

Evolution must explain ecology (and vice versa)

Viral-host systems are a great place to look for the math that is biology because the numbers are large & (recently) observable.

total number of virions on Earth $\sim 10^{31}$
estimated population size of one viral gene $\sim 10^{24}$
number of viral species $> 10^8$

In the last minute, 1-10 million metric tons of microbes were blown to bits by viruses...

Evol/Ecology Roles of Viruses/Phage

- Most common predator on the planet
 $\sim 10^7$ phage ml^{-1} vs. $\sim 10^{-19}$ great white sharks ml^{-1}
- Major players in global C cycling
 - increase respiration
 - decrease primary production

Transduction and lysogenic conversion

- 10^{25} - 10^{28} base pairs of DNA per year in the oceans
- *Vibrio cholerae*
- moving DNA
- Increase microbial diversity
 - "kill-the-winner" & Red Queen

About 10 virus-like particles (VLPs) per microbial cell in all ecosystems

- most are phage that kill Bacteria-

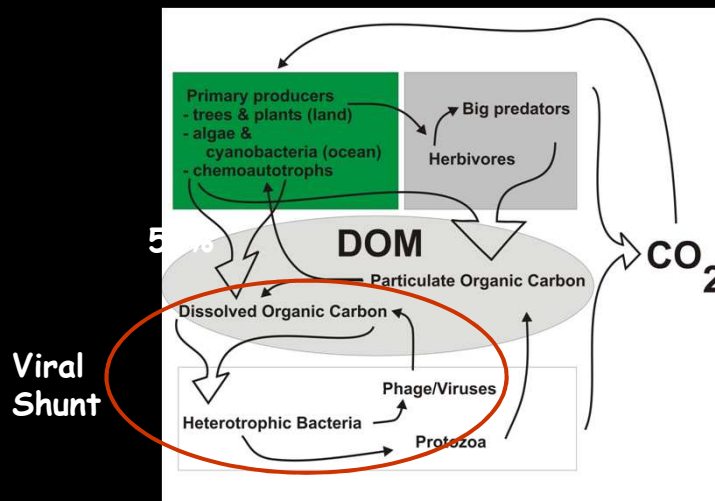
Capture microbes and VLPs on 0.02 Anodisc

↓
Stain with SYBR-Gold

↓
Count with epifluorescent microscope



Predators control microbial abundance



10 viruses per cell in all ecosystems
~10³¹ viruses on the planet

Why 1 million microbes per ml?

Microbes are obligate osmotrophs

- they must "drink" their food from a dissolved phase

In the ocean, most of the food is contained in the Dissolved Organic Matter (DOM) phase

- heterotrophic microbes are eating DOM
- DOM is not generally used by other organisms

DOM consists of both nutrients and energy

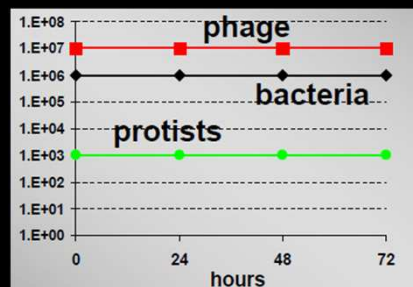
There is enough DOM that microbial communities are not limited by energy

- individuals within the community may be energy or nutrient limited!

Marine microbial population sizes are relatively constant over time

Filter	Removes
GF/F	large Eukaryotes
0.45 μm	nanoflagellates
0.2 μm	most bacteria
0.02 μm	viruses

100 ml GF/F + 3 ml GF/F
↓
Count microbes, viruses, and protists every 24 hrs



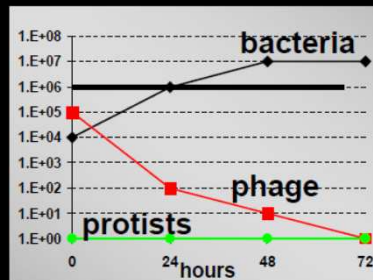
Adapted from Wilcox & Fuhrman (1994) Mar Ecol Prog Ser

Marine microbial population sizes increase if predators are removed

Filter	Removes
GF/F	large Eukaryotes
0.45 μm	nanoflagellates
0.2 μm	most bacteria
0.02 μm	viruses

100 ml 0.02 μm + 3 ml 0.45 μm

↓
Count microbes, viruses, and protists every 24 hrs



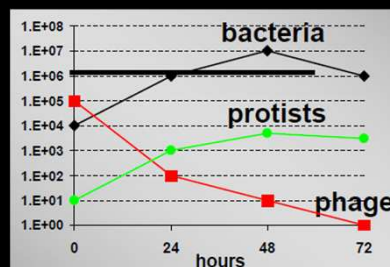
Why do viruses in the inoculum not "catch up" to microbial community?

Protists alone can control microbial populations

Filter	Removes
GF/F	large Eukaryotes
0.45 μm	nanoflagellates
0.2 μm	most bacteria
0.02 μm	viruses

100 ml 0.02 μm + 3 ml GF/F μm

↓
Count microbes, viruses, and protists every 24 hrs



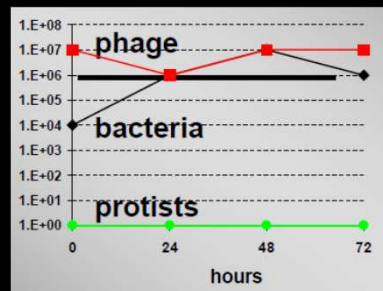
Viruses alone can control microbial populations

Filter	Removes
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0.2 μm	most bacteria
0.02 μm	viruses

100 ml 0.2 μm + 3 ml 0.45 μm

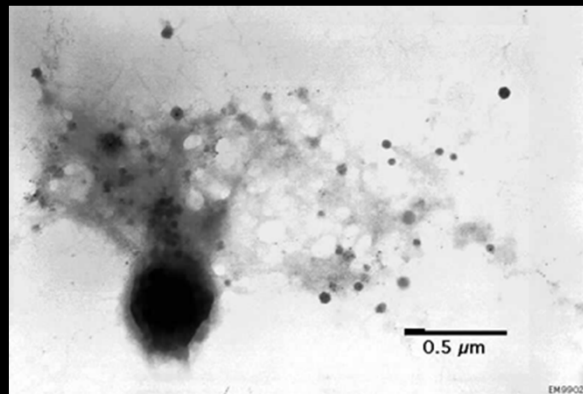


Count microbes, viruses, and protists every 24 hrs



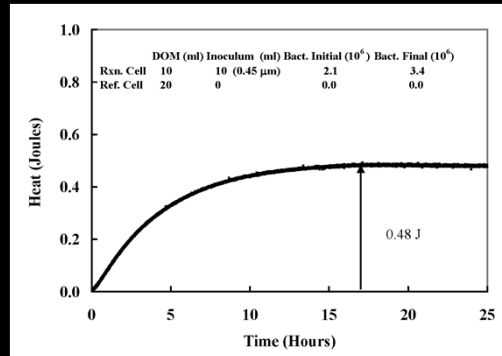
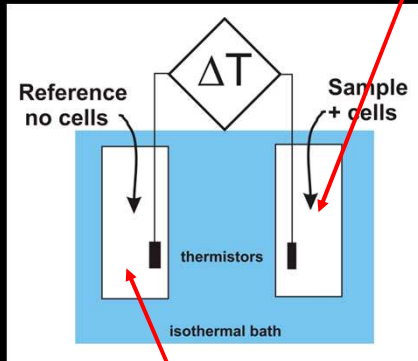
Viral lysis produces more DOM

- feeds other microbes



Calorimetry of marine microbial communities

FOOD = 19 ml of 0.02 μm filtered seawater (DOM)
 +
 INNOCULUM = 1 ml of 0.45 μm filtered seawater (microbes)



FOOD = 19 ml of 0.02 μm filtered seawater (DOM)

Viruses lower standing stock (top-down) & increase work output of system

FOOD = 18 ml of 0.02 μm filtered seawater (DOM)
 +
 INNOCULUM = 1 ml of 0.45 μm filtered seawater (microbes)

1 ml viral concentrate

1 ml killed viral concentrate

expm	Test Cell		Phage	Reference Cell		Phage	Differential Heat Output (Joules)
	10^6 init. Count	10^6 final Count		10^6 init. Count	10^6 final Count		
6a	0.1	16.7	live	0.1	22.8	Autoclave	1.37
6b	0.4	5.6	live	0.4	8.8	Autoclave	0.63
6c	0.9	17.5	live	0.9	21.4	Autoclave	0.44

Viruses lower standing stock

& increase work output of system

Heat output with viruses - heat output no viruses =

expt.	Test Cell			Reference Cell			Differential Heat Output (Joules)
	10 ⁶ init. Count	10 ⁶ final Count	Phage	10 ⁶ init. Count	10 ⁶ final Count	Phage	
6a	0.1	16.7	live	0.1	22.8	Autoclave	1.37
6b	0.4	5.6	live	0.4	8.8	Autoclave	0.63
6c	0.9	17.5	live	0.9	21.4	Autoclave	0.44

>0.5 Joules per 20 ml per 36 hrs

or ~10²³⁻²⁴ Joules in the world's ocean every year

This is equivalent to all the energy stored in Earth's fossil fuels...

Peter Salamon has a thermodynamics model of this system.

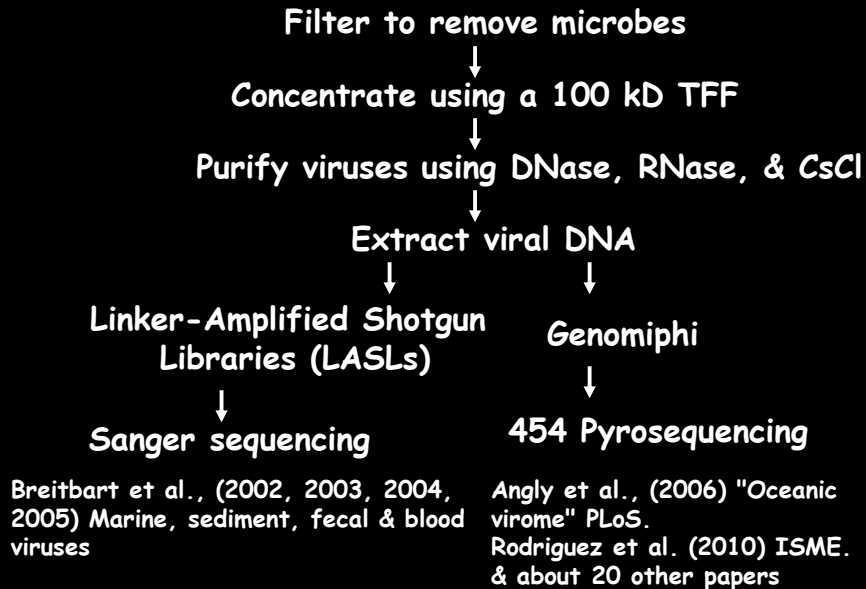
Ecological Roles of Marine Phage

- Most common predator in the ocean
~10⁷ phage ml⁻¹ vs. ~10⁻¹⁹ great white sharks ml⁻¹
- Major players in global C cycling
 - increase respiration
 - decrease primary production

Transduction and lysogenic conversion

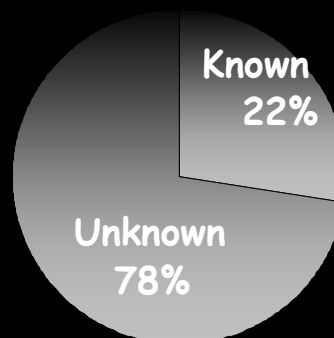
- 10²⁵-10²⁸ base pairs of DNA per year in the oceans
- *Vibrio cholerae*
- moving DNA
- Increase microbial diversity
 - "kill-the-winner" versus "rich-get-richer"

Metagenomics of viral communities



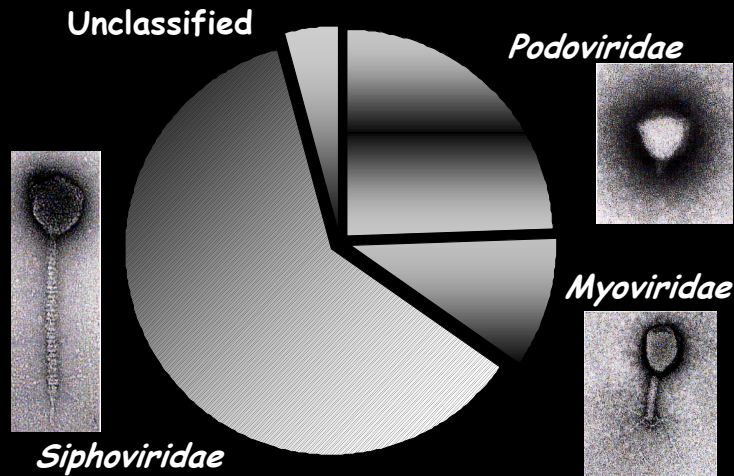
Most uncultured viral sequences are "Unknowns"

metagenomic
fragments tblastx
against NR
"known" $E < 0.001$

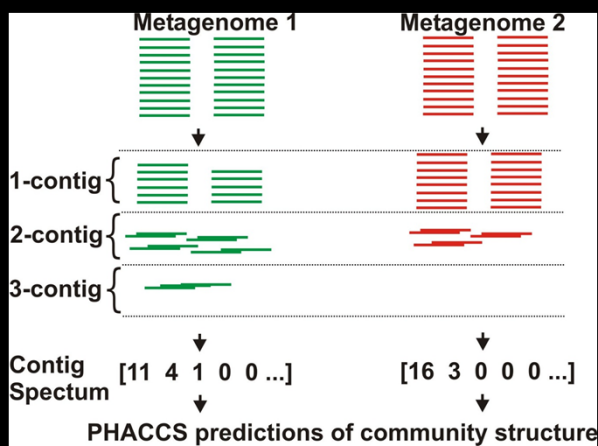


Viromes represent the largest region of unexplored sequence space on Earth

Siphophages are the most successful genome arrangement on the planet



Viral community structure from metagenomes

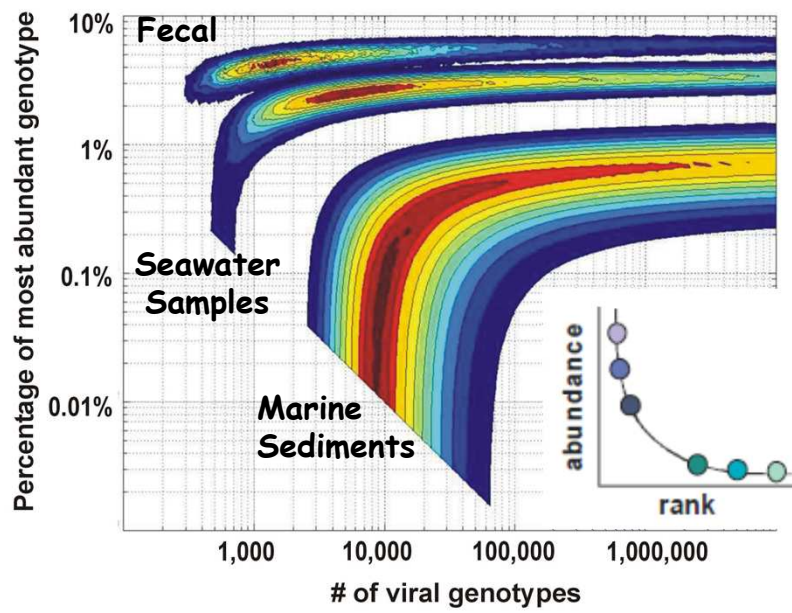


Metagenomic genotype or "species"

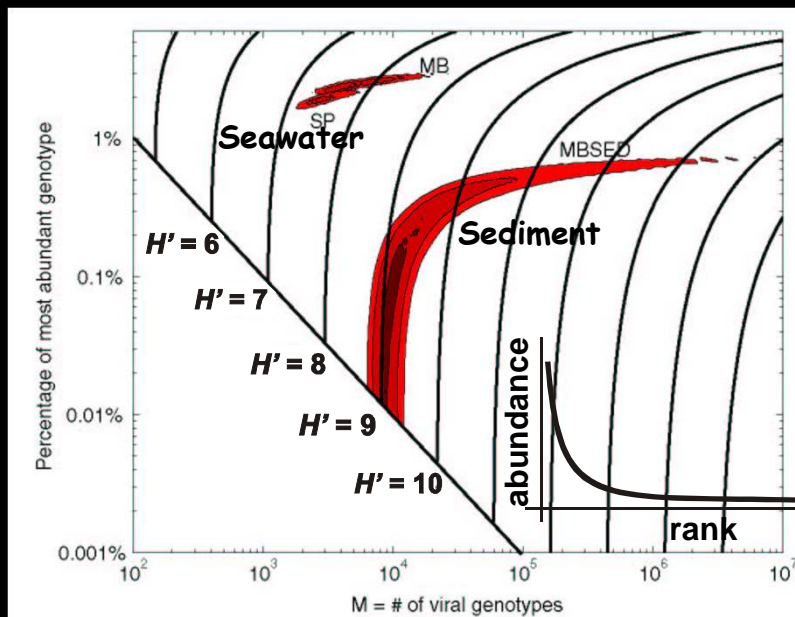
The more overlaps, the lower the diversity of the community

Breitbart et al., (2002) "Marine viral metagenomes" PNAS. 99:14250-14255.

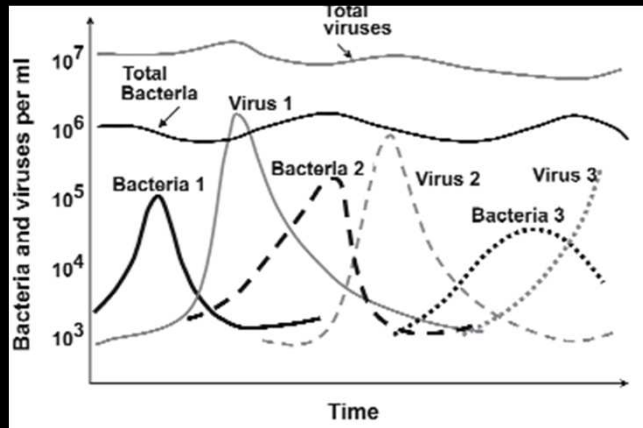
Viral diversity is the highest ever observed



Phage diversity is the highest reported



Kill-the-Winner/Lotka Volterra dynamics



Viruses attack specific strains of cells

Need time series data in the "same" ecosystem
(i.e., stable geochemistry)

Freshwater
aquaculture pond
- 4 time points



Linlin Li

San Diego Bay
solar saltern system



Low salinity
- 3 time points

High salinity
- 4 time points

Medium salinity
- 5 time points

Beltran
Brito
Rodriguez



Metagenomes of VLPs and microbes

Microbial fraction (100 - 0.45/0.2 microns size range)

- Bacteria, Archaea, small protists, some viral-like particles

Viral fraction (0.2 micron - 100 kD size range; CsCl)

- mostly viruses and phage

Extract DNA

Pryosequencing by 454 Life Sciences

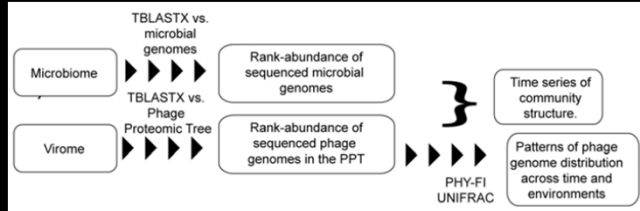
3.3 million reads



Matt Haynes



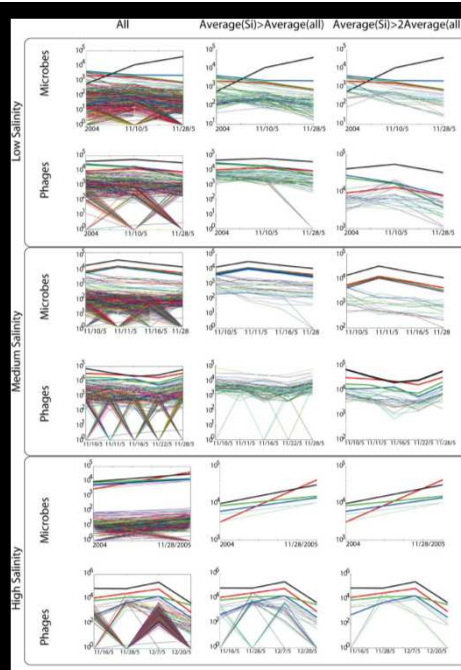
Rob Edwards



In the same environment
the dominant microbial
and viral taxa are stable
over time

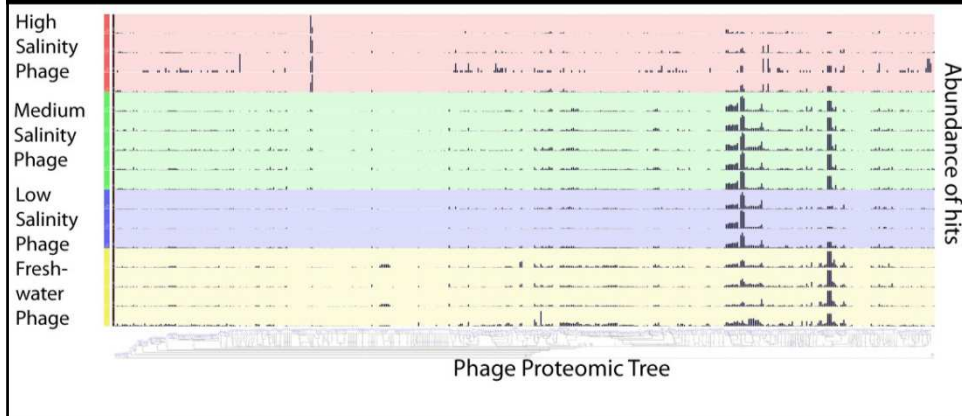
viromes and
microbiomes from
different ponds at
various times
↓
tblastx against
database of complete
genomes
↓
top hits used to
calculate relative
abundances

relative abundance



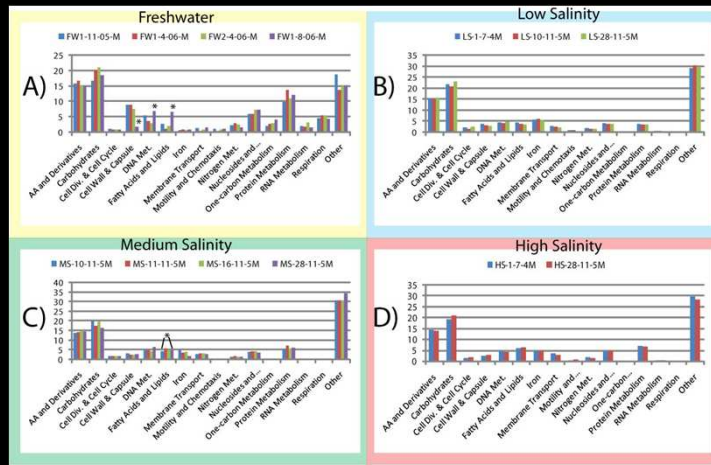
time points

Each environment has an unique viral (& microbial) community



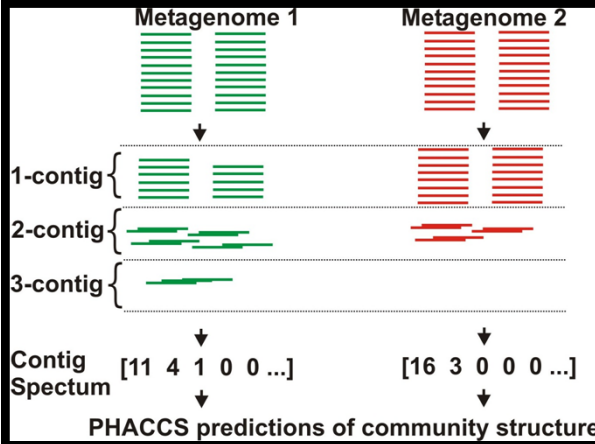
viromes from different ponds at various times → tblastx against database of complete phage genomes → assign taxonomy based on top hit & plot relative abundance on PPT

Metabolic potential is stable over time



microbiomes from different ponds at various times → blastx against SEED database → assign metabolic potential based on top hits (XIPE for stats)

At level of tblastx and blastx, microbial and viral communities appear stable over time...what happens at population levels?



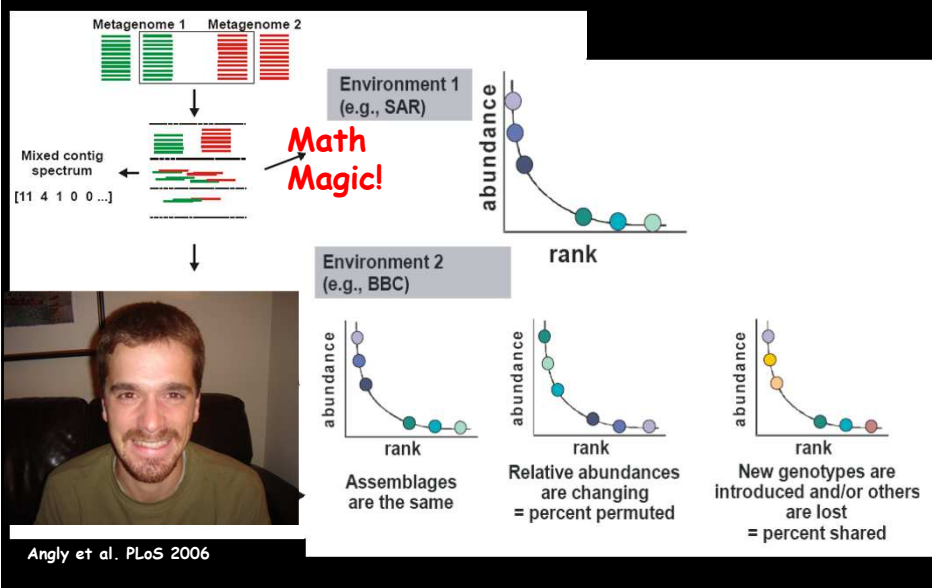
Metagenomic genotype by assemblies

= 99% identity over ≥ 35 bps

The more overlaps, the lower the diversity of the community

Breitbart et al. (2002) PNAS

Maxiphi - How many genotypes are shared between metagenomes?



Dynamics at genotype level: there is complete change over of viral communities at different times

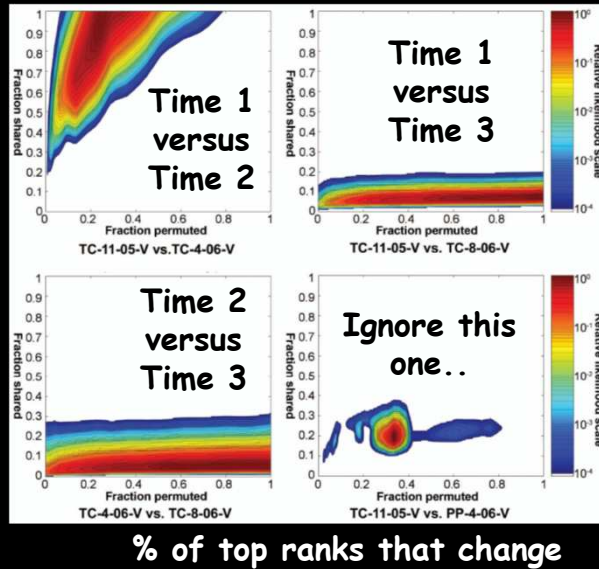
viromes from freshwater pond at various times

pairwise X-contig analysis of samples

Maxiphi

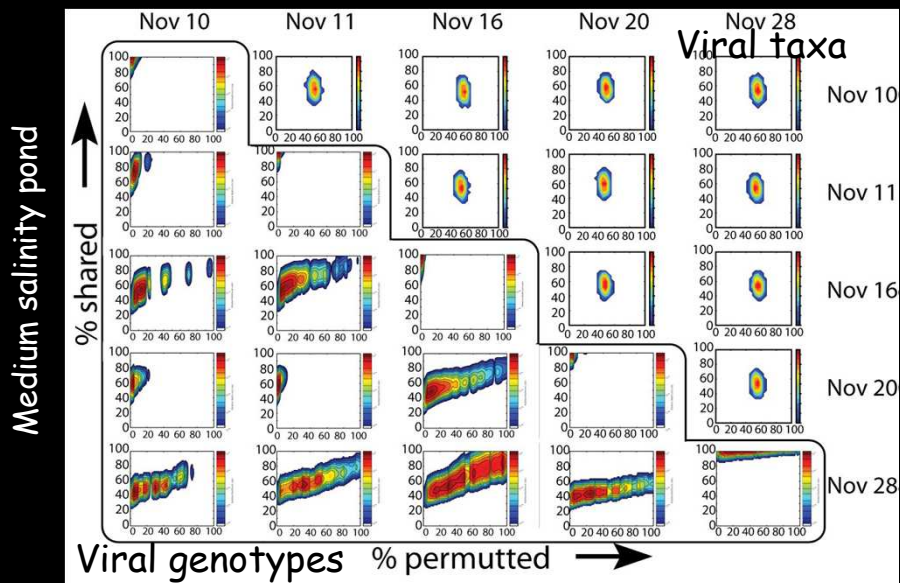
% of genotypes that change

Aquaculture ponds



% of top ranks that change

Rapid turn over of phage genotypes is the rule for all 4 ecosystems



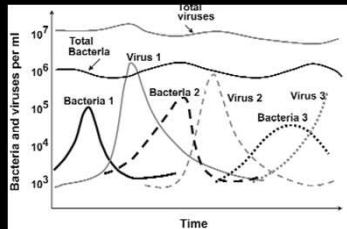
How does this work?

We know what to expect in a particular environment,

Different times in Salterns...
 Predictive power of metagenomes ...
Prochlorococcus & SAR11 in the open ocean...
 Seasonal cycling of taxa...

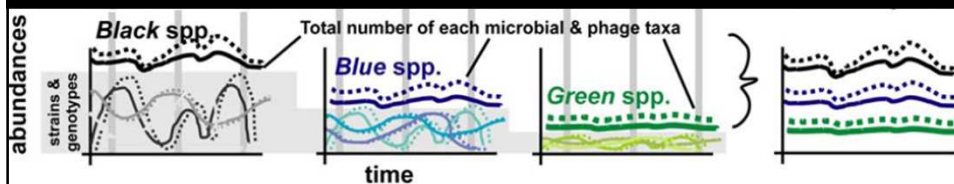
but the viruses should be wiping the dominant microbes out...

Kill-the-Winner



Phage attack specific microbes

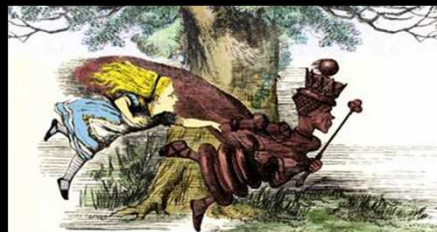
Rapid change over of closely related viral and microbial strains



Red Queen

Evolutionary model that predator-prey pairs need to constantly adapt to each other

Should be able to see this at the level of the genomes...



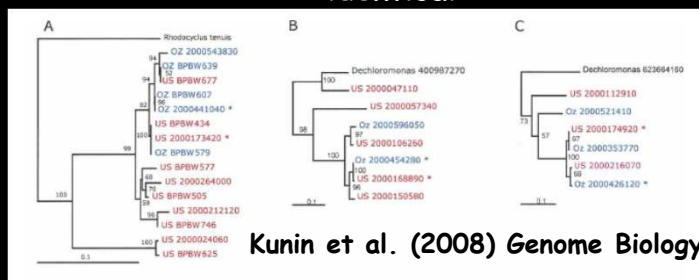
"The Red Queen has to run faster and faster in order to keep still where she is. That is exactly what you all are doing!"

Sludge metagenomics

Sludge bioreactors enriched in a single bacterial species *Candidatus Accumulibacter phosphatis* (CAP)

Microbial metagenomes from Australia (OZ) & USA
Viral metagenome from USA

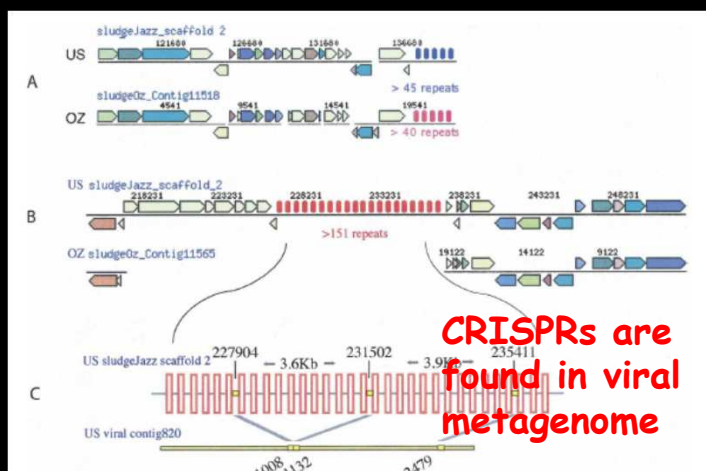
The CAP "genomes" from OZ and USA were 96-97% identical



Differences between the CAP strains in OZ and USA metagenomes are responses to predation

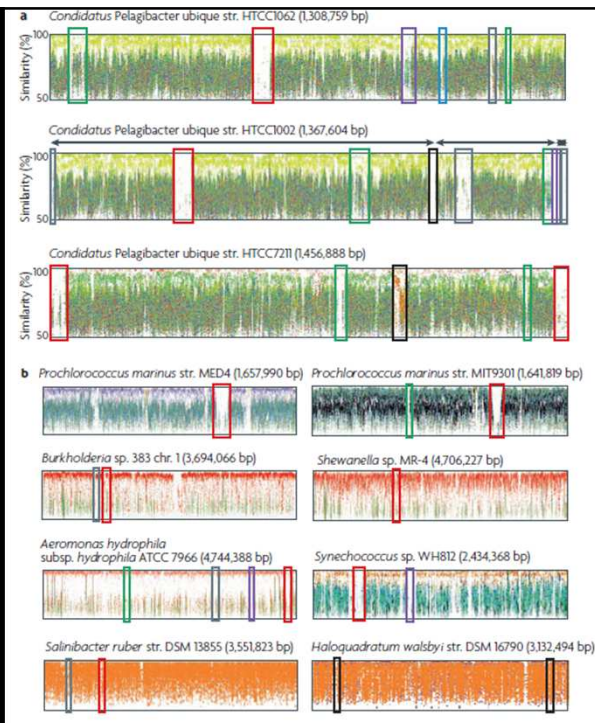
- A) Phage Attachment - EPS is different in OZ and USA
- B) Anti-phage CRISPR elements

microbomes from OZ and USA sludge ponds
↓
blastn comparisons to CAP genome "recruitment"
↓
identify regions of variation (e.g., deletions, substitutions, etc)



Primary defenses against predation are evident all environments analyzed...

Green=Pili
 Red=O antigen in LPS
 Purple=large proteins
 Blue=EPS
 Grey=transmembrane



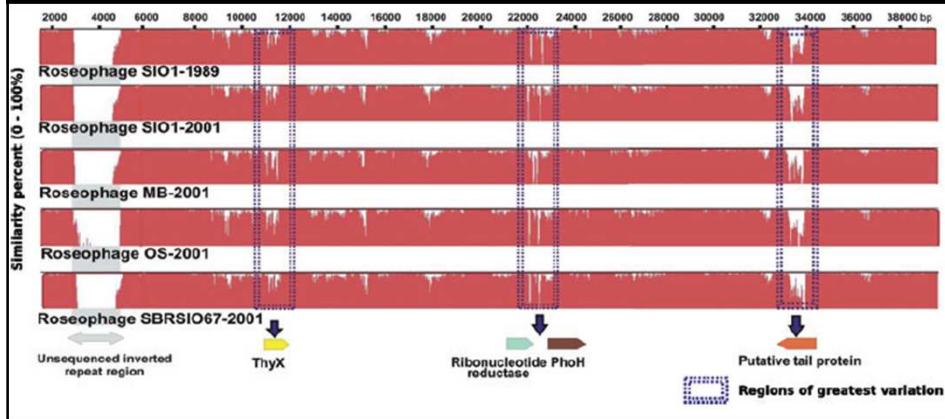
Roseophage SIO1-like Isolates
 - cultured on *Roseobacter* SIO67
 - selected by PCR and sequencing over ~600 bp

Collection site	Isolate	Collection year	Host range
	OS-2001	2001	<i>Roseobacter</i> SIO67
	SBR SIO67-2001	2001	<i>Roseobacter</i> SIO67, <i>Roseobacter</i> GAI-101
	SIO1-1989	1989	<i>Roseobacter</i> SIO67
	SIO1-2001	2001	<i>Roseobacter</i> SIO67
	MB-2001	2001	<i>Roseobacter</i> SIO67

12 years between collections (100's of generations)
 50 kilometers between sites

Resequenced 5 genomes using overlapping PCR products

Comparison of Roseophage SIO1-like genomes



Identity between SIO1-like genomes were 96-99%

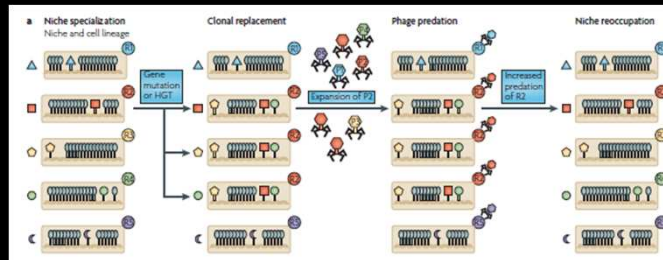
No rearrangements

Most changes in coding regions

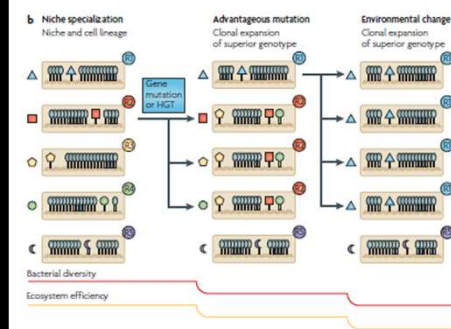
Purifying selection indicated by dS/dN ratios

Tail fiber protein is most variable ORF...**Red Queen**

Constant Diversity



Sweeps



Rohwer Lab - SDSU

Beltran Brito-Rodriguez
Dana Willner (UQueens)
Matt Haynes
Mike Furlan
Nichole Hanson
Linda Wegley Kelly
Tracey McDole
Katie Barott
John Mokili
Yanwei Lim
Allison Gregg
Lance Boling
Florent Angly (Uqueens)

Edwards Lab - SDSU

Rob Edwards
Robert Schmieder

Math Guys - SDSU

Peter Salamon
Ben Felts
Jim Nulton
Barb Bailey

Gordon Lab - WU

Jeffery Gordon
Alejandro Reyes

Doug Conrad - UCSD

Paul Rainey - Massey Uni

The \$\$\$:)



Roche/454 Life Sciences

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