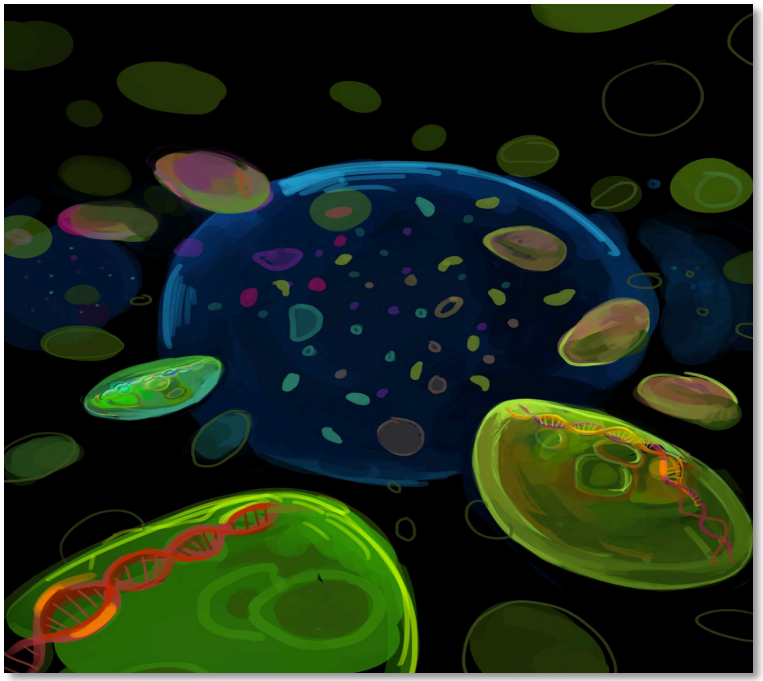
Prochlorococcus

An adventure in Microbial Oceanography (or the power of the model system approach)



Carly Sanker, MIT

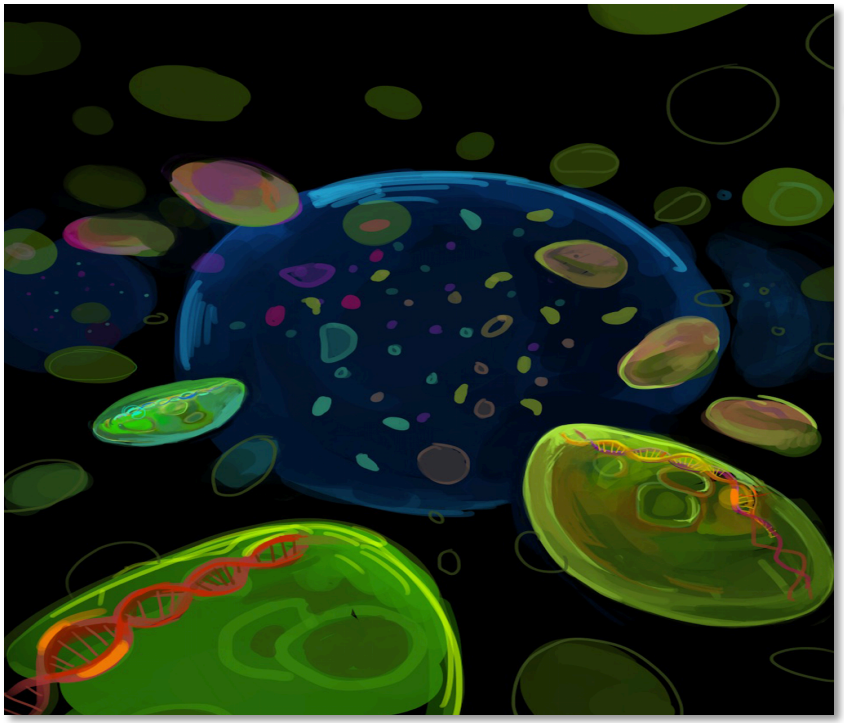
Penny Chisholm

C-MORE Summer Course

June 4, 2014

Prochlorococcus

An adventure in Microbial Oceanography (or the power of the model system approach)



Carly Sanker, MIT

A self-centered presentation!

C-MORE Summer Course

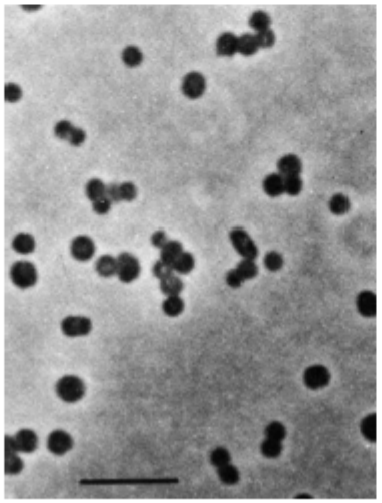
June 4, 2014

Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology

...The BIG discovery - began the paradigm shift

Waterbury *et. al.* 1979



. 1 Phase contrast photomicrograph of *Synechococcus* sp. rain Syn-48) illustrating general cell morphology (scale bar, 5.0 μm).

JOHN B. WATERBURY
STANLEY W. WATSON
ROBERT R. L. GUILLARD
LARRY E. BRAND

Department of Biology,
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Woods Hole, Massachusetts 02543

Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

IN marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms[1–3]. We report here the widespread occurrence of a small, marine, chroococcalean cyanobacterium belonging to the genus *Synechococcus*.

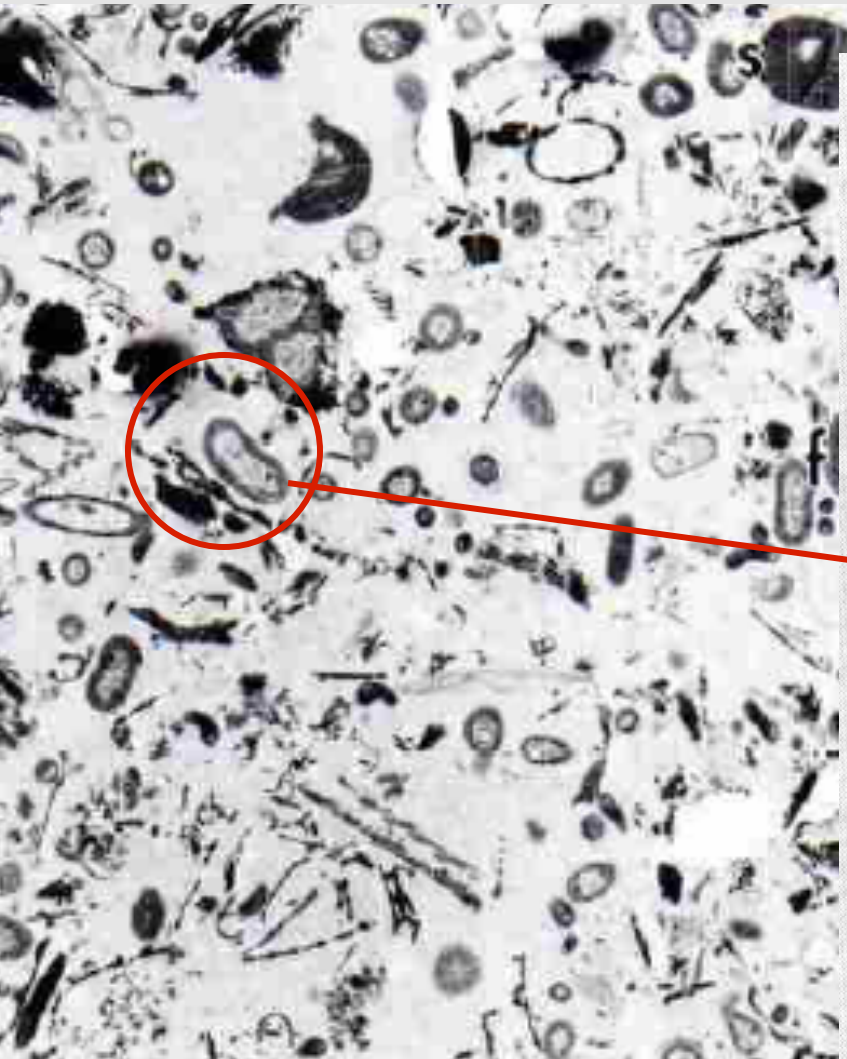
Natural water samples were filtered through 0.2 μm Nuclepore filters, counterstained with Irgalan black[4]. The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450–500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyanobacteria fluoresce orange and can be distinguished from phytoplankters that fluoresce red.

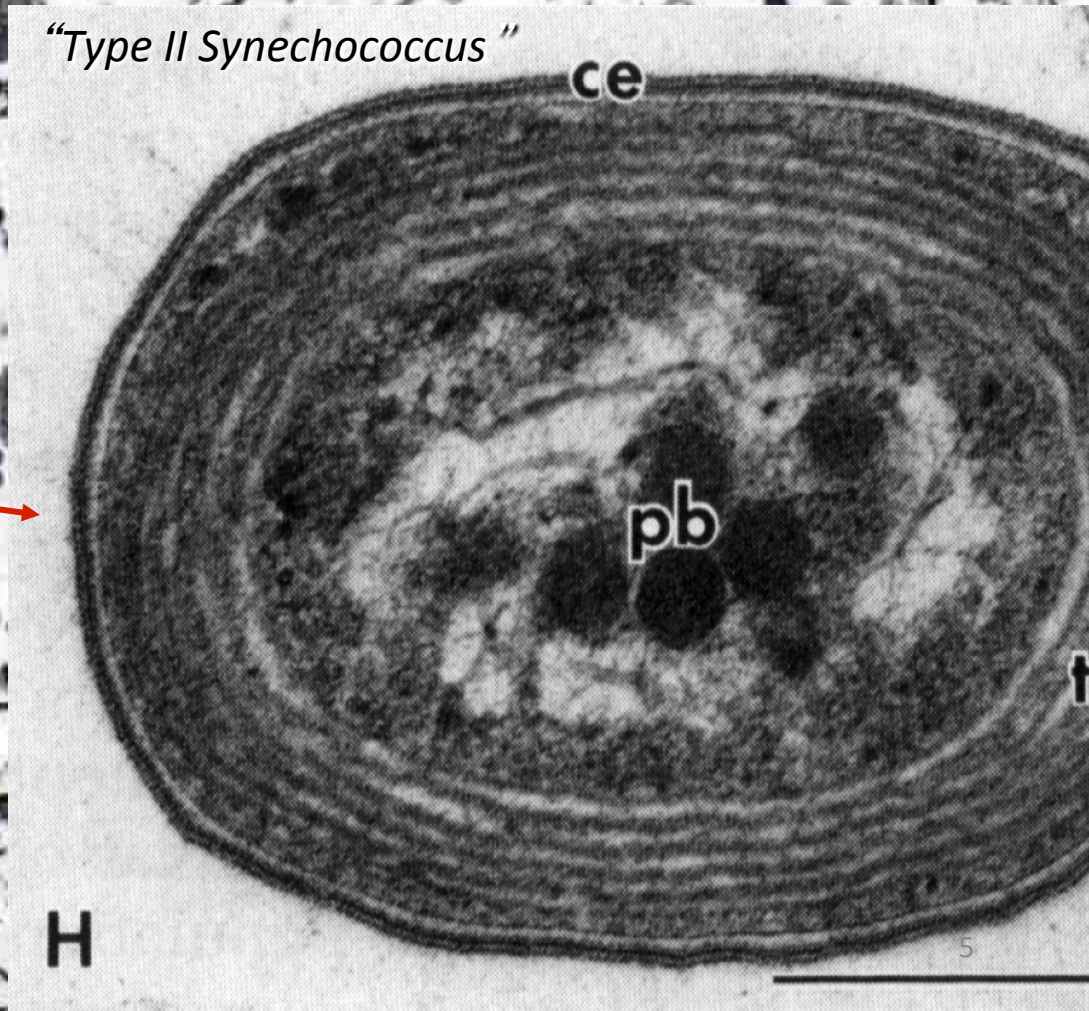
Phycoerythrin-rich unicellular cyanobacteria were observed at seven stations in the Arabian Sea in January 1977, at three stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor. In the relatively rich waters of the Arabian Sea and off the coast of Peru, the population varied from 10^4 to 10^5 cells ml^{-1} within the euphotic zone (Table 1). The greatest number of cells was found within the top 20 m of the water column, with occasional cells being observed as deep as 400 m. In contrast, the surface sample collected from Slope Water north

Johnson and Seiburth 1979

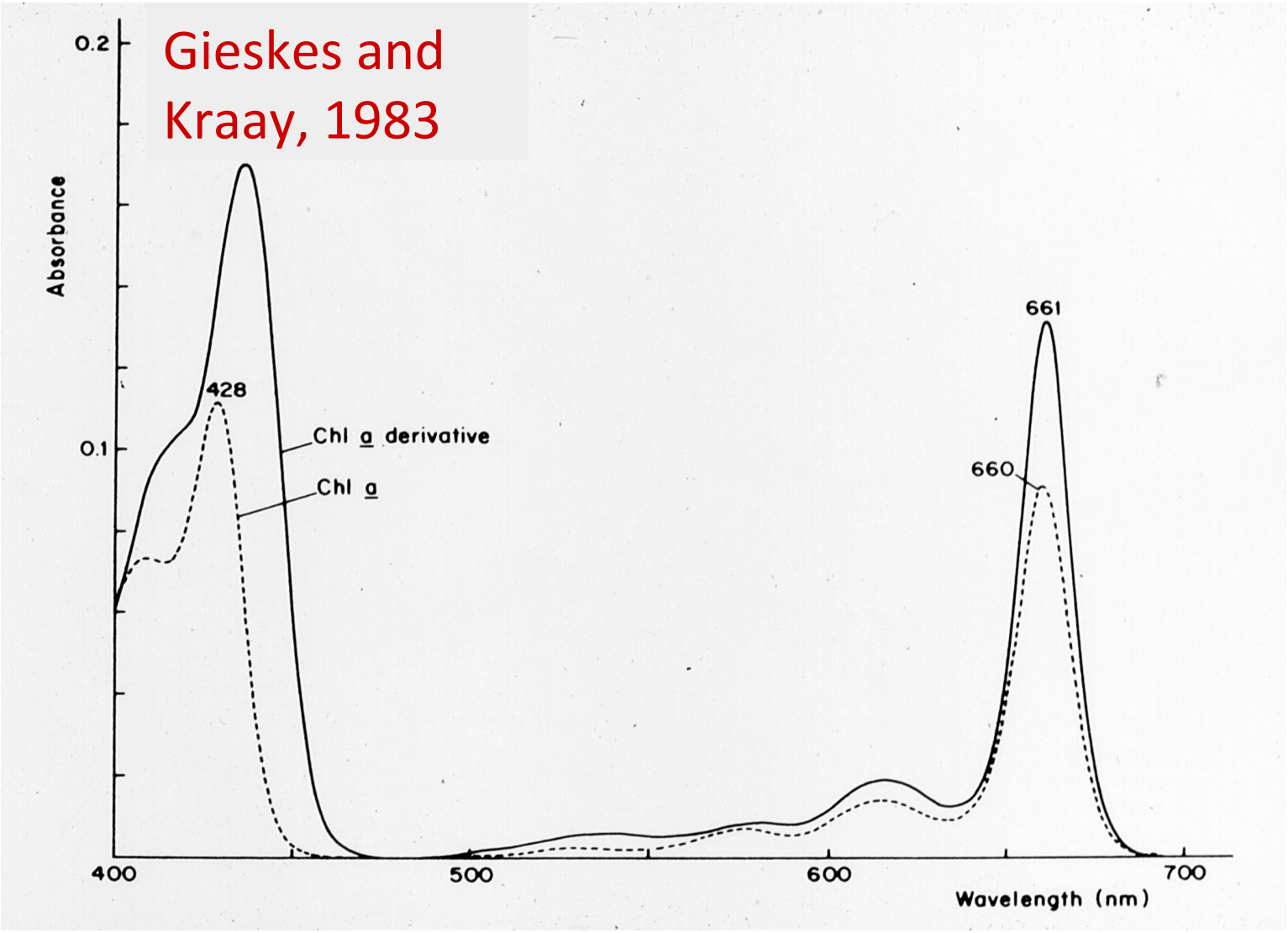
Chroococcoid cyanobacteria in the sea:
A ubiquitous and diverse phototrophic biomass.
Limnology and Oceanography 24(5):928-935.

That same year…

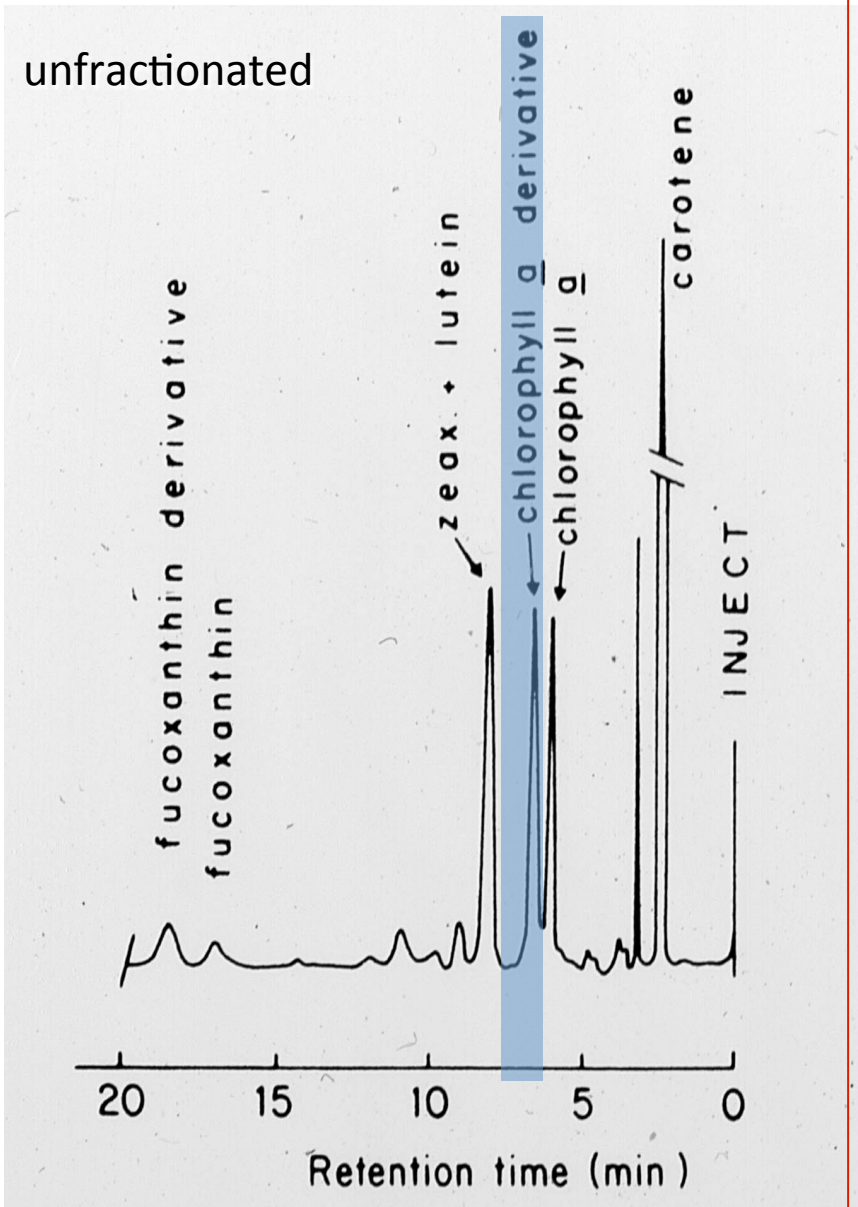


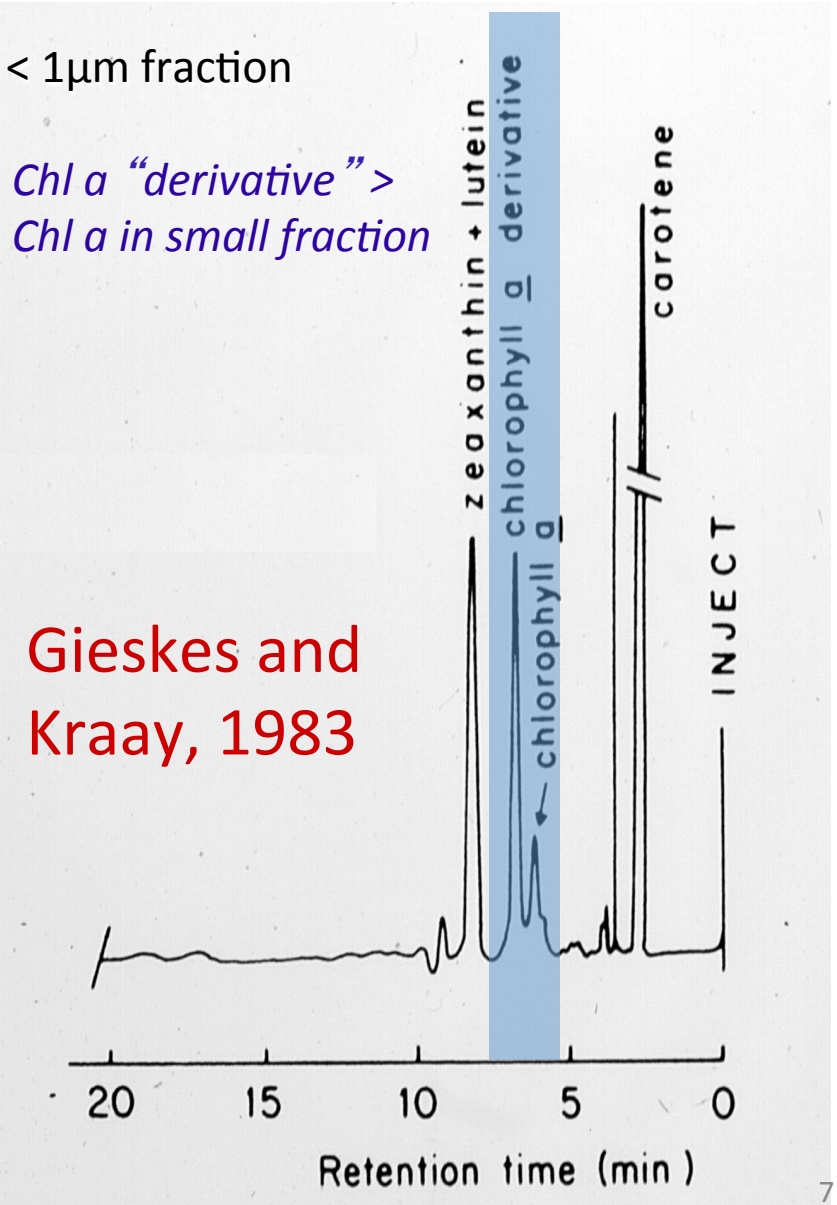


Meanwhile, a few years later, a new pigment was discovered…



And that pigment is enriched in the < 1µm fraction

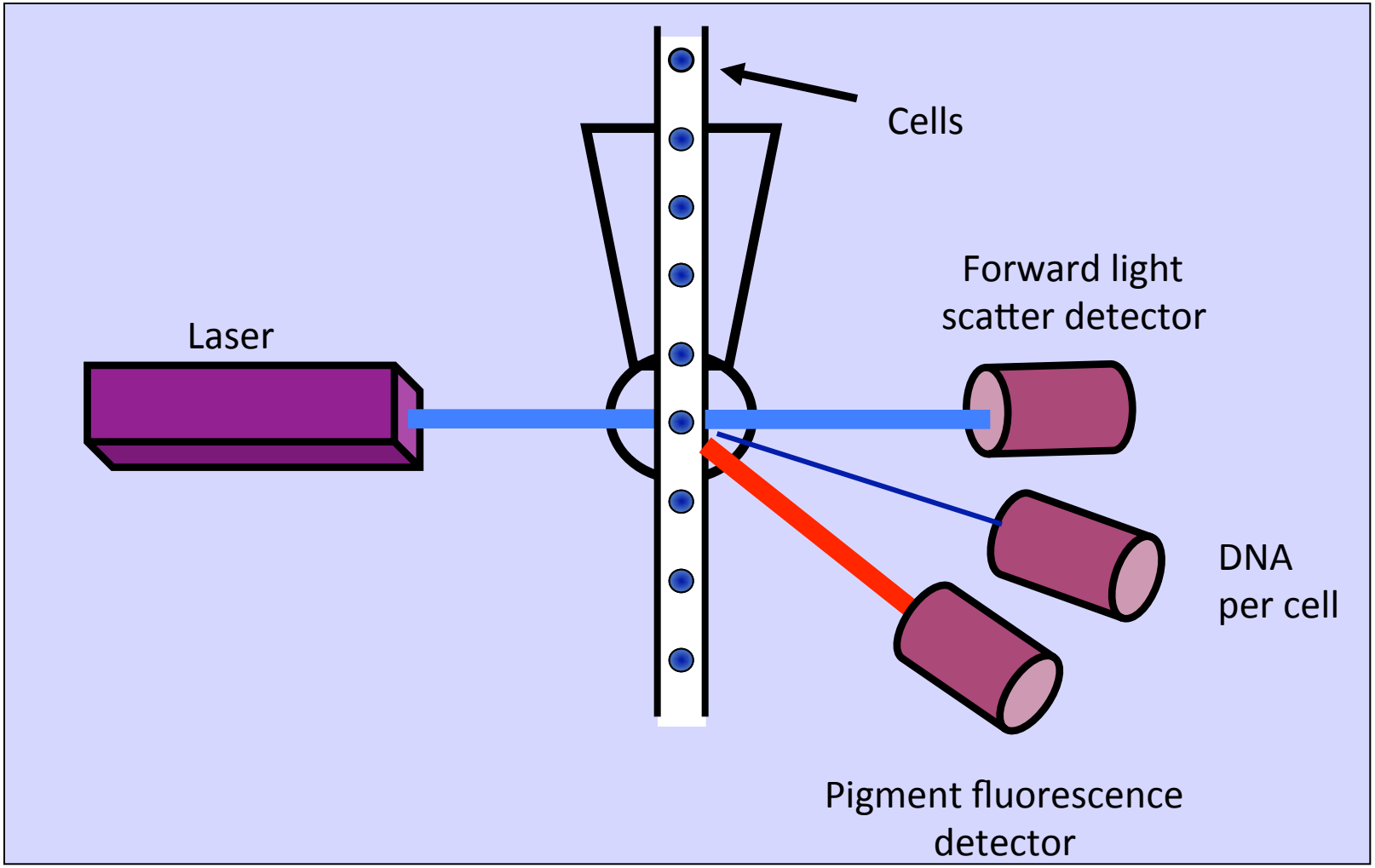




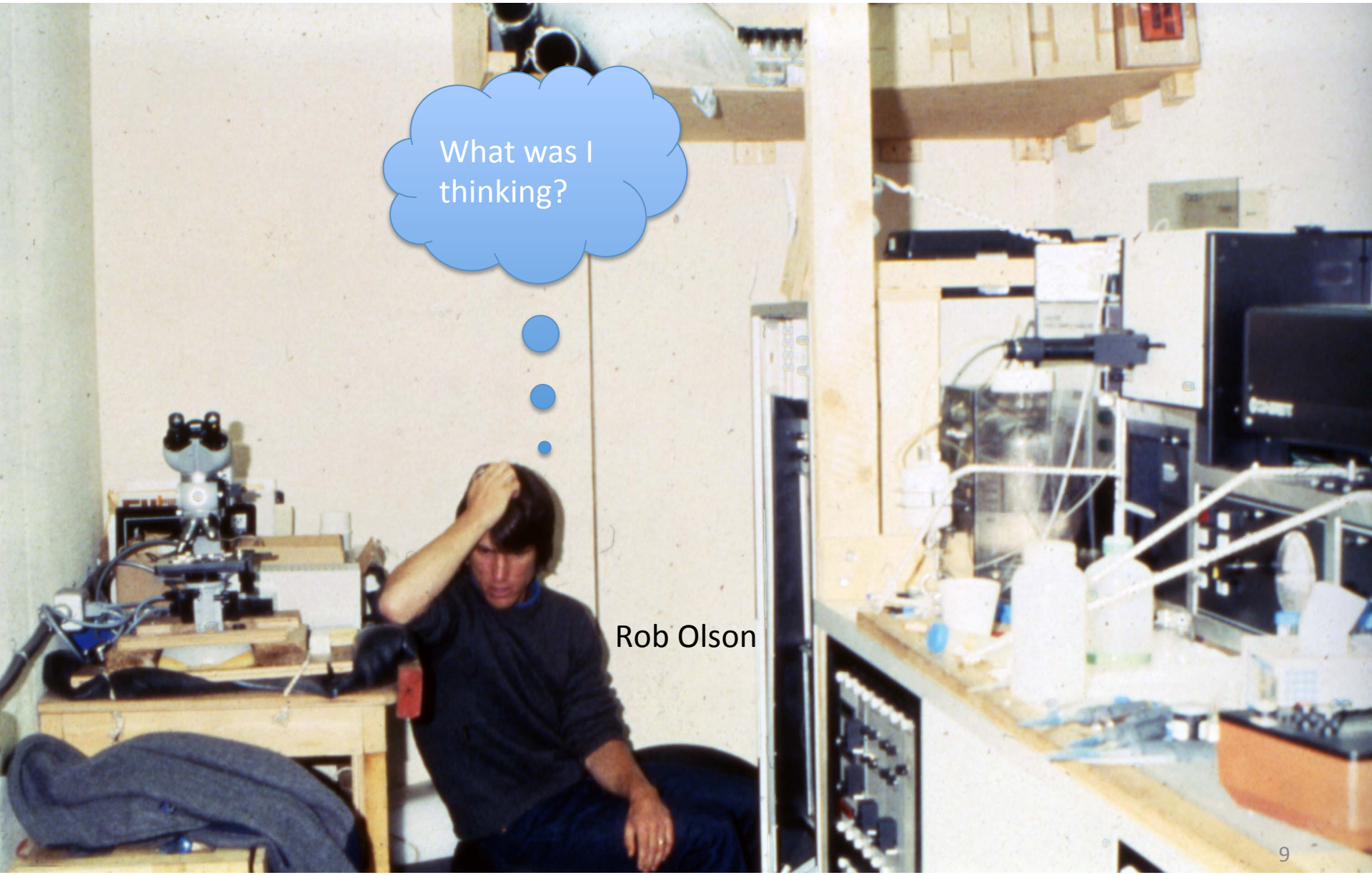
Gieskes and Kraay, 1983

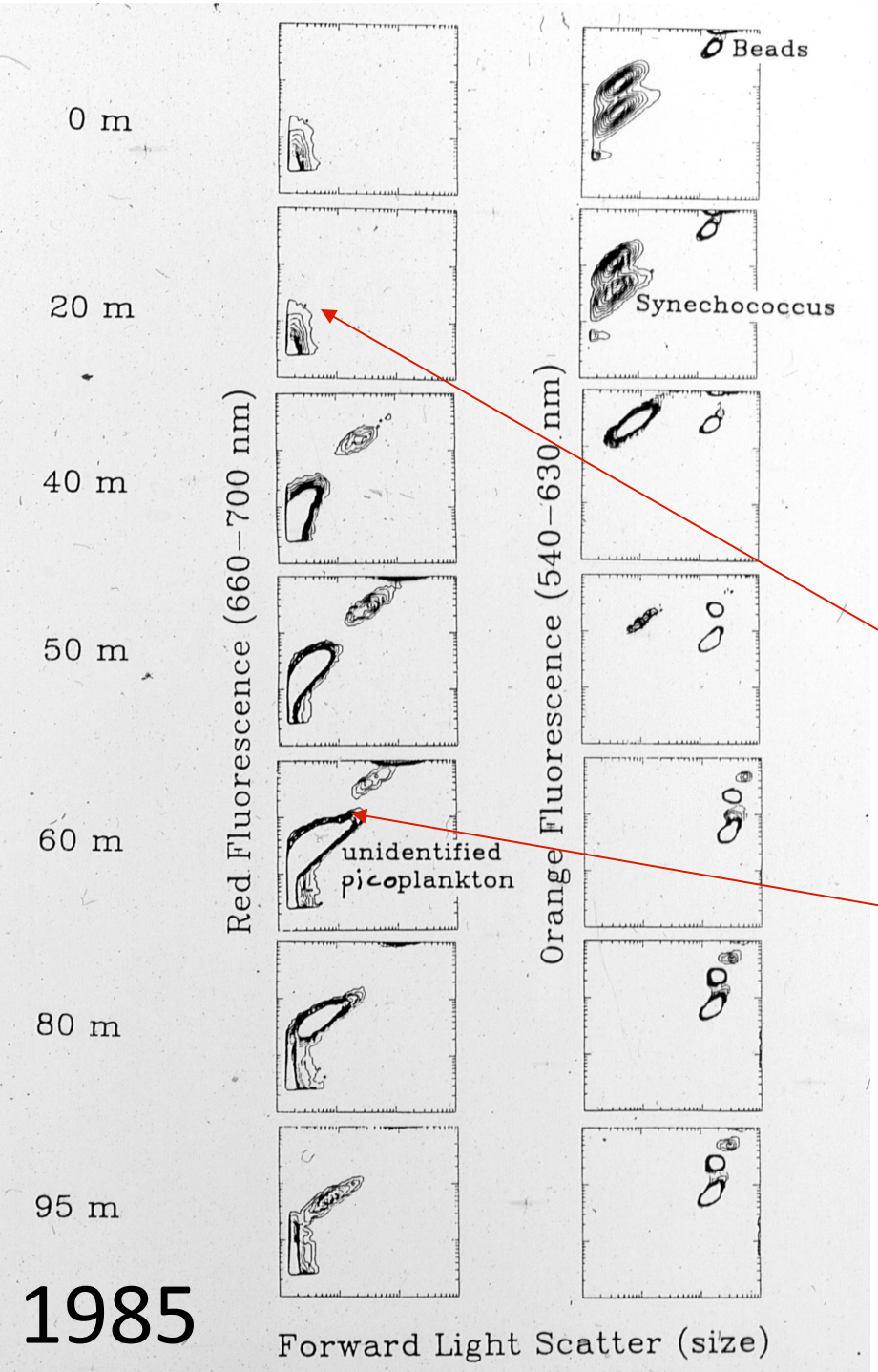
Enter Flow Cytometry…..

...for the study of single cells



And someone with the courage to take it to sea…





We set out to study *Synechococcus*, and noticed something else…

The noise

Emerging from the noise!

Based on their fluorescence excitation/emission spectrum - suspected chl b, typical of green algae.
Called them "**Little Greens**"

Photo by R. Olson

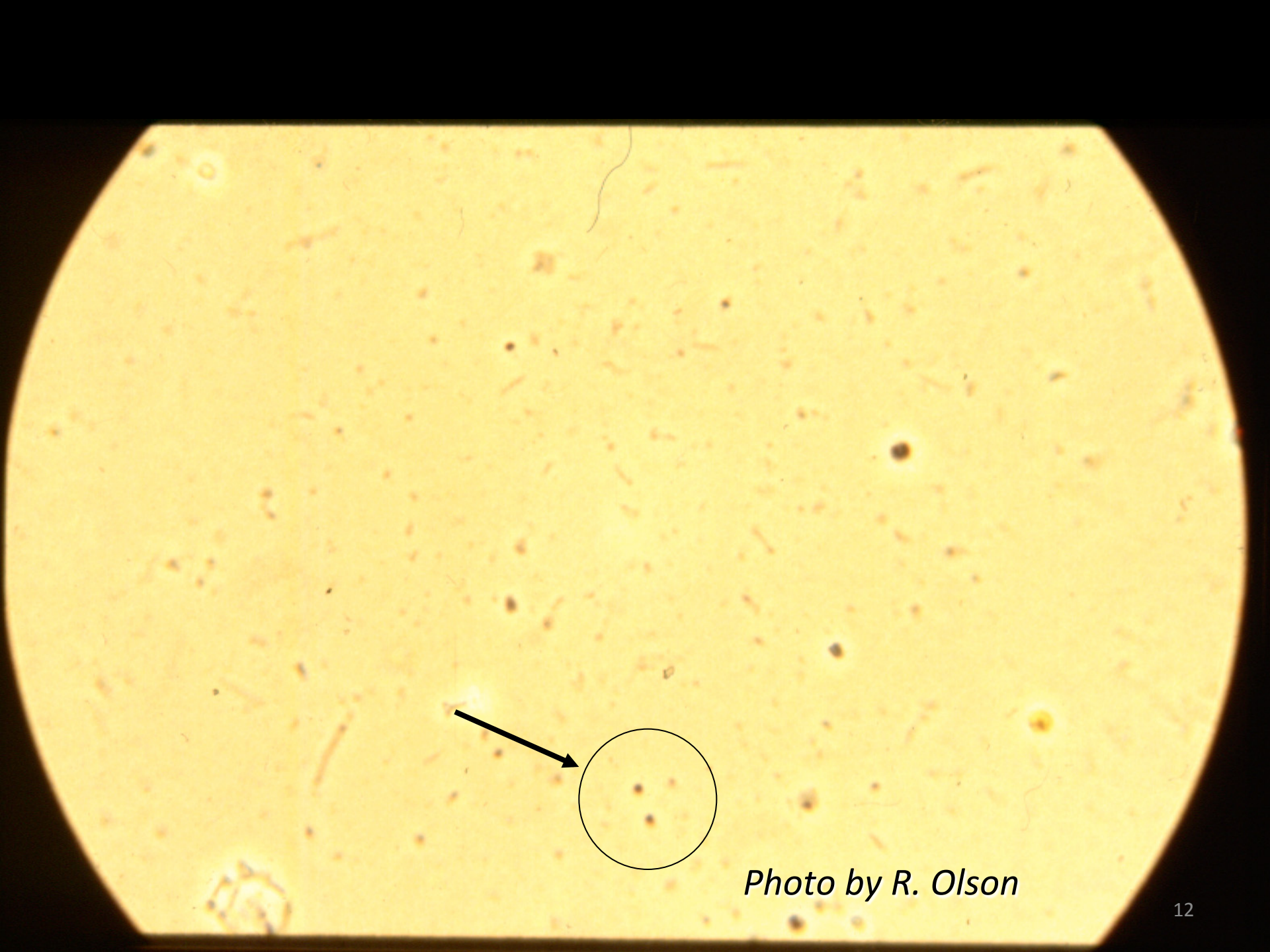
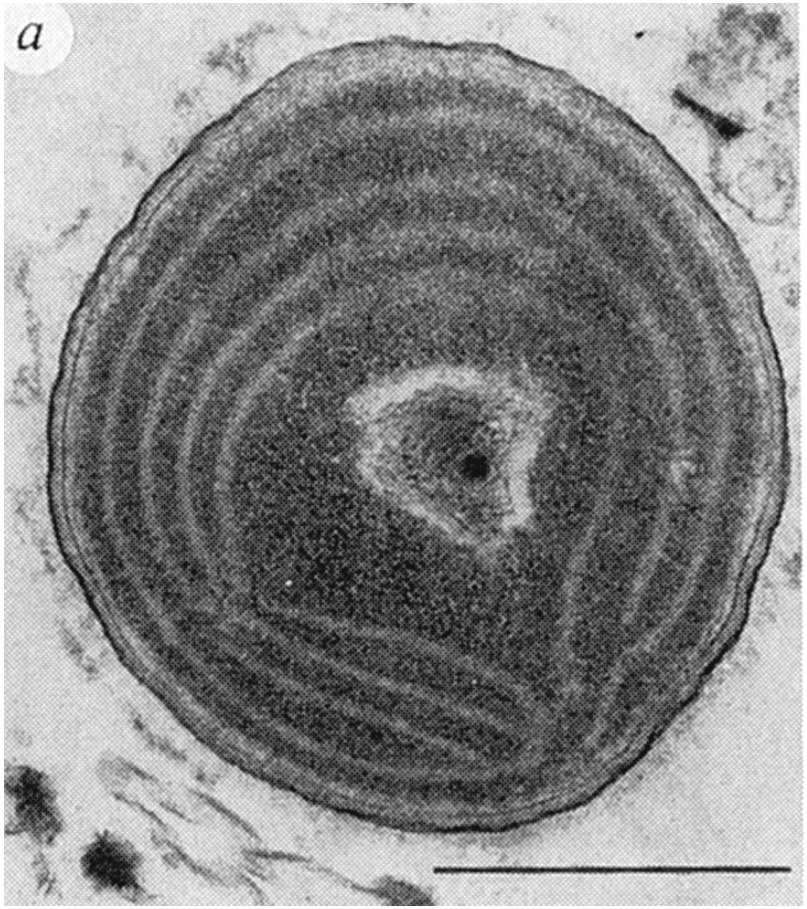


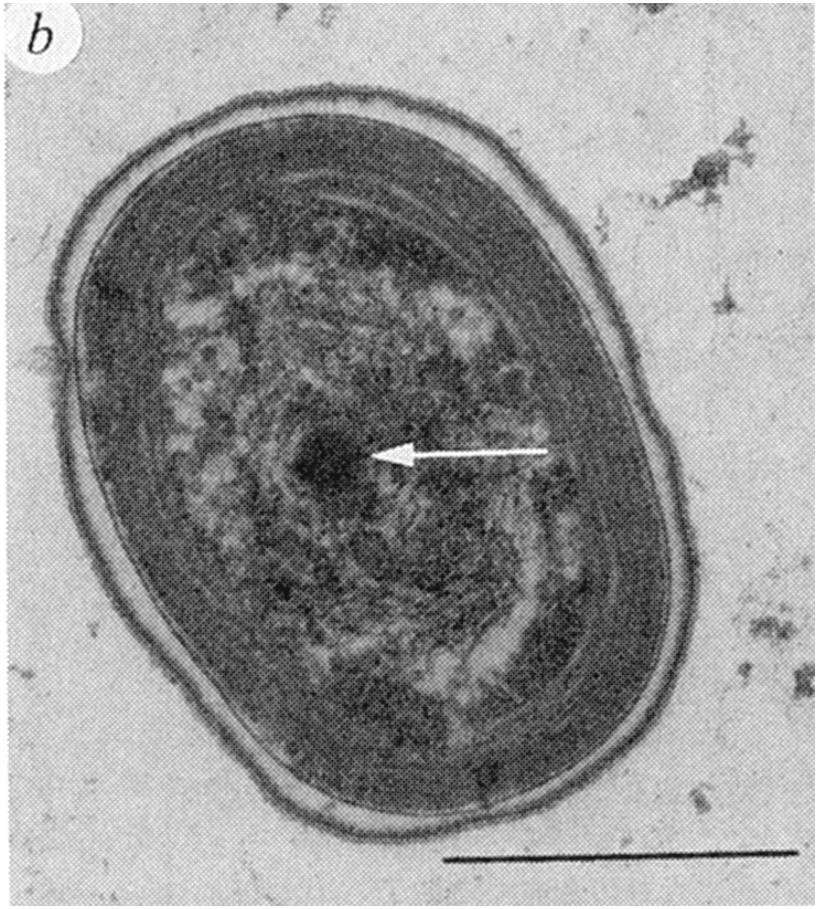
Photo by R. Olson

Finally, and electron micrograph – It's a prokaryote!

Synechococcus



"Little Greens"

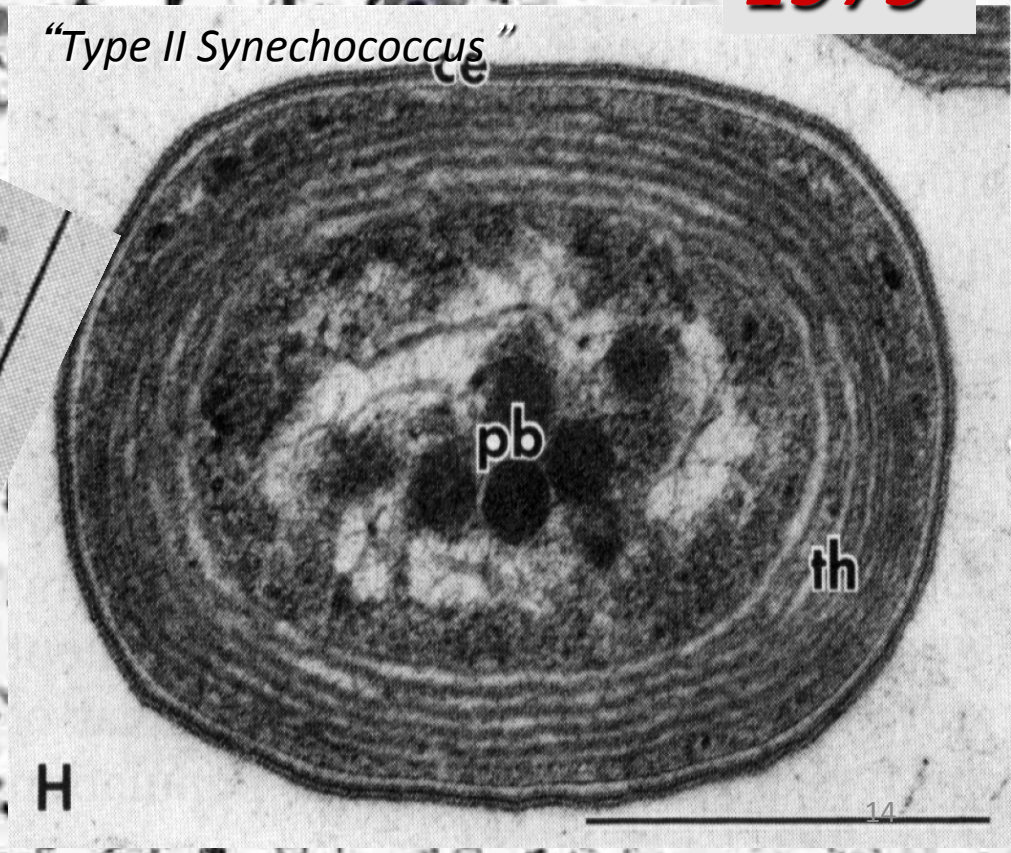


John Waterbury

CLOSURE I: Johnson and Sieburth's *"Type II Synechococcus" was the same as our cells*

1985

1979



What is their pigment composition?

Divinyl Chl

Ralph Goericke

HPLC pigment analysis of unidentified picoplankton

	0.8 μm filtrate (〉95% pure)	FCM sort
chlorophyll a_1	2.0 fg cell^{-1}	present
chlorophyll b_1	2.7 fg cell^{-1}	present
zeaxanthin	0.6 fg cell^{-1}	
α carotene	0.3 fg cell^{-1}	
lutein	not detected	
chlorophyll a_2	not detected	
β carotene	not detected	

= divinyl chl a

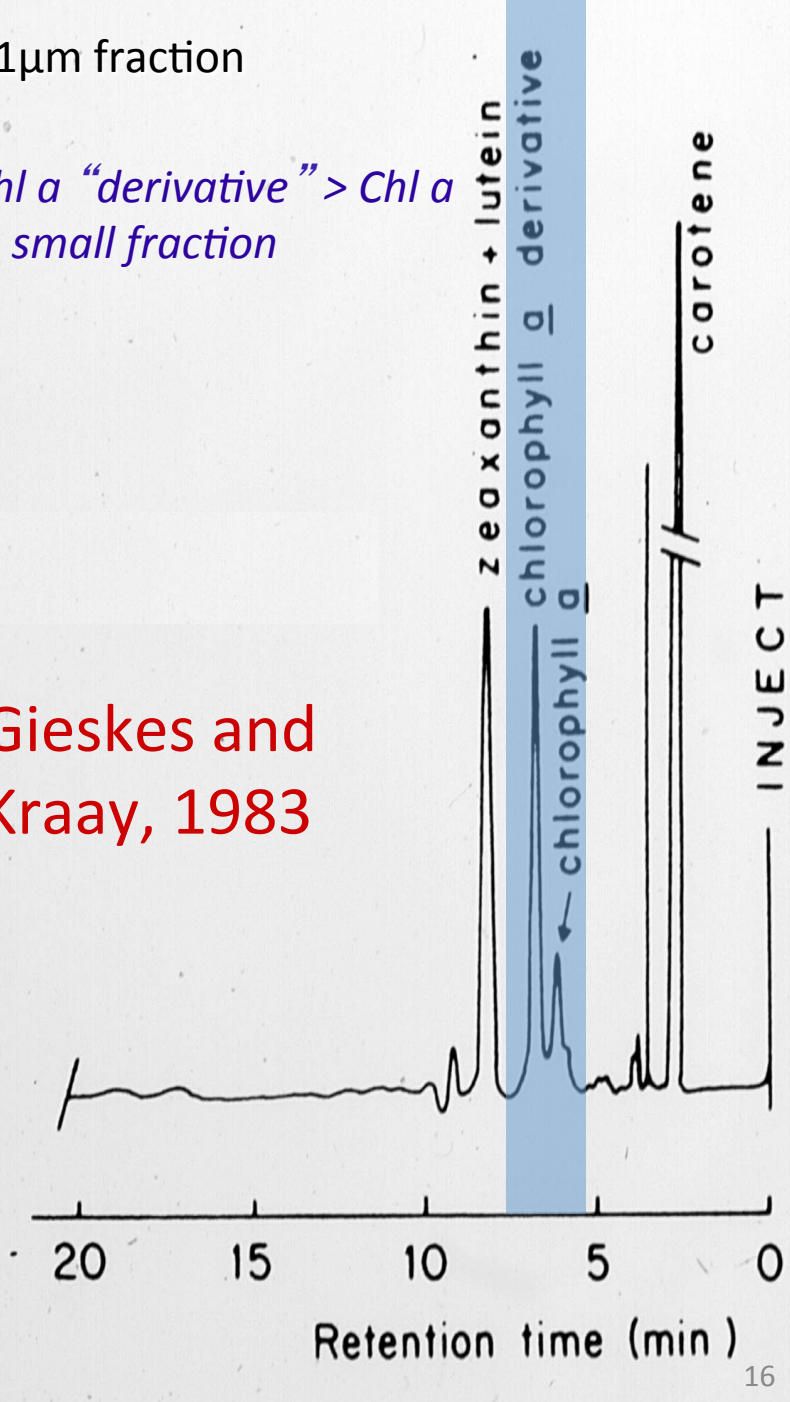
= divinyl chl b

CLOSURE II:

Gieskes and Kraay's "chlorophyll *a* derivative" was the divinyl chlorophyll *a* of the "Little Greens"

< 1µm fraction

Chl a "derivative" > Chl a in small fraction



Gieskes and Kraay, 1983

But what do we call them?

What else is prokaryotic and has Chlorophyll b?

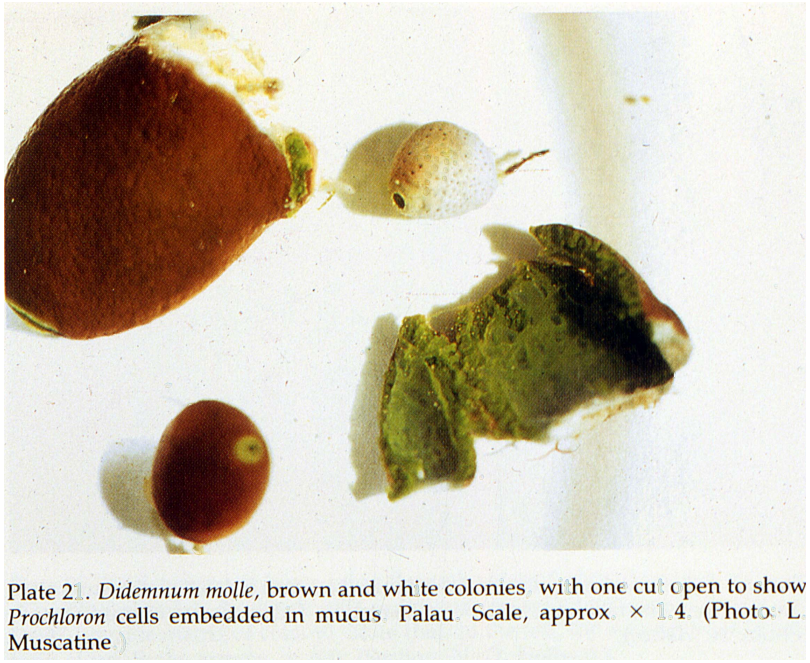
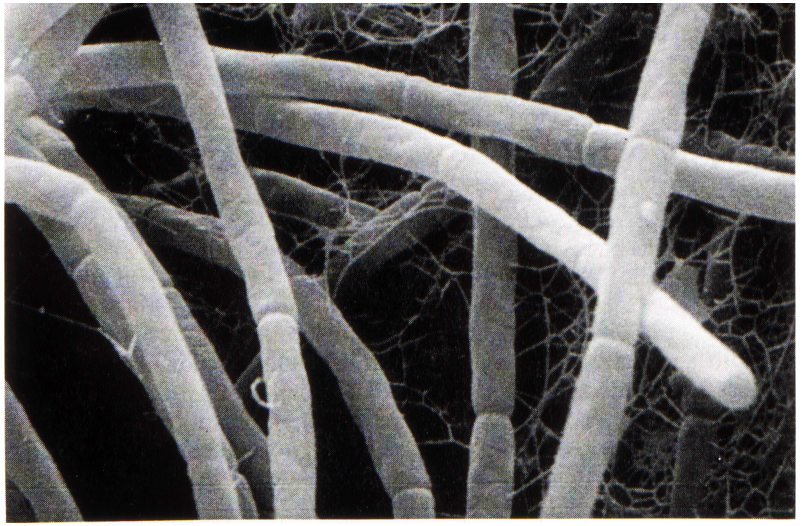


Plate 21. *Didemnum molle*, brown and white colonies with one cut open to show *Prochloron* cells embedded in mucus. Palau. Scale, approx. × 1.4. (Photo: L. Muscatine.)

Prochloron



Prochlorothrix

So we called them "Prochlorophytes"

1988: The Birth Announcement

A novel free-living prochlorophyte abundant in the oceanic euphotic zone

Sallie W. Chisholm, Robert J. Olson*, Erik R. Zettler*, Ralf Goericke†, John B. Waterbury* & Nicholas A. Welschmeyer†

48-425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
* Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
† Harvard University, Cambridge, Massachusetts 02138, USA

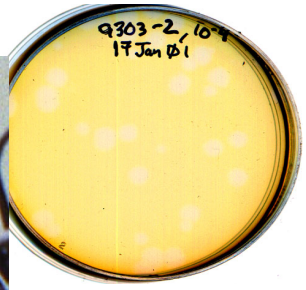
1992 Cultures

Brian Palenik

John Waterbury

Freddie Valois

Sometime later…..



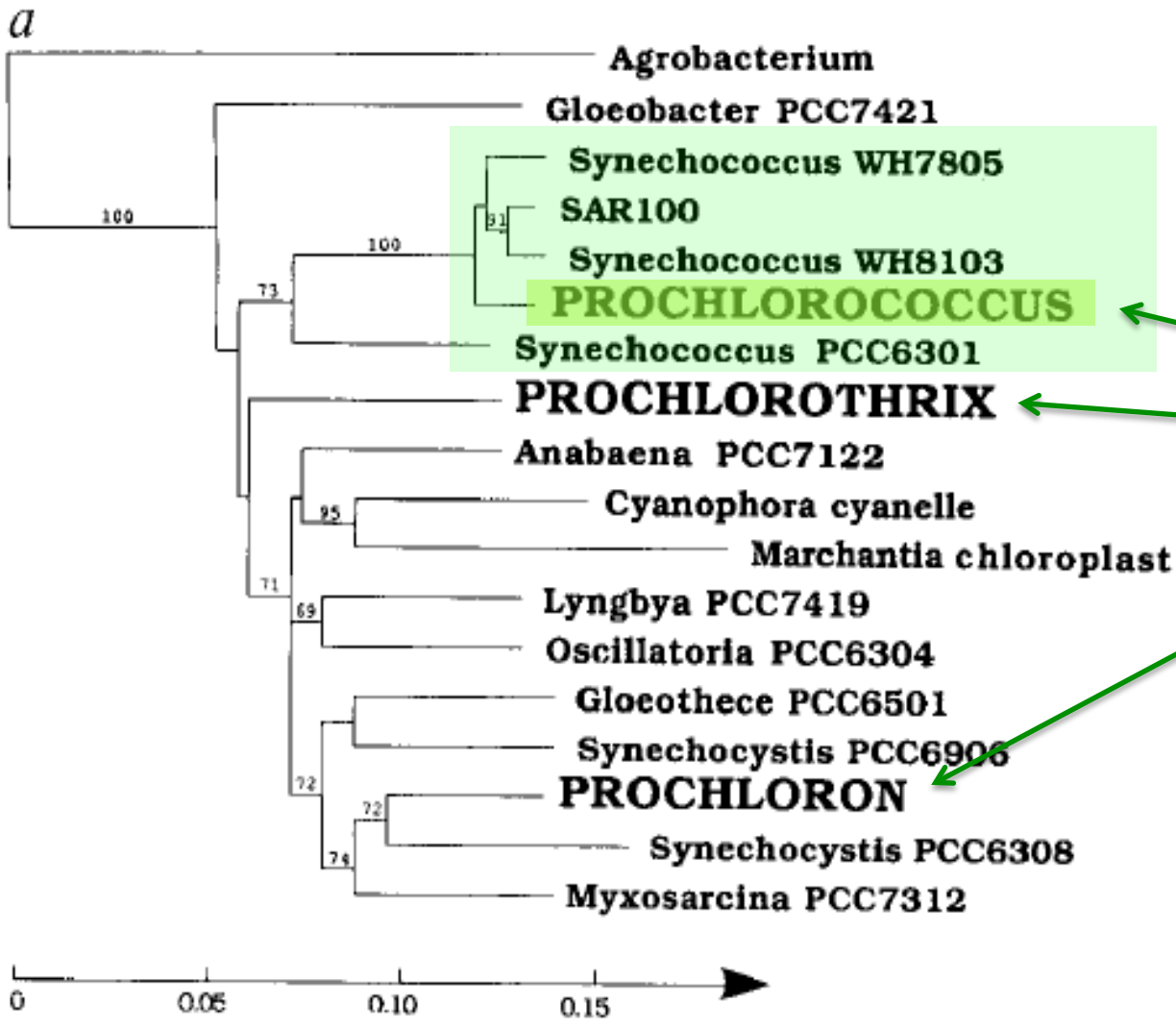
Mak Saito

Lead to the name: *Prochlorococcus*

("coccoid prokaryote with chl b")

Enter Molecular Phylogeny: We were wrong!

It *IS* closely related to *Synechococcus,* and not to *Prochloron* or *Prochlorothrix*



Chl b trait evolved independently in these three groups

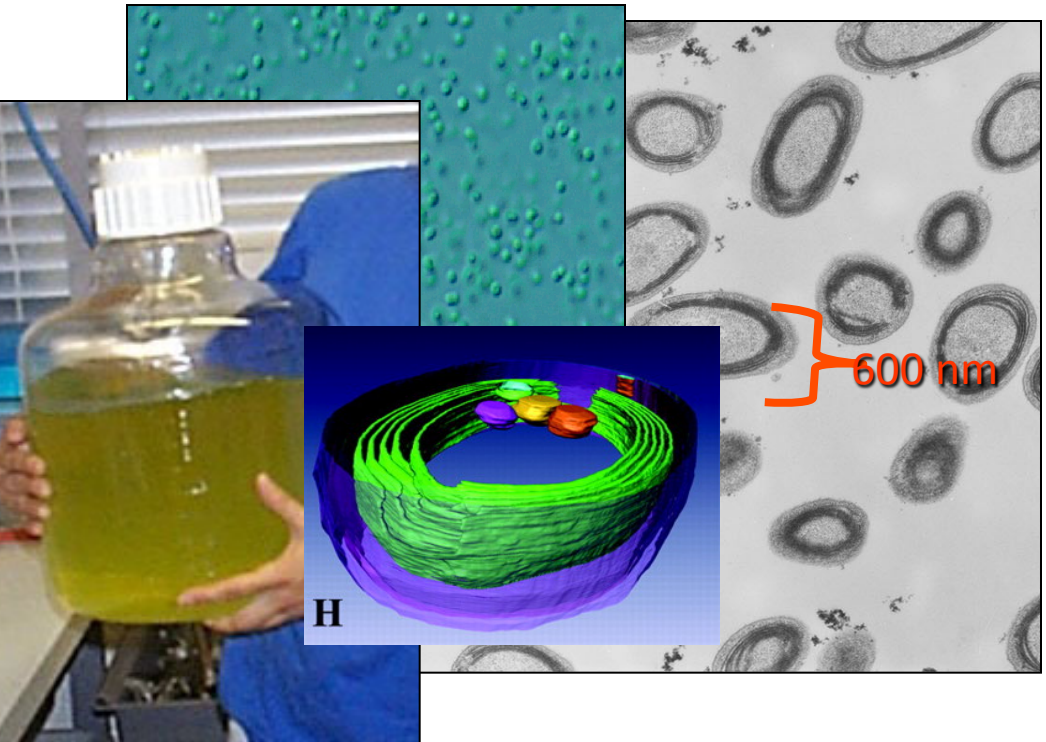
Ena Urbach

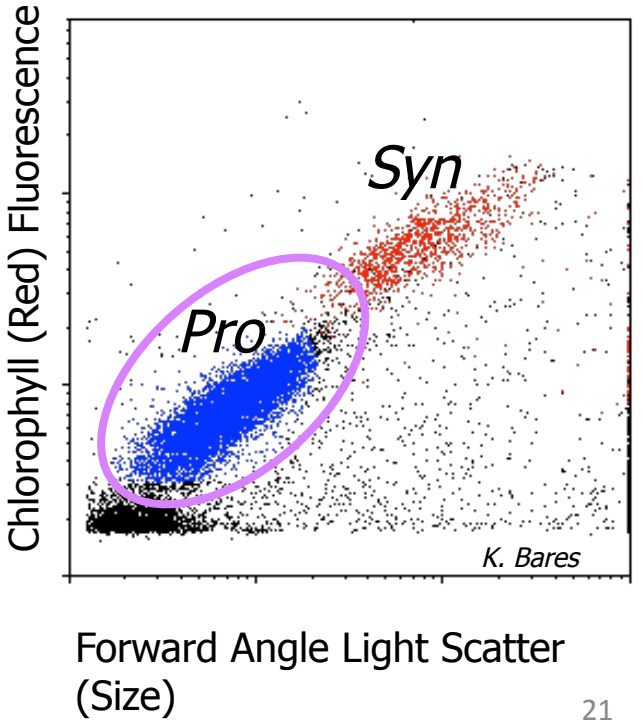
Mitch Sogin

Brian Palenik

What exactly is *Prochlorococcus*?

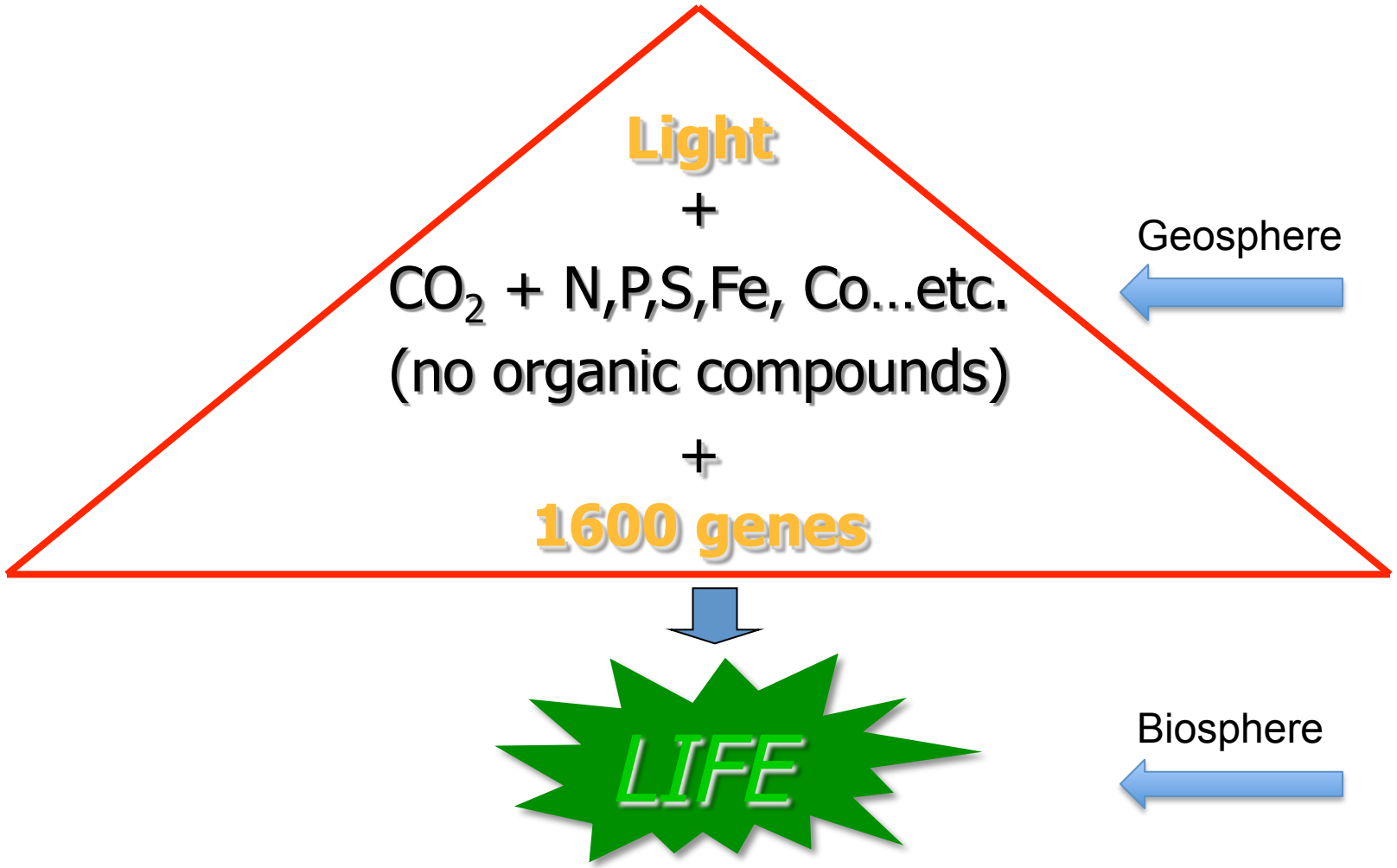
- Smallest cell in the oceans that fluoresces red
- Contains Divinyl Chl a and Chl b
- Oceanic cyanobacterium, 0.6 - 0.8 µm diameter
- Smallest (size and genome), and most abundant photosynthetic cell on Earth



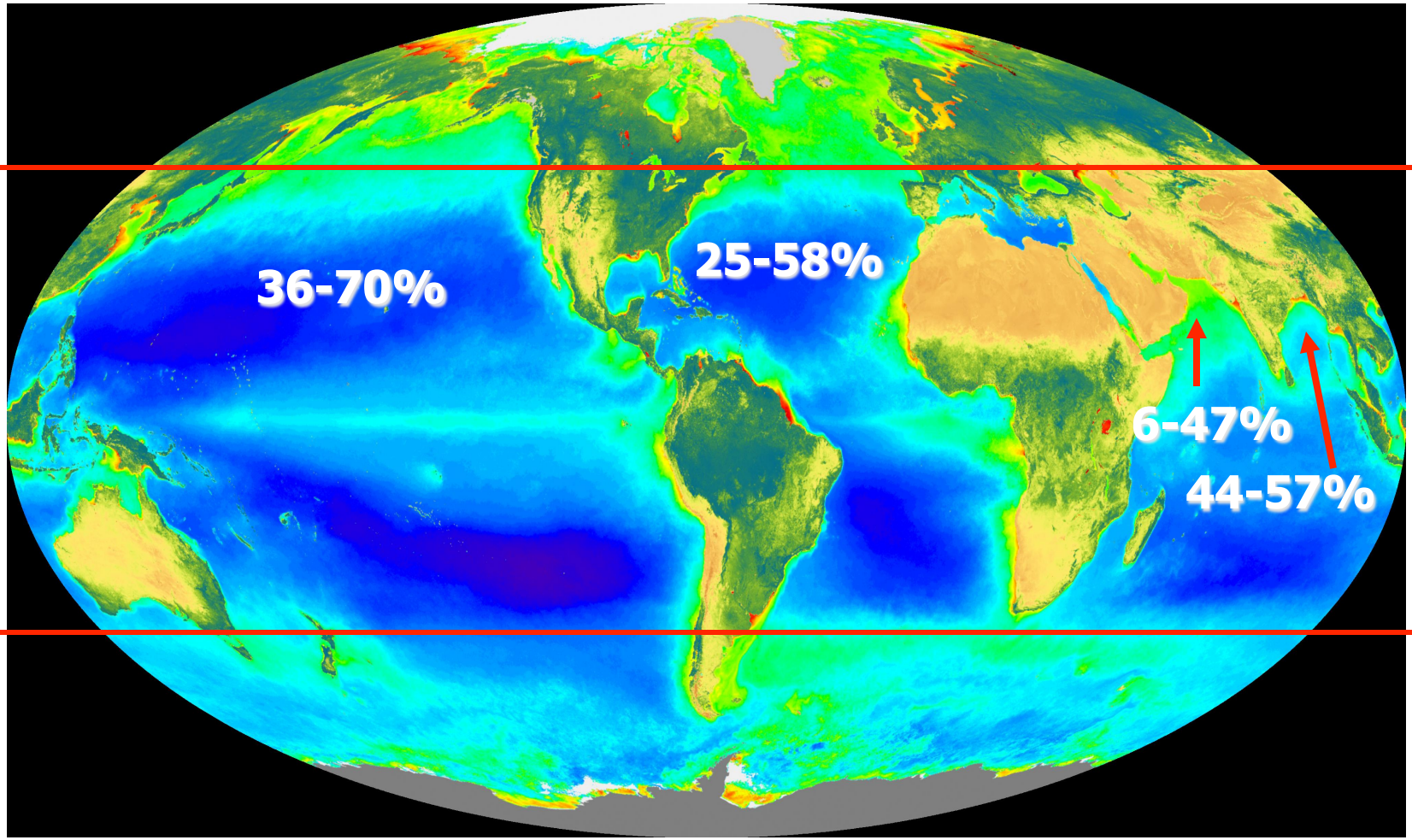


Prochlorococcus is the 'minimal life' form:

Smallest amount of information that can make life from scratch



Can be significant fraction of ocean chlorophyll

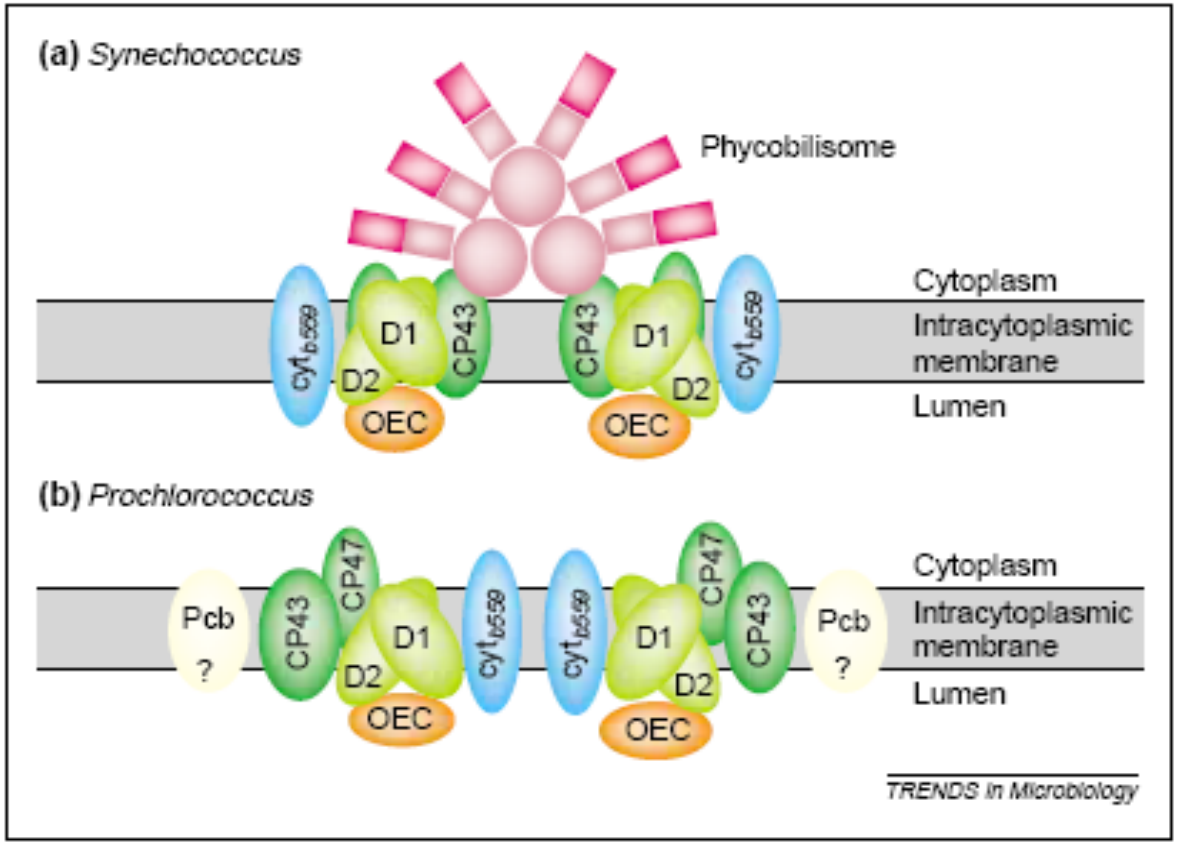


Record concentration:

700,000 cells ml^{-1}

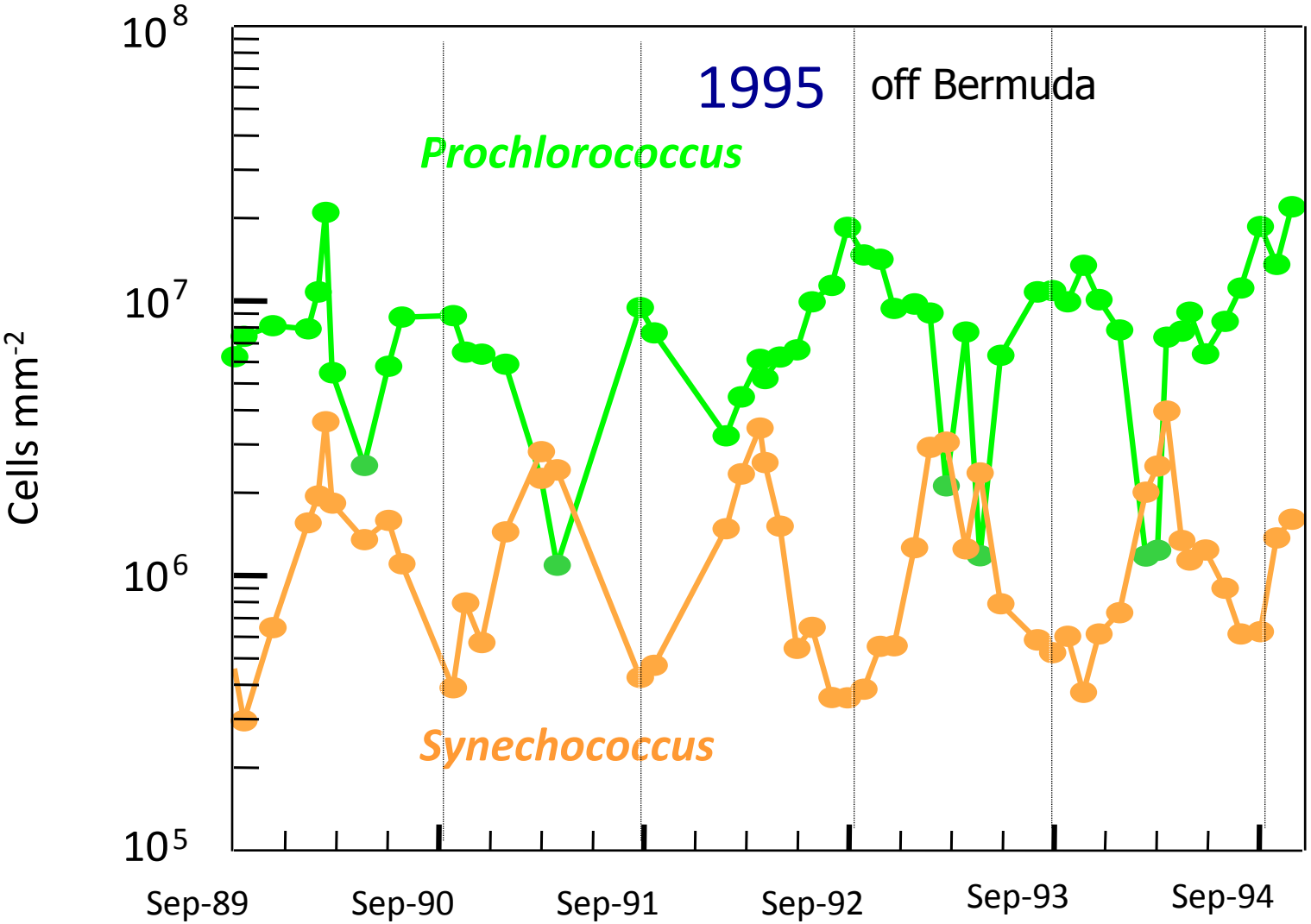
Global photosynthesis ≈ 5 Gt C yr^{-1}

Prochlorococcus is basically a slightly smaller *Synechococcus,* with a different light harvesting system



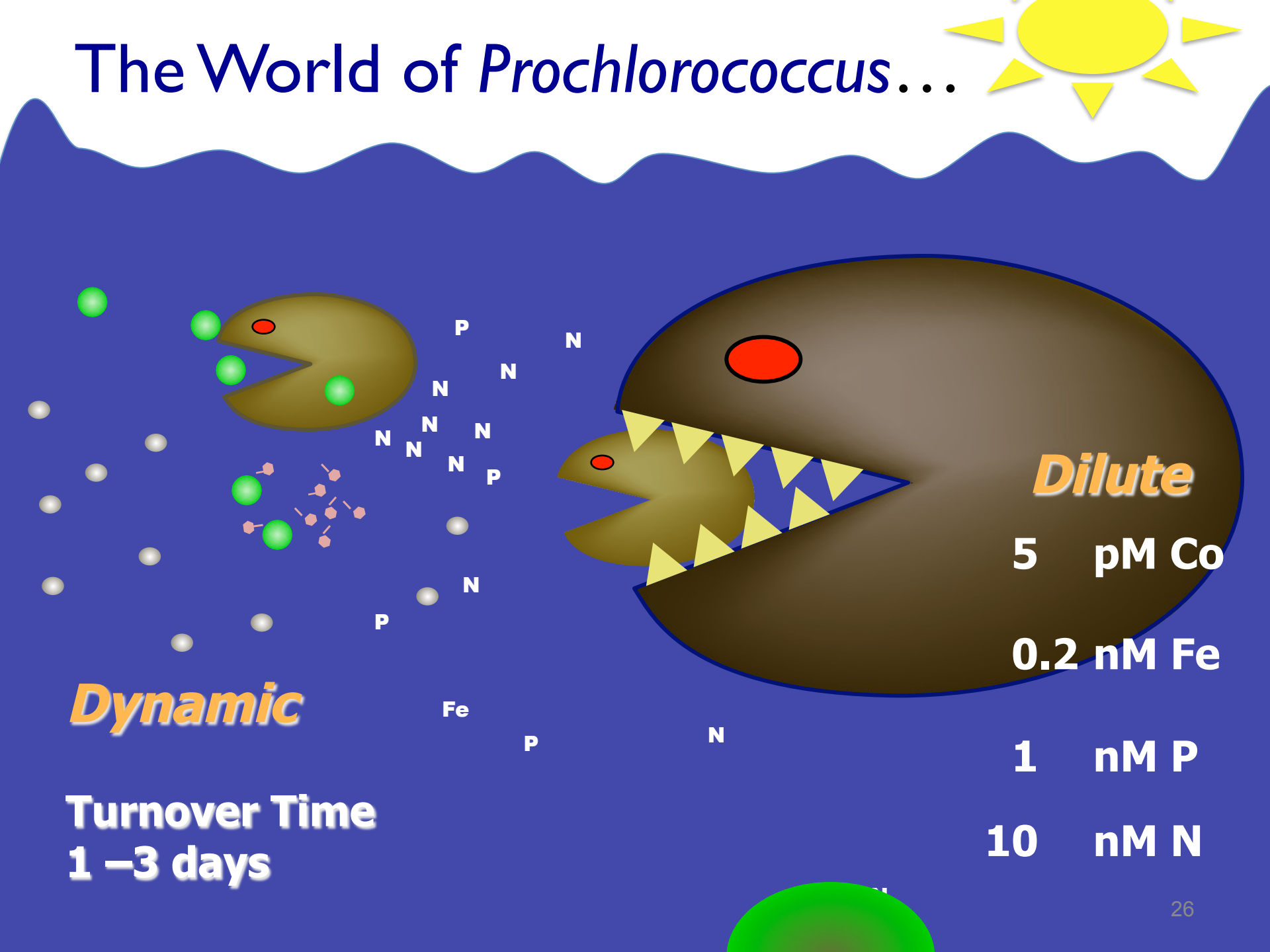
Ting et al 2006

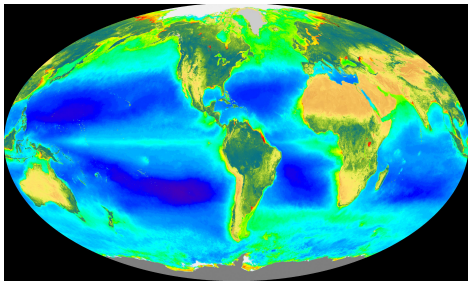
They share (and partition) the "small size bin"



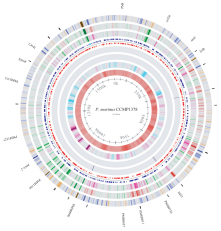
DuRand et al 2001

The World of *Prochlorococcus*…

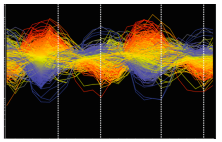




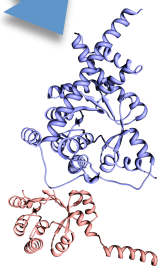
Biosphere



Genes



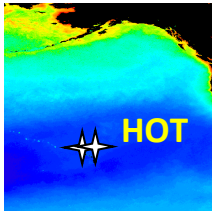
Transcripts



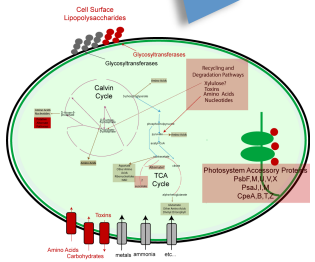
Proteins

Our Goal:

Study *Prochlorococcus* at all scales of organization

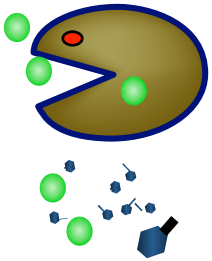


Ecosystem



Cell

Population

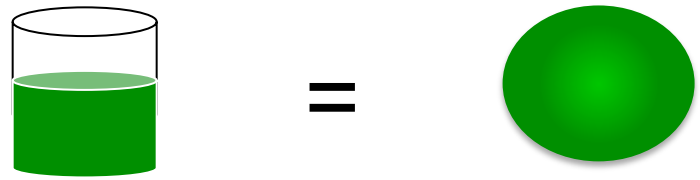


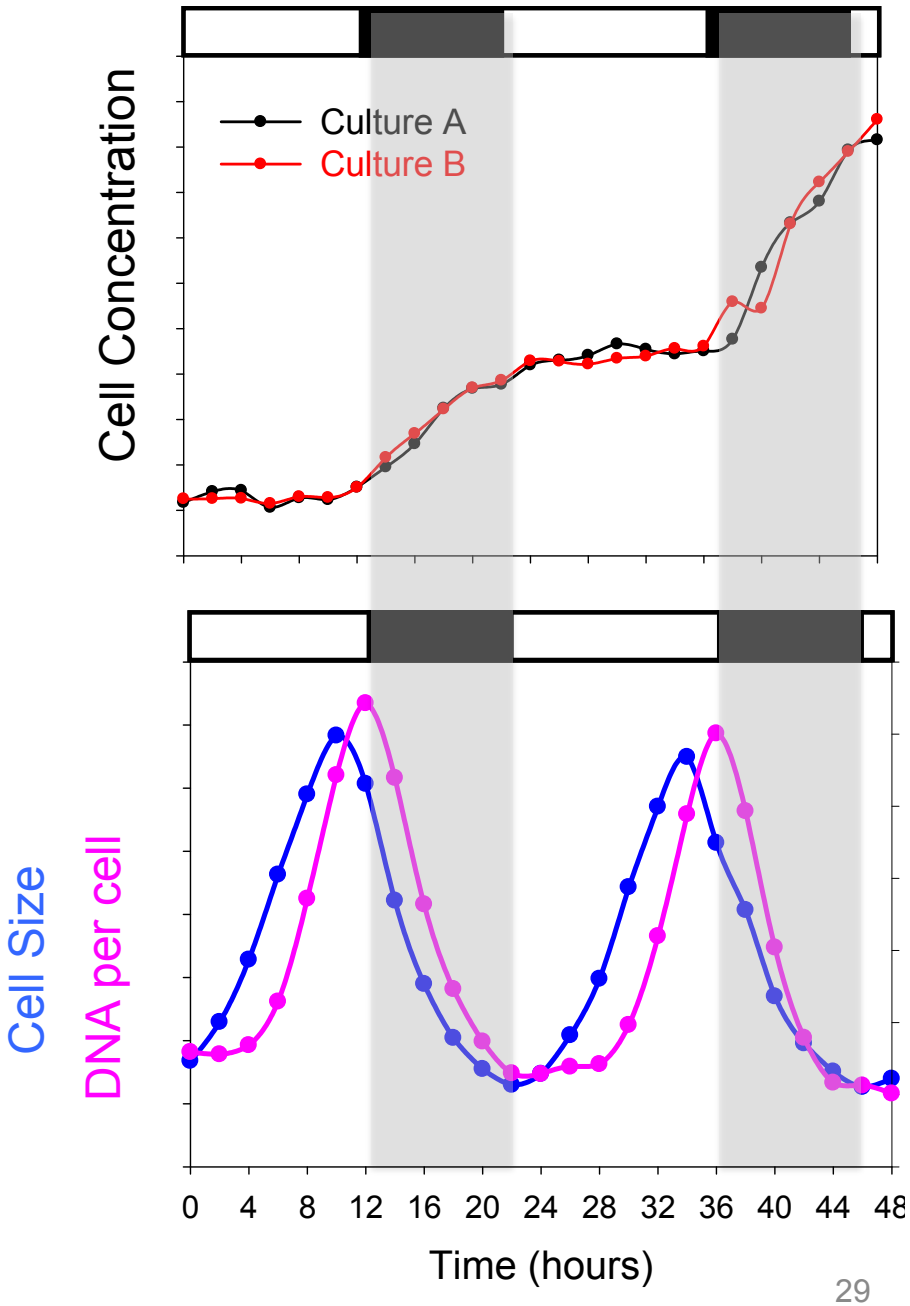
Food Web

Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology

Tight Cell Cycle Synchrony

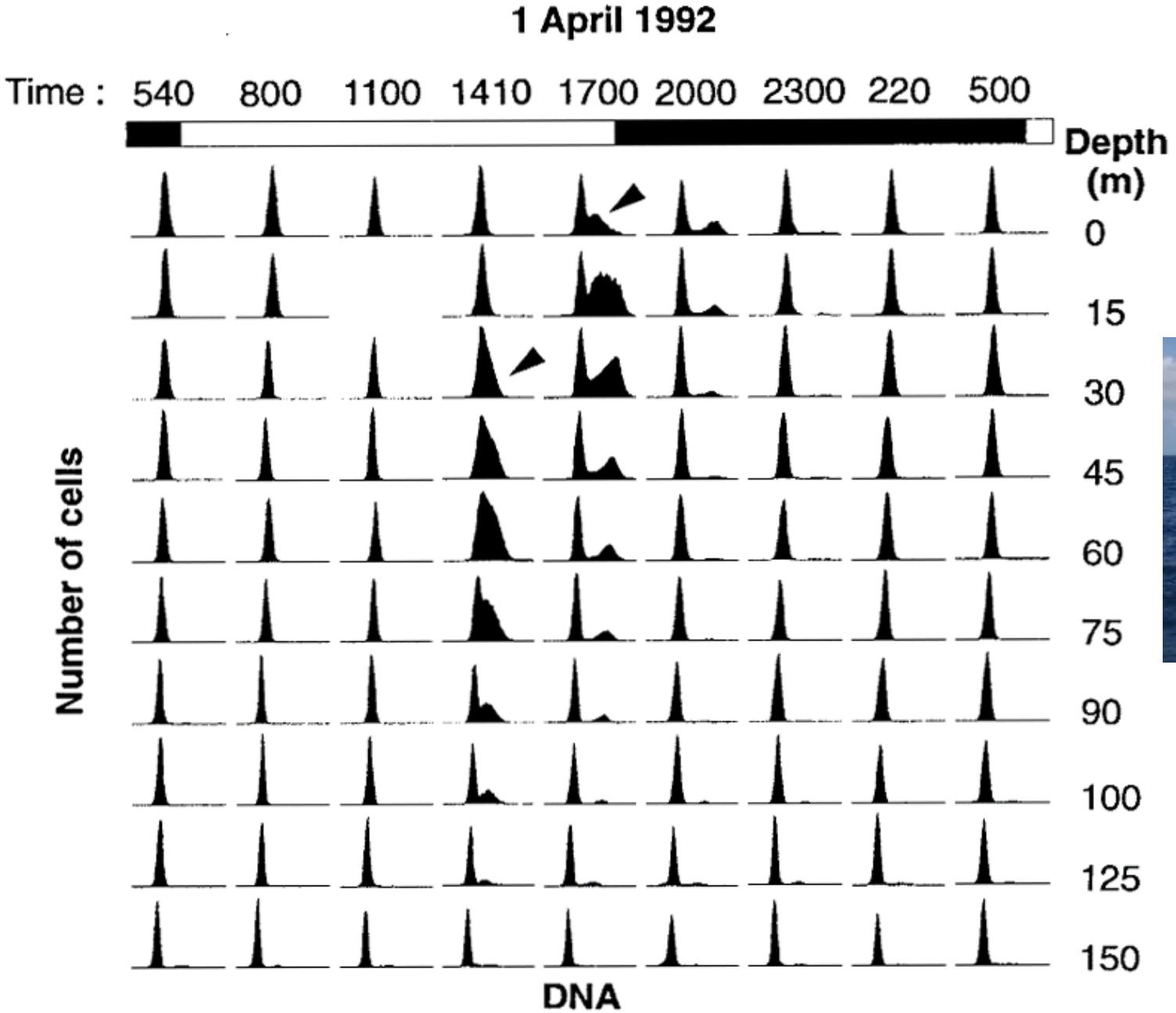




Zinser, Lindell et al. 2009

Can see it growing in the wild

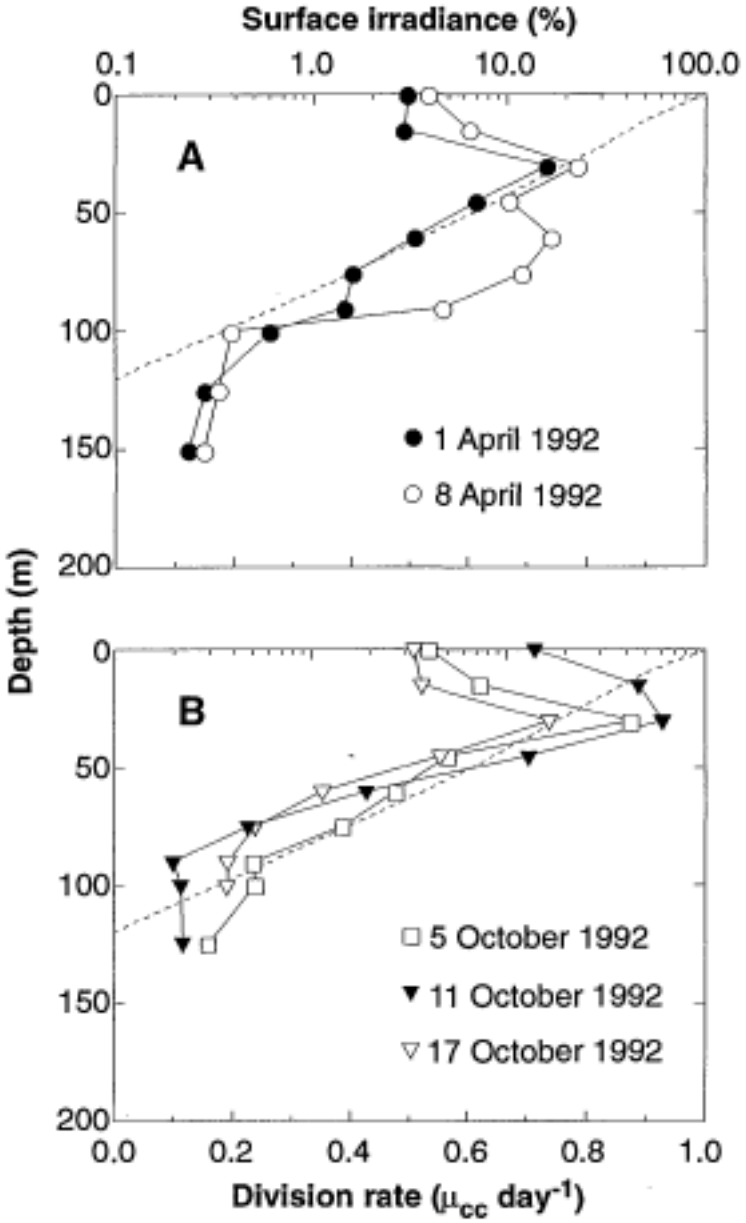
Equatorial Pacific

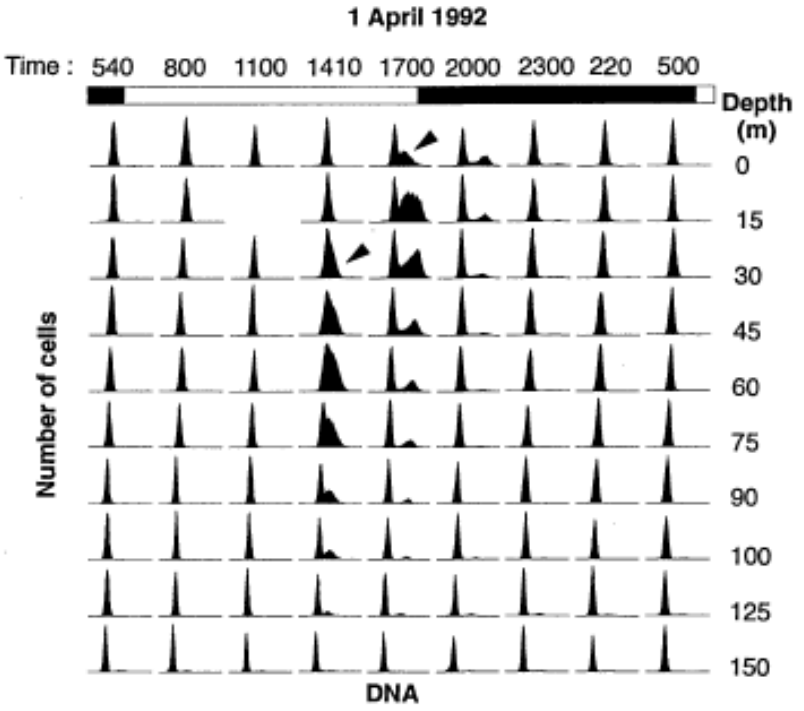


Daniel Vaulot

Prochlorococcus growth rate as a function of depth

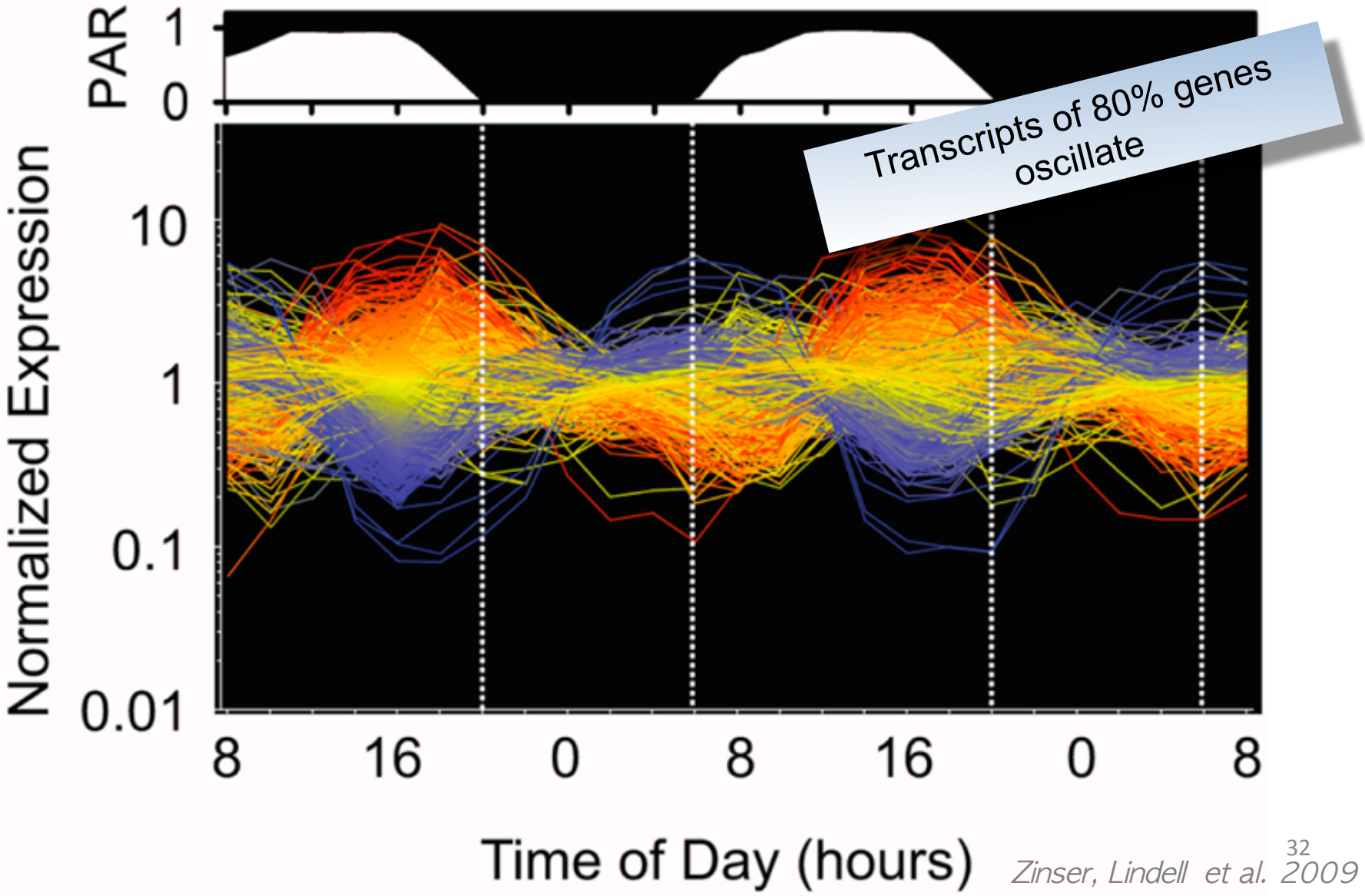
(measured by cell cycle progression)





Vaulot et al 1995

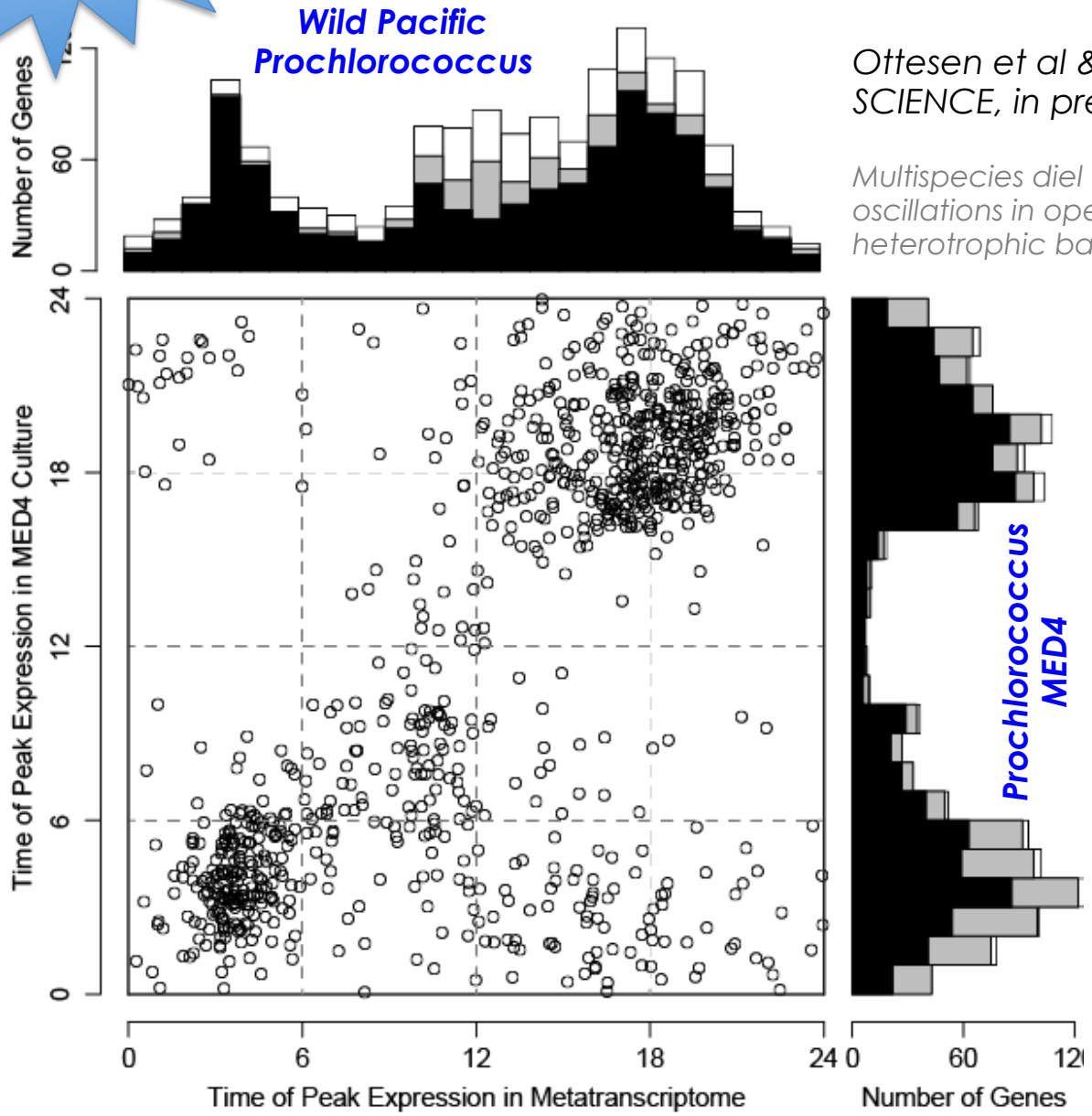
Gene expression highly choreographed



Zinser, Lindell et al. 2009

… in the wild too!

News flash!



Ottesen et al & DeLong, SCIENCE, in press

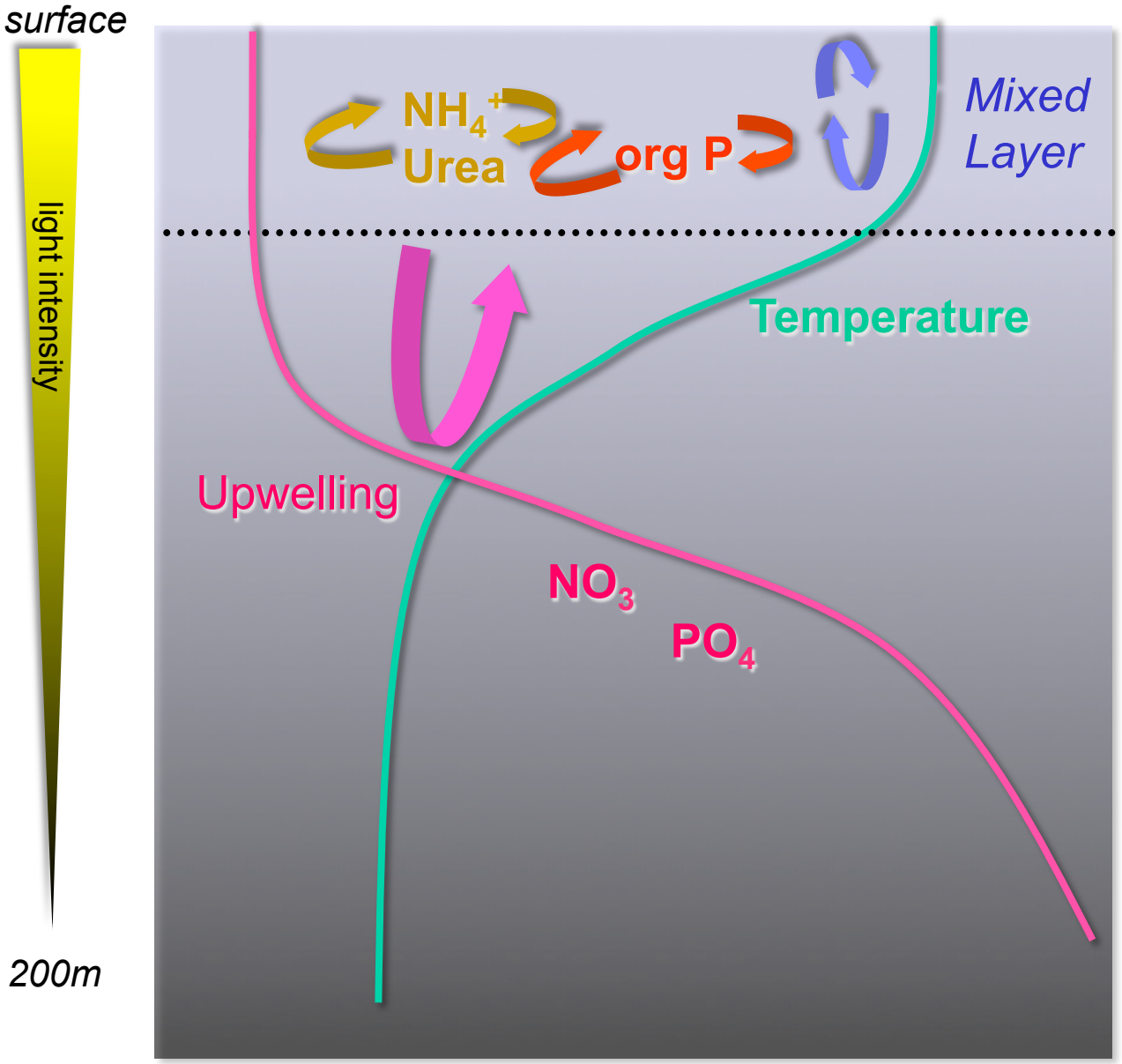
Science AAAS

Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages

Zinser et al & Chisholm 2009

Choreography of the transcriptome, photophysiology and cell cycle of a minimal photoautotroph

Reminder: Structure of the ocean habitat



…physical and chemical gradients =

Niche dimensions!

Overview

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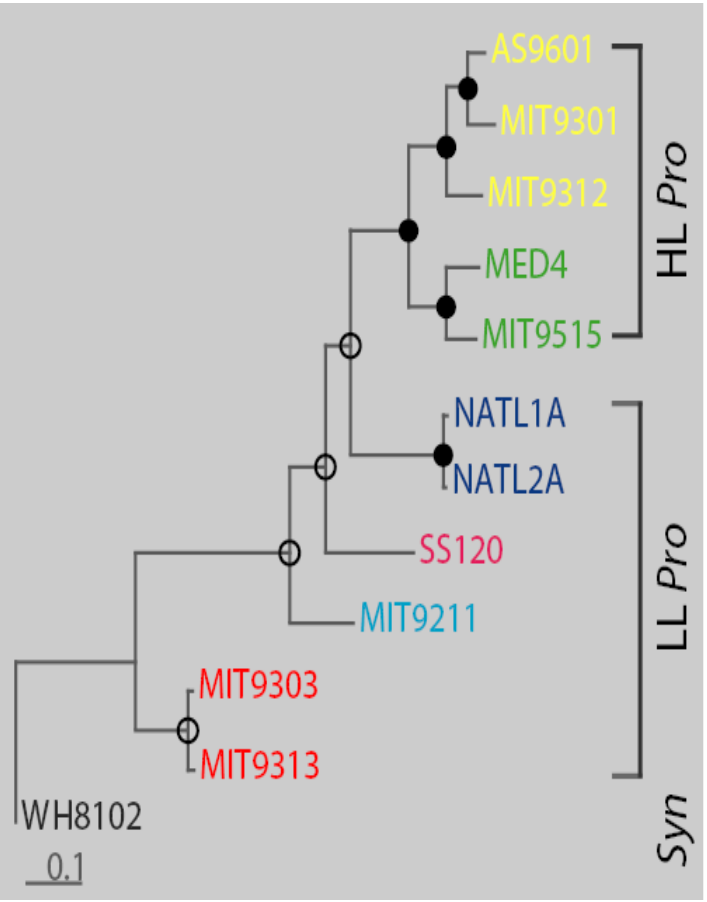
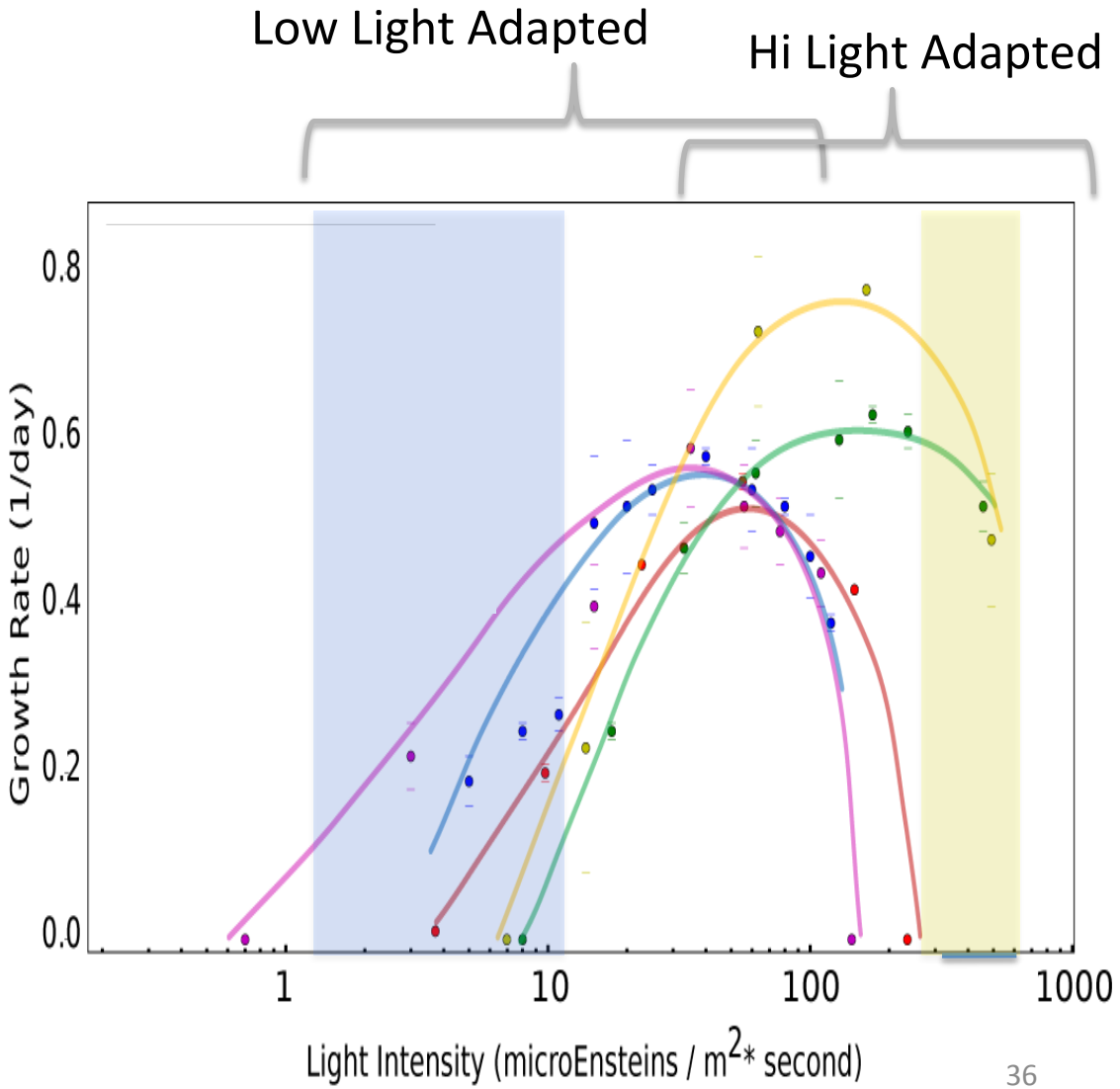
Light adaptation defines ecotypes…

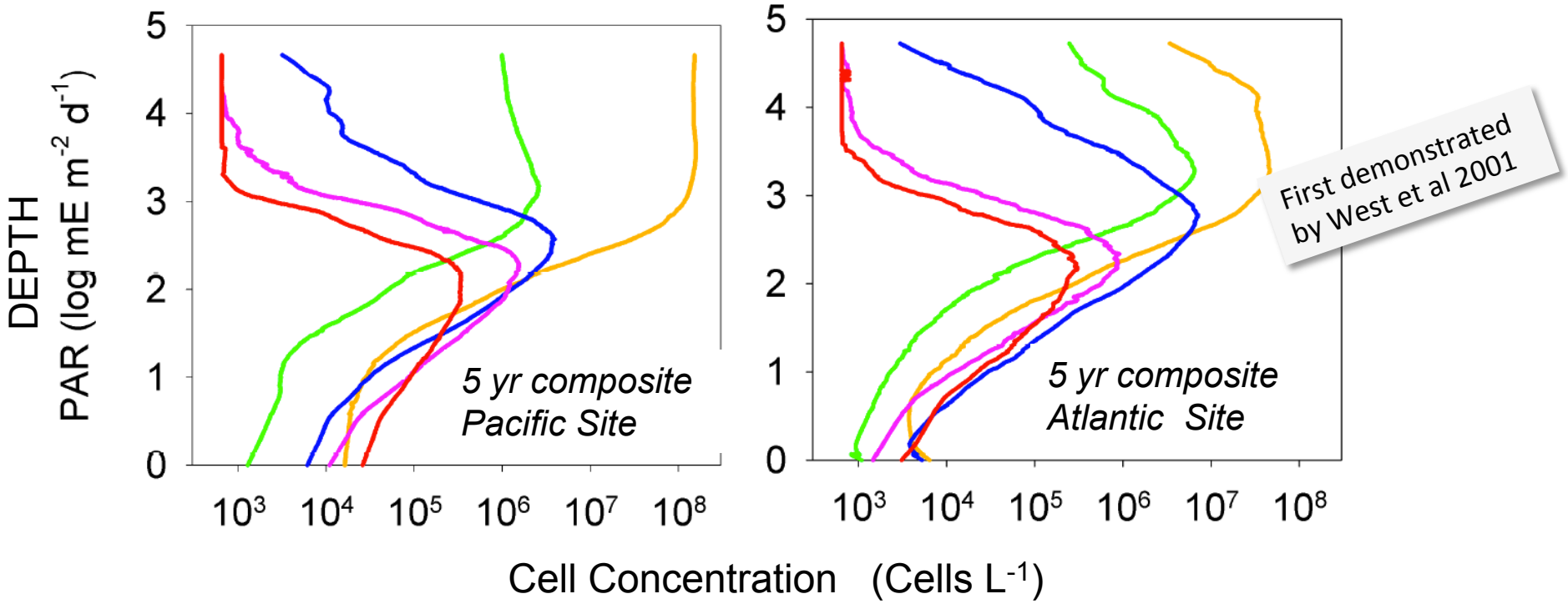
Whole Genome Phylogeny
(matches rRNA ITS phylogeny)

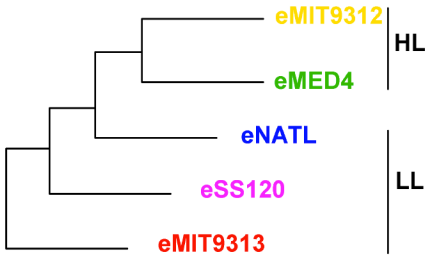




Moore, Rocap, Kettler and more, 1990 - 2006

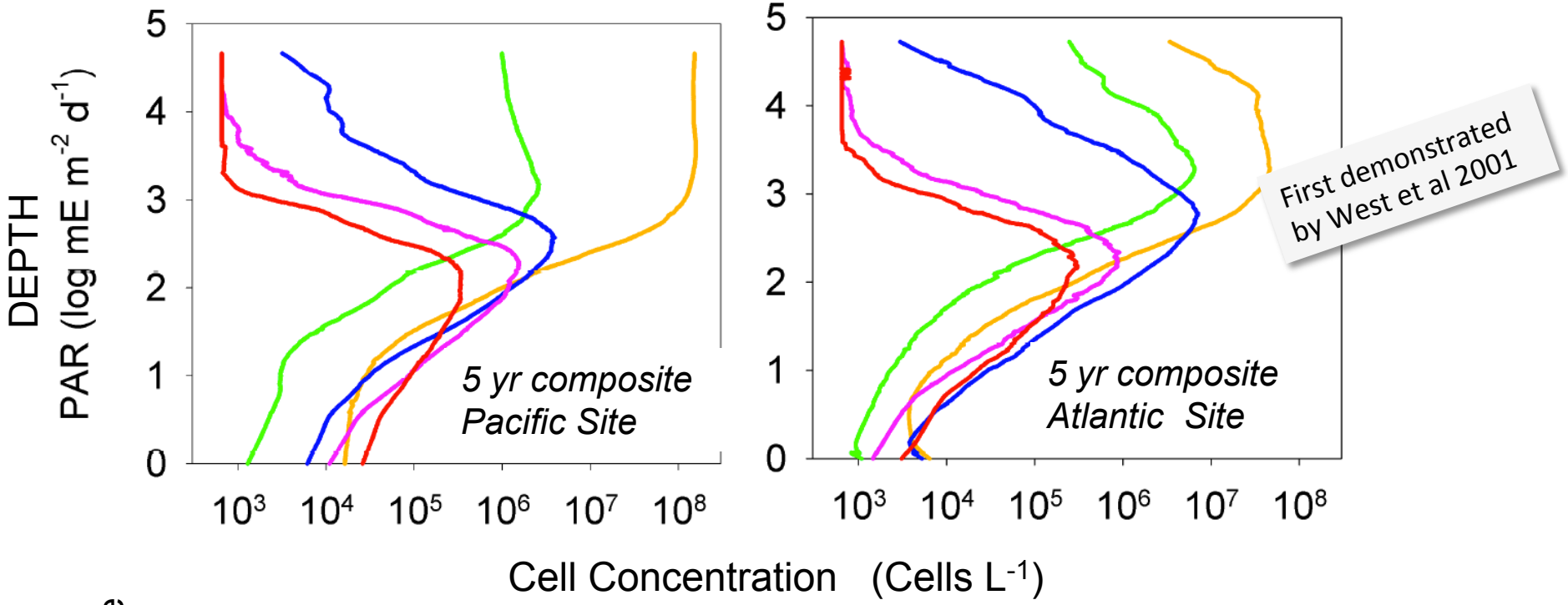
…leading to niche differentiation



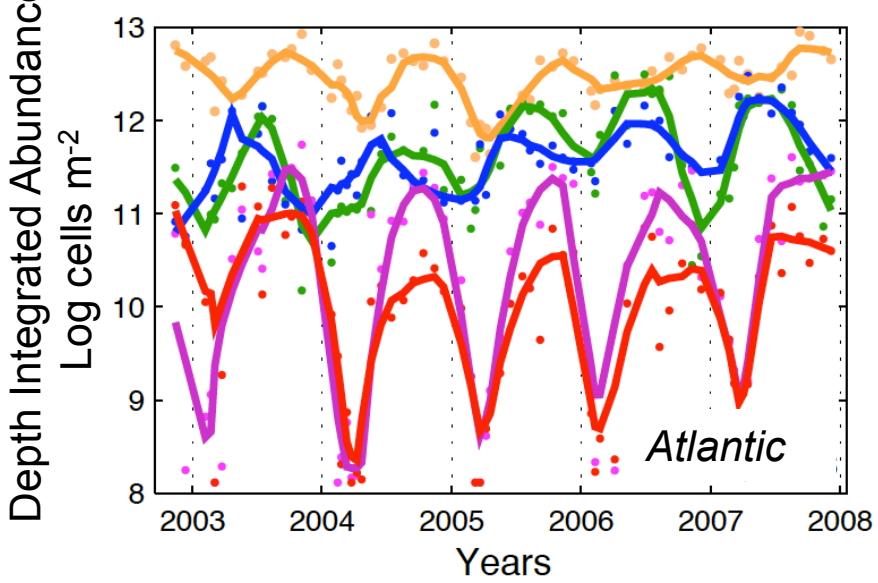


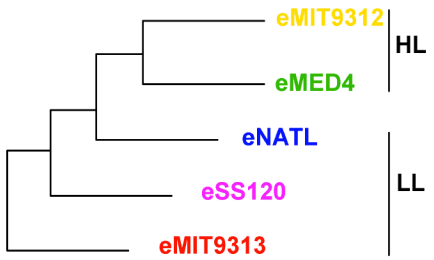
Malmstrom et al 2010

…leading to niche differentiation



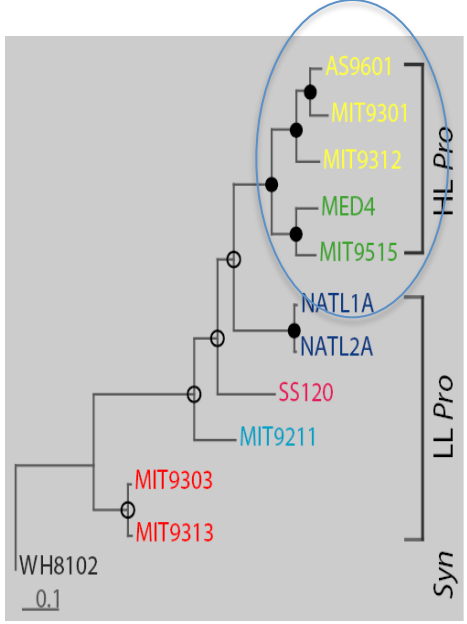
First demonstrated by West et al 2001

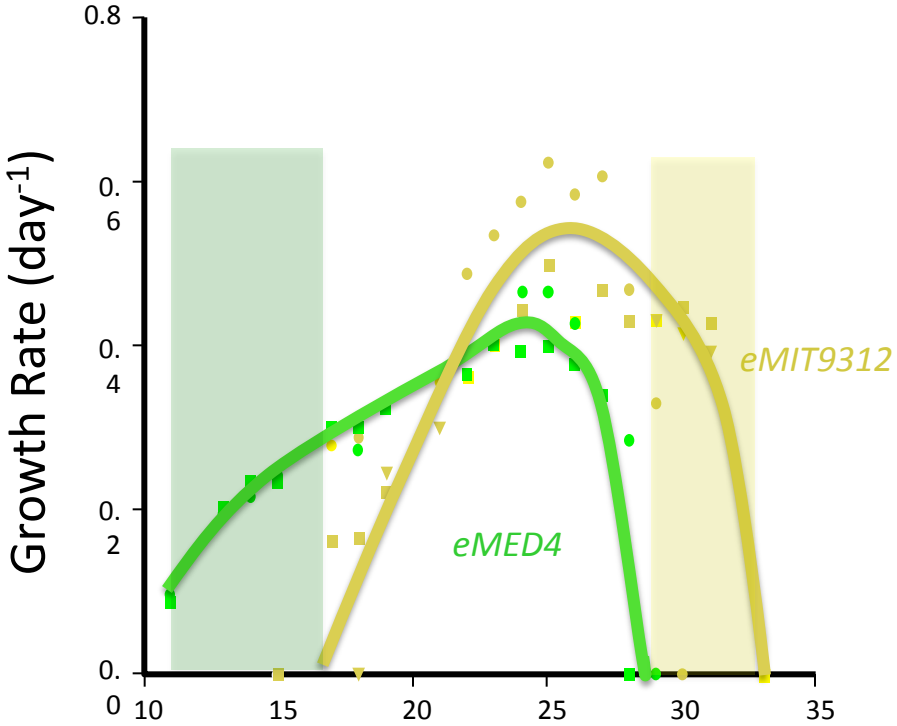


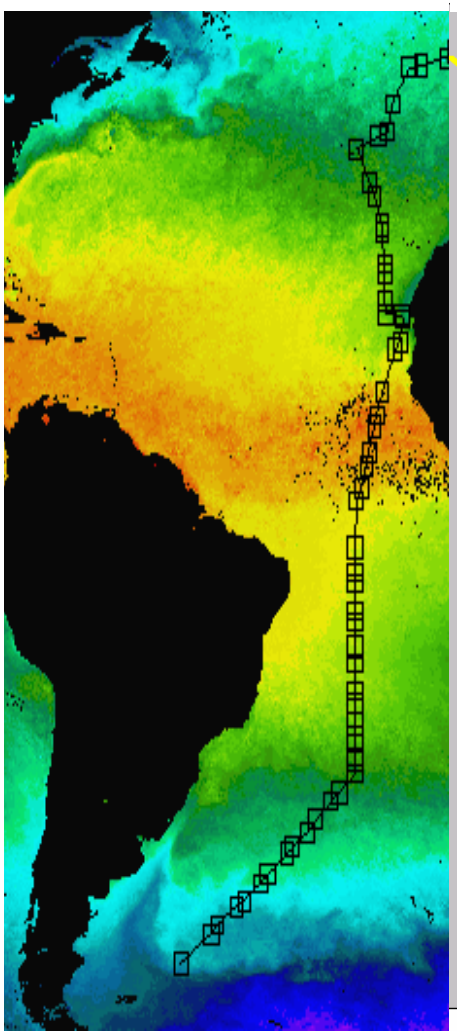


Malmstrom et al 2010

Temperature differentiates the two High Light ecotypes

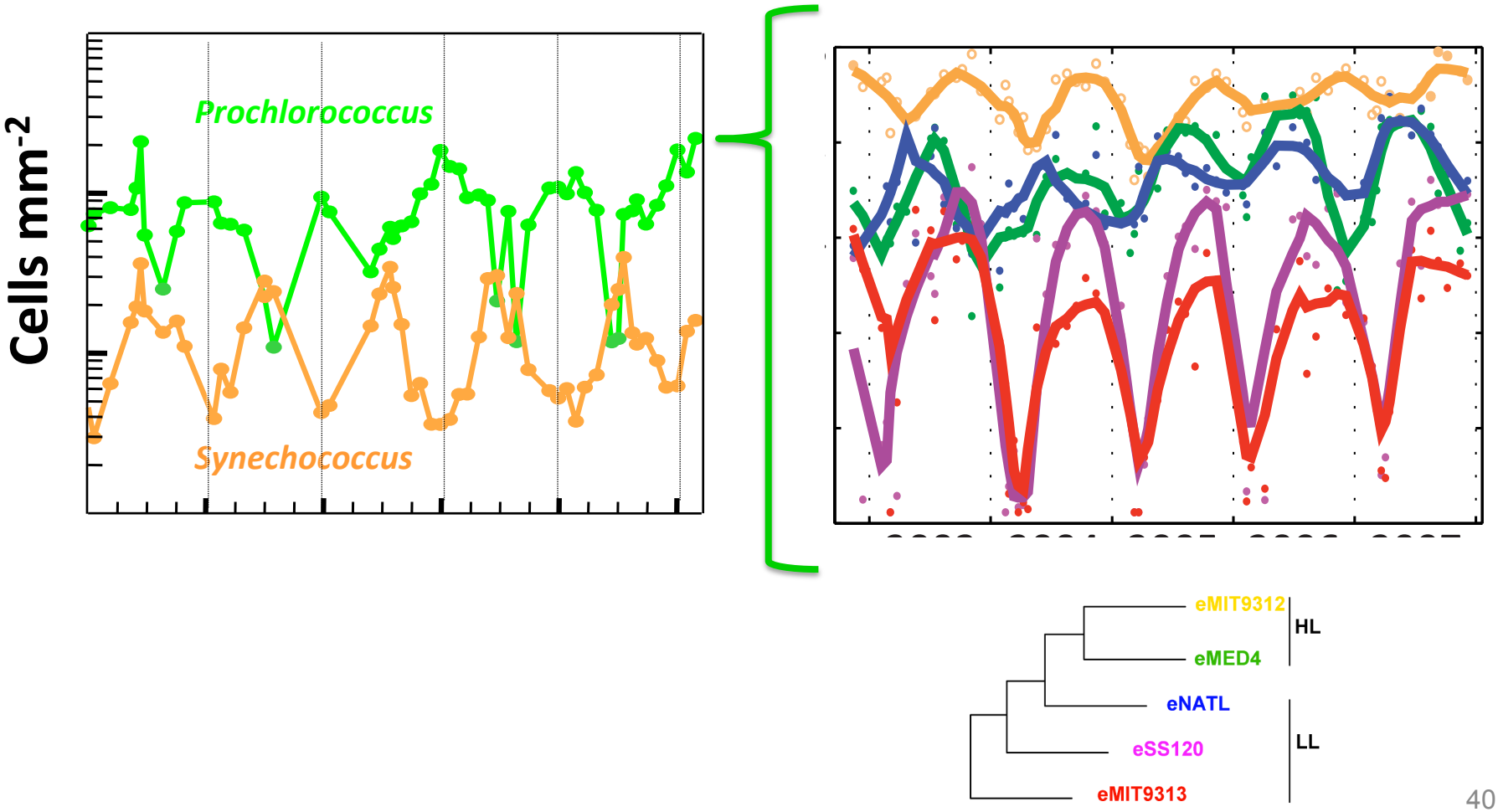






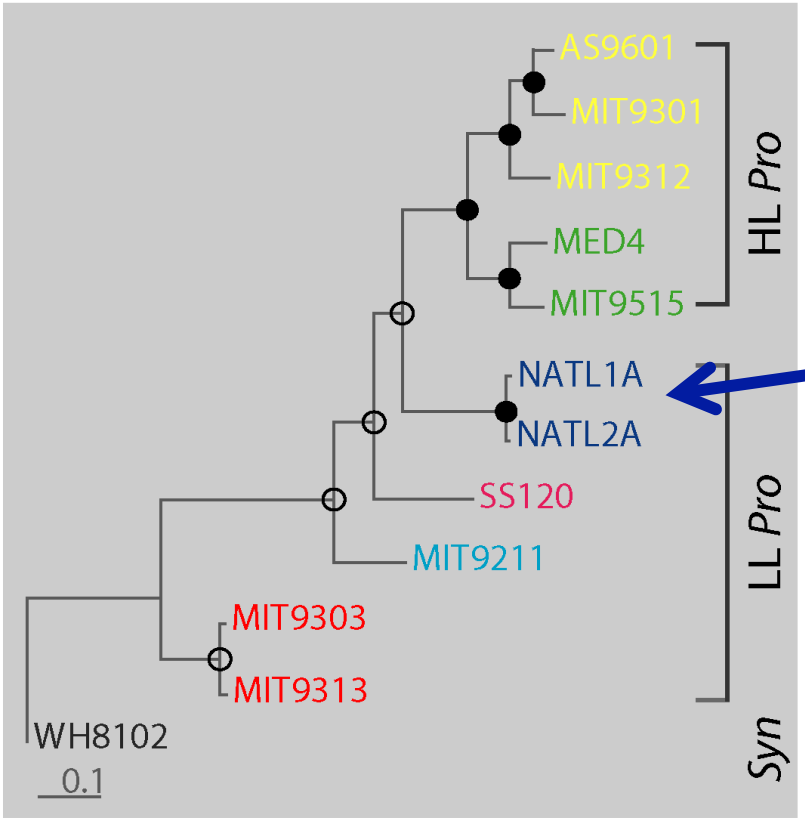
Johnson, Zinser et al, 2006

So there are meaningful layers, within layers, of diversity (leads to stability)

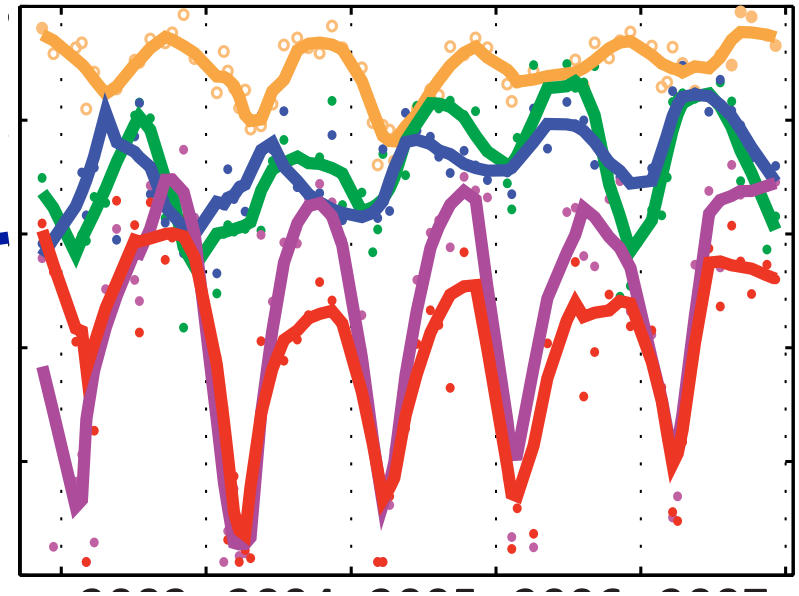


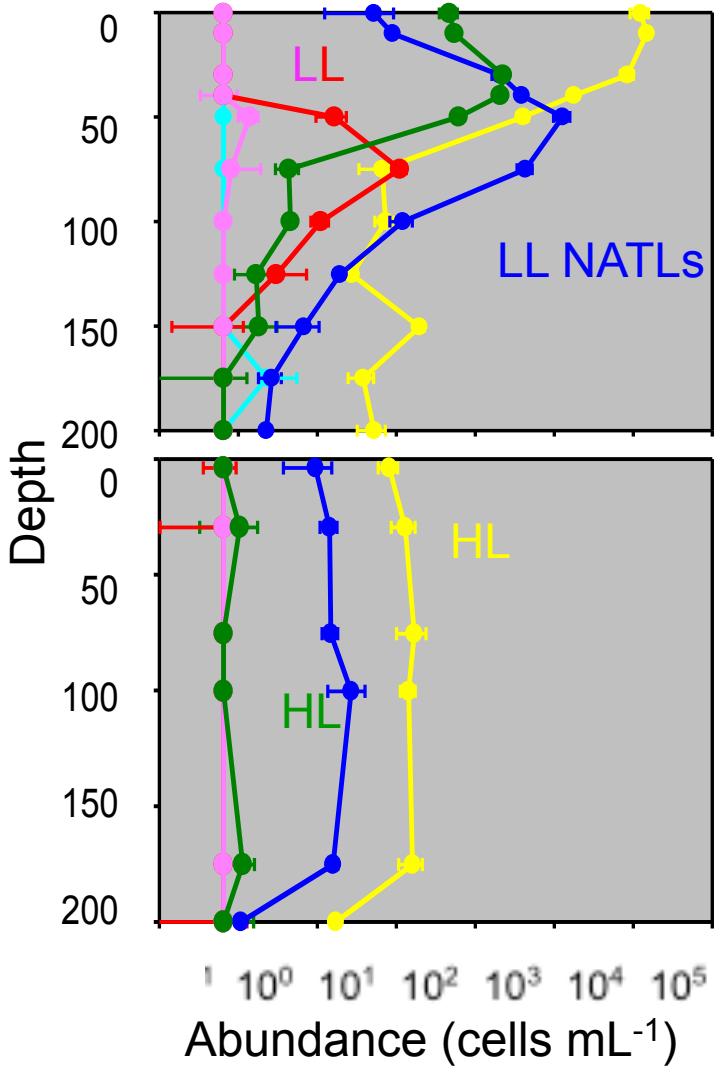
"The NATLs" are very interesting

Not HL-Adapted, but intermediate taxonomic position



Prochlorococcus ecotypes

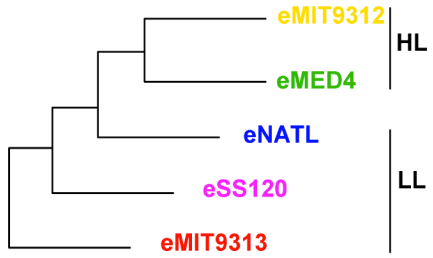




NATLs appear to:

handle high light better than other LL ecotypes…

…and deep mixing better than some HL ecotypes

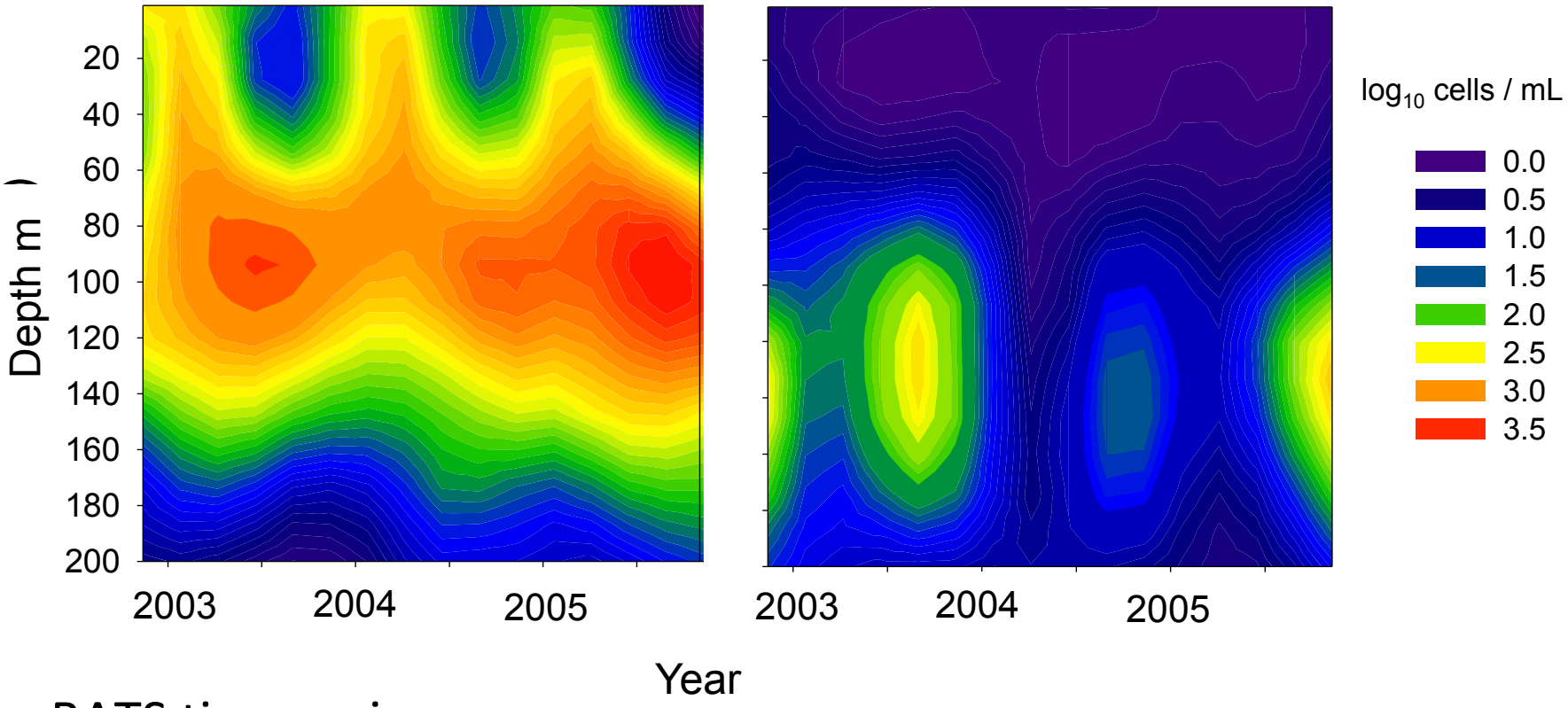


Zinser et al 2007

HYPOTHESIS: NATLs better adapted to fluctuating light

NATLs

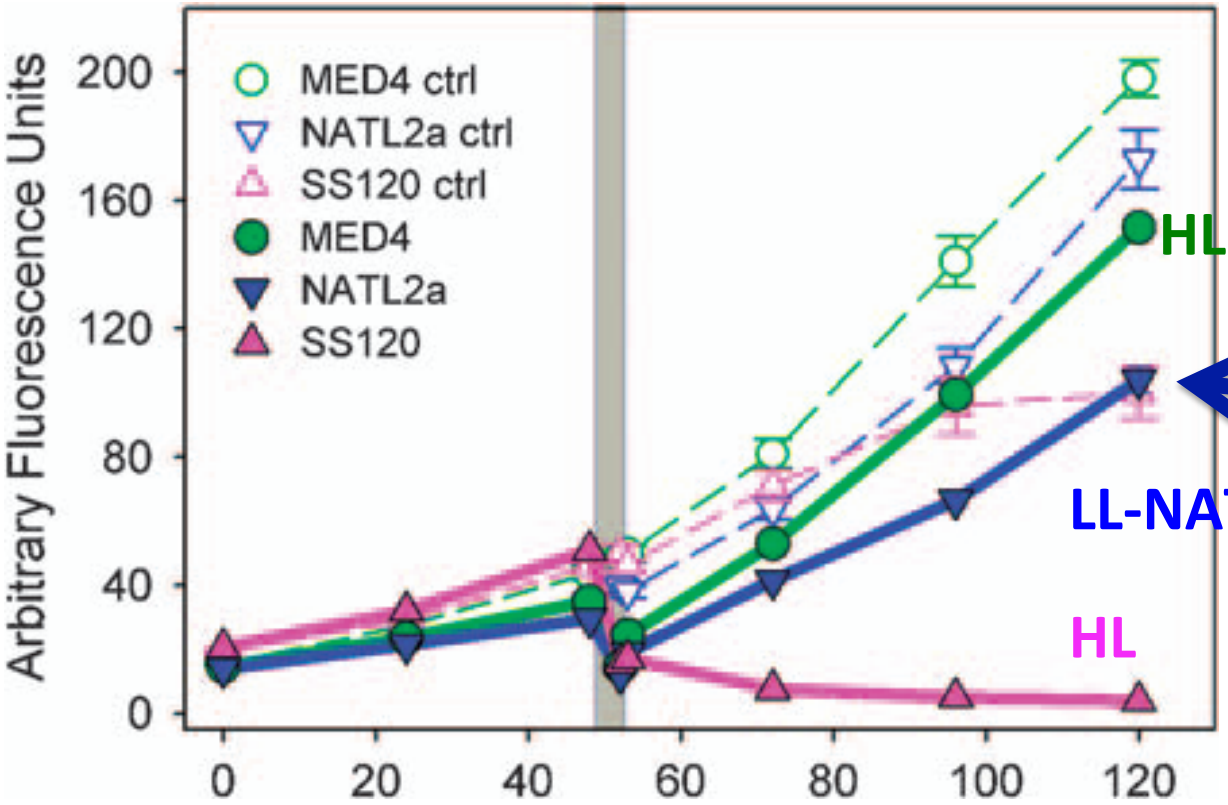
True LL Adapted



BATS time series

Malmstrom et al 2010

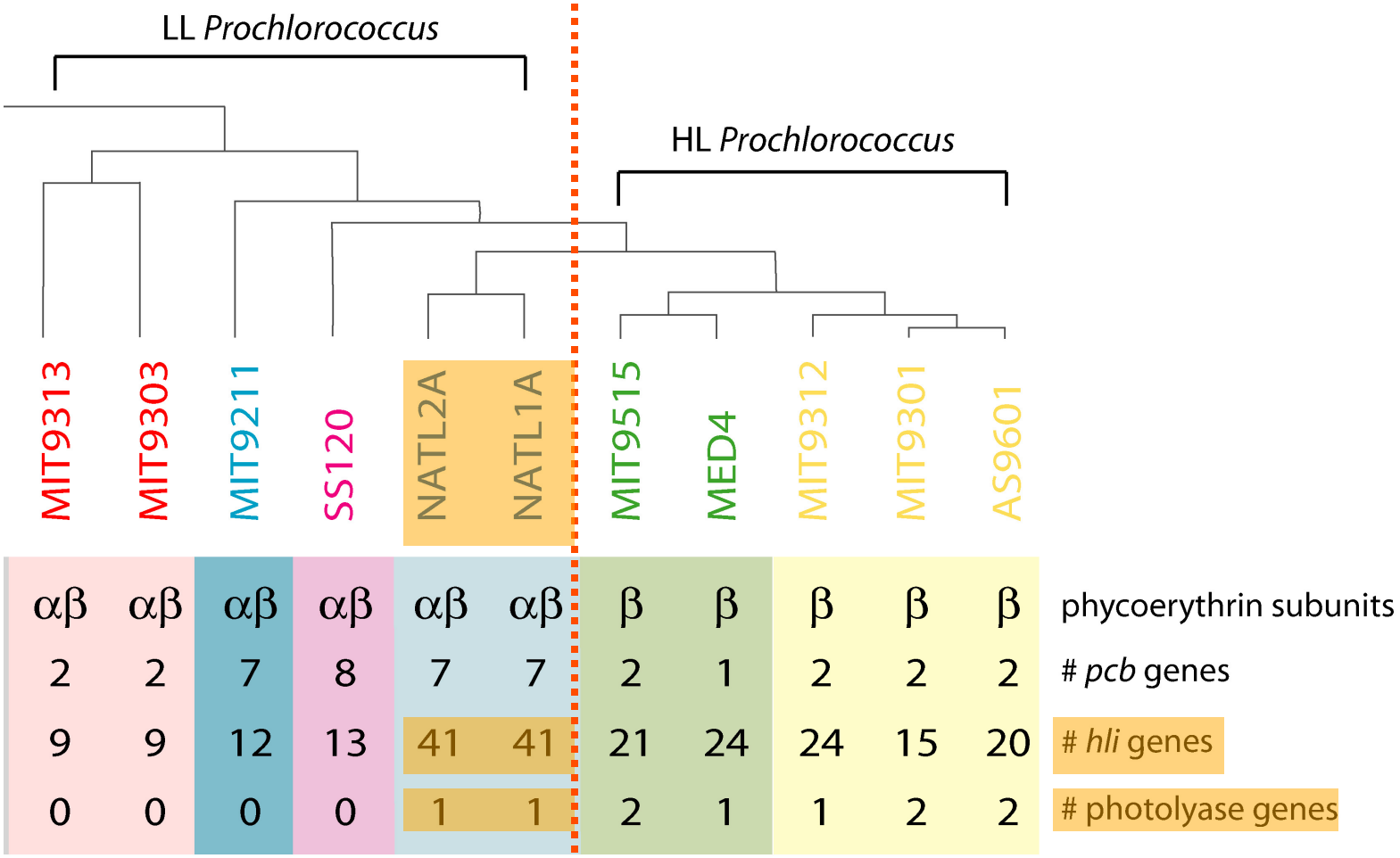
Back to the lab…



"The NATLs" are more resistant to light shock than other LL strains

Malmstrom et al 2010

Flashing forward: What do the genomes tell us?



Coleman and Chisholm 2007

45

Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
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    - Phosphorus
    - Nitrogen
    - Iron
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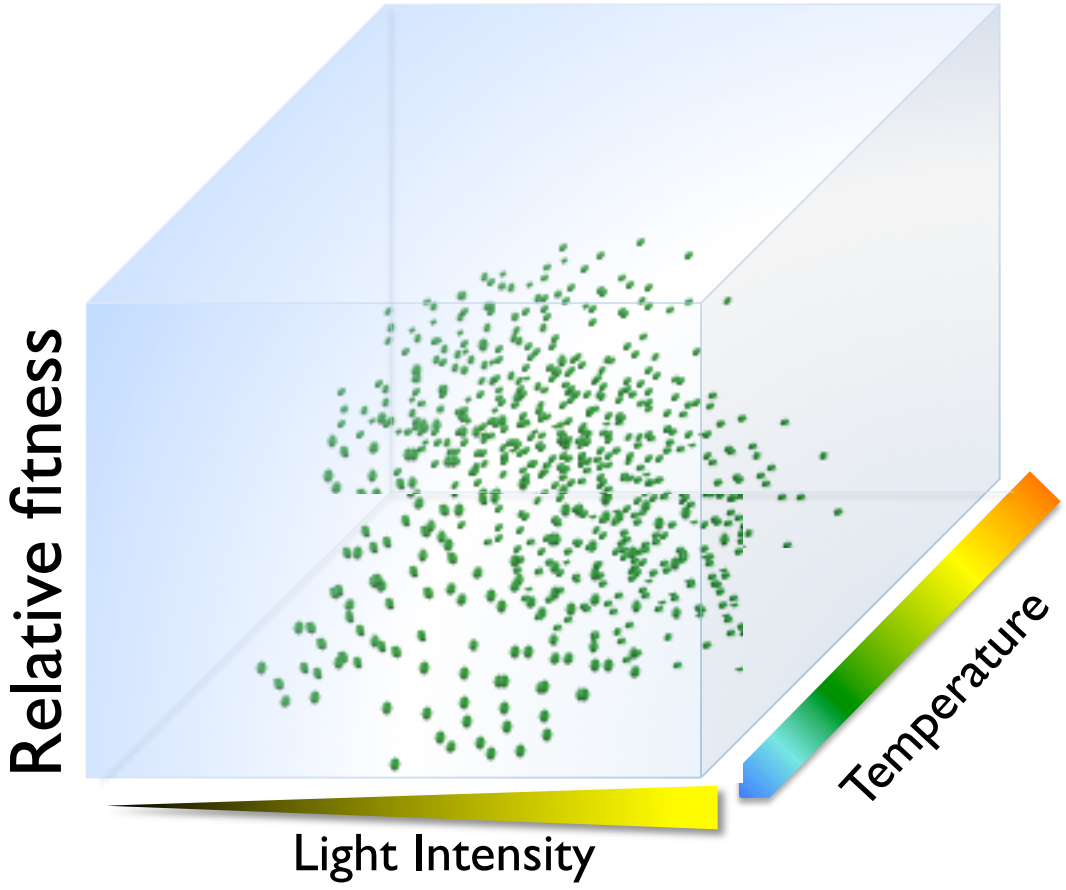
- Marine Vesicles
- Integrative Systems Biology

Beginning to understand *Prochlorococcus* niche differentiation in *two dimensions*



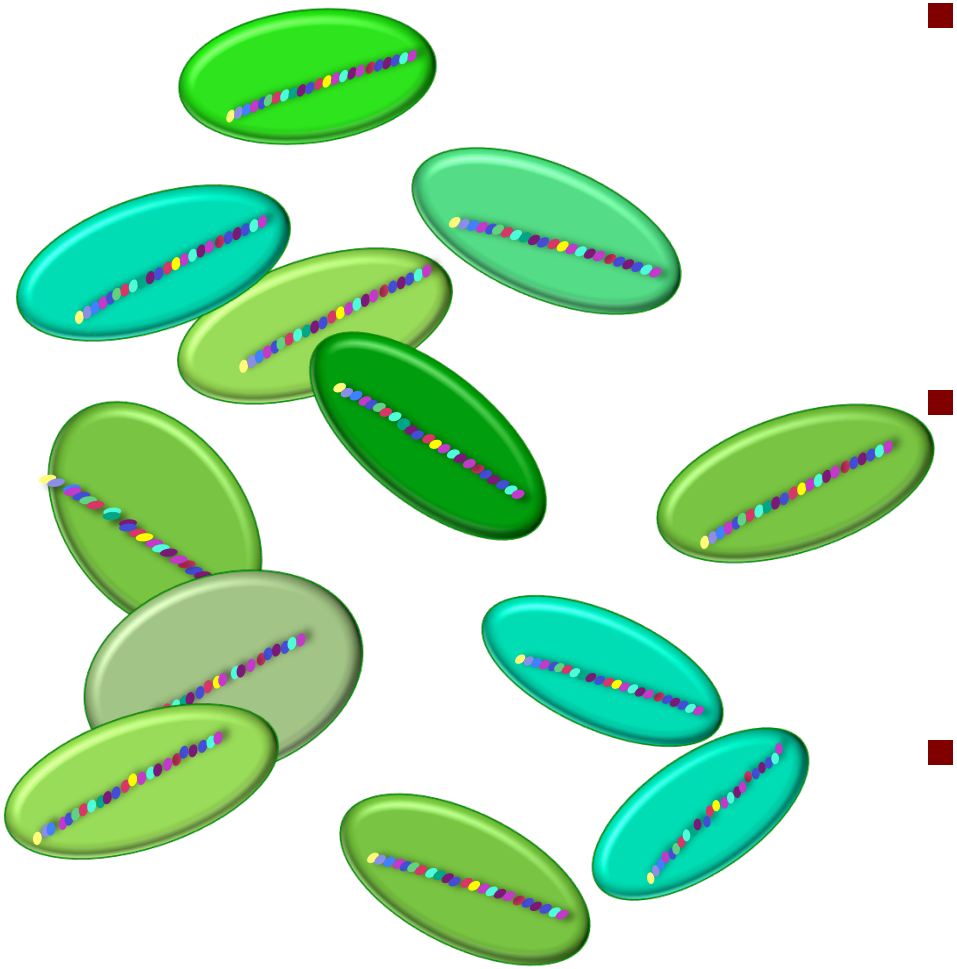
BUT Remember…

Ecological Niche:

***n**-dimensional*
hypervolume

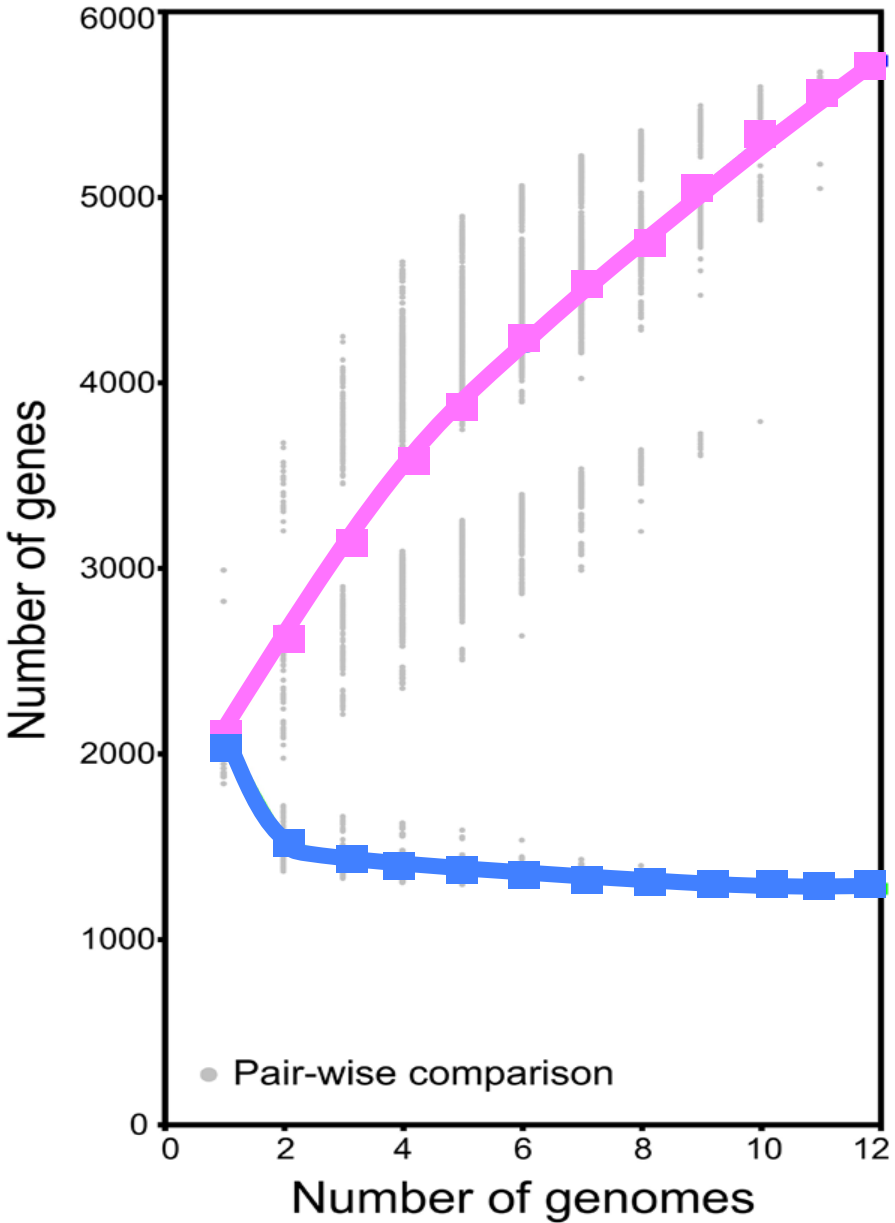
n-2 dimensions to go!

Enter Genomics:
Genomes of 13 *strains (now 45)*



- What genes are "core" i.e. shared by all?
- How many unique genes in the gene pool of *ALL Prochlorococcus*?
- Their functions?

The core and flexible genomes – 12 strains



5736

'Flexible Genes'
(w/ 45 genomes -12,000)

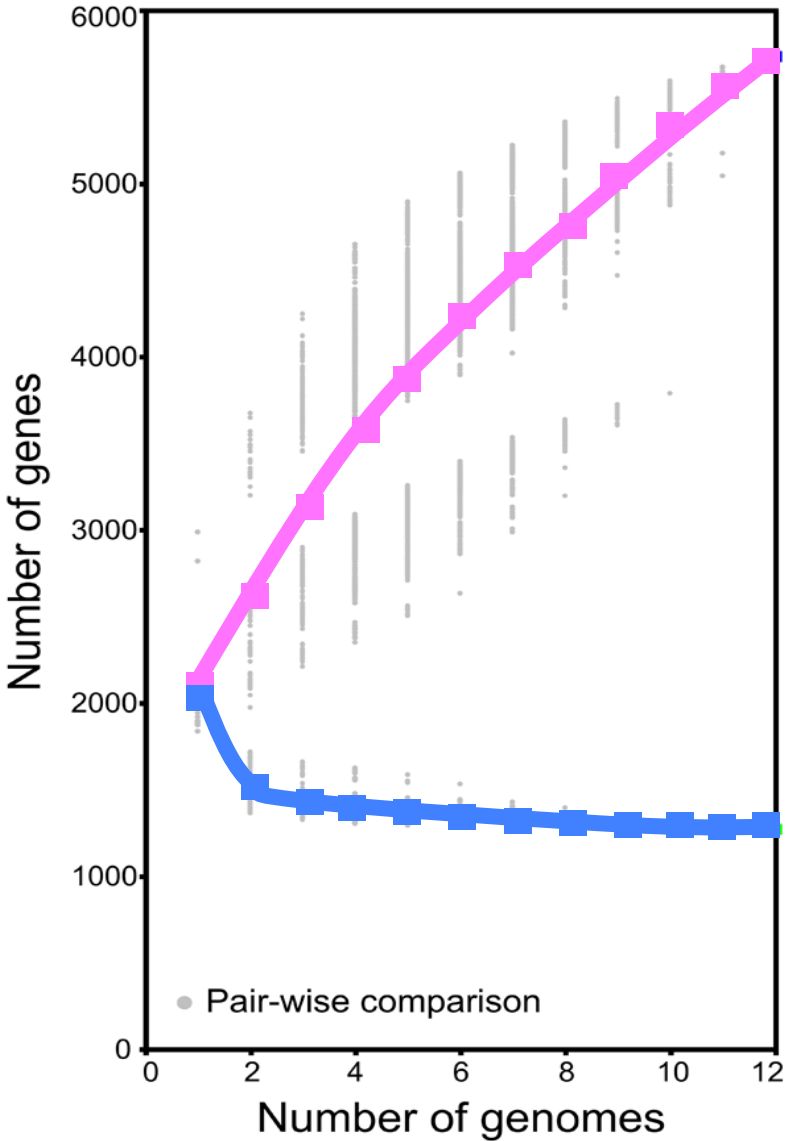
What is the global pan-genome?

1250

Shared Core Genes
(now about 1000)

Kettler, Martiny et al. 2007

Global Pan-genome is huge



Total of 57,792 genes

1250 Core Genes

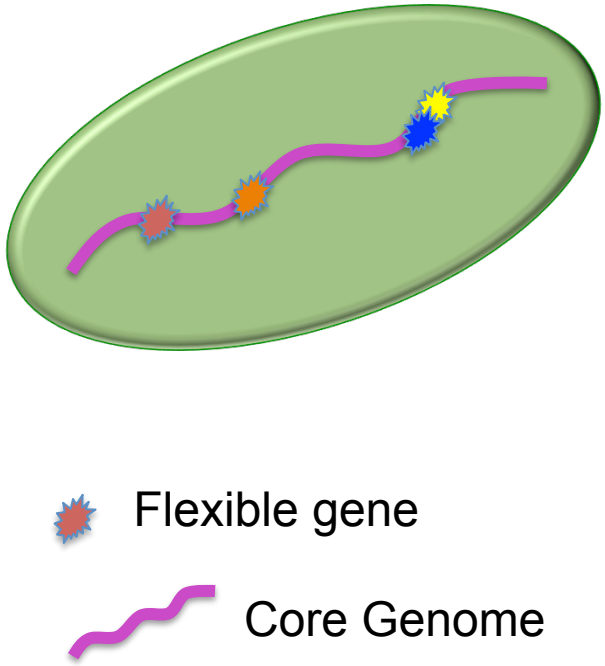
Baumdicker et al 2012. The infinitely many genes model for the distributed genome of bacteria. Genome Biol Evol. doi: 10.1093/gbe/evs016

FLEXIBLE genes give us clues as to environmental pressures

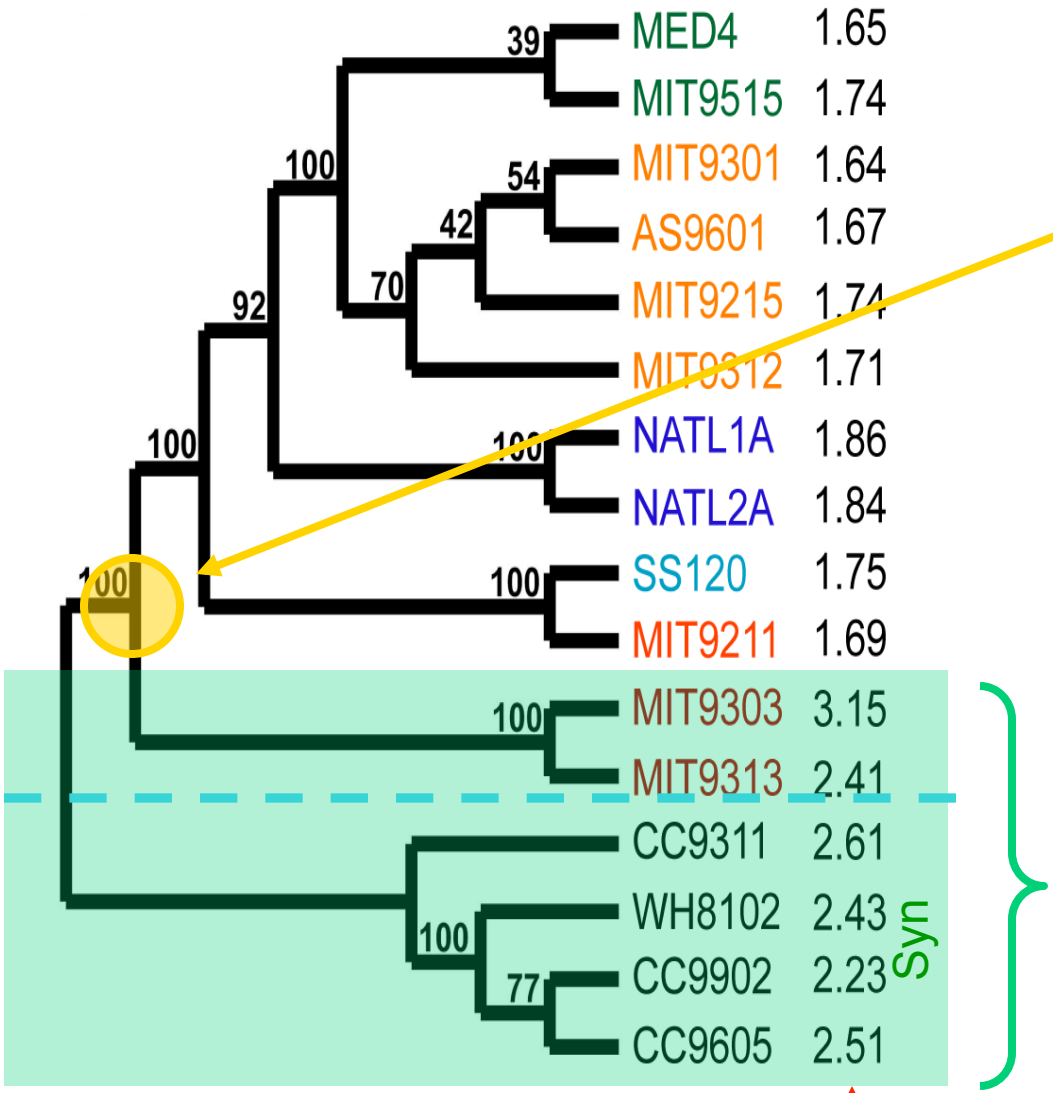
- Viral defense
- Nutrient acquisition
- Stress responses

- 80 % from distant phyla

➢ a lot of gene swapping going on out there!



Genome size in *Prochlorococcus* is variable



Systematic Genome Reduction? NO

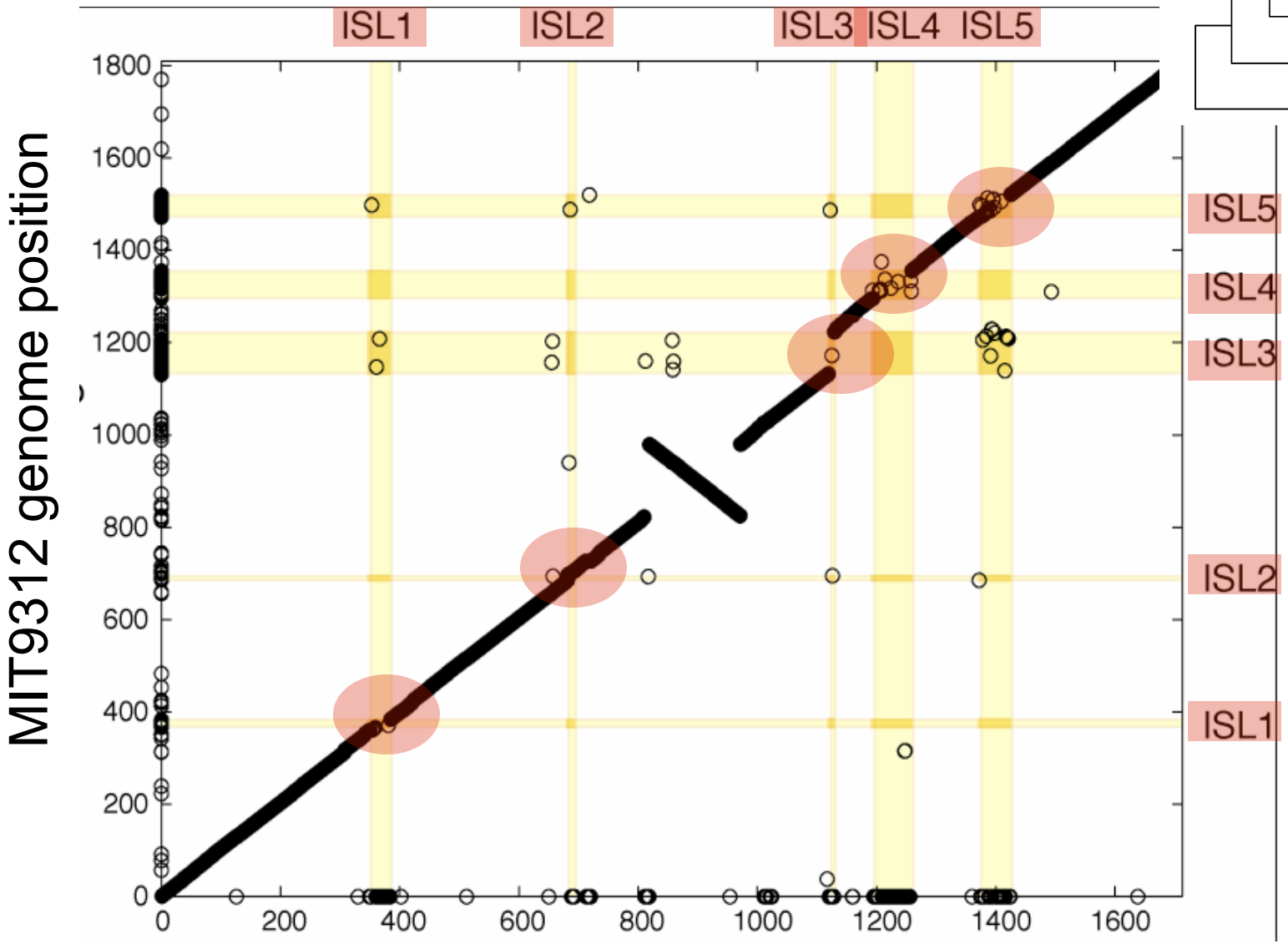
small genomes

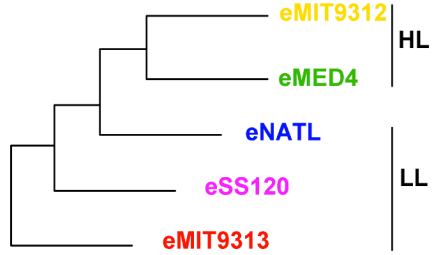
Genes are gained and lost – with most of the action in the "leaves of the tree"

large genomes

Kettler, Martiny et al. 2007

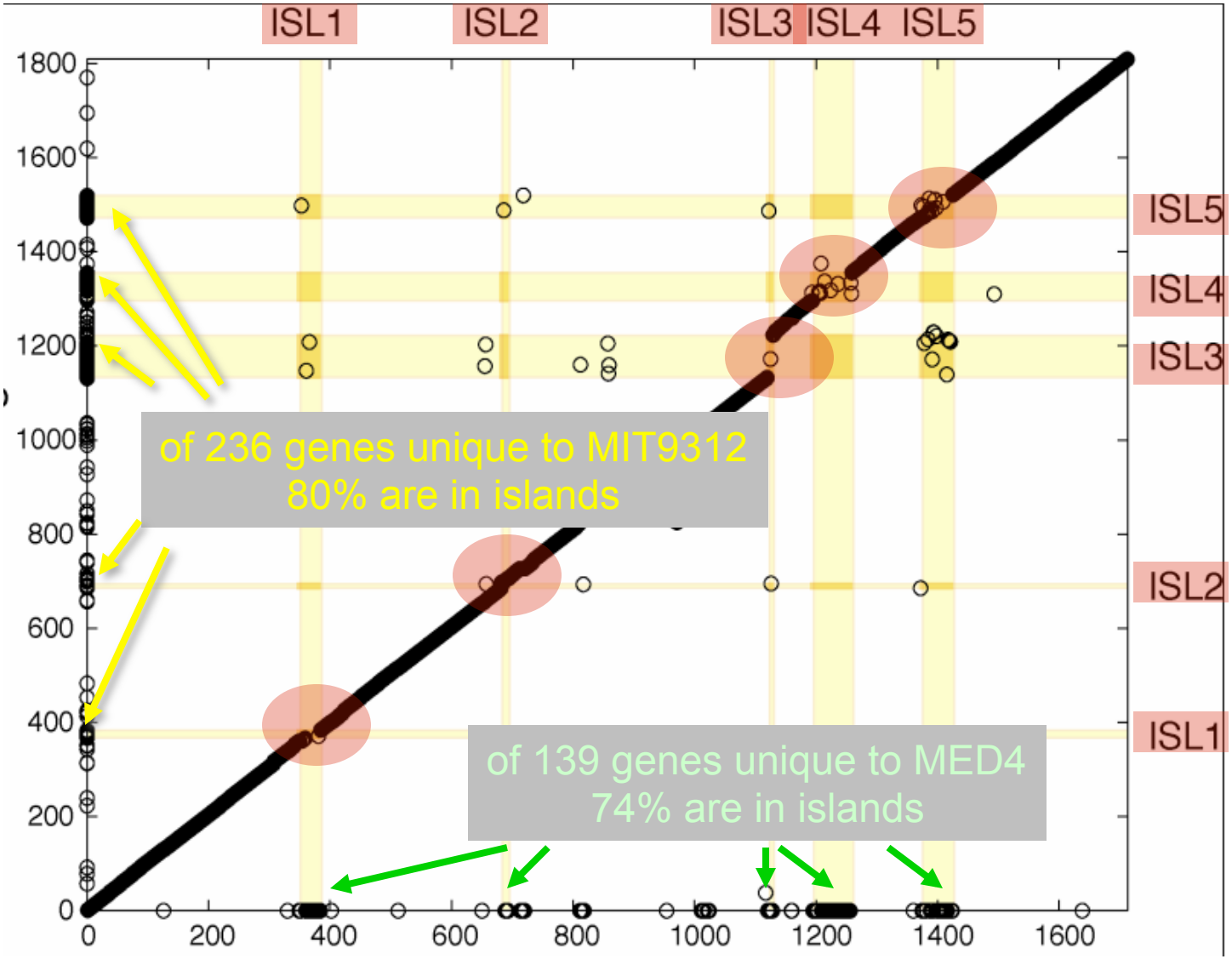
Variability focused in genomic islands





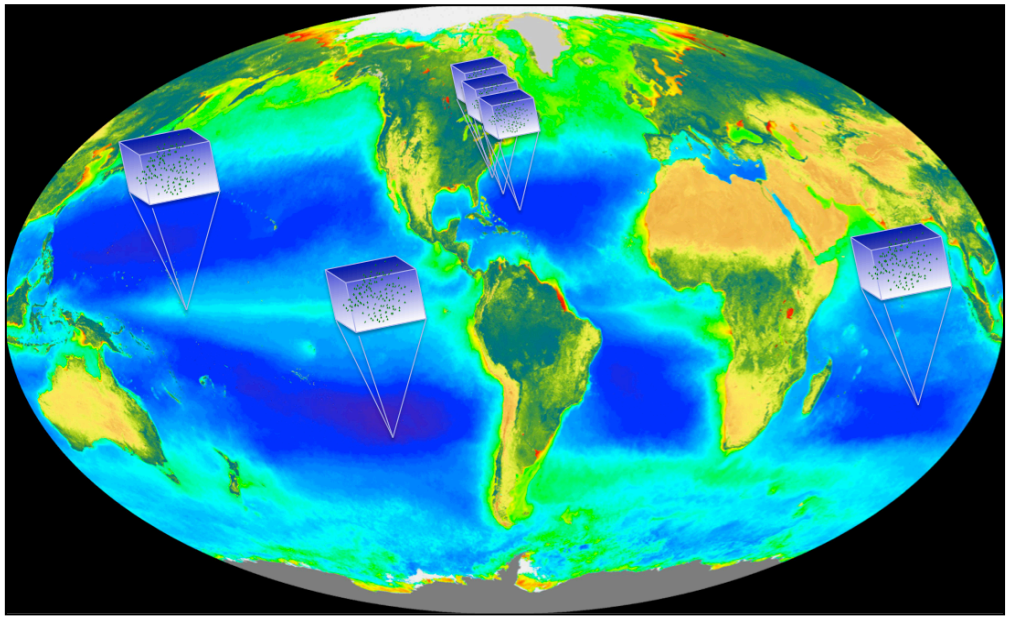
Coleman et al. 2006

Variability focused in genomic islands

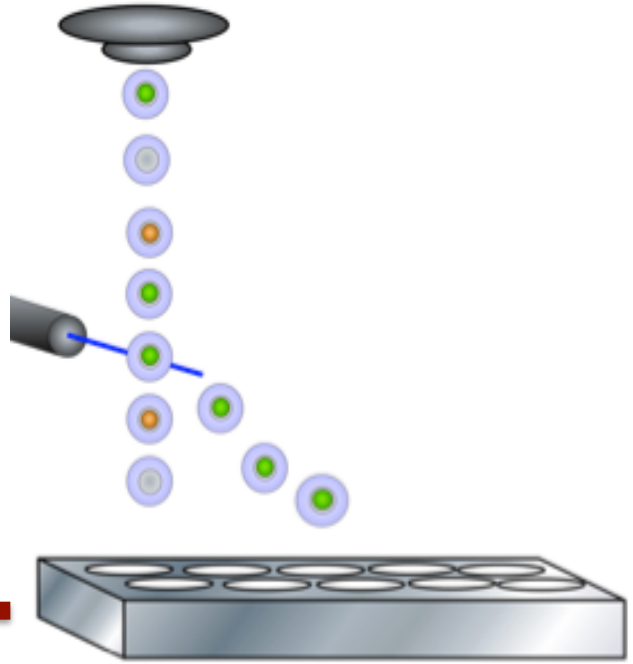


Coleman et al. 2006

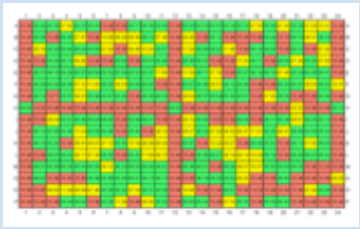
Exploring the full diversity through single cell genomics of wild *Prochlorococcus*



Flow Sort individual cells



DNA Amplification

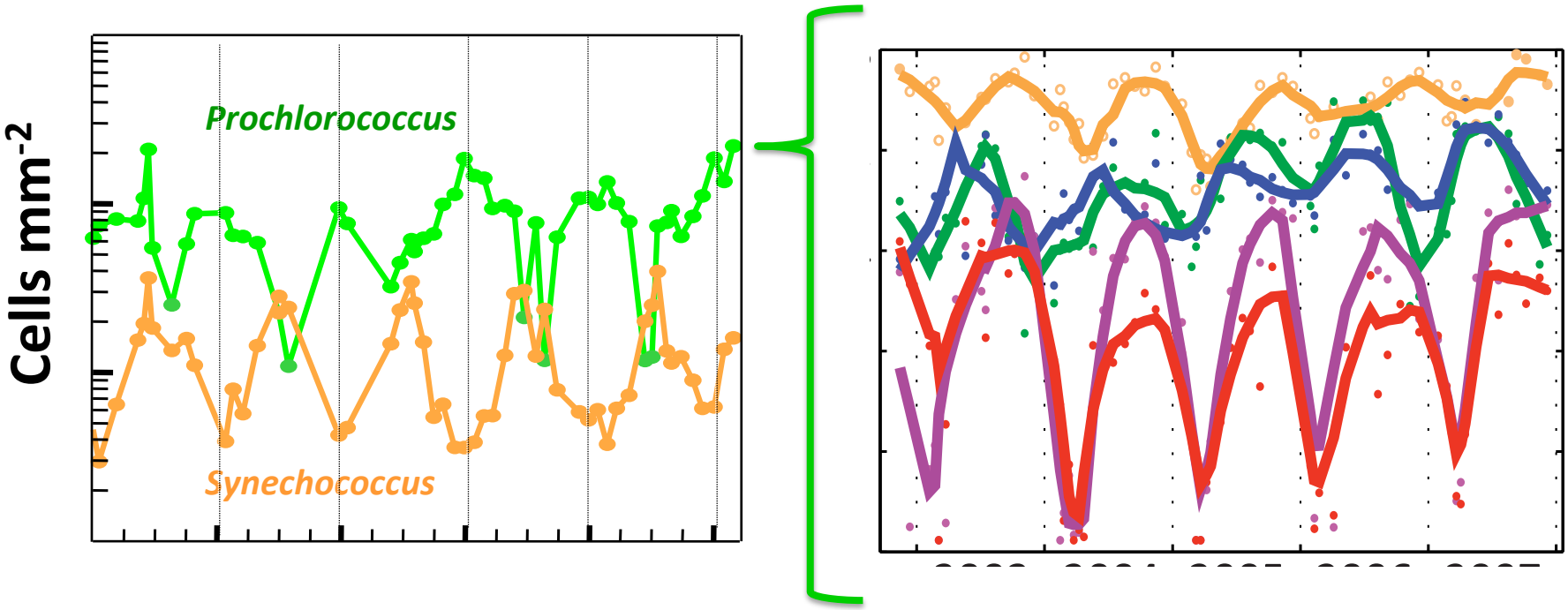


Select cells for sequencing

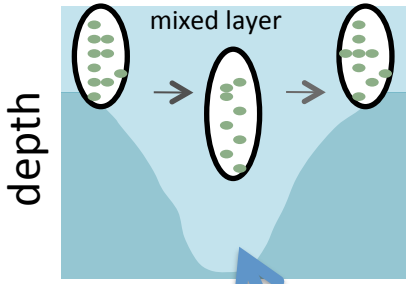
Sequence Genomes

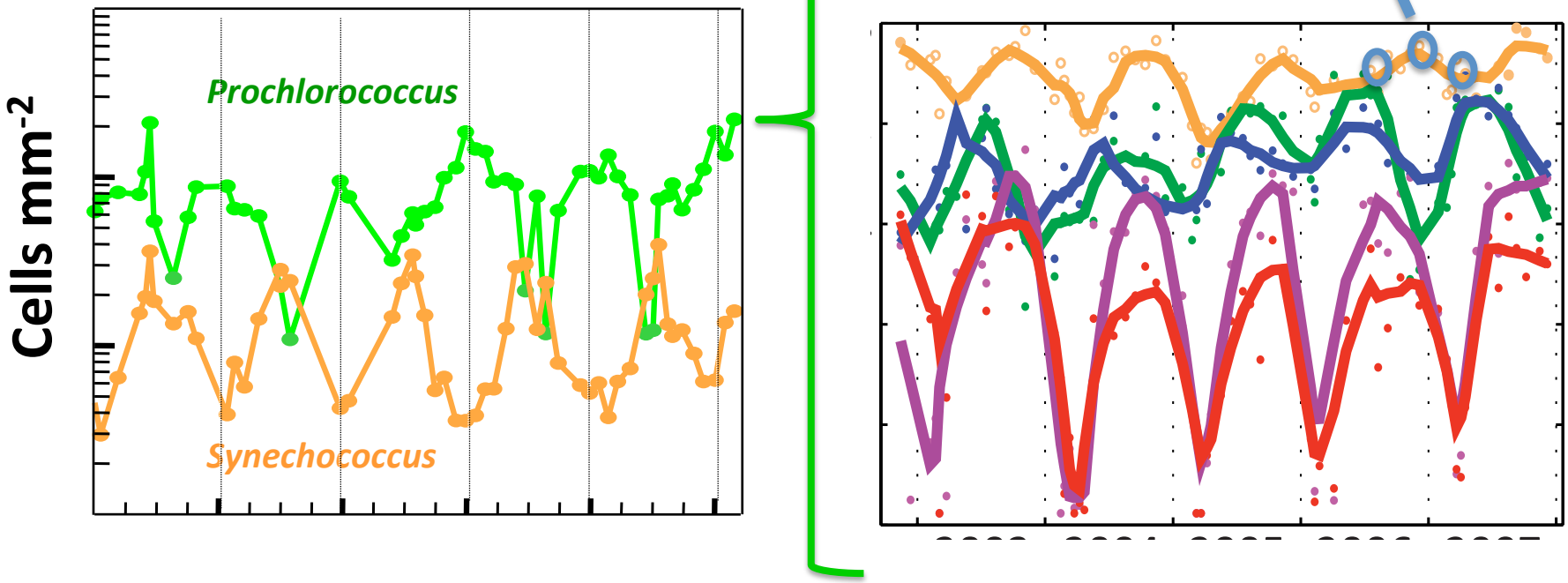
Rodrigue, Malmstrom, et al & Chisholm 2009

Remember this?

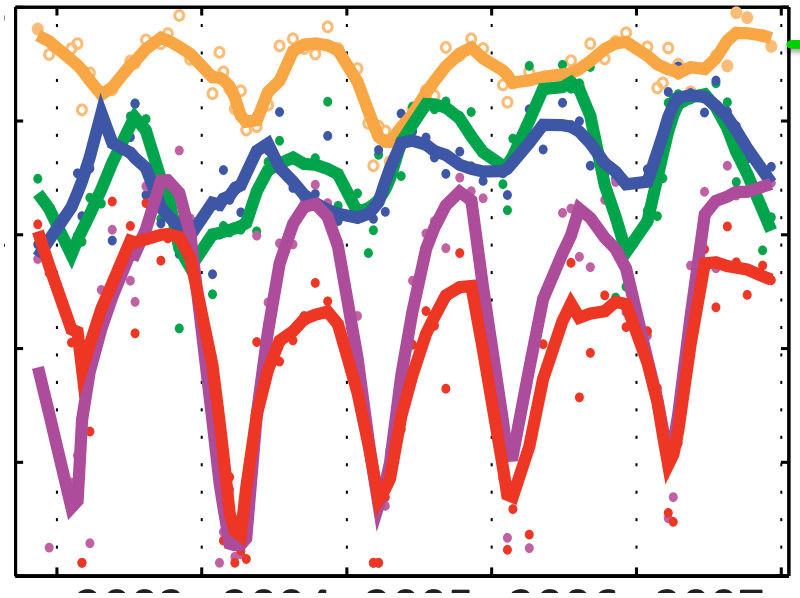


Thousands of single cell rRNA ITS sequence, and hundreds of (partial) genomes





Astounding Diversity!
(layer upon layer…leading to stability)



REPORTS

Single-Cell Genomics Reveals Hundreds of Coexisting Subpopulations in Wild *Prochlorococcus*

Nadav Kashtan,[1]* Sara E. Roggensack,[1] Sébastien Rodrigue,[1,2] Jessie W. Thompson,[1] Steven J. Biller,[1] Allison Coe,[1] Huiming Ding,[1,3] Pekka Marttinen,[4] Rex R. Malmstrom,[5] Roman Stocker,[1] Michael J. Follows,[6] Ramunas Stepanauskas,[7] Sallie W. Chisholm[1,3]*

- Roughly 5000 new genes added to pan-genome
- 'Genomic backbones' (core allele variation + associated flexible genes)

Kashtan et al 2014

Astounding Diversity!
(layer upon layer…leading to stability)

- Populations well-mixed 10 km^2 x 3 m (1 week)
- Each backbone sub-population > 10^{13} cells
- ≈ Effective Population size

REPORTS

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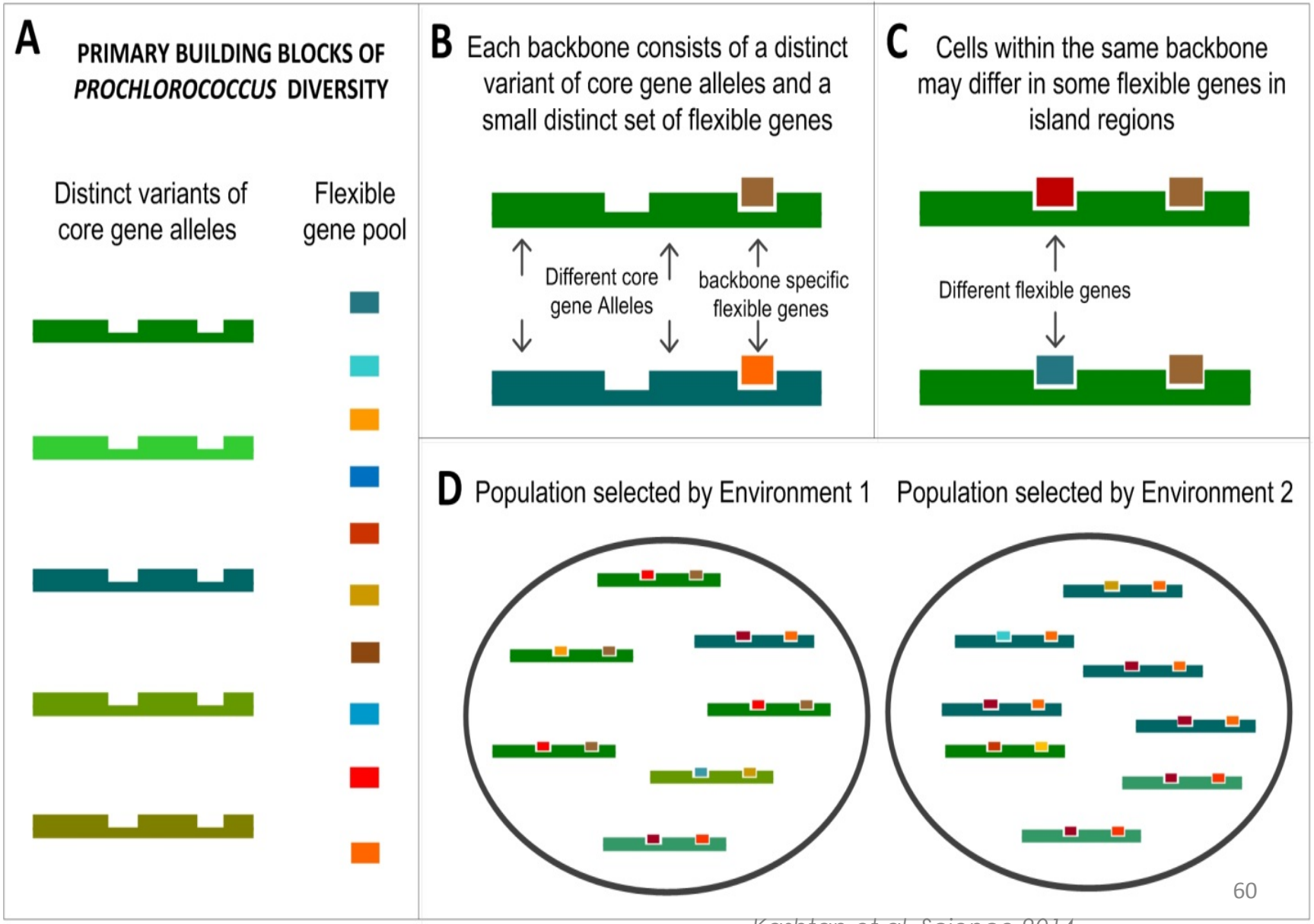
Kashtan et al 2014

The assembly of diversity



Kashtan et al Science 2014

Overview

- History
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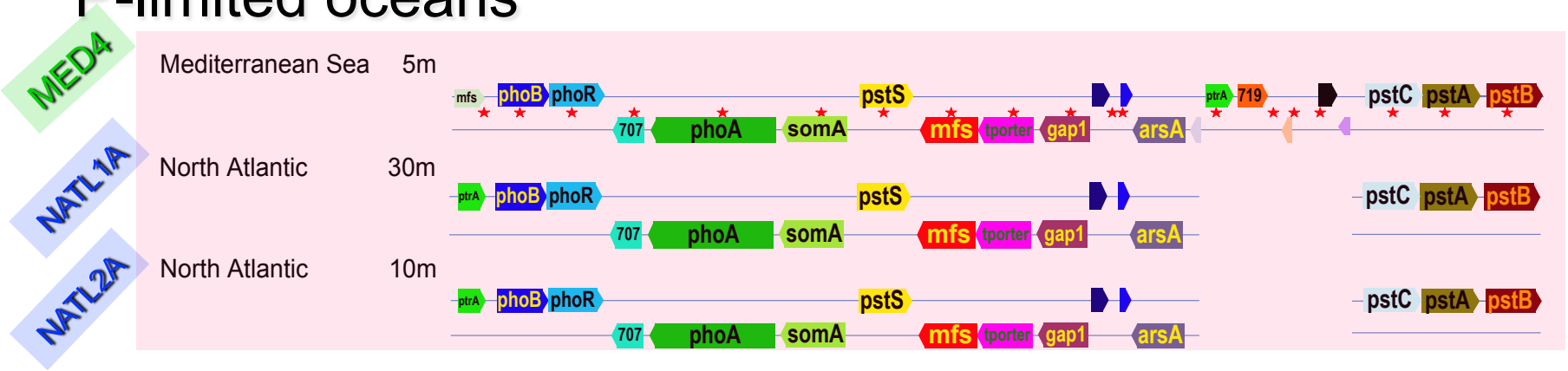
Prochlorococcus is P–challenged

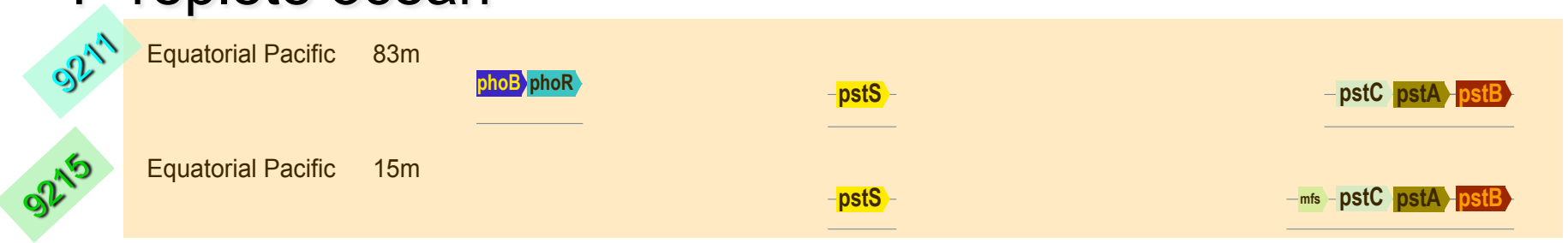
- 13 amole P per cell
- N:P Ratio higher than Redfieldian (21-62)
  (Bertilsson et al 2003)
- Sulfo- instead of Phospho- lipids
  (Van Mooy et al 2006)
- 96% P is in nucleic acids
  (Waldbauer 2010)

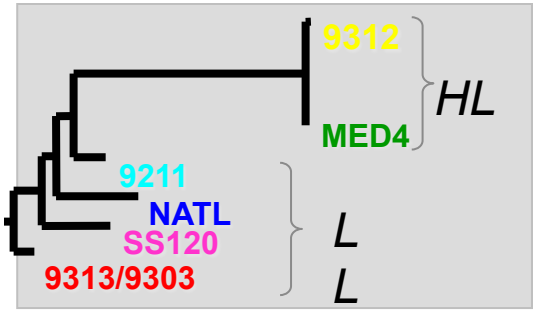
phoBR genomic region (phosphorus acquisition) in different strains is dictated by ocean of origin and *not* ribotype

P-limited oceans



P-replete ocean

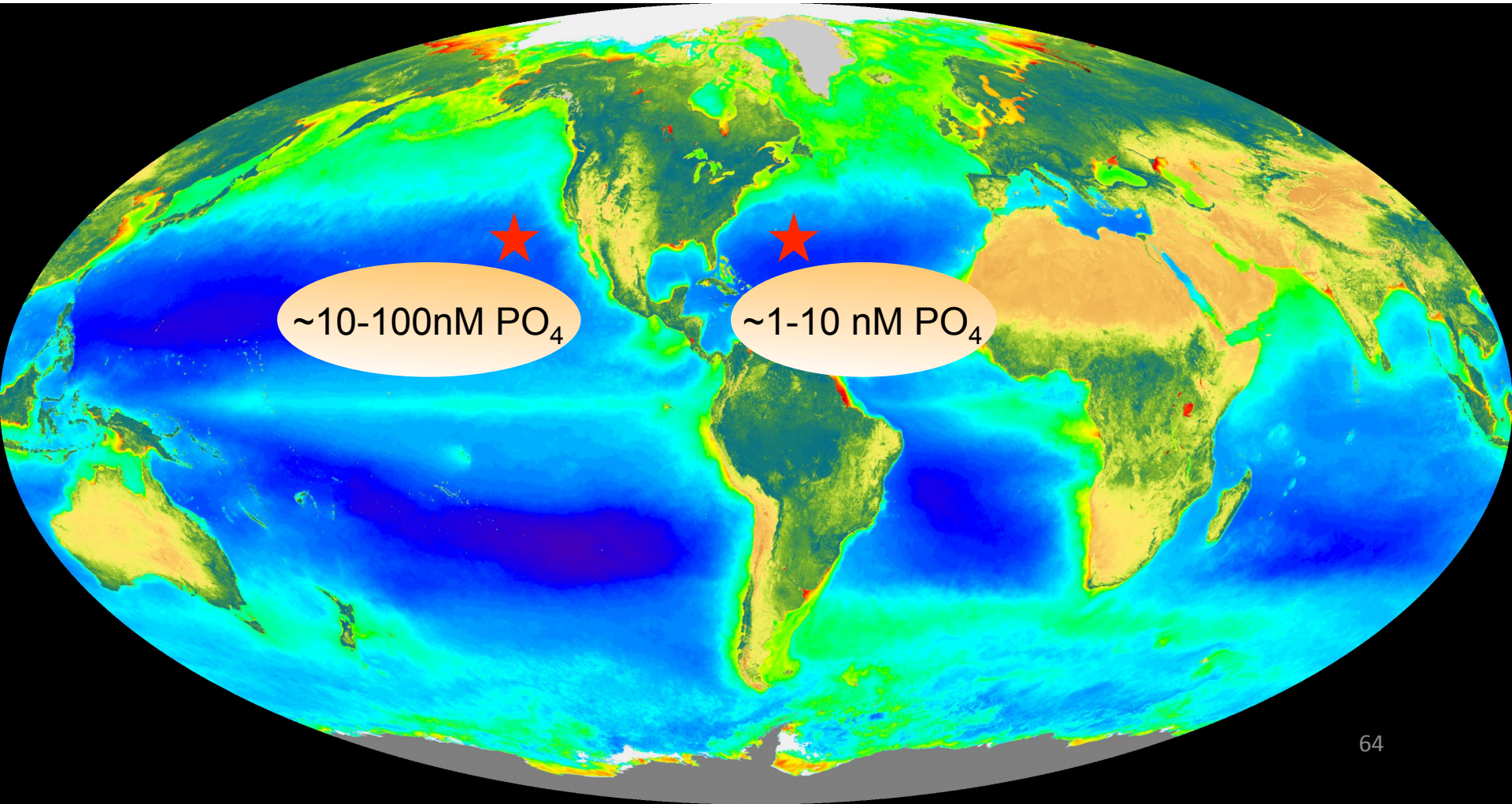




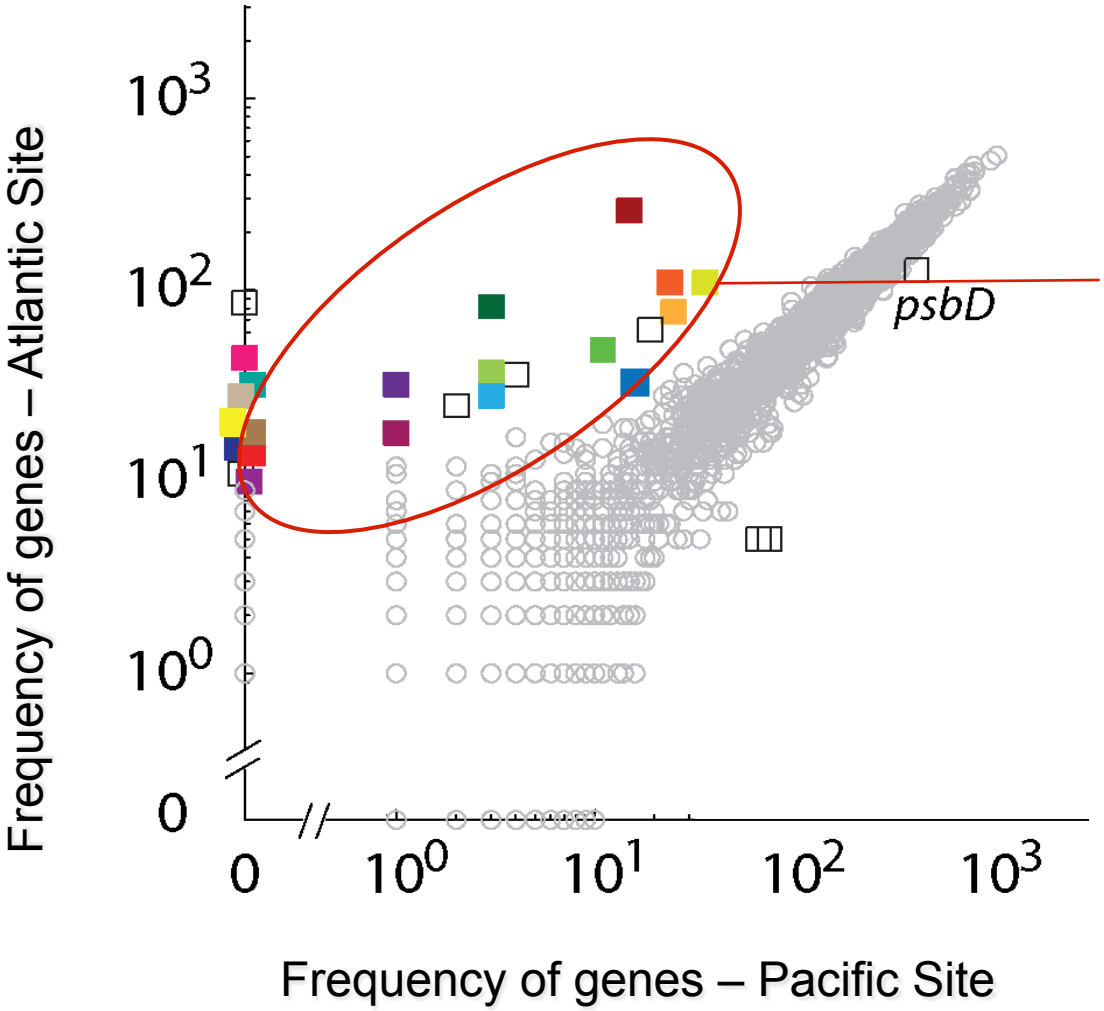
Martiny, Coleman et al 2006

Martiny, et al 2009

Back to the oceans…
Comparing the *Prochlorococcus* populations in the Atlantic and Pacific



For *Prochlorococcus*, the only major difference between the two ecosystems is …



P- acquisition genes

+

Arsenate reductase and arsenite efflux genes

Coleman and Chisholm 2010

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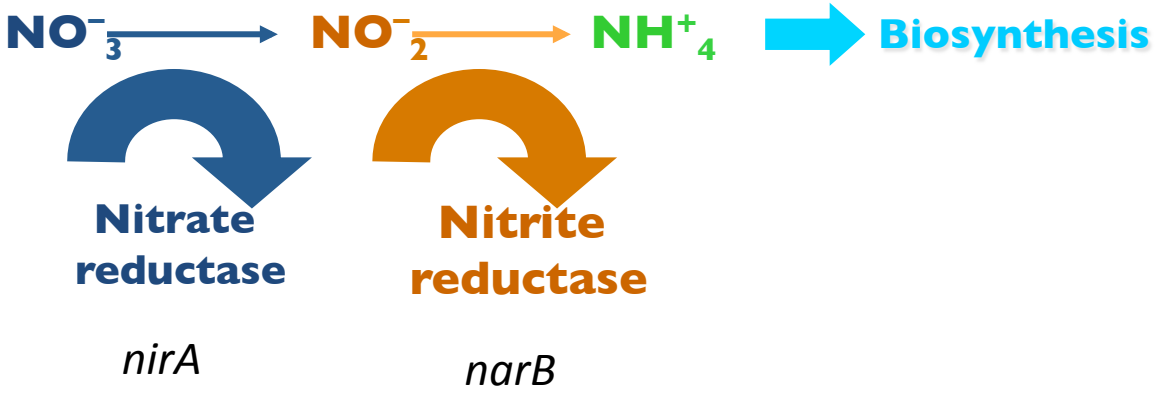
Prochlorococcus and N sources:

Synechococcus (like "the ancestor")



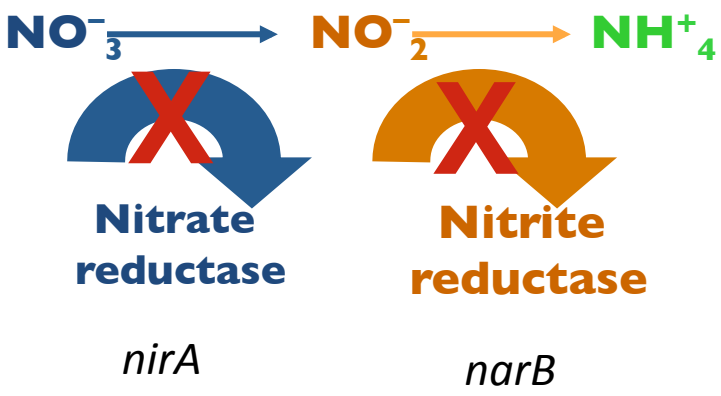
The *Prochlorococcus* dogma (based on cultured isolates):

- All *Prochlorococcus* can use NH_4
- Some can use NO_2
- **None can use NO_3 (odd…)**

Moore et al 2002

Prochlorococcus and N sources:

Synechococcus (like "the ancestor")



13 Genomes confirmed loss of reductase genes in Prochlorococcus

Moore et al 2002

Prochlorococcus and N sources:

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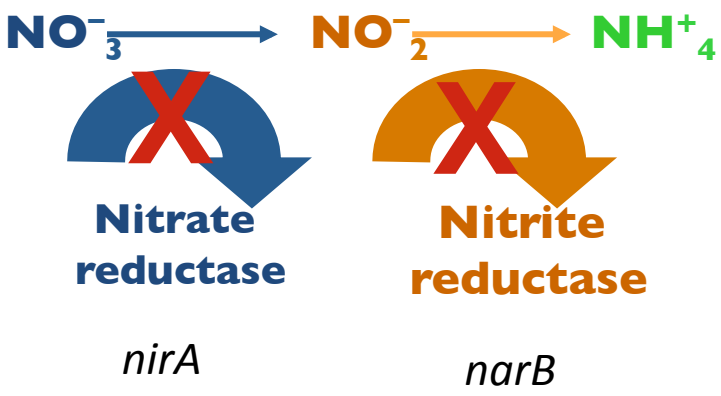


13 Genomes confirmed loss of reductase genes in Prochlorococcus

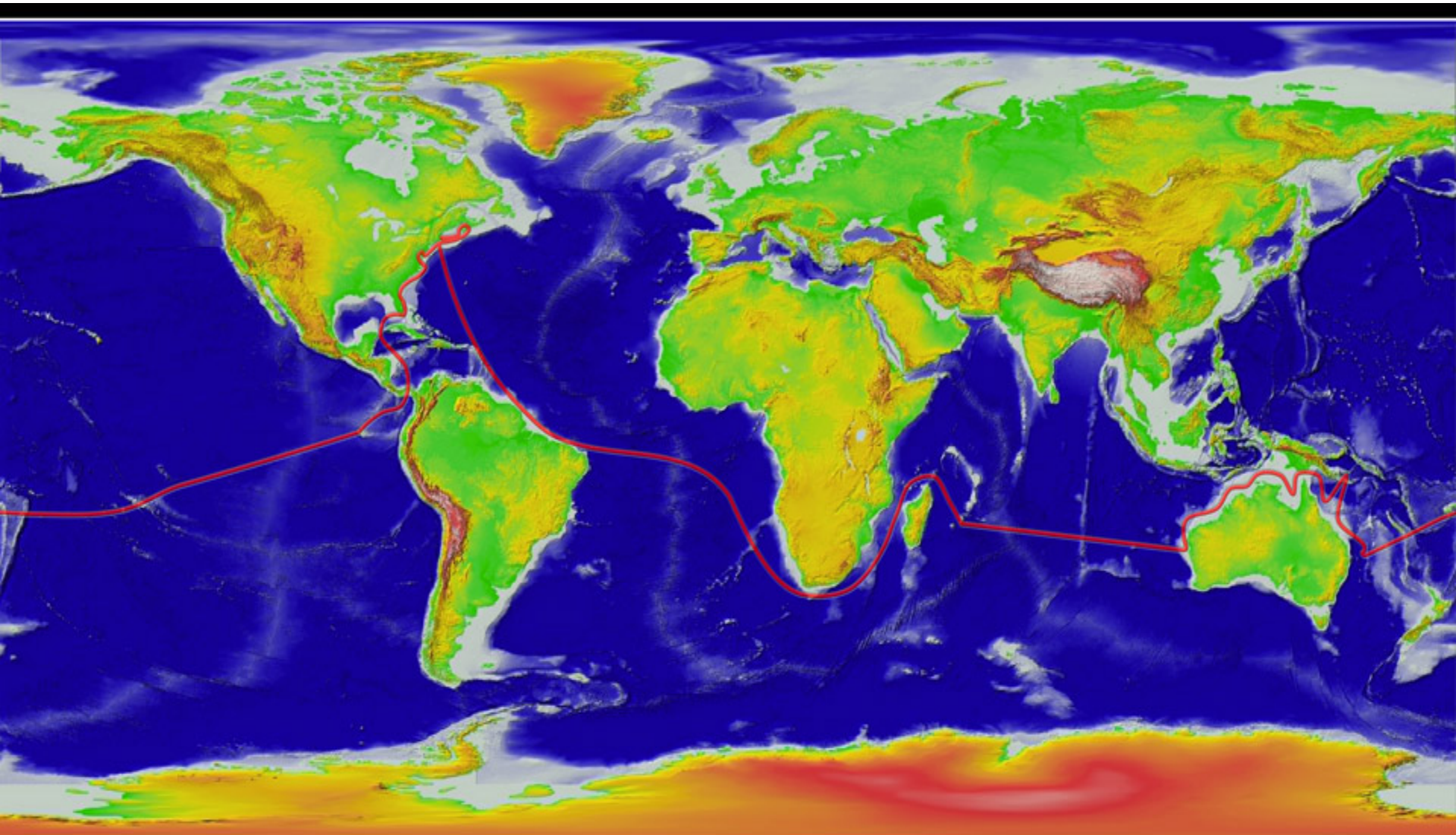
BUT… There was some evidence for NO^3 utilization

CULTURES Williams EZ, Campbell L, DiTullio G. (1999). ASLO Aquatic Sciences Meeting – reported on NO_3 utilizing *Prochlorococcus*

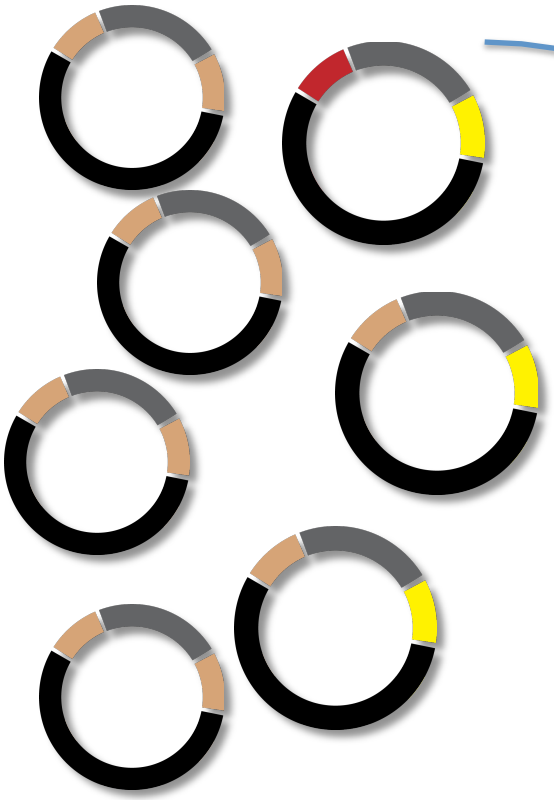
WILD Casey JR, Lomas MW, Mandecki J, Walker DE. (2007). *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophysical Research Letters 34: L10604.

Turning to GOS again…

Adam Martiny and Paul Berube

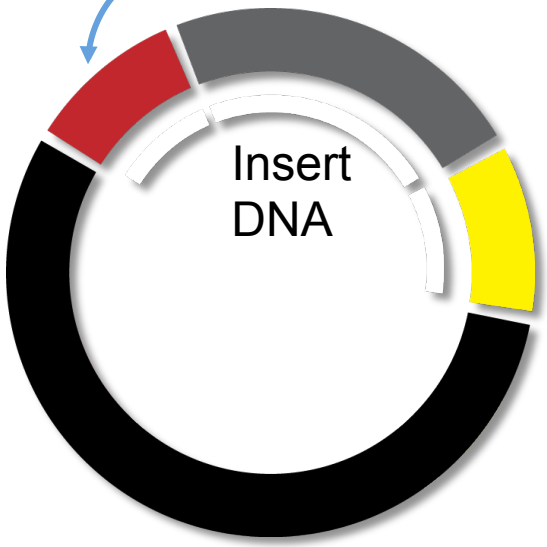


- Recruit all fragments with *narB* gene
- Find those containing known *Prochlorococcus* gene



Cloned DNA fragments from the wild (GOS)

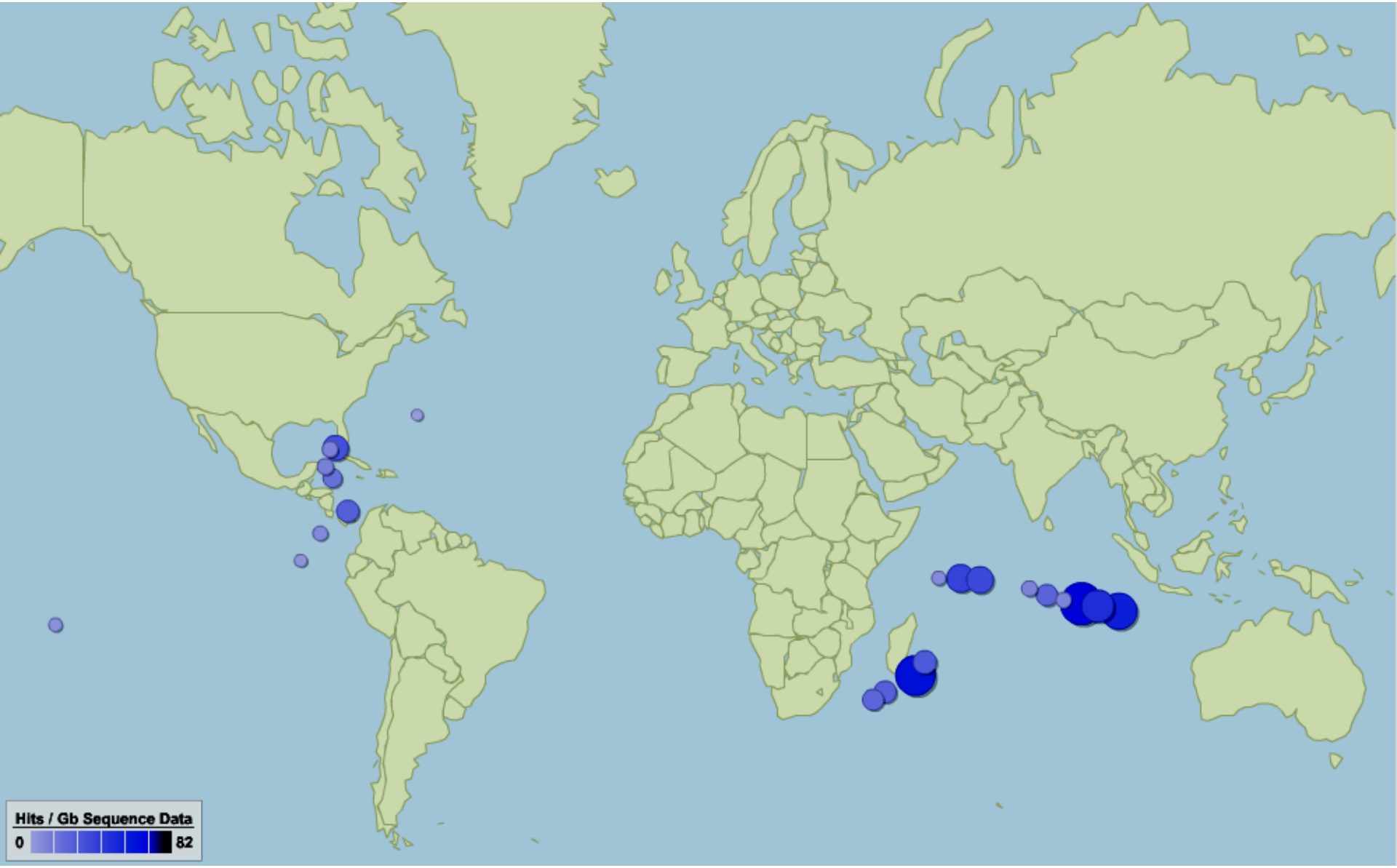
Similar to sequence from *Prochlorococcus* reference genome



Nitrate reductase gene on fragment with known *Prochlorococcus* gene

Martiny et al 2009

Abundance distribution of *Prochlorococcus narB* genes in GOS (they are not everywhere)



Martiny et al 2009

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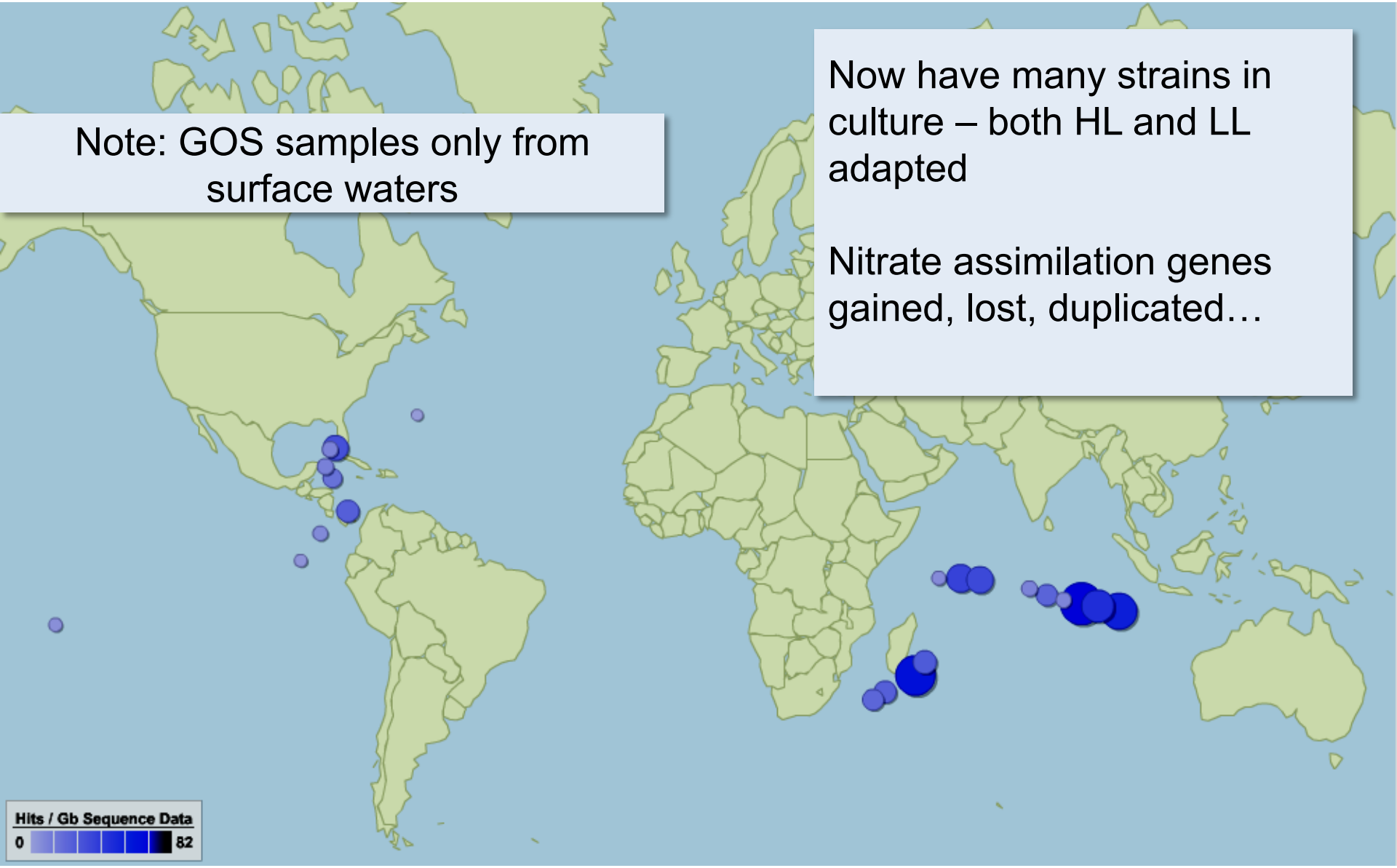
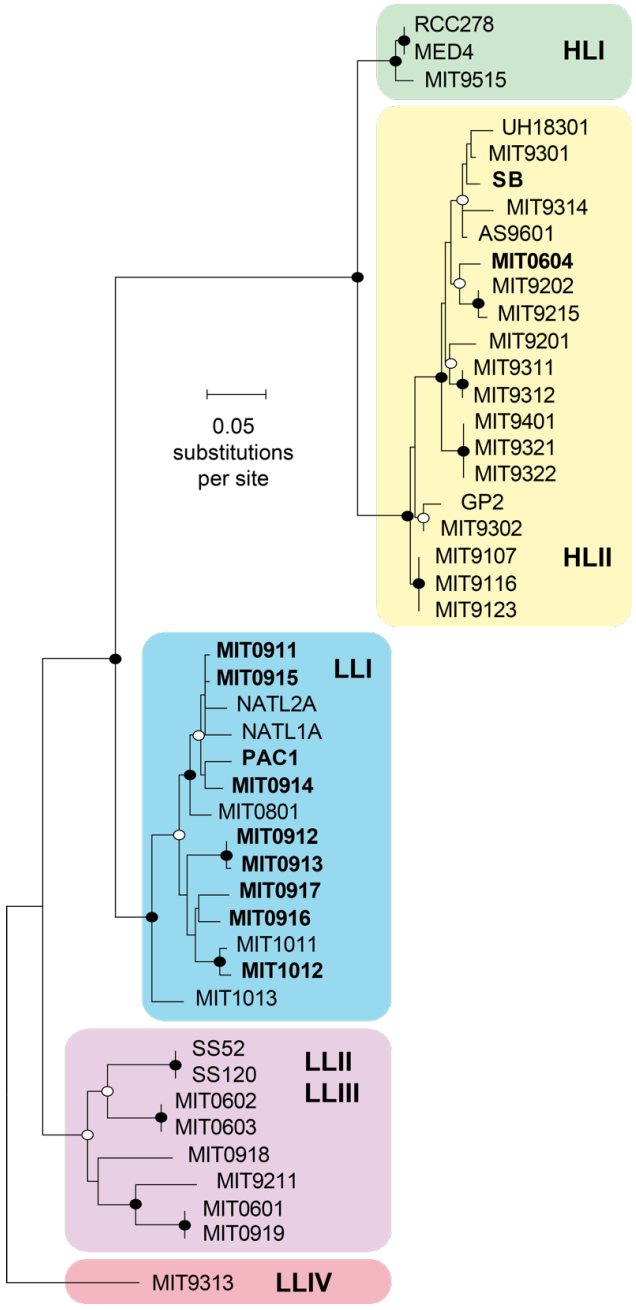


Martiny et al 2009

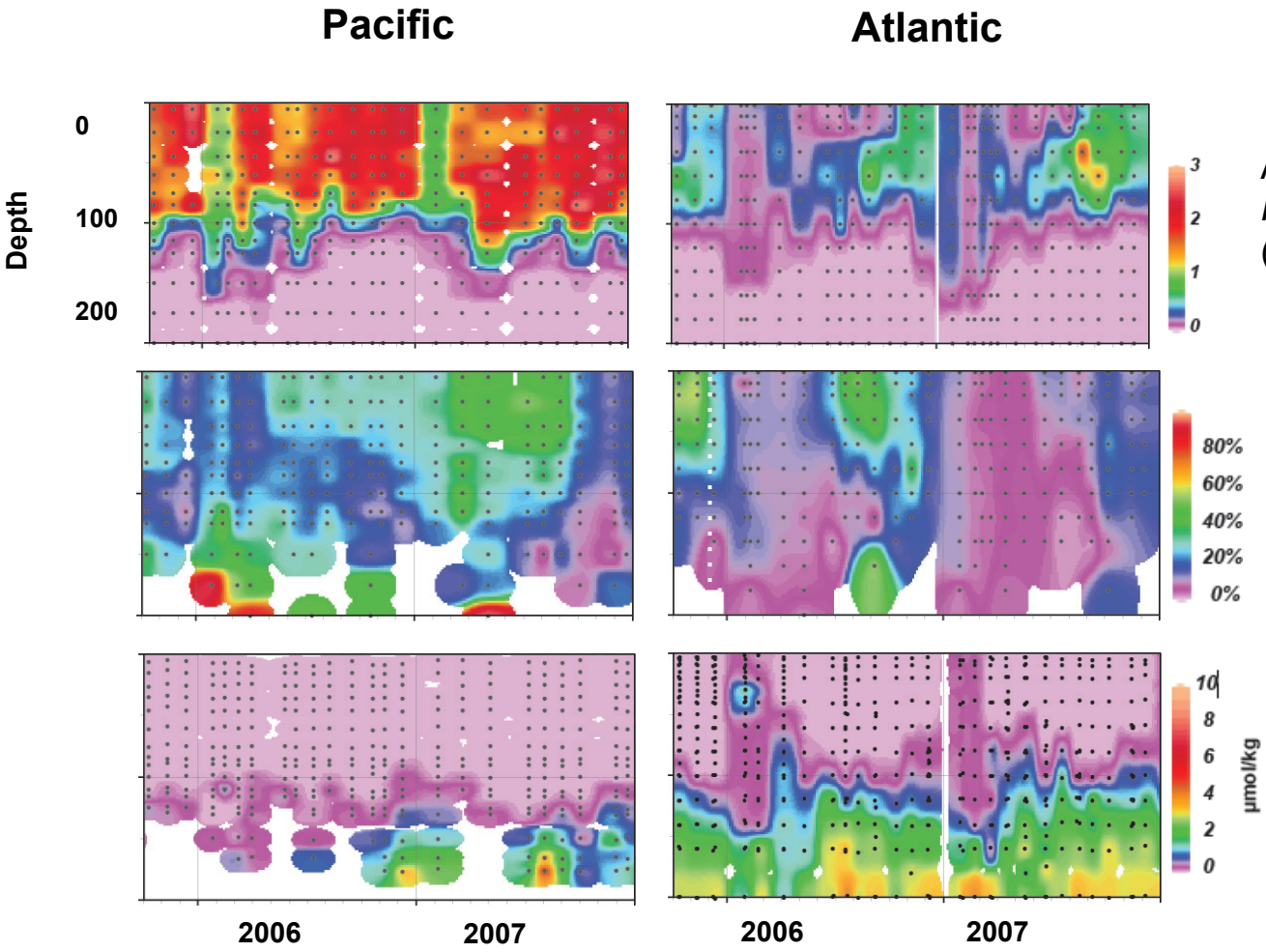


Prochlorococcus isolates (bold) that can utilize nitrate – i.e. contain narB (a number are axenic)

- We suspect that all clades will ultimately have representatives
- This trait, like P-acquisition, does not follow taxonomy

Berube et al. in prep

Dynamics of *narB* - containing *Prochlorococcus* in the wild (they are a fraction of the population)



Abundance High Light-Adapted *Prochlorococcus* Clade HLII (10^5 cells/ml)

% HLII cells that contain *narB* (can utilize nitrate)

Nitrate + Nitrite

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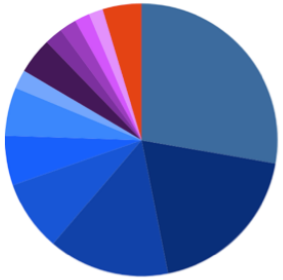
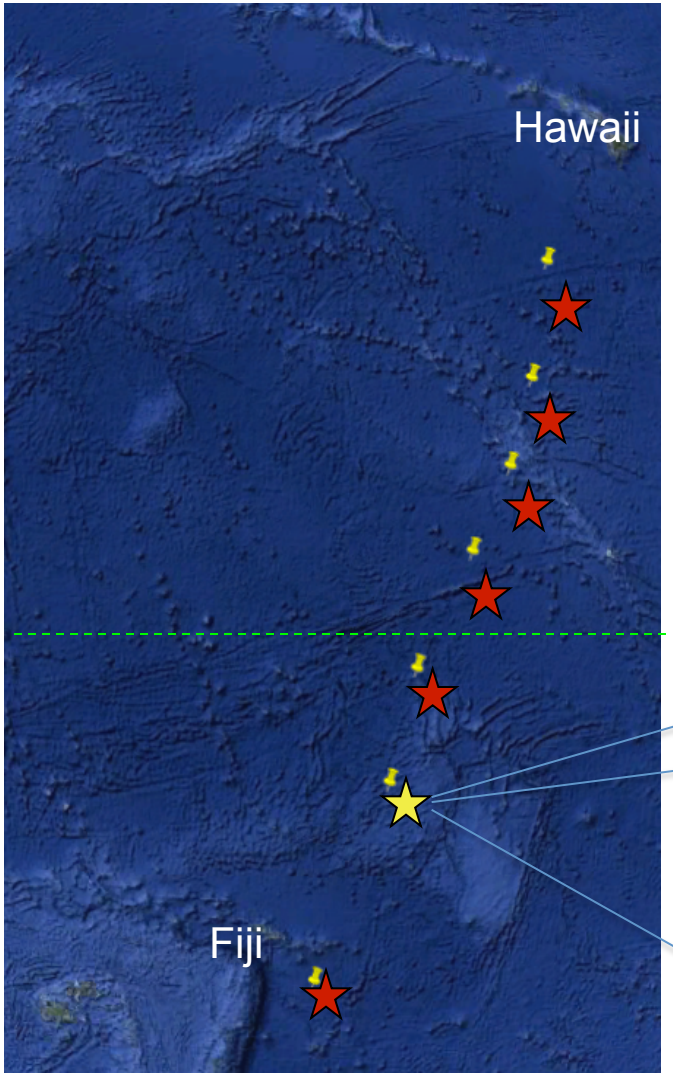
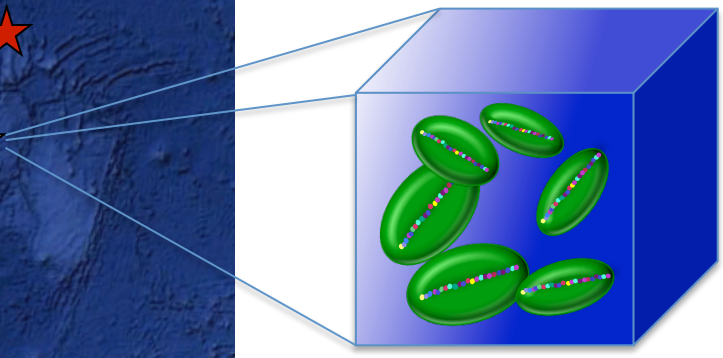
Genomes of 5 Wild *Prochlorococcus* Cells



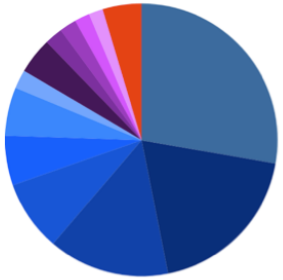


- Expanded recruited GOS reads by 15%
- Added to **hundreds of new genes** to *Prochlorococcus* pan-genome
- New functions



Malmstrom, Rodrigue, 2012

Genomes of 5 Wild *Prochlorococcus* Cells



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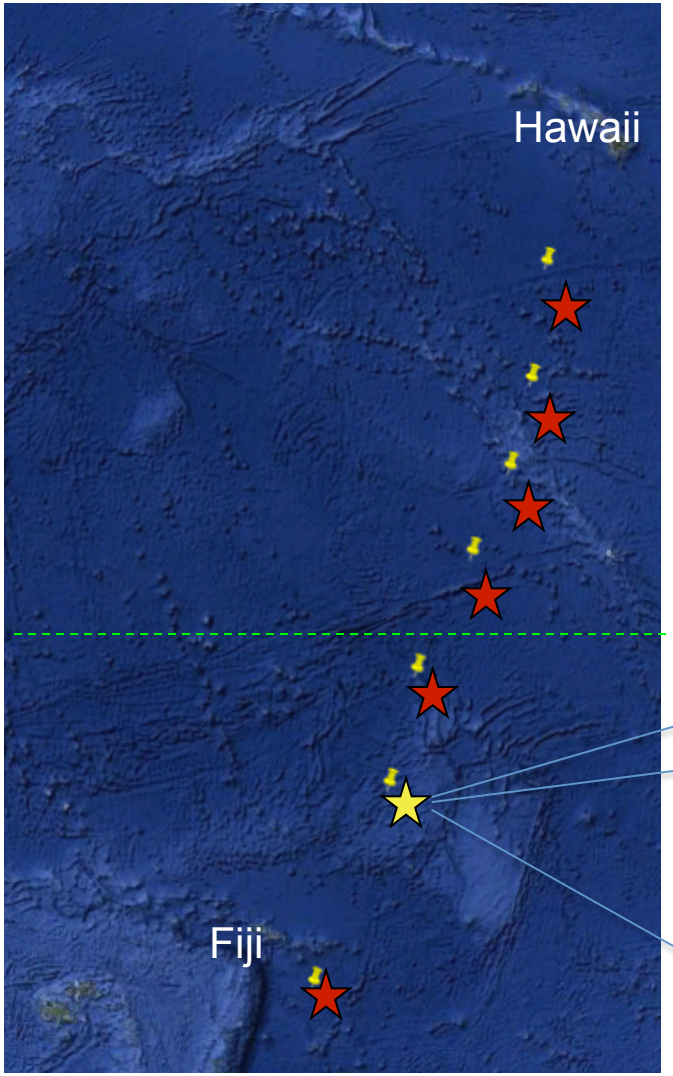
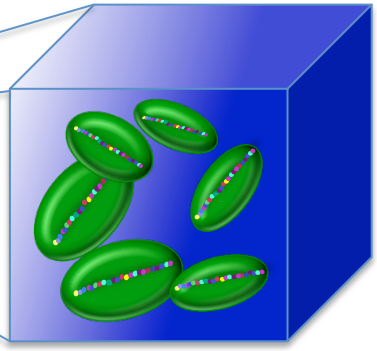
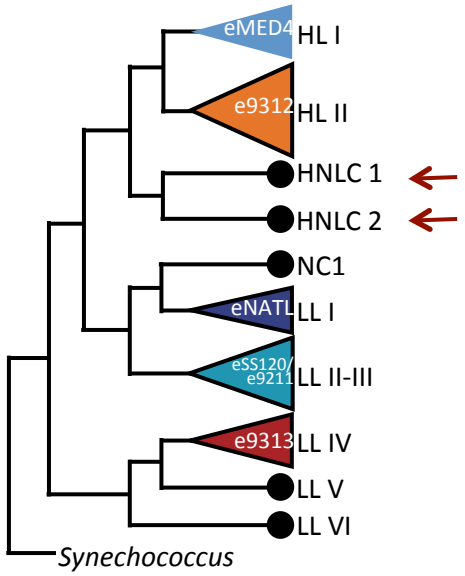


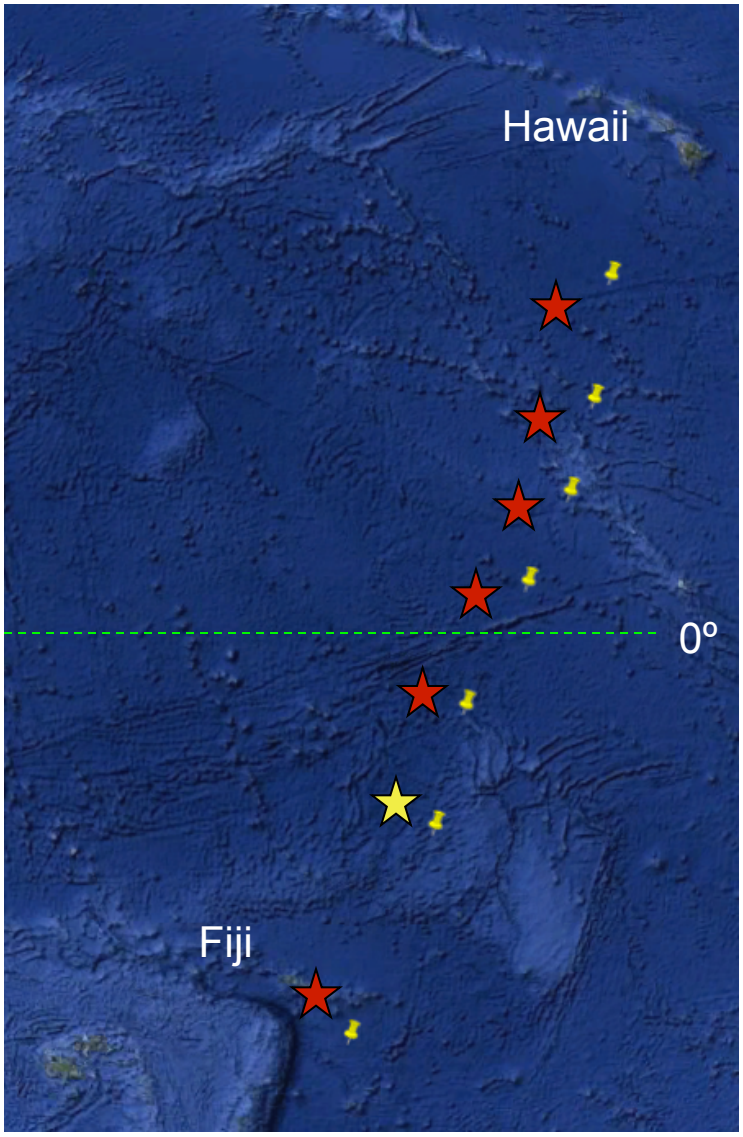


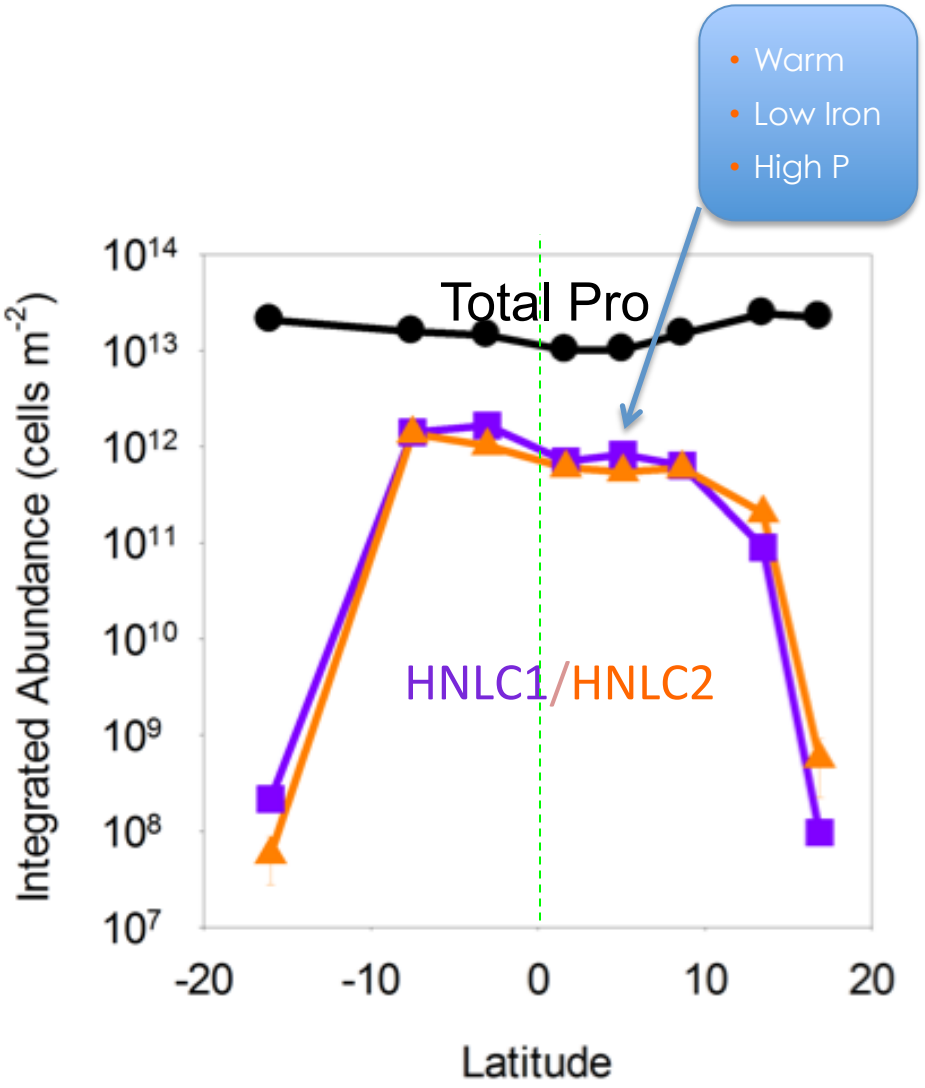


Malmstrom, Rodrigue, 2012

Rusch et al 2010, West et al 2011

New HL clades restricted to equatorial waters





Malmstrom, Rodrigue et al, 2012

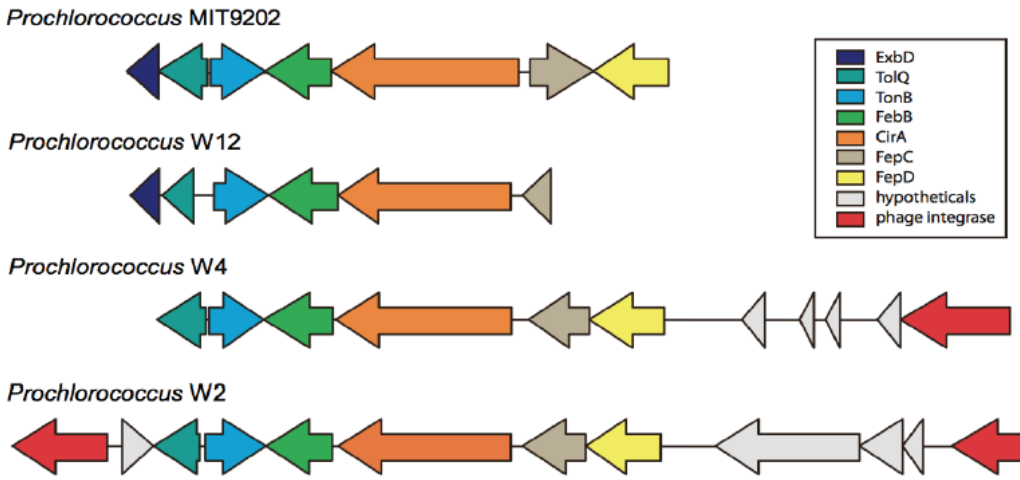
Siderophore transport genes found in wild cells, and in one of our cultures

Culture - MIT9292

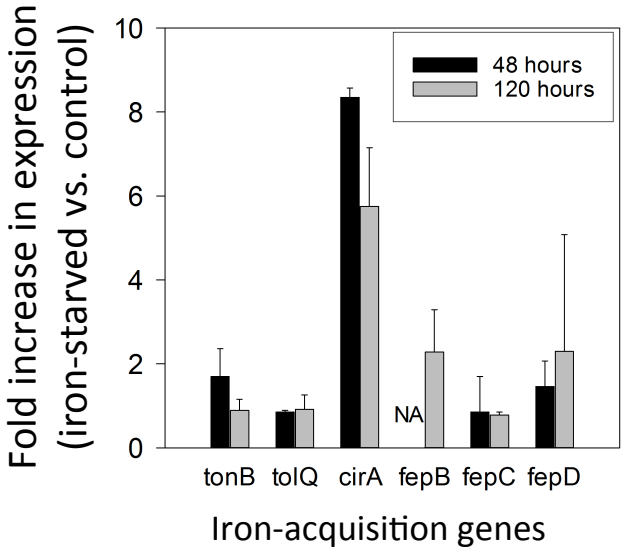
Wild single cell

Wild single cell

Wild single cell



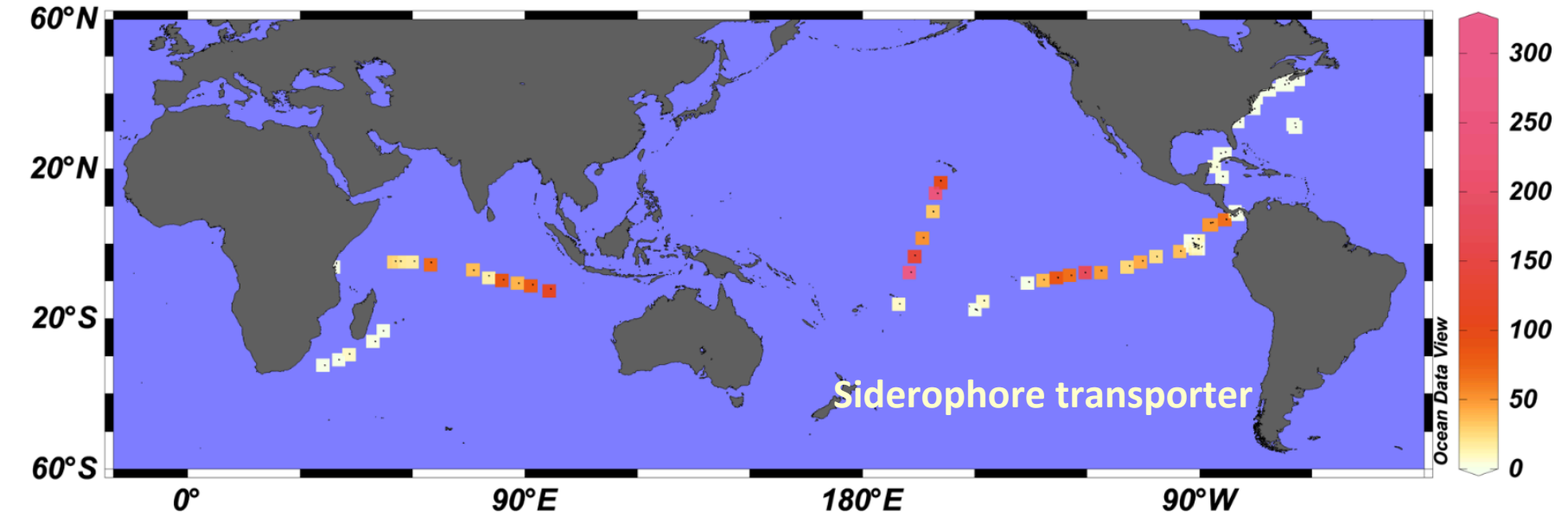
Phage integrase

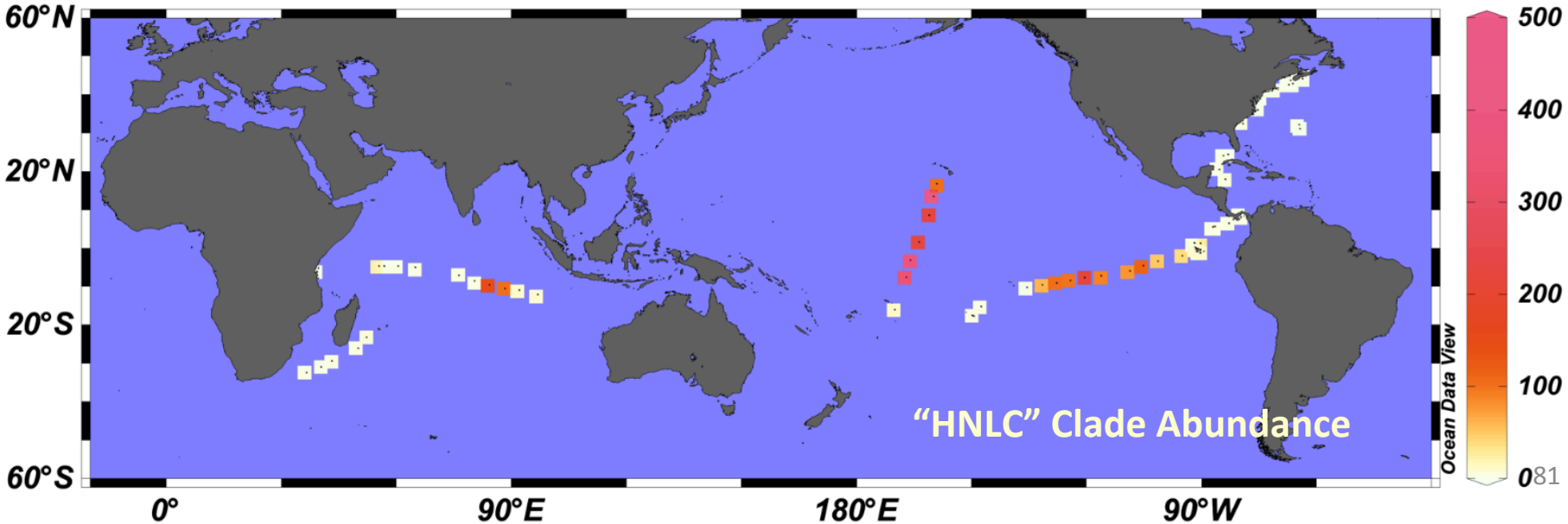


Genes are expressed under conditions of iron starvation in cultures

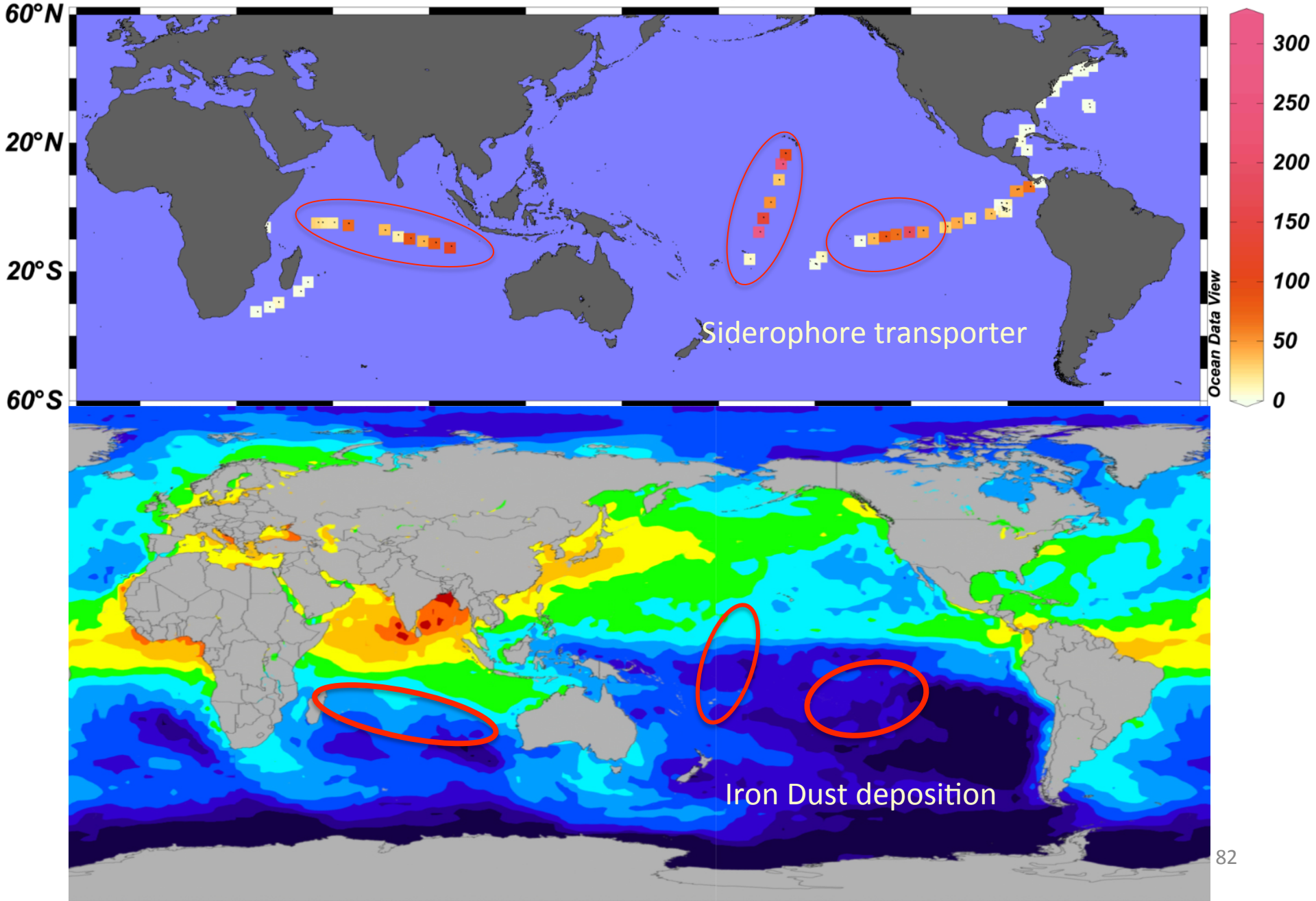
Malmstrom, Rodrigue et al, 2012

Global distributions through GOS metagenomics





Makes sense, so far…



Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
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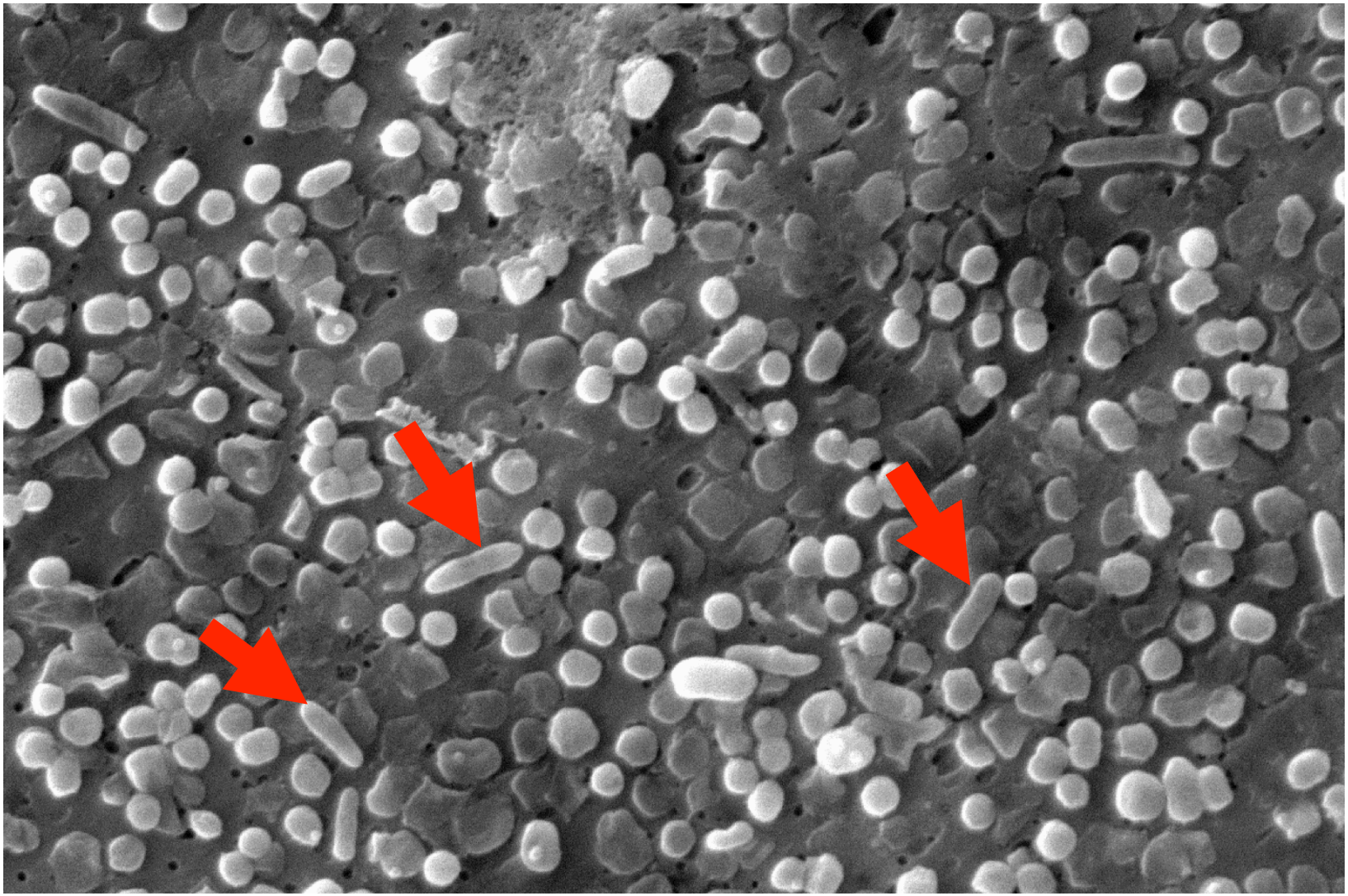
Prochlorococcus loves its heterotrophs

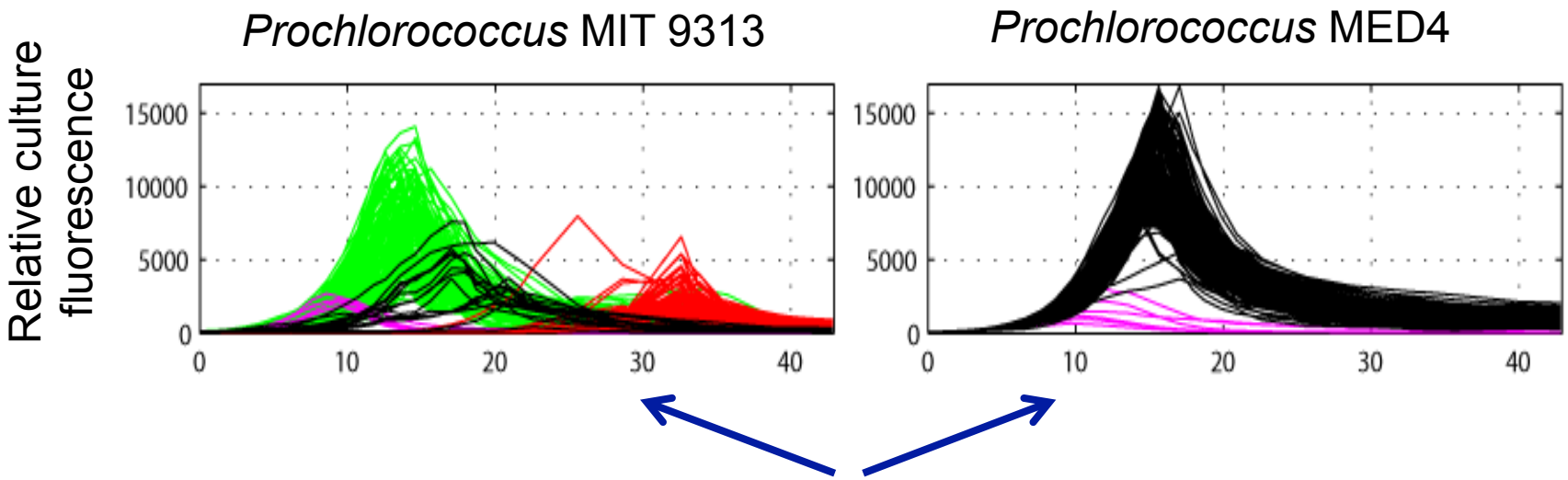


1 μm

Photo by Anne Thompson

…*but not ALL* heterotrophs

No effect of co-culture
enhanced
inhibited

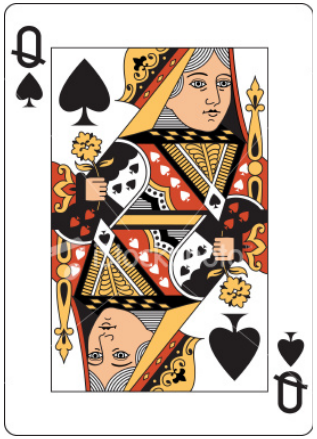


…and different strains respond differently to the same suite of heterotrophs
(a lifetime of PhD theses!)

Daniel Sher

Sher et al, 2011. ISME Journal Feb 2011 5(7):1125-1132. doi: 10.1038/ISMEJ.2011.1

The Black Queen Hypothesis



Jeffrey Morris. Richard Lenski and Erik Zinser

OPINION/HYPOTHESIS

The Black Queen Hypothesis: Evolution of Dependencies through Adaptive Gene Loss

J. Jeffrey Morris,[a,b] Richard E. Lenski,[a,b] and Erik R. Zinser[c]

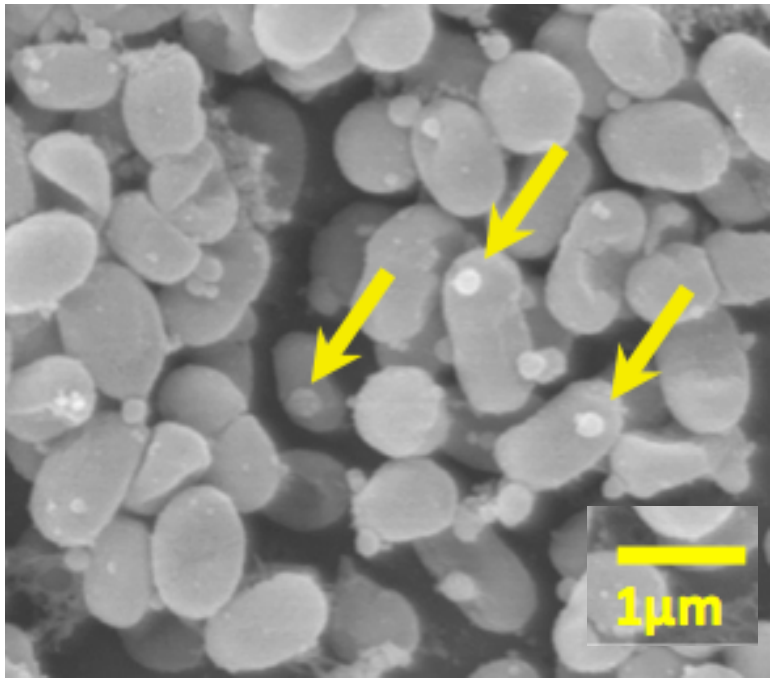
Michigan State University, East Lansing, Michigan, USA[a]; BEACON Center for the Study of Evolution in Action, East Lansing, Michigan, USA[b]; and University of Tennessee, Knoxville, Tennessee, USA[c]

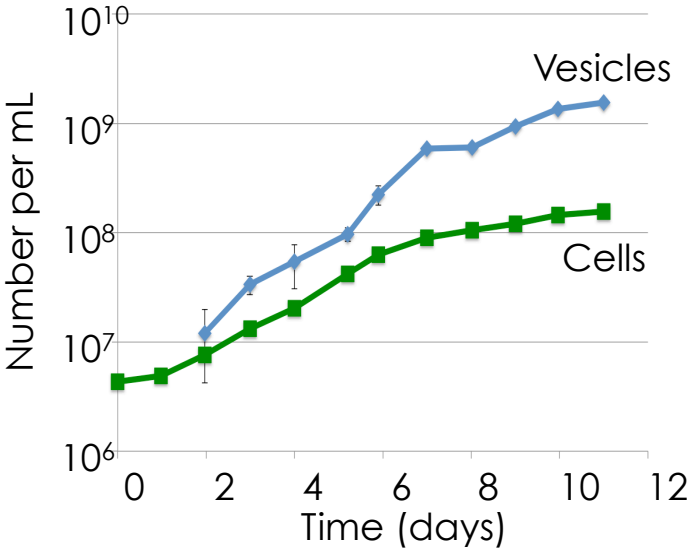
Evolution generates "beneficiaries" of reduced genomic content [*Prochlorococcus*] dependent on leaky "helpers," [heterotrophs that leak catalase-peroxidase] perhaps explaining the observed non-universality of phototrophy, stress resistance, and other cellular functions…

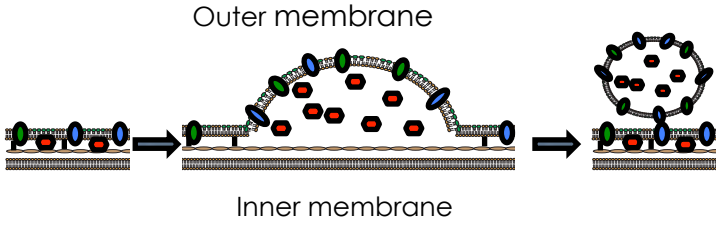
It's a metabolic marketplace…
…a meta-metabolic web

J. Jeffrey Morris, Richard E. Lenski and Erik R. Zinser. 2012. mBio 3(2) doi:10.1128/mBio.00036-12.

Prochlorococcus reveals another new ocean feature







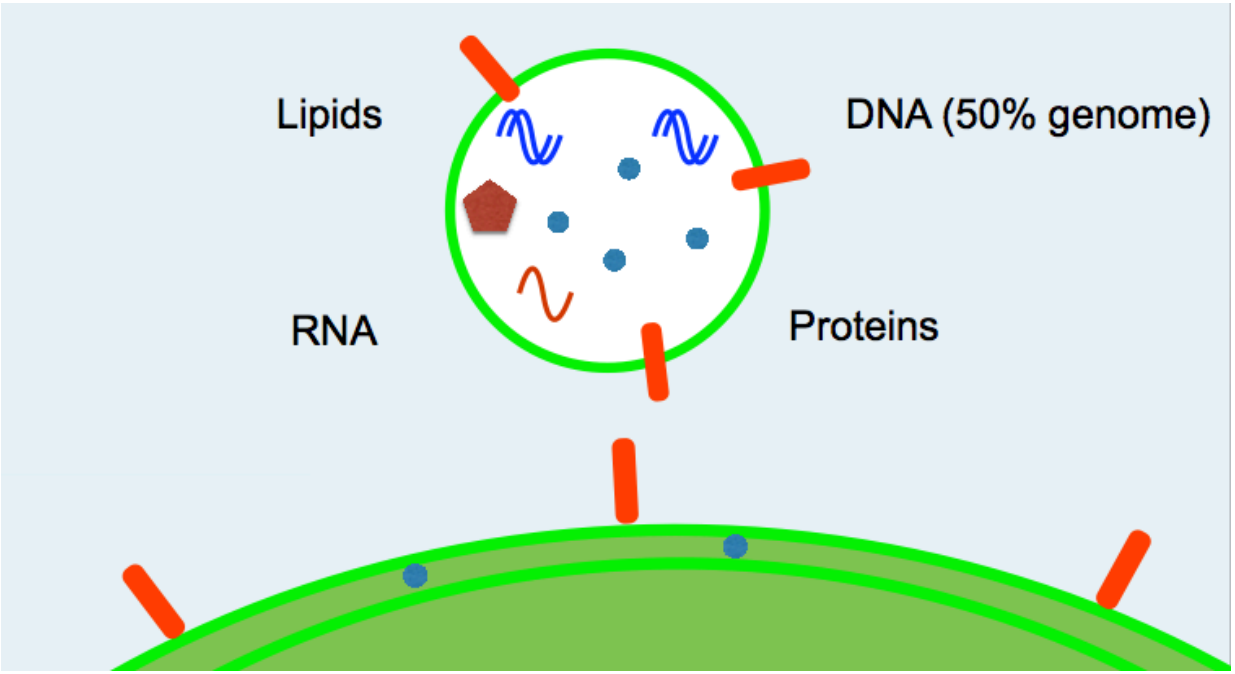
After Ellis and Kuehn (2010) *MMBR*, Schertzer and Whiteley (2012) *mBio*

- 2-5 vesicles per cell per generation
- Stable for weeks

Biller et al 2014

- Global production ~10^{27}-10^{28} vesicles/day (0.1 – 1 megatonnes carbon)

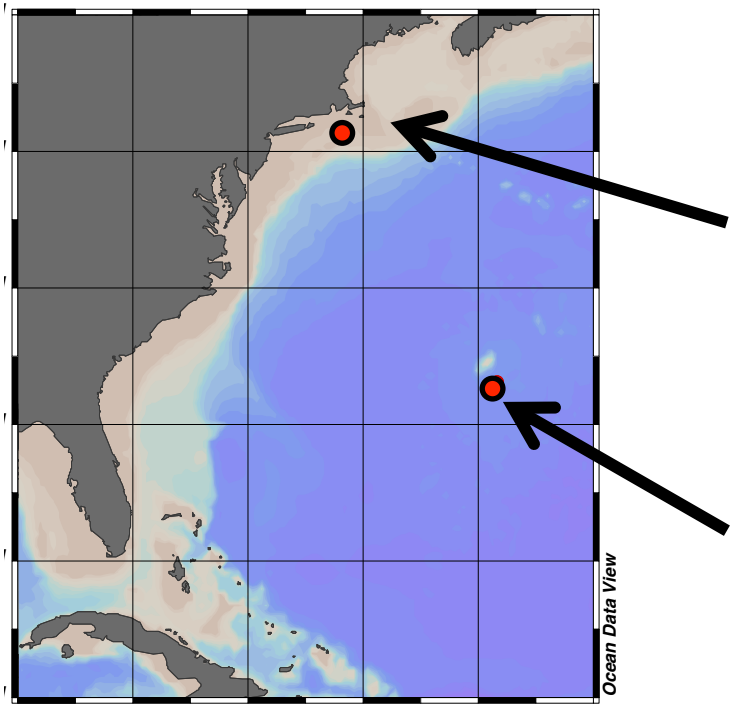
- Vesicles contain interesting cargo

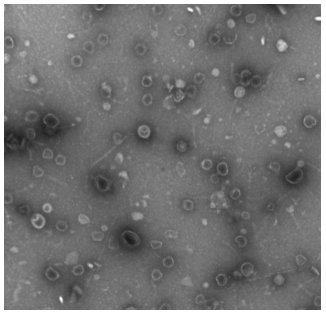


Prochlorococcus points the way!

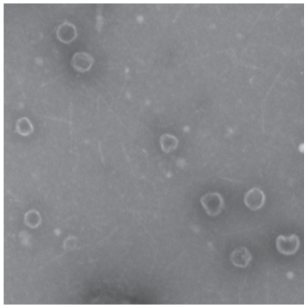
Many ocean microbes produce vesicles

- A new dimension of ocean biogeochemistry





Coastal



Open ocean

Vesicles contain DNA from…

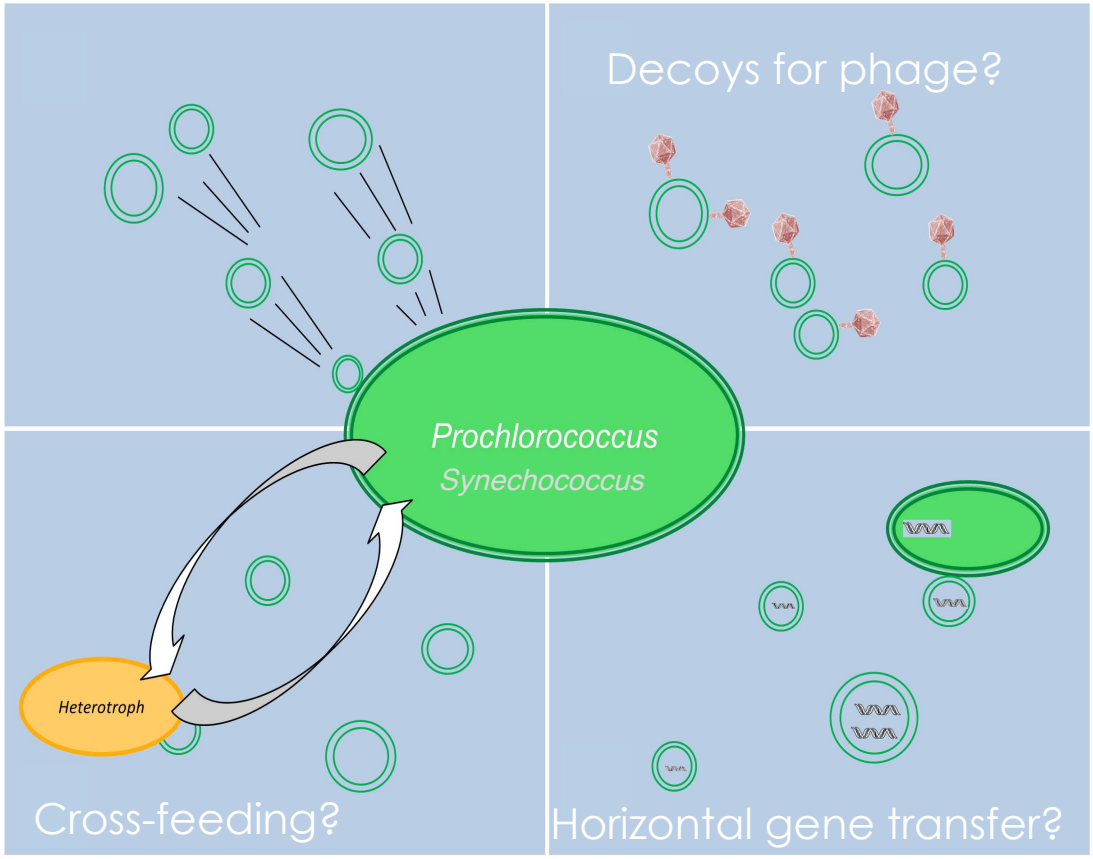
34 phyla across all prokaryotes

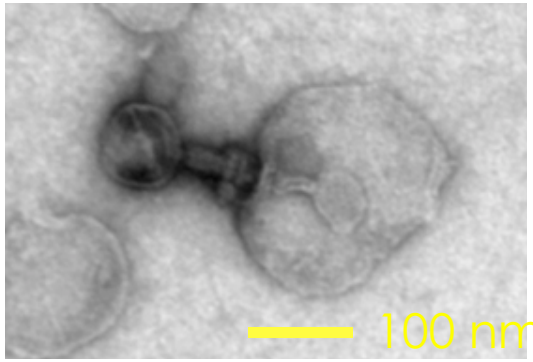
Vesicles abound

10^5 - 10^6 mL^{-1}

Biller et al. 2014

What is the function of vesicle release?
Why should *Prochlorococcus* release valuable resources?





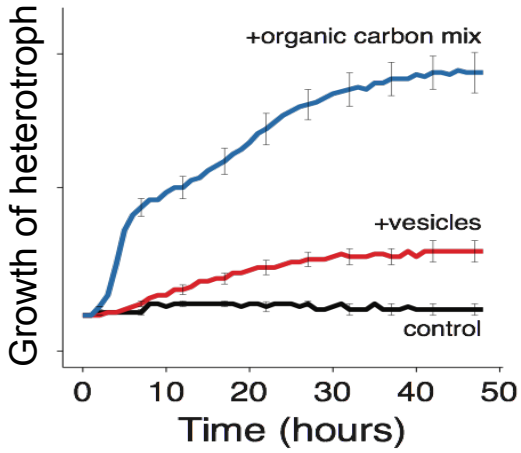


Image from Dave Scanlan

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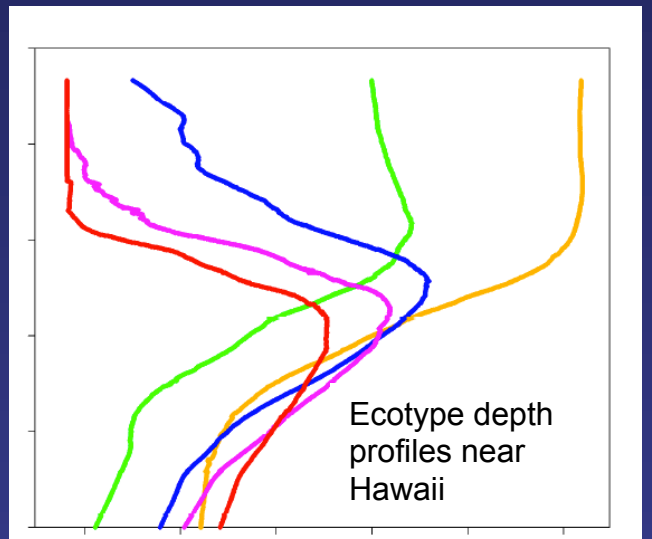
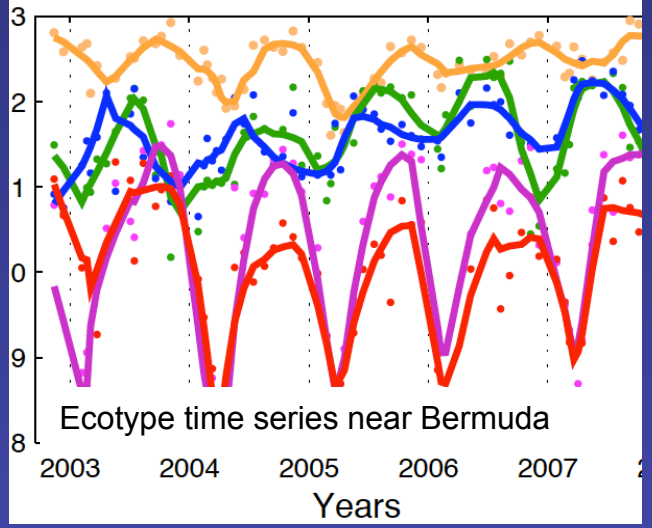
The micro-scale complexity is humbling…

Have come to view the system as
A loose network of
'dissolved information'
temporarily housed
in microbes and viruses

…the stability of the emergent patterns is awe inspiring

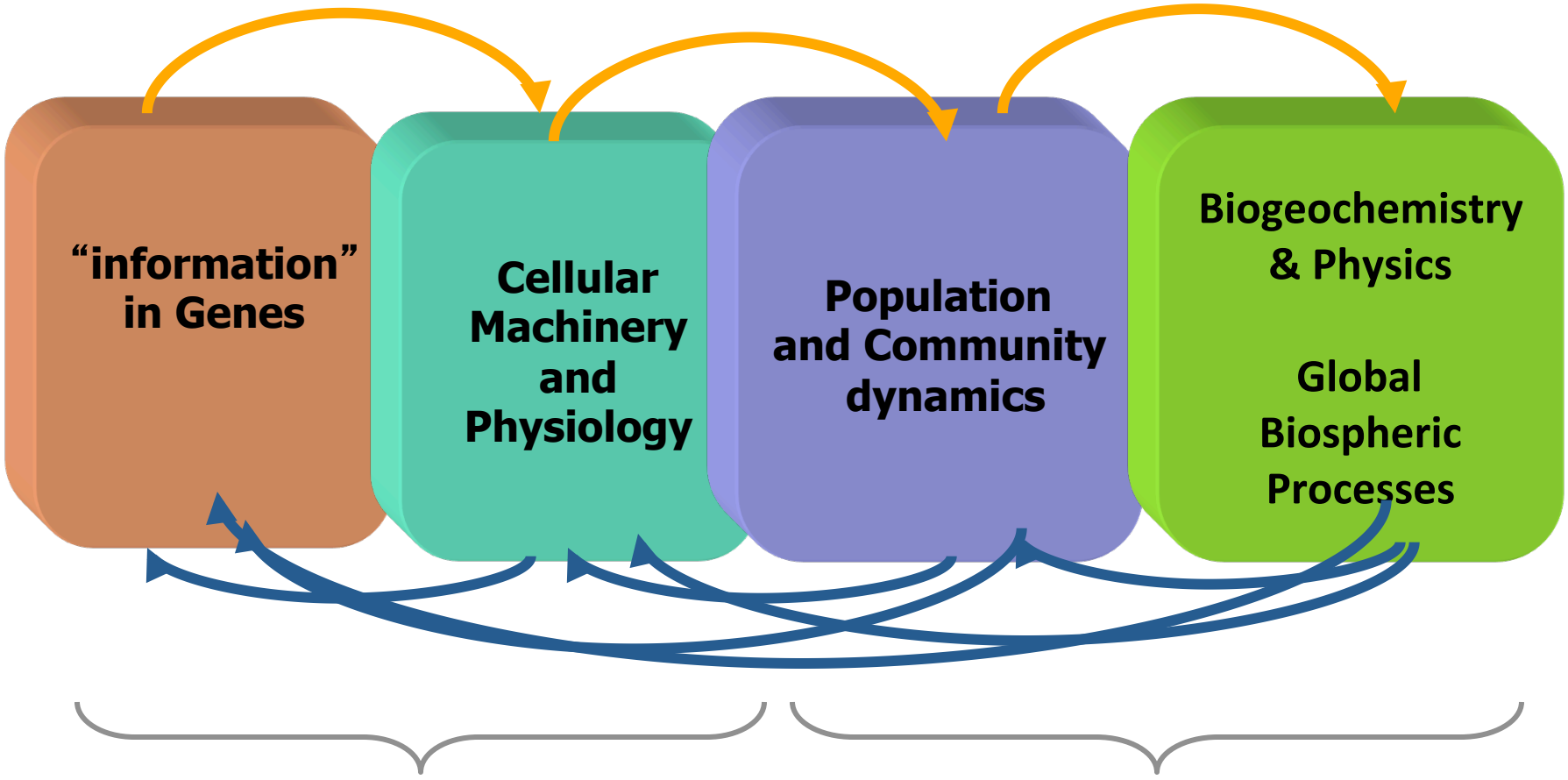




It's a co-evolved, self-organizing, COMPLEX SYSTEM…

What are the assembly rules?

Toward a "New Biology"
Integrative Systems Biology



"Our task now is to resynthesize biology;

…put the organism back into its environment;

…connect it again to its evolutionary past;

…and let us feel that complex flow that is organism, evolution, and environment united."

– Carl Woese, 2004

"A New Biology for a New Century," in *Microbiology and Molecular Biology Reviews*, June 2004 (http://dx.doi.org/10.1128/MMBR.68.2.173-186.2004);

Many thanks to…

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Nadav Kashtan
Steve Biller

Adam Martiny
Kun Zhang
Paul Berube
Jake Waldbauer
Luke Thompson
Qinglu Zeng
Libusha Kelly
Peter Weiglele

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Mak Saito
Eric Webb
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Ed DeLong
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Bruce Birren
Matt Henn

And many more!