



CINVESTAV  
Unidad Irapuato



# The hidden diversity of bacteria and emergent properties in synthetic communities

Quorum  
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U Miami

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# Today's talk:

A primer on Microbiology: Its all bacteria!  
DNA at the origin of life and biodiversity

## Cuatrociénegas, Coahuila

- Geology, ponds in a desert, and phosphorus limitation
- Diversity: How we study it through DNA
- Is Cuatrocienegas a *Lost world?*

## Community assembly, focus on the genus *Bacillus*

- Interactions, food web-like

## Synthetic Ecology

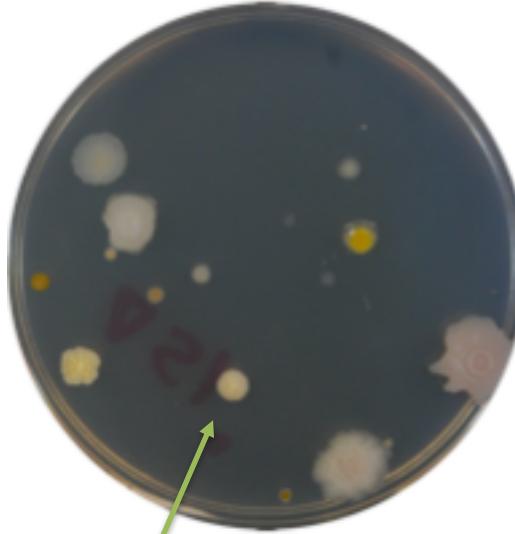
- How bacterial competition influences the structure of bacterial communities
- Emergent properties and early response to antagonism

Bacteria grow exponentially and divide every 40 min  
Each colony on these Petri dishes started as a single cell  
“last night”

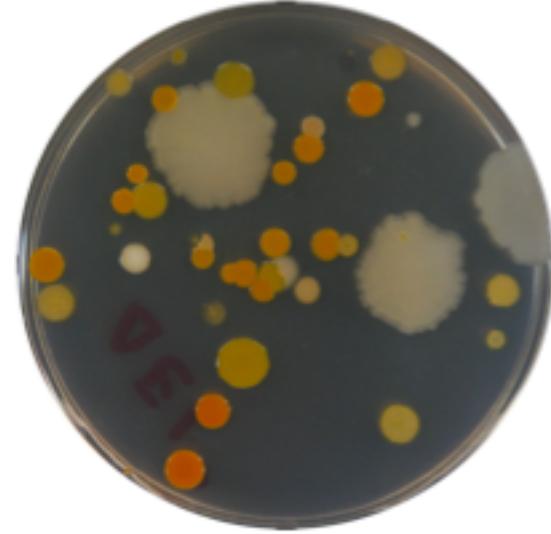
Soil sample



Water sample



Sediment sample



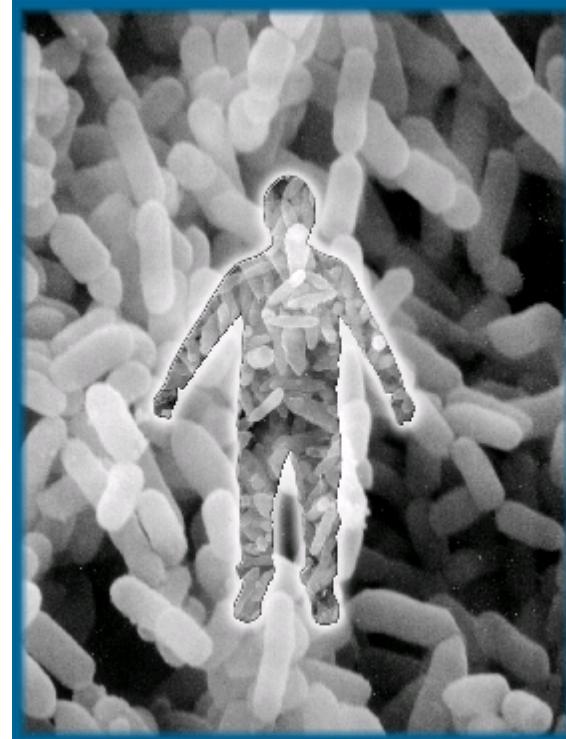
Colony  
100 million bacteria

Community: a group of species

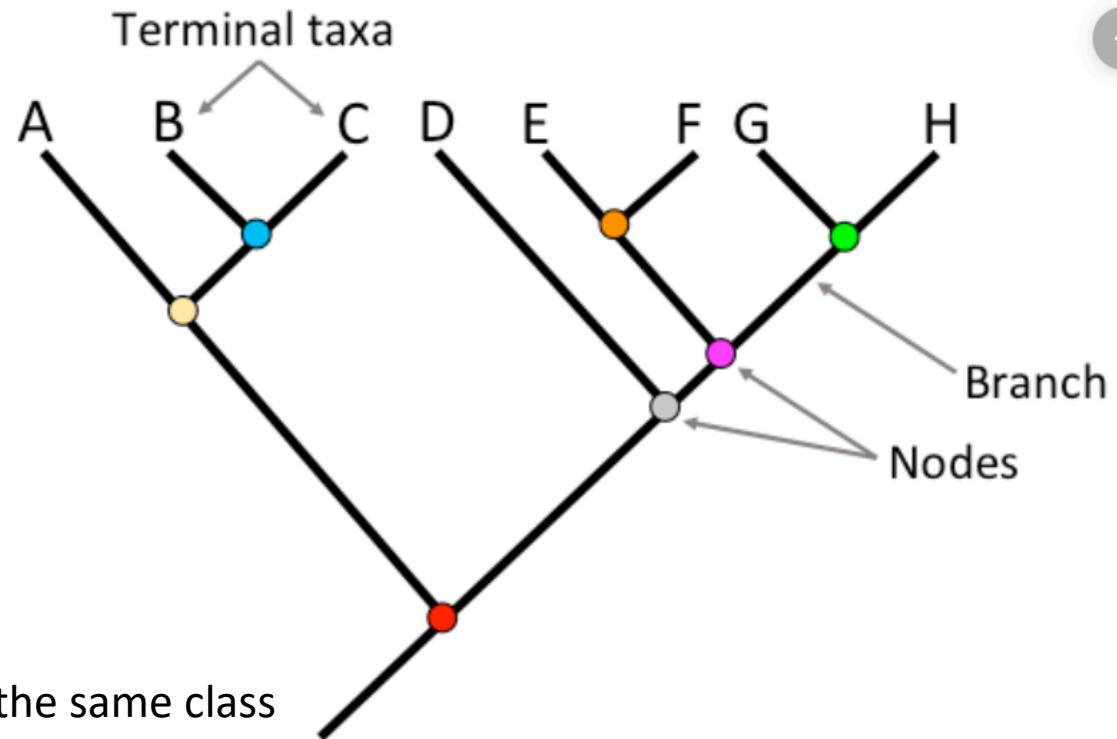
All cultured in Marine Medium in a Petri dish

# Your body has more bacteria than cells

- Human microbiome:  
All the bacteria in your body
- 3,000,000,000,000 bacteria (3 times more than human cells)
- How can we count them?



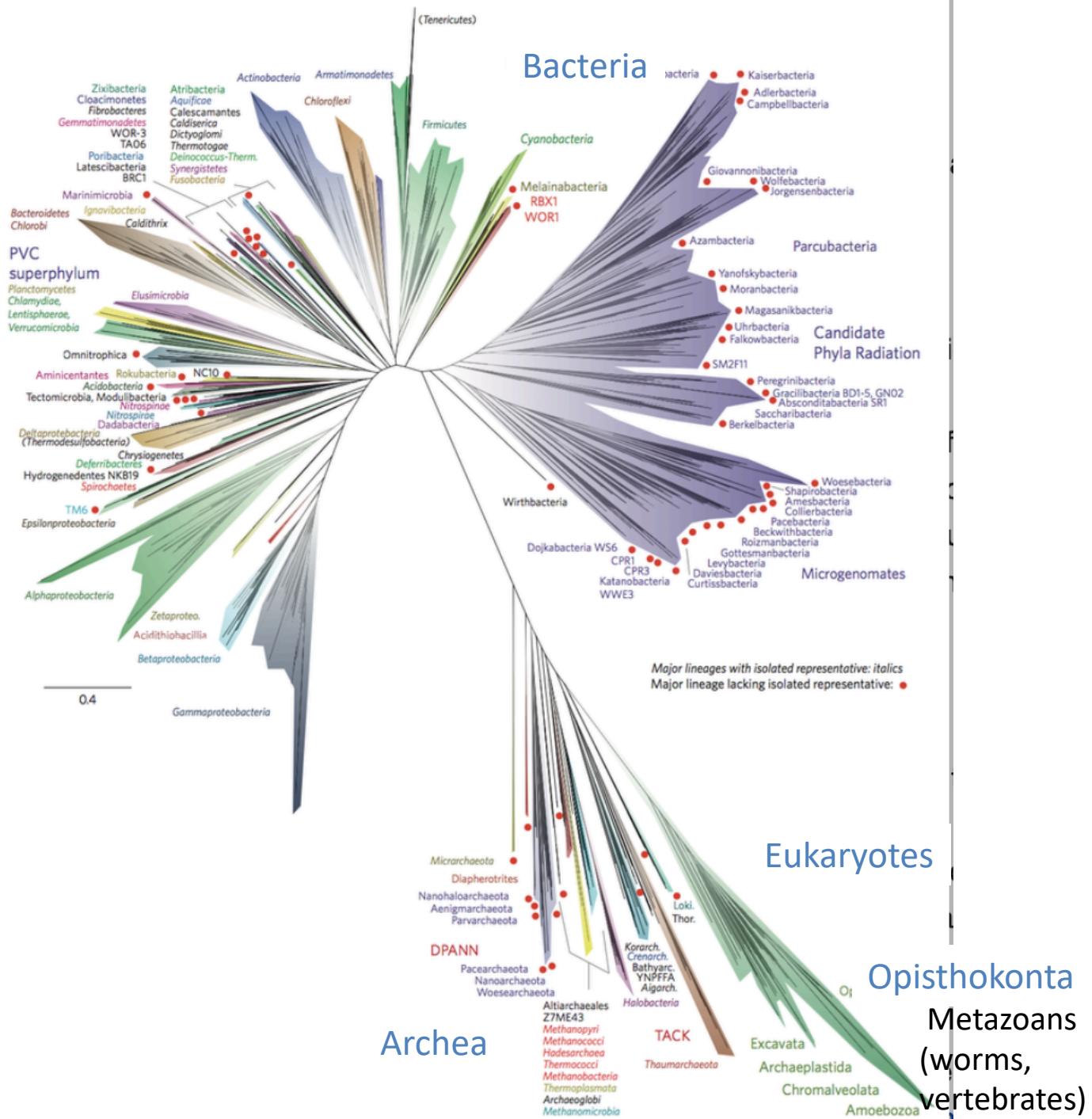
# Evolutionary studies through DNA



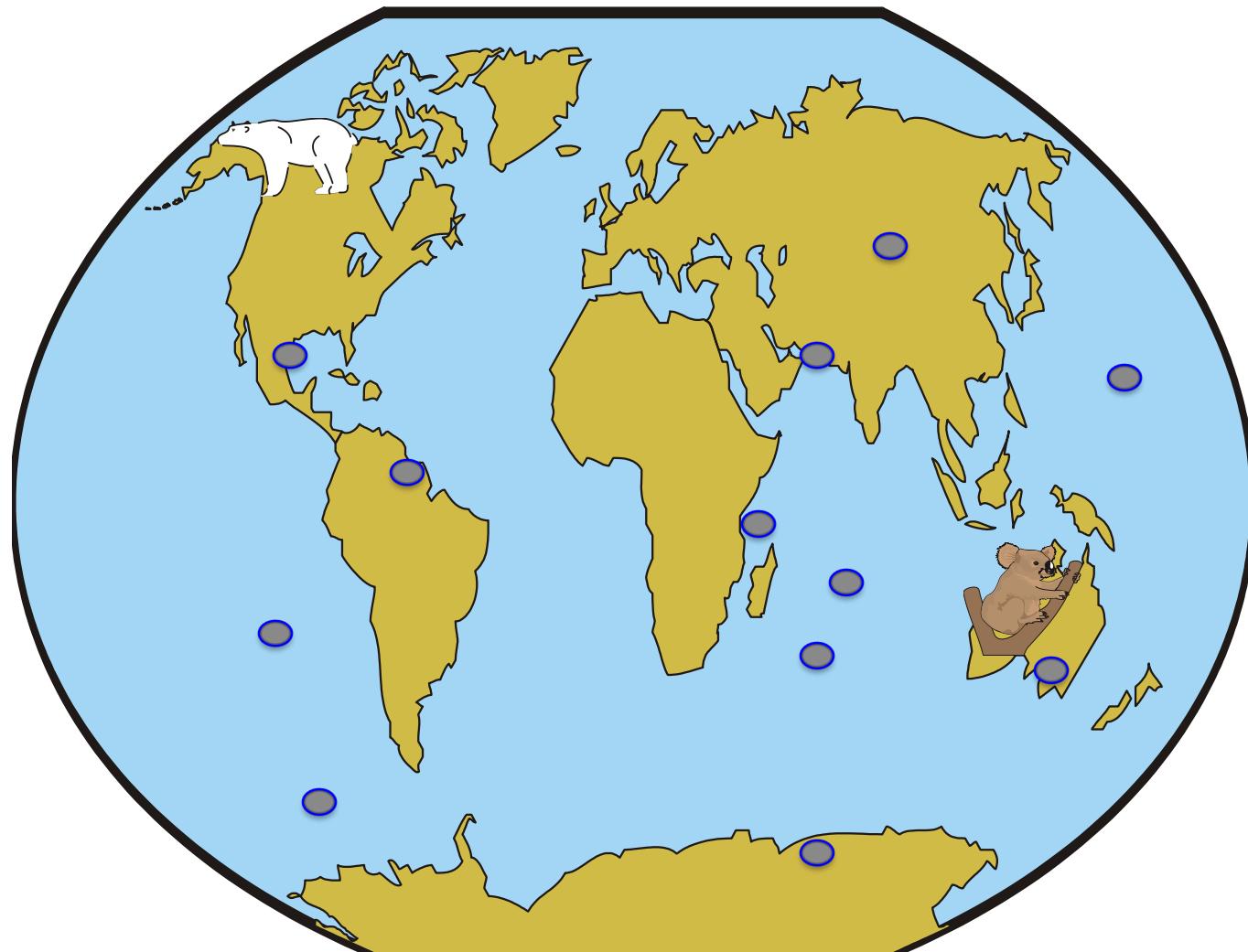
The affinities of all the beings of the same class have sometimes been represented by a great tree. I believe this simile largely speaks the truth.

— Charles Darwin

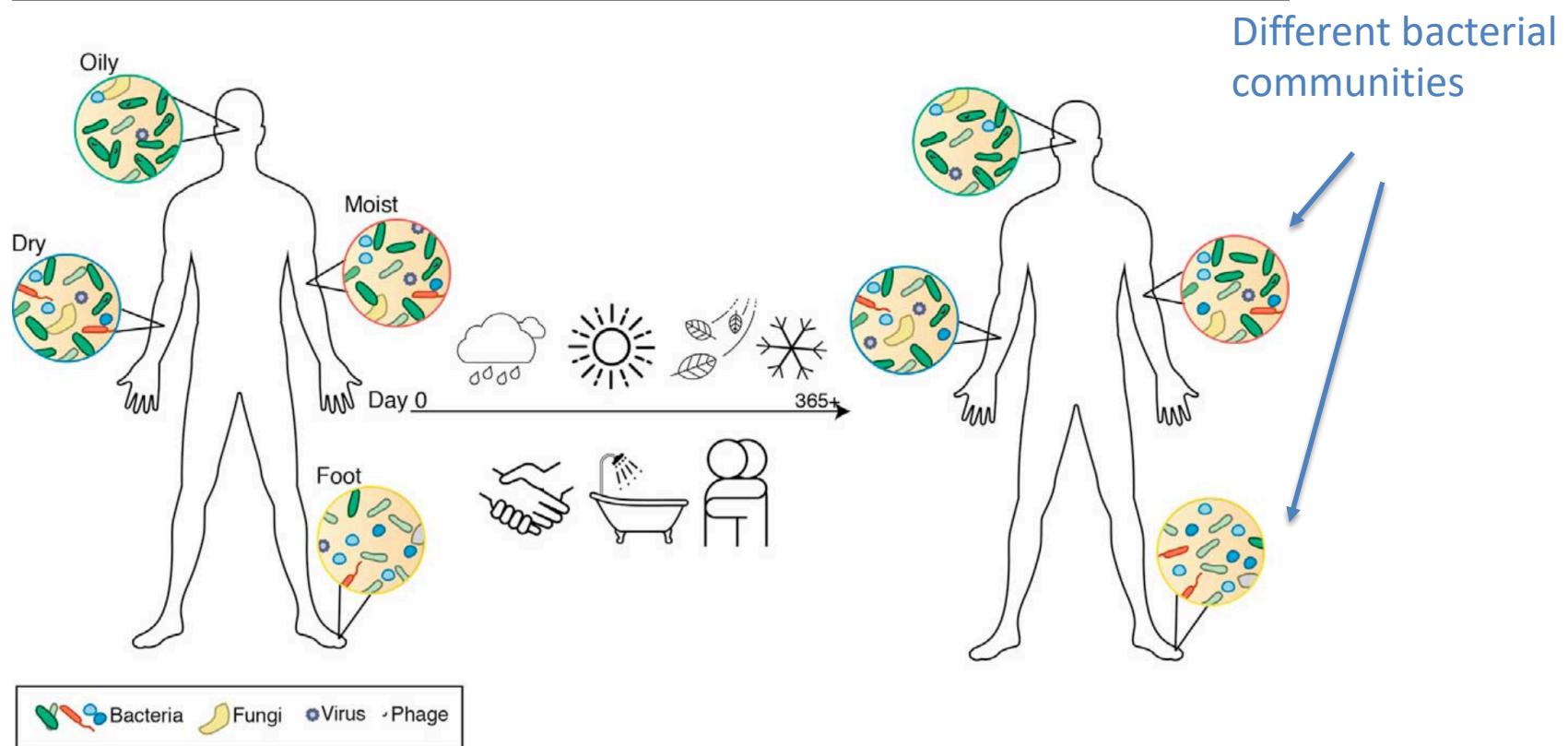
# The tree of life



# Dispersion of organisms on Earth

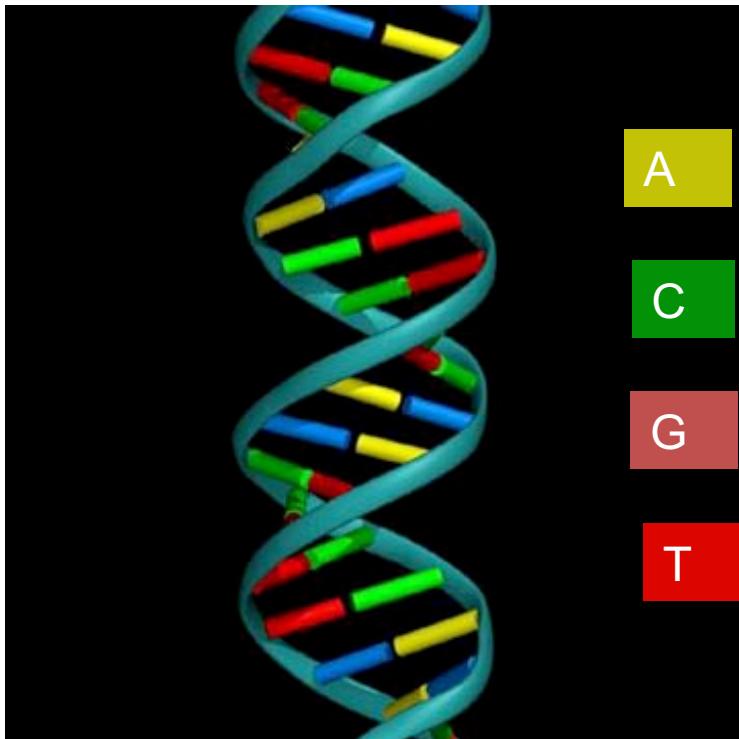


# How can our skin microbiome be stable?



- Cell, Oh and Byrd et al.: "Temporal Stability of the Human Skin Microbiome"

# The DNA molecule explains the Planet Earth's diversity



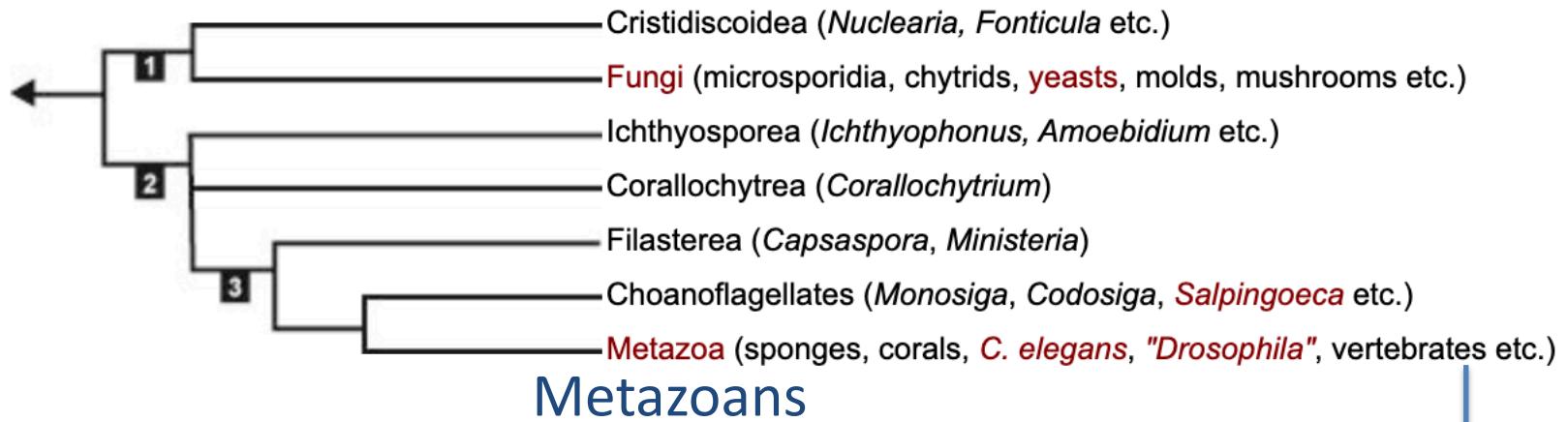
Obtain “the sequence” of any organism’s DNA (the information for all their genes = the genome)

The new taxonomy (count, classify)

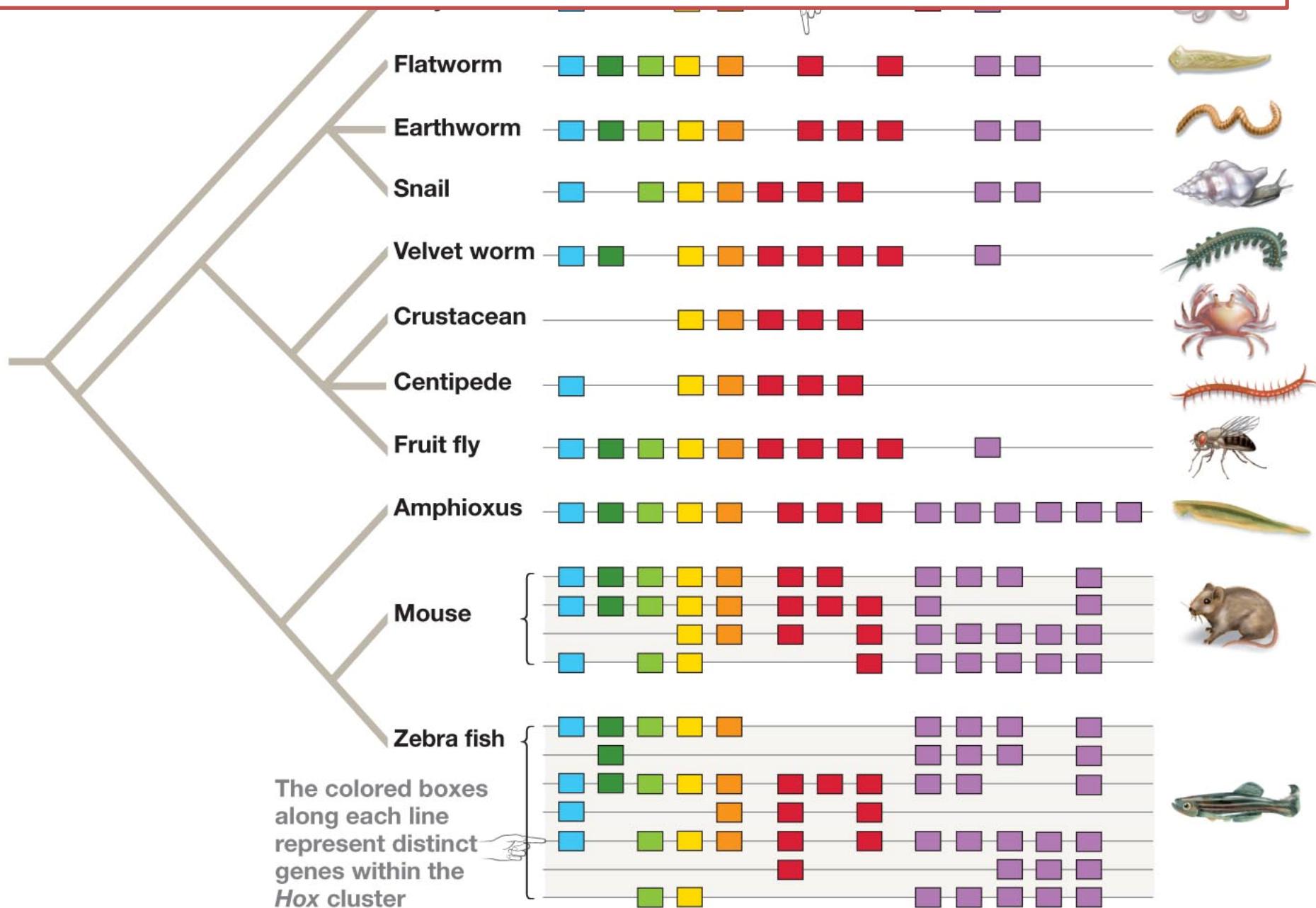
Obtain valuable information of its family relations (past millions of years)

Deduce evolutionary patterns and functions

# We are but a tiny part of the biodiversity: Opisthokonta



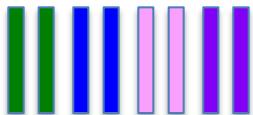
# Metazoan Evolution, a combinatorial of genes



# Barcodes to identify bacterial species: 16S ribosomal gene is de oficial I.D.

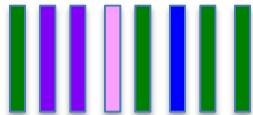
Aislado 1

GGAACCTT



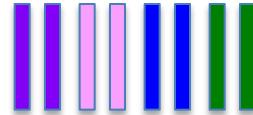
Aislado 2

GTTCGAGG



Aislado 3

TTCCAAGG



DATA BASE

TTCCAAGG



*E. coli*

GGAACCTT



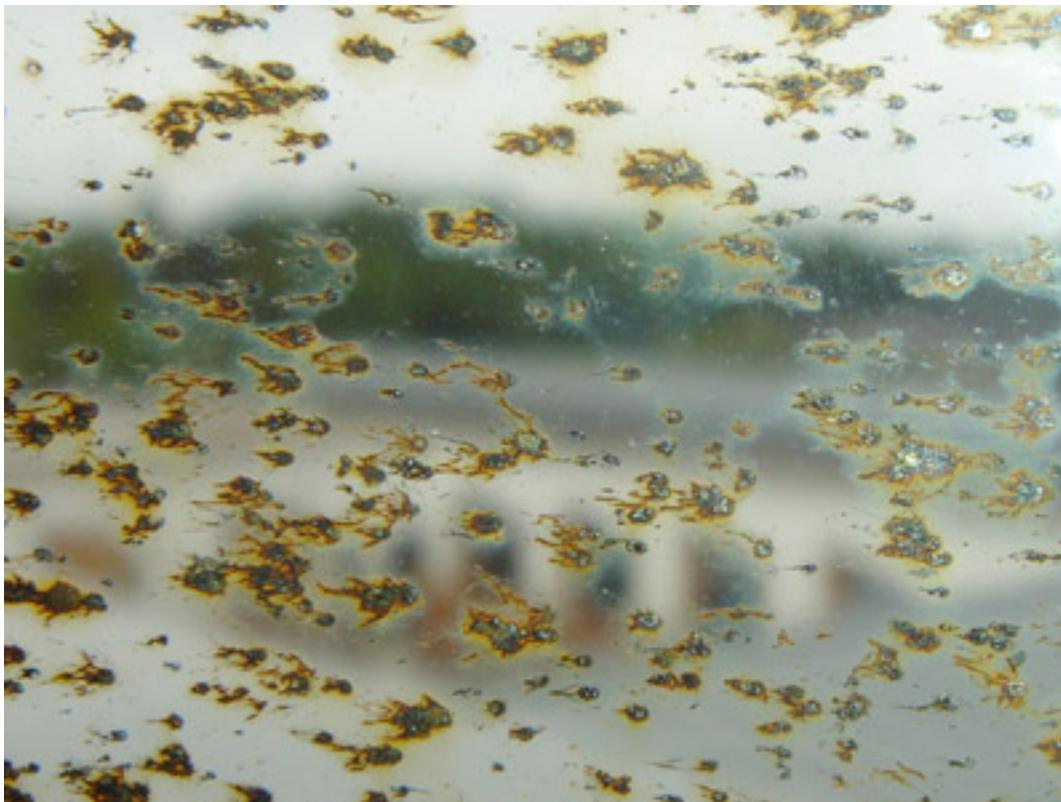
*Acetobacter*

GTTCGAGG



*Campylobacter*

# Metagenome, when you can't count one by one: windshield spatter analysis



Insect's diversity in a given transect

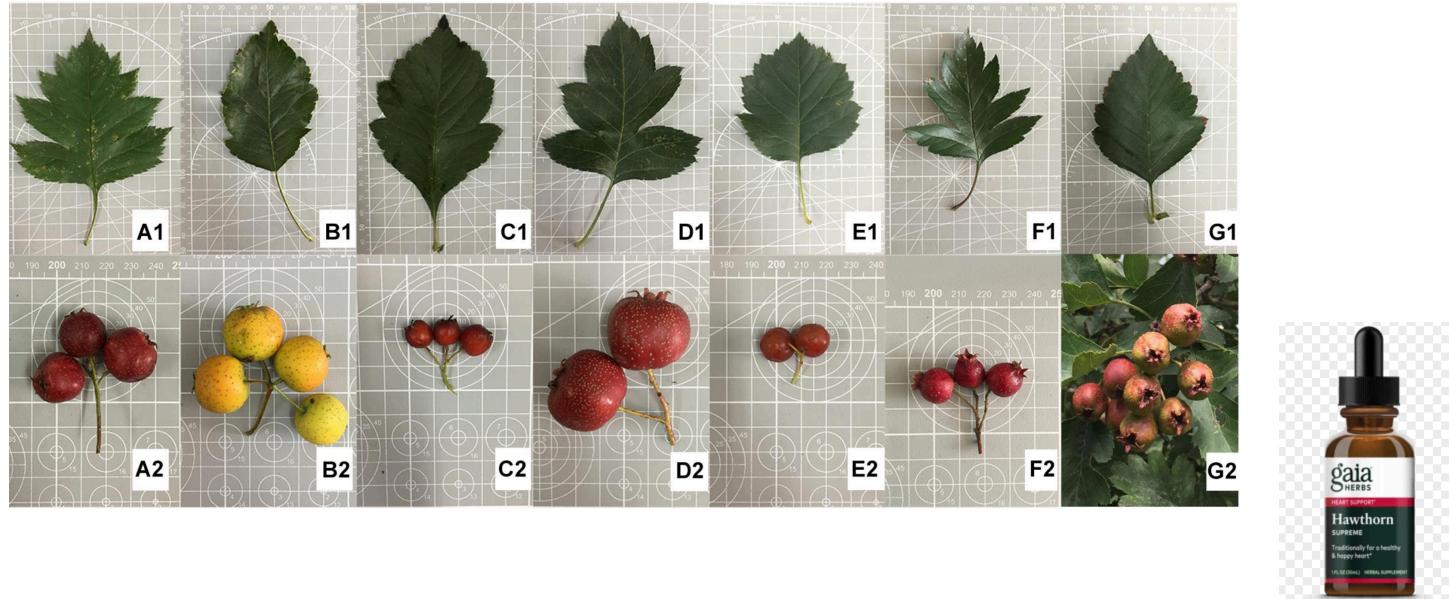
Collect the death bugs, sequence their DNA

Check database for identity

# A huge task to describe life's diversity

“The several hundred kinds of howthorns laugh at the single name they are forced to share”

The overstory, R. Powers



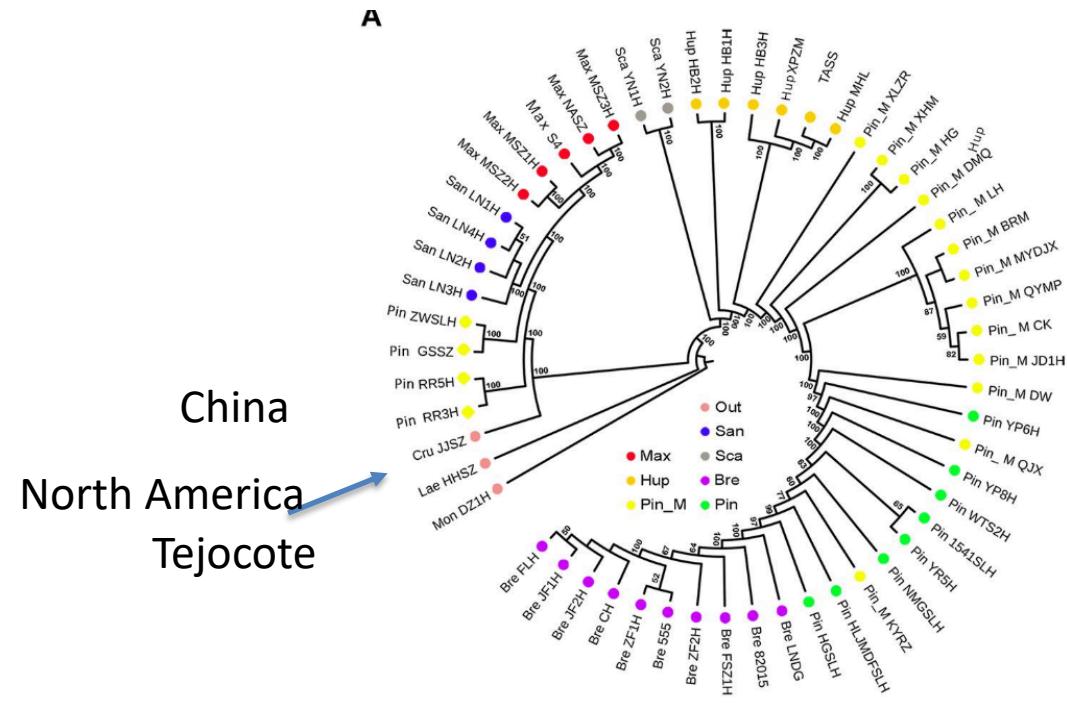
(Howthorns: in spanish, espinos)

# Sequences are aligned and “trees” are constructed to reflect genetic relationships

C

		60	65	75	80	140	145	230	235	245	
	<b>ATCC 14581 (*1)</b>	TTATAT .....	TTTGAT	TATTCA	ACCAAT	AACAGTATGTT					
	<b>IFO 12108 (*1)</b>	TTATAT .....	TTTGAT	TATTCA	ACCAAT	AACAGTATGTT					
	<b>KCCM 12503 (*3)</b>	TTATAT .....	TTTGAT	TATTCA	ACCAAT	AACAGTATGTT					
	<b>KCCM 11745 (*1)</b>	TTATAT .....	TTTGAT	TATTCA	ACCAAT	AACAGTATGTT					
	<b>ATCC 41415 (*1)</b>	TTATAT .....	TTTGAT	TATTCA	ACCAAT	AACATATGTT					
	<b>QMB 1551</b>	TTACAT .....	TTTGAC	TATCTA	ACCAAT	AACAAATATGTT					
	<b>ATCC 19213 (*9)</b>	TTACAT .....	TTTGAC	TATCTA	ACCAAT	AACAAATATGTT					
	<b>KCCM 11776 (*6)</b>	TTACAT .....	TTTGAC	TATCAA	ACCAAT	AACATTATGTT					
	<b>ATCC 15451 (*4)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACAAATATGTT					
	<b>ATCC 14945 (*7)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACATATGTT					
	<b>KCTC 2194 (*8)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACATATGTT					
	<b>ATCC 21916 (*8)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACATATGTT					
	<b>KCCM 11934 (*6)</b>	TTACAT .....	TTTGAC	TATTAA	A	ACCAAT	AACATATGTT				
	<b>KCTC 3712 (*2)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACAGTATGTT					
	<b>ATCC 10778 (*5)</b>	TTACAT .....	TTTGAC	TATTAA	ACCAAT	AACAGTATGTT					
	<b>KCCM 11761 (*5)</b>	TTACAT .....	TTTGAC	TATTGA	ACCAAT	AACAGTATGTT					
	<b>KCCM 11938 (*5)</b>	TTACAT .....	TTTGAC	TATTGA	ACCAAT	AACAGTATGTT					

# The diversity and evolutionary history of howthorns told by their DNA



Xiao Du et al. Frontiers in  
Plant Science 2019

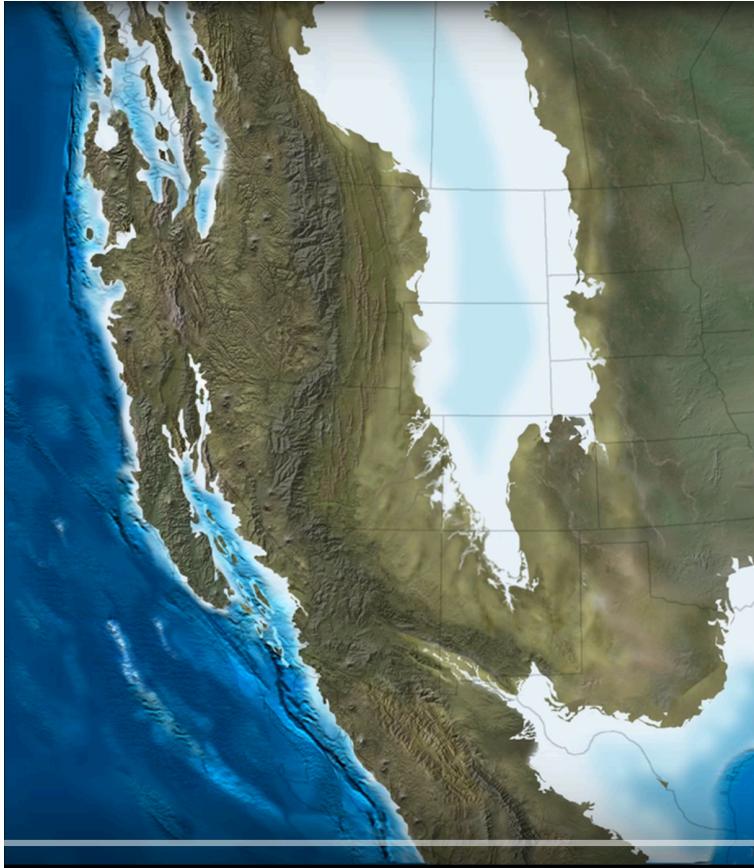
Evolutionary history of Chinese *Crataegus* (Bayesian clustering algorithm with admixed models, [Hubisz et al., 2009](#))

One of the main objectives in the field of microbial ecology is to be able to project a future scenario of microbial community structure and functions in a changing environment

# Cuatro Cienegas, Coahuila, Mexico



# Where was Coahuila 100 million years ago?



Today



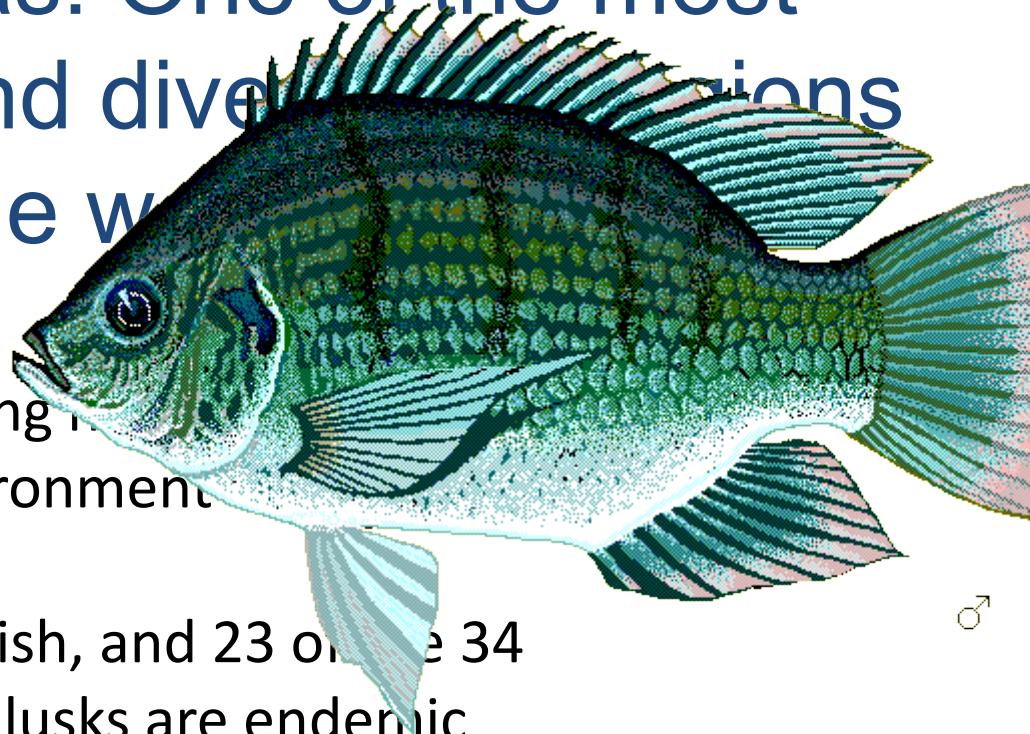
Coahuila

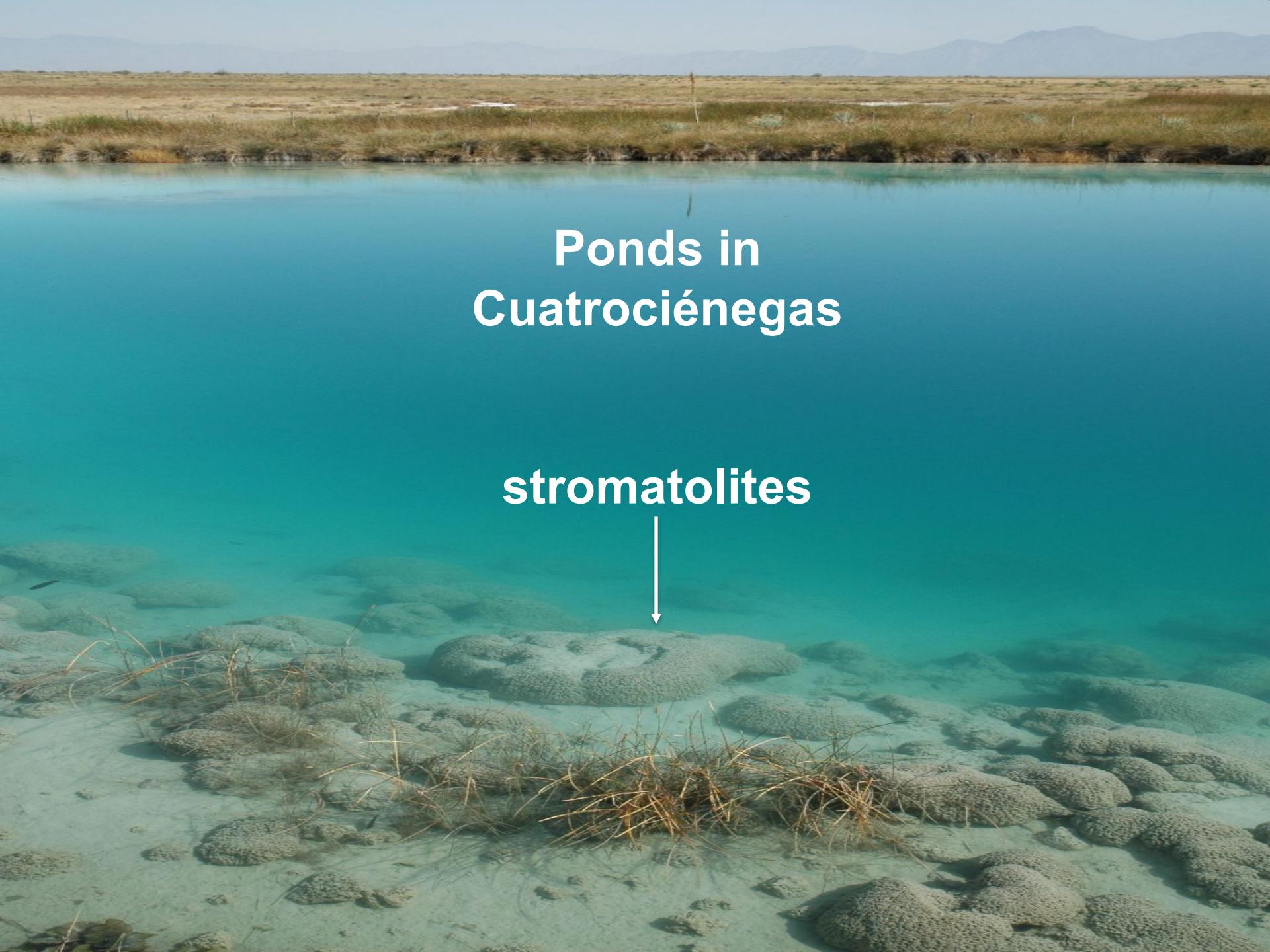
# Cuatro Ciénegas: One of the most biologically rich and diverse regions in the world

An interior basin containing rain-fed pools in a desert environment

Half of the 20 species of fish, and 23 of the 34 species of freshwater mollusks are endemic (i.e. *Cichlasoma minckleyi*)

Extremely low levels of phosphorus limit size of living organisms in ponds but not diversity...there is an amazing diversity of bacteria



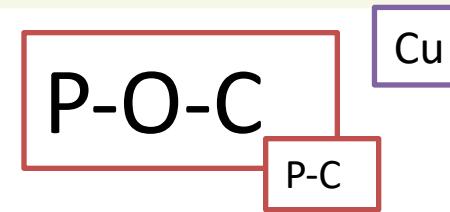
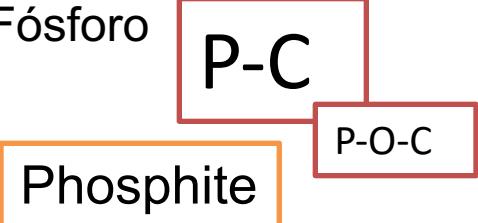
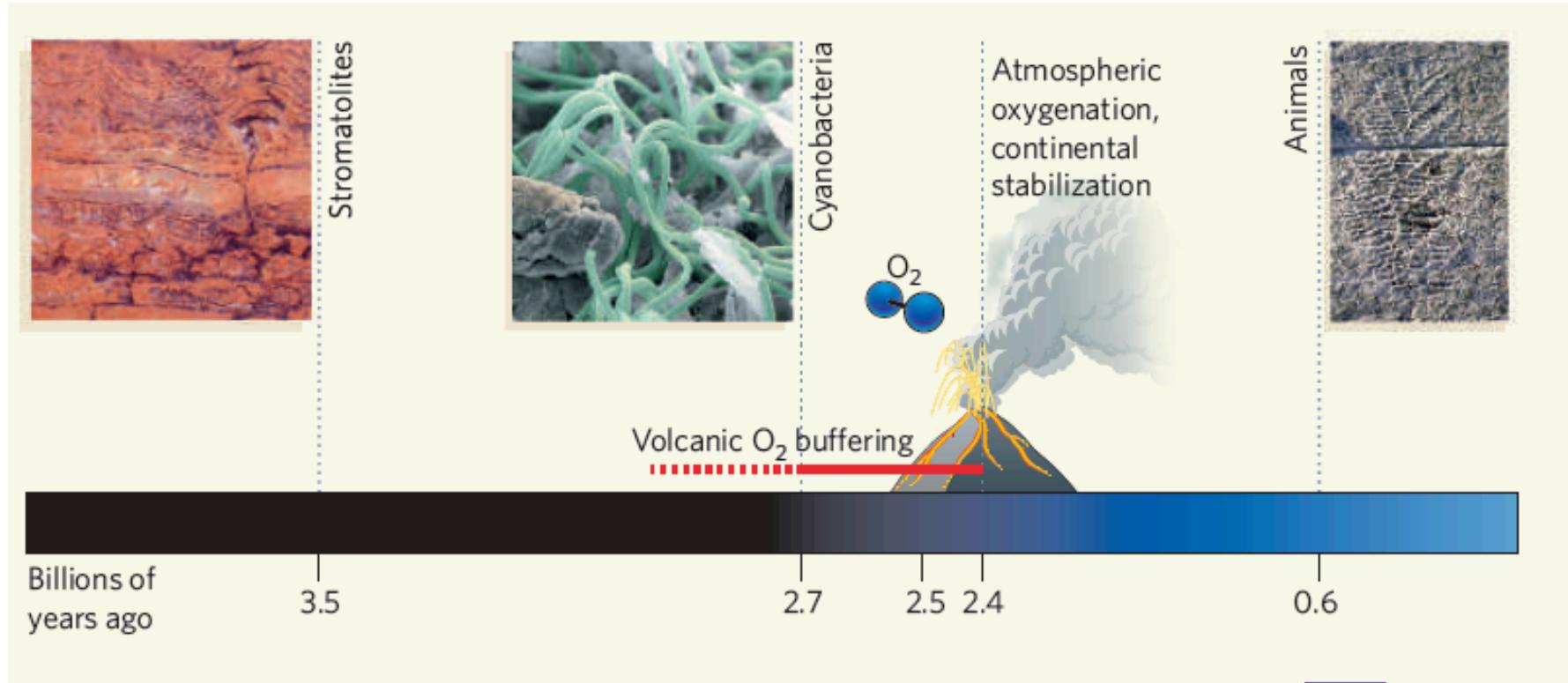
A photograph of a shallow, turquoise-colored pond. The water is clear enough to see the rocky bottom, which is covered in small, rounded, light-colored stones. In the background, there is a dry, grassy plain leading to a range of mountains under a clear sky.

# Ponds in Cuatrociéneas

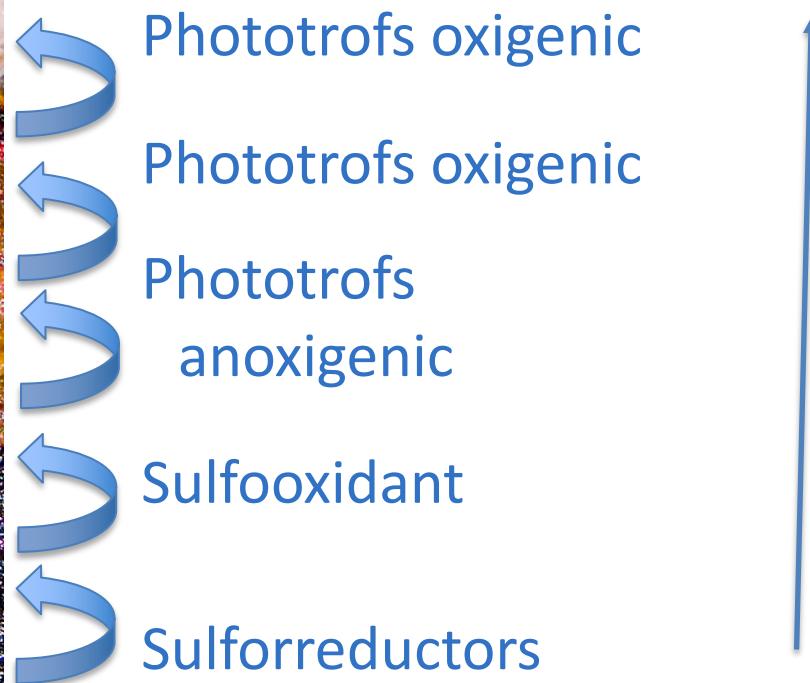
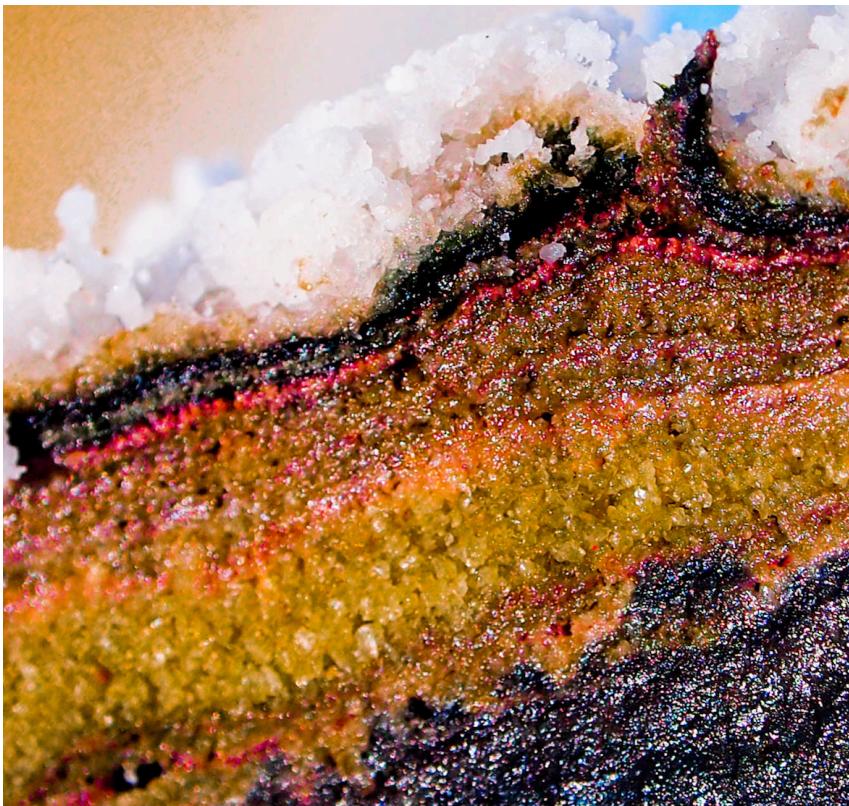
**stromatolites**



# Stromatolites: evidence of appearance of life on Earth



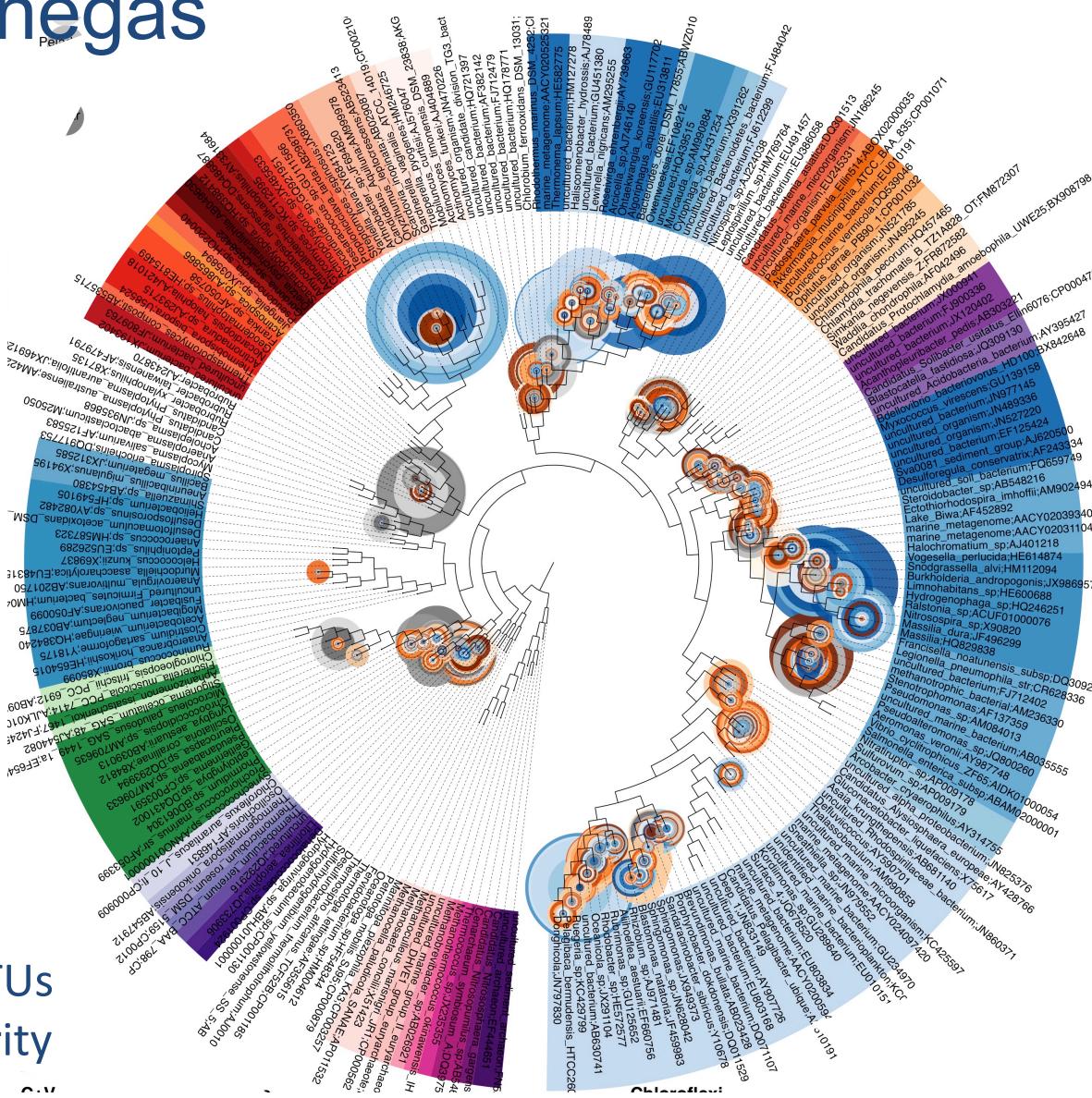
# Microbial mats: The Earth's biochemistry invented through evolution



Evolution of the planet's chemistry and its atmosphere

# Bacteria diversity at the Churince water system in Cuatrocienegas

Has 57 out of the 86 known Bacteria phyla, = 66.28% of the world known biodiversity



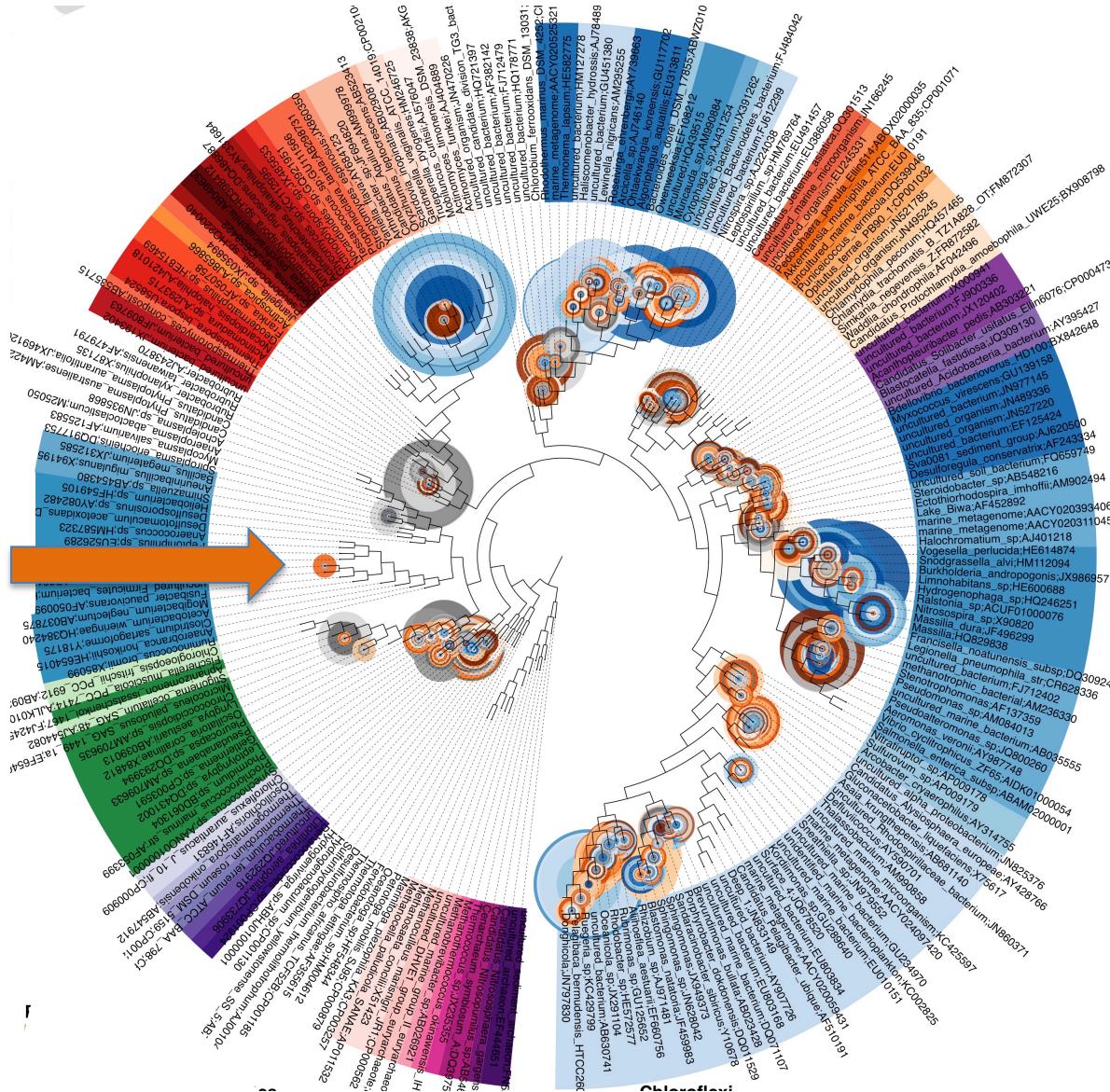
16 S rRNA analysis, OTUs defined at 97% similarity

Metagenomic data, eLIFE 2018

# Now I'll tell you about my favorite bacteria: the *Bacillus* genus

Our group has a collection of about 1800 *Bacillus* strains from Cuatrocienegas

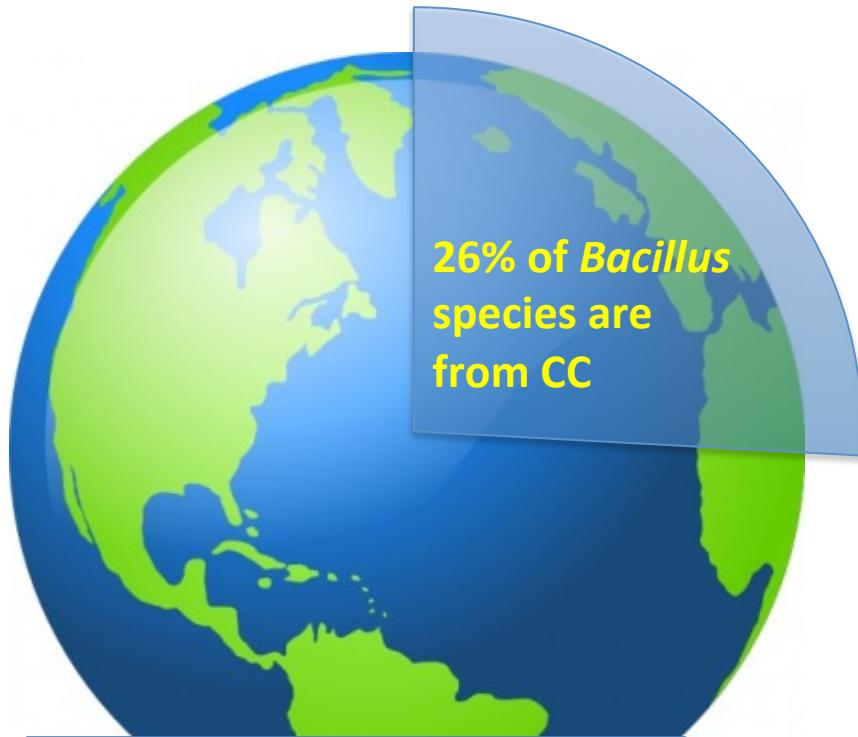
## *Bacillus*



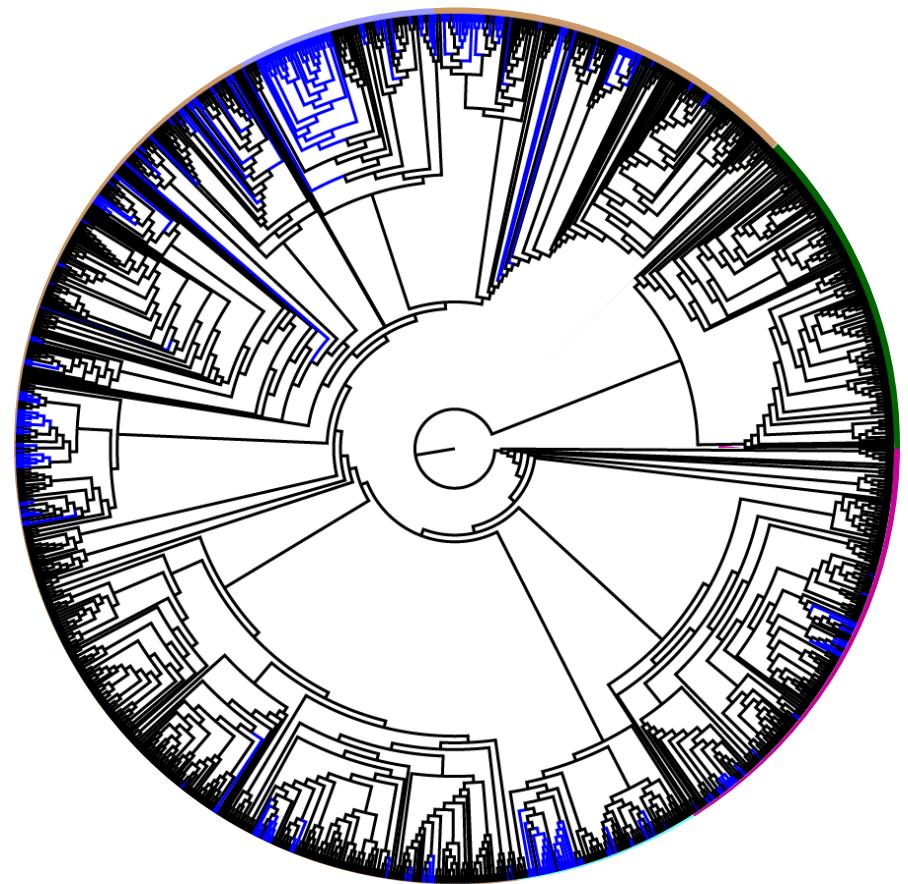
# At Cuatro Cienegas we uncovered 25% of all known *Bacillus* species diversity

WORLD: 1019 OTUs

CCC: 265 OTUs



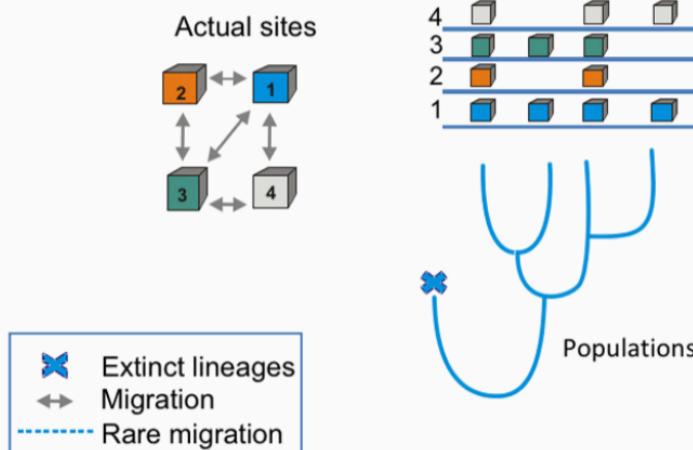
Souza et al. 2018. The lost world of Cuatro Cienegas Basin, a relictual bacterial niche in a desert oasis eLIFE, accepted



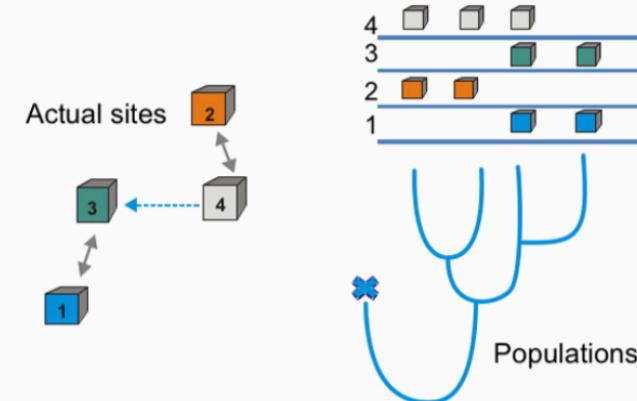
Dendrogram of the 1284 strains of the genus *Bacillus* reconstructed from 16S rRNA. All taxa have a sequence divergence over 97%.

# Conceptual frame-work of species diversification: name of the game, limit gene flow

## A Everything is everywhere and the environment selects

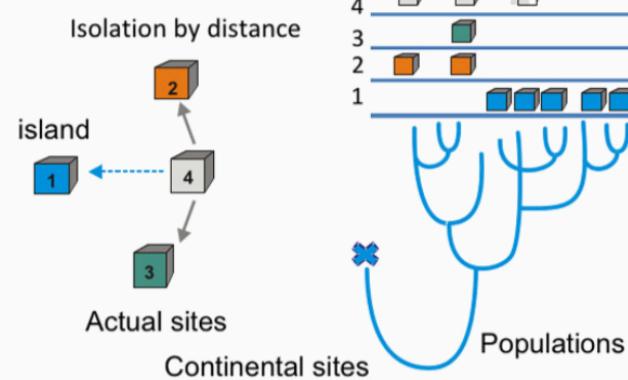


## B Isolation by distance



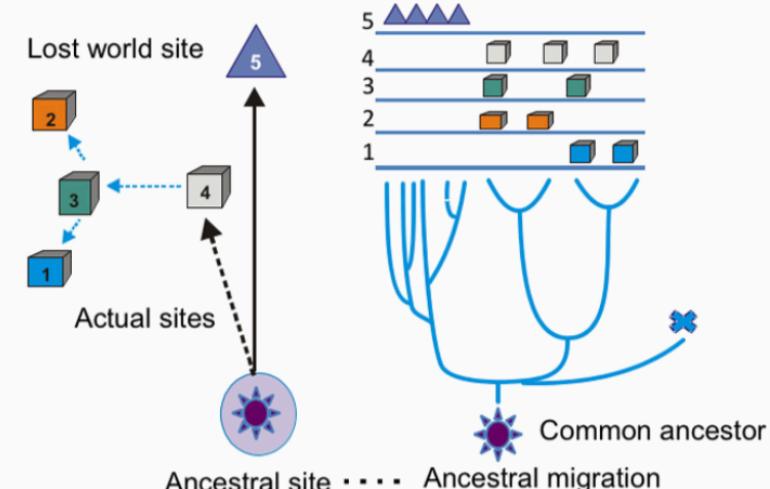
occurs in most plants and animal phyla

## C "Galapagos island" model, Isolation and diversification



Island refuge  
 adaptive radiation due to isolation

## D "Lost World" model, ancestral Isolation and diversification



ancestral isolation and diversification

# The core of my work: understanding how microbial communities are ensambled

## Stochastic Model

(birth, death, extinction, speciation)

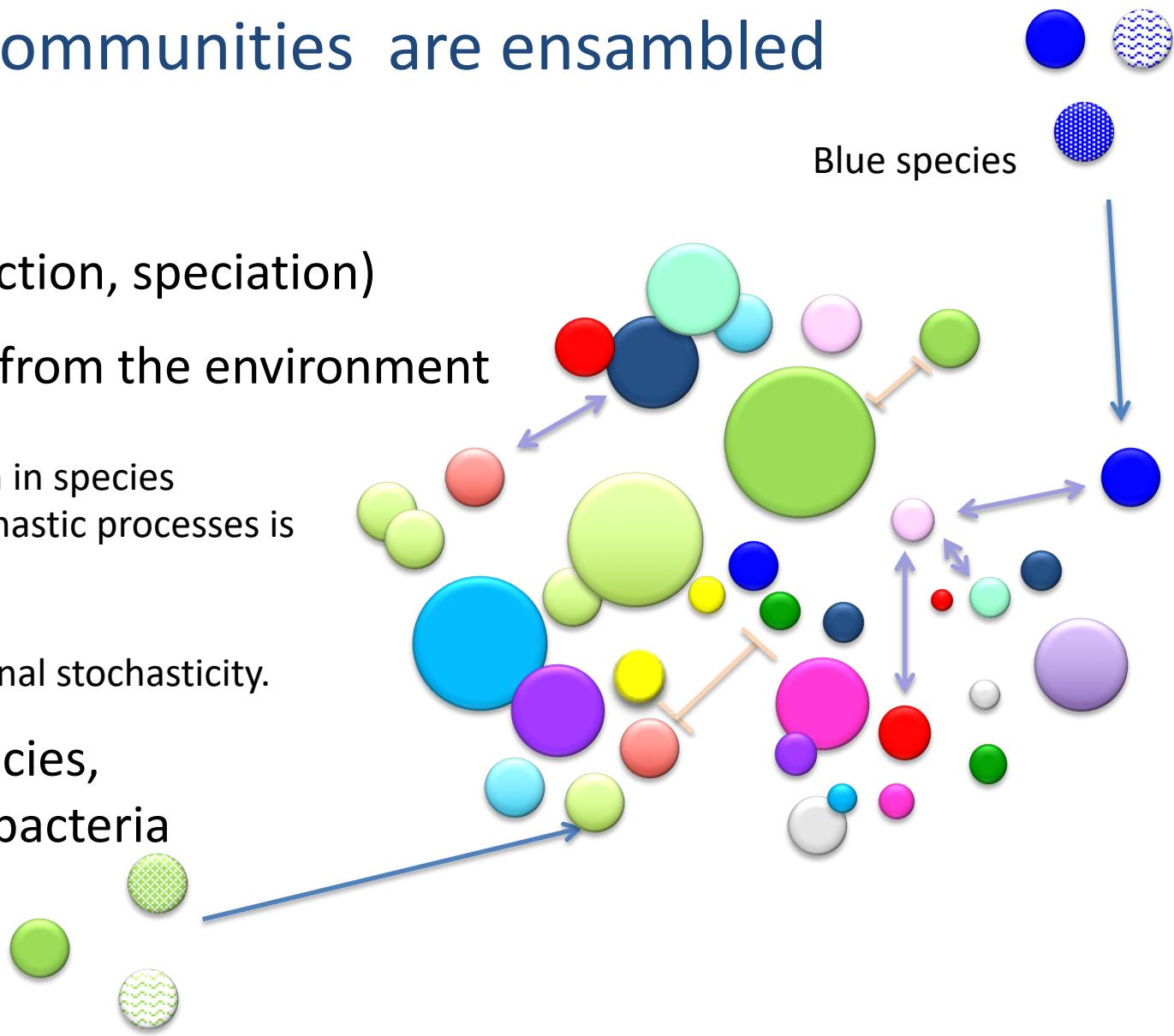
Random selection from the environment

The site- to-site variation in species composition due to stochastic processes is unpredictable.

This is called Compositional stochasticity.

Equivalence of species,  
any green or blue bacteria  
will do

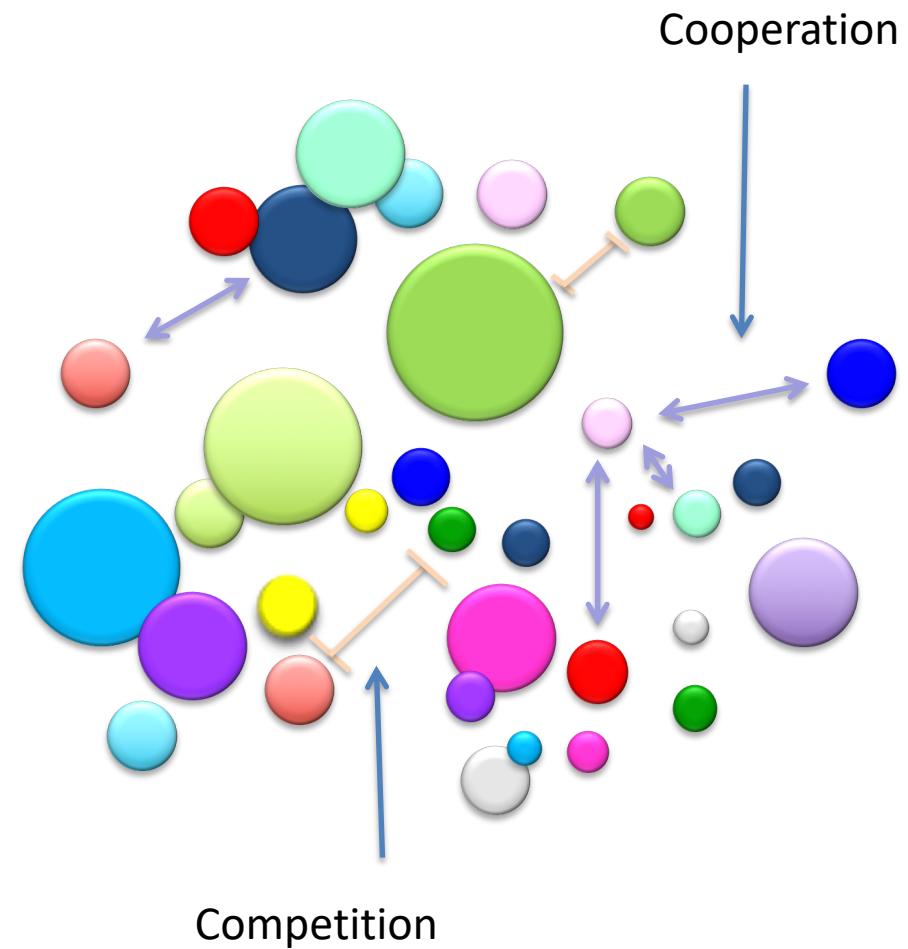
Green species



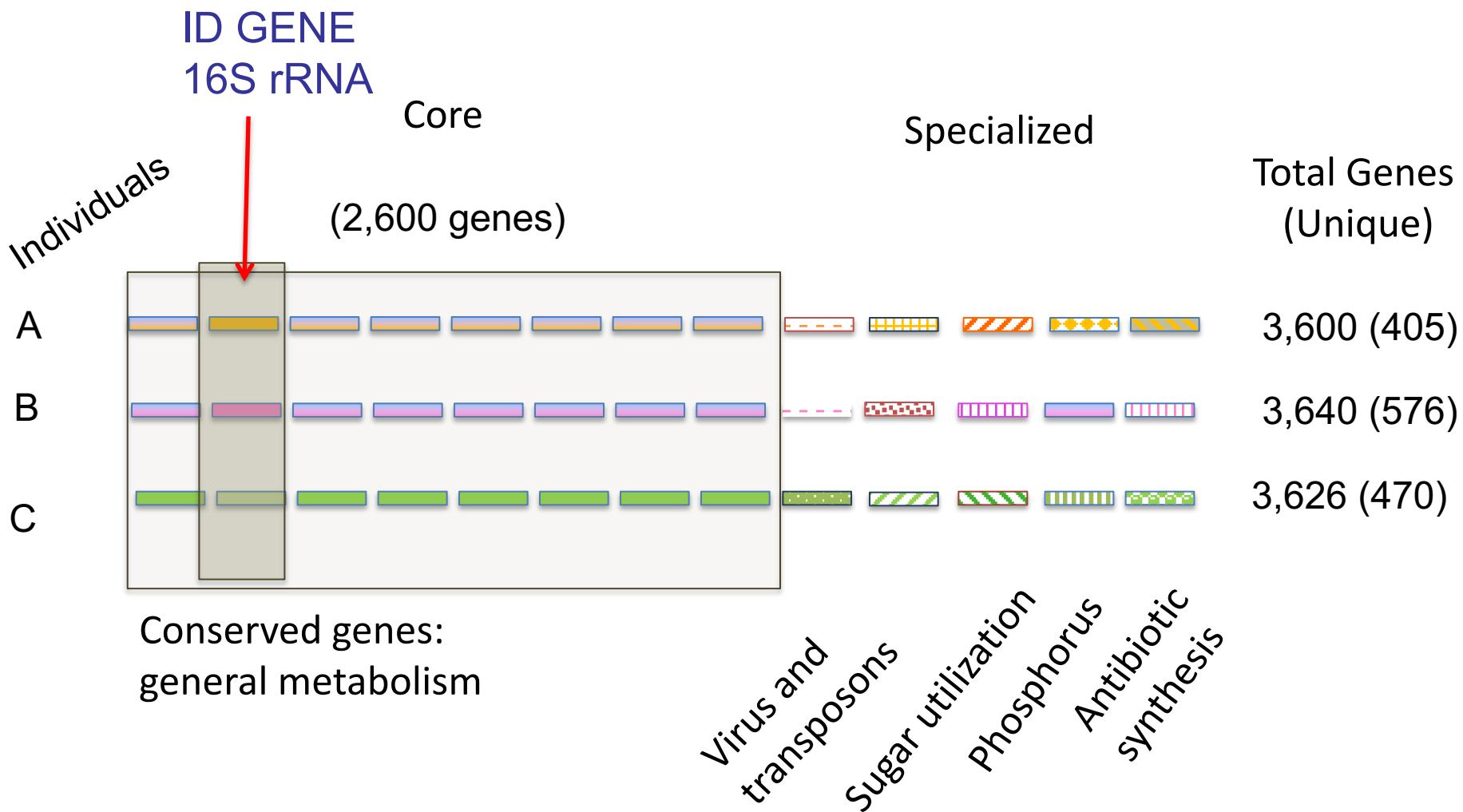
# Complex network of interactions in communities lead to competitive phenotypes

## Deterministic model

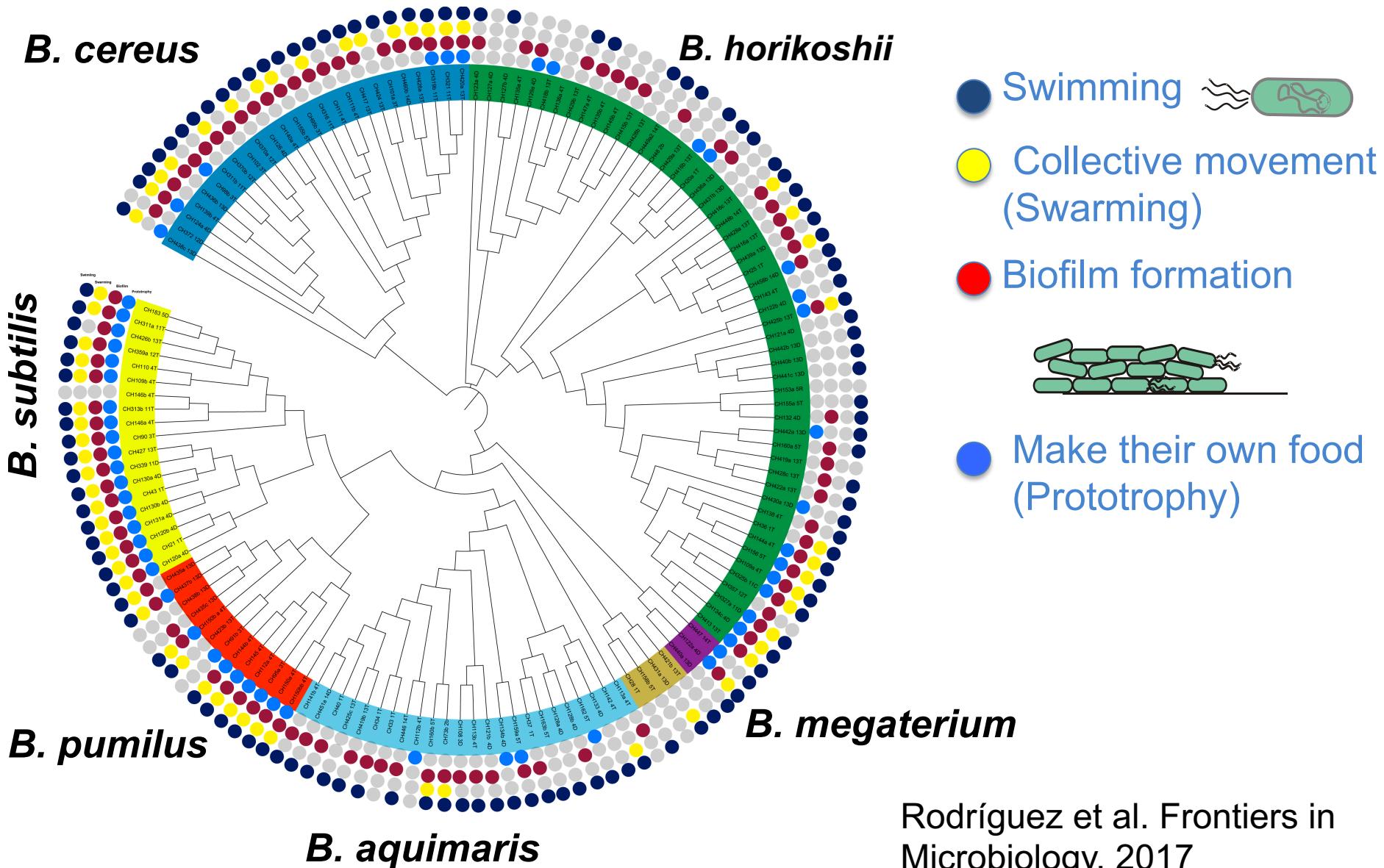
Niche theory: all species/individuals are ecologically and functionally different and environments play important roles in governing species abundance and distribution



# To understand bacterial diversity we have to count species and genes



# Functional diversity supports a deterministic model



Rodríguez et al. Frontiers in  
Microbiology, 2017

# How significant is diversity in a deterministic model?

## Enzymes (genes) are public goods

Phosphorus utilization is a distributed trait

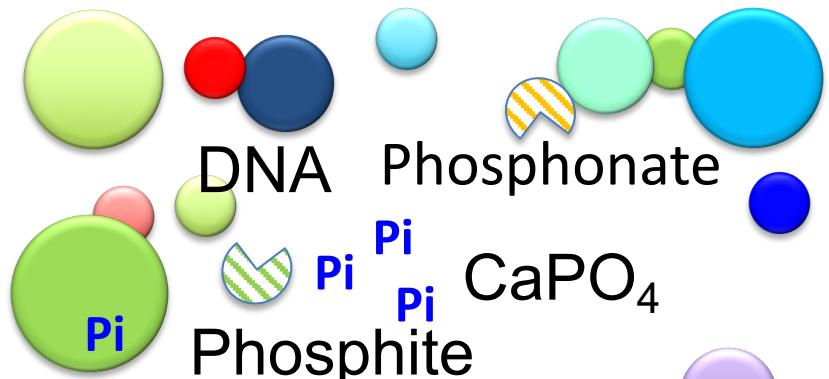
Genes for phosphorus utilization transfer between the bacteria in the community

The enzymes are public goods: some bacteria contribute but everybody benefits

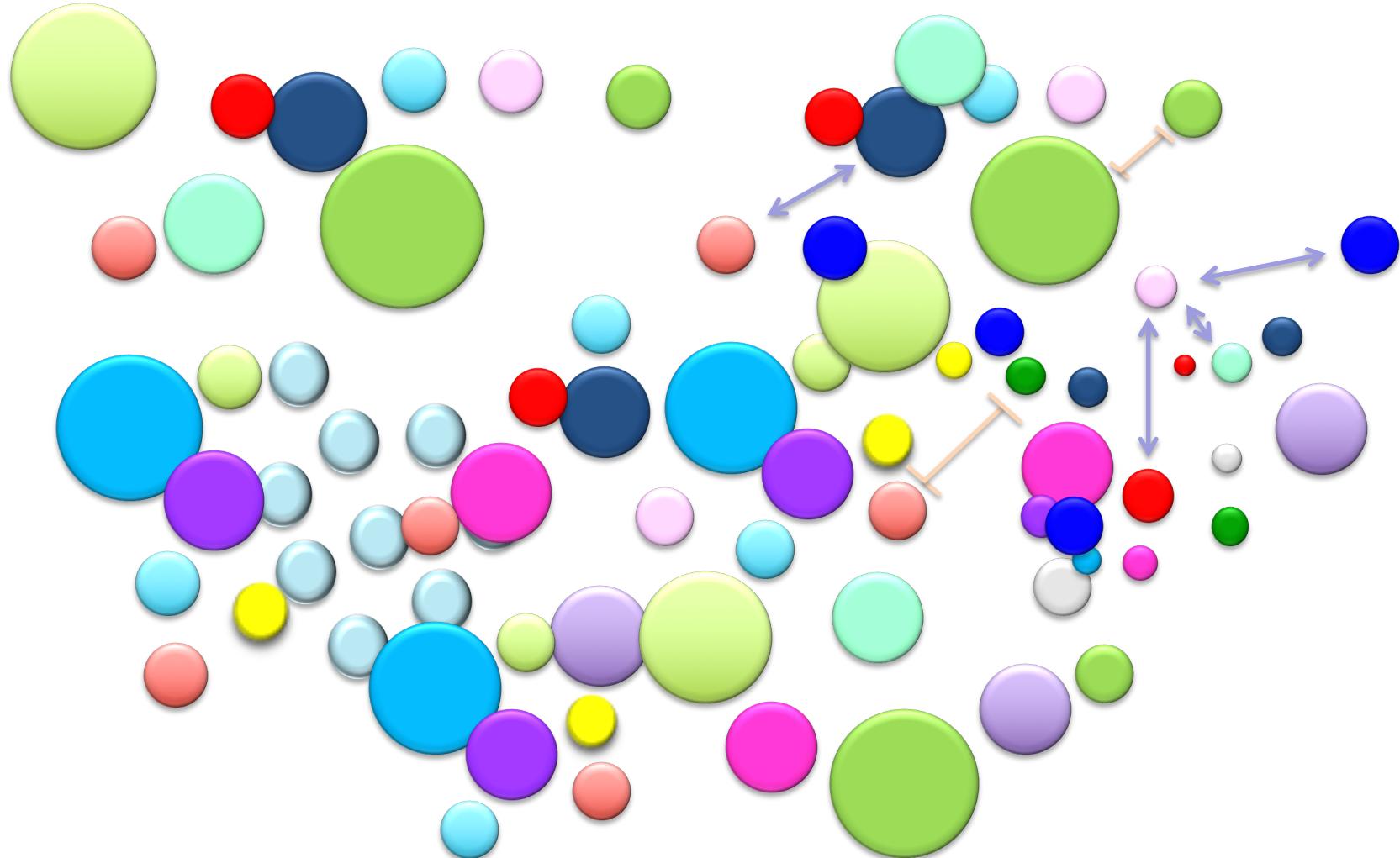
Expanded capabilities for P utilization

**The environment is modified!**

All is converted to edible Pi outside the cells



At what scale should communities be studied? Will each simple be different?



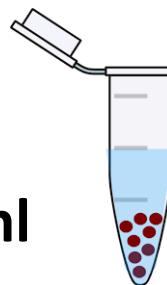
# Microbial Ecology: Evaluate interactions among members of sediment communities

Deterministic hypothesis

Community structure is influenced by antagonistic interactions?

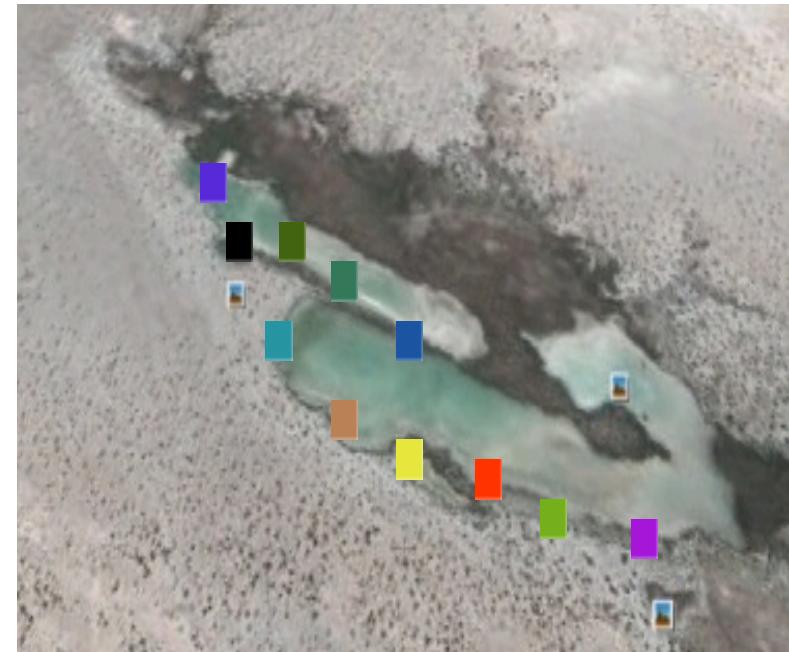
Compare interference within-site and across-site, interactions would be different.

**Sample: 0.05 ml**



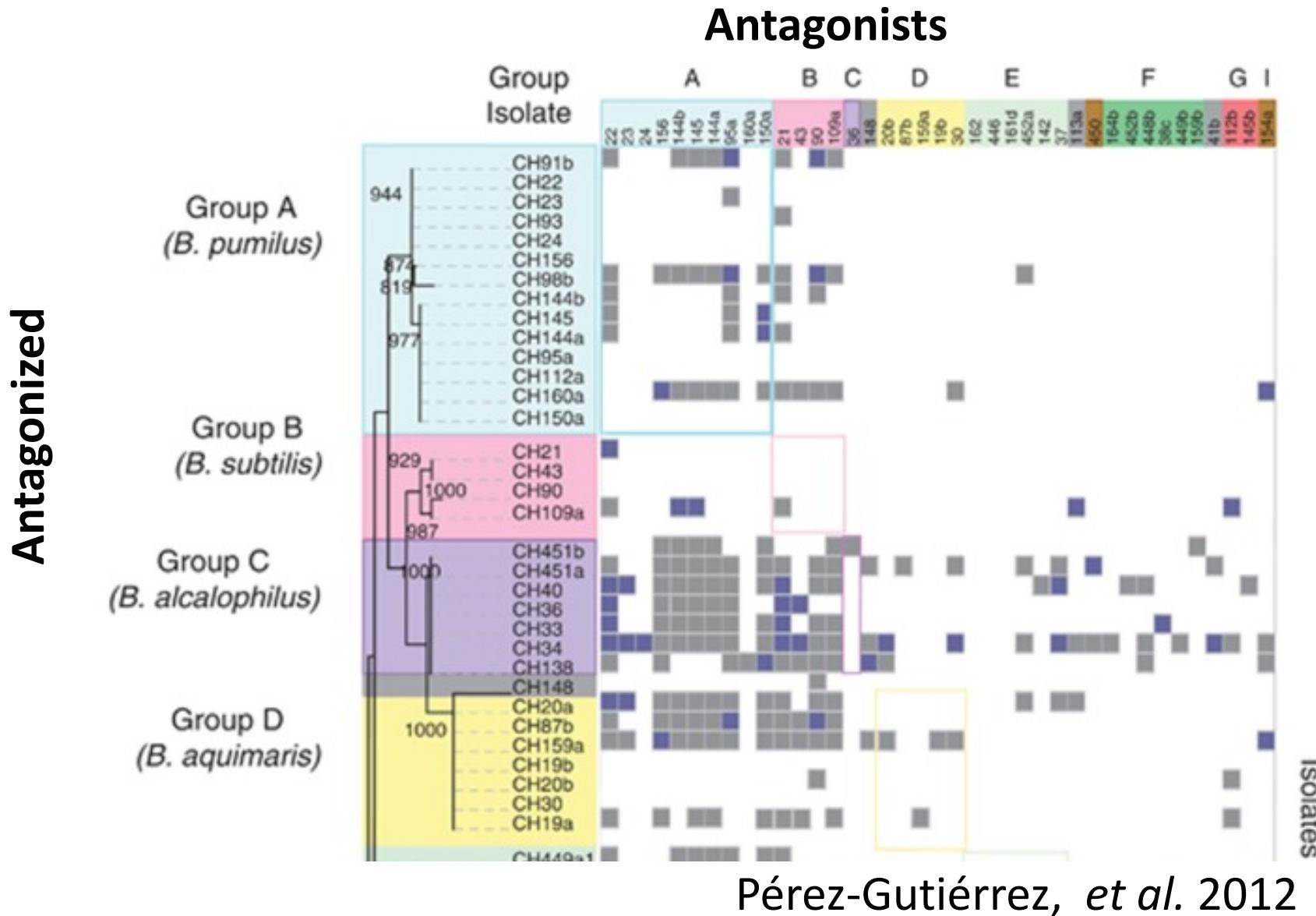
Relevant for diffusion of metabolites

**The Churince water system 350 m long**



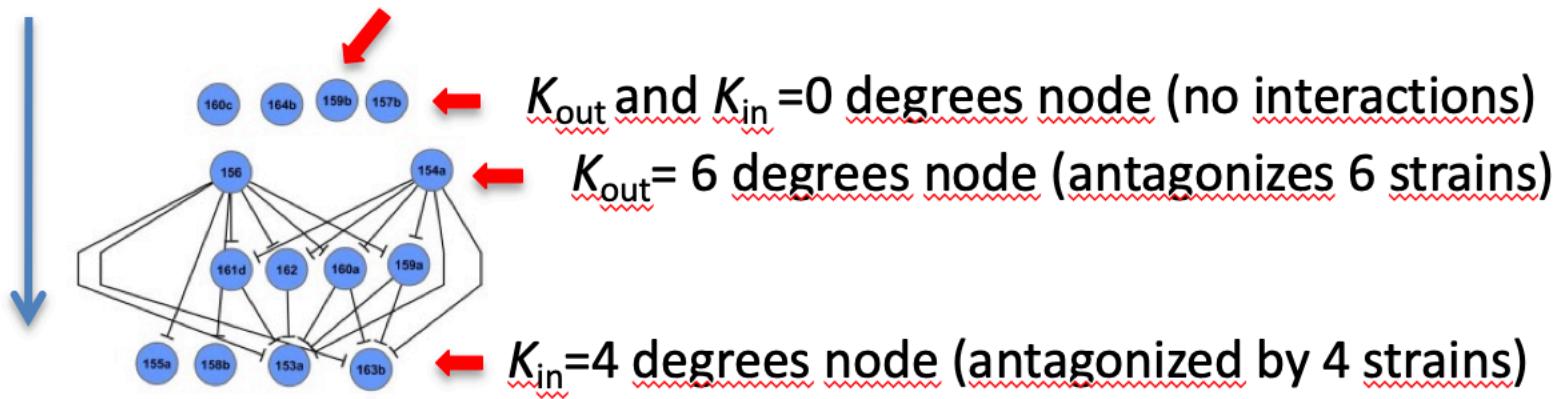
Approx. 30 m between consecutive sampling sites

# Antagonistic interactions



# Network representation of antagonisms

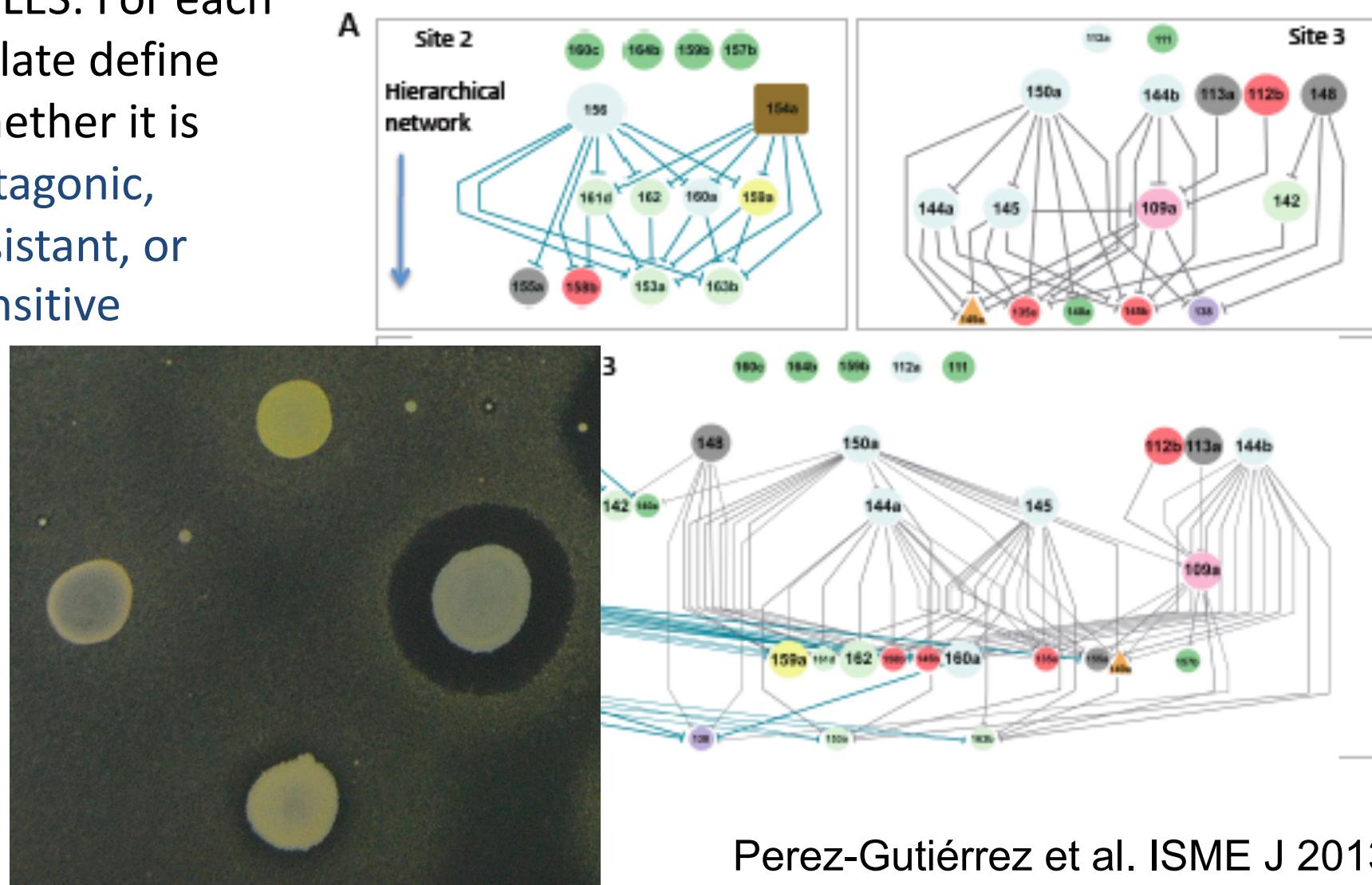
Each strain is a node



Hierarchical network  
shows  
Directionality

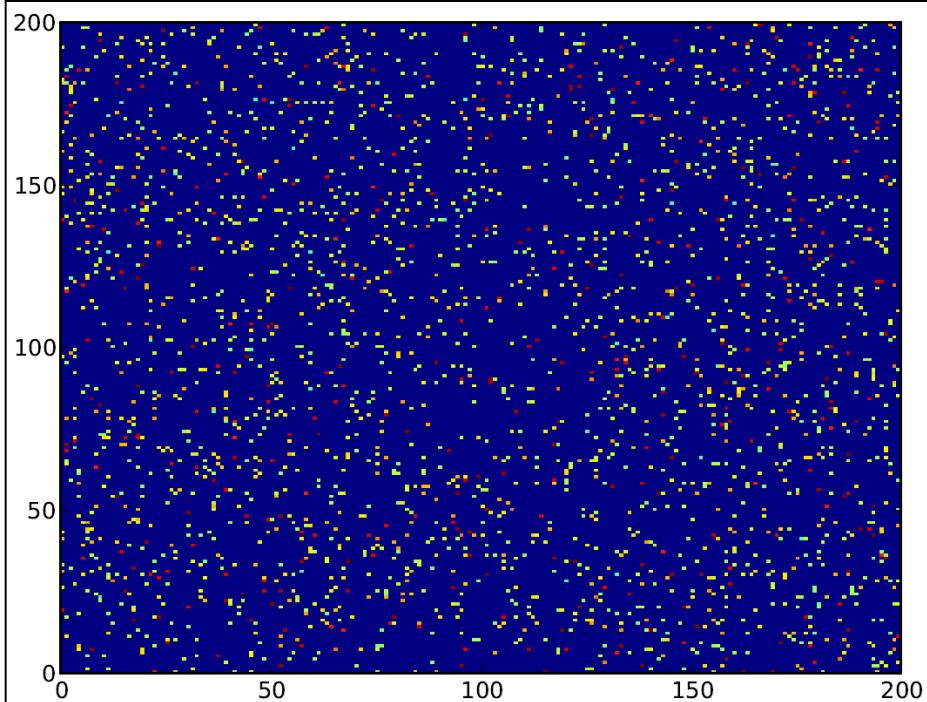
# Networks of antagonistic interactions in *Bacillus* spp.

ROLES: For each isolate define whether it is antagonistic, resistant, or sensitive

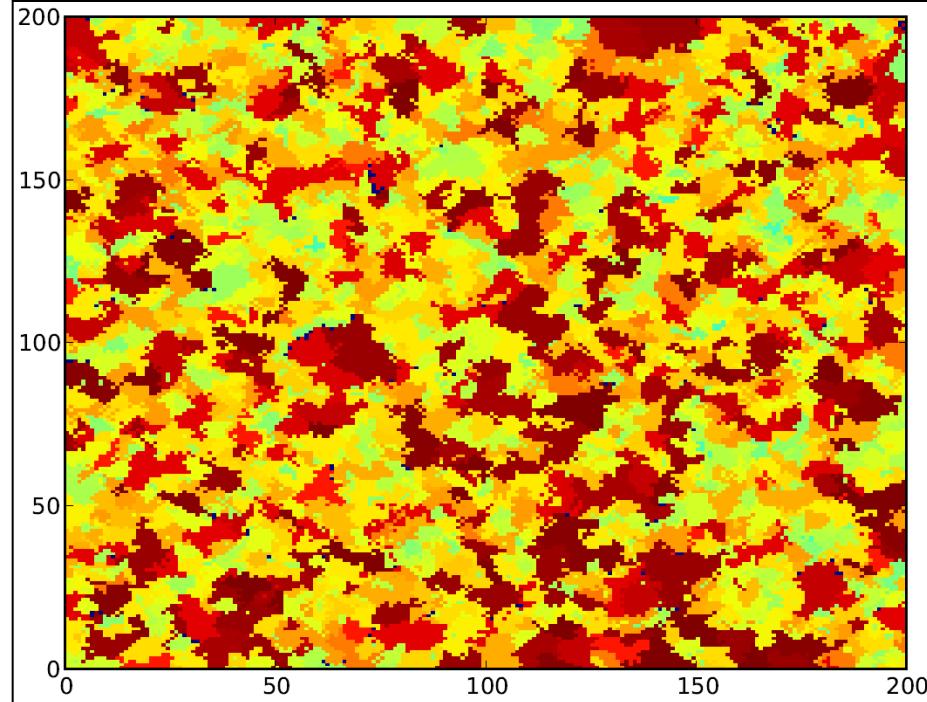


# In a structured space antagonist bacteria don't lead sensitive bacteria to extinction

● Antagonistic    ● Sensitive    ● Resistant



Initial state

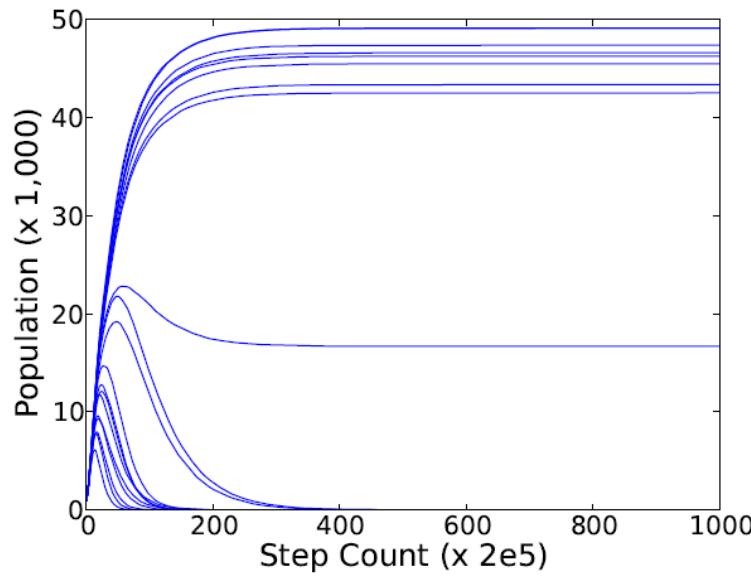
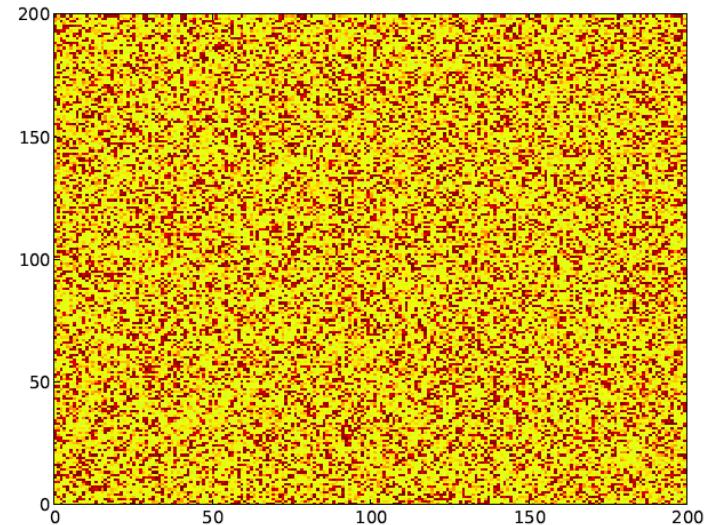
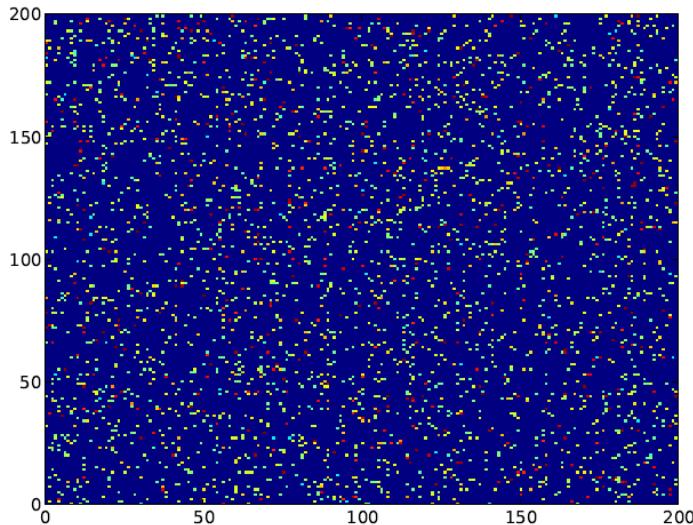


Final state

Cellular automaton, simulation of the spacial organization of isolates with different antagonism/resistance properties

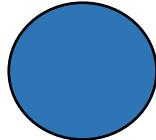
Zapien *et al.* Frontiers in Microbiology 2015

# Perturbations result in the extinction of sensitive bacteria, structure is essential

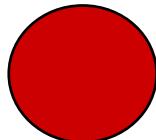


# Synthetic Ecology

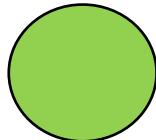
**Resistant and  
non  
Antagonistic**



**Resistant and  
Antagonistic**

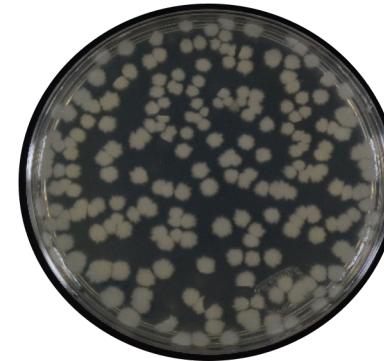


**Non resistant and  
non antagonistic**

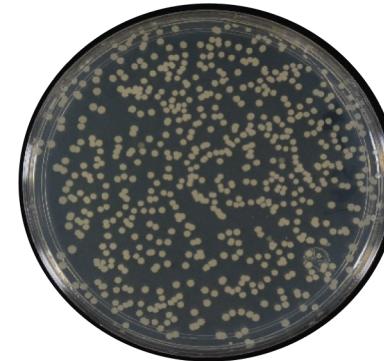


- Different colony phenotypes to count even in mixtures
- Different species to identify their genes in transcriptome

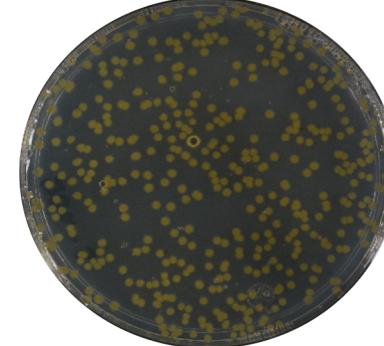
111 (*B. cereus*)



145 (*B. pumilus*)

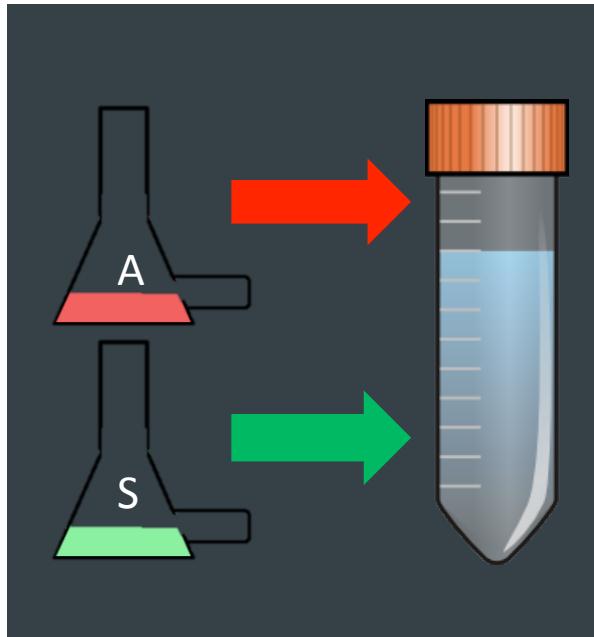


20a (*B. horikoshii*)



# Setup of interaction experiments

Double interactions



Triple interactions

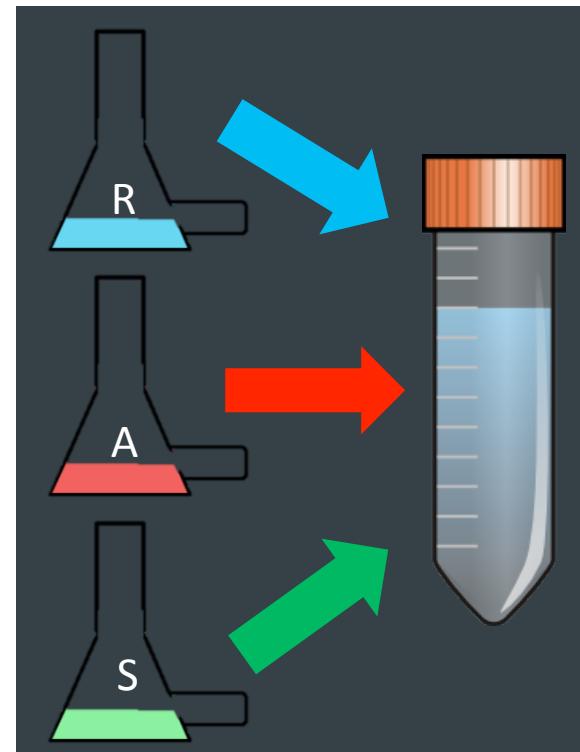
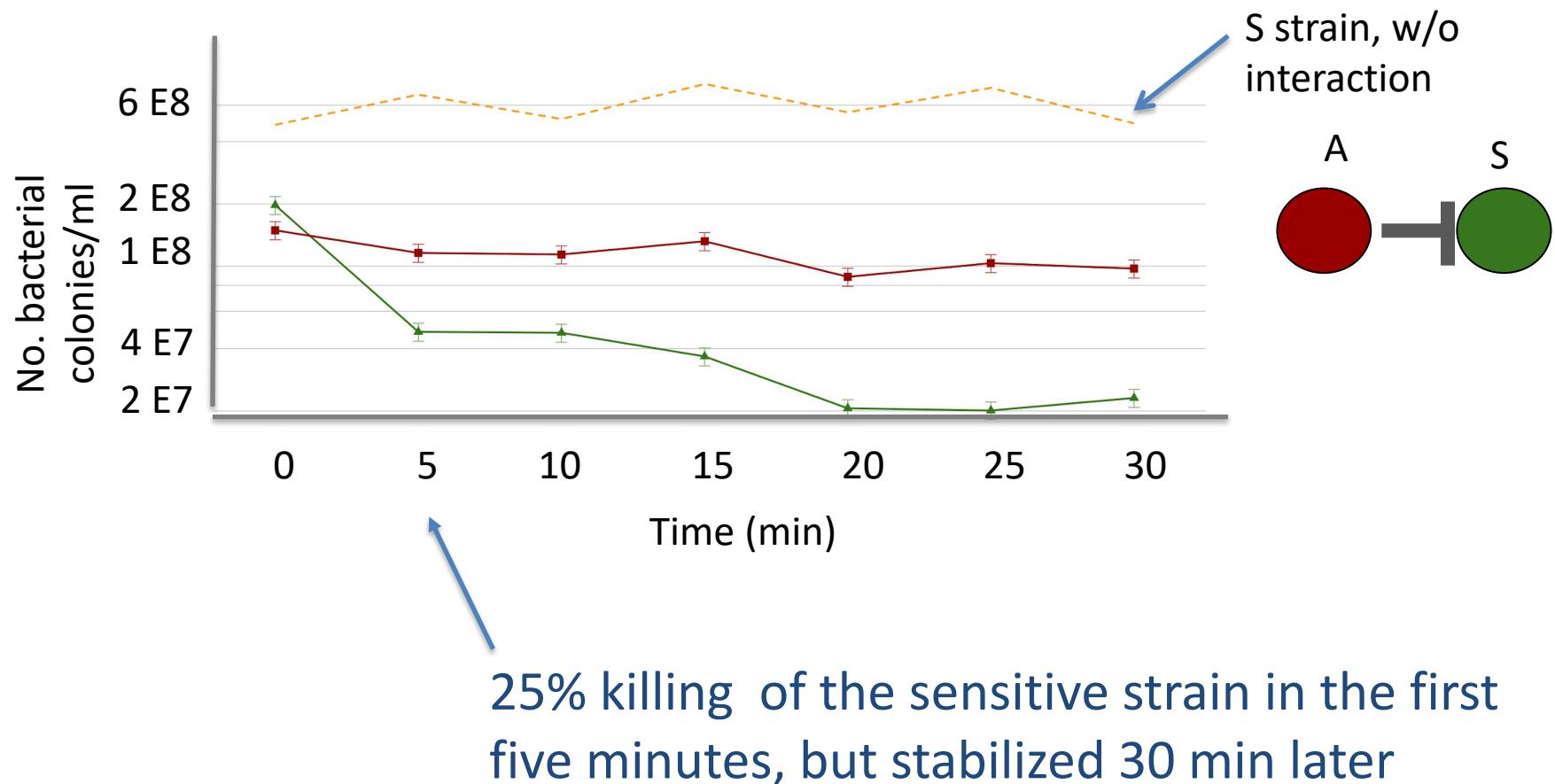


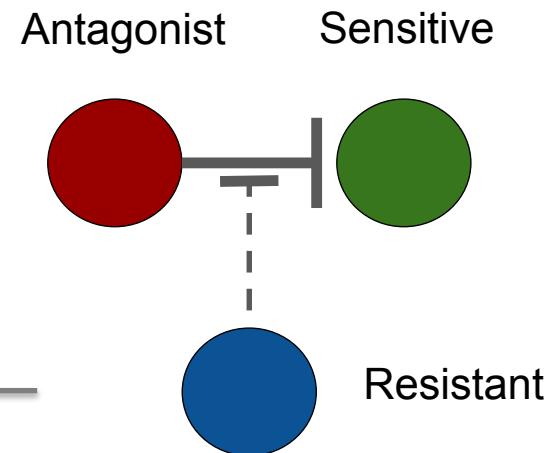
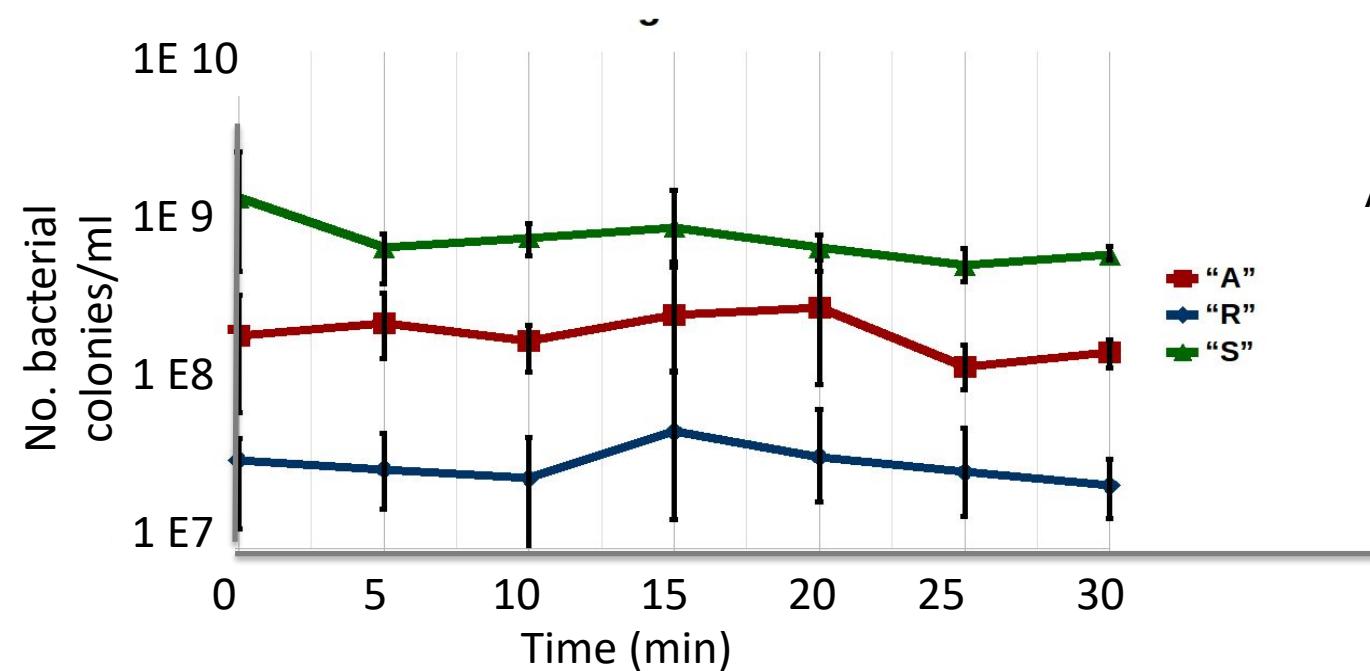
Plate on Marine Medium Agar after 0, 5, 15, etc. min

# Rapid killing of the sensitive strain in a non-structured environment, followed by stabilization

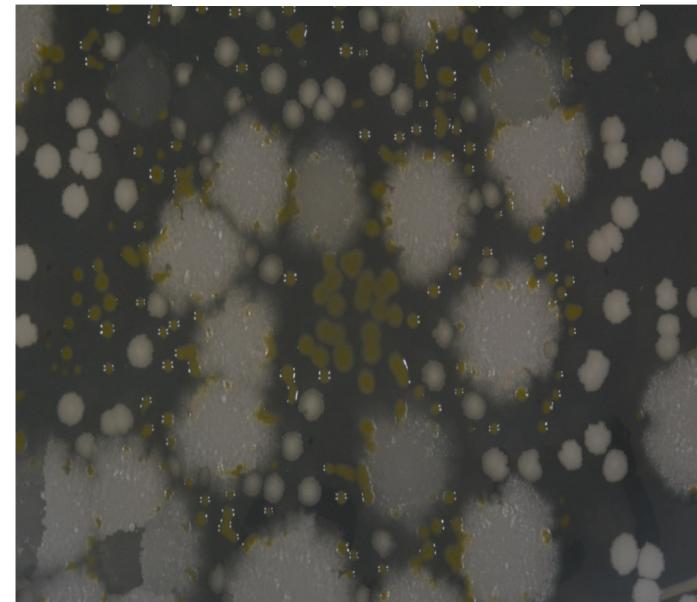
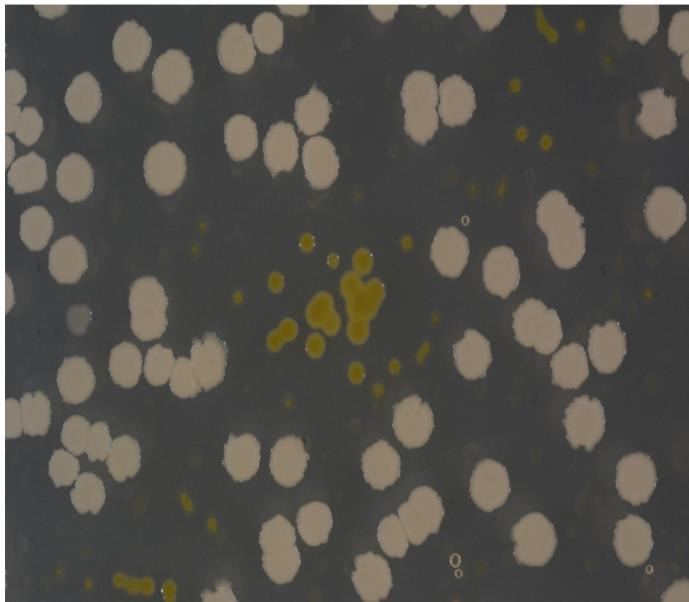
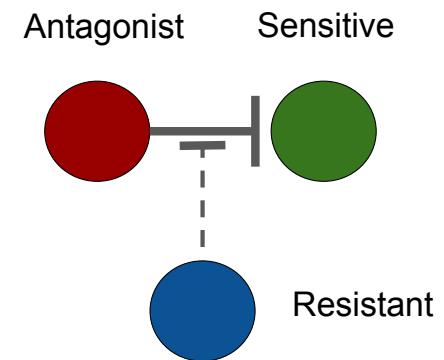
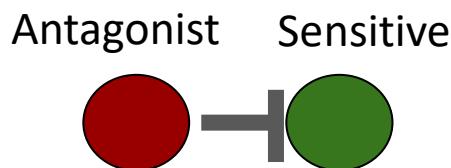


# Emergent property 1

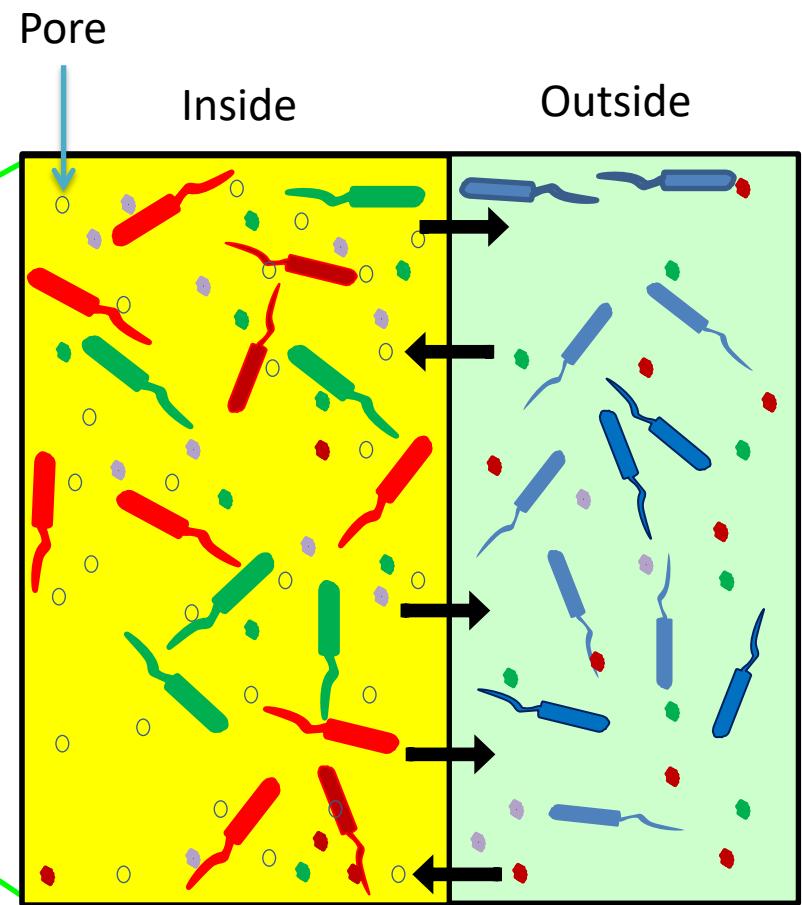
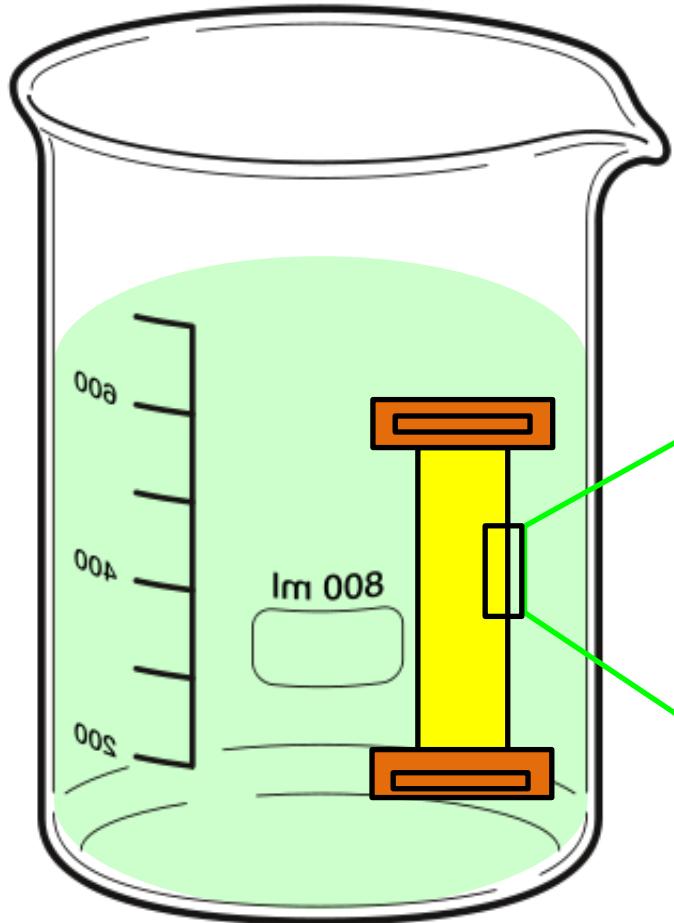
Killing is prevented in the presence of the resistant



# Islands of resistance in a structured environment, similar to cell automaton

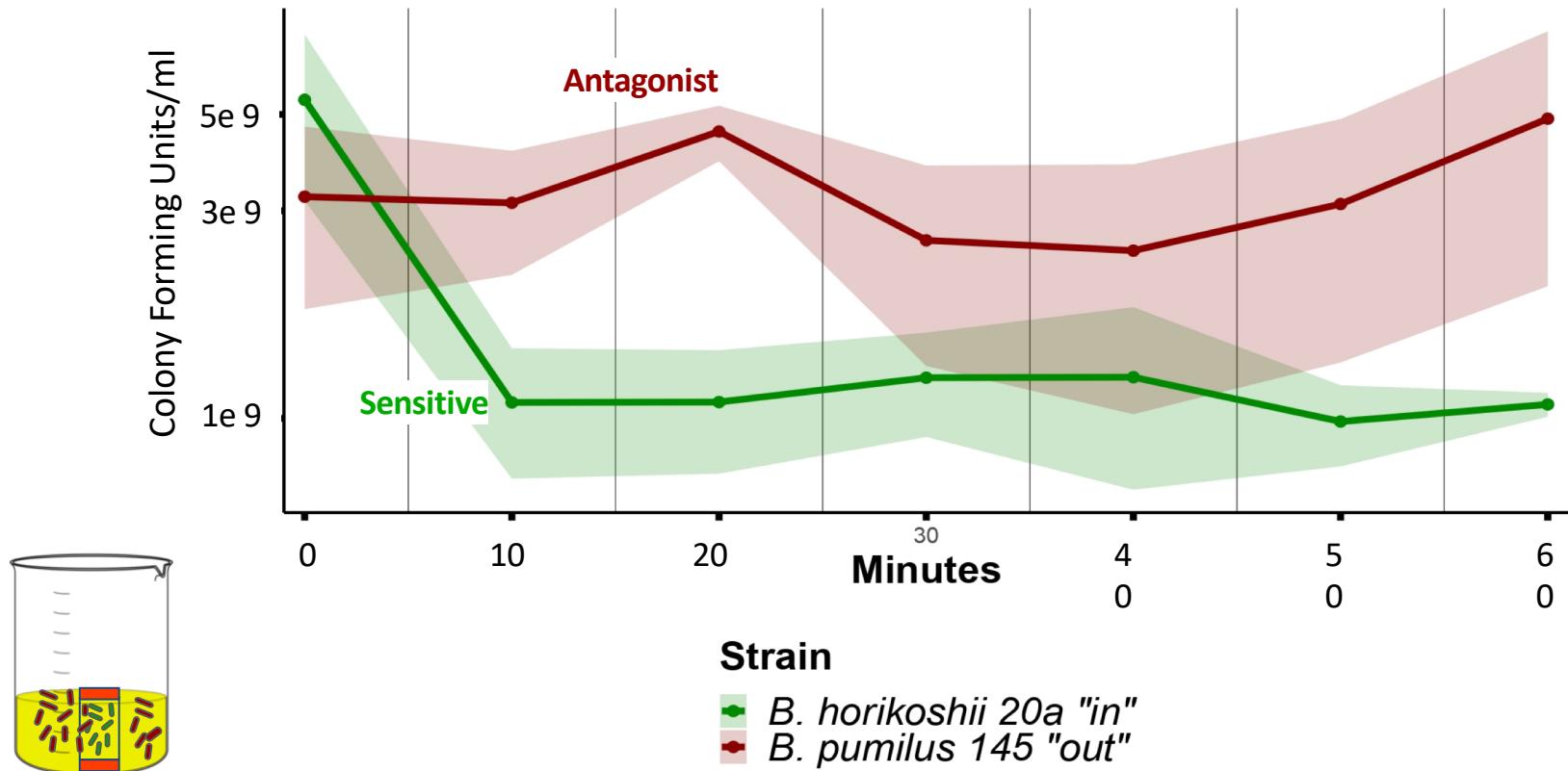


# Small molecules determine antagonism

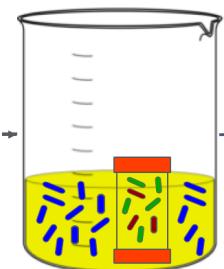
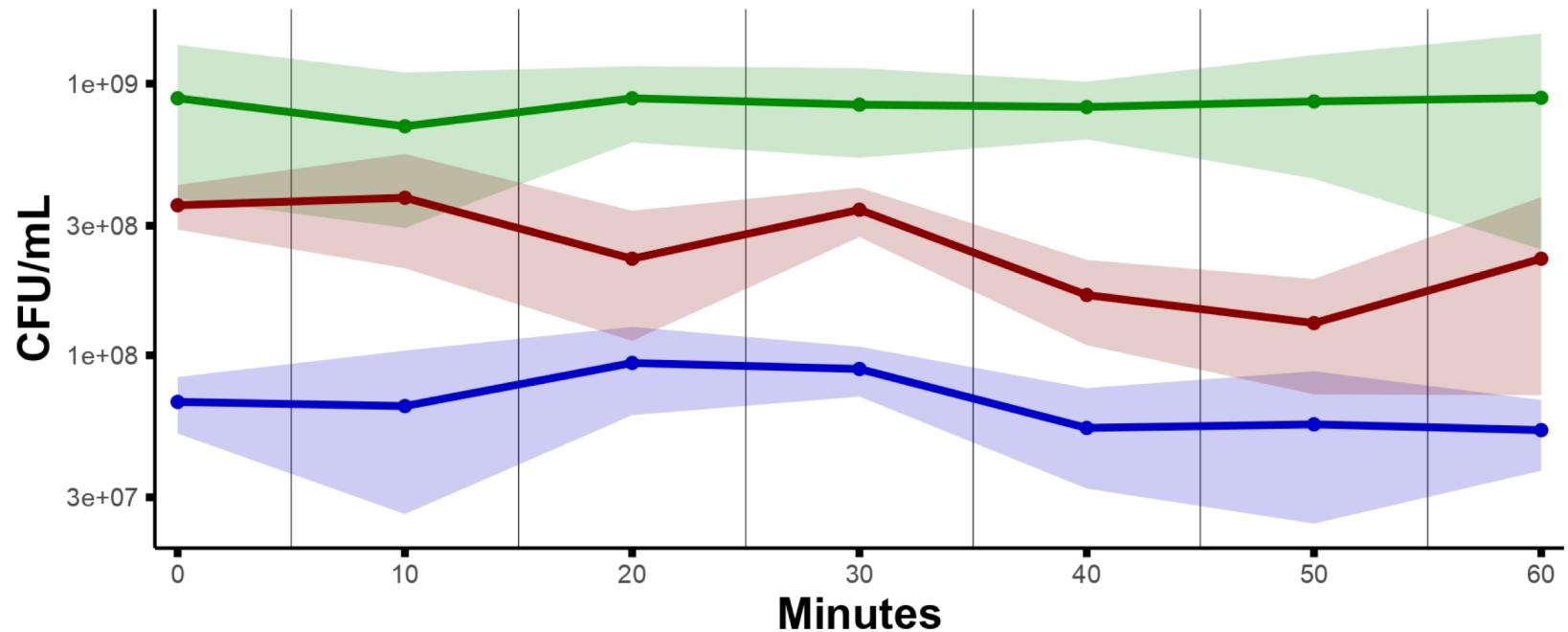


Separate bacterial species with a membrane that allows molecules to cross: bacteria will be

## Killing (10 min) followed by stability d



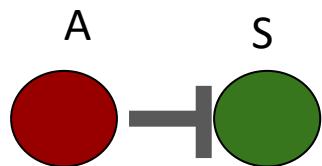
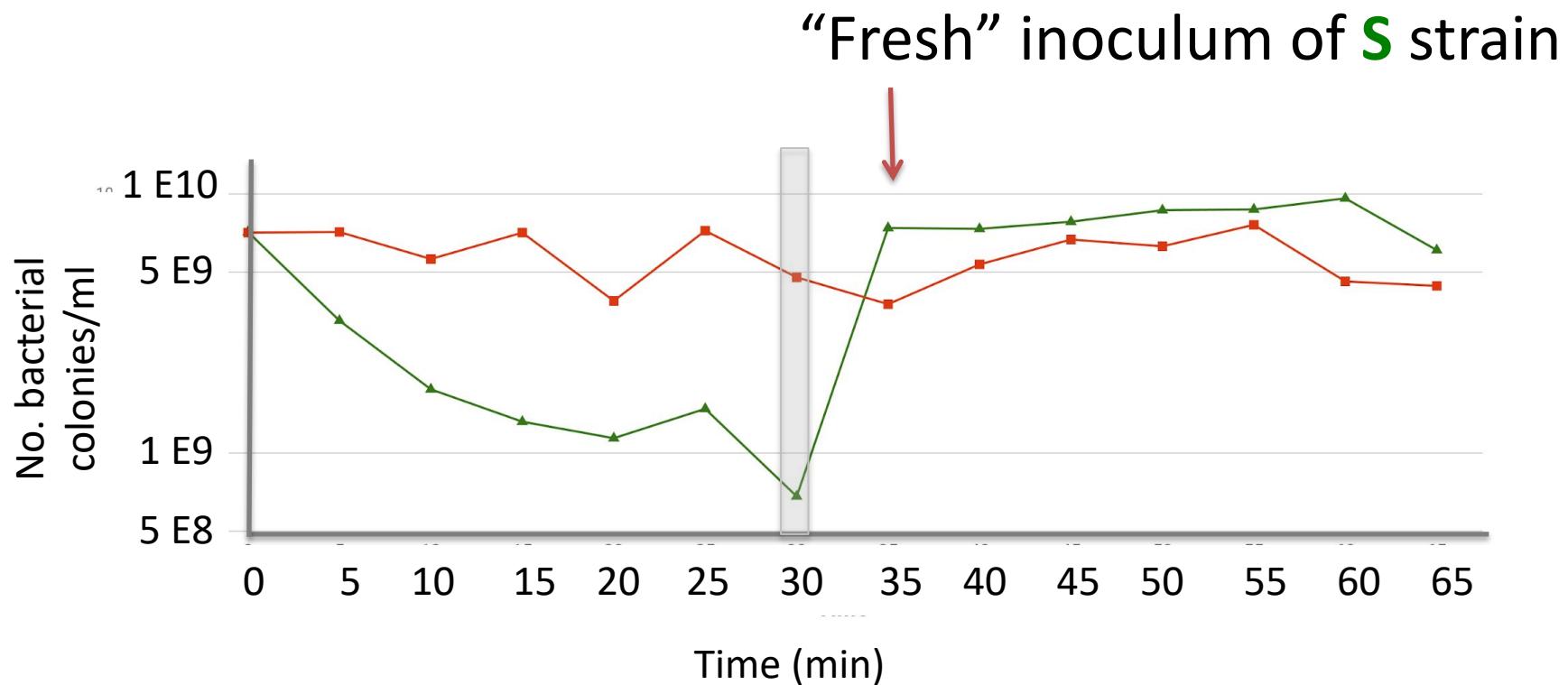
In a triple interactions **S** is no longer killed



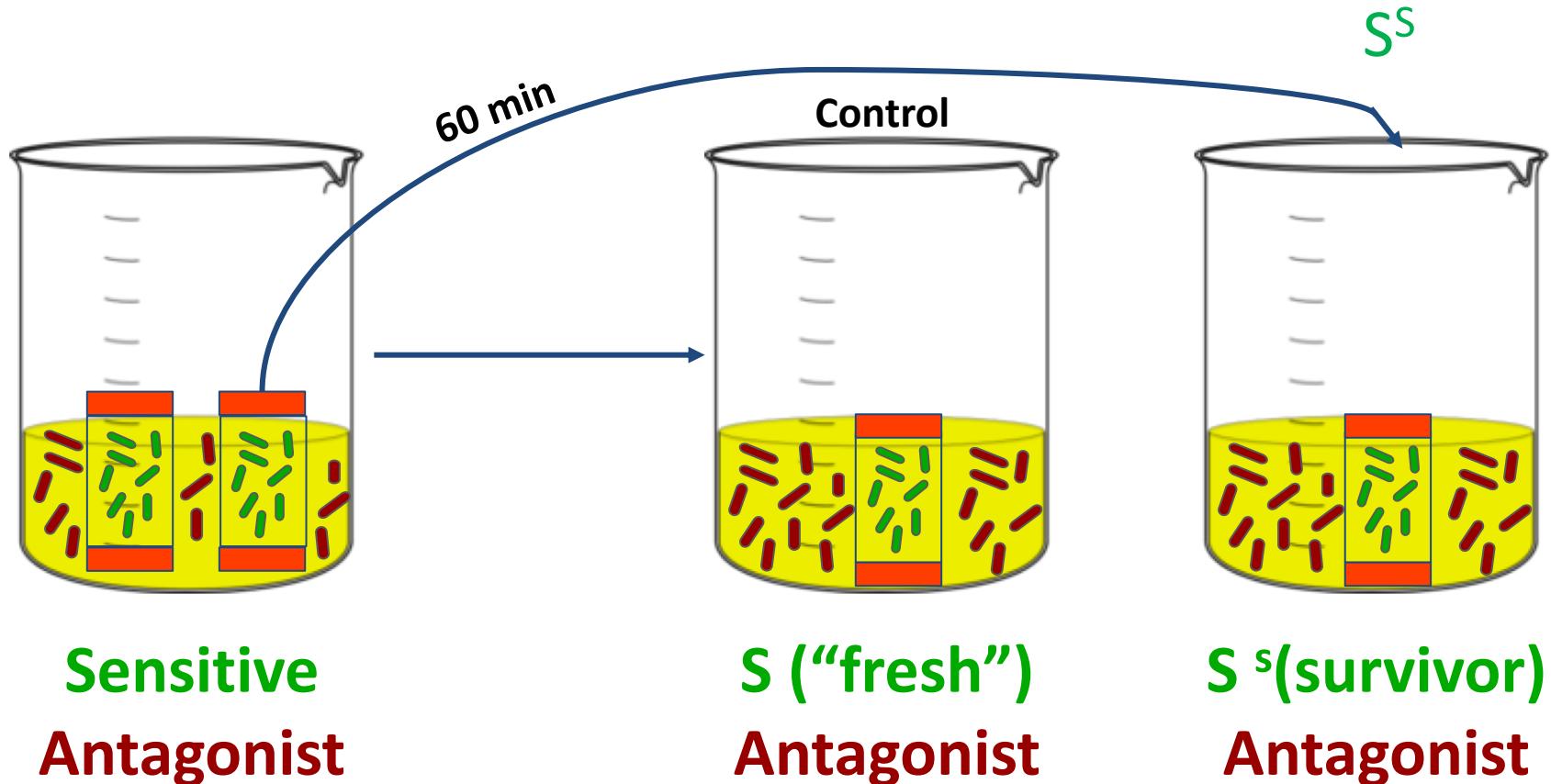
### Strain

- *B. cereus* 111 "out"
- *B. horikoshii* 20a "in"
- *B. pumilus* 145 "in"

# Emergent property 2: Stability in the S X A interaction

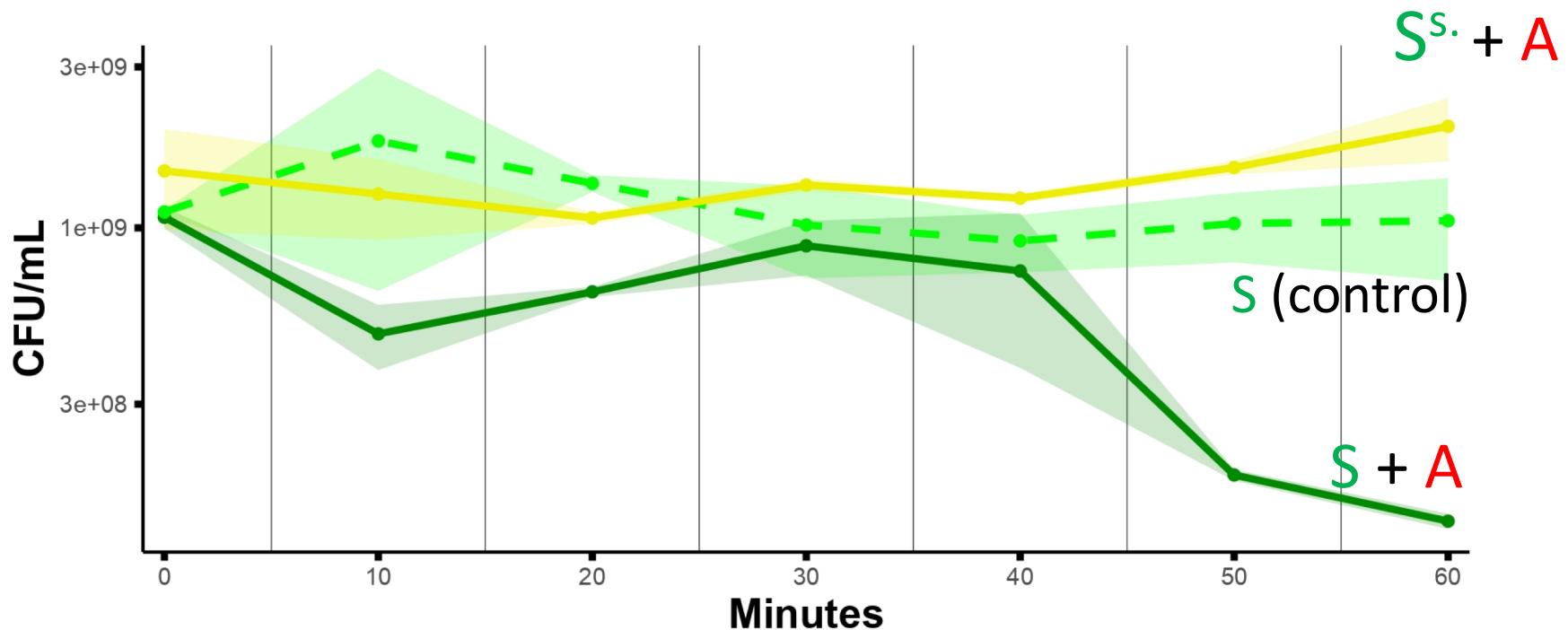


# surviving $S^S$ is transferred to Antagonist



(we know that 75%  
survive  $S^S$ )

## Emergent property 3: $S^S$ cells can tolerate A

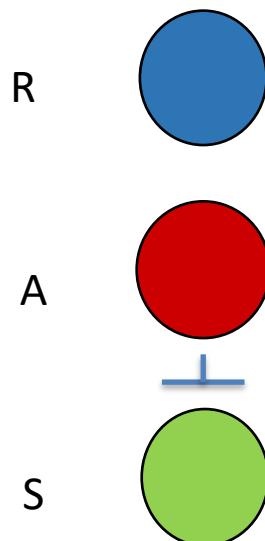


### Strain

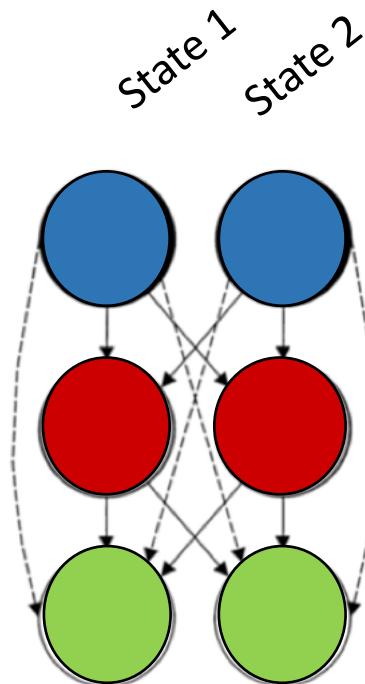
- $B. horikoshii$  20a "in"
- $B. horikoshii$  20a ctrl
- $B. horikoshii$  20a survivor

# Complexity of the study of communities

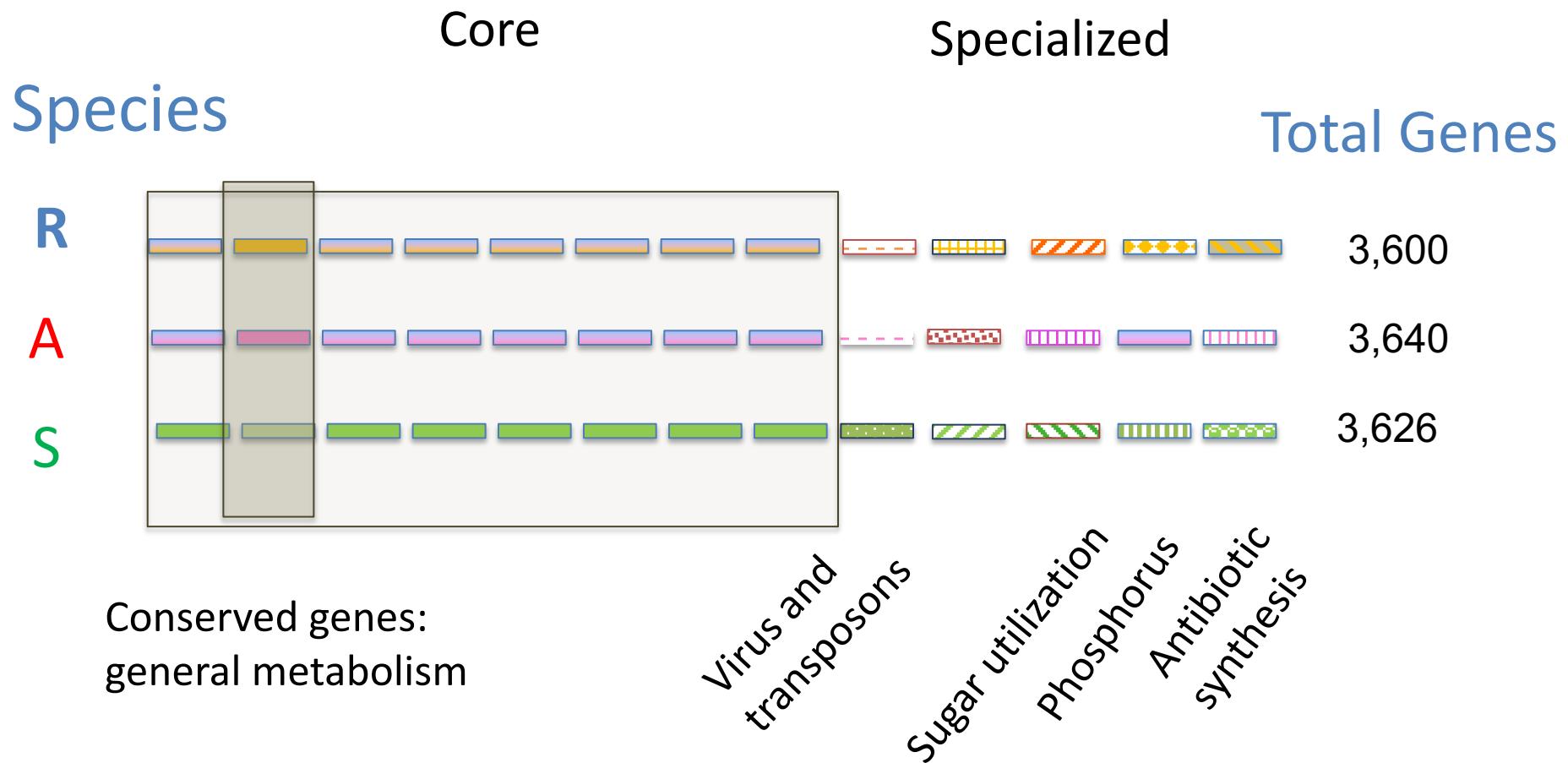
This was the model



This is now the model



# Bacterial diversity: count species and genes



# Genes can be “ON or “OFF”

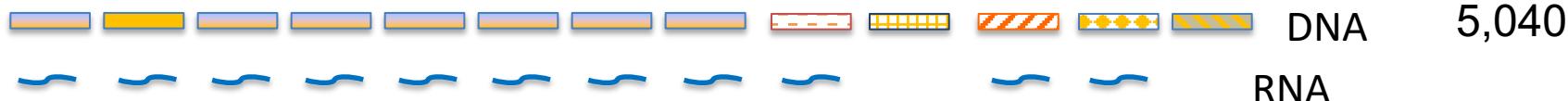
## RNA means ON genes, reflecting ecological importance

Species

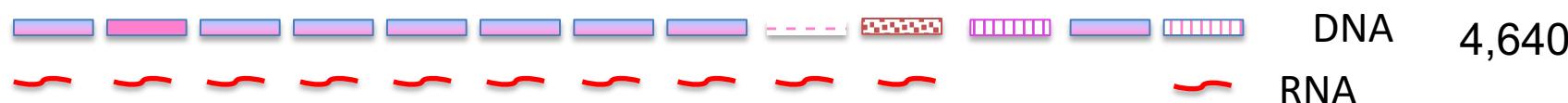
Core

Specialized

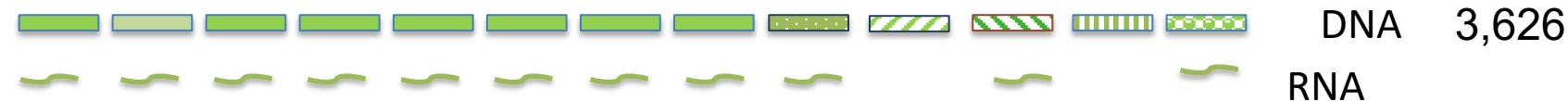
R



A



S

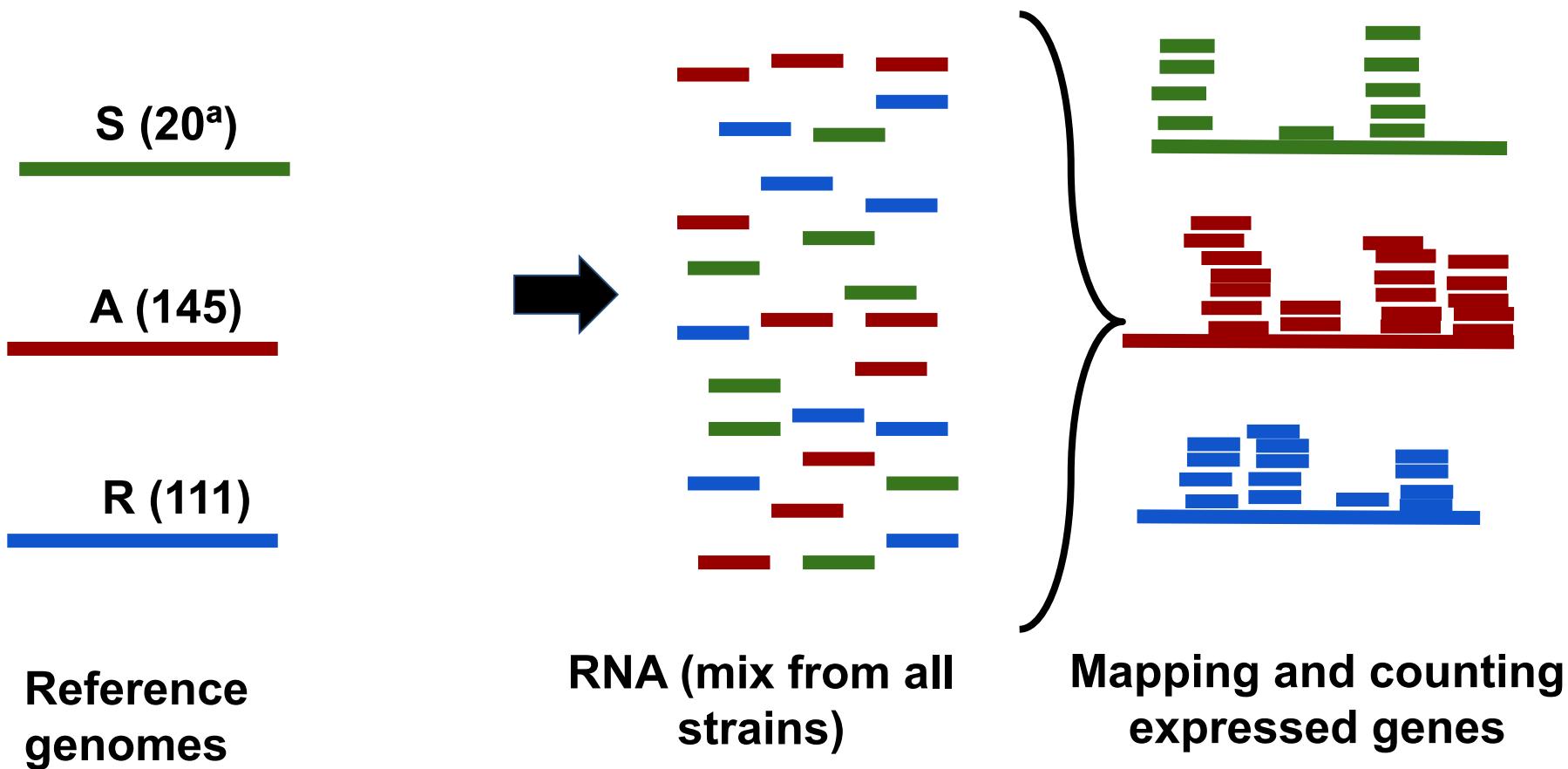


?

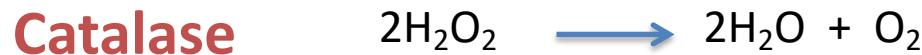
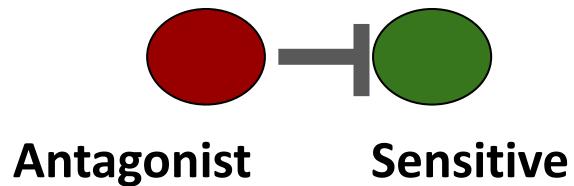
?

?

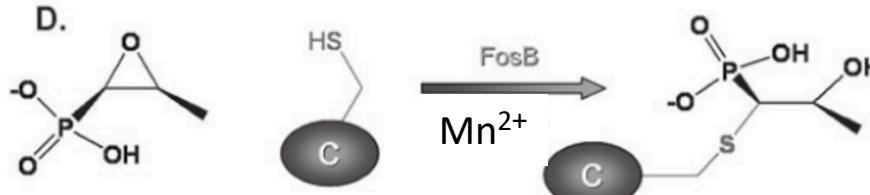
# Map the RNA immediate response (5 min) of the interactions



# Sensitive strain response to antagonism after 5 min: Desintoxication response!



FosB



Inactivate antibiotics

Fosfomycin ring is opened and the antibiotic is inactivated

Addition of a thiol cofactor to fosfomycin. L-cysteine is probably the physiological thiol donor.

## Perspectives

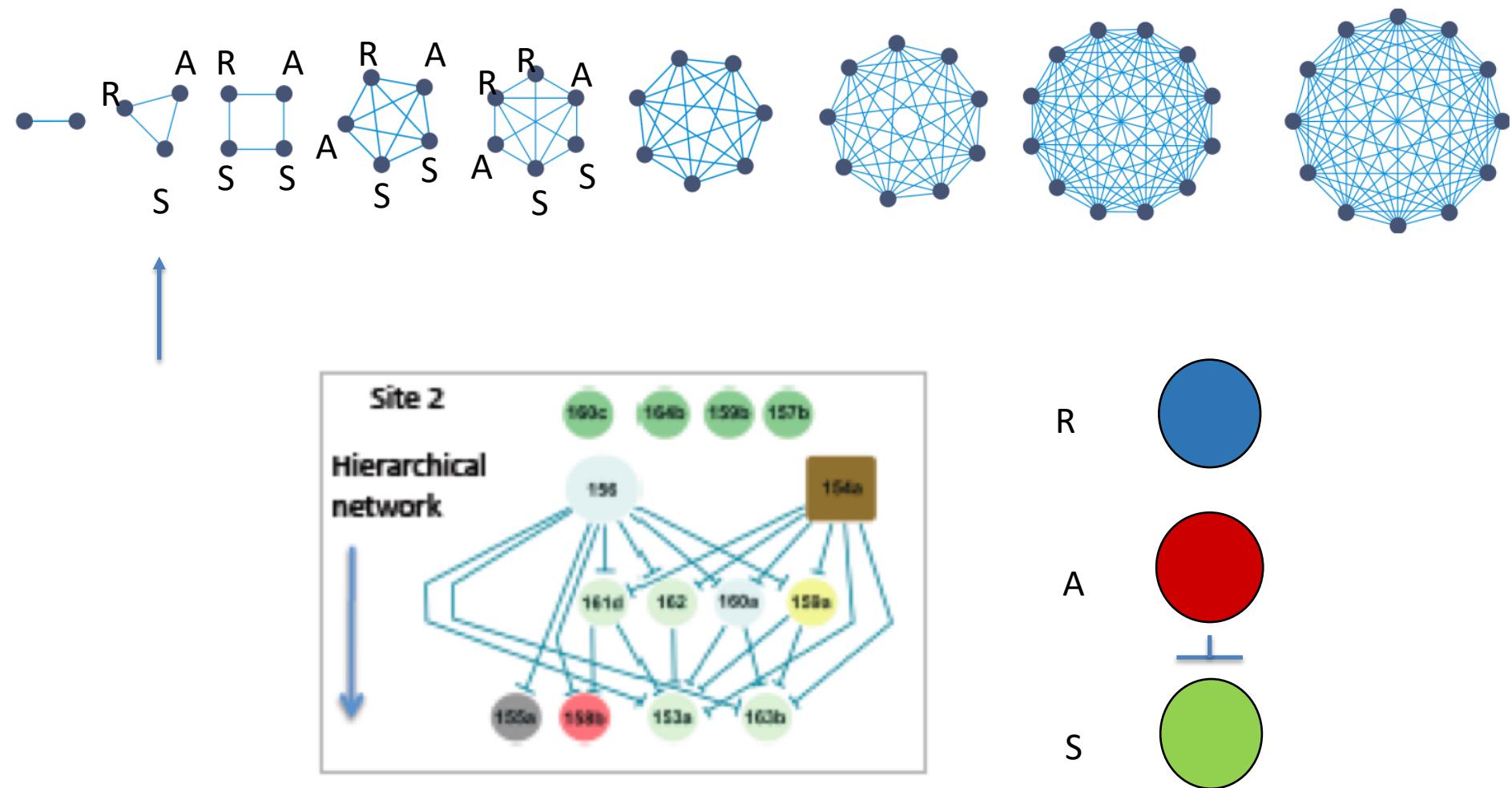
Understanding of microbial competition will allow us to better predict the behaviour of bacteria in microbial communities (industrial, environmental, and medical purposes)

Identification of functional traits important for microbial community assembly and ecosystem functioning

Explore competition in a combined nutrient limiting-antibiotic interaction

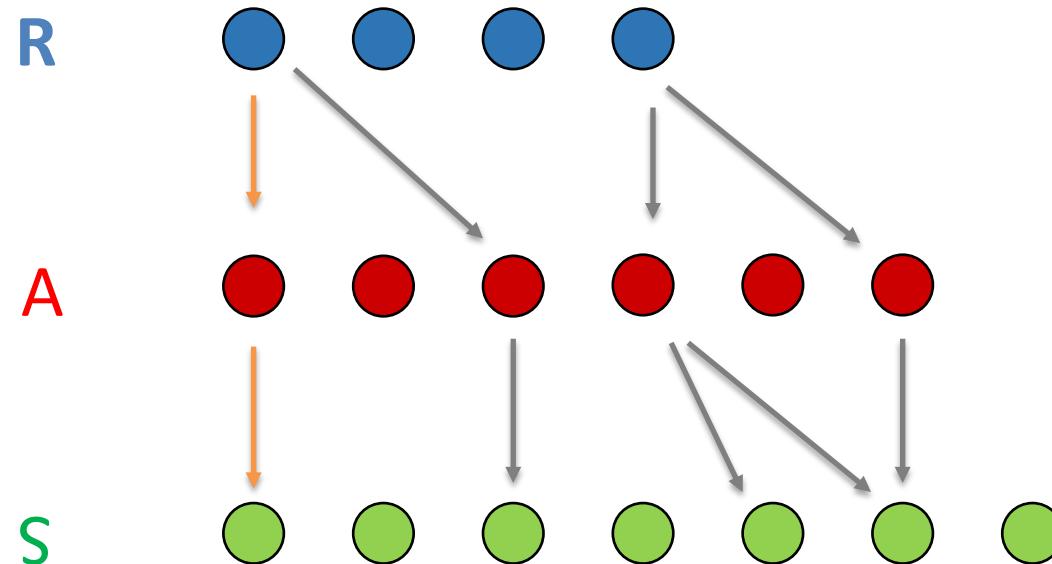
Determining when and under which conditions a microbial community will be more sensitive to stochastic processes

# Is stability maintained adding more strains...how many?



# Can stable combinations be predicted?

Species



# Cuatrociénegas, an endangered ecosystem



Feb 2011



Oct 2011

What happened to the water at the Churince?

# Archean domes, more to explore about ancient life



# Science for high school students

Molecular Biology laboratory  
Inauguration at the  
Cuatrocienegas high school



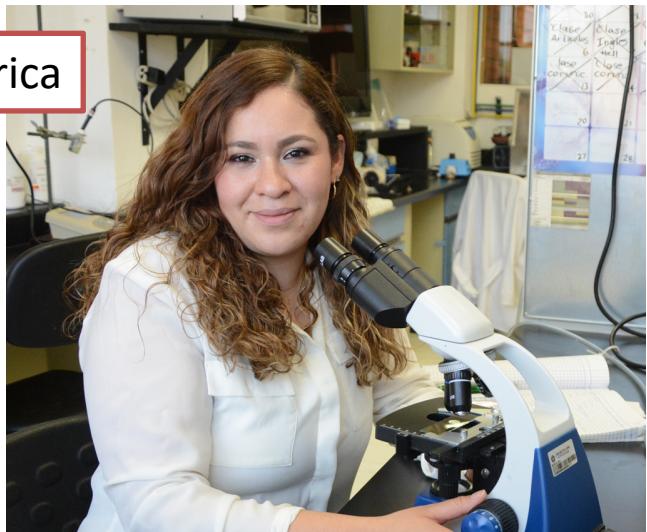
Since 2012  
Anual workshops

Taller Ciencia Viva at  
CINVESTAV and at  
Cuatrocienegas

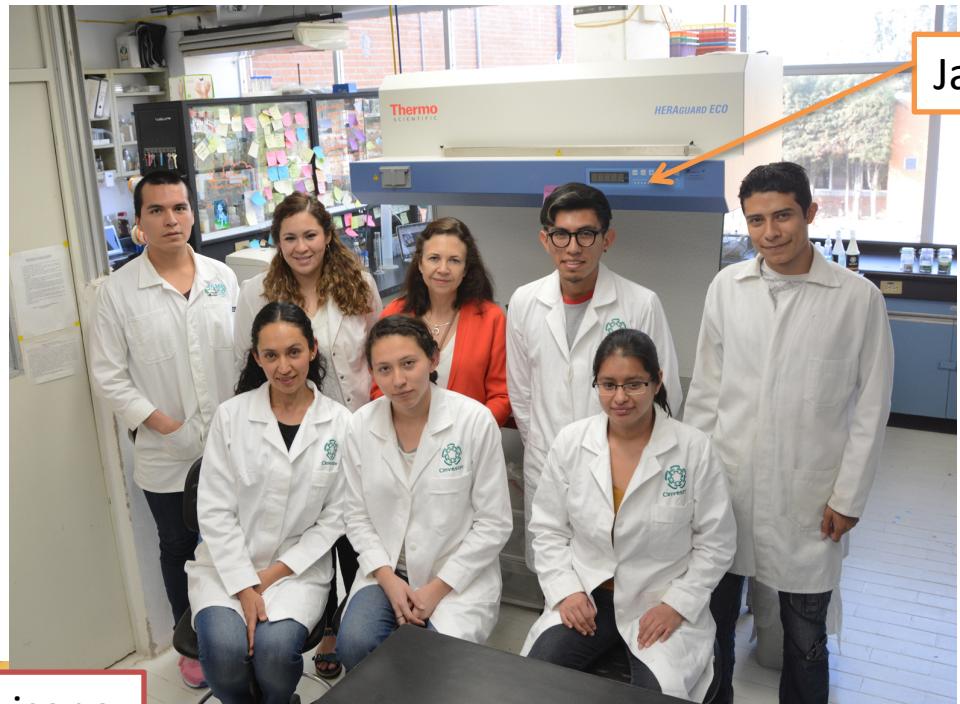


Anual workshops since 2003

Africa



Jaime



Valeria Souza

Mike Travisano



Bernardo





Cinvestav Irapuato



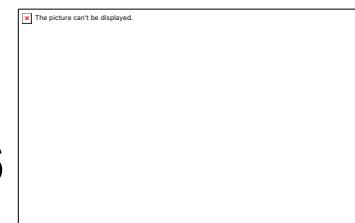
**Africa Islas**  
**Zulema Gómez-Lunar**  
**Ma. Dolores Torres**  
Diana Barceló  
Enrique Hurtado  
**Bernardo Aguilar**  
**Jaime Ortega**  
**Eugenia Zarza**  
**Román U. Zapián**  
Ismael Hernández

**UNAM**  
Instituto de Ecología  
**Valeria Souza**  
**Luis Eguiarte**  
**Luis David Alcaraz**  
UNAM CIECO  
**Felipe García**  
ENES Morelia  
**Yunuen Tapia**

**U Minnesota:**  
**Mike Travisano**

**Wilfrid Lauriel U:**  
**Gabriel Moreno**

Multidisciplinario  
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Básica 2013  
Fronteras 2016



WWF-Alianza Carlos  
Slim to V. Souza