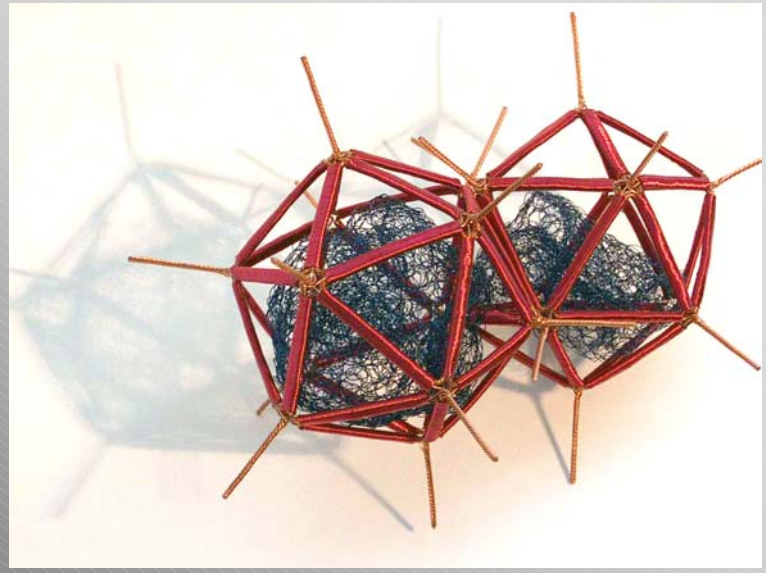
Viral biodiversity, ecology, evolution, & a little coral stuff…



Phage art by Jennifer Jo Walrath

Forest Rohwer

San Diego State University
&
Center for Microbial Sciences

Ecological roles of phage

- **Most abundant predators**

  ~10^7 viruses per ml surface seawater
  estimated 10^{31} viruses on the planet

- **Major players in global C cycling**

- **Increase microbial diversity**

  - "Kill-the-Winner”

- **Transduction and lysogenic conversion**

  - 10^{25}-10^{28} base pairs of DNA per year in the oceans
  - cholera toxin

Cloning viral communities

Filter to remove microbes (0.16 μm)

↓

Concentrate using a 100 kD TFF

↓

Purify phage using DNase, RNase, & CsCl

↓

Extract phage DNA

↓

Linker-Amplified Shotgun Libraries (LASLs)

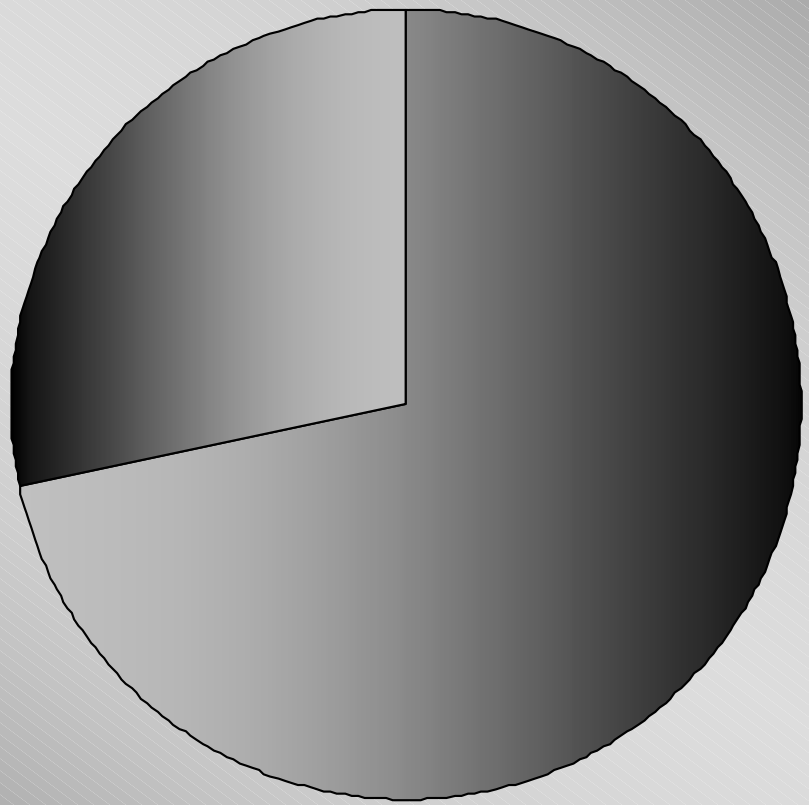
David Mead - Lucigen™

↓

Sequence

Breitbart et al., (2002) "Marine water viruses" PNAS. 99:14250-14255.
Breitbart et al., (2003) "Fecal viruses" J Bact. 85 (20). 6220-6223.
Breitbart et al., (2004) "Marine sediment viruses" Proc Royal Society B. 271. 565-574.

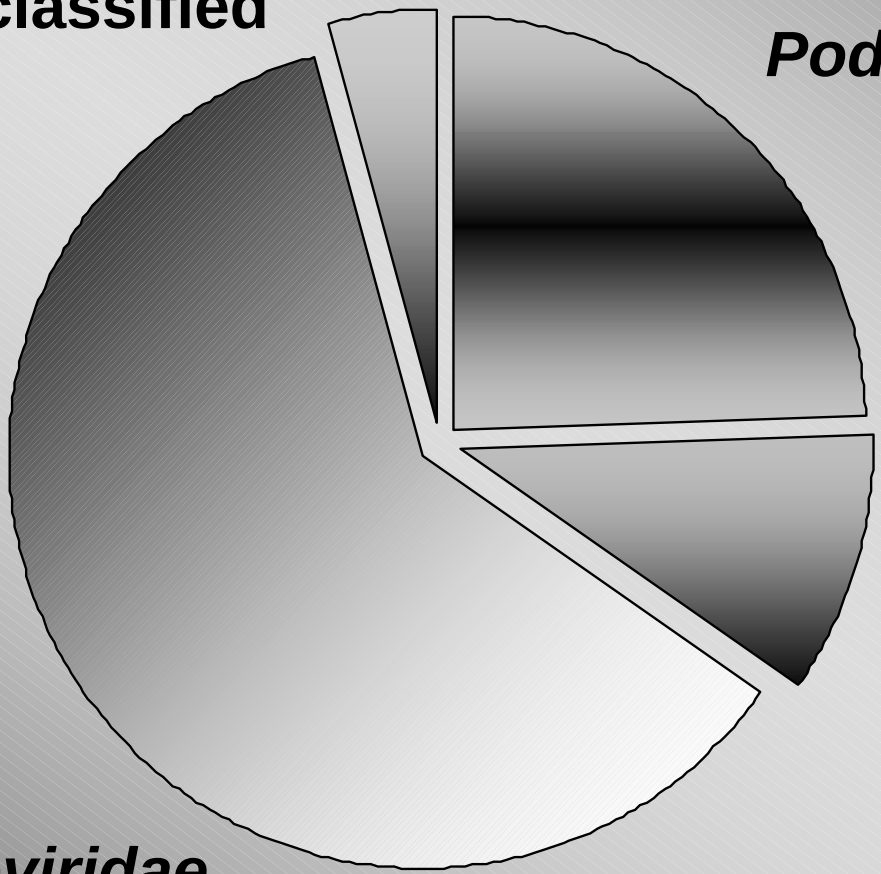
Most uncultured phage sequences are "Unknowns"

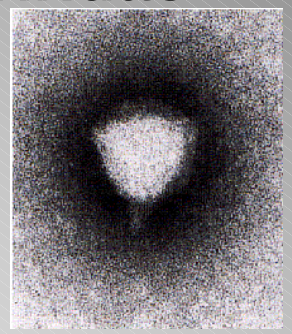


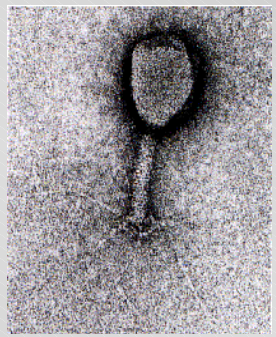
<0.0002% of the global phage metagenome has been sampled

Rohwer (2003) Global phage diversity. Cell. 113.
> preview for Pedulla et al. (2003) Origins of highly mosaic mycobacteriophage genomes

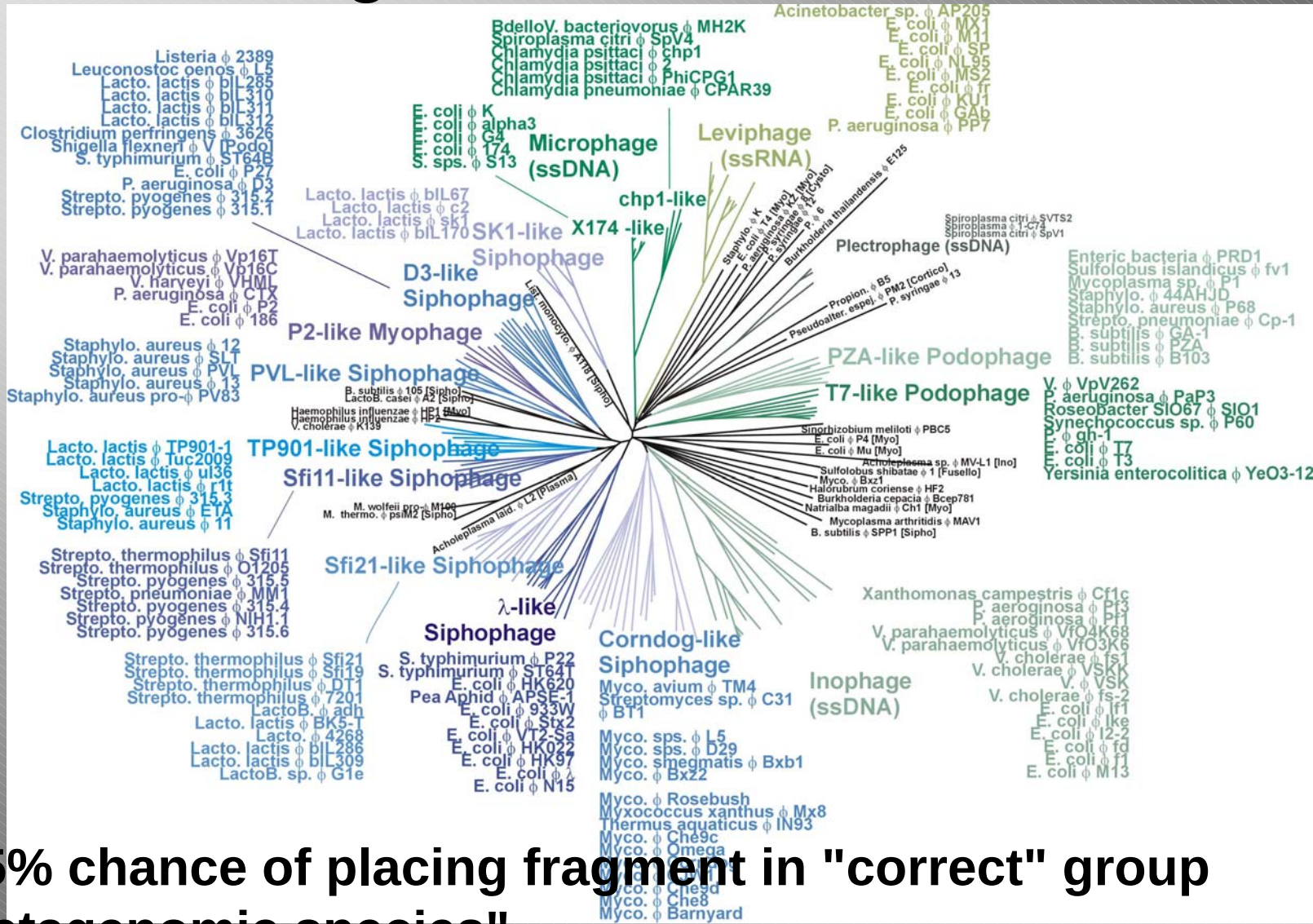
Siphophage are the most common type of phage







Phage Proteomic Tree II

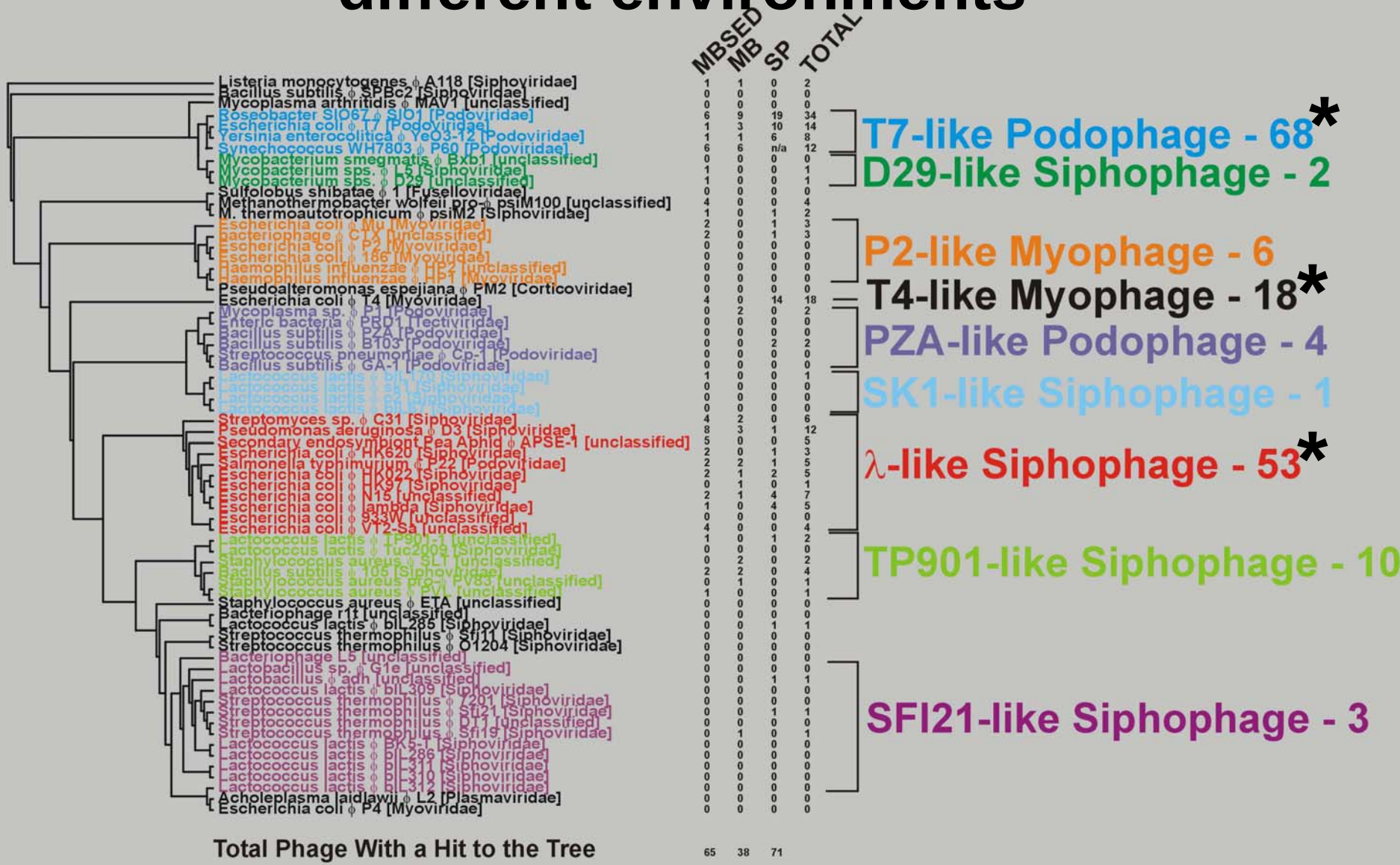


~95% chance of placing fragment in "correct" group "metagenomic species"

Edwards & Rohwer (2005) Viral Metagenomics. Nature Reviews in Microbiology. 3 (6). 504-510.

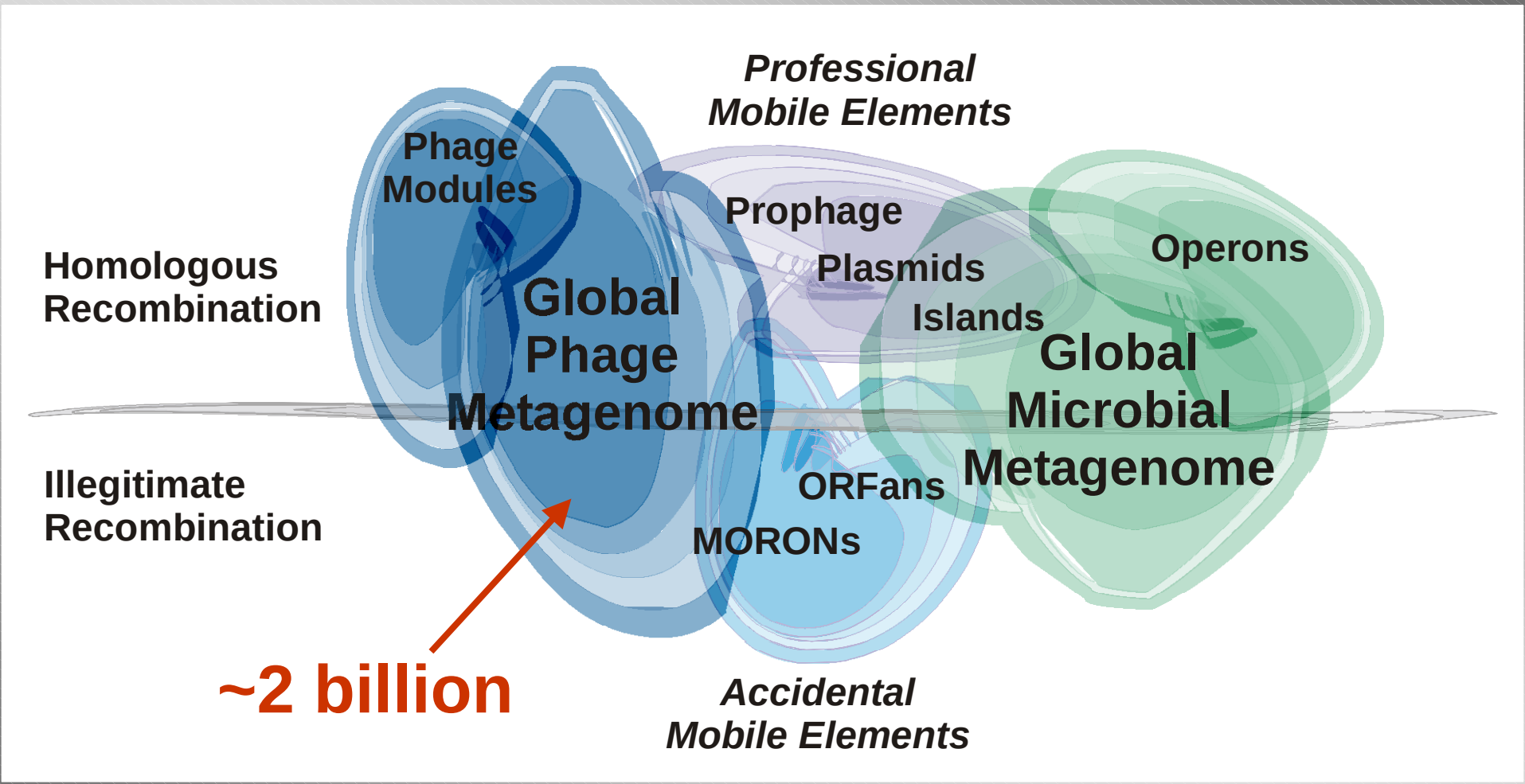
Rohwer & Edwards (2002) "The Phage Proteomic Tree…" J Bact. 184 (16). 4529.

Distinct phage groups dominate in different environments



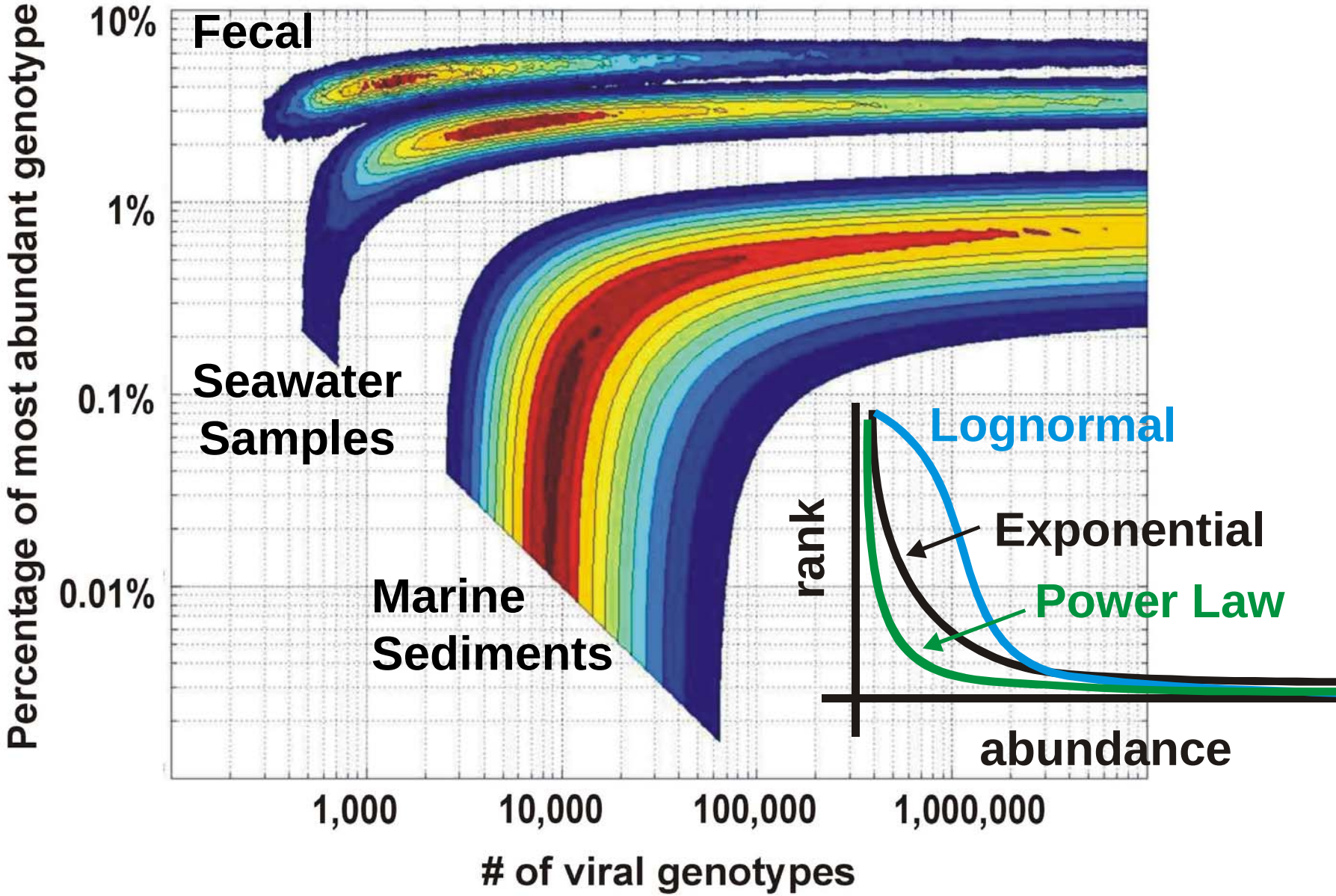
Metagenomic “signatures” of different environments

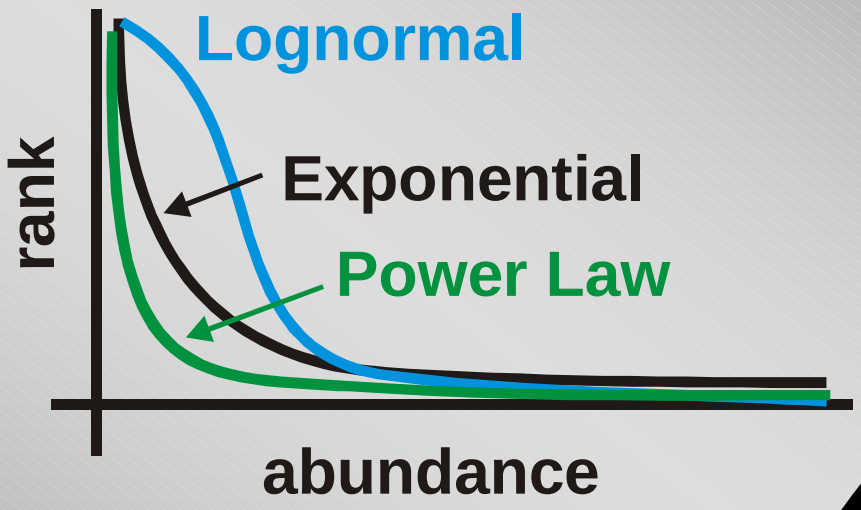
Quantum landscape of genes



Breitbart & Rohwer (2005) Here a Virus, There a Virus, Everywhere the Same Virus?. Trend in Microbiology. 13 (6). 278-284.

Viral community structure





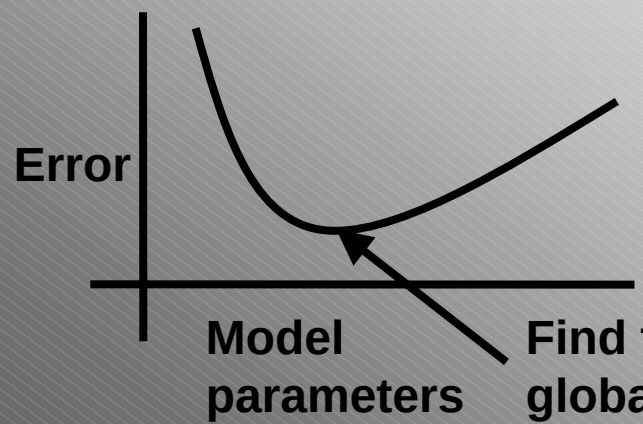
Determine the actual contig spectrum of the sample

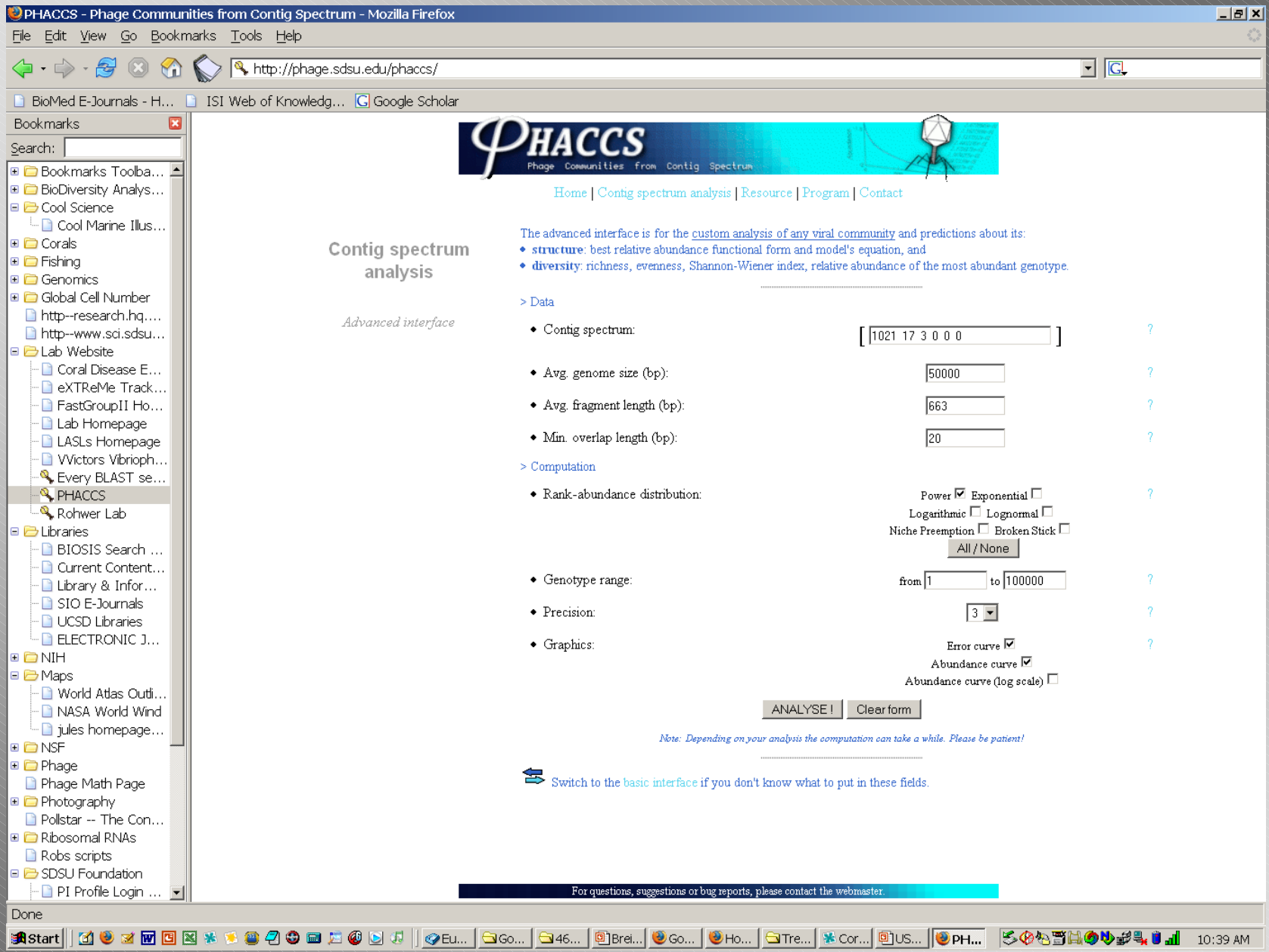
Predict a contig spectrum using a species abundance model

Compute the error between the actual and predicted

Adjust the parameters in the species abundance model to minimize errors

Continue this procedure to obtain the smallest error





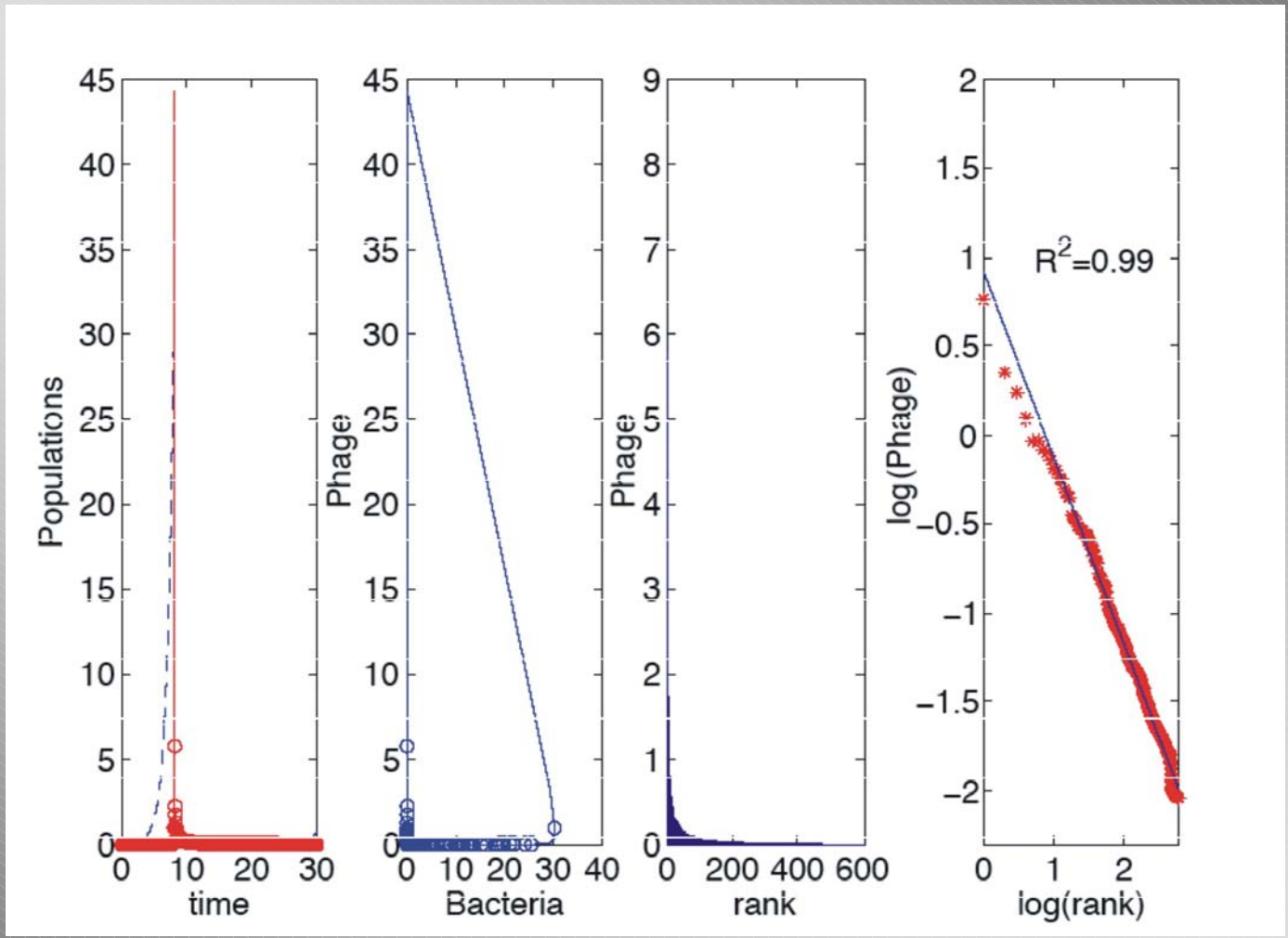
Angly et al. (2005) PHACCS, an online tool for estimating the structure and diversity of uncultured viral communities using metagenomic information. BMC Bioinformatics. 6:41

Power Law is the best explanation (so far)

Model	% most abundant	Error	Number of species	Parameters of the model
Power Law	2.65	2.11	7420	B=0.73062
Exponential Law	0.42	16.2	7500	B=0.0042156
Logarithmic	3.02	2.81	1560	B=1.8054
Broken Stick	0.78	14.6	960	No additional parameters
Niche Preemption	0.476	38.1	8200	K=0.0047582
Lognormal	2.77	2.31	43110	Sigma=2.2237

Fewer "species"
Models of community structure

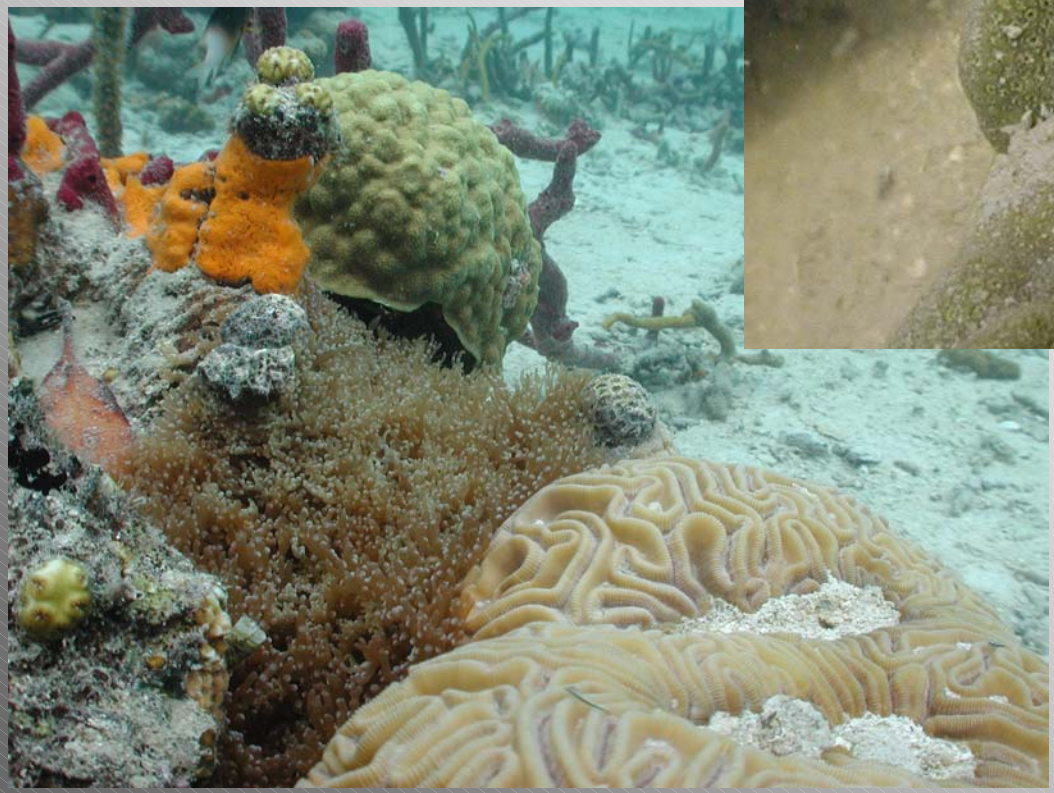
Generalized Lotka-Volterra



"Kill-the-Winner"

Corals are heavily colonized by prokaryotes

~10^7-10^8 prokaryotes per cm^2



Wegley, et al. 2004. Coral-associated Archaea. MEPS. 273. 89-96.

Diploria strigosa, *Porites astreoides*, & *Montastraea franksi* from Bermuda and Panama (2 different years)

Isolate total DNA

↓

PCR amplify 16S rDNAs from Bacteria

↓

Make library

↓

Sequence 5' 550 bp from ~ 100 clones per sample

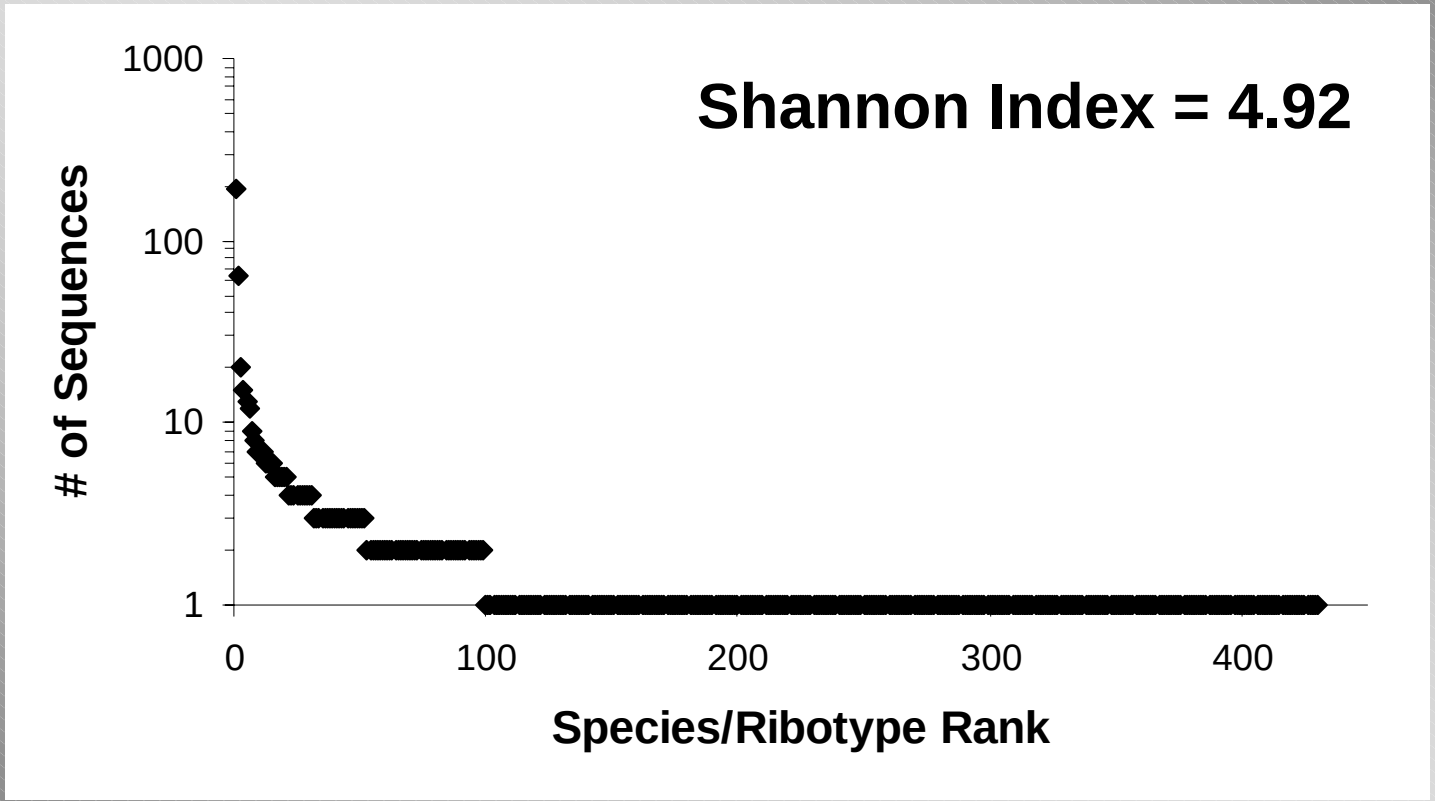
↓

Analyze with FastGroup, ClustalW, and BLAST

- total of 1,178 rDNA seqs from 14 samples

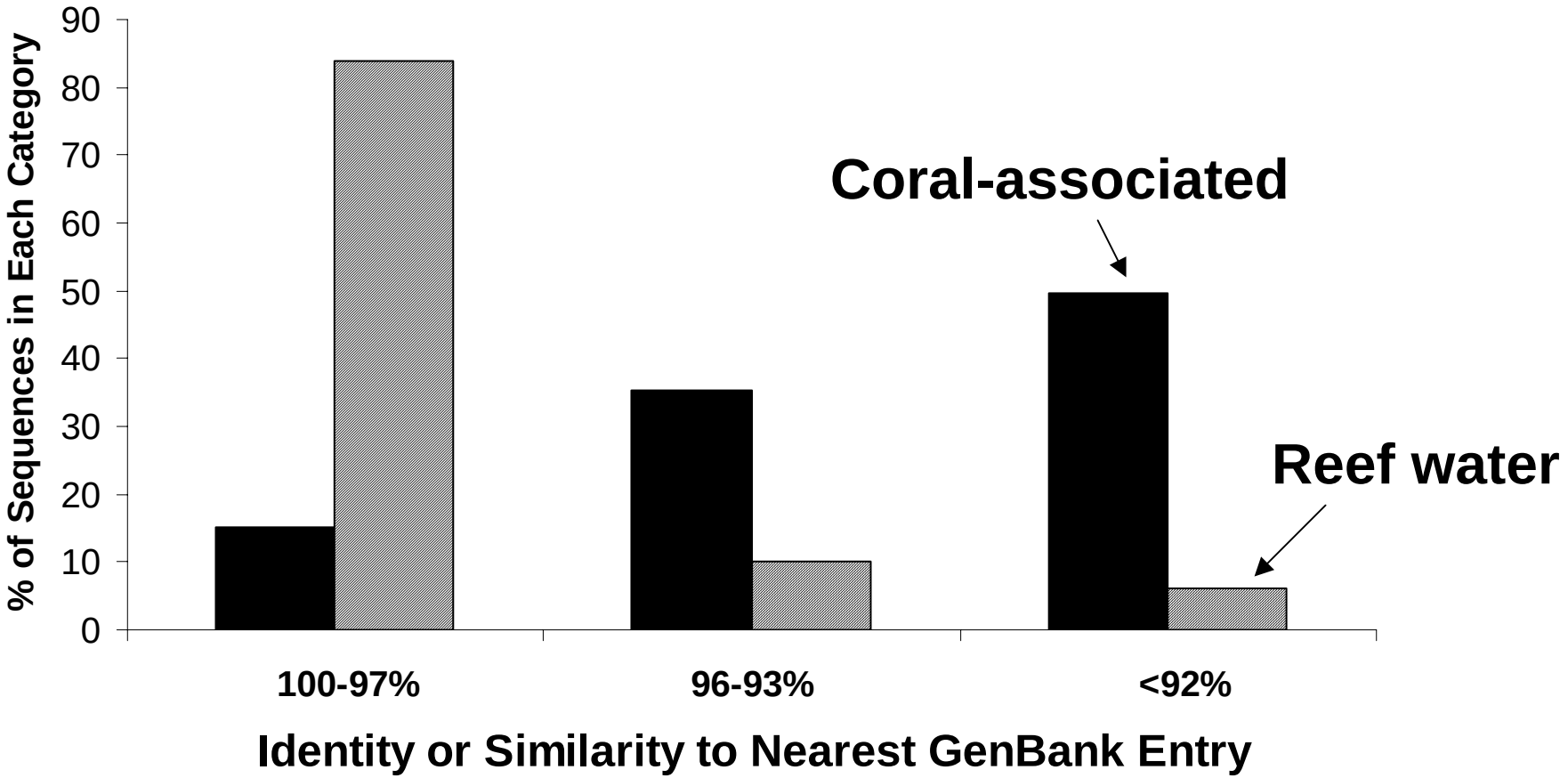
Consider sequences with ≥97% identity as the same ribotype

Diversity of coral-associated bacteria is very high

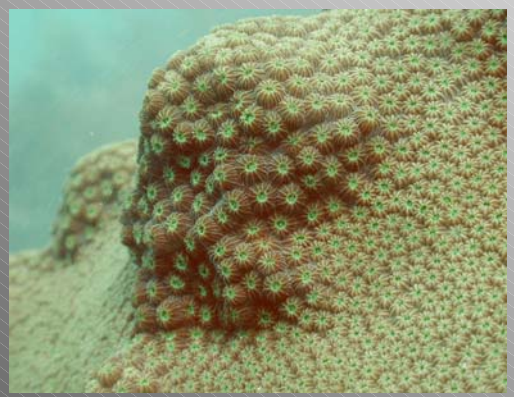


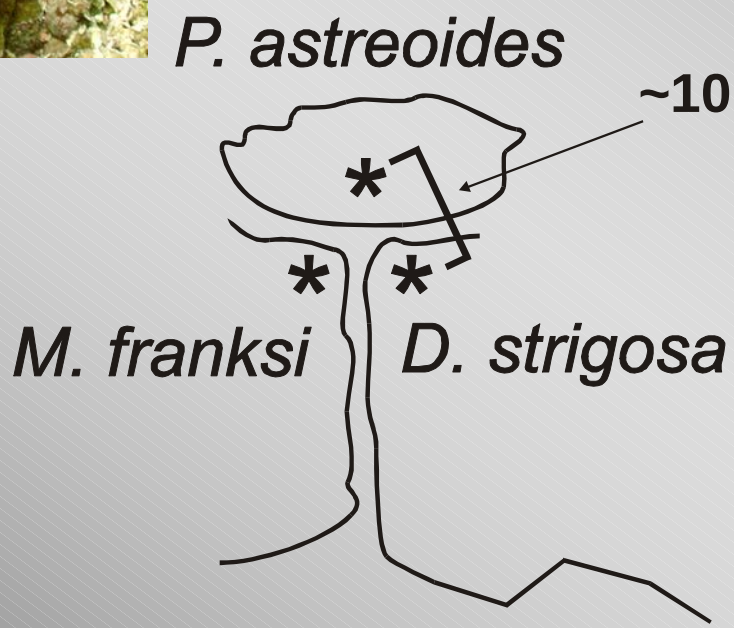
- 430 distinct bacterial ribotypes from 14 corals
- Chao2 predicts 6,000 ribotypes

Most coral-associated bacteria are novel genera &/or species



Corals located next to each other have unique microbiotas





Three corals from Bermuda shared 1 out of 101 ribotypes
Three corals from Panama shared 0 out of 123 ribotypes

Coral-bacteria associations are maintained over time and distance

	Porites astreoides					*Diploria strigosa*				*Montastraea franksi*					AVE
	BER1	BER2	PAN4	PAN5	PAN5	BER1	BER2	PAN4	PAN5	BER1	BER2	BER2	PAN2	PAN5	
Alpha	9	1	9	**0**	10	7	11	21	26	25	34	15	19	47	17
Gamma	13	5	2	14	22	13	15	26	18	20	12	35	15	20	16
PA1 (Gamma)	40	92	72	77	27	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	22
CFB	13	**0**	**0**	**0**	7	43	28	17	26	8	3	6	19	9	13
BC	**0**	**0**	**0**	7	15	32	24	35	26	12	4	11	4	11	13
Cyano	6	**0**	17	2	8	**0**	12	**0**	**0**	10	4	2	7	10	6
Other	18	2	1	**0**	11	5	10	3	3	25	42	31	37	2	14
n	61	143	88	54	73	75	126	81	81	87	82	70	62	95	

- **PA1 is a γ-proteobacteria always associated with *P. astreoides***
- **Cytophaga-Flexibacter-Bacteroides (CFB) and Bacillus-Clostridium (BC) groups are preferentially associated with *D. strigosa***
- **Diversity is higher in Panamanian samples, which parallels metazoan biodiversity gradients**

Conclusions

- **The coral holobiont harbors all three Domains of life**
- **Different coral species have unique bacterial populations**
- **Specific bacteria-coral associations are maintained over geographic and temporal space**
- **Individual coral colonies have microniches that harbor distinct bacteria**
- **For every coral colony:**

1 coral animal:2-3 zoox species:60-? prokaryotic species

Rohwer Lab - SDSU

Mya Breitbart
Beltran Rodriguez-Brito
Pat McNairnie
Florent Angly
Linda Wegley
Veronica Casas
Jon Miyake

Math Guys - SDSU

Peter Salamon
Joe Mahaffy
Bjarne Andresen
James Nulton
Ben Felts
David Bangor

Rob Edwards - SDSU

Anca Segall - SDSU

David Mead - Lucigen

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