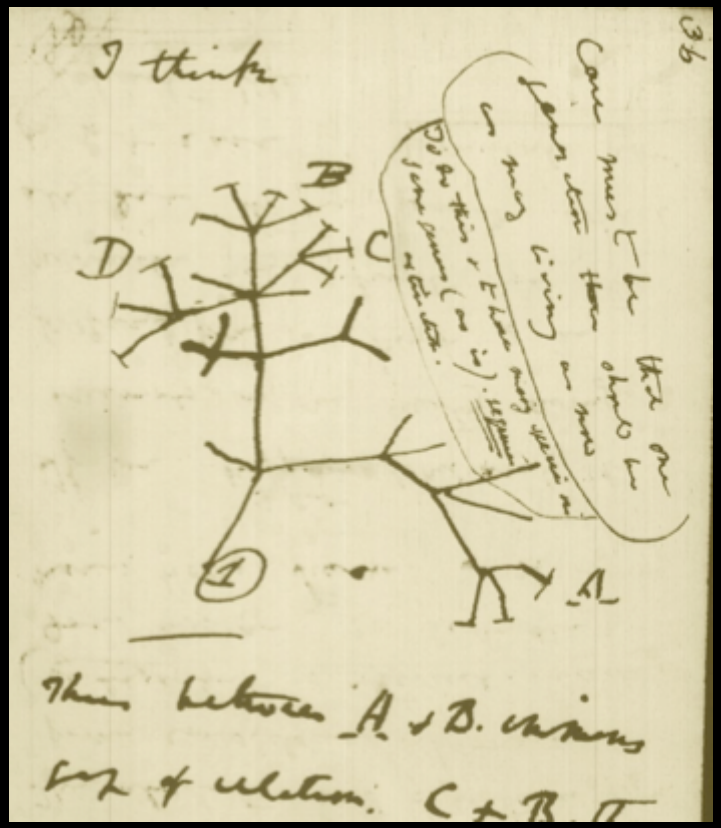
Reliving the Past: Experimental Evolution of Major Transitions in the History of Life

V Cooper (UNH), S Copley (UColorado), P Gerrish (UNM), M Herron (UM), M Kinnersley (UM), J McCutcheon (UM), S Miller (UM), E Smith (SFI), G Sherlock (Stanford), P Sniegowski (Penn)

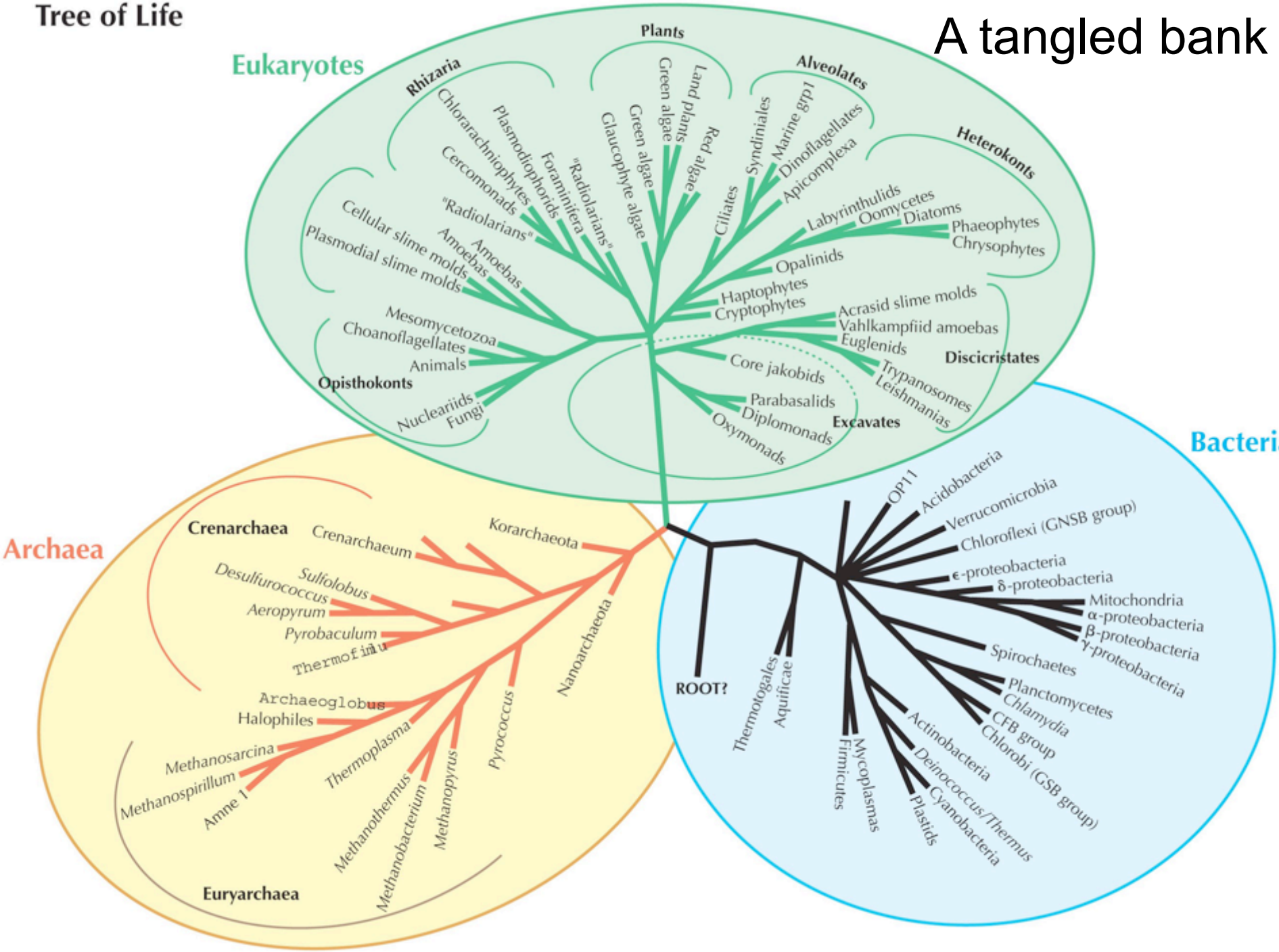
A tangled bank

It is interesting to contemplate **a tangled bank** clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us.

Darwin, 1859



A tangled bank



A tangled bank

It is interesting to contemplate **a tangled bank** clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us.

These laws lead to a **Struggle for Life**, and as a consequence to Natural Selection

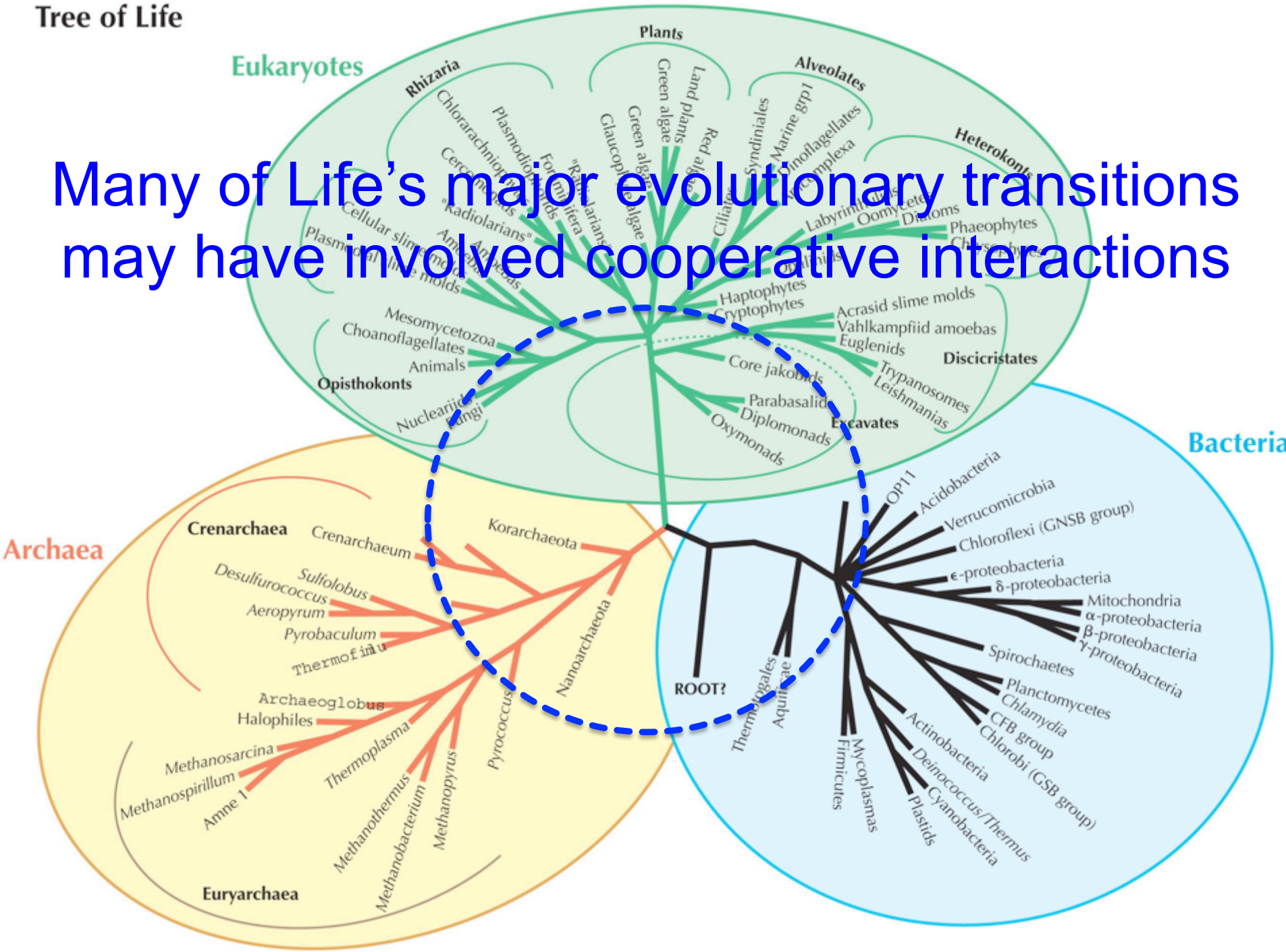
Nature Red in Tooth and Claw

G Stubbs ca.1780

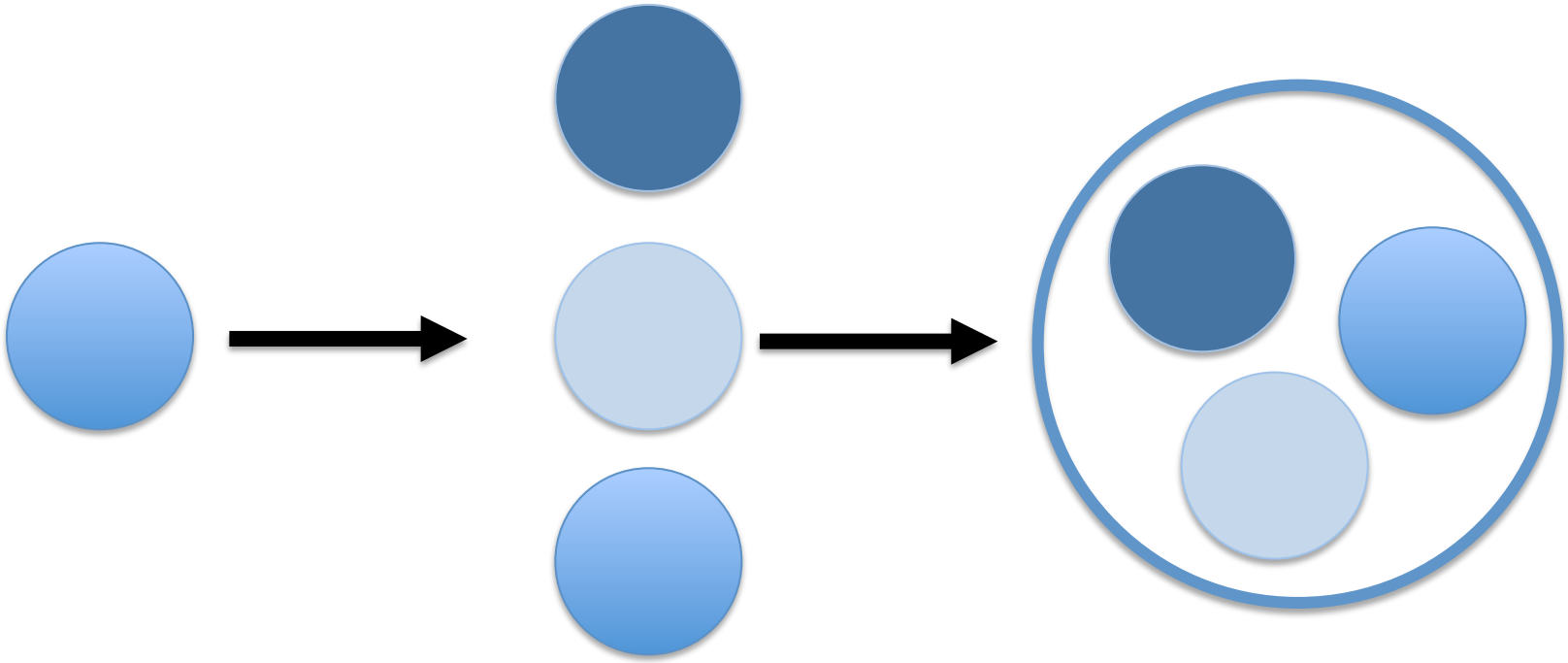
The Peaceable Kingdom

E Hicks 1834

Many of Life's major evolutionary transitions may have involved cooperative interactions

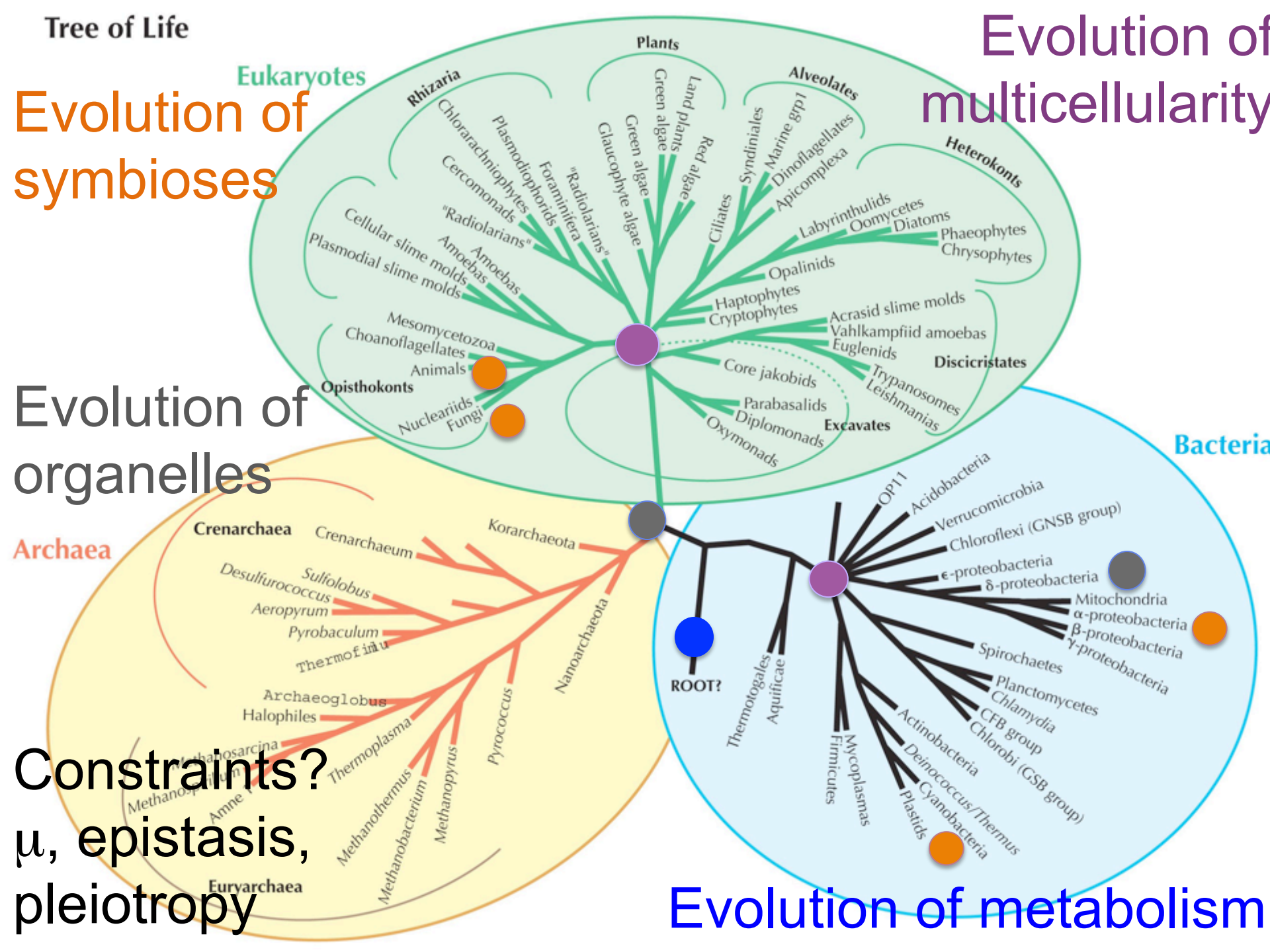


Major Evolutionary Transitions: From simple units to more complex wholes



Interdependence
Autonomy

Szathmary and Maynard-Smith, 1995


Tree of Life
Evolution of symbioses
Evolution of multicellularity
Evolution of organelles
Constraints?
μ, epistasis, pleiotropy
Evolution of metabolism
Eukaryotes
Plants
Rhizaria
Alveolates
Heterokonts
Chlorarachniophytes
Plasmodiophorids
Foraminifera
"Radiolarians"
Cercomonads
"Radiolarians"
Cellular slime molds
Amoebas
Amoebas
Plasmodial slime molds
Green algae
Green algae
Glaucophyte algae
Land plants
Red algae
Ciliates
Syndiniales
Marine grp1
Dinoflagellates
Apicomplexa
Labyrinthulids
Oomycetes
Diatoms
Phaeophytes
Chrysophytes
Opalinids
Haptophytes
Cryptophytes
Acrasid slime molds
Vahlkampfiid amoebas
Euglenids
Discicristates
Trypanosomes
Leishmanias
Core jakobids
Parabasalids
Diplomonads
Oxymonads
Excavates
Mesomycetozoa
Choanoflagellates
Animals
Opisthokonts
Nucleariids
Fungi
Archaea
Crenarchaea
Crenarchaeum
Korarchaeota
Sulfolobus
Desulfurococcus
Aeropyrum
Pyrobaculum
Thermofilum
Nanoarchaeota
Archaeoglobus
Halophiles
Thermoplasma
Methanosarcina
Methanothermus
Methanobacterium
Methanopyrus
Pyrococcus
Euryarchaea
Bacteria
OP11
Acidobacteria
Verrucomicrobia
Chloroflexi (GNSB group)
ε-proteobacteria
δ-proteobacteria
Mitochondria
α-proteobacteria
β-proteobacteria
γ-proteobacteria
Spirochaetes
Planctomycetes
Chlamydia
CFB group
Chlorobi (GSB group)
Actinobacteria
Deinococcus/Thermus
Cyanobacteria
Plastids
Mycoplasmas
Firmicutes
Thermotogales
Aquificae
ROOT?

Reliving the Past: Experimental Evolution of Major Transitions in the History of Life

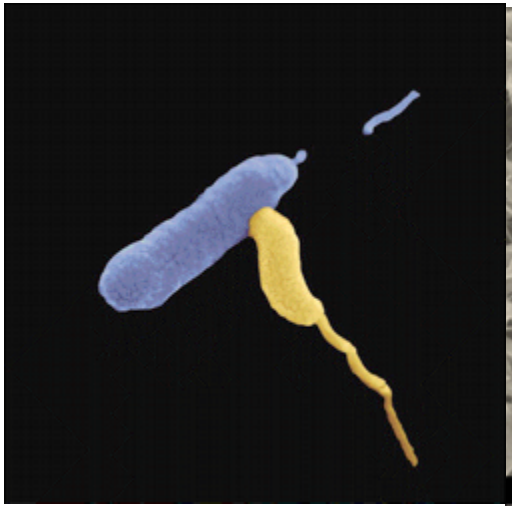
Recapitulate and study in the lab
transitions from simple units to complex wholes

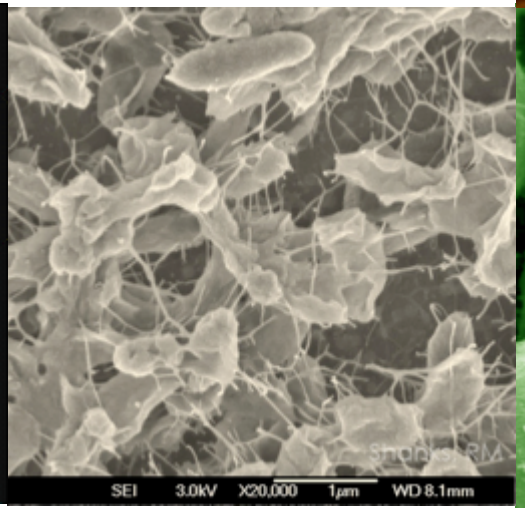
How will we do it?

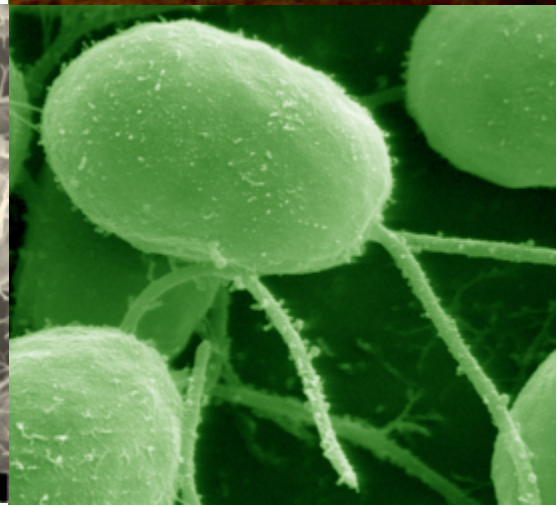
Laboratory experimental Evolution

Laboratory experimental evolution

- Specify selection pressure
- Control and replicate trials
- MET involved microbes, which are ideal models
  - Short gen times, large population sizes
  - Easy to manipulate genetically
  - Using continuous culture we can watch evolution in real time
  - Cryopreserve a living “fossil record”
  - Rapidly, cheaply sequence clones, popns



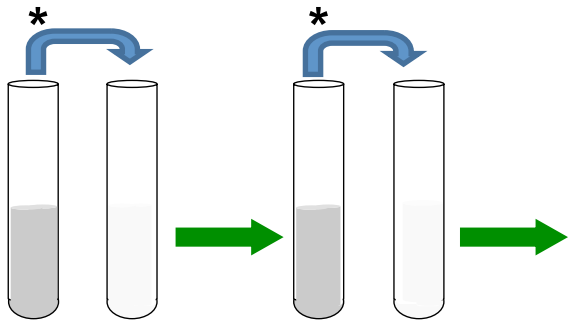


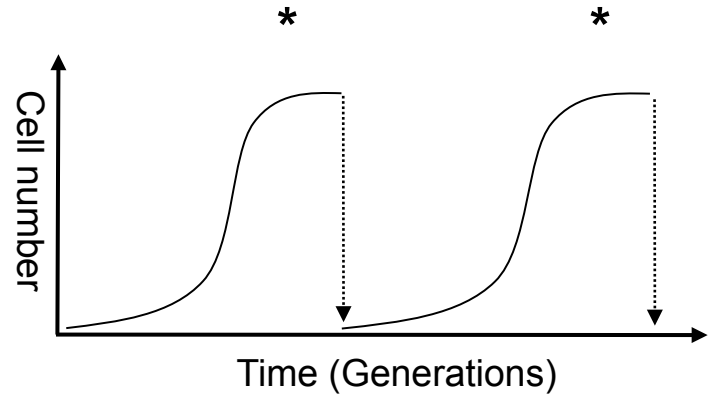


Experimental microbial evolution

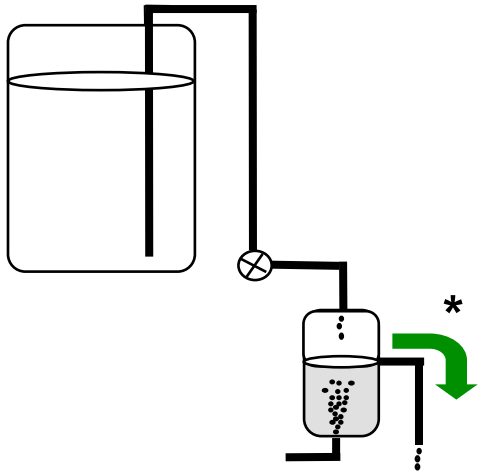
2 modes of continuous culture

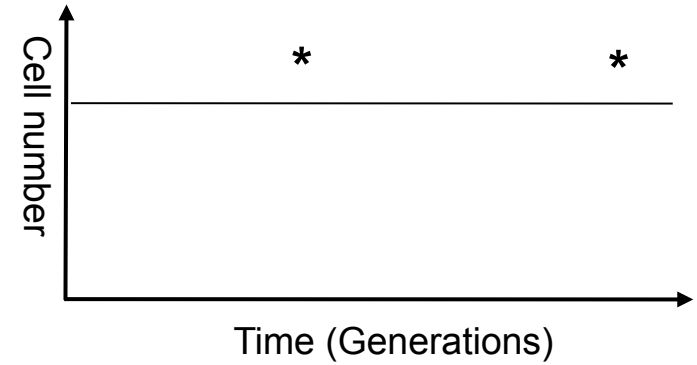
Propagate by serial transfer





Propagate under nutrient limitation (chemostat)





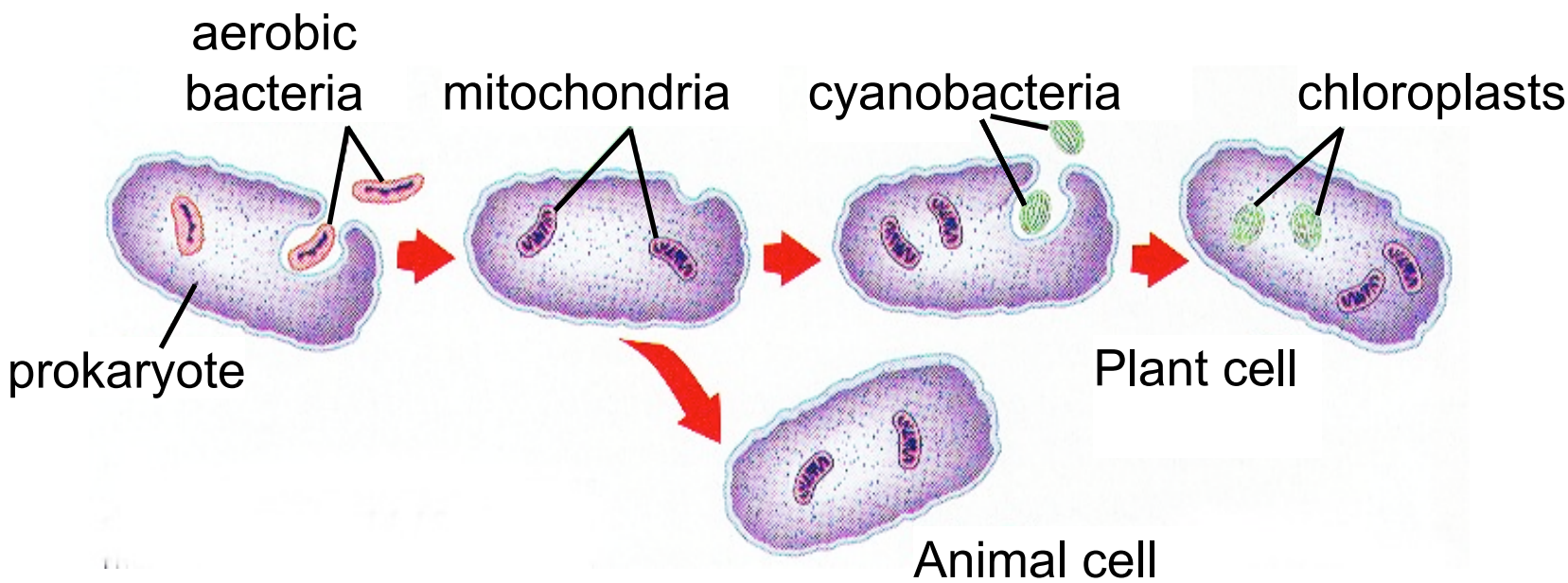
*Archive samples for genetic and genomic analysis.

2 representative projects will be carried out by current and former NPP fellows

Project #4 (Margie Kinnersley)

How did the Eukaryotic cell come to be, specifically the cell having a mitochondrion?

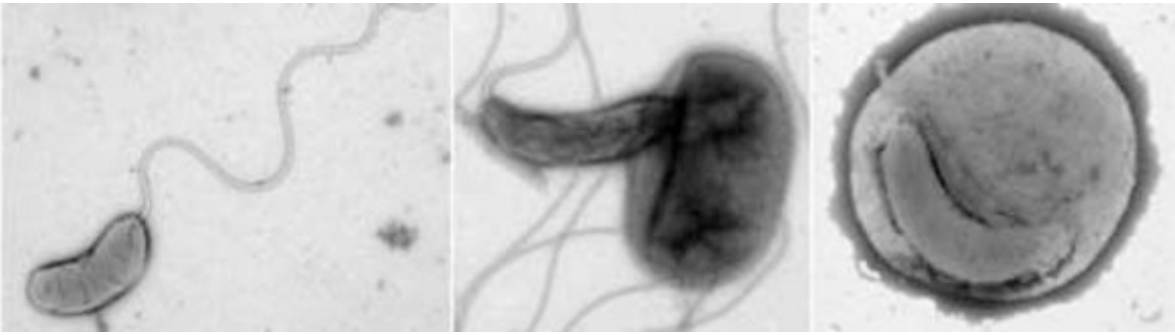
Eukaryogenesis via endosymbiosis



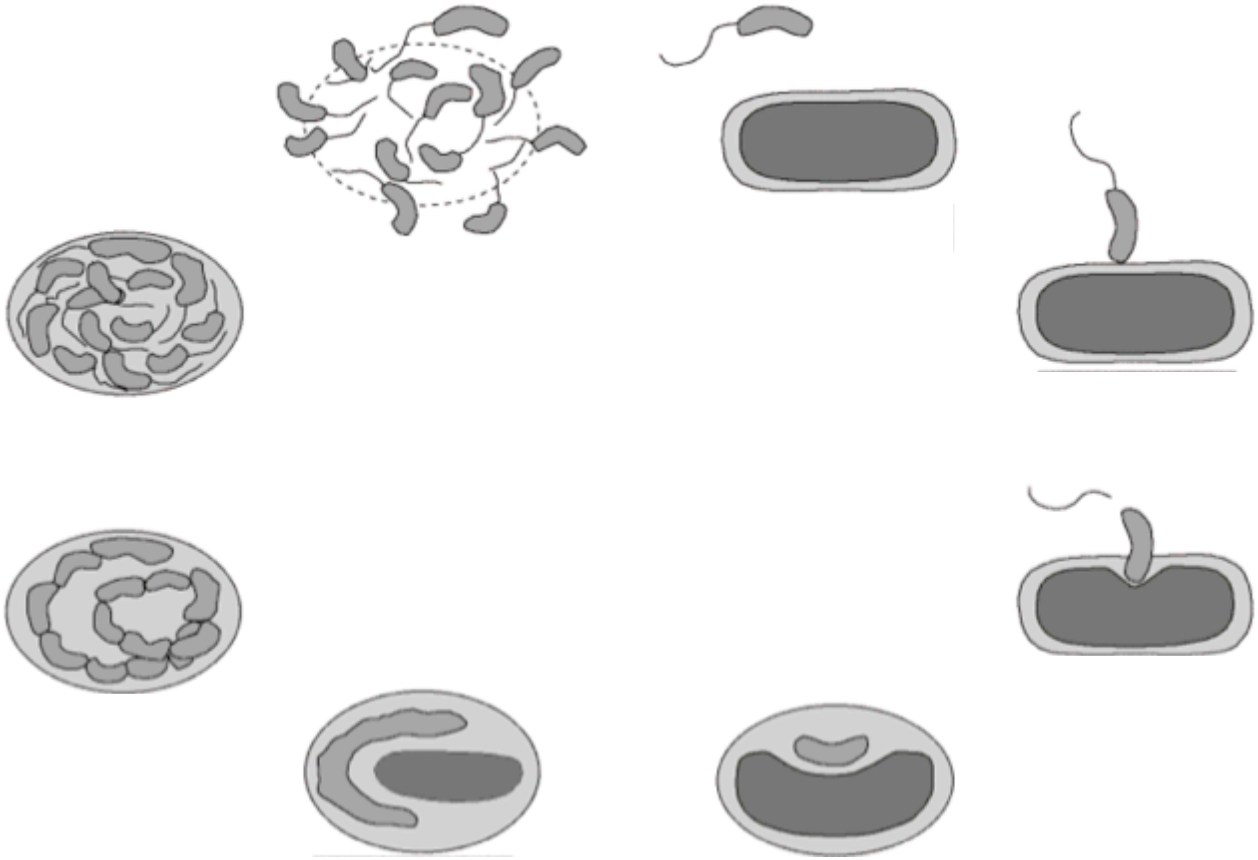
Major problem, recognized by Margulis

No extant prokaryote capable of phagocytosis. Perhaps mitochondria arose from a predator-prey relationship?

A bacterial predator, Bdellovibrio bacteriovorus



http://www.alumni.nottingham.ac.uk/netcommunity/page.aspx?pid=2680

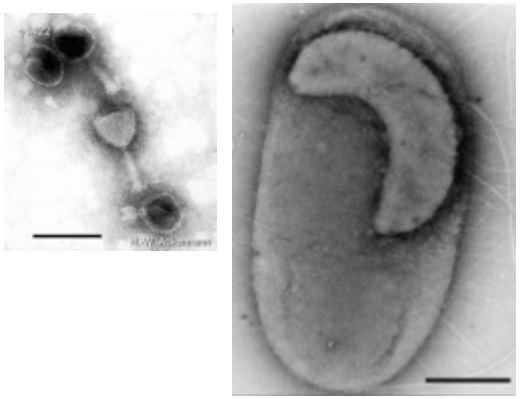


http://www.mpg.de/495902/pressRelease20040123?filter_order=L

Experimental co-evolution of E. coli and its predator, Bdellovibrio bacteriovorus

Stacking the odds:

- Respiratory proficient Bdellovibrio
- Respiratory deficient E. coli
- Make external environment inhospitable with bdellophage



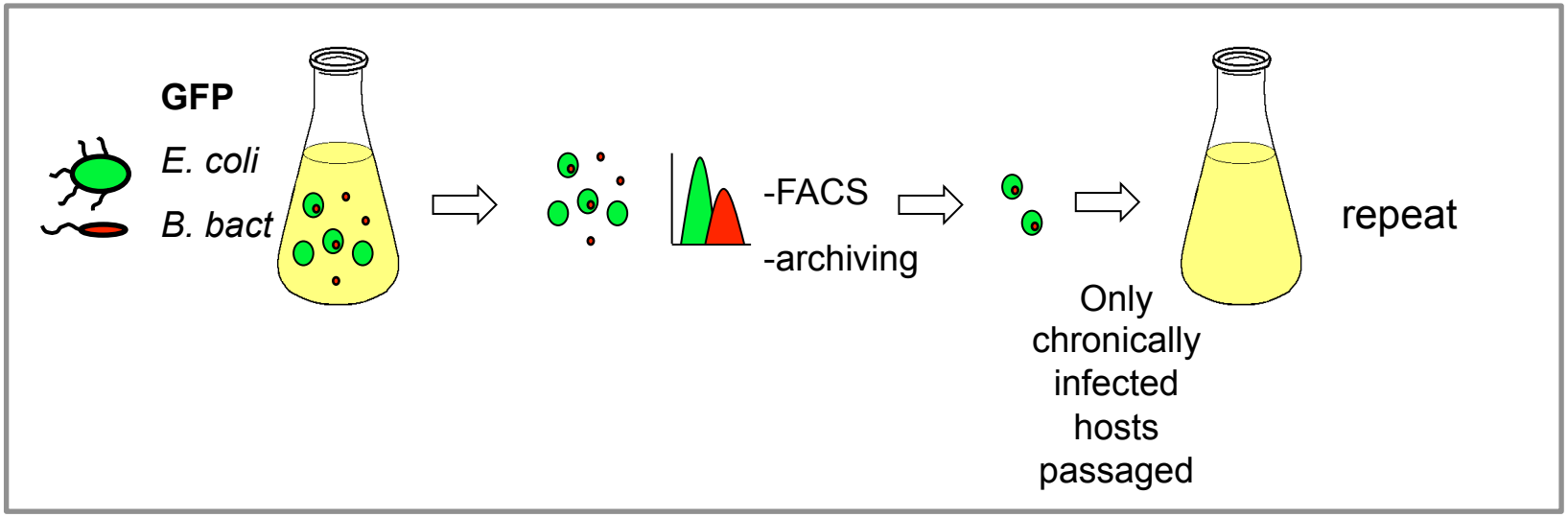
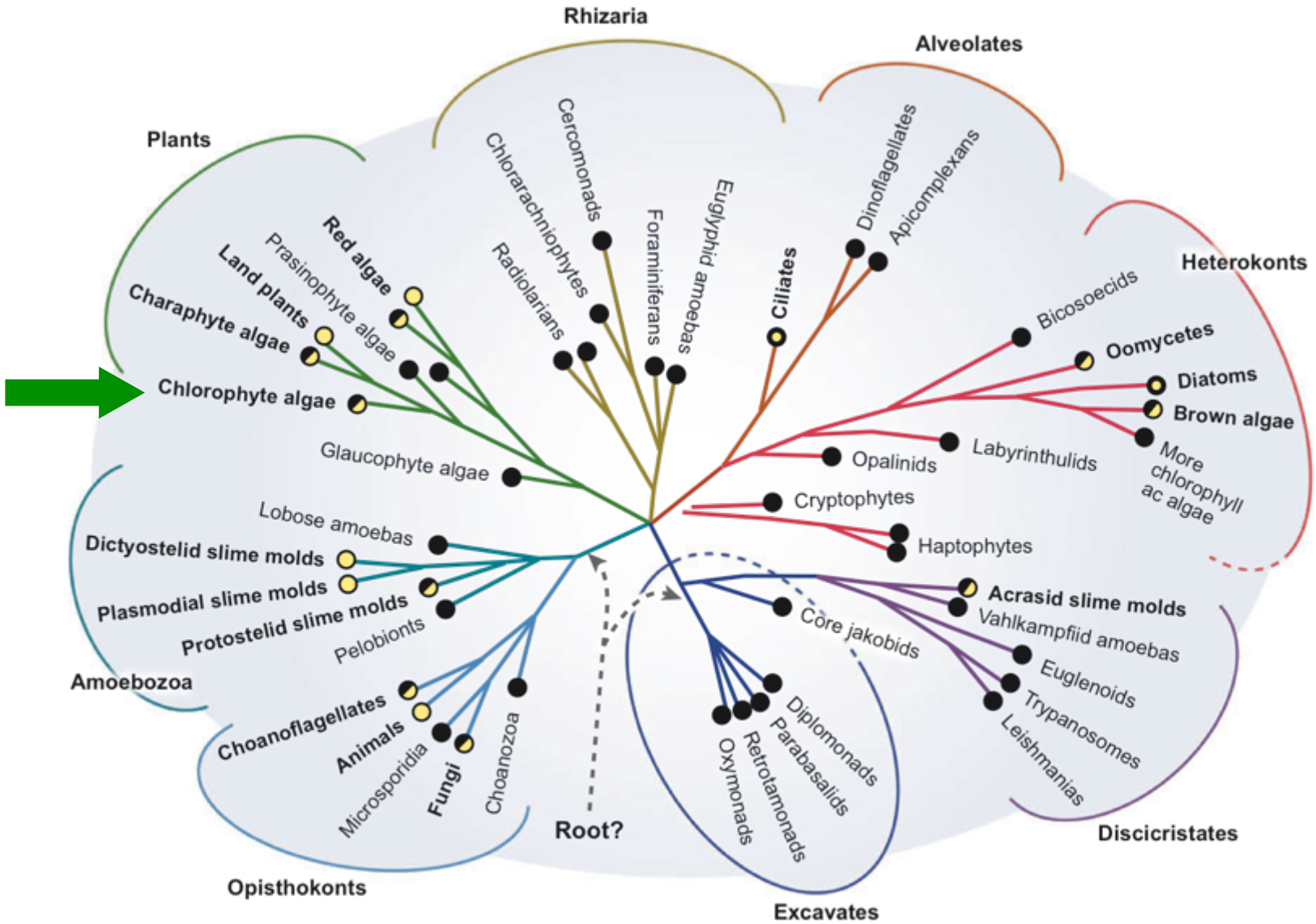


Image *B.bacteriovorus* in *E. coli from* Lerner et al. PLoS Pathog 8(2): e1002524.
http://ictvdb.bio-mirror.cn/WIntkey/Images/em_myovi_phi1422-HWA.htm

Project #6 (Matt Herron)

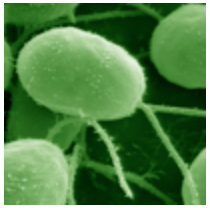
How did multicellularity arise?

Multicellularity has evolved at least 25 times

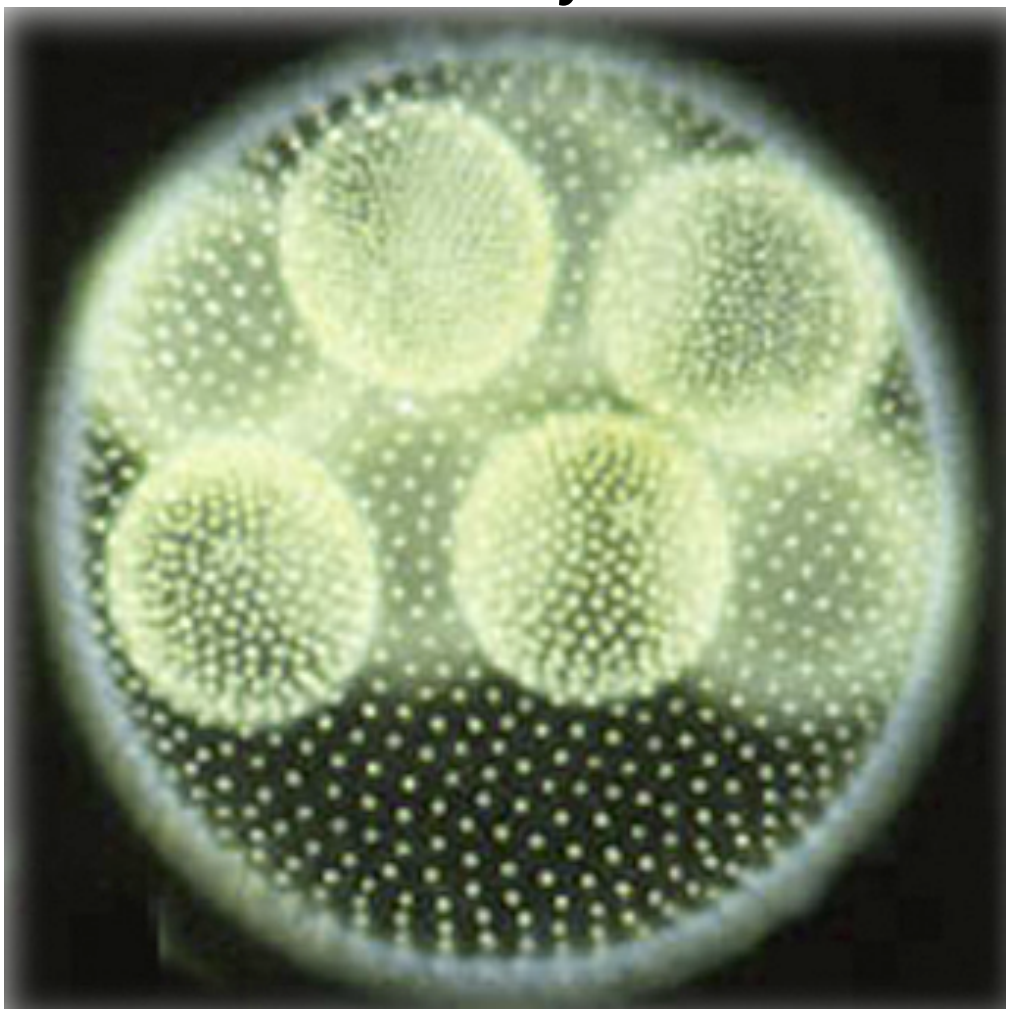


Grosberg & Strathmann 2007 *Ann. Rev. Ecol. Evol. Syst.* 38:621

Can we experimentally evolve something that begins to resemble *Volvox* from *Chlamy*?

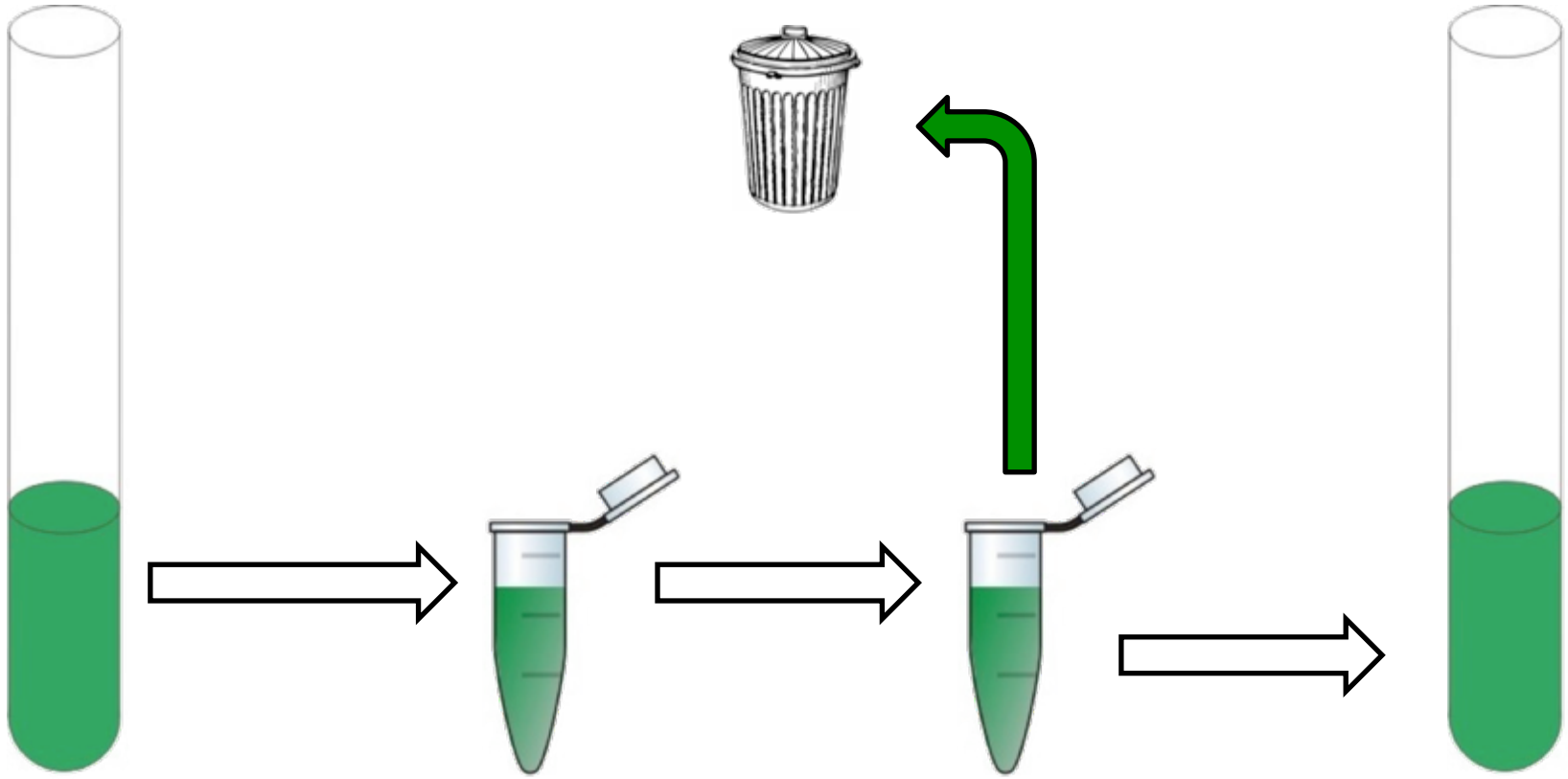


Chlamydomonas
a unicellular alga



Volvox
a multicellular, differentiated alga closely related to *Chlamy*

Select for large size via centrifugation



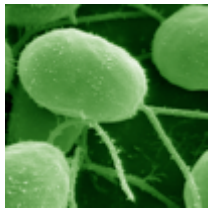
72 hour incubation

centrifuge 100g For 5 seconds

discard upper 90% of medium

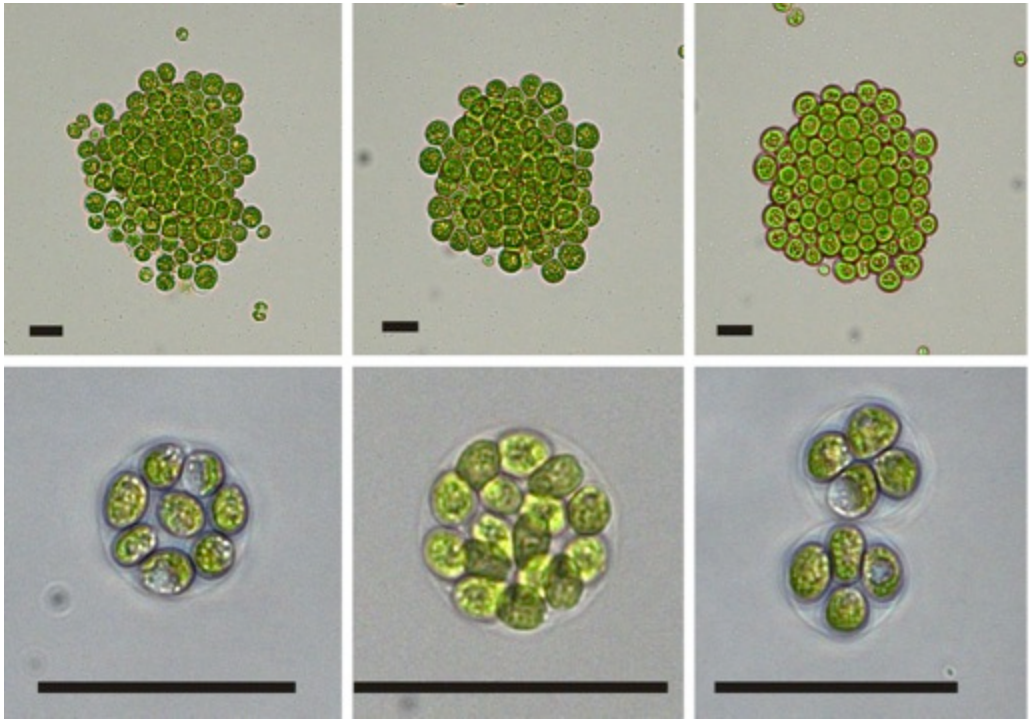
transfer to fresh medium

Can we experimentally evolve something that begins to resemble *Volvox* from *Chlamy*?



Chlamydomonas
a unicellular alga

Ecology
Genetics



TOP centrifugation
BOTTOM predation

Chlamydomonas
evolved under selection for large size

Ratcliff, Herron et al. 2013 *Nature Commun*
Herron, Ratcliff et al. In Prep (WGS, RNA-Seq, BSA)

Overarching Question

How have complex networks/associations evolved?

Smith
Santa Fe Inst

Gerrish
New Mexico

Question 1: **Evolution of metabolism**

Question 2: **Evolution of eukaryotes**

Question 3: **Evolution of symbioses**

Question 4: **Evolution of multicellularity**

Question 5: **Evolution of the hereditary apparatus**

Copley
Project 1:
Inefficient generalist enzymes
Colorado

McCutcheon
Project 2:
Functionality Through reduction
Montana

Miller
Project 3:
RecA dupli-cation
Montana

Kinnersley
Project 4:
Eukaryo-genesis
Montana

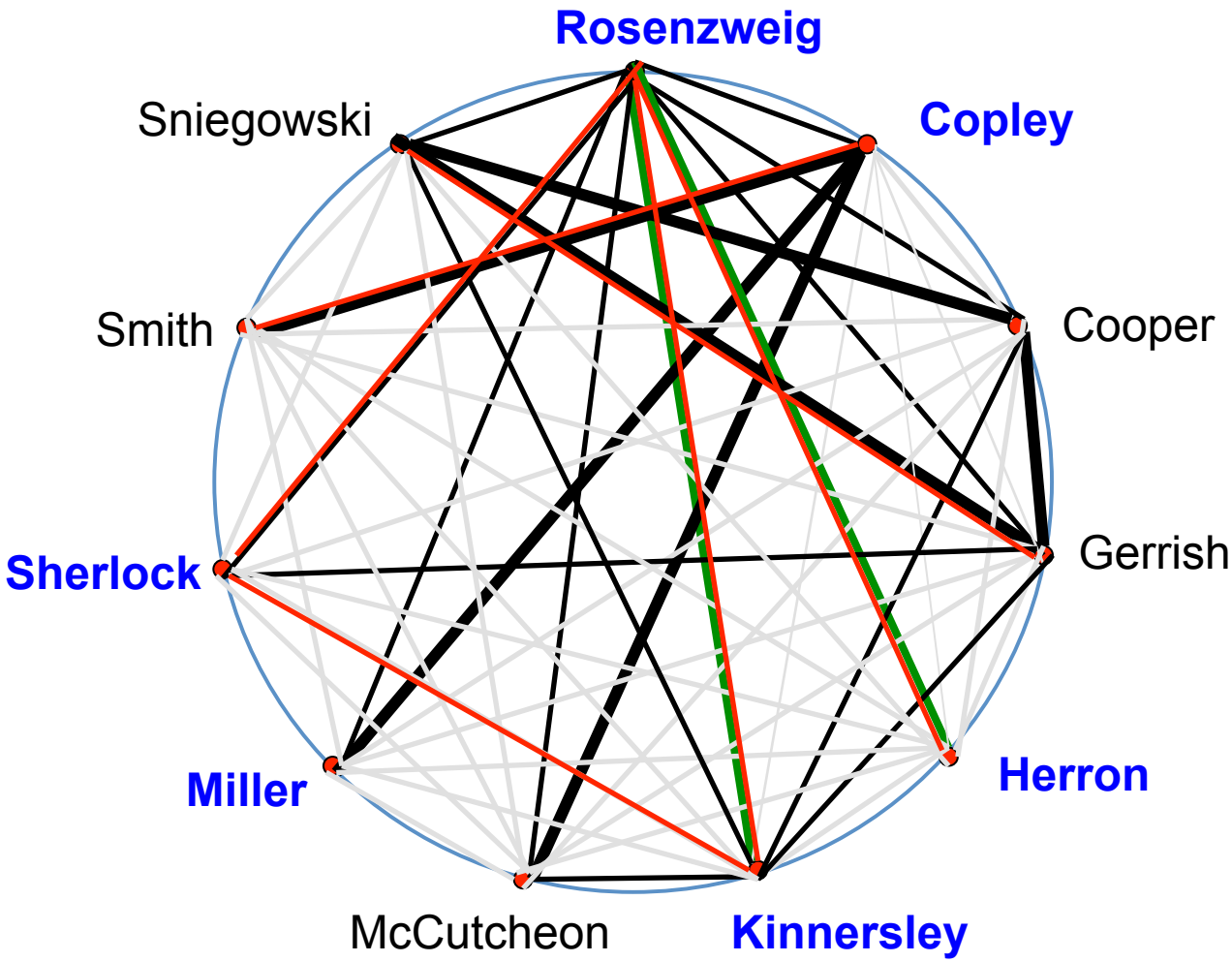
Cooper
Project 5:
Adaptation in biofilms
New Hampshire

Herron
Project 6:
Multi-cellularity
Montana

Sniegowski
Project 7:
Error rate
Penn

Sherlock
Project 8:
Limits to optimality
Stanford

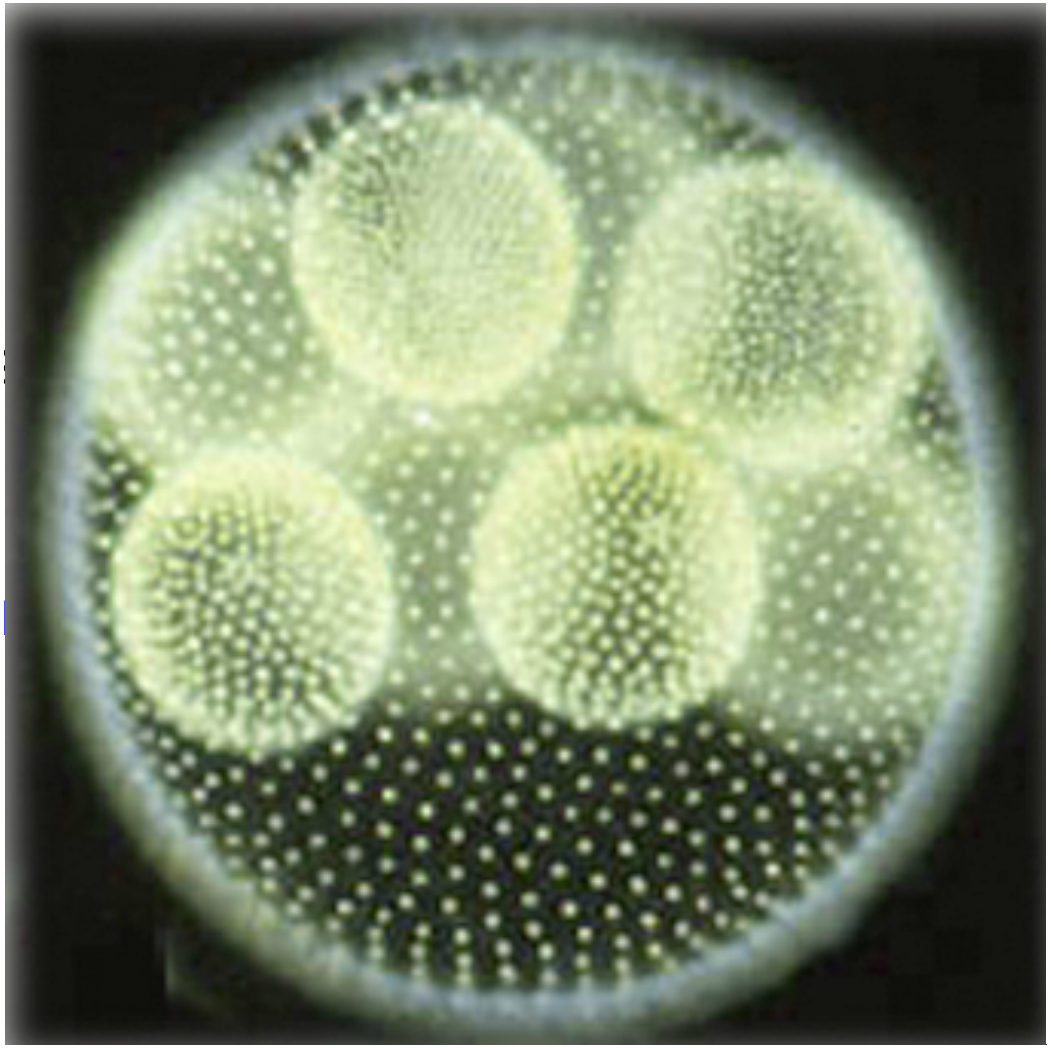
UM CAN7 team is itself a cooperative system



Collaborations
Strong interactions/possible future collaborations
Common interests

Current or former NASA funding
Mentoring relationships
Previous publications together

UM CAN7 team is itself a cooperative system



Collaborations
Strong interactions/possible future collaborations
Common interests

Current or former NASA funding
Mentoring relationships
Previous publications together

UNIVERSITY OF MONTANA

A NASA Astrobiology Institute
Reliving the Past:
Experimental Evolution of Major Transitions in the History of Life

SANTA FE INSTITUTE

Thank you!

APPENDIX

Rationale and overarching goal(s) of each UM Cycle 7 project

Project #1 (Copley)

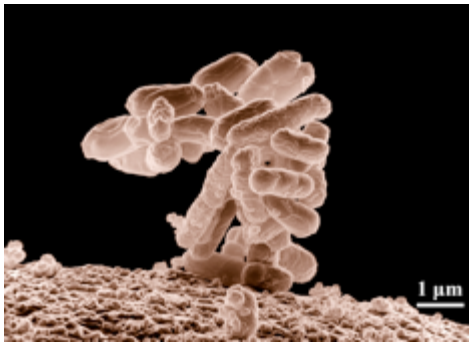
Where do new enzymes come from?

Also,

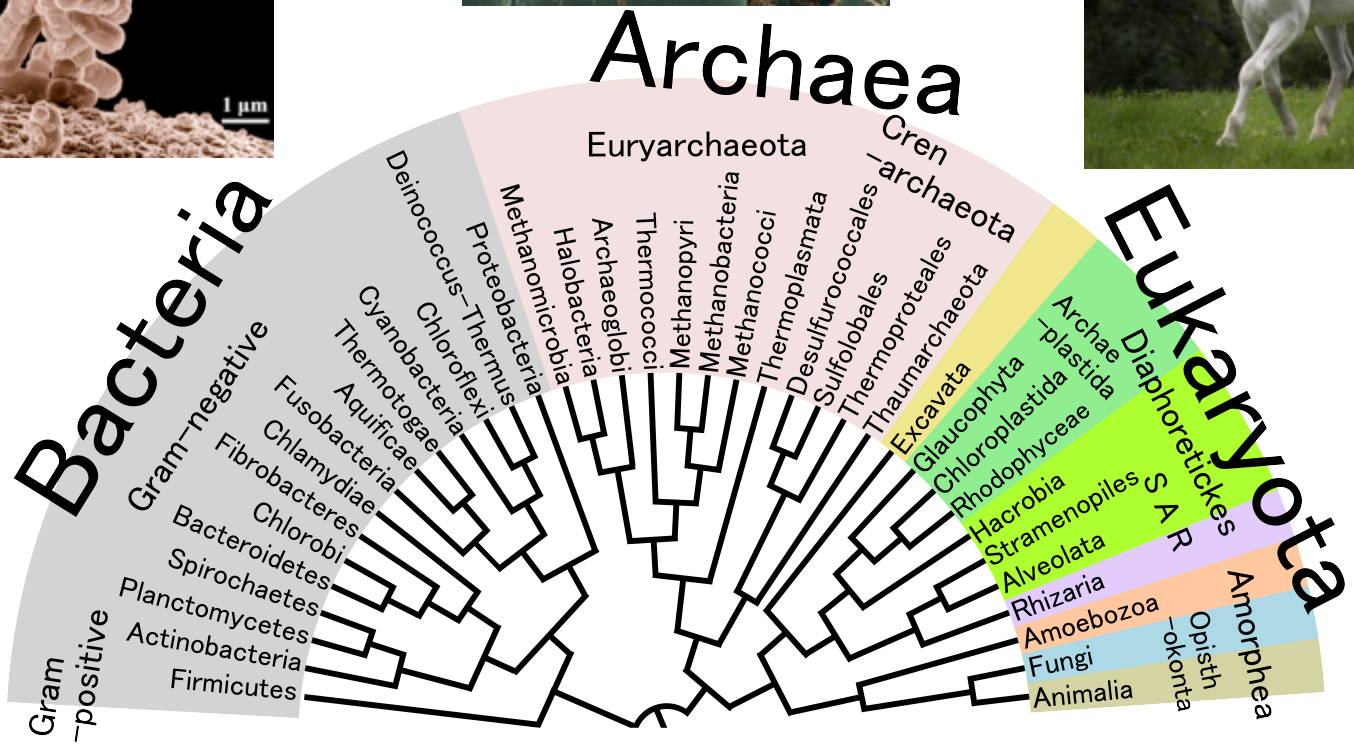
Project #2 (Miller)

Project #3 (McCutcheon)

~5000



20,000-30,000



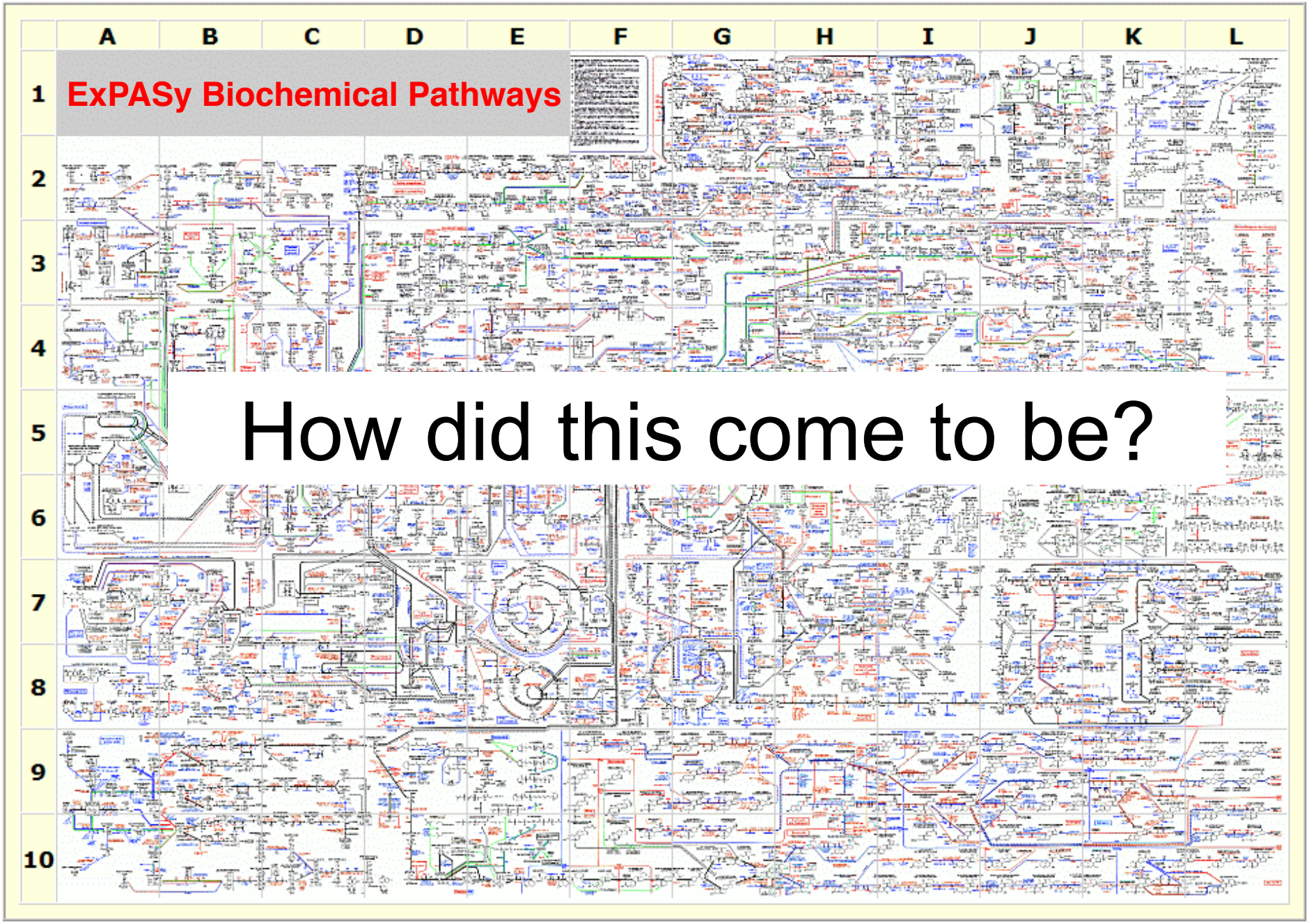
LUCA

Last **U**niversal **C**ommon **A**ncestor

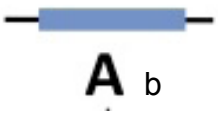
A few 100 protein-coding genes

Res. Microbiol. 157, 57-68, 2006

From 100s to many 1000s of genes that form networks



How do we get from one enzyme to two?

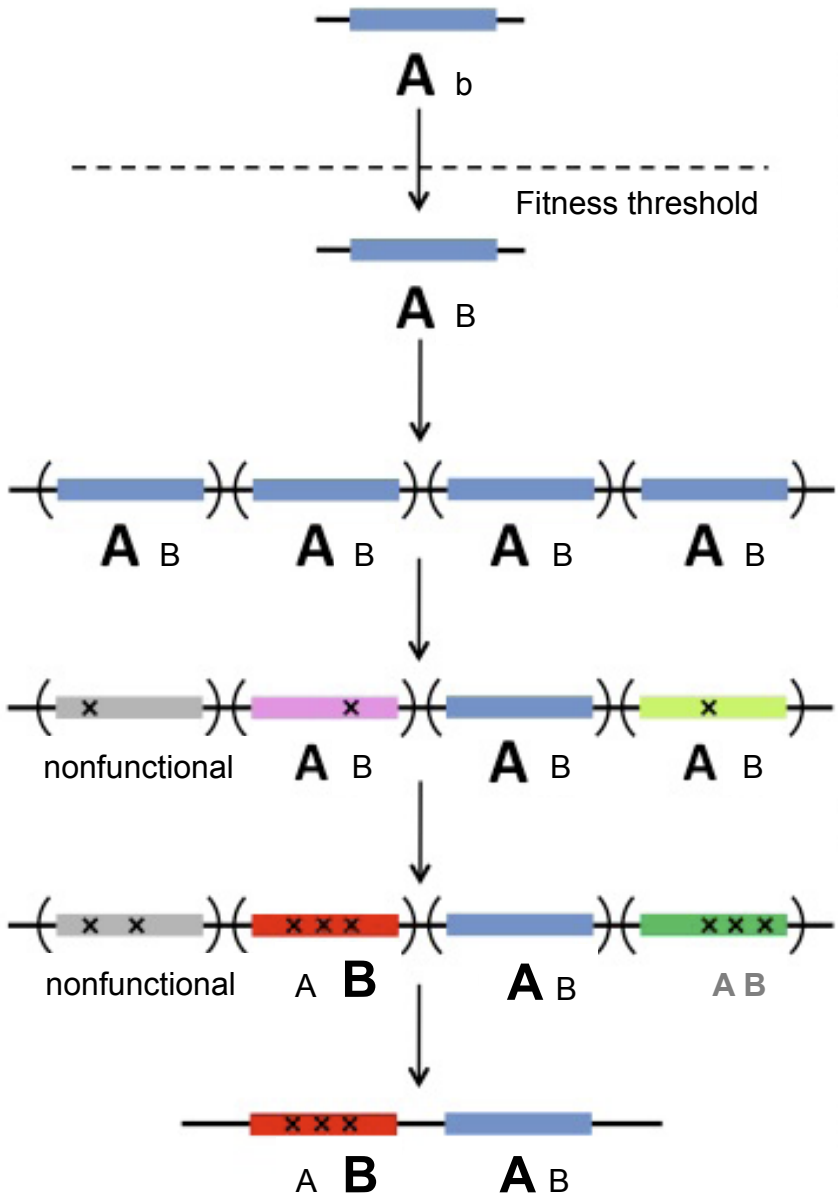


From one generalist or
'promiscuous enzyme'

To two specialist enzymes

Testing the IAD Model

Innovation → Amplification → Divergence



Activity B becomes important for fitness

- Environmental change
- Promoter mutation
- Mutation ↑B

Duplication / Amplification

Point Mutations

Recombination

Loss of copies
Specialized enzymes

Project #2 (McCutcheon)

Function by reduction: do extant symbiont enzymes recapitulate ancient metabolic generalists?

Function by reduction: do extant symbiont enzymes recapitulate ancient metabolic generalists?

Proteins encoded by insect endosymbionts are among the most unusual and rapidly evolving known across the tree of life.

The activities and structural characteristics of these proteins have been little studied.

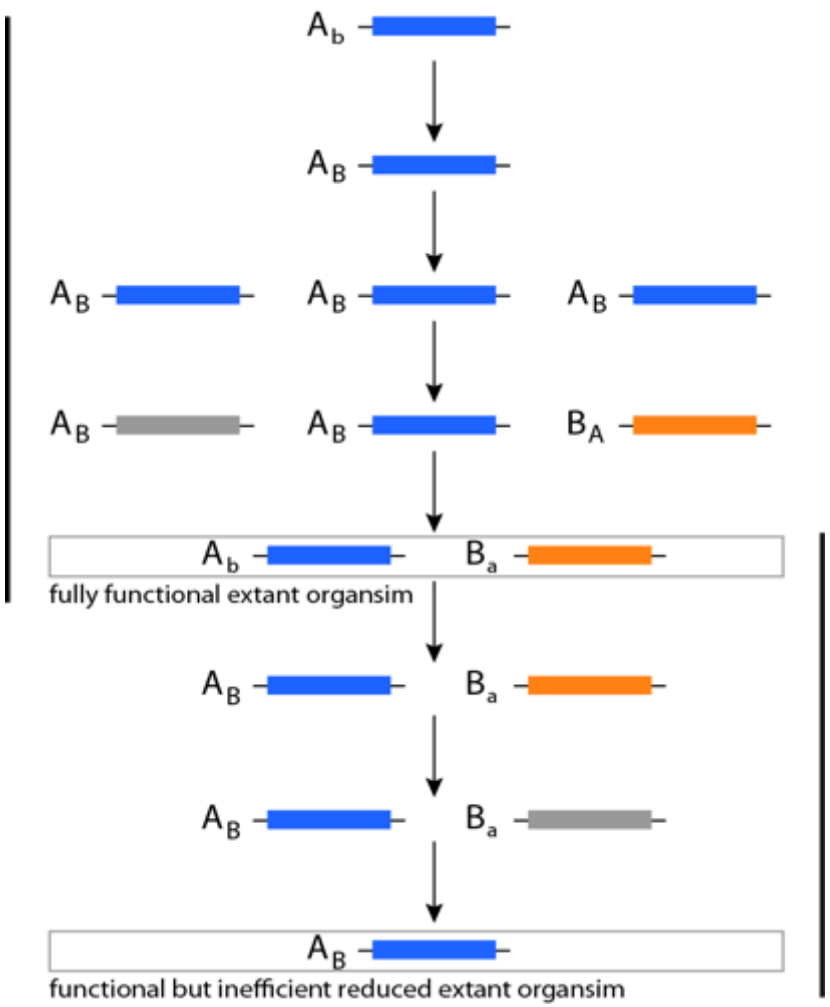
The goal of this project is to gain functional understanding of these unusual proteins.

Specifically, we will test the hypothesis that extreme genome reduction combined with rapid sequence evolution results in generalist enzymes.

These proteins may provide insight into how ancient generalist enzymes functioned.

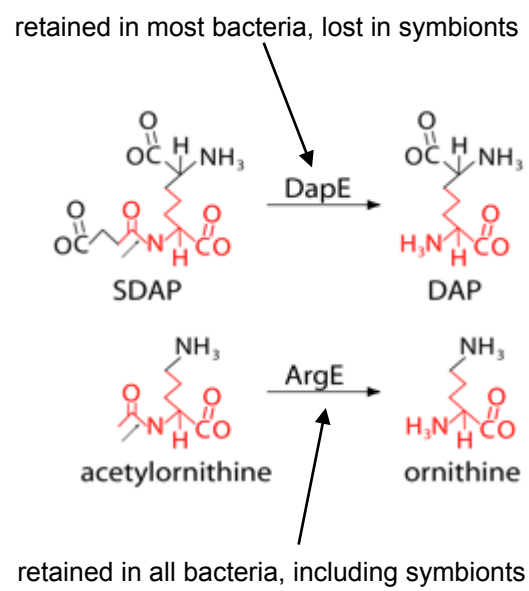
We will first test this by testing whether putative symbiont generalist enzymes (A_B) can perform the chemistries of extant specialist enzymes (A_b and B_a).

Multi-functionality
Through
Reduction
(co-I McCutcheon)



Innovation
Amplification
Divergence
(co-I Copley)

We will test whether symbiont ArgE can perform the chemistries of specialist DapE and ArgE.

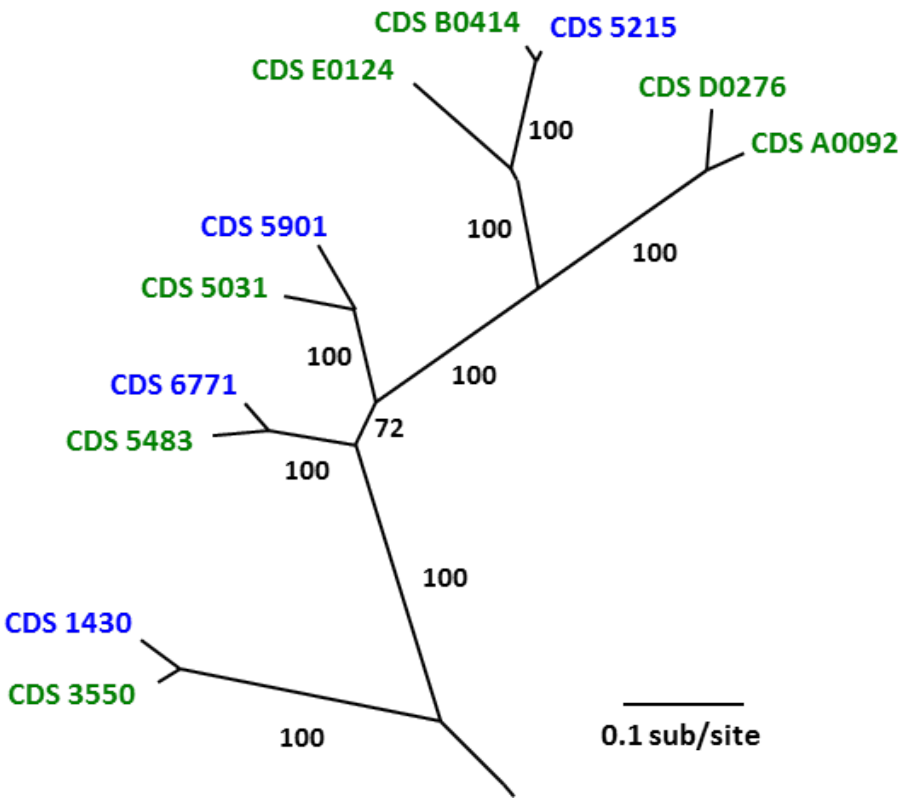


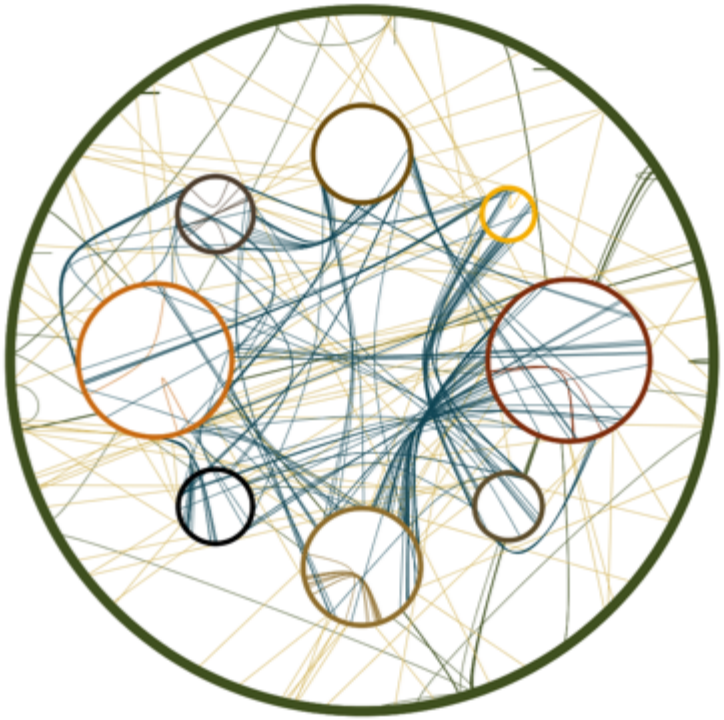
Project #3 (Miller)

Does *recA* copy number control the extent of recombination and the tempo of genome evolution?

Project 3 (Miller)
***recA*, recombination & genome evolution**

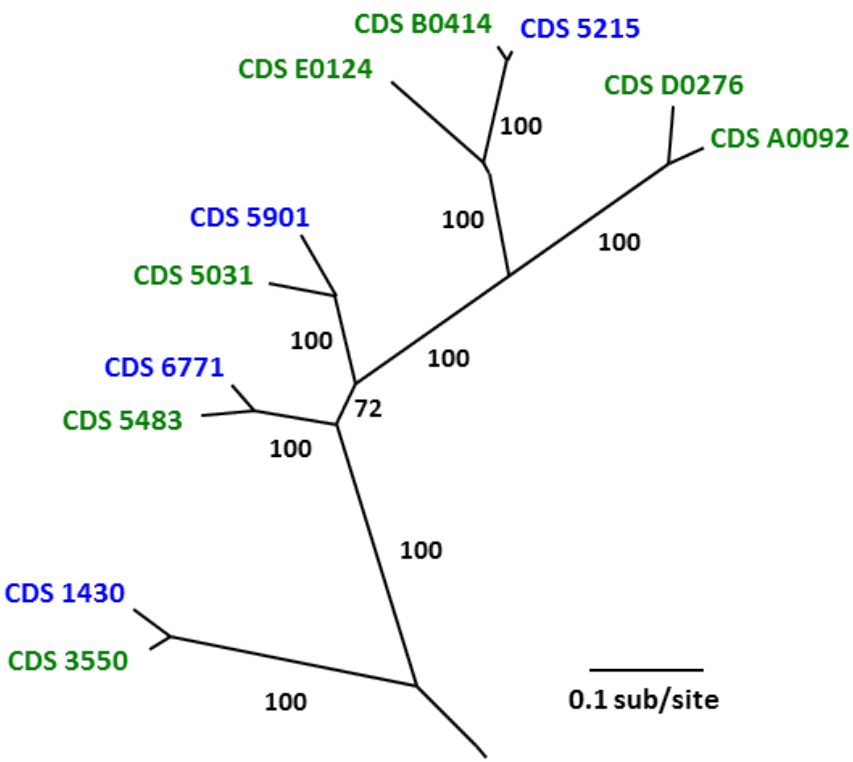
- Chl *d*-producing cyanobacteria (*Acaryochloris*) have large, dynamic genomes with 10x greater duplication rates than other bacteria
- Linked to extraordinary number of gene copies encoding the multifunctional recombinase protein RecA

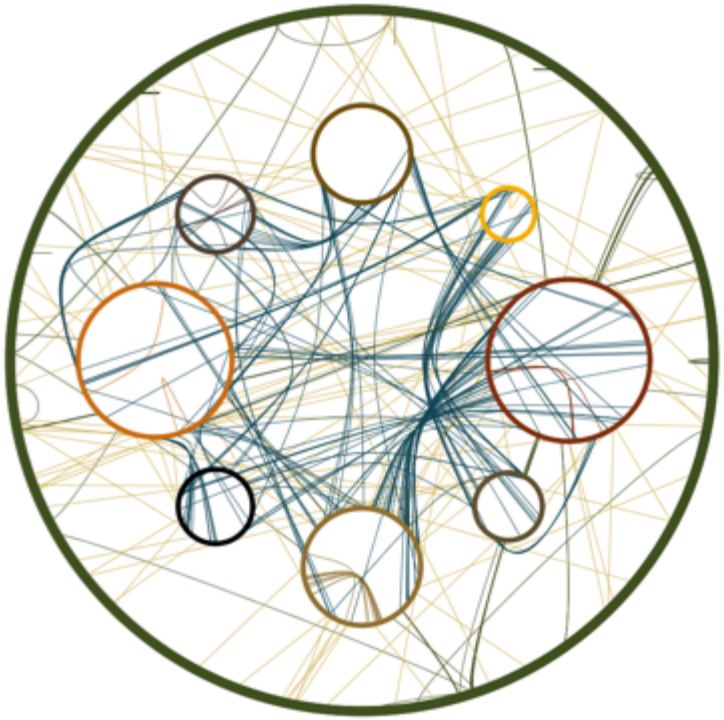




recA, recombination & genome evolution

- Use this system to address central questions regarding the balance among creative, stabilizing and destabilizing contributions of the ancient process of recombination to genome evolution & fitness
- Functional characterize *Acaryochloris* RecAs, manipulate *recA* copy number, test effects on recombination & fitness

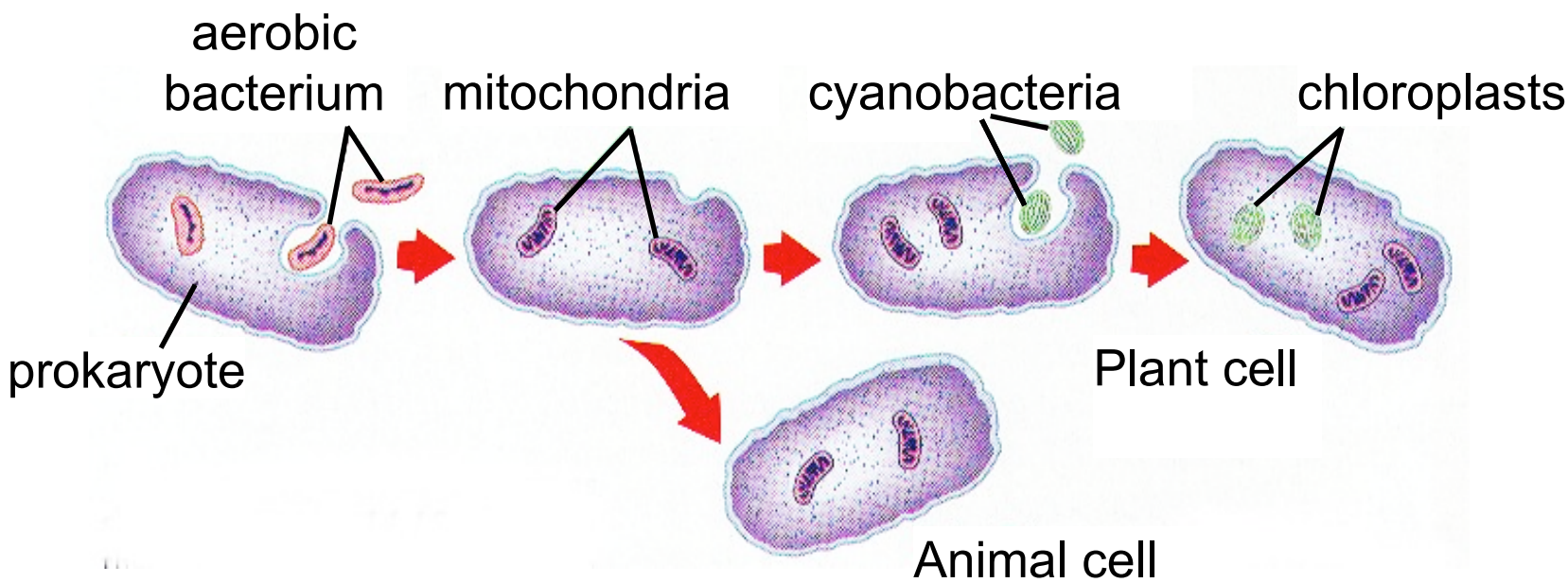




Project #4 (Kinnersley)

How did the Eukaryotic cell come to be, specifically having a mitochondrion?

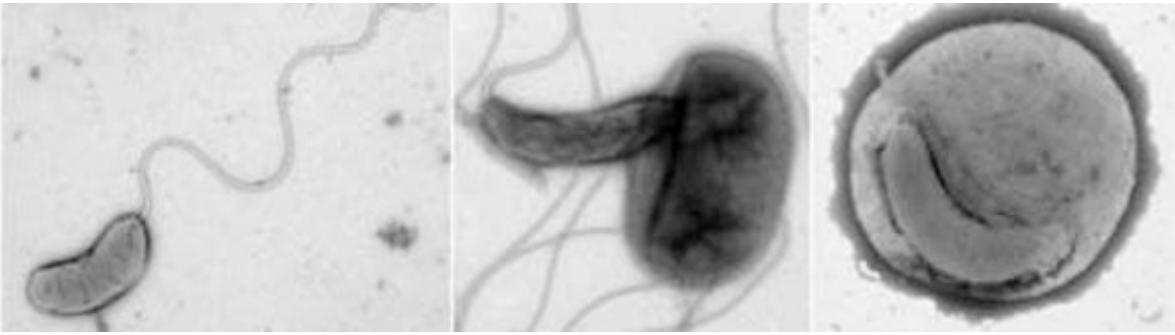
Eukaryogenesis via endosymbiosis



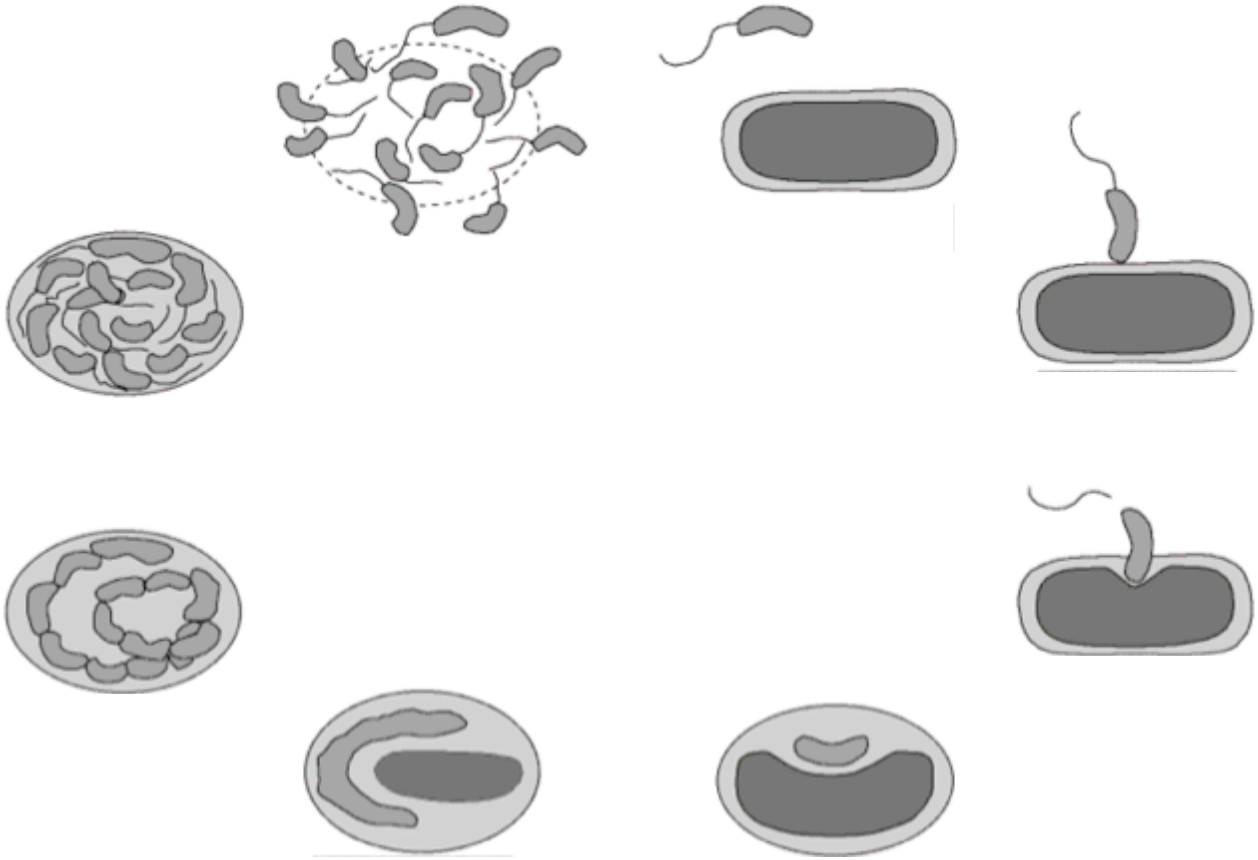
Major problem, recognized by Margulis

No extant prokaryote capable of phagocytosis. Perhaps mitochondria arose from a predator-prey relationship?

A bacterial predator, Bdellovibrio bacteriovorus



http://www.alumni.nottingham.ac.uk/netcommunity/page.aspx?pid=2680

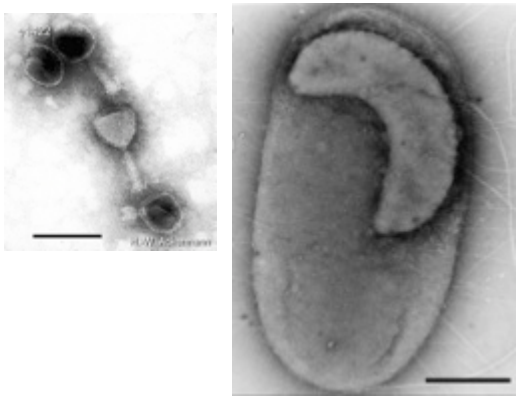


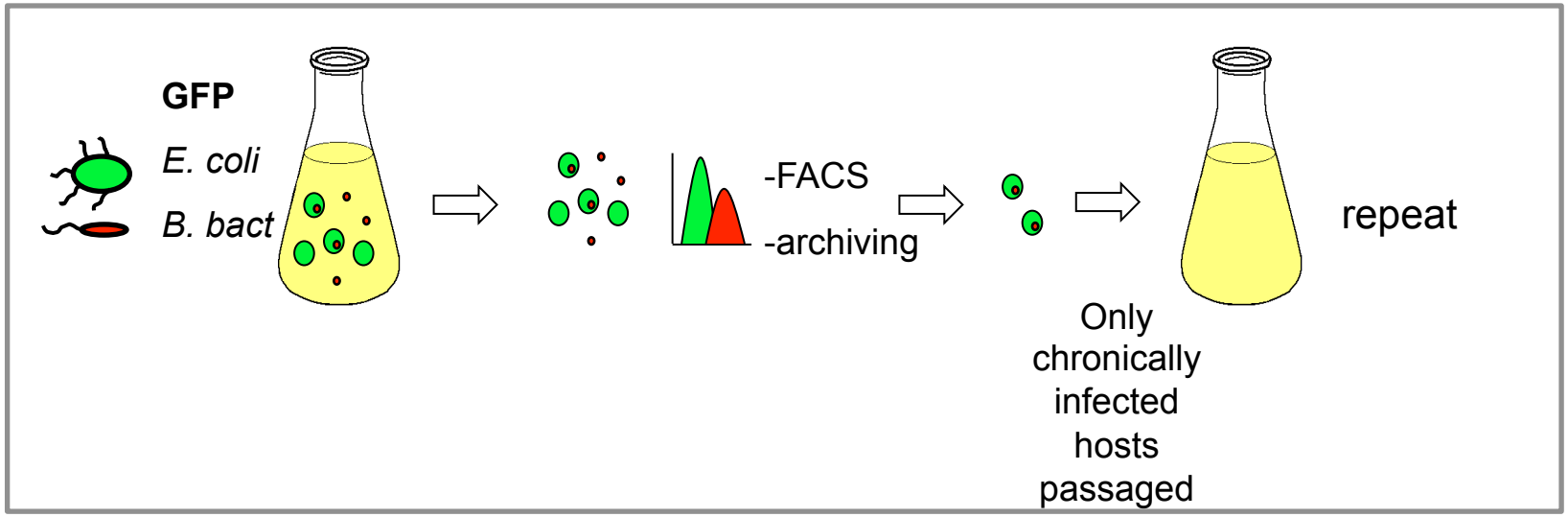
http://www.mpg.de/495902/pressRelease20040123?filter_order=L

Experimental co-evolution of E. coli and its predator, Bdellovibrio bacteriovorus

Stacking the odds:

- Respiratory proficient Bdellovibrio
- Respiratory deficient E. coli
- Make external environment inhospitable with bdellophage





Establish a model system that captures the essential features of mitochondriogenesis

Project #5 (Cooper)

Synergy & division of labor in microbial biofilms: A prelude to multicellularity?

Adaptation, mutation supply, and evolution of synergy in biofilm communities

Rationale: Biofilms are surface-associated communities that represent the dominant form of microbial life and likely reflect the origins of multicellularity. Surface growth preserves diversity, leading to a range of possible interactions. The forces that govern the balance of these interactions are mostly unknown yet are central to understanding the evolution of biocomplexity.

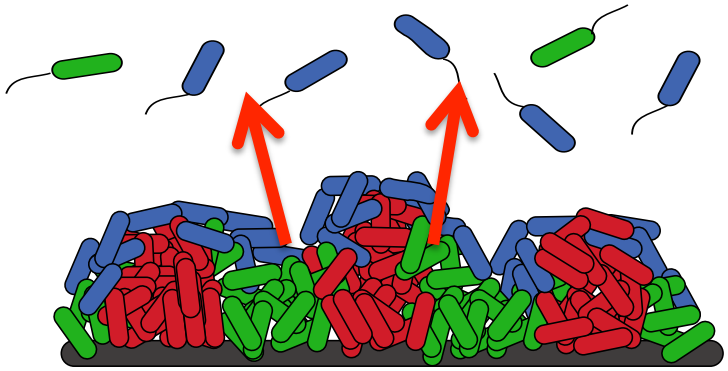
Hypothesis: community stability and productivity relate to the beneficial mutation supply rate.

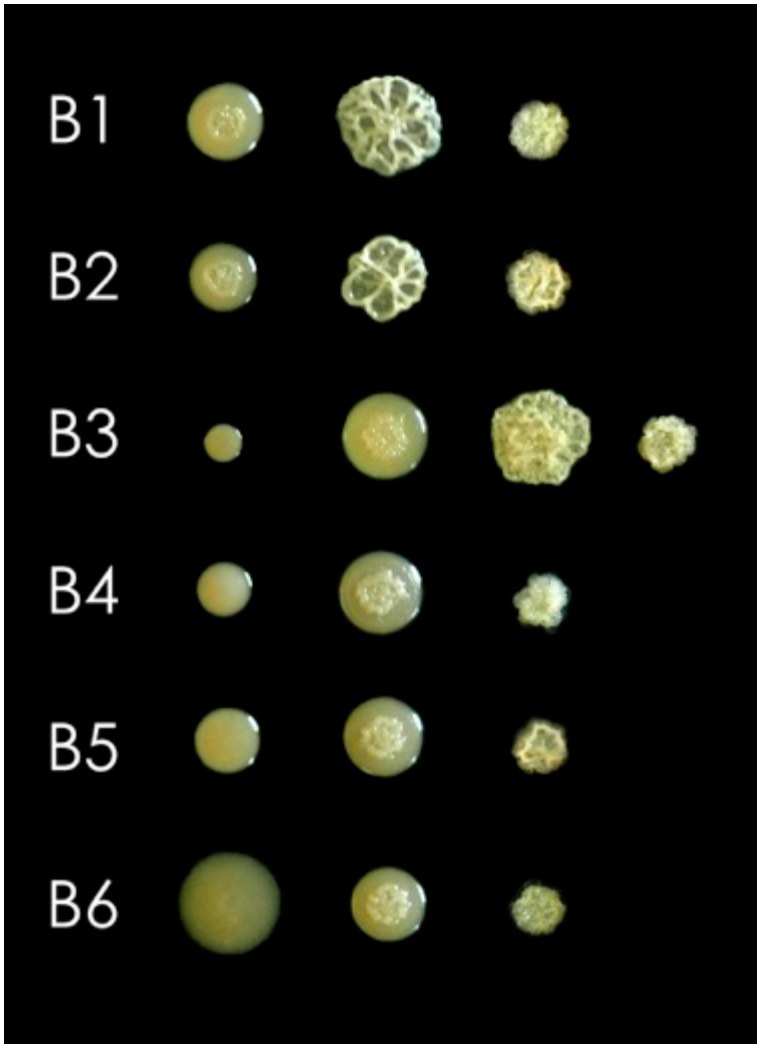
Goals:

1. **Determine how genotype fitness affects probability a population can be invaded by mutator genotypes**.
2. **Determine how environmental structure and biotic interactions affect mutator invasion.**
3. **Identify the mutations that drive adaptation amidst the variation generated by mutators and test their functional roles.**

Experimental design

- Experimental evolution selects for “reversibly sticky” biofilms growing on plastic beads
- Predictable diversity evolves = ecological roles → synergistic communities
- Population genomics and constructed communities will reveal how/why these synergies evolve





Also see Traverse et al. http://www.pnas.org/content/110/3/E250.short

Project #6 (Herron)

The evolution of complexity via multicellularity and cellular differentiation

Project #6:
The evolution of complexity via multicellularity and cellular differentiation

The evolution of multicellularity set the stage for unprecedented increases in biocomplexity.

Goals:

- Observe the evolution of multicellular complexity in real time in a species that has never had a multicellular ancestor
- Ascertain the genetic bases underlying the evolution of multicellularity
- Evaluate the roles of plasticity and genetic assimilation in the evolution of multicellularity

Methods

- Selection for increased size using centrifugation
- Biotic selection using a filter-feeding predator
- Genetic investigations using whole genome sequencing, RNA-Seq, and bulked segregant analysis
- Measure changes in reaction norms over evolutionary time
- Measure possible forms of cellular differentiation

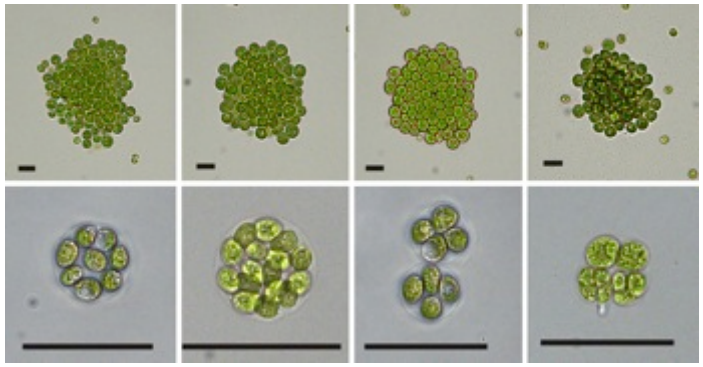


Figure 1 Multicellular structures from the centrifugation (top row) and predation (bottom row) experiments. All scale bars = 50μm.

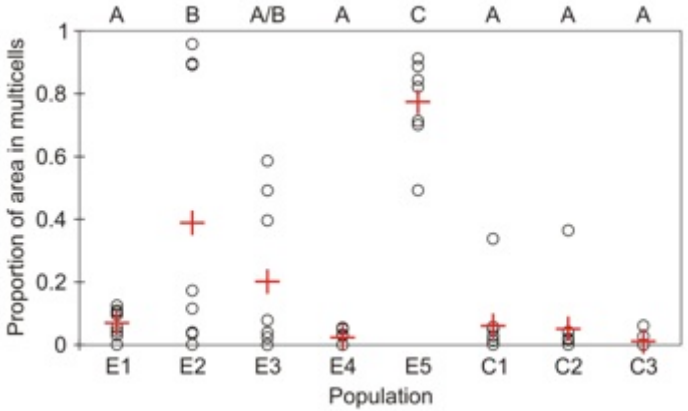


Figure 2 Proportion of cells in multicellular structures from 5 experimental (E1-E5) and 3 control (C1-C3) populations in the predation experiment. Circles represent estimates for each isolate. Red crosses represent means of 8 random isolates per population.

Project #7 (Sniegowski and Gerrish)

Error rate and the origin and early evolution of life: Exploring the relationship between mutation, recombination and cooperation

Project 7 Error Rate and the Origin and Early Evolution of Life: Exploring the Relationship between Mutation, Recombination and Cooperation

Our theoretical and experimental work predicts that genomic mutation rate is prone to rise to levels that cause extinction when recombination is absent (see **Figures 1** and **2**). We hypothesize that the major transition to complex life depends on stabilization of the mutation rate via recombination, which then sets the stage for the evolution of complexity. We will test this hypothesis with a combination of experimental evolution and theoretical approaches.

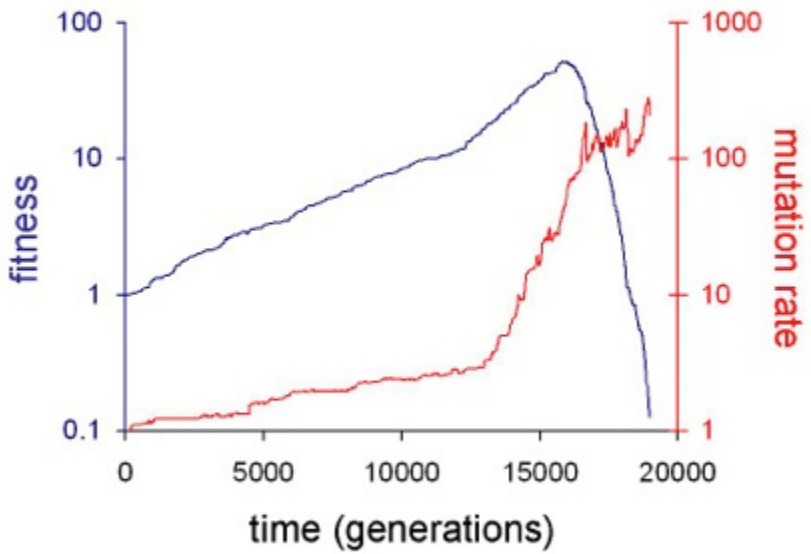


Figure 1. Simulation of an evolving asexual population in which adaptation drives elevation of the mutation rate—to the point where the population goes extinct (Gerrish et al 2007).

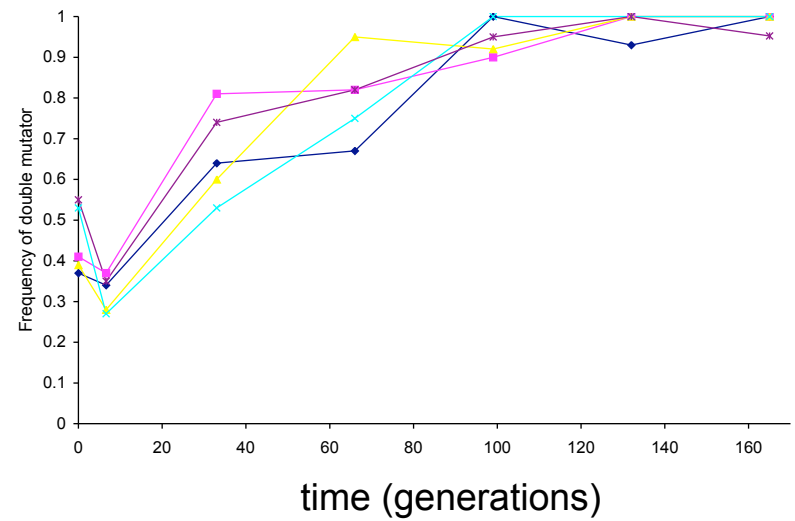


Figure 2. Experimental result in which an *E. coli strain* with an extraordinarily high mutation rate spreads in the absence of recombination (Gentile et al. 2011).

Project 7: Experimental & theoretical approaches

<u>Experimental:</u> Propagate long term (LTEE) and short term (STEE) experimental populations of *E. coli* using batch transfer (see Figure 3. Assay mutation rate evolution using strains with and without recombination conferred by integrated F plasmids.

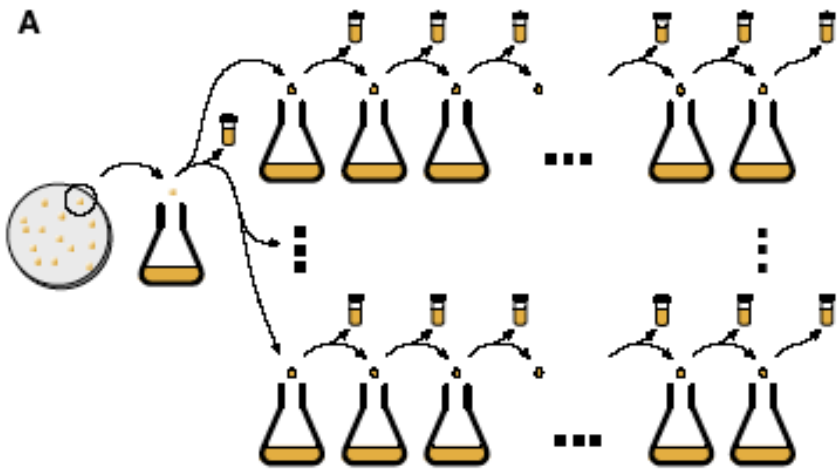


Figure 3 Experimental evolution by serial propagation: replicate populations, founded from one or more ancestors (including ancestors differing in mutation and recombination rates) can be propagated for thousands of generations. Periodically, samples of the populations are archived in the freezer; subsequent analyses examine evolution of fitness, mutation rates, and recombination.

<u>Theoretical:</u> Simulations and analytical theory complement the experimental approach by investigating broader ranges of parameter space and suggesting avenues for further experimental work.

Project #8 (Sherlock)

What are the limits to optimality in adaptive evolution?

Limits to optimality in adaptive evolution

- Rationale: Founding genotype may profoundly influence subsequent evolution, for example due to epistasis.

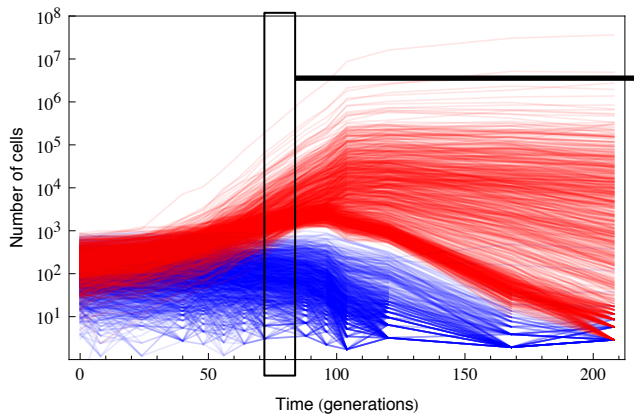
- Our aims are to precisely determine how genotype may influence:

  (1)The rate at beneficial mutations (U_b) appear

  (2)The distribution of their fitness effects (DFE),

  (3)The identity of beneficial mutations.

Experimental Design

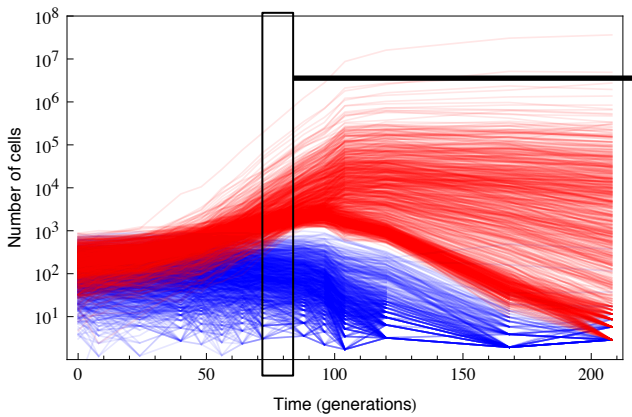


2. Select 4 adaptive clones

1. Evolve barcode lineage tagged population

3. Add new lineage tags

Experimental Design

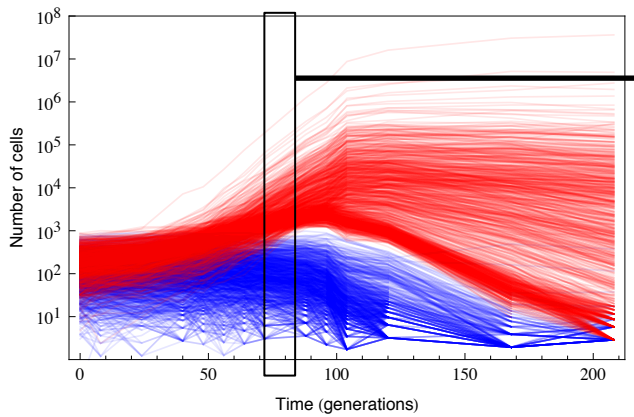


2. Select 4 adaptive clones

3. Add new lineage tags

4. Further evolve lineage tagged population

Experimental Design

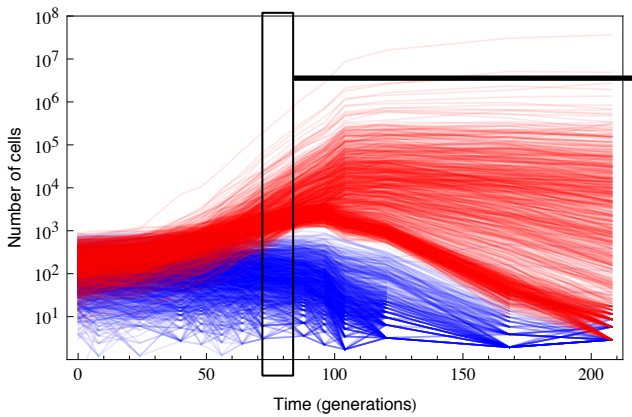


5. Sequence 240 beneficial clones, determine U_b and DFE

4. Further evolve lineage tagged population

3. Add new lineage tags

Experimental Design



2. Sequence 240 beneficial clones, determine U_b and DFE

3. Add new lineage tags

4. Evolve lineage tagged population