Evolutionary Synthetic Ecology

Microbial Ecology Perspective

Natural Selection provides avenues for efficient Engineering & Design

Michael Travisano
Ecology, Evolution & Behavior
BioTechnology Institute
UMN

SYNTHETIC ECOLOGY

Extending synthetic biology from the design of genetic circuits to the engineering of ecological interactions, [...] linking processes at the cellular level to the collective behavior at the system level.

Shou et al. 2007

Using Natural Selection to Design & Engineer Synthetic Ecology Systems

Goals: Product synthesis

BioDegradation

Ecological issues: Cooperation and Conflict among members

Stability

Design Efficiency

Interaction Schematic : Two Species

INTERACTIONS

+	positive
0	neutral
-	negative

Effect of Species 1 on 2 (columns) / **Effect of Species 2 on 1** (rows)

	+	0	-
+	mutualism	facilitation	predation
0	facilitation	neutrality	
-	predation		competition

Interaction Schematic : Two Species

INTERACTIONS

- \+ positive
- 0 neutral
- \- negative

Effect of Species 1 on 2

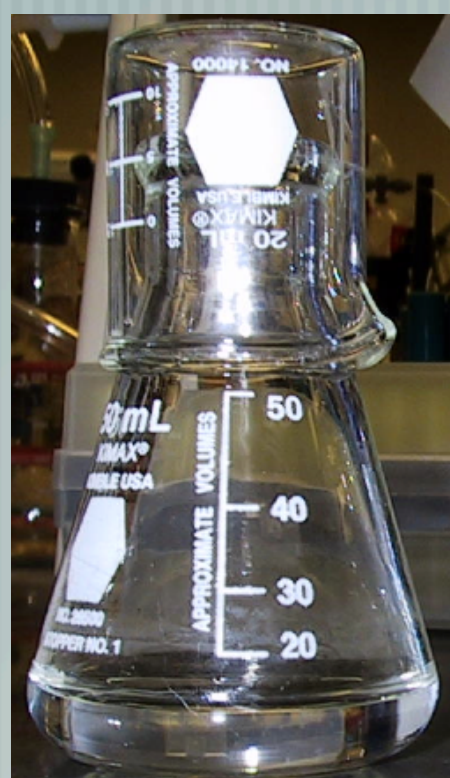
Effect of Species 2 on 1	+	0	-
+	mutualism	facilitation	predation
0	facilitation	neutrality	
-	predation		competition

Facilitation: Cross-feeding & Environmental Engineering

Specialist 1 consumes glucose releases acetate
Specialist 2 consumes acetate

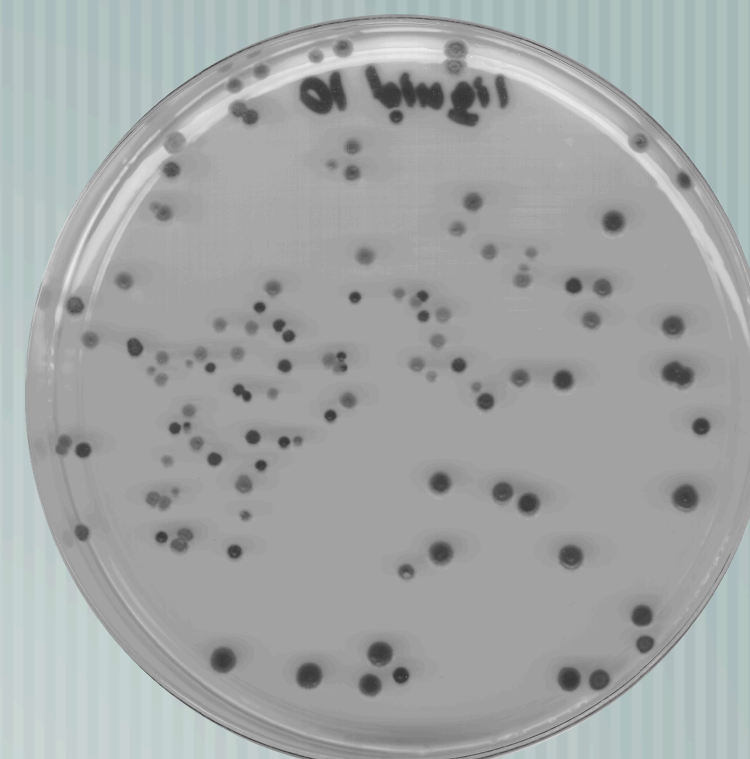
Evolve a single *E. coli* genotype
in a glucose minimal medium

Loss of acetate specialist



Mass Action Environment
Stable Coexistence

Helling et al. 1987



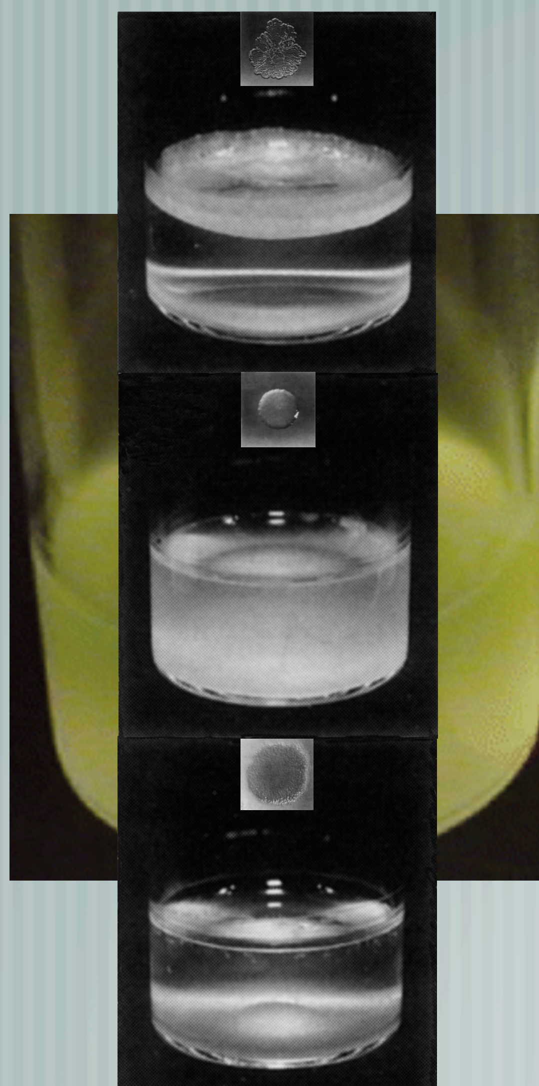
Spatially Structured Env.
Not Stable

Saxer, Doebeli & Travisano 2009

Facilitation: Cross-feeding & Environmental Engineering

Evolve a single Pseudomonas genotype in a static rich broth medium

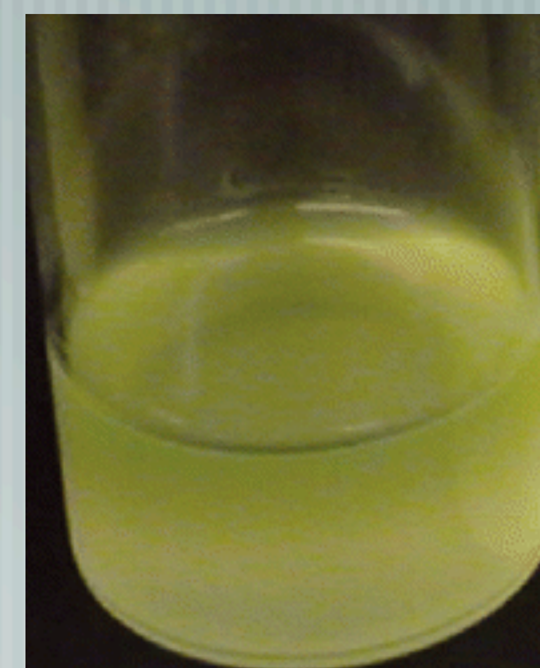
3 Specialists: Top, middle & bottom



Rainey & Travisano 1998

Start Shaking the tube →

Back to one genotype



Interaction Schematic : Two Species

INTERACTIONS

+ positive

0 neutral

Effect of Species 1 on 2 (columns); Effect of Species 2 on 1 (rows)

	+	0
+	mutualism	facilitation
0	facilitation	

Mutualism: Cross-feeding

E. coli consumes lactose excretes acetate

To test the effect of mass action, bacteria were added to a 125-mL flask with 10 mL of lactose M9 minimal media. Every 24 h 100 μL was transferred to a new flask (100-fold dilution). Three replicates were carried out with initial frequencies of 99.99% methionine excreters and 0.01% nonexcreters.

After every passage, the number of *E. coli* and *Salmonella* were determined by plating on LB plates with X-gal. To determine the frequency of excreters and nonexcreters, 30 Salmonella colonies were stabbed onto a lawn of *E. coli* on a lactose plate with X-gal. If an isolate was an excreter a blue colony formed on the plate, otherwise no colony appeared.

Results

At the start of the study, cultures of the bacteria were unable to grow together (Fig. 1, left). A specific selection regime was used to evolve cooperative methionine excretion in *Salmonella*, thereby allowing community growth.

EVOLUTION OF *SALMONELLA* WITH HIGH METHIONINE EXCRETION

HPLC measurements indicated that initially *Salmonella* excreted very low levels of methionine (0.005 ± 0.002 mM methionine in overnight glucose culture). A two-step process was used to acquire cooperative *Salmonella*. First, an established chemical technique was used to select overproduction of methionine. Resistance to the methionine-analog ethionine has been shown to cause a constitutive expression of the methionine pathway (Lawrence et al. 1968). It was anticipated that selection on ethionine plates would be sufficient to create cooperative *Salmonella*, but methionine excretion levels were no higher than ancestral *Salmonella* as measured by cross-feeding assays (Fig. 1, middle) and HPLC.

An indirect selection method was then used to select for increased methionine excretion by *Salmonella*. Lactose minimal plates were seeded with 10^7 each of met-*E coli* and ethionine-resistant *Salmonella* and allowed to grow for three days at 37°C. The three-day plate contained little visible growth, but was scraped and an aliquot was spread on a new plate. After five days on the second plate, several large colonies appeared, containing both *E. coli* and *Salmonella*. The *Salmonella* in these colonies were a mutant that excreted high levels of methionine thus enabling the *E. coli* to grow. Assays of methionine levels in spent media confirmed an approximate 15-fold increase (0.08 ± 0.02 mM) in methionine excretion by these *Salmonella* mutants (Fig. 1; Methods). High excretion mutants arose twice in 10 replicates (multiple colonies forming on the second plate within a replicate were conservatively deemed one evolutionary origin as they could have come from a single mutant on the first plate). The second mutant performed identically in cross-feeding assays, but was not measured with HPLC.

Ten indirect selection replicates were also initiated with wild-type *Salmonella*. No evolution of high methionine excretion was observed in these cases. This suggests that the ethionine treatment facilitated the evolution of methionine excretion.

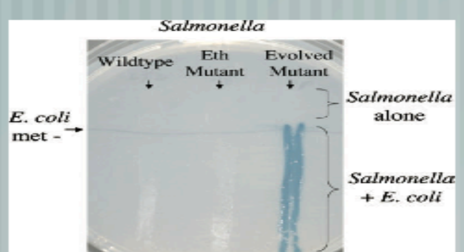


Figure 1. Cross-streaks of the three types of *Salmonella* across *E. coli*. *E. coli* was streaked horizontally across the plate. *Salmonella* was then streaked vertically from top to bottom. "Wild-type" indicates the initial *Salmonella typhimurium*. "Eth mutant" indicates the ethionine-resistant mutant. "Evolved mutant" indicates the methionine-excreting mutant that arose on plates with *E. coli* and was used in experiments. The blue line is bacterial growth where the methionine-producing *Salmonella* was streaked across *E. coli*.

METHIONINE EXCRETION IS COSTLY

To determine whether methionine excretion impaired *Salmonella* fitness, mutant *Salmonella* were competed against wild-type *Salmonella* in acetate minimal media. In these conditions, *E coli* were absent and the *Salmonella* grew according to their intrinsic metabolic abilities. Any fitness effect of methionine excretion would lead to reduction in growth of methionine excreters and therefore an increase in the frequency of wild-type *Samonella*. In liquid, the wild-type swept from an initial frequency of 2% to near fixation in one transfer, a selection coefficient (s) of −0.43 ± 0.05 for methionine excretion. The selection coefficient of methionine excreters in comparison to nonexcreting ethionine mutants is −0.37 ± 0.06. This result suggests that there was a cost associated with ethionine resistance, and an additional cost was associated with methionine excretion.

The apparent cost of methionine excretion distinguishes *Salmonella's* excretion from that of *E. coli*. *E. coli*'s excretion is beneficial for the bacteria independent of other species, whereas *Salmonella*'s excretion clearly is not. I use the term cooperation to describe *Salmonella's* excretion as it benefits another species, and is not beneficial to *Salmonella* in the absence of interspecific

Evolved Salmonella consumes acetate excretes Met

Harcombe 2010

Spatial Structure stabilizes the interaction

feedback. This definition of cooperation as an adaptation that is selected because it helps a recipient follows West et al. (2007b).

COOPERATION IS SUPERIOR IN A STRUCTURED ENVIRONMENT

The evolutionary fate of cooperative versus noncooperative *Salmonella* was tested in a structured environment. *E. coli* and *Salmonella* were plated together on lactose minimal plates at a density of 5×10^7 each. Initially, the *Salmonella* population consisted of 99% wild-type and 1% cooperative methionine excreters. Over four transfers (approximately 20 generations), cooperative methionine excreters spread through the population to greater than 80% (Fig. 2A). Coincident with the increase in cooperators, the density of bacteria on the plates after 48 h increased by more than 15-fold (Fig. 2E). This result demonstrates that, on lactose plates, the fitness cost of high methionine excretion by *Salmonella* is overcome by the fitness gained from receiving more food from enhanced *E. coli* growth.

% Met secreters

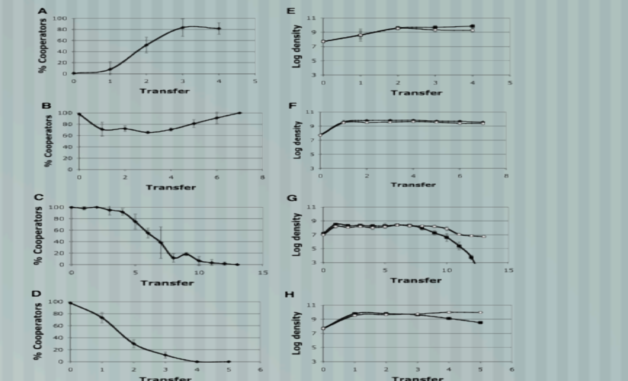


Figure 2. Dynamics of the system with variation in reciprocation and spatial structure. Graphs A–D are the percentage of cooperators in the *Salmonella* population. Graphs E–H are the log density of *E. coli* (filled squares) and *Salmonella* (open circles). A and E are the results from communities grown on lactose plates when cooperators were initially rare. B and F are the results from communities grown on lactose plates when cooperators were initially common. C and G are the results from communities grown on acetate plates. D and H are the results from communities grown in lactose flasks with no spatial structure. Error bars represent the standard deviation.

Mass Action leads to rapid loss of cooperation

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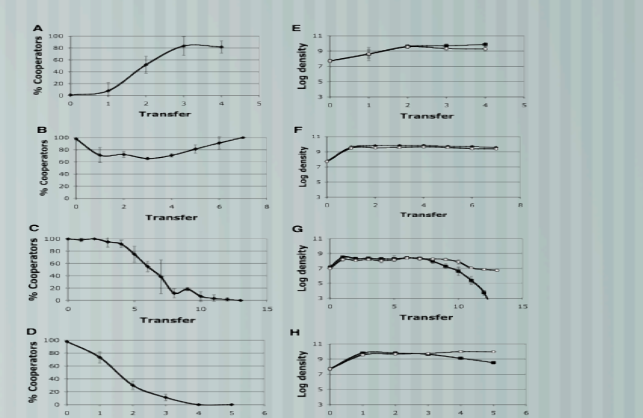

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Stability Summary

Stable Community

	Facilitation	Mutualism
Cross-feeding	Mass Action	Spatial Structure
Environmental Engineering	Spatial Structure	Spatial Structure

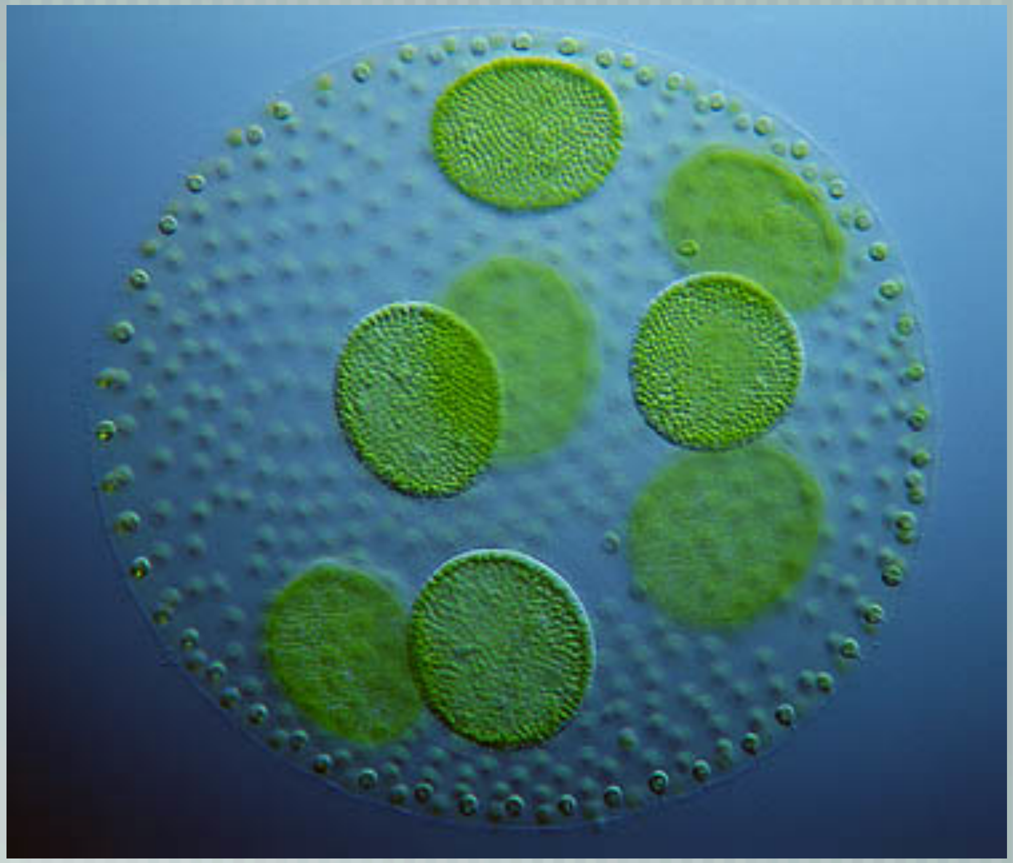
Instability Caused by Ecological Breakdown

Cheaters = Evolutionary Breakdown

Application: Evolve a complex cooperative system

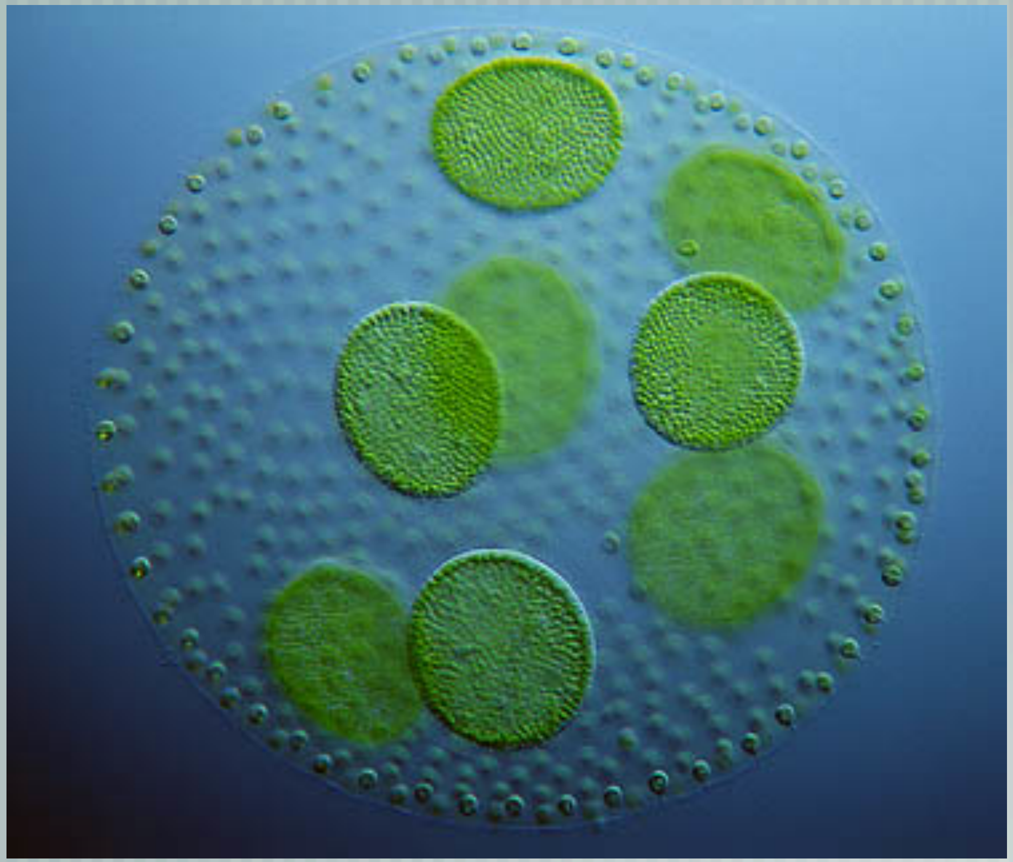
Unicellularity

Multicellularity



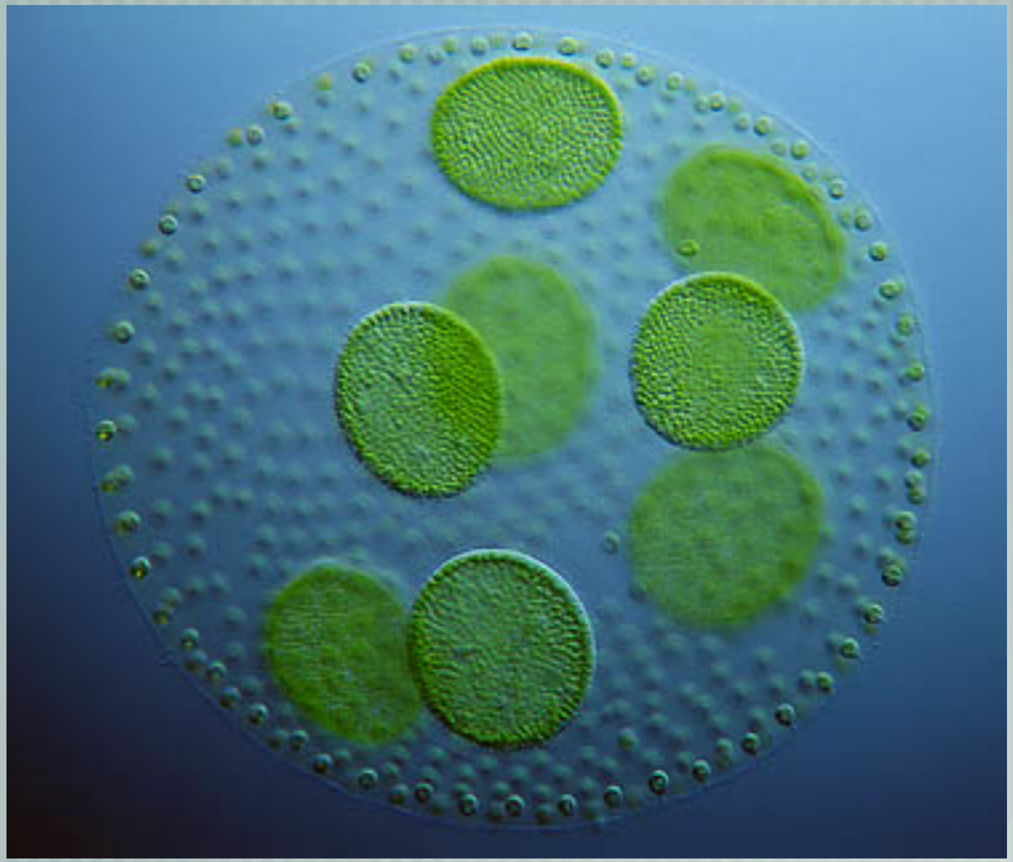
Stability: Mutualism criteria

Multicellular organisms are mutualists



Spatial structure likely to be important
(counter examples myxo and dicty)

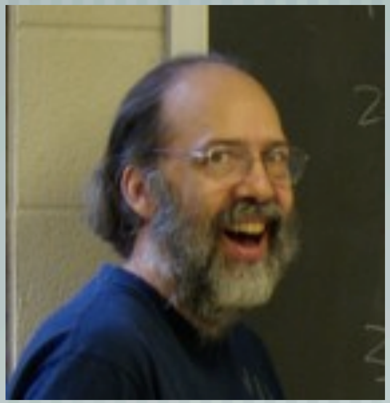
Ecological conditions: Mass Action



Selective conditions that promote the evolution of spatial structure within a mass action environment

Our Team

Will Ratcliff



R. Ford Denison

Mark Borrello

Johnathon Fankhauser

Mitch Hoverman
Kristin Jacobsen
Amanda muehlbauer
Jennifer Pentz

Genome Sequencing
Molly Burke (UCI)
Kenny Beckman (BMGC)

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