blooms)

Boom-and-bust model parameters are **easy to measure**

To model batch growth one needs to know:

- The maximum growth rate $g_i^{(\max)}$ on each resource
- The lag times τ_{ij} and

dilution factor D>1

- The resource hierarchy or co-utilization ratio

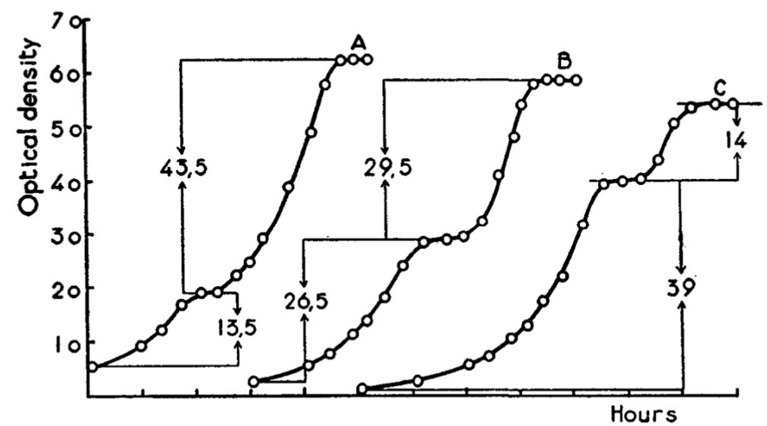


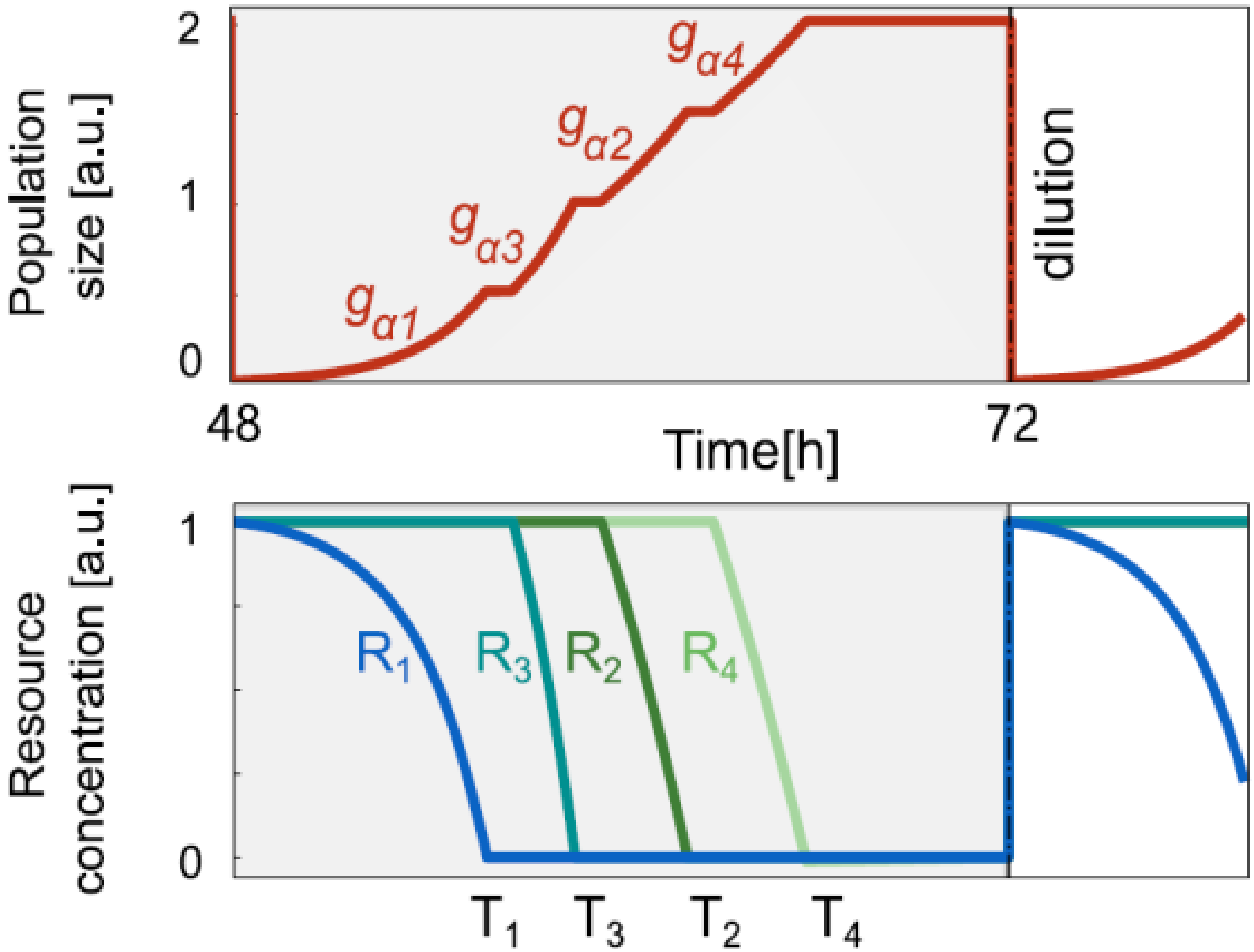
FIG. 9.—Diauxie. Growth of *E. coli* in synthetic medium with glucose+sorbitol as carbon source.
The figures between arrows indicate total growth corresponding to each cycle.
(*a*) Glucose 50 µg. per ml.; sorbitol 150 µg. per ml.
(*b*) Glucose 100 µg. per ml.; sorbitol 100 µg. per ml.
(*c*) Glucose 150 µg. per ml.; sorbitol 50 µg. per ml.
Total growth corresponding to first cycle is proportional to glucose concentration. Total growth of second cycle is proportional to sorbitol concentration (11).

Monod (1949) The Growth of Bacterial Cultures. *Annual Reviews in Microbiology*

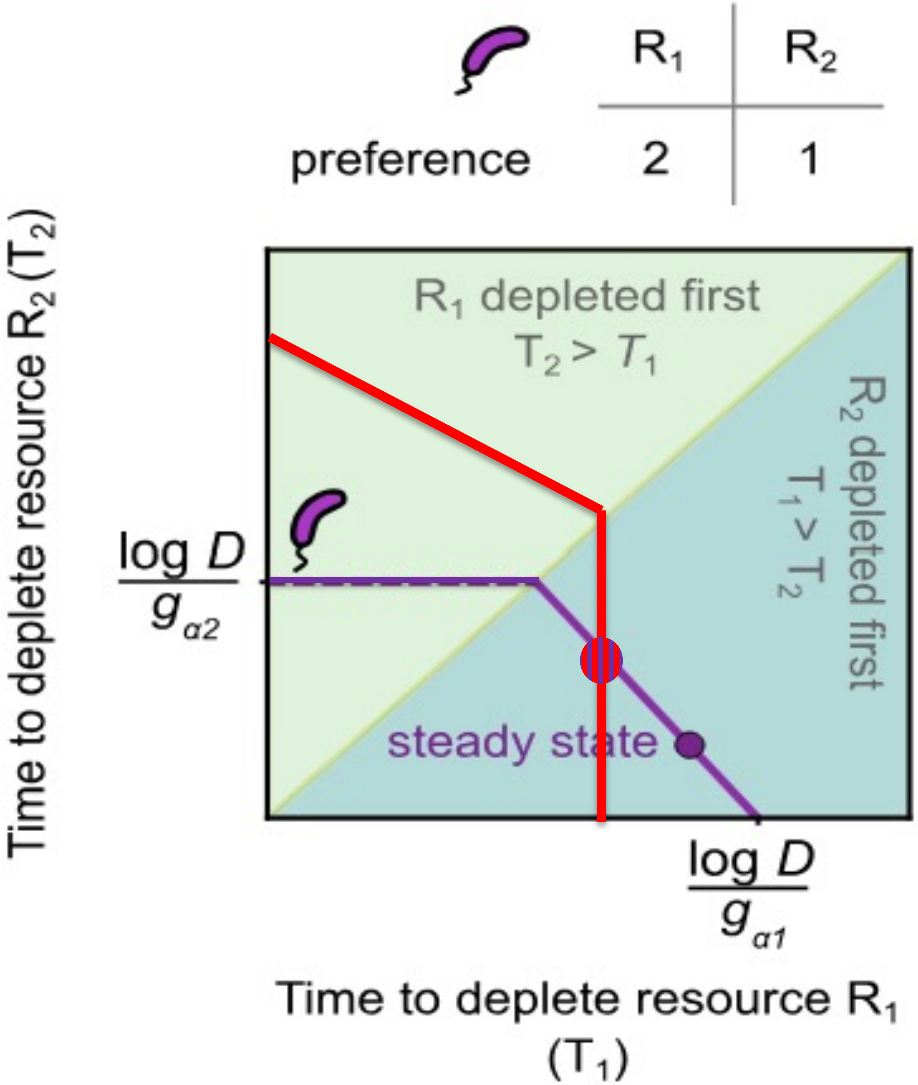
Use case scenario

- Your experimentalist friend has a favorite set of n_S species: **the "dream team"**
- He needs to keep **all species alive** in a serial passage (dilution) experiment
- We can provide the following services:
  - Determine if these species can in principle stably coexist on a given set of $\boldsymbol{n_R}$ resources
  - Design the resource ratio $\boldsymbol{R_1}:\boldsymbol{R_2}:\boldsymbol{R_3}$ for this community to successfully assemble
  - **Bonus.** Tune the resource ratio for a desired species abundance ratio $\boldsymbol{N_1}:\boldsymbol{N_2}:\boldsymbol{N_3}$

The boom phase is defined by resource depletion times T_i



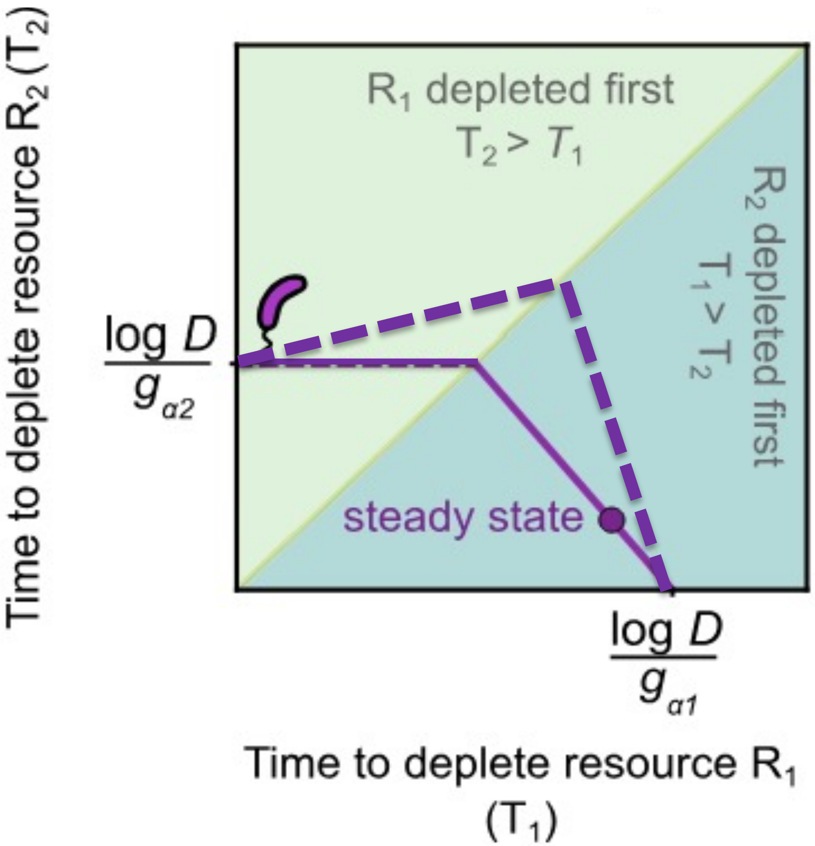
How to generalize Tilman's graphical method of from chemostats to serial dilution?

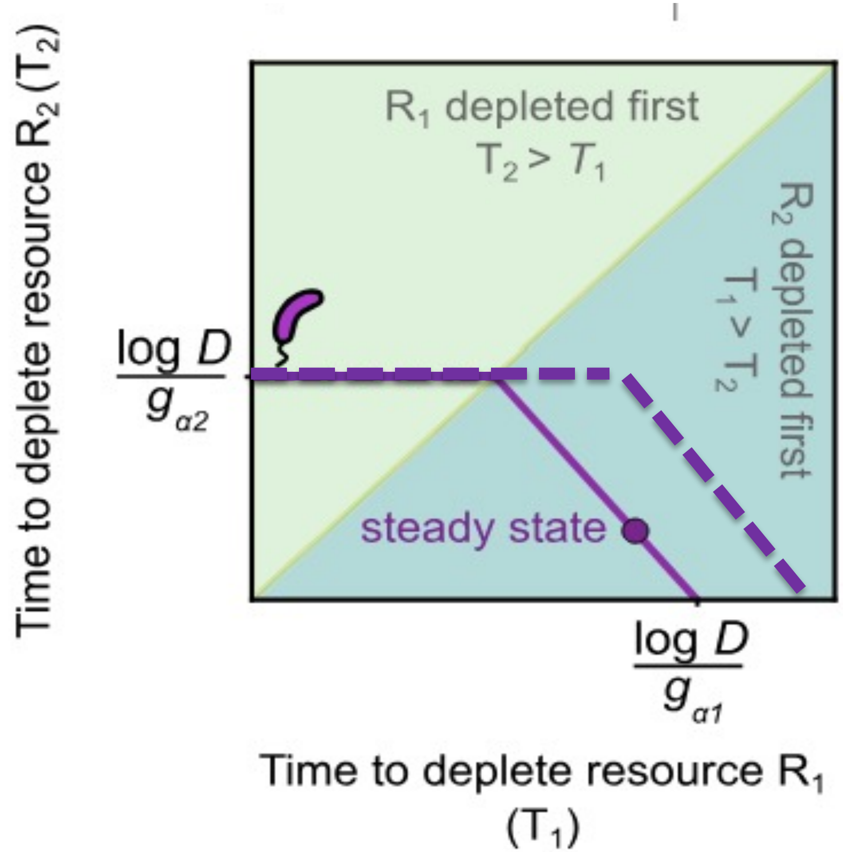


$$\exp(g_2 T_2) = D$$

$$T_2 = \log D / g_2$$

Wang, Z., et al. *Nature communications*, *12*(1), 6661 (2021)



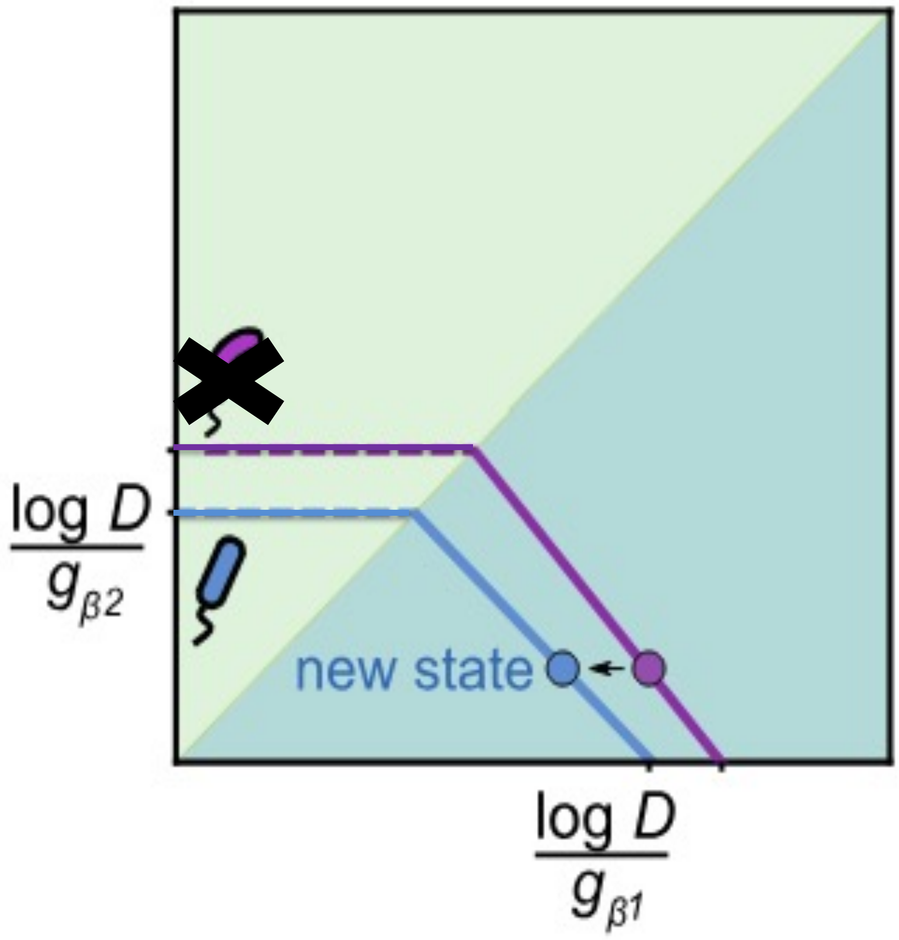


can be generalized for co-utilization

can be generalized for lags

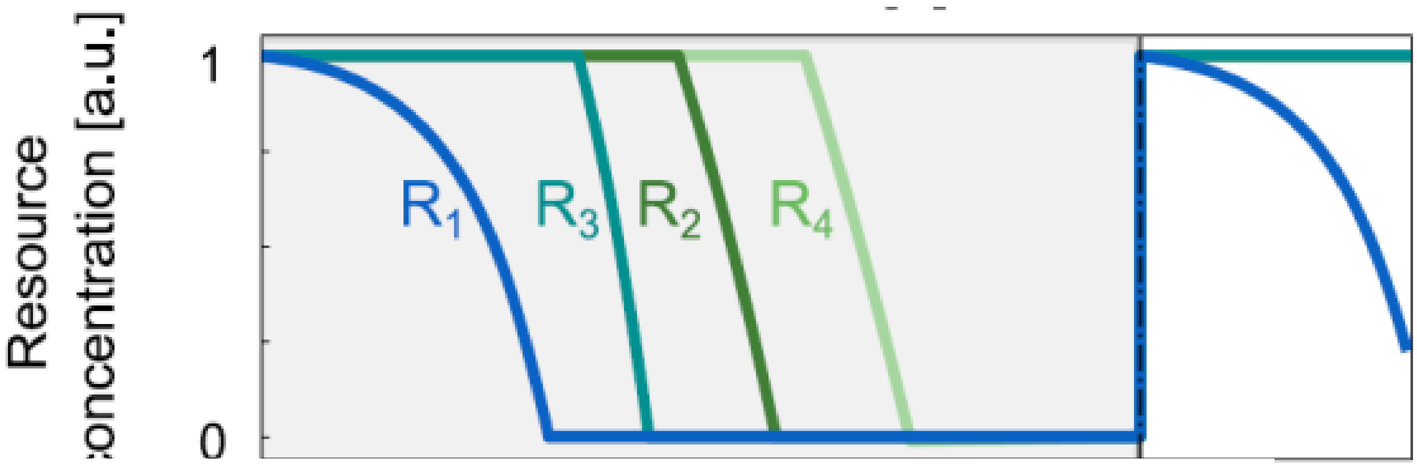
Wang, Z., et al. *Nature communications*, *12*(1), 6661 (2021)

Feasibility: can these species in principle coexist? These two cannot



Wang, Z., et al. *Nature communications*, *12*(1), 6661 (2021)

Change from resource depletion times to duration of temporal niches



Temporal niches t_1 | t_2 | t_3 | t_4 |

- For n resources there are **2^n-1 possible temporal niches**
- Resource depletion order (e.g., T_1<T_3 <$T_{2\ldots}$<T_n shown) determines which **n temporal niches** (t_1,t_2 ,t_3,…t_n) are realized
- To test feasibility, one needs to go through **all of n! possible depletion orders**

Feasibility of the steady state

- $G_{\alpha i}$ is the growth rate of bug α in niche *I*
- Fix the depletion order $T_1 < T_3 < T_2 \ldots < T_n$
- Without lags: $\sum_i G_{\alpha i}\, t_i = \log D$
- With lags: $\sum_i G_{\alpha i}\,(t_i - \tau_{\alpha i}) = \log D$
- $\sum_i G_{\alpha i}\, t_i = \log D + \sum_i G_{\alpha i}\, \tau_{\alpha i} = \log D_\alpha$

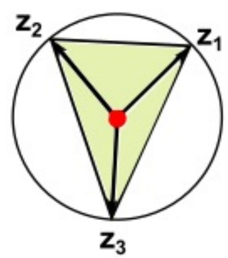
  can be formally solved as

$$\boldsymbol{t_i} = \hat{G}^{-1} \cdot \overrightarrow{\mathbf{log}\, D}_\alpha$$

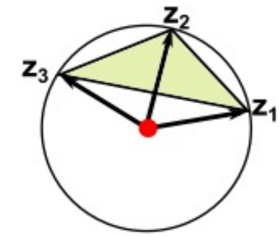
- The steady state is feasible if all $t_i > 0$
- $|\det(G)|$ = stability against variations in lags
- We separately test for dynamical stability

Feasibility of a random community with identical hierarchies

$$\vec{z}_i = \begin{bmatrix} z_{\alpha i} \\ z_{\beta i} \\ z_{\gamma i} \end{bmatrix} \sim \begin{bmatrix} g_{\alpha i} \\ g_{\beta i} \\ g_{\gamma i} \end{bmatrix} - \begin{bmatrix} \mu_{\alpha i} \\ \mu_{\beta i} \\ \mu_{\gamma i} \end{bmatrix}$$



Feasible assembly (promoted by tradeoffs)



Unfeasible assembly (promoted by correlated growth rates)

$$P_{\text{feasible}}(n_S, n_R) = 1 - \left(\frac{1}{2}\right)^{n_R - 1} \cdot \sum_{j=0}^{Ns-2} \binom{n_R - 1}{j}$$

$$P_{\text{feasible}}(n_S, n_S) = \left(\frac{1}{2}\right)^{n_S - 1}$$

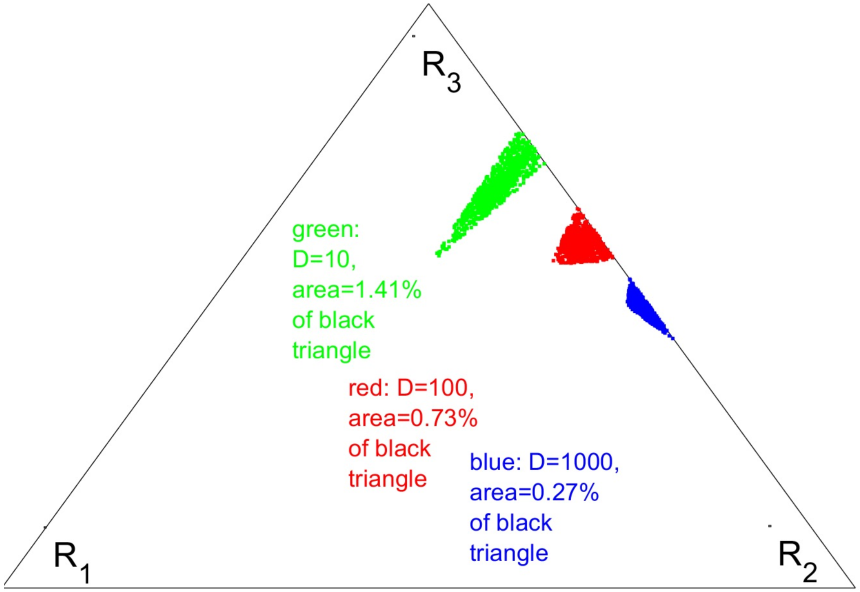
P_{feasible}~*0.5* when ***n_R=2n_S***

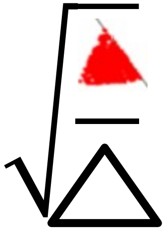
Tradeoffs promote the likelihood of assembly (see Posfai et al PRL 2017)

Supebugs suppress the likelihood of assembly (*V. natriegens* will win)

Z Wang, A Goyal, S Maslov, bioRxiv, July 2023

At what resource concentration ratios is this coexistence possible?



Normalized structural stability= 

Z Wang, A Goyal, S Maslov, bioRxiv, July 2023

Can we use our

engineered assembly methods to

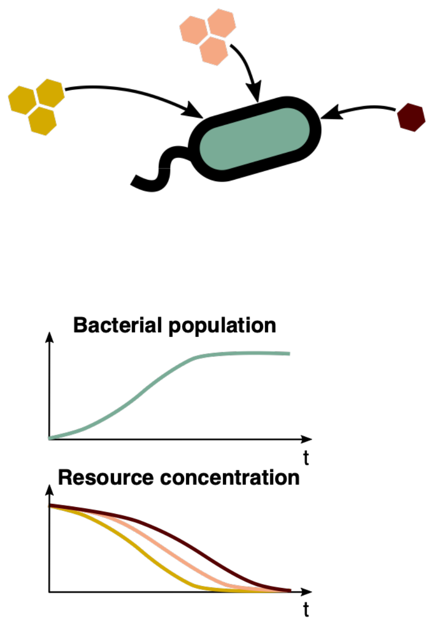
compare the success of

different metabolic strategies

in **real-life ecosystems?**

Microbial metabolic strategies

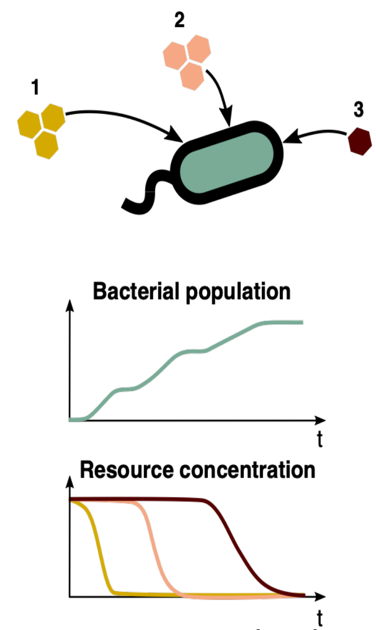
Coutilization of substitutable resources



MacArthur model, 1960s

See recent papers from Mehta, O'Dwyer, Tikhonov, Wingreen labs, and many others

Sequential utilization of substitutable resources



There is no standard model for communities

We developed the methods: Maslov lab 2018, 2021, Gore lab, 2023

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Current Opinion in Microbiology

Hierarchical and simultaneous utilization of carbon substrates: mechanistic insights, physiological roles, and ecological consequences

Hiroyuki Okano[1], Rutger Hermsen[2] and Terence Hwa[1]

CrossMark

Bacteria grown on a mixture of carbon substrates exhibit two utilization patterns: hierarchical utilization (HU) and simultaneous utilization (SU). How and why cells adopt these different behaviors remains poorly understood despite decades of research. Recent studies address various open underlying these utilization patterns. Here we review recent experimental and modeling studies that have provided fresh insights into this classical topic.

Hierarchical utilization (HU) refers to cases in which the

- **Regulatory mechanisms**: known well in model organisms. But may need 282 variables/476 parameters to describe

- **Physiological role**: understood through proteome allocation → growth optimization. But species **do not always optimize** proteome allocation

- **Ecological consequences**: poorly understood

How do we choose growth rates in our model?

- $g_i^{(max)} \rightarrow \lambda_i$.

we follow the notation from Hermsen et al. MSB (2015)

-

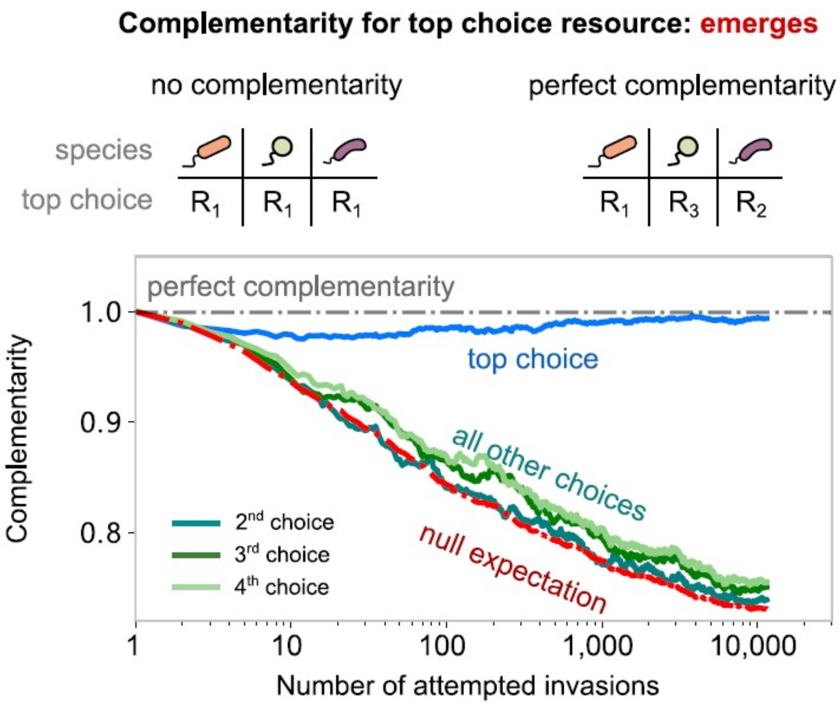
- $\frac{\lambda_i}{1-\lambda_i/\lambda_c} = N(0.5,0.1)$, $\lambda_c = 1$

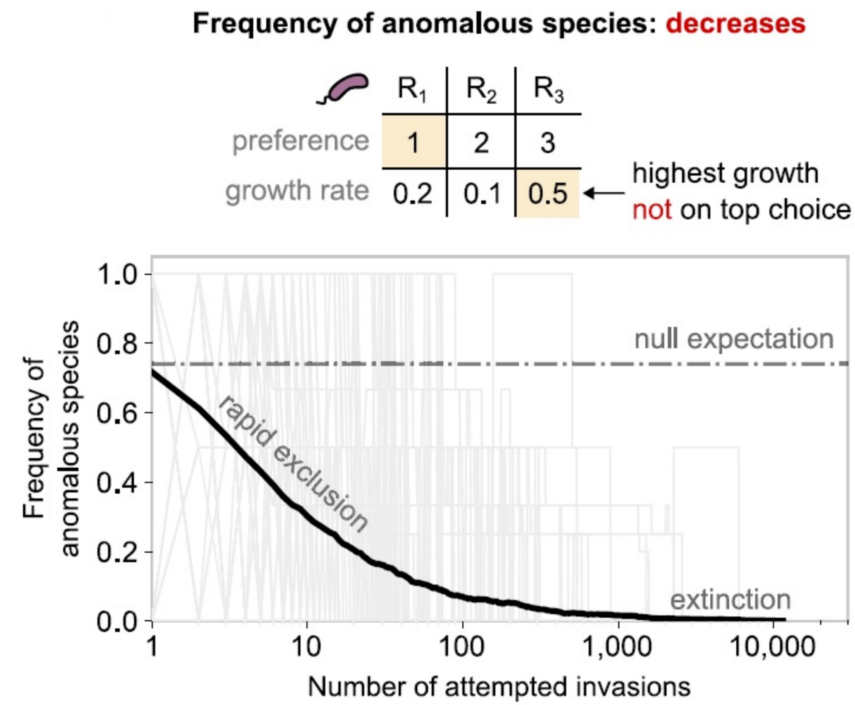
- $\lambda_{ij} = \frac{\lambda_i+\lambda_j-2\lambda_i\lambda_j/\lambda_c}{1-\lambda_i\lambda_j/\lambda_c^2}$ for co-utilizing species

Metabolic strategies we compare

- **``Smart'' sequential utilization**:
  - resource hierarchy matches that of growth rates
- **Random sequential utilization**:
  - resource hierarchy is not correlated with growth rates
- **``Top smart'' sequential utilization**:
  - top resource has the fastest growth rate. Other resources – random hierarchy (inspired by Z Wang, et al Nat Comm 2021)
- **Co-utilization strategy**:
  - proteome equally allocated among the remaining resources. Growth rate – average among growth rates on these resources

Mature communities have complementary resource preferences and no “anomalous species”





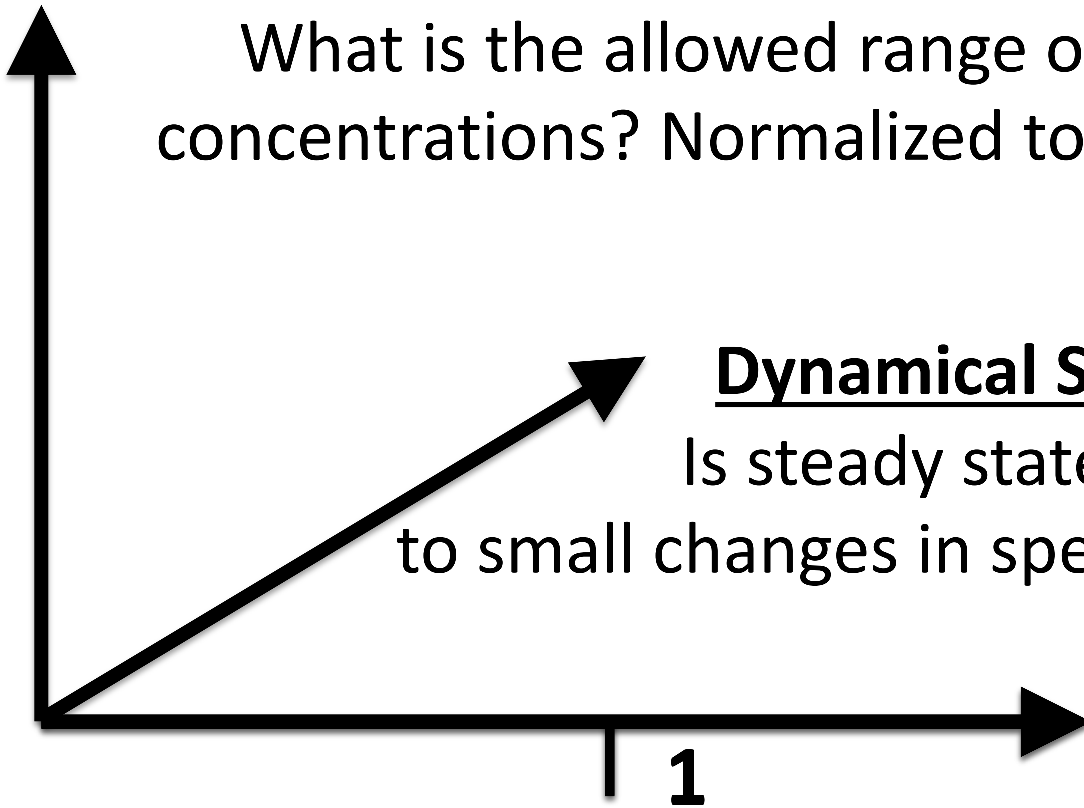
Only top resource is complementary

Anomalous species = top preference is not the fastest growth resource

Wang Z, Goyal A, Dubinkina V, George AB, Wang T, Fridman Y, **Maslov S,** . Nat Comm. 2021

Structural stability

What is the allowed range of resource concentrations? Normalized to a single ratio



Dynamical Stability

Is steady state stable to small changes in species abundances

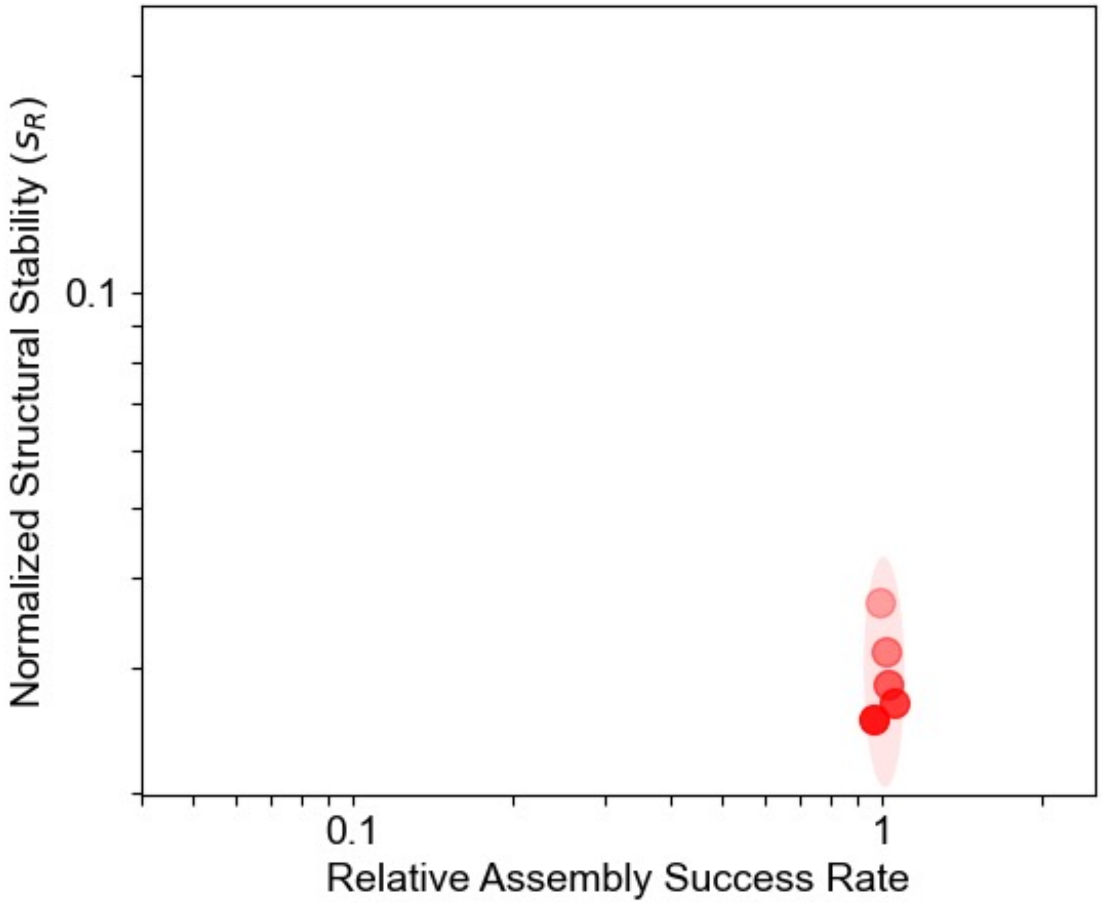
Feasibility + Dynamical Stability

Feasibility

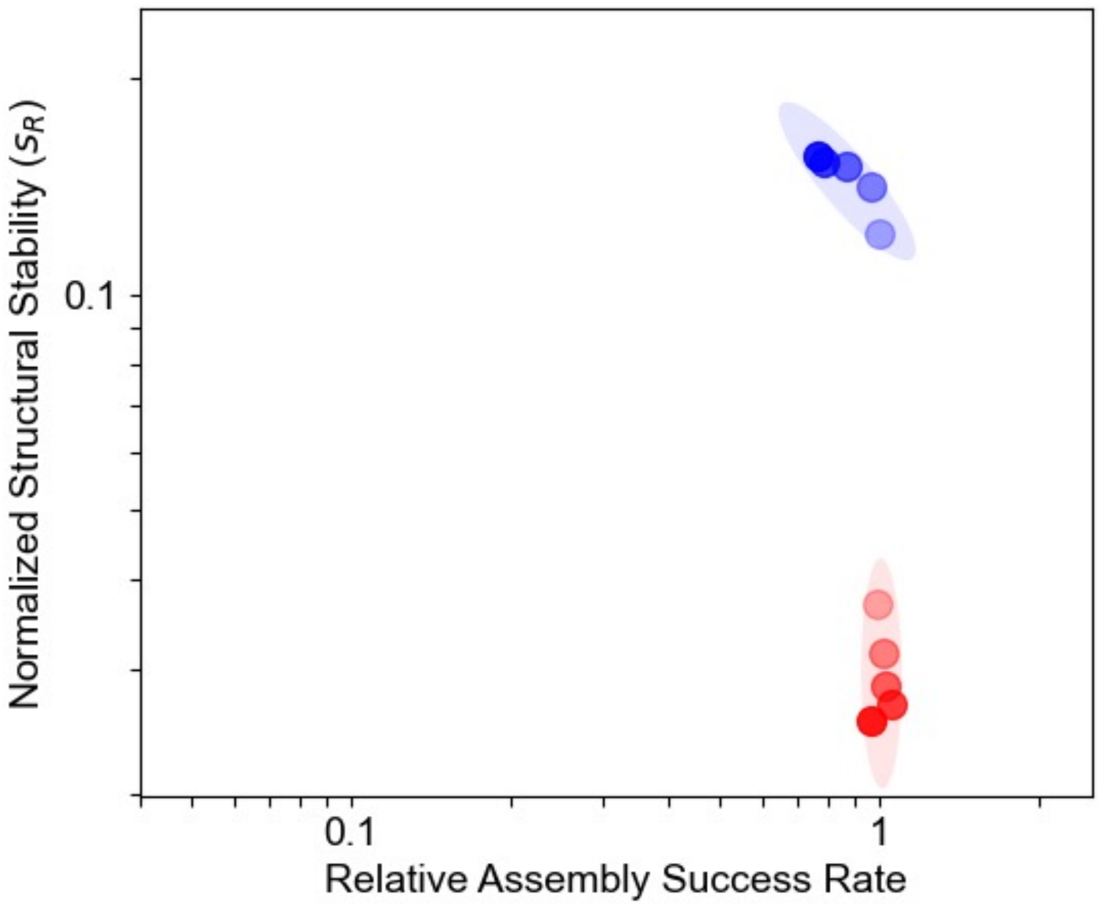
How likely is that the "dream team" can be **stably** assembled?

How likely is that the "dream team" can be assembled?

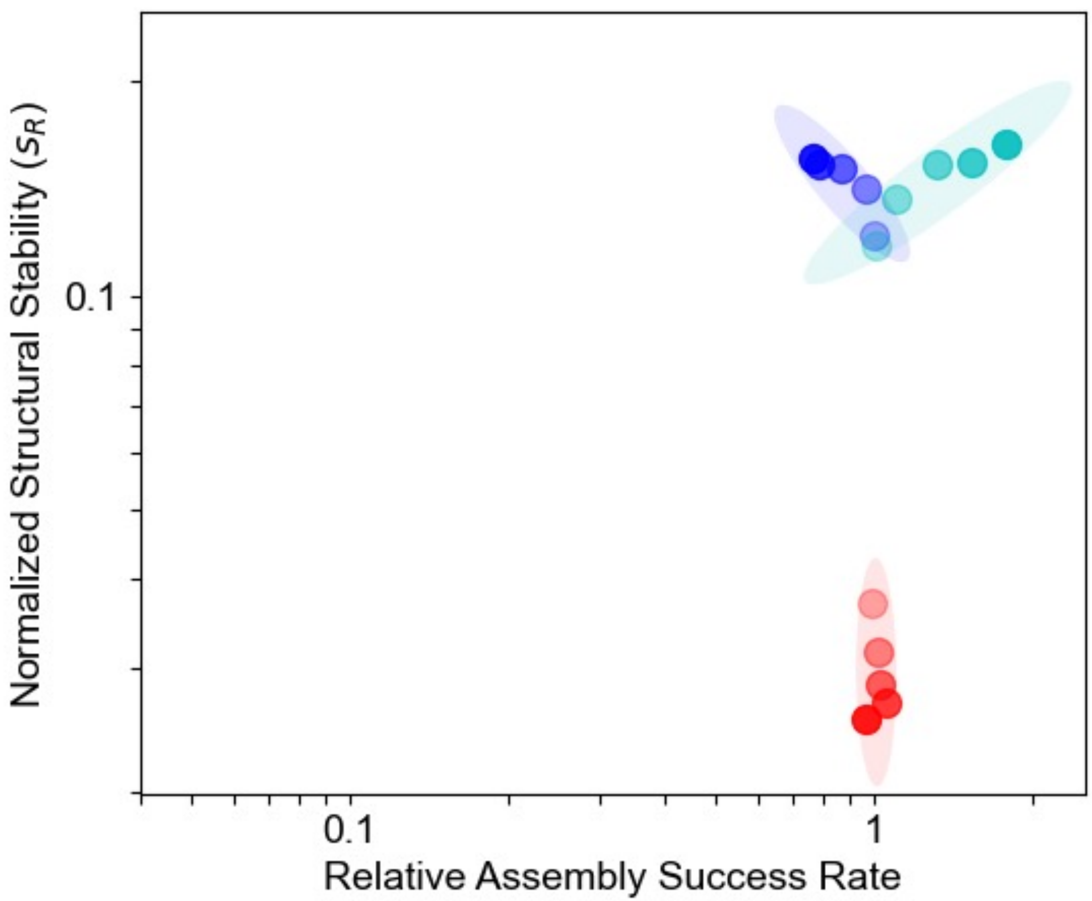
Normalized to random: $\frac{1}{2^{n_s-1}} \rightarrow 1$



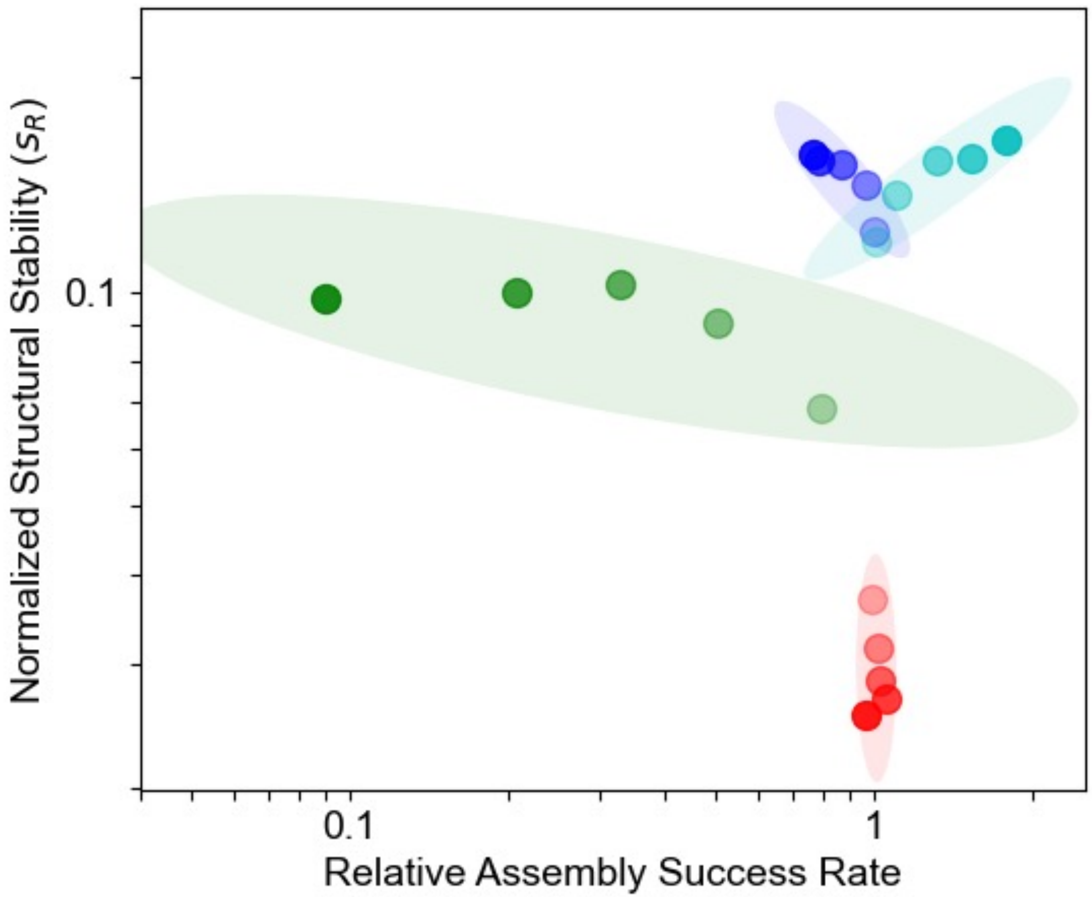
Normalized Structural Stability (s_R)
0.1
0.1
1
Relative Assembly Success Rate



Normalized Structural Stability (s_R)
0.1
0.1
1
Relative Assembly Success Rate

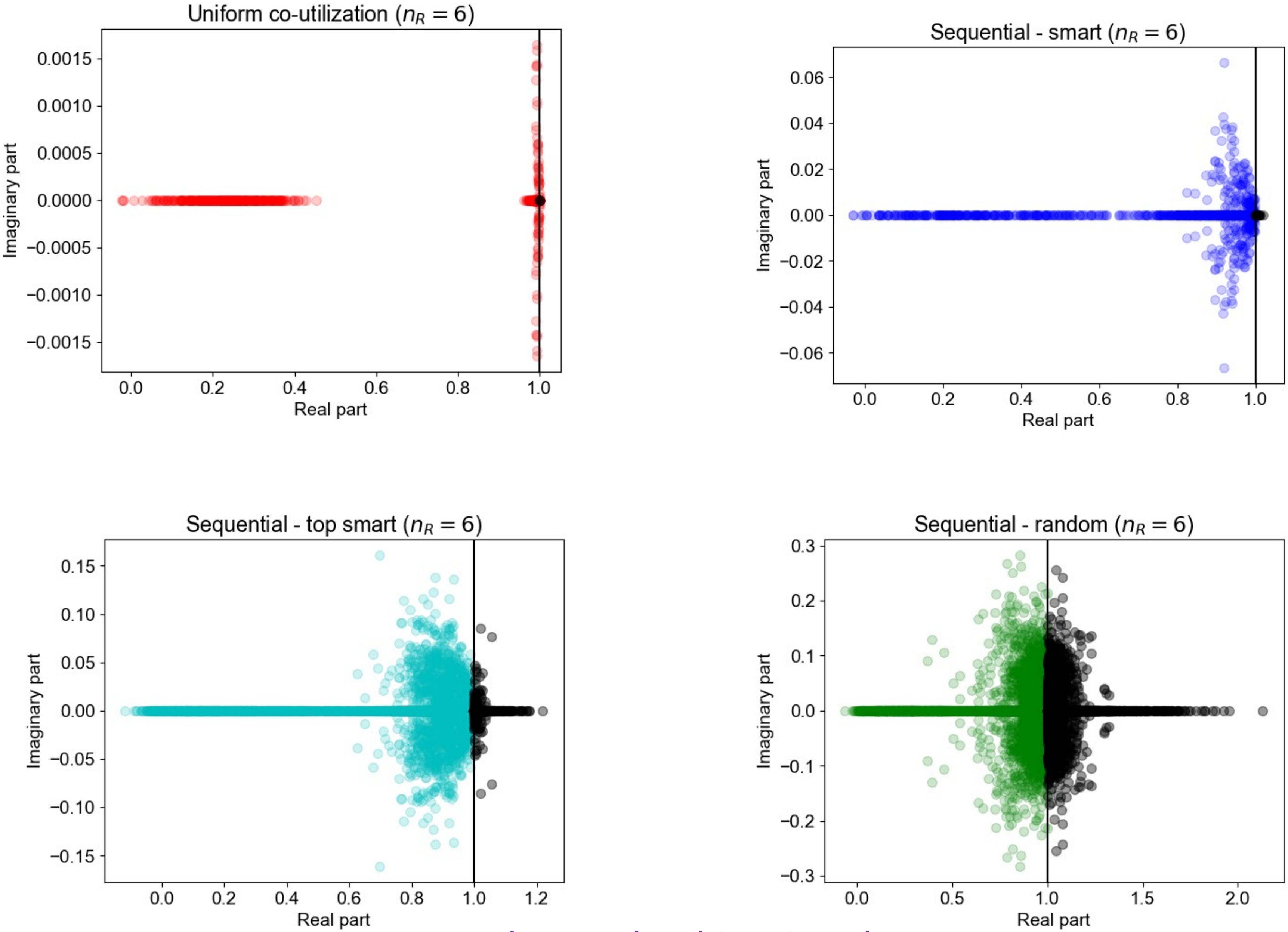


Normalized Structural Stability (s_R)
0.1
0.1
1
Relative Assembly Success Rate



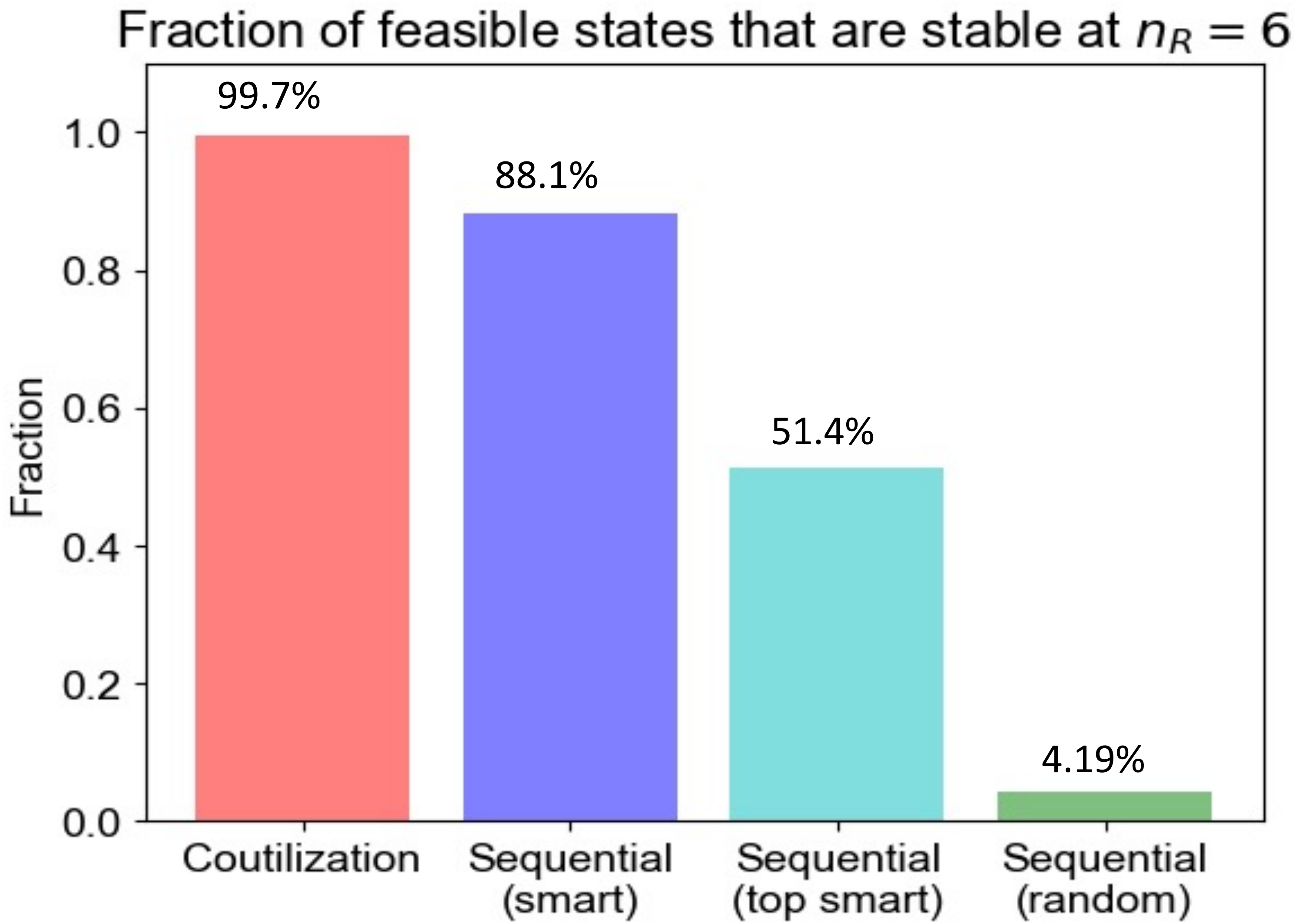
Normalized Structural Stability (s_R)
0.1
0.1
1
Relative Assembly Success Rate

Dynamical stability spectra



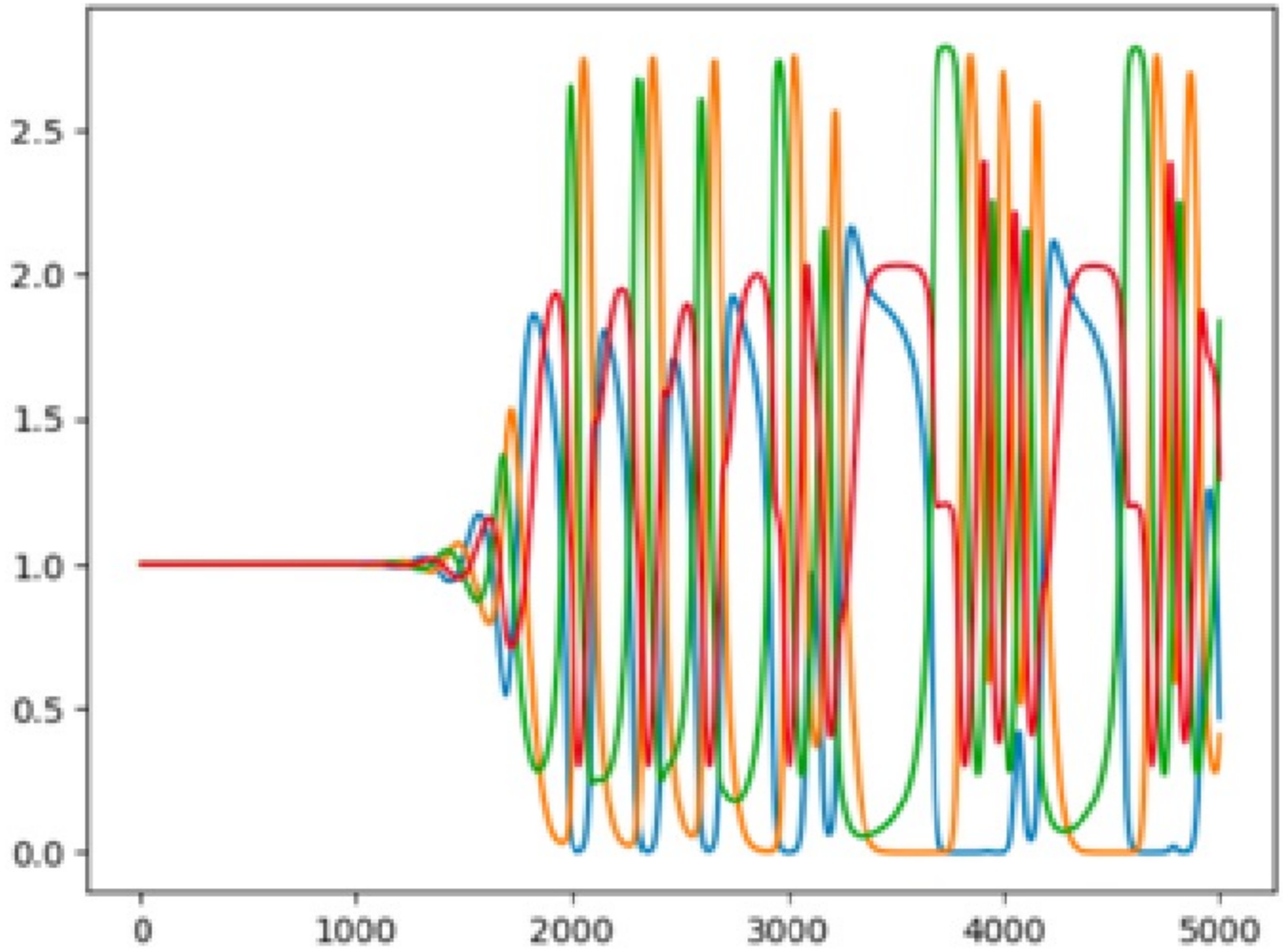
Z Wang, A Goyal, S Maslov, bioRxiv, July 2023

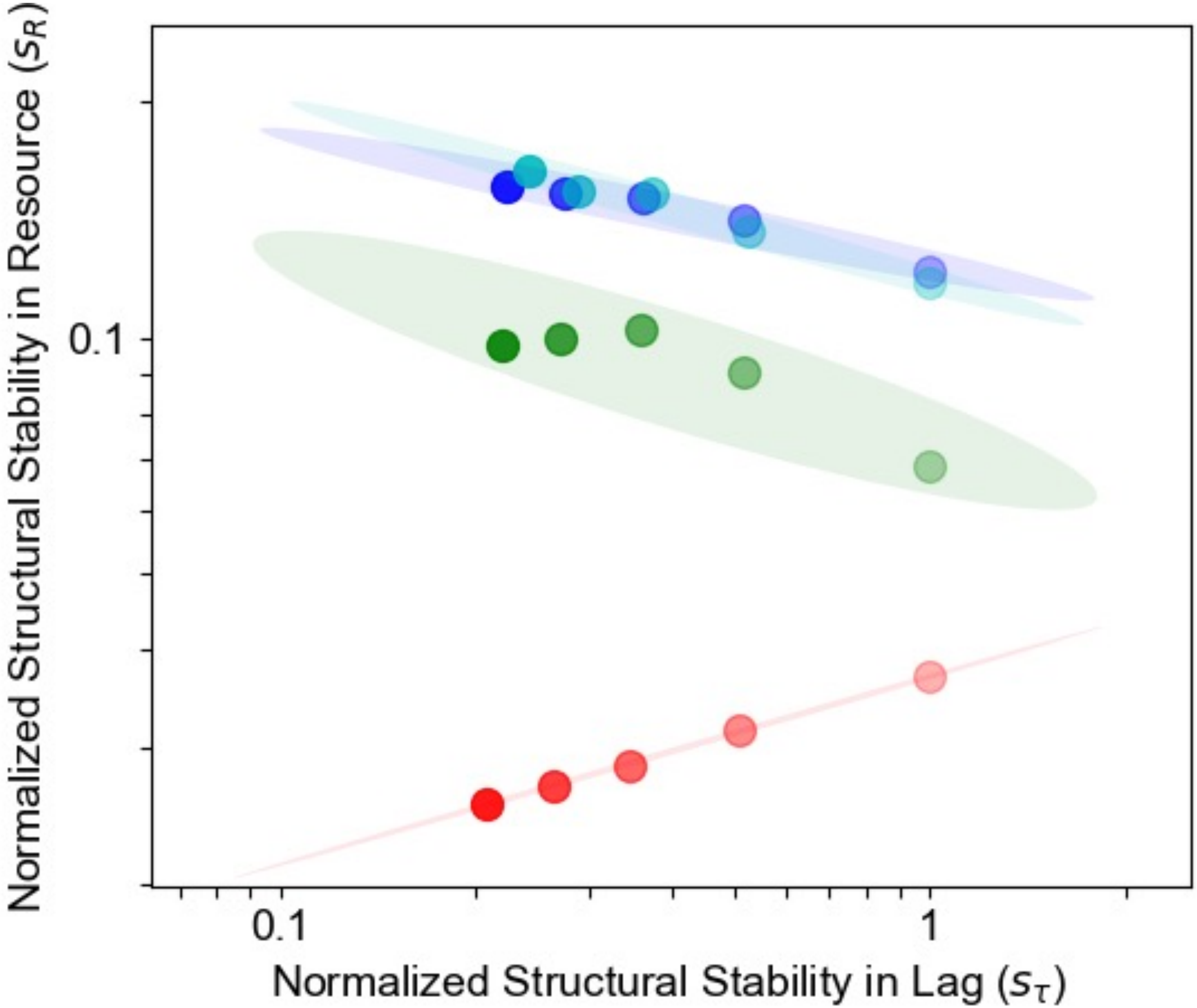
Dynamical stability fraction



Chaotic solutions are possible

but rare: <1 out of 10,000



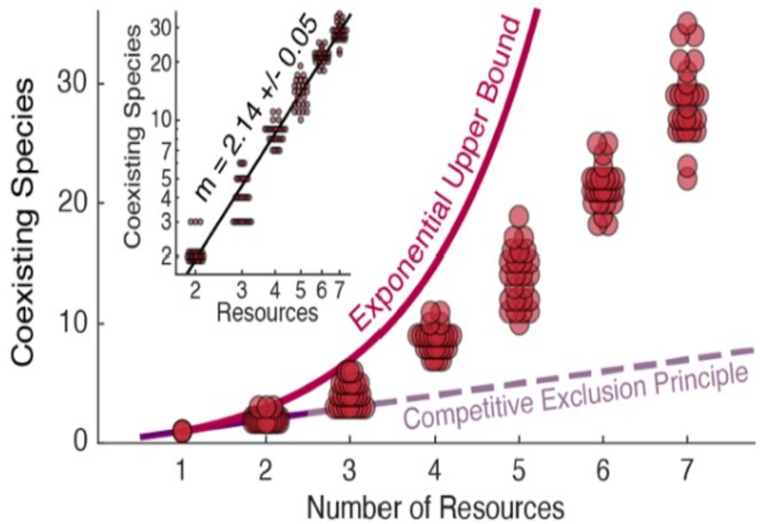


Normalized Structural Stability in Resource (s_R)
0.1
0.1
1
Normalized Structural Stability in Lag (s_τ)

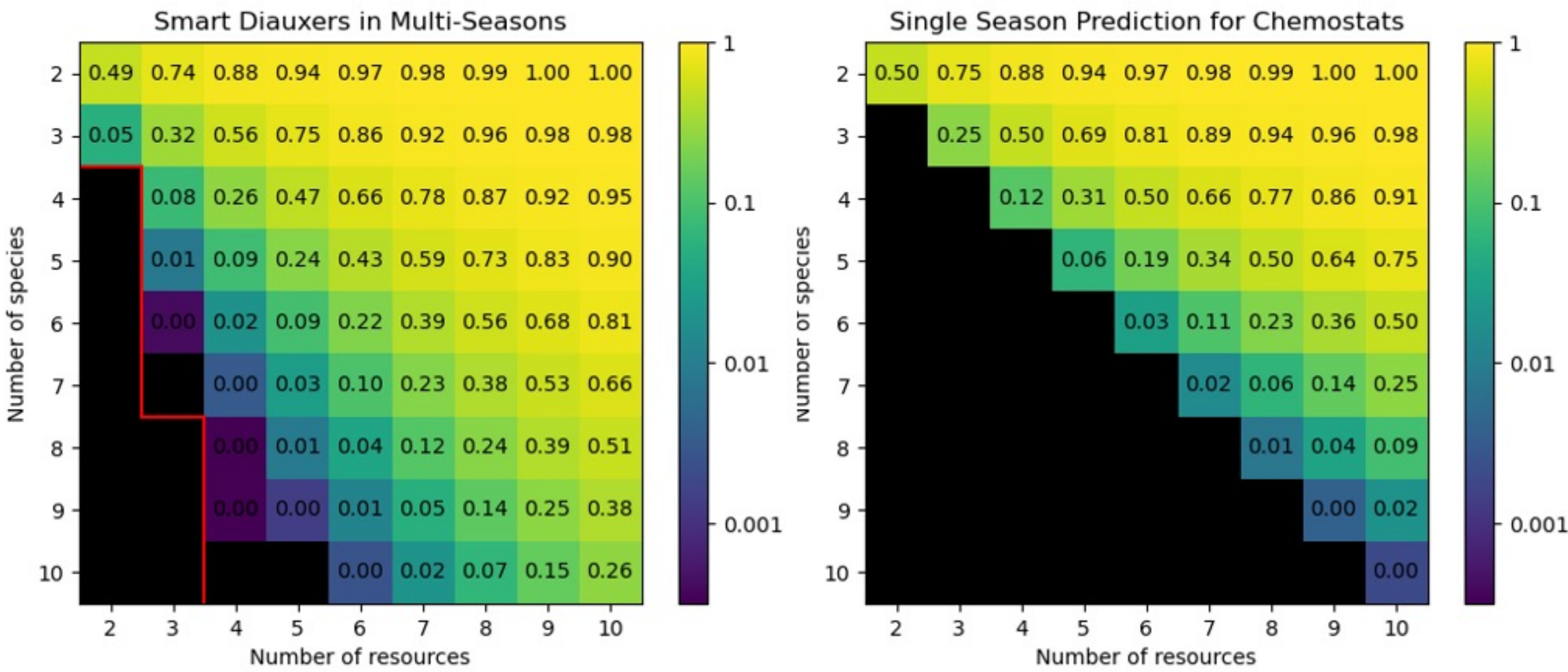
What if the number of resources you have access to is too low?

Robots to the rescue!

- Blox Bloxham et al., bioRxiv (2023):
  - In principle: $n_{species} \leq 2^{n_{resources}} - 1$
  - In practice in randomly fluctuating environments: $n_{species} \sim (n_{resources})^2$
- We can beat it by using robots to add prescribed different nutrient ratios at different cycles



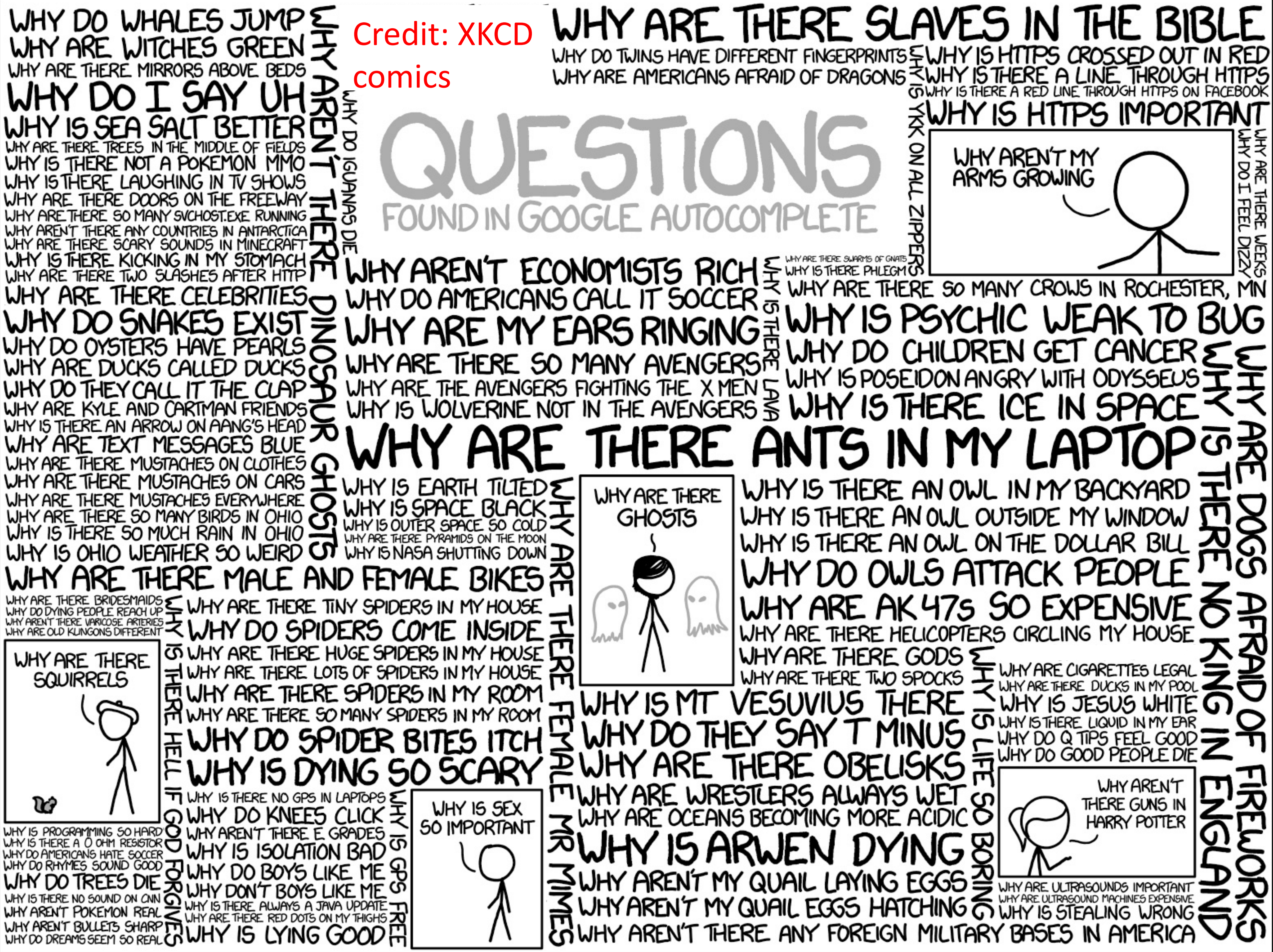
Success rates of assemblies for smart diauxers



Z Wang, A Goyal, Y. Fu, Y. Fridman, S Maslov, in preparation

Take home messages and open questions

- **We can engineer resource ratios** for a given set of species to co-exist in **boom-and-bust environments**
  - Bonus 1: we can make any desired relative species abundances
  - Bonus 2: we can break the competitive exclusion principle: $n_{species} \leq n_{resources}$ potentially going up to $n_{species} = 2^{n_{resources}} - 1$
- **We can compare different metabolic strategies to each other**. Next steps:
  - **Ecology and evolution** of the optimal tradeoff between shorter lags and reduced growth rate due to pre-allocated enzymes
  - Include **crossfeeding diauxie** (glucose-acetate)
  - Can we explain deviations from optimal hierarchy by the success of the **top smart sequential strategy**?



Credit: XKCD comics