Microevolutionary dynamics of subeafloor Methanothermococcus populations in high-temperature vent fluids from the Mid-Cayman Rise

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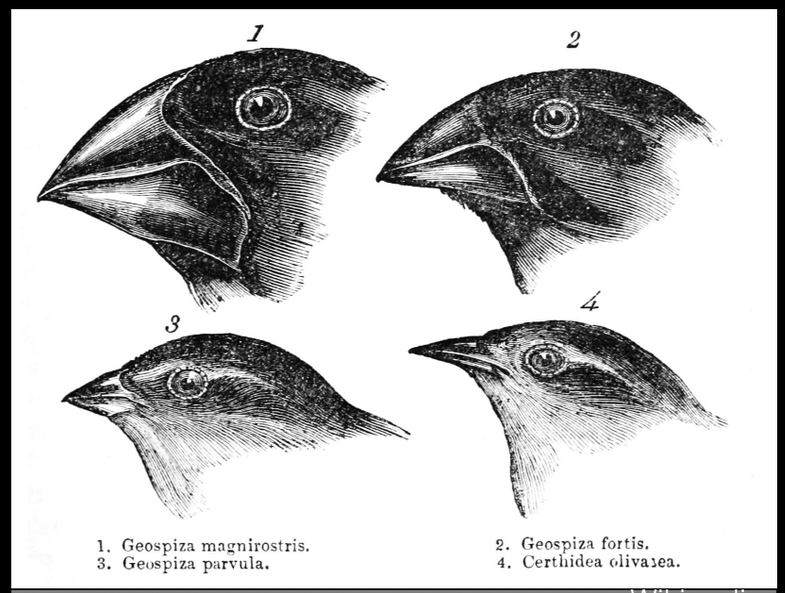
Motivating Questions

How do deep subsurface archaea and bacteria <u>evolve</u>?

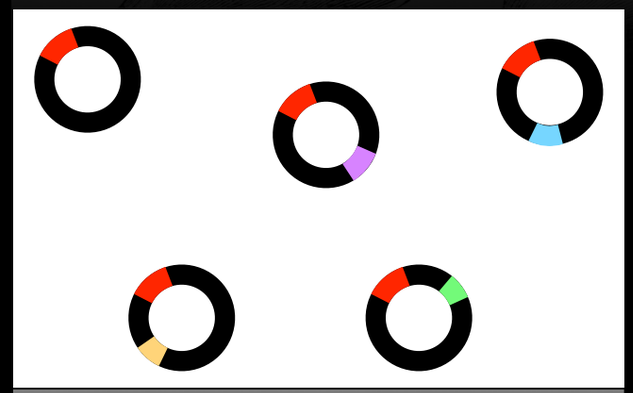
To what extent do chance (genetic drift) and necessity (natural selection) drive the evolution of subsurface microbes?

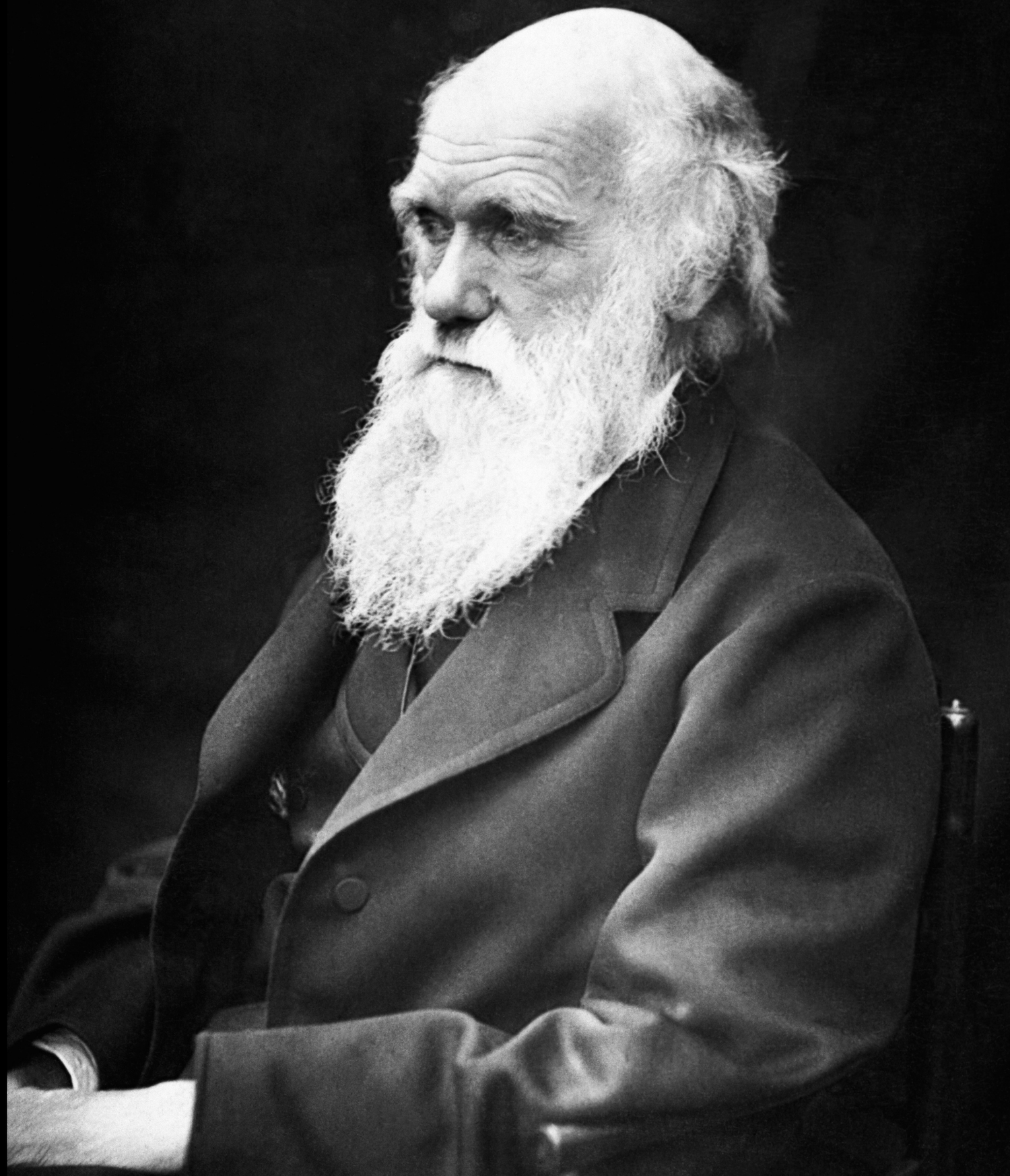
How does the environment mold microbial evolution?

Natural variation can help us understand evolution

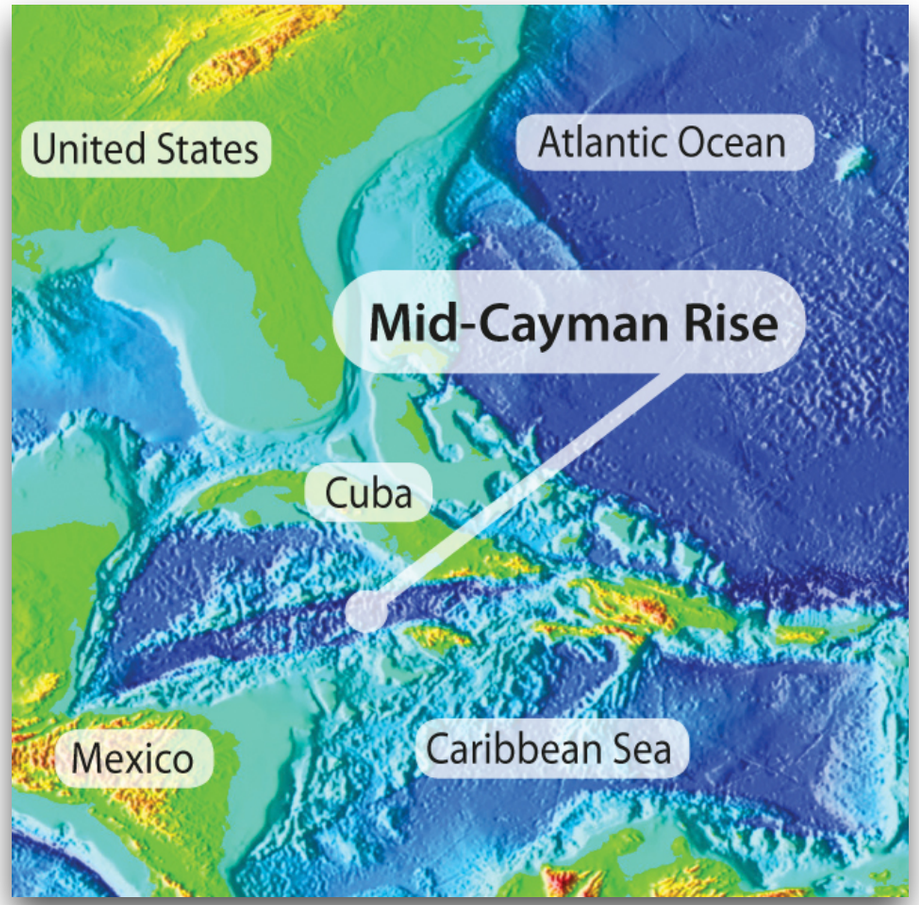


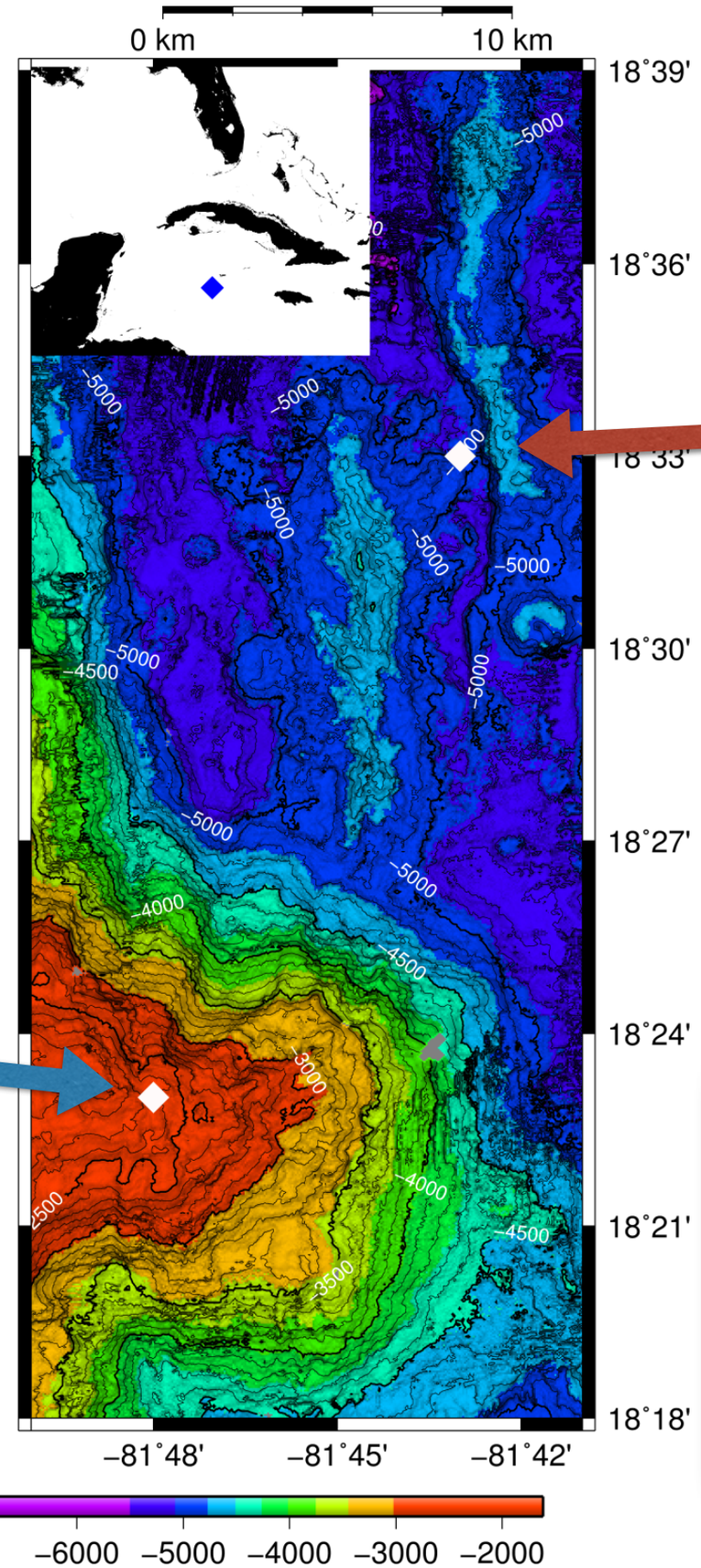
Wikipedia





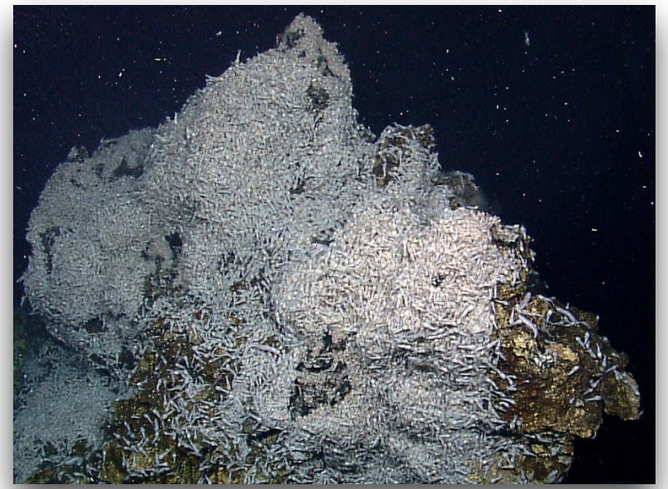
Study site: The Mid-Cayman Rise





Piccard 4950 m, mafic

Von Damm 2350 m, ultramafic

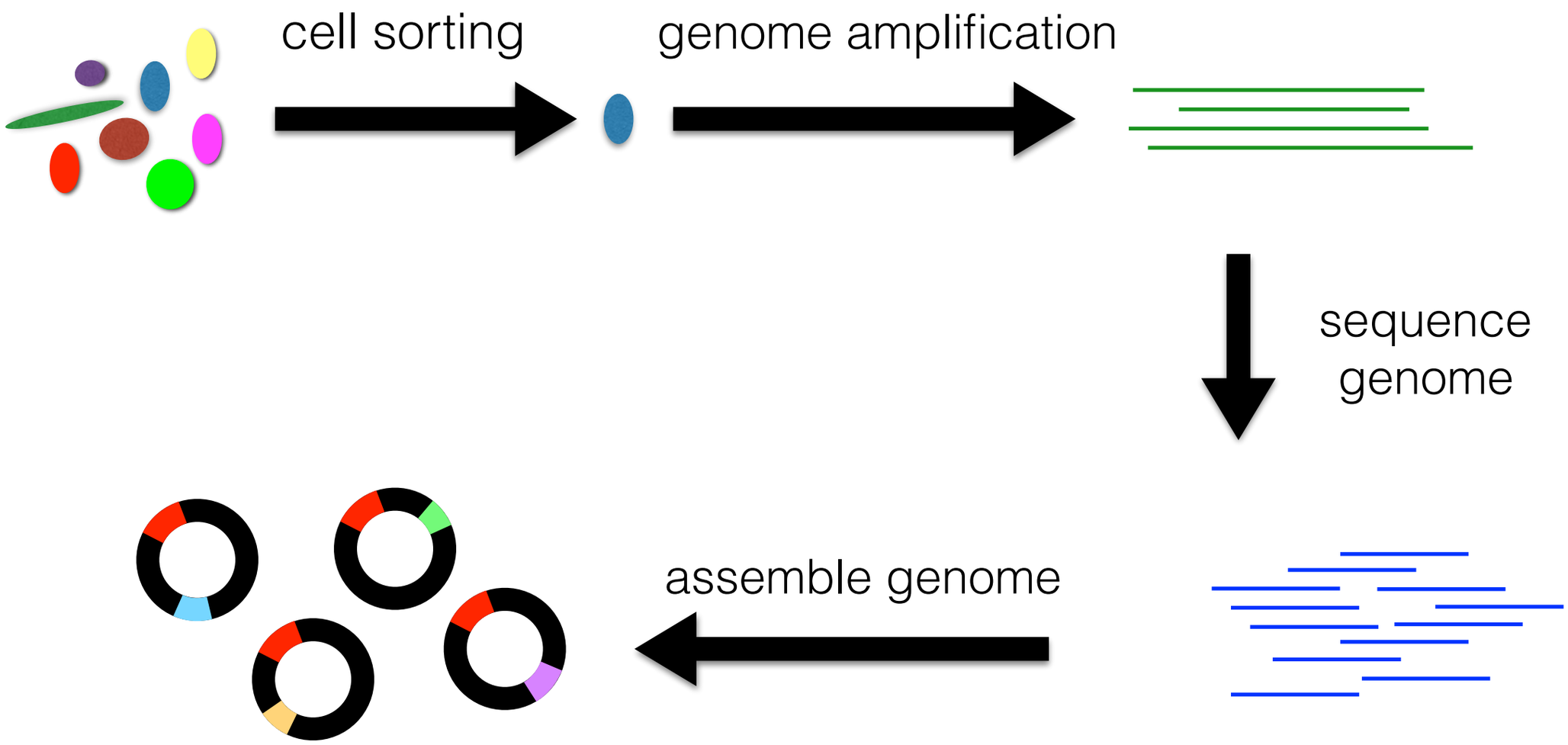


NOAA Okeanos Explorer Program

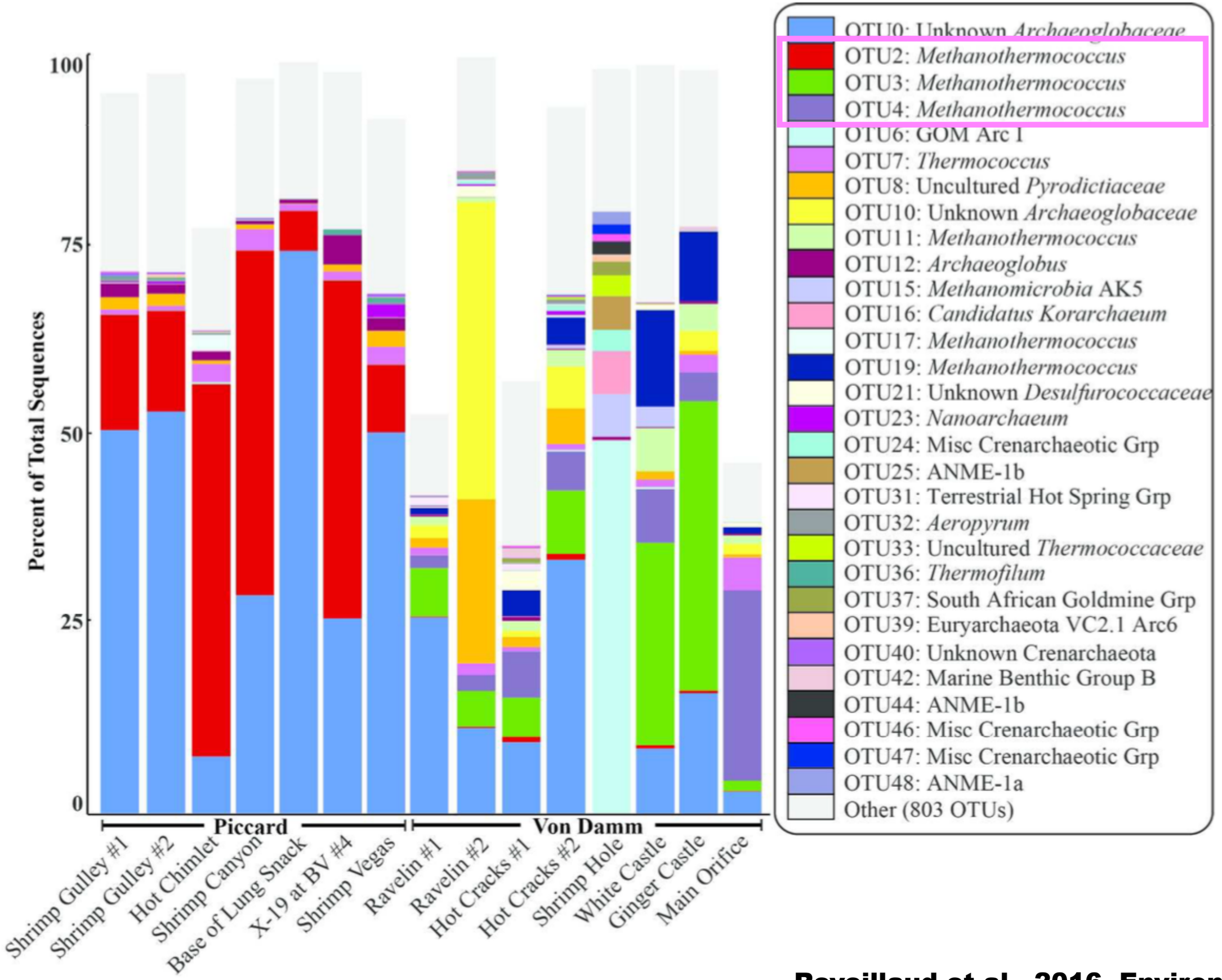
Kinsey and German, 2013, EPSL

Single cell genomics

Sort and sequence genomes of individual cells



Focus on Methanothermococcus



Reveillaud et al., 2016, Environ. Micro.

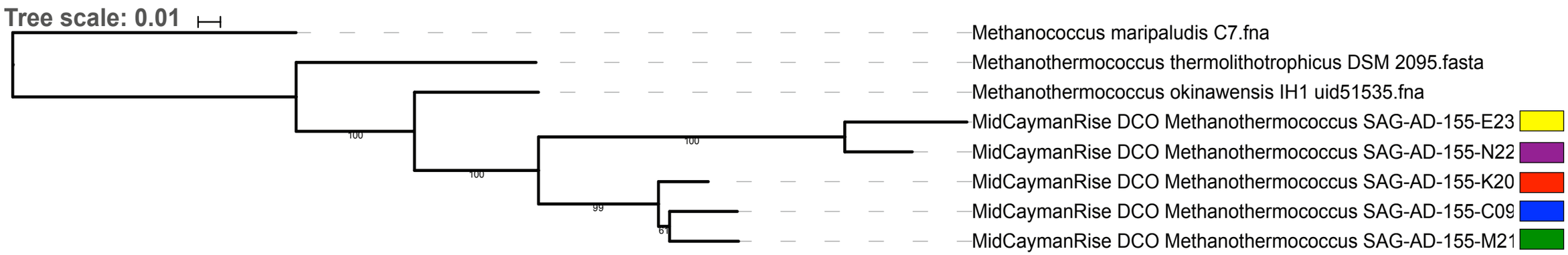
Methanothermococcus SAGs sequenced

Name	Completeness (%)	Number of scaffolds >1kbp	Assembly size	N50	Number of genes
AD-155-C09	84.27	125	1,740,890	25,888	1474
AD-155-E23	80.5	51	1,043,924	56,319	1221
AD-155-K20	16.01	25	188,232	9,430	205
AD-155-M21	67.77	107	932,247	18,049	937
AD-155-N22	55.15	99	856,655	16,384	866

all *Methanothermococcus* strains from **Ginger Castle** vent at **Von Damm**, collected in 2012

supported by a DCO Census of Deep Life Sequencing Grant, 2015

Phylogenetic relatedness

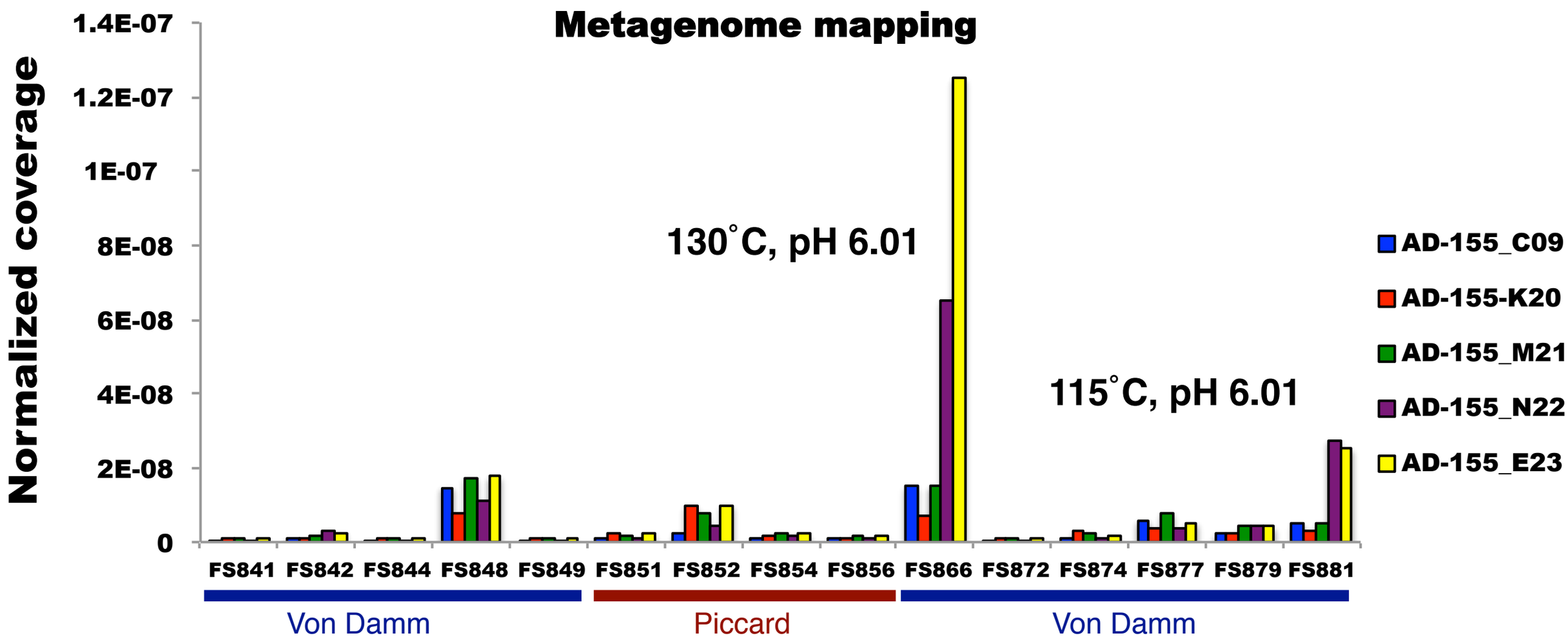


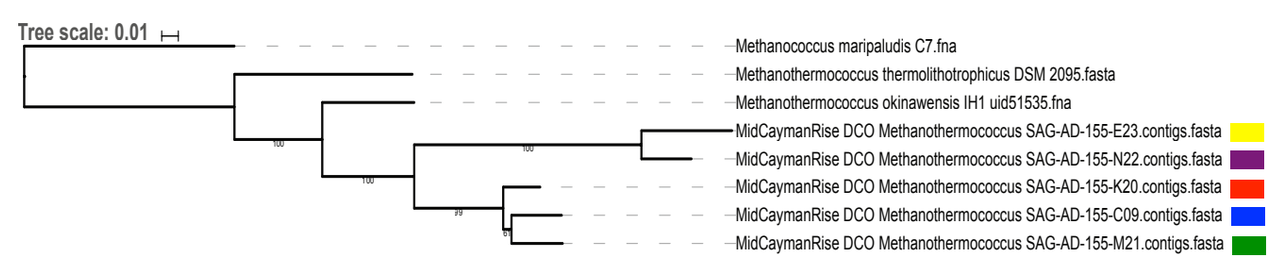
Single-copy universal marker genes identified and aligned with PhyloSift

Tree constructed using RAxML, 100 bootstraps.

Methanothermococcus strains are different from each other

Some Methanothermococcus strains are more abundant in specific samples: evidence for selection?





Using SNVs to look at variation <u>within</u> populations in samples

Methanothermococcus SAG sequence

overall coverage

GTAGTAACGATTACGGAAGACGGCTTACGATCAAGATTTACAT

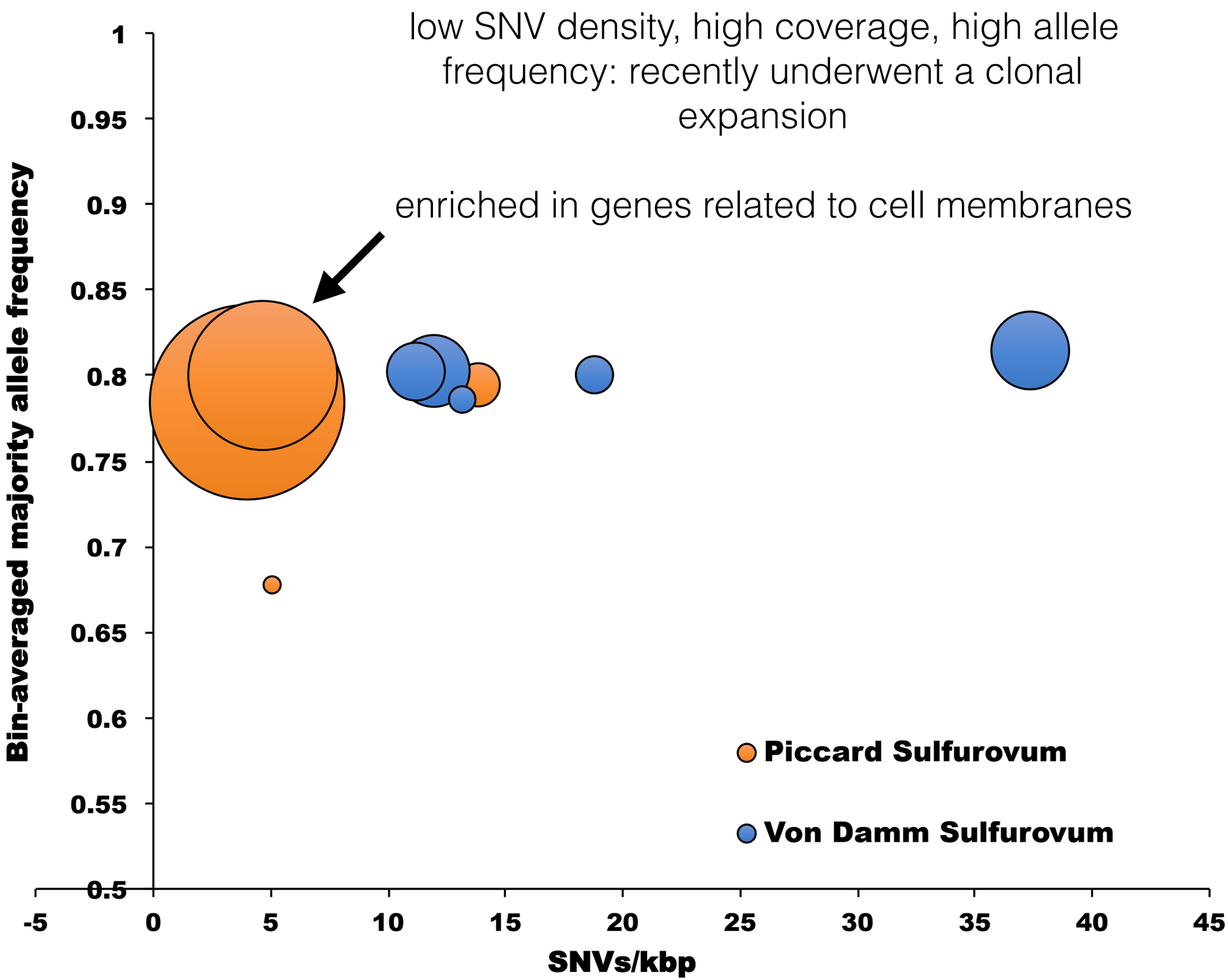
AGTAACGATTA
GTAACGATTAC
AACTATTACG
ACTATTACGG
TATTACGGA

GGCTTACGATCAA
GCTTATGATCAAC
CTTATGATCAACC
TTATGATCAACCG
TTATGATCAACCG

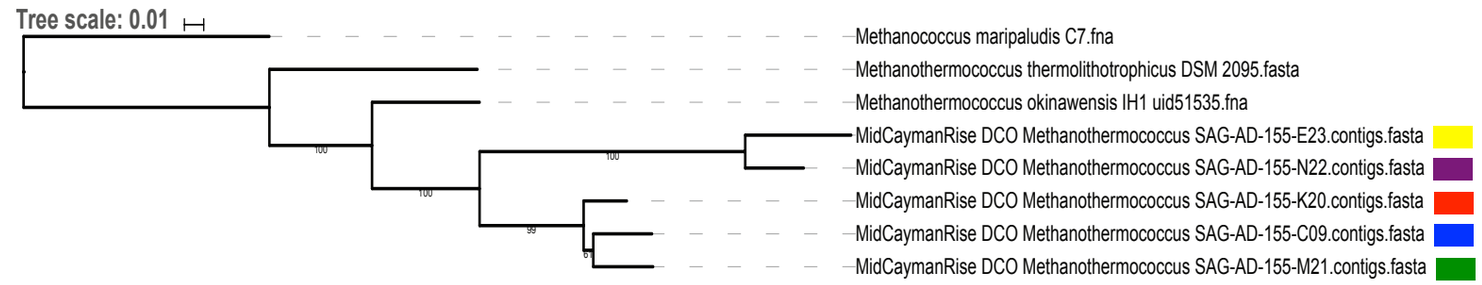
SNVs evenly represented in population

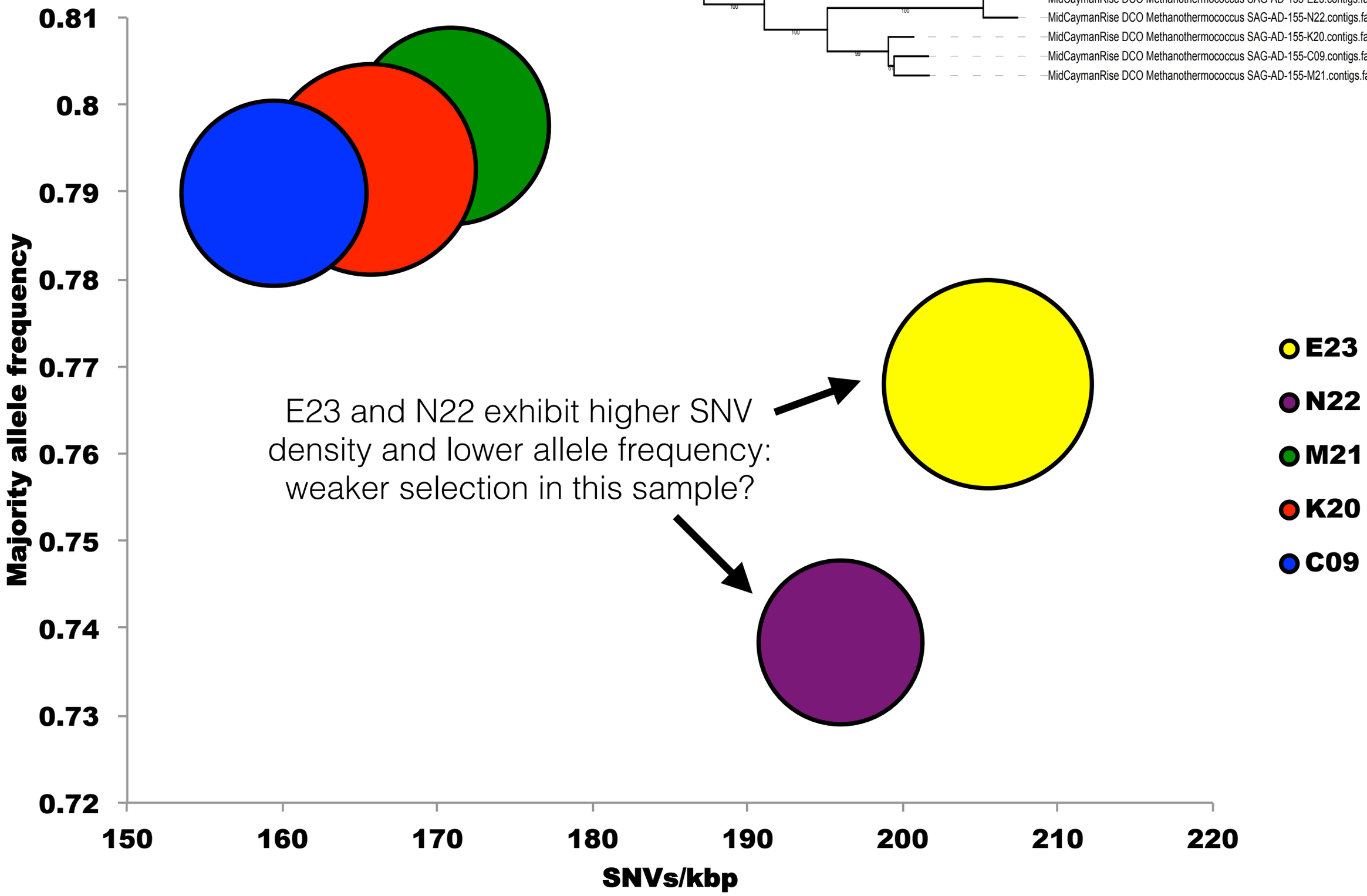
dominant SNV

Case study: Sulfurovum from the Mid-Cayman Rise

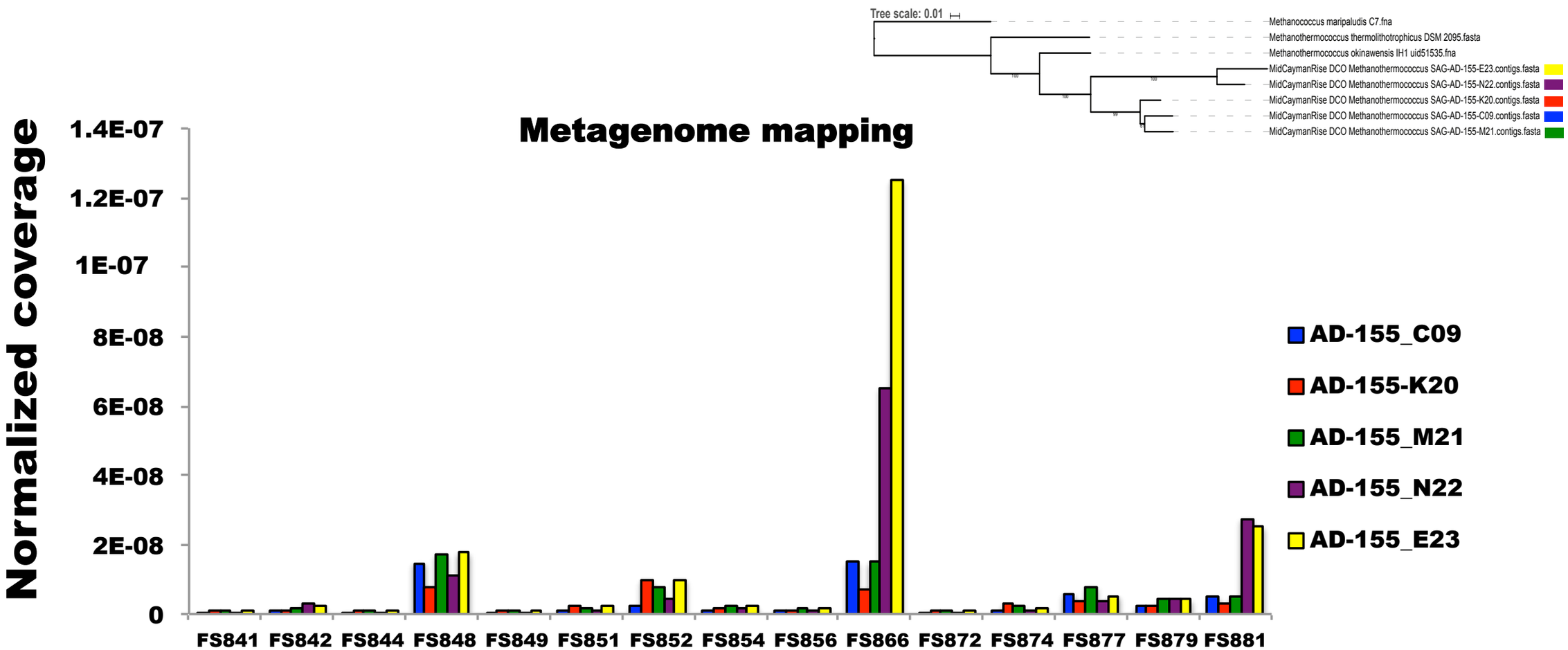


Identifying single nucleotide variants <u>within</u> Ginger Castle populations





Is there a difference in gene content?



… unclear.

C09 + **M21**: genes related to restriction modification

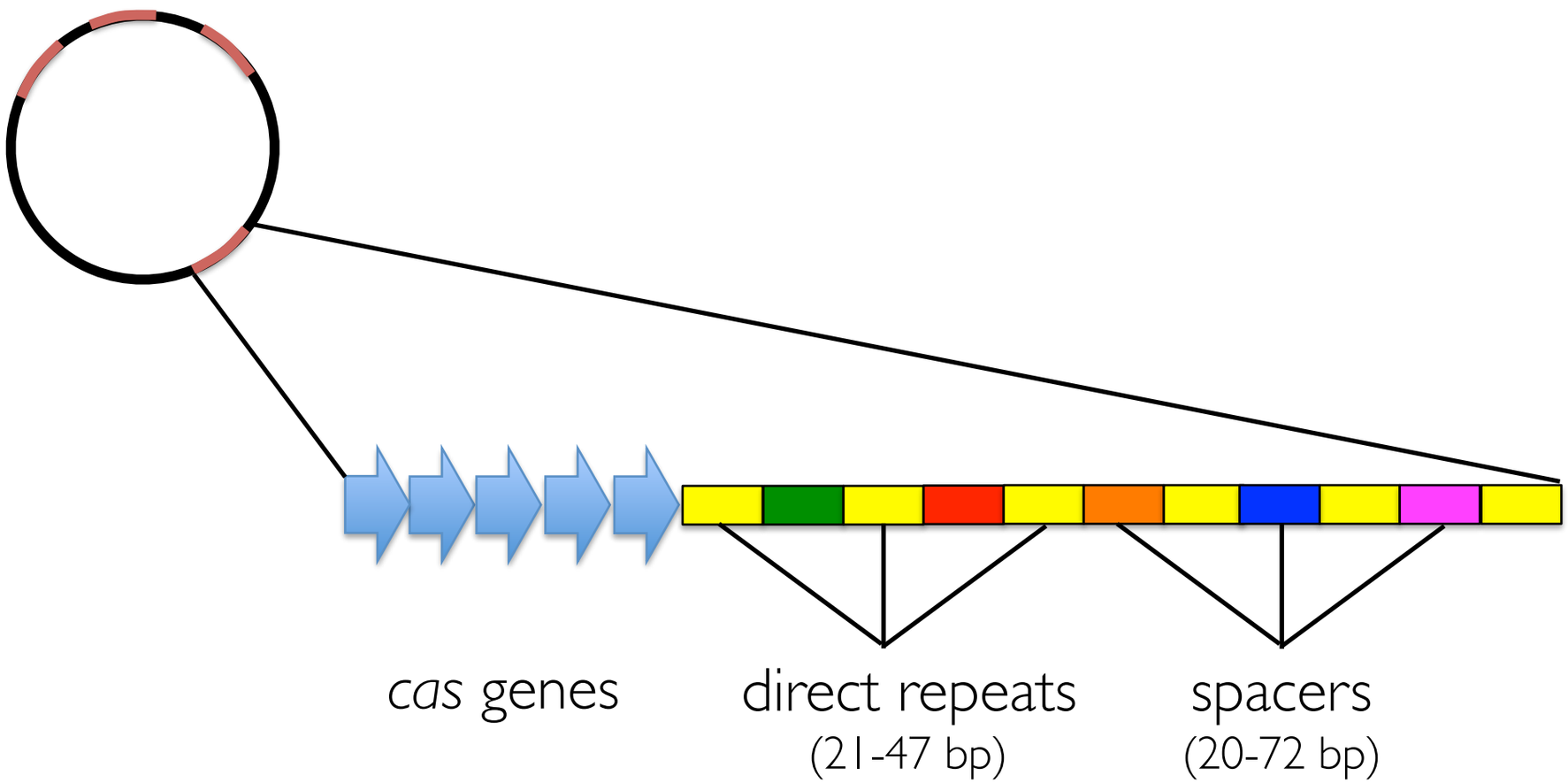
E23 + **N22**: genes related to cell motility, cell membrane

SAG incompleteness = a problem!

CRISPR: Clustered Regularly Interspaced Palindromic Repeats

A prokaryotic viral immunity system

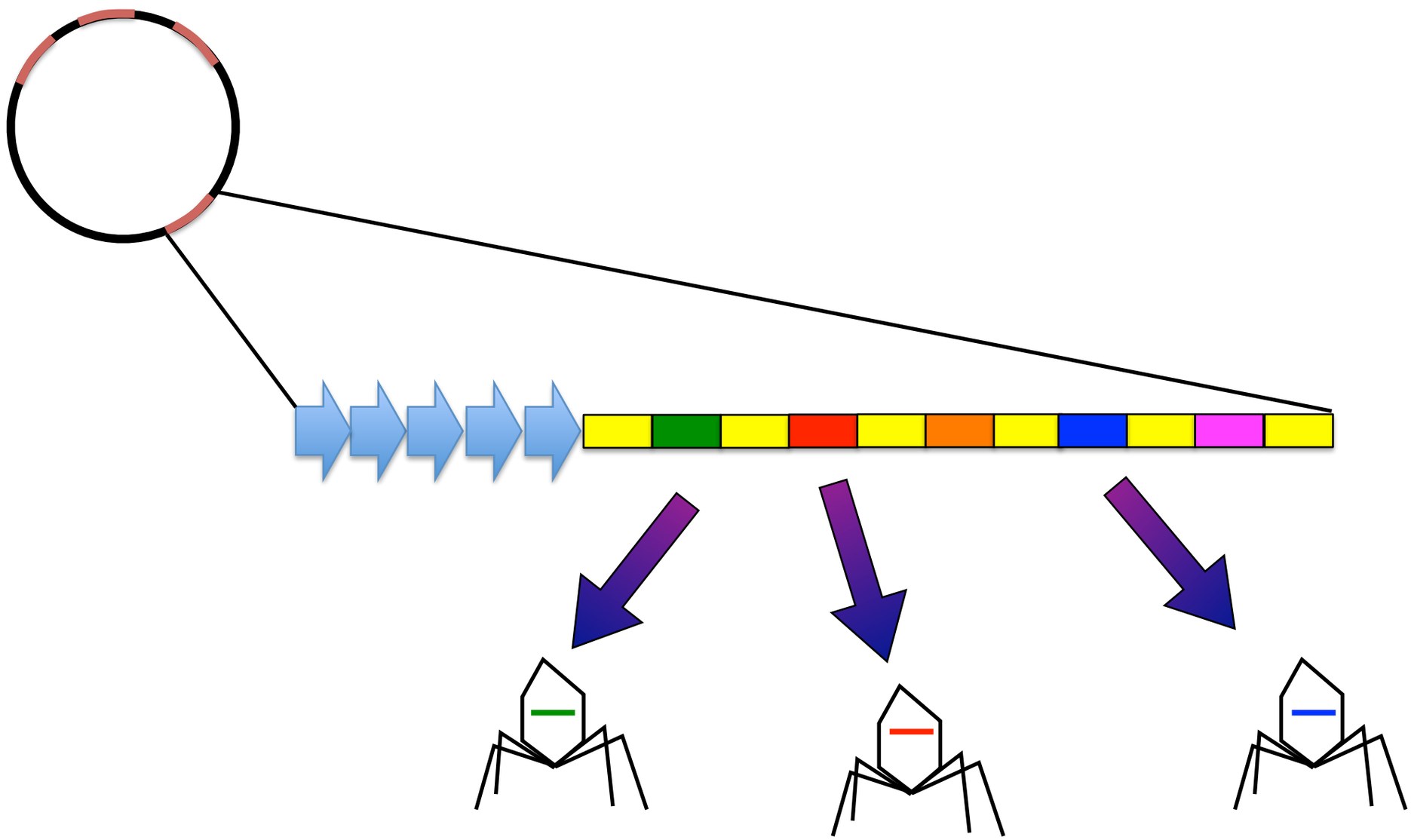
40% of Bacteria have CRISPRs
90% of Archaea have CRISPRs

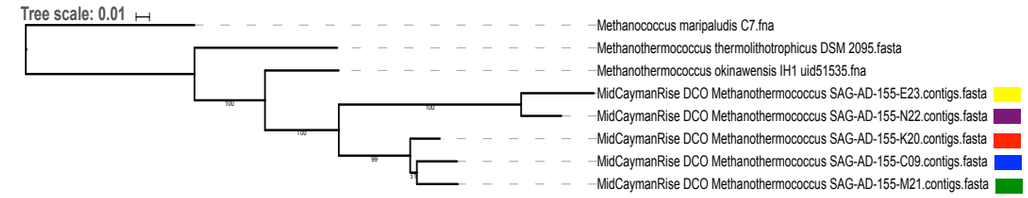


Barrangou et al (2007) Science; Labrie, Samson, and Moineau (2010) Nat Rev Micro.

CRISPR: Clustered Regularly Interspaced Palindromic Repeats

A prokaryotic viral immunity system





Methanothermococcus SAG AD-155-C09

CRISPR 1 DR: GTTTCCATCCCACAATGGTCTGATTTTAAC

CRISPR 2 DR: GTTTCCATCCCACTATGGTCTGATTTTAAC

CRISPR 3 DR: GTTTCCATCCCACAATGGTCTGATTTTAAC

CRISPR 4 DR: GTTAAAATCAGACCATTGTGGGATGGAAAT

CRISPR 5 DR: GTTTCCATCCCACAATGGTCTGATTTTAAC

CRISPR 6 DR: GTTAAAATCAGACCATTGTGGGATGGAAAC

Methanothermococcus SAG AD-155-E23

No CRISPRs found.

Methanothermococcus SAG AD-155-K20

CRISPR 1 DR: CACGCCCCCGTGAGAGGGCGAAC

Methanothermococcus SAG AD-155-M21

CRISPR 1 DR: GTTTCCATCCCACTATGGTCTGATTTTAAC

CRISPR 2 DR: GTTTCCATCCCACAATGGTCTGATTTTAAC

CRISPR 3 DR: GTTTCCATCCCACAATGGTCTGATTTTAAC

CRISPR 4 DR: GCTTCGCTCGTAAATGGTCTGATTTTAAC

CRISPR 5 DR: GTTAAAATCAGACAATTGTGGGATGGAAAC

CRISPR 6 DR: GTTTCCATCCCACTATGGTCTGATTTTAAC

Methanothermococcus SAG AD-155-N22

No CRISPRs found.

Presence of CRISPR loci corresponds to phylogeny

—> E23 and N22 have other mechanisms for avoiding viral infection?

Only a few shared spacers between strains

—> some cross-infection, but not much

Many different, unique spacers

—> CRISPR loci are active

—> *Methanothermococcus* viruses appear to be diverse

Each box = 1 spacer
Each SAG assigned a different color
If spacer unique to that SAG, it is given that color
If spacer matches a spacer in another SAG, colors match
If spacer matches another spacer in same SAG, there is a circle inside the box that matches the color of the matching spacer.

Conclusions

Big-picture question: What drives evolution in the vent subsurface?

Results so far:

- Some Methanothermococcus strains are more abundant in some samples than others
- Evidence for differential selection on different strains across samples
- Viruses may play an important role in differentiating strains

Ongoing work:

- Quantify selection (and drift?)
- Examine the pangenome: are there genomic islands that uniquely distinguish one strain from another?

Acknowledgements

Huber lab, MBL

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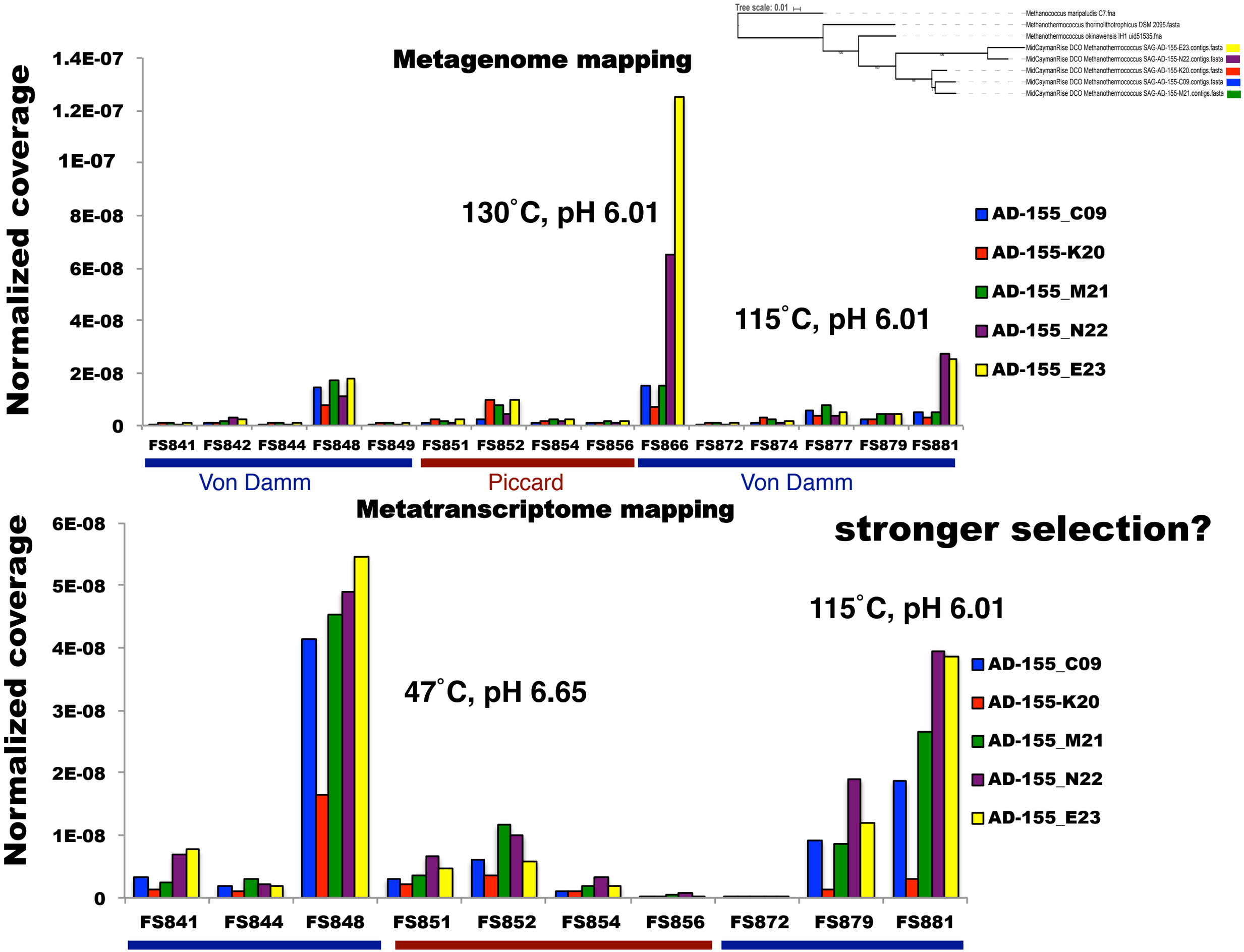
Deep Carbon Observatory
C-DEBI
NASA Astrobiology Institute
NASA ASTEP
R/V Atlantis crew
Falkor crew
NSF
Schmidt Ocean Institute

Keck Sequencing Center at MBL

DEEP LIFE

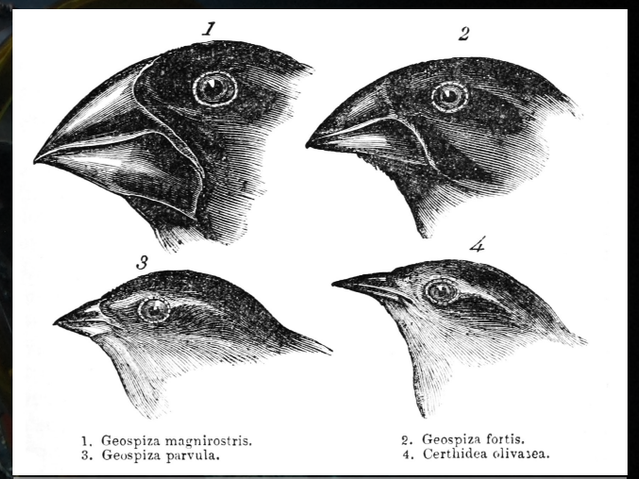
CENTER for DARK ENERGY BIOSPHERE INVESTIGATIONS

OCEAN EXPLORATION & RESEARCH


Tree scale: 0.01
Methanococcus maripaludis C7.fna
Methanothermococcus thermolithotrophicus DSM 2095.fasta
Methanothermococcus okinawensis IH1 uid51535.fna
MidCaymanRise DCO Methanothermococcus SAG-AD-155-E23.contigs.fasta
MidCaymanRise DCO Methanothermococcus SAG-AD-155-N22.contigs.fasta
MidCaymanRise DCO Methanothermococcus SAG-AD-155-K20.contigs.fasta
MidCaymanRise DCO Methanothermococcus SAG-AD-155-C09.contigs.fasta
MidCaymanRise DCO Methanothermococcus SAG-AD-155-M21.contigs.fasta
Metagenome mapping
Normalized coverage
1.4E-07
1.2E-07
1E-07
8E-08
6E-08
4E-08
2E-08
0
130˚C, pH 6.01
115˚C, pH 6.01
AD-155_C09
AD-155-K20
AD-155_M21
AD-155_N22
AD-155_E23
FS841 FS842 FS844 FS848 FS849 FS851 FS852 FS854 FS856 FS866 FS872 FS874 FS877 FS879 FS881
Von Damm
Piccard
Von Damm
Metatranscriptome mapping
stronger selection?
Normalized coverage
6E-08
5E-08
4E-08
3E-08
2E-08
1E-08
0
115˚C, pH 6.01
47˚C, pH 6.65
AD-155_C09
AD-155-K20
AD-155_M21
AD-155_N22
AD-155_E23
FS841 FS844 FS848 FS851 FS852 FS854 FS856 FS872 FS879 FS881

Big-picture question

What is the nature of variation within and among *Methanothermococcus* strains isolated from a single hydrothermal fluid sample?

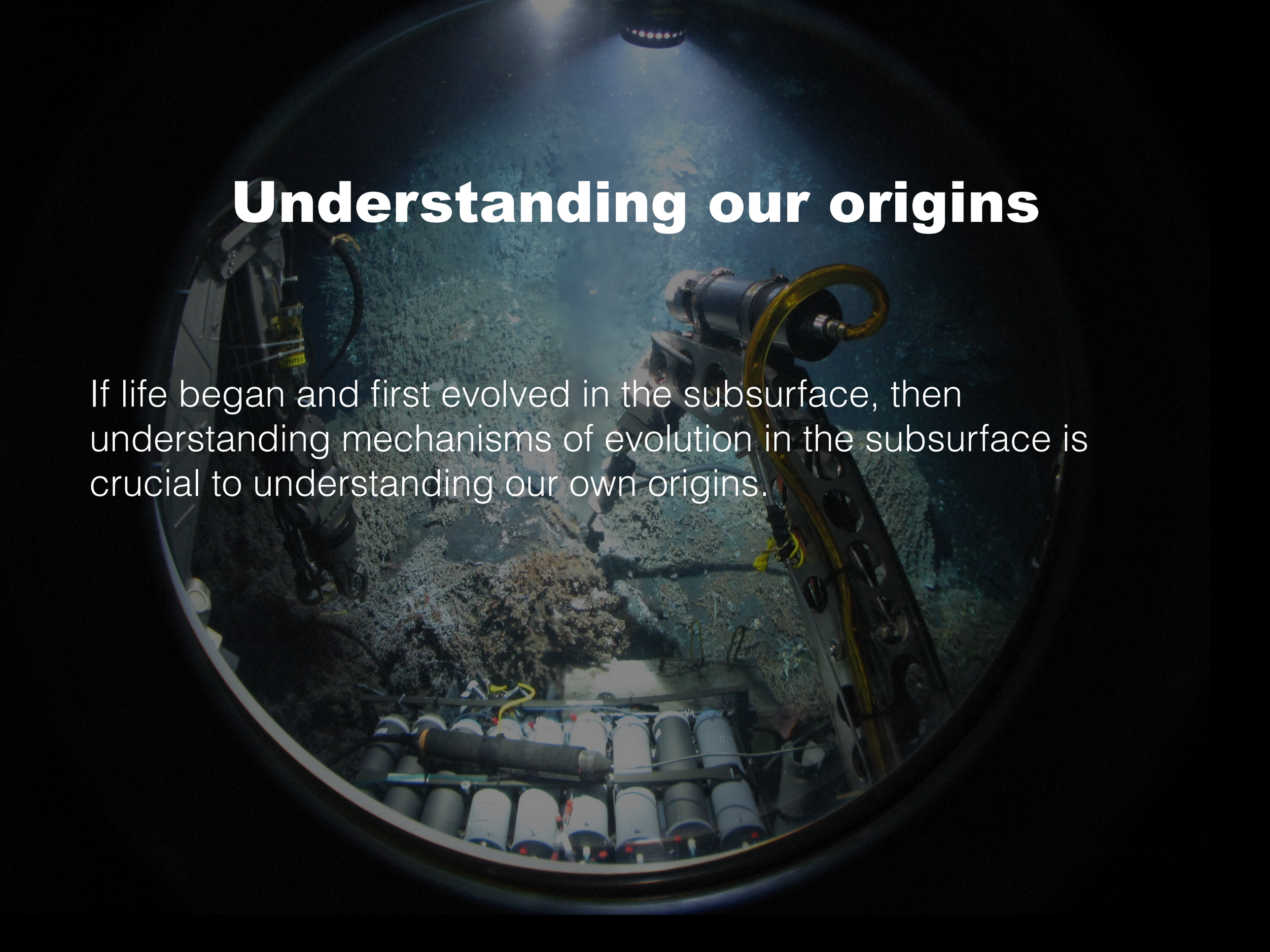


More specific questions:

- Are some strains are more abundant or more highly expressed than others? Why?
- Do we see variation at the nucleotide level? At the gene level?
- Do we see evidence of horizontal gene transfer, mobile elements, viruses?

Samples from the Mid-Cayman Rise, 2012-2013

Sample Name	Year	Vent Field	Vent Name	T (˚C)	Cells/ml	pH	Mg (mmol/kg)
FS841	2012	Von Damm	Old Man Tree	114	NC	5.89	14
FS842	2012	Von Damm	Ravelin #2	86	NC	6.26	15.8
FS844	2012	Von Damm	Shrimp Hole	50	2.69E+05	7.55	47.1
FS848	**2012**	**Von Damm**	**Ginger Castle**	**47**	**1.08E+05**	**6.65**	**37.3**
FS849	2012	Von Damm	Main Orifice	109	2.97E+04	7.13	47.4
FS866	2013	Von Damm	near Main Orifice	130	3.72E+05	6.01	27.3
FS872	2013	Von Damm	Shrimp Hole	30	1.54E+05	7.61	53.0
FS874	2013	Von Damm	Twin Peaks	140	4.02E+05	6.00	15.5
FS877	2013	Von Damm	Shrimp Buttery	131	1.81E+05	6.22	27.3
FS879	2013	Von Damm	Hot Cracks #2	29	1.12E+05	6.91	48.0
FS881	2013	Von Damm	Old Man Tree	114	9.41E+04	6.01	19.0
FS851	2012	Piccard	Hot Chimlet, BVM	106	5.45E+04	6.98	52.2
FS852	2012	Piccard	Shrimp Canyon, BVM	44	5.20E+04	6.45	49.1
FS854	2012	Piccard	Marker X-19 at BV #4, BVM	18	7.27E+04	6.79	51.2
FS856	2012	Piccard	Shrimp Gulley #2, BSM	108	1.40E+05	6.66	50.9

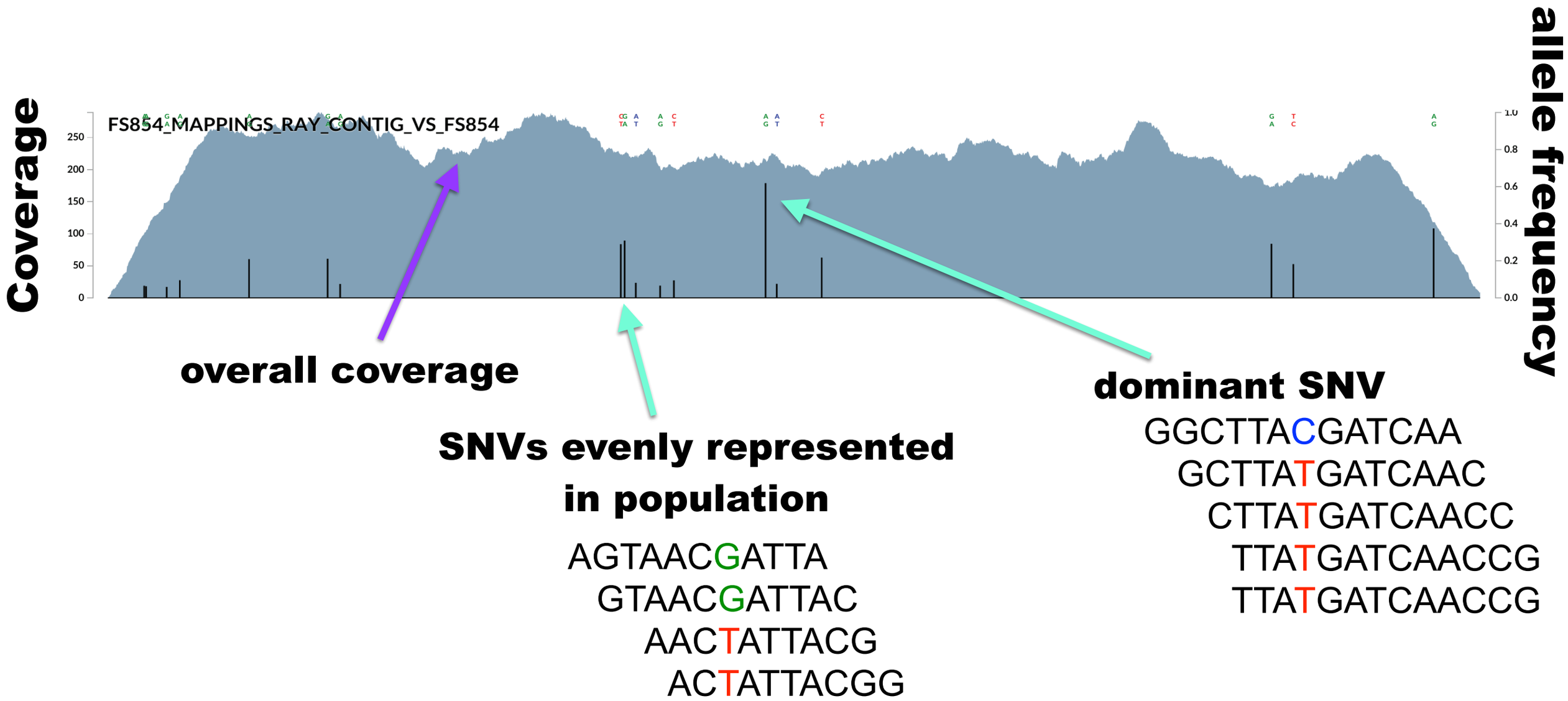


Understanding our origins

If life began and first evolved in the subsurface, then understanding mechanisms of evolution in the subsurface is crucial to understanding our own origins.

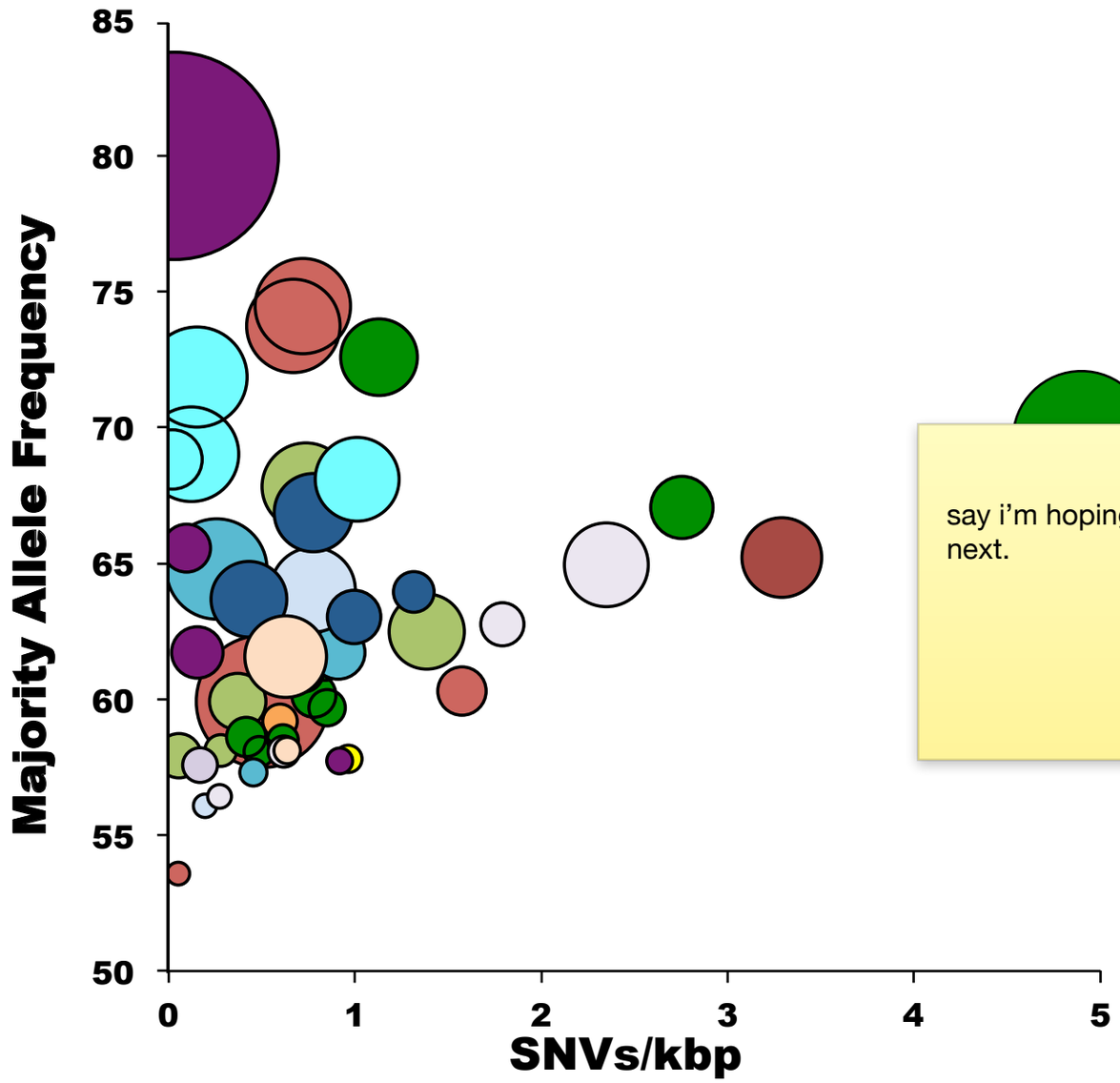
A closer look:

Natural variation within populations through single nucleotide variants (SNVs)

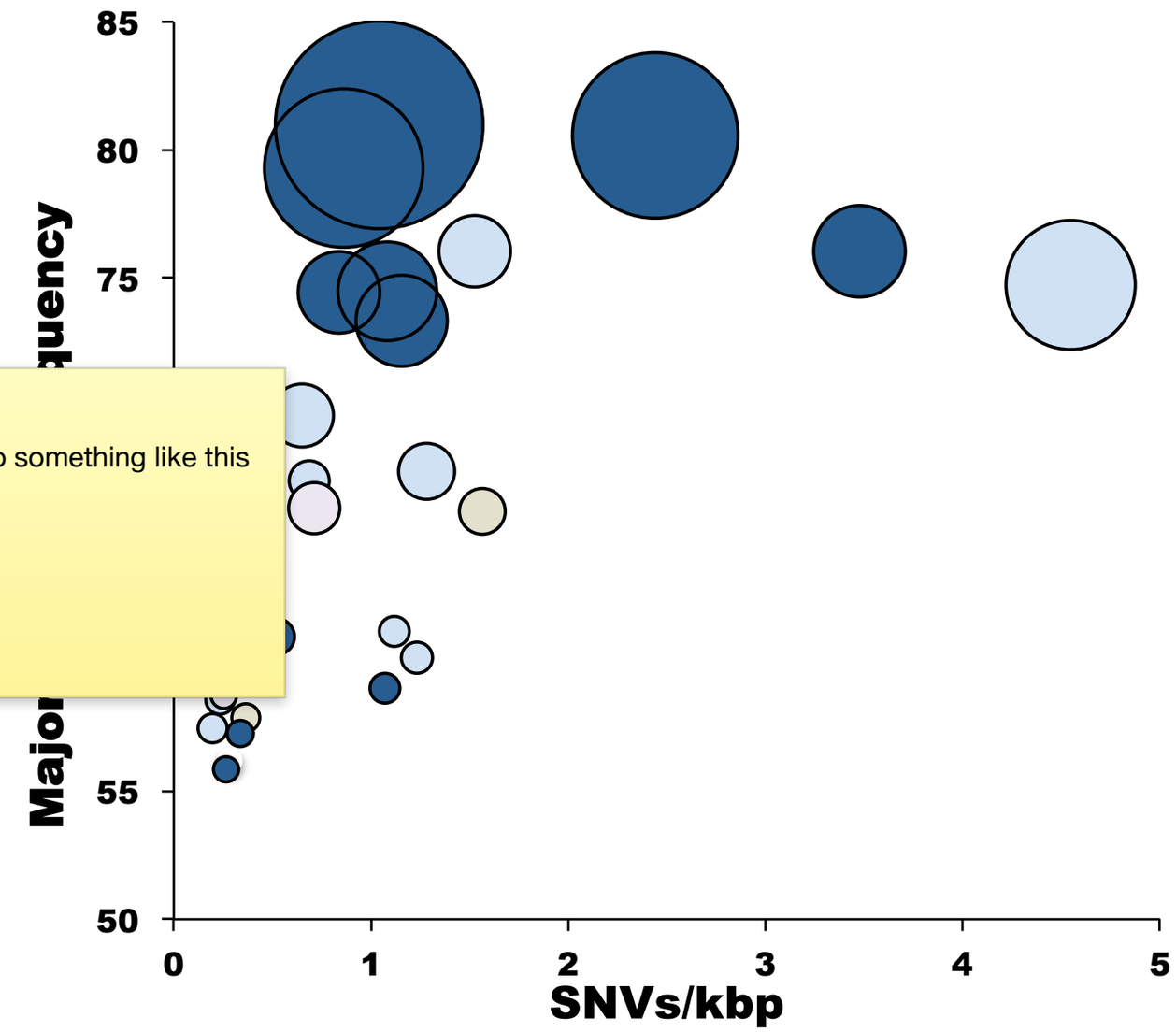


Patterns of variability within microbial populations

Von Damm

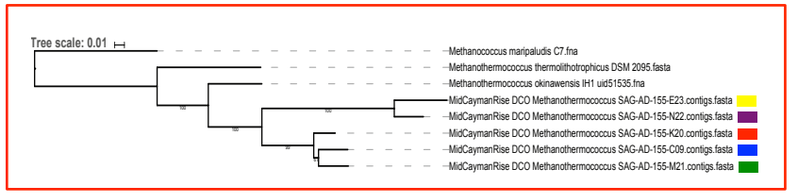
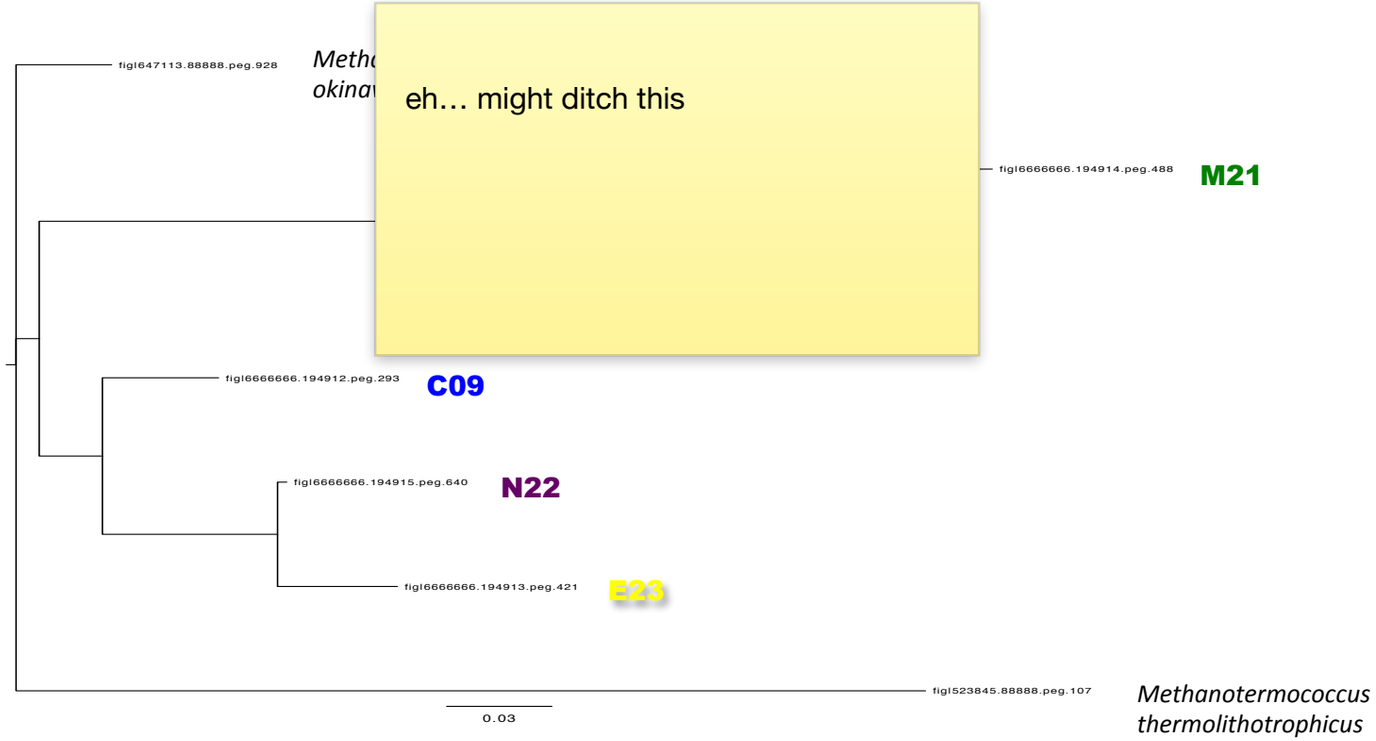


Piccard



say i'm hoping to do something like this next.

- Aquificales
- Caldiarchaeum
- Candidate Division SR1
- Candidatus Micrarchaeum
- Chloroflexi
- Crenarchaeota
- Deltaproteobacteria
- Epsilonproteobacteria- Other
- Epsilonproteobacteria- Sulfurovum
- Euryarchaea- Other
- Euryarchaea- methanogens
- Firmicutes
- Gammaproteobacteria
- Nanoarchaeota
- Thermodesulfobacteria

Examining gene relatedness

mcrA