

My Story and The Gut Microbiome

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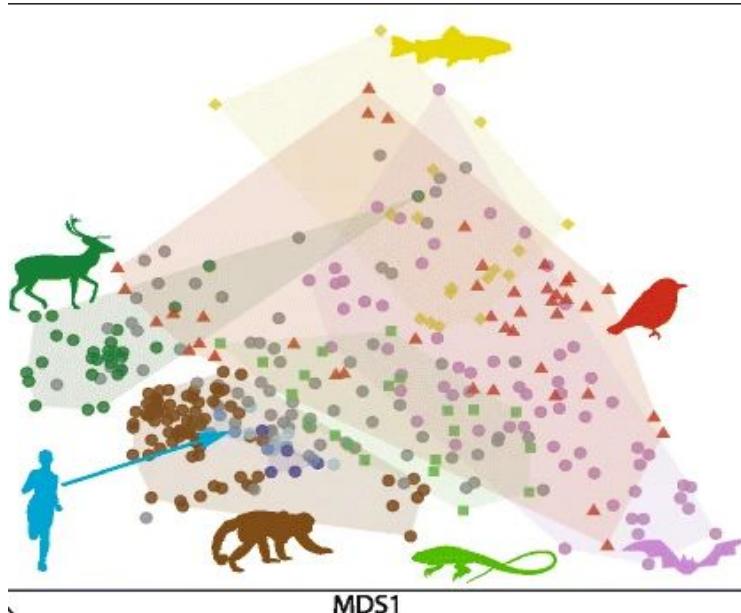
Microbiology - the origins of life

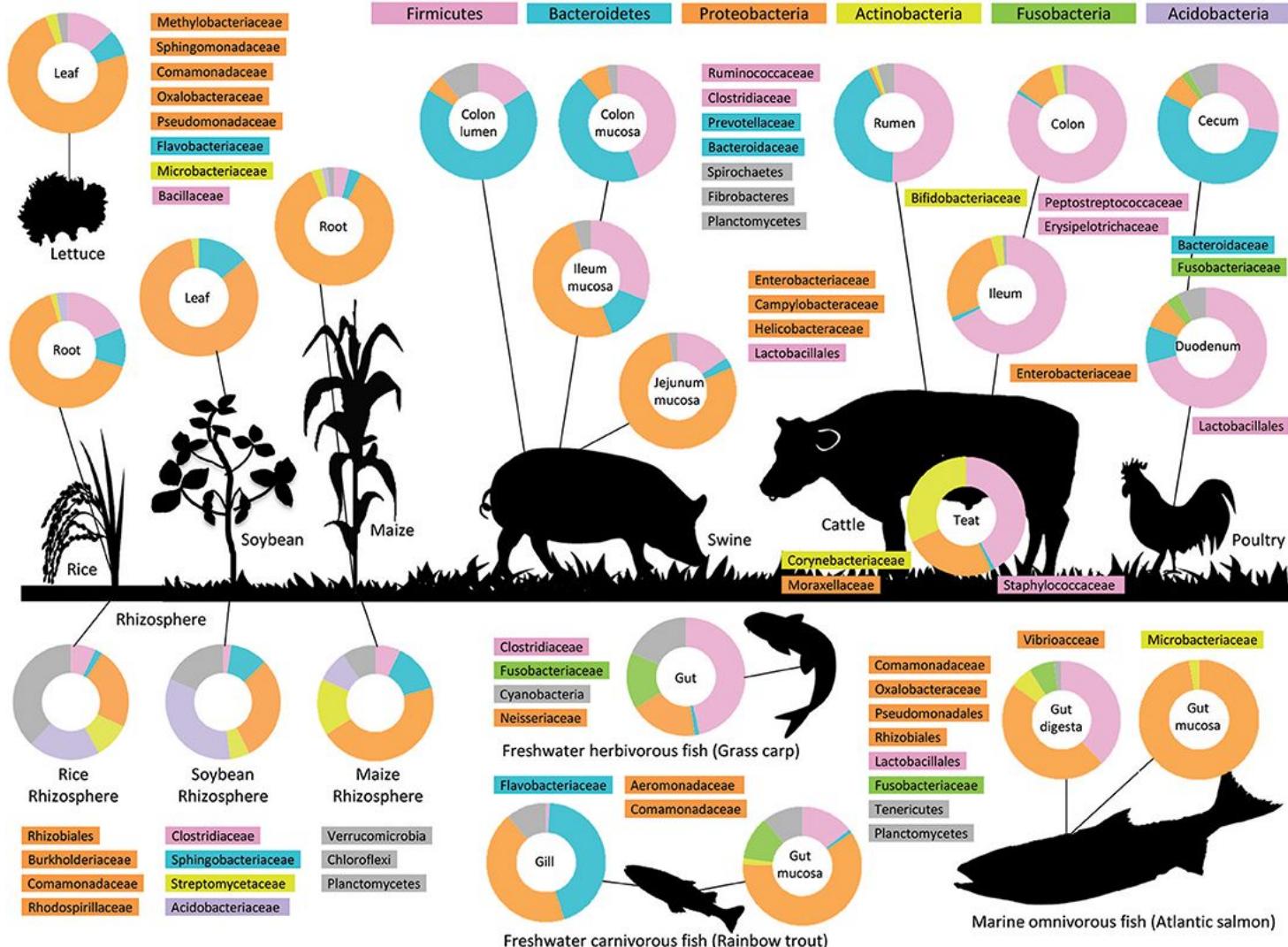
- Microbes first form of life ~ 4 billion years ago.
 - One reason there are so many diverse bacterial metabolic pathways.
- Abiogenesis: Earth forms (4.54Bya), Oceans (4.4Bya), evidence of self-replicating RNA in early hydrothermal vent formations.
- Multicellular life not until ~2.2 billion, upon oxygenation of atmosphere. The two have evolved symbiotically since then.
 - Endosymbiotic theory: organelle evolution based on specialized microbes.



Microbes evolve with Eukaryotes

- As humans have gained access to more complex and diverse energy sources, microbiome has adapted metabolically to this.
- The rise of Abx → increase in bacterial resistance
- Rhizobacteria: nitrogen-fixing for root systems



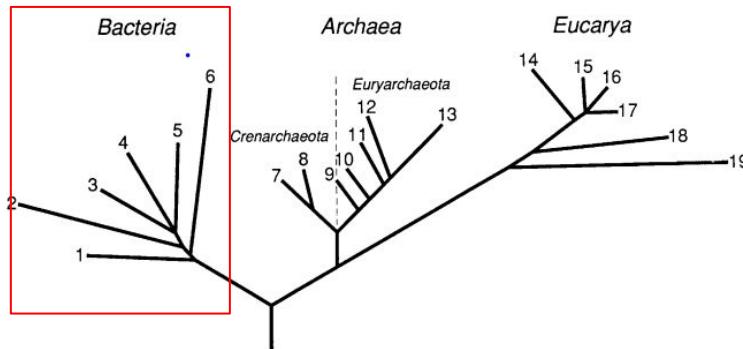


Microbiome Overview

- Similar to how ‘Genome’ refers to all of an organism’s genes, the ‘Microbiome’ refers to all of the microbes living in and on a host.
- Microbiome can include bacteria, viruses, archaea, protozoa, fungi, etc. Although the word ‘microbiome’ is typically used in the context of bacterial communities.
- Human microbiome: skin microbiome, mouth microbiome, and gut microbiome are just 3 of the many functionally and ecologically distinct communities of microbes that live in and around us.

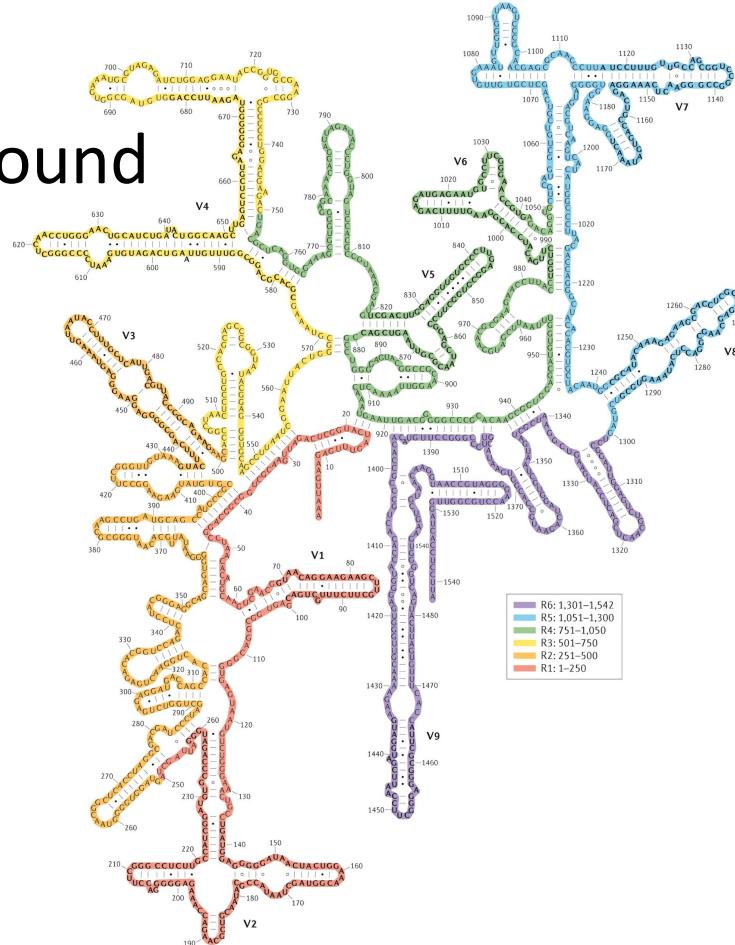
Microbiome Research: Background

- Microscopy and culturing both important roles but the advent of sequencing is main reason why microbiome research activity has grown exponentially in past 20 years.
- In Carl Woese and George Fox's seminal 1977 Paper (1), they described a third domain of life, Archaea, based upon short gene sequences of the 16S rRNA gene.
- Important in establishing the 3 domains of life that we now recognize today, even more important in establishing a way to define bacterial species, phylogenetically.



Human Microbiome Research: Background

- Sequencing of the 16S rRNA gene becomes common:
Why 16S rRNA?
 - Encodes for the small subunit of a bacterial ribosome.
 - Highly conserved gene.
 - Generally, a 97% sequence similarity threshold is used to differentiate species.
 - 9 variable regions (V1-V9)
 - Most modern workflows will sequence the V4 region or the V3-V4 regions



Human Microbiome Research: Workflow

DNA EXTRACTION - gDNA is extracted from feces, blood, tissue, etc.



PCR - The 16S rRNA gene V4 region (250bp) is amplified. Usually special primers with indexes and Illumina adapters flanking the gene of interest are used.



Normalization/PCR cleanup - The pure, amplified sequences need to be roughly the same concentration so each sample is sequenced equally.



QC - Checking that the length of the amplicon is correct and checking what concentration they can be loaded onto the MiSeq at.



Sequencing - Samples are sequenced, usually on an Illumina MiSeq. Spike in controls determine error rates, Q30.

Human Microbiome Project: Goals

- Previous knowledge existed of human microbiome being important, but identities and functional roles largely unknown; 2007 HMP introduced by NIH.
- Initial Goals:
 - Development of a reference set of 3,000 isolate microbial genome sequences.
 - Initial 16S & mWGS metagenomic studies to generate an estimate of the complexity of the microbial community at each body site, providing initial answers to the questions of whether there is a "core" microbiome at each site.
 - Demonstration projects to determine the relationship between disease and changes in the human microbiome.
 - Development of new tools and technologies for computational analysis, establishment of a data analysis and coordinating center (DACC), and resource repositories.
 - Examination of the ethical, legal and social implications (ELSI) to be considered in the study and application of the metagenomic analysis of the human microbiota.

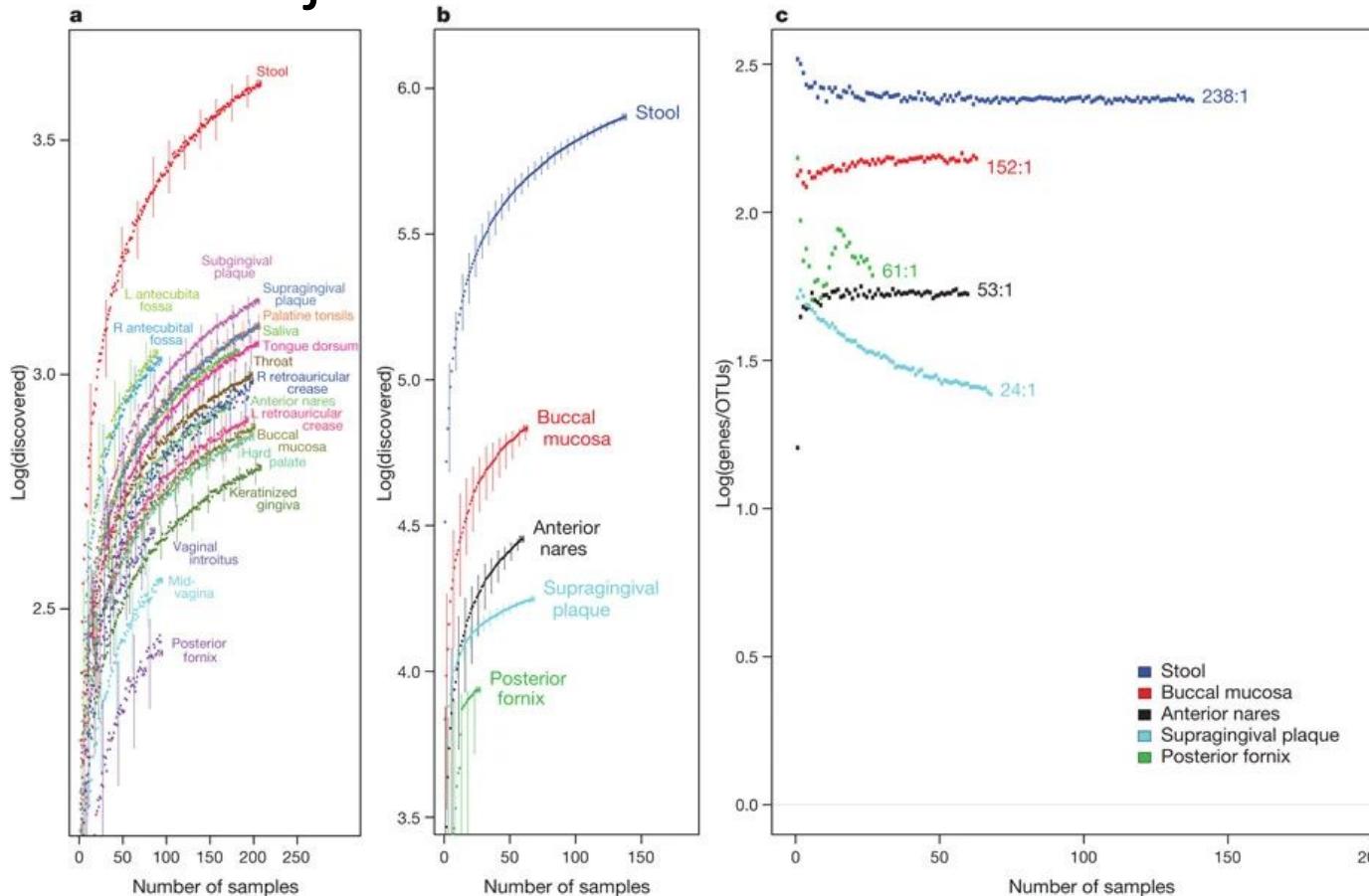
(www.hmpdacc.org/hmp)

Human Microbiome Project: Outcomes

- Officially concluded in 2017
- 300 Humans sampled at 5 sites: oral cavity, nasal cavity, skin, GI tract, urogenital tract.
- 2200 Reference strains isolated and sequenced.
- Over 650 papers, cited 70,000+ times.
- Two landmark papers published in Nature (Methe et al. 2012, Huttenhower et al. 2012); thousands of papers followed using the publicly available information.
- Significant contributions in how we think about the human microbiome's role in nutrient uptake from food, metabolism of drugs, development and activity of immune system, even brain development and behavior.

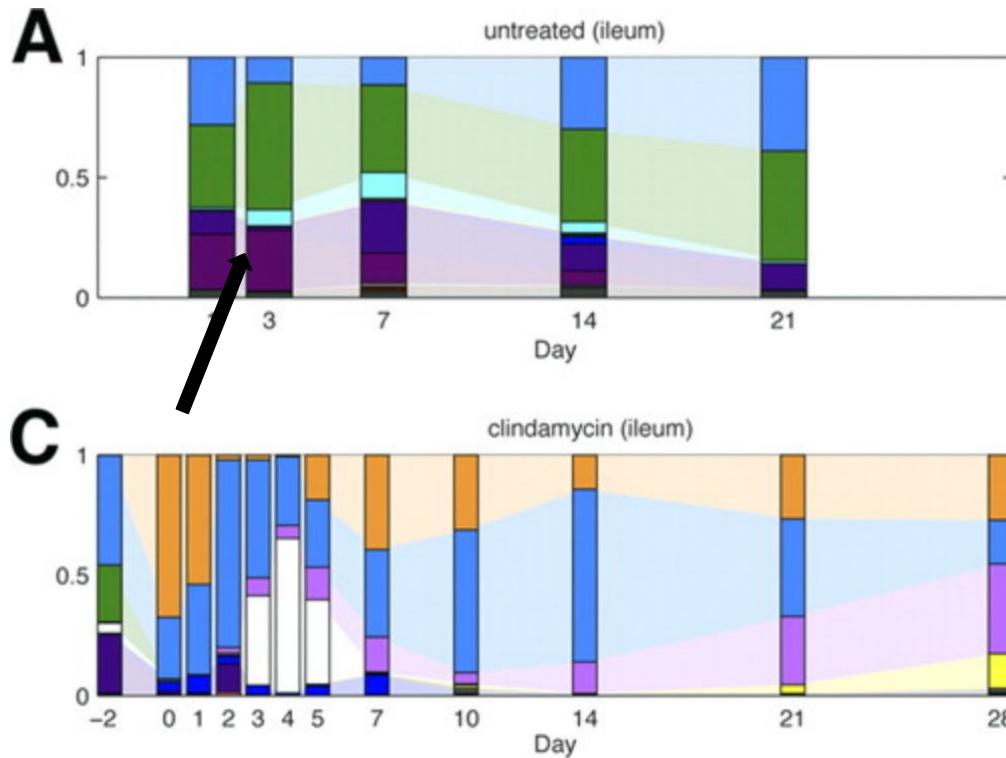
Human Microbiome Project: Outcomes

- Largest microbiome dataset at the time: immense interpersonal variation, each body site had distinct microbiome; informed future research.
- The quality-controlled standardized pipelines developed for analyzing the metagenomic and 16S data provided framework for future studies.



Antibiotics (Abx)

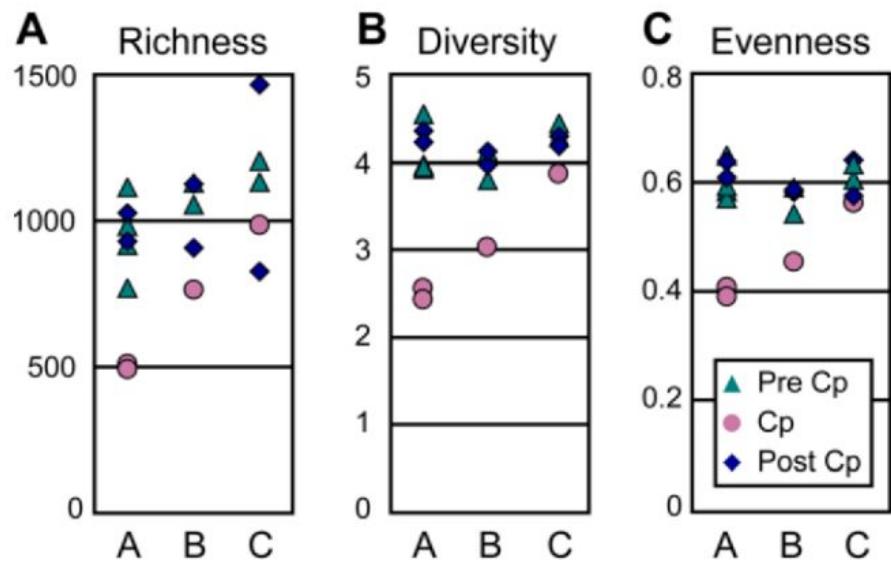
- Antibiotics, while good for fighting infection, also kill off many native microbiome members.
- Many of these native microbiota are essential to our health. One of the reason strong Abx result in diarrhea is because our gut is trying to restore equilibrium.
- Different Abx result in different changes to the microbiota depending on mechanism to disrupt growth.



The community of a healthy mouse small intestine (ileum) starts the same, but after Clindamycin treatment, the community composition is drastically different.

Antibiotics (Abx)

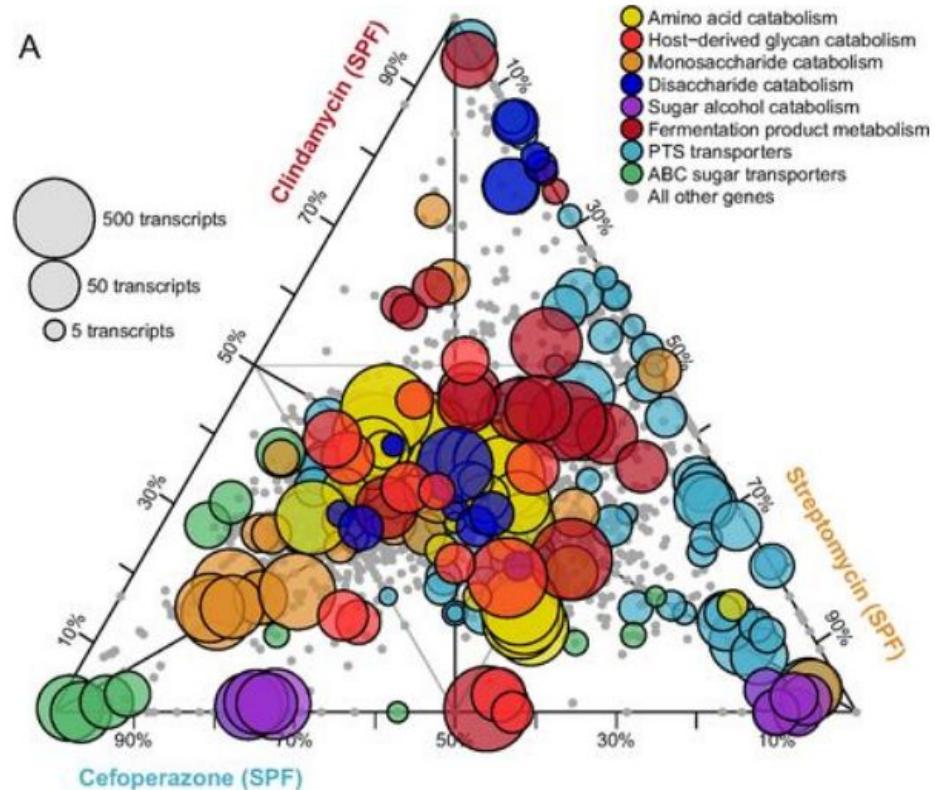
- Abx can be either broad spectrum (all bacteria) or target certain types of bacteria (gram positive vs. gram negative).
- Different classes of Abx have different targets: cell wall, DNA synthesis, enzyme interference, etc.
- Abx lead to decreases in species richness () and diversity(). This disruption can lead to susceptibility to further infection. (**Opportunistic Pathogens**)



In the bacterial communities of 3 healthy individuals, A, B, and C the pre- and post- ciprofloxacin (Cp) treatment is shown.

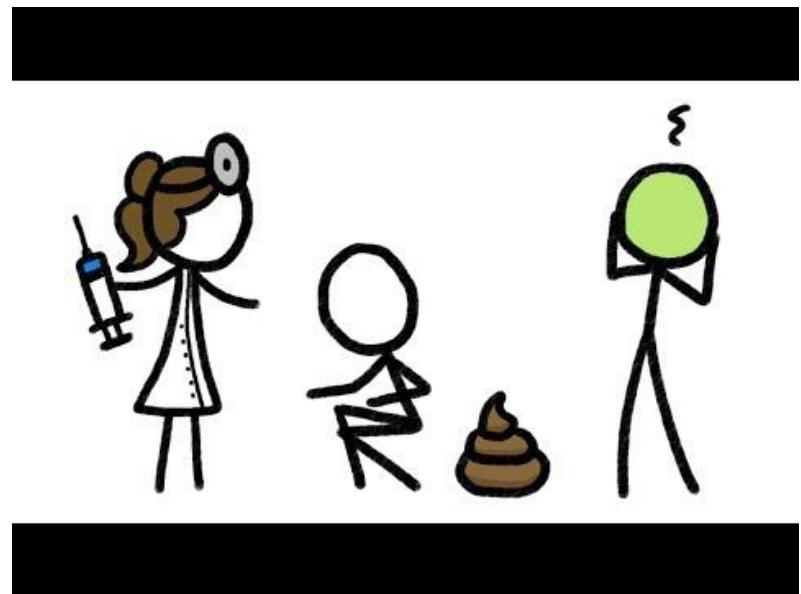
Opportunistic Pathogens

- *C. difficile* infection is the most common hospital-acquired infection in the U.S. and the main risk factor for disease is antibiotic exposure.
- To the right: Each color of circle represents a different functional nutrient niche (bacterial metabolism), each point of the triangle represents a different immunocompromised community (due to Abx). Communities that are disturbed differently lose different metabolic functions that allow for colonization of a pathogen.
- Can be thought of as filling different metabolic holes that were left by the antibiotics.



FMT = Fecal Microbiota Transplantation

- FMT is a relatively new investigational treatment that aims to restore protection from opportunistic pathogens in immunocompromised individuals.
- Feces from a healthy relative donor, screen it for pathogens, administered via colonoscopy, naso-enteric tube, or capsules.
- By restoring some of the important microbiota that were wiped out by Abx, and increasing diversity of the microbiome, opportunistic pathogens have less opportunity to colonize.
- Also being investigated as a treatment for IBS and Crohn's disease.



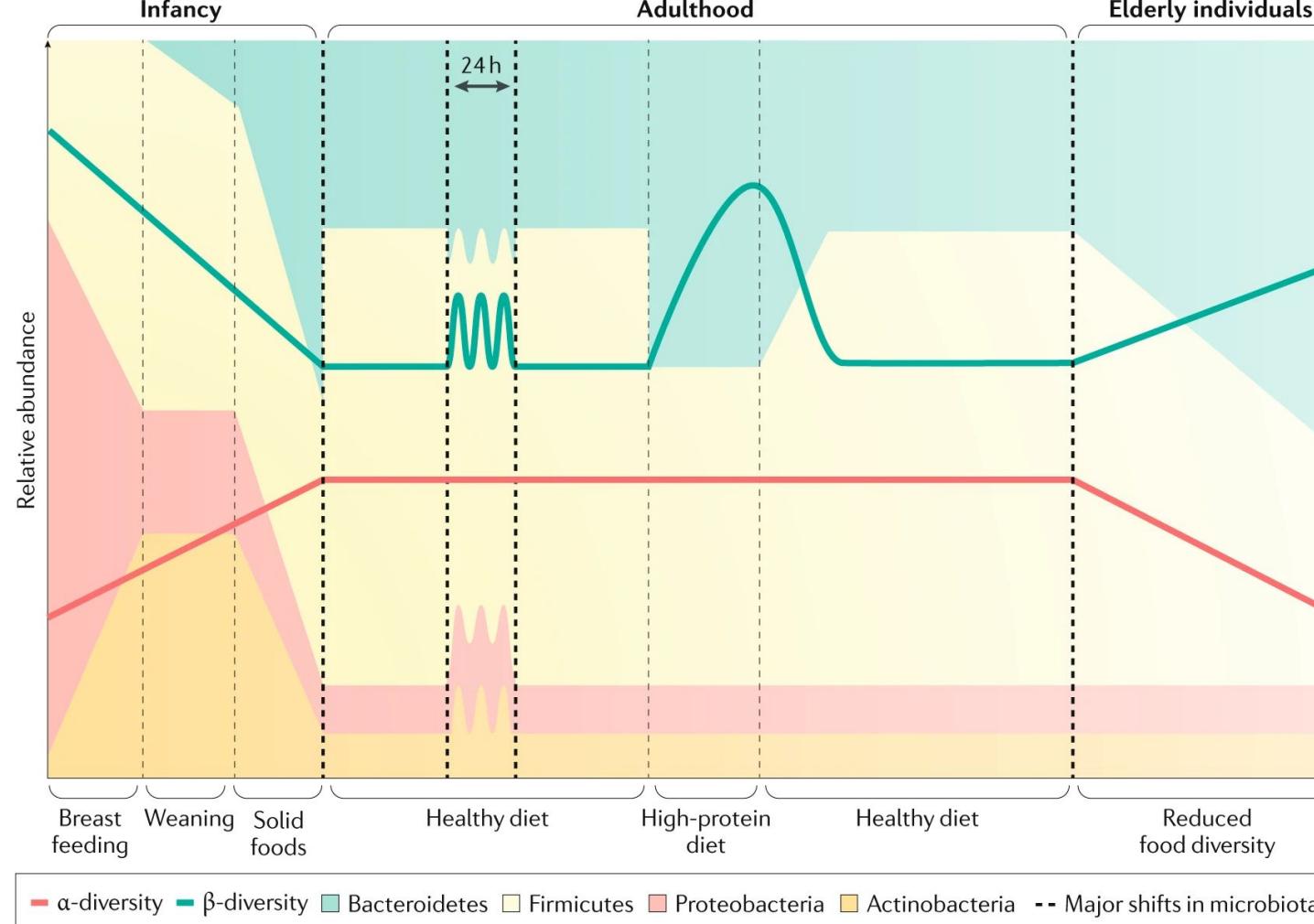
YouTube video via MinuteEarth

Microbiome and Diet

- Most complex plant polysaccharides are not digested by humans; enter the colon as a potential energy source for gut microbes.
- In early 80's Dr. Abigail Salyers' research group: ability of bacteria to metabolize plant substrates and mucins. Proposed that by limiting fiber intake, could trigger induction of enzymes capable of degrading the intestinal mucin layer as energy source instead, affecting human health and even colon cancer.
- Not until 2005 that Dr. Jeffrey Gordon's research group showed in mice that different enzymes are expressed in fiber-rich and fiber-depleted diets and in a fiber depleted diet, leads to significant reduction in colonic mucus layer thickness and increases susceptibility to disease .
- While there is no one 'healthy microbiome' - fiber is clearly an important part of keeping gut microbes happy.

Microbiome and Diet

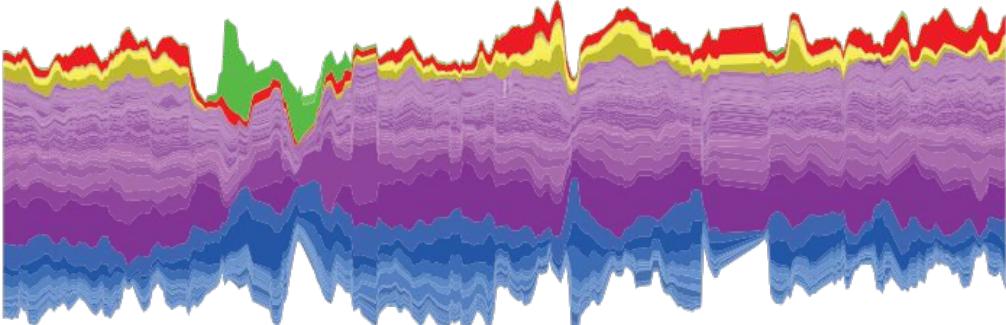
- The same year as the mucus wall discovery, Jeffrey Gordon's group showed that a mouse model of obesity had an altered ratio of the two main phyla present in the gut; the *Bacteroidetes* and the *Firmicutes*. Diversity is important to a 'healthy' gut microbiome.
- A functional analysis of these microbiomes revealed that an obesity-associated microbiota had an increased capacity for energy harvest from food, and this phenotype could be transferred through FMT.
- These led to multiple re-analysis of publically available datasets to translate to human health and the findings were mixed. Diversity and evenness measures/number of bacterial species and obesity status did have significant associations, albeit relatively weak.



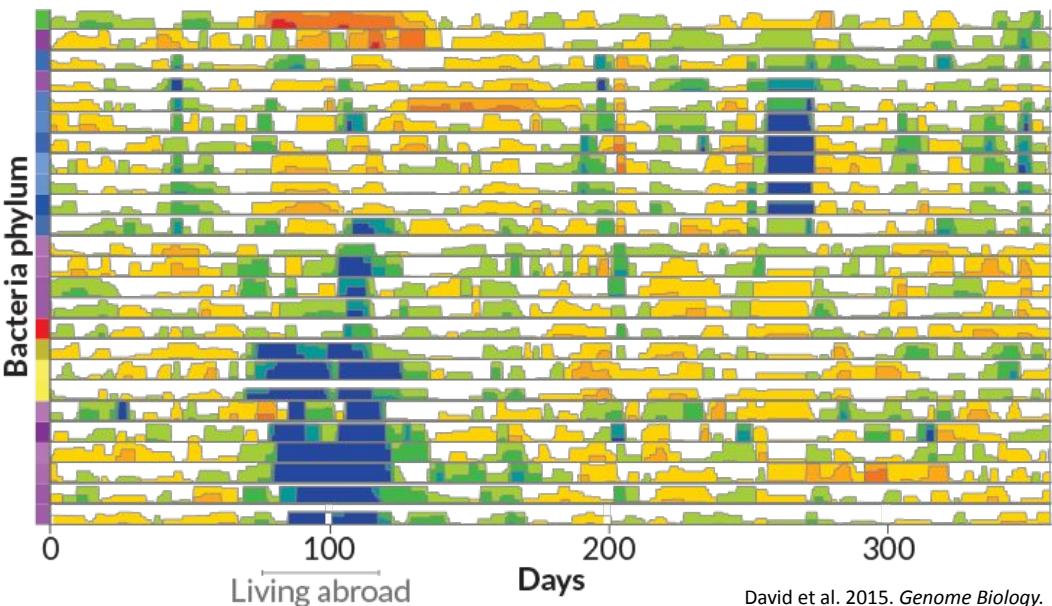
Microbiome and Diet

- In 2014, Lawrence David published a paper where he tracked his microbiome every day for one whole year. Evidence of lifestyle and diet interaction with the microbiome was evident: In the stacked plots shown to the right, where each color is indicative of a different type of bacteria, the only major changes to community structure occurred when he was living abroad (the green from days 71 to 122); likely a result of dietary and climatic change.

Relative gut bacteria abundance, by phylum



Change in gut bacteria abundance, by bacteria type



The Gut-Brain-Axis

- The human gut microbiome has been linked to a wide range of neurological conditions: People with IBS report high rates of depression. People on the autism spectrum have higher rates of digestive issues. And people with Parkinson's are prone to constipation. These are just a few examples.



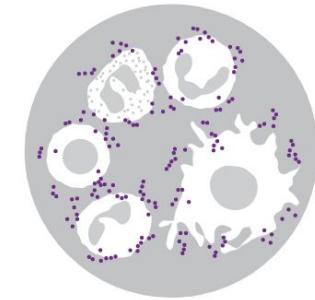
Substances secreted by microbes into the gut may infiltrate **blood vessels** for a direct ride to the brain.



Microbes prompt **neuropod cells** in the gut lining to stimulate the vagus nerve, which connects directly to the brain.



More indirectly, microbes activate **enteroendocrine cells** in the gut lining, which send hormones throughout the body.



Even more indirectly, gut microbes influence **immune cells** and **inflammation**, which can affect the brain.

Germ-Free Animals

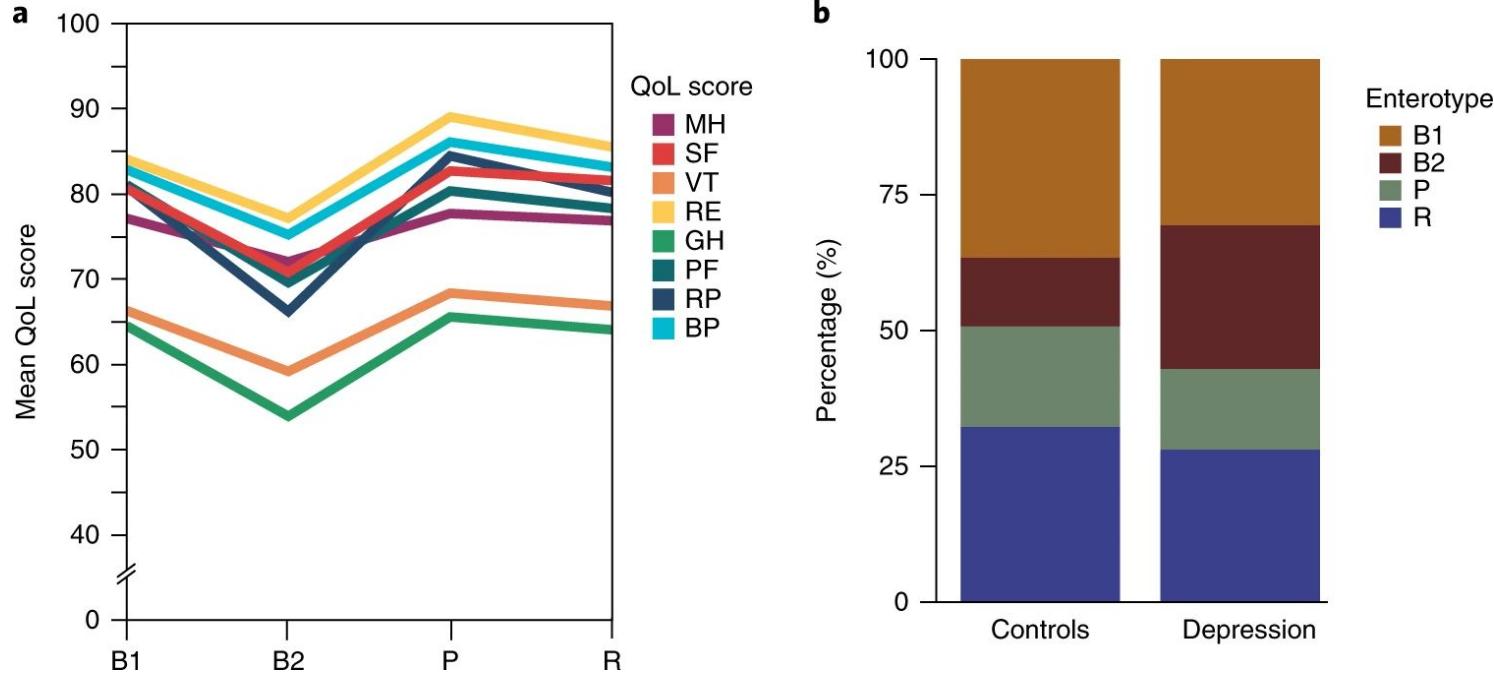
- Most common model used to do microbiome research.
- Germ free animals are raised under sterile conditions to prevent exposure or colonization of contaminating microbes.
- Germ free animals first introduced in the 1960's for use in dietary studies, but soon scientists (Schaedler et al. 1965) realized they could transfer bacterial cultures of interest into the mouse via gavage or injection study effect.
- Offspring will inherit those colonized strains.



*Photo courtesy of Taren M. Thron,
California Institute of Technology*

Gut-brain-axis: Humans

- Almost all gut-brain-axis work to date has been performed on model animals.
- One of the first large-scale human studies was conducted in 2019 by Jeroen Raes' research group as part of the Flemish Gut Project:
 - They found that out of the 173/1054 participants that reported depression, their microbiomes consistently were absent of two types of bacteria: *Coprococcus* and *Dialister*
 - To confirm that this is not population-dependent, the group analyzed publicly available data from a Dutch study that sampled microbiomes from 1064 people - out of those Dutch participants that reported depression, the same two bacteria were also absent!
 - They found that *Coprococcus* seems to have pathway related to dopamine, although they have no evidence how this might protect against depression.
 - *Coprococcus* also makes anti-inflammatory substance butyrate, and increased inflammation is implicated in depression. These factors have led to the microbe being formulated in probiotic supplements.

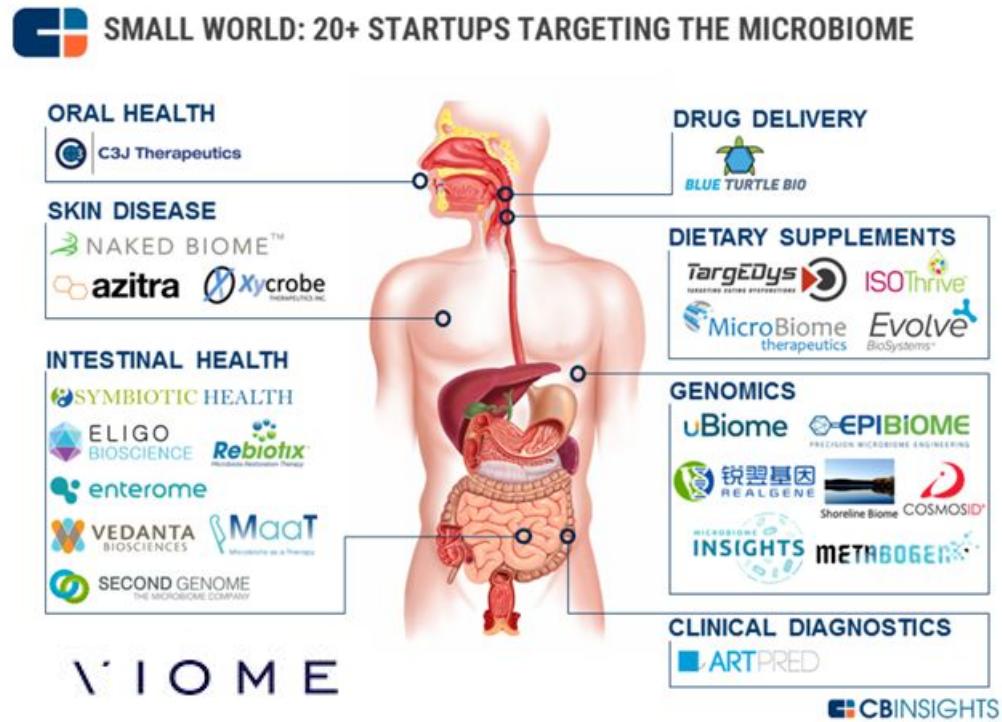


The Raes group used enterotyping and quality of life scores (QoL) to look at differences between depressed and non-depressed groups.

Enterotypes are a classification of a bacterial ecosystem living within a host. As you can see, depression leads to a visible difference in bacterial ecosystem living in the participants. And all 10 QoL score were unevenly distributed across the 4 enterotypes they defined.

Future Direction

- Big focus on precision medicine: as sequencing costs continue to come down, 'omics -based health information will become normal.
- Microbiome clearly plays significant role in many aspects of human health.
- Personal microbiomes can better inform treatment for infectious disease, diet, drugs prescribed to a patient, and best practices for personal hygiene.



Metagenomic Analysis

As has been previously shown, the microbiome is an extremely complex community that performs many functions. Often times, a transcript level analysis of some of the key components is needed to determine function changes. Other times, a population level, metagenomic analysis may be needed to gather more ecological information than 16S may provide. Below are a couple programs that QC, assemble, bin, and predict community members from metagenome data.

- MEGAN ([here](#))
 - Works like a ‘glue’ to combine taxonomic tools, functional analysis, and statistics.
- PiCRUST (<http://picrust.github.io/picrust/>)
 - Uses taxonomies from 16S sequences to predict function
- HUMAnN2 (<https://huttenhower.sph.harvard.edu/humann2>)
 - Custom pipeline for transcript-level analysis of metabolic potential of microbiomes

16S Analysis

For analyzing 16S amplicons that your lab has generated at either the operational taxonomic unit (OTU) level or amplicon sequence variant (ASV) level, there are 3 commonly cited software programs available below. Each has different options for QC, aligning to a database, clustering, and assigning taxonomies.

- Mothur (<https://mothur.org/>)
- QIIME/QIIME2 (<https://qiime2.org/>)
- DADA2 (<https://benjineb.github.io/dada2/>)



ASV's

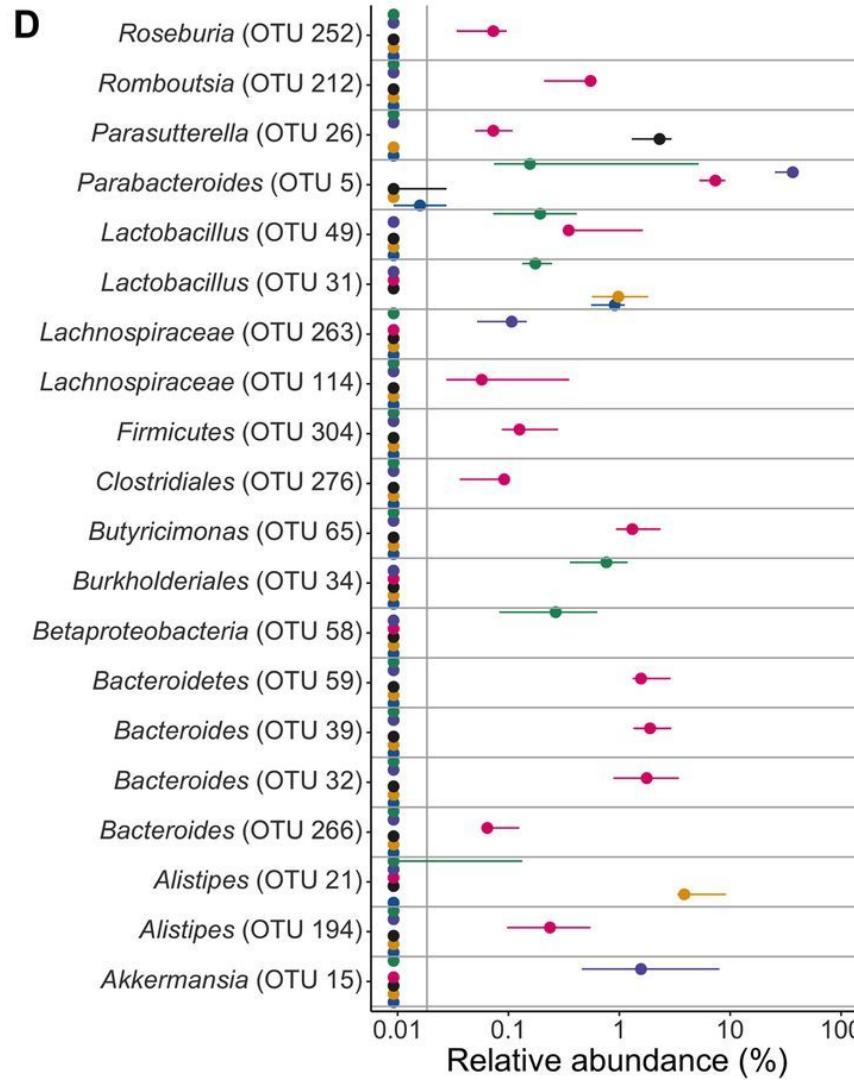
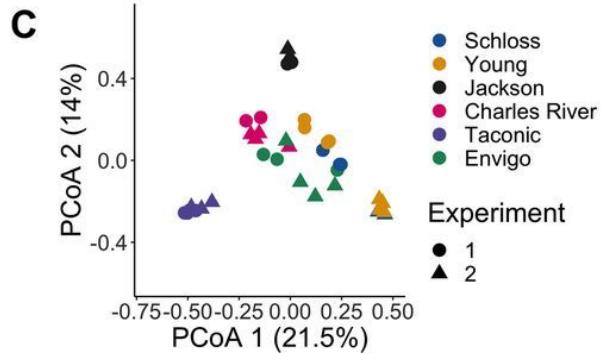
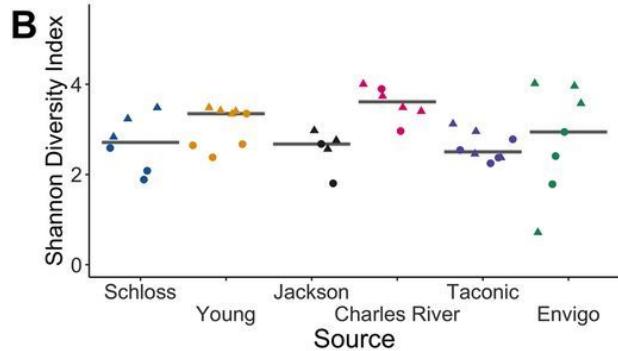
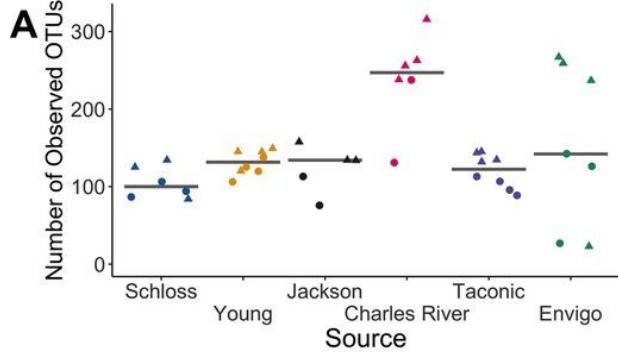
Total Community



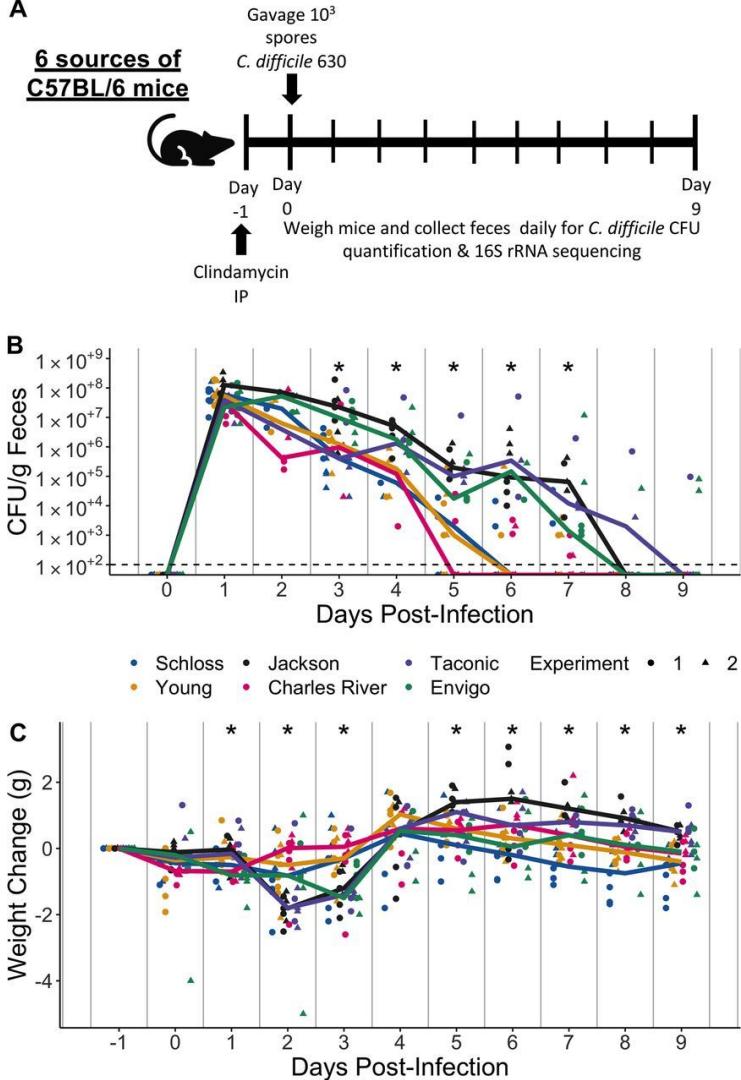
OTU Clusters

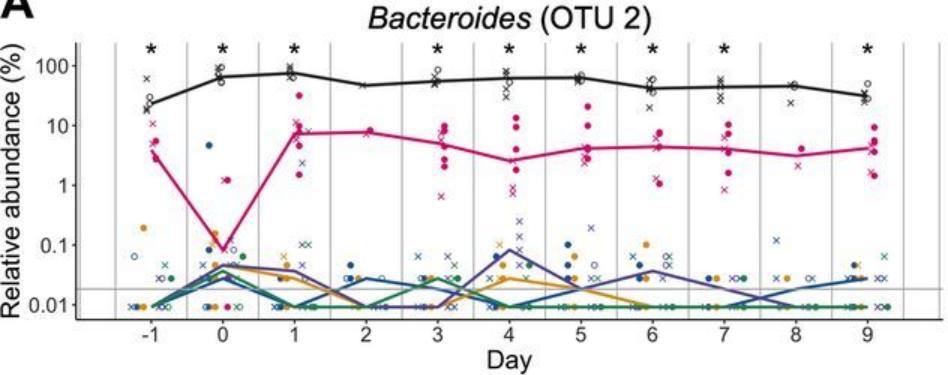
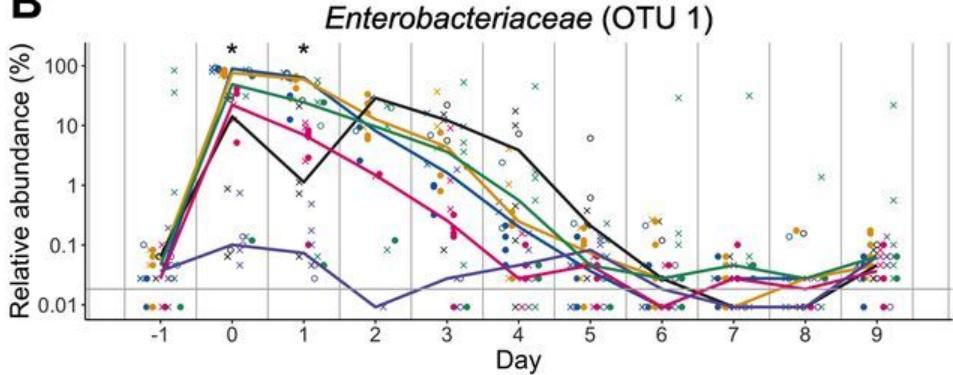
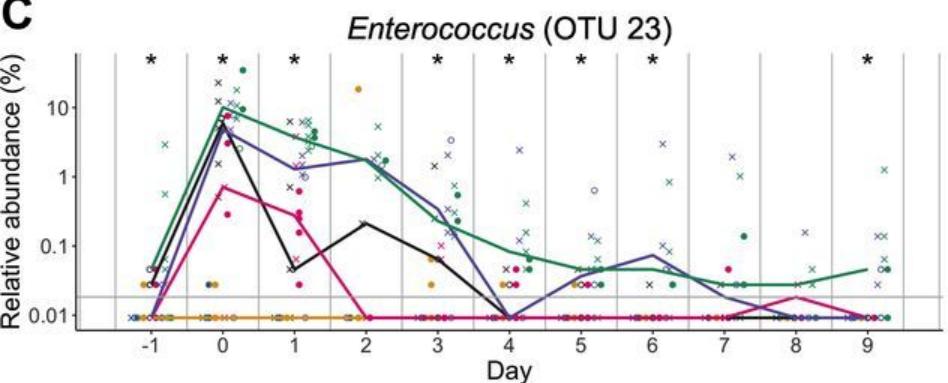
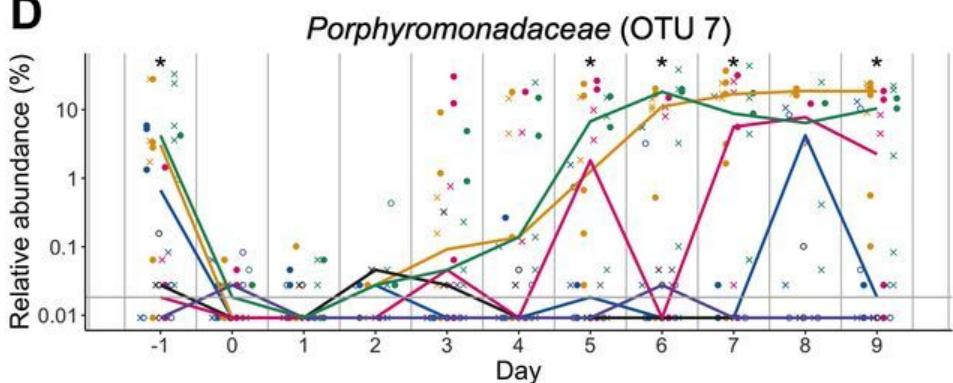
Representative Set





- Out of 268 OTU's, 20 were significantly different across vendors.
- Diversity was clearly different across all vendors.
- Does this lead to different resistance to opportunistic pathogens?
- Does this lead to differing abilities to clear infection?



A**B****C****D**

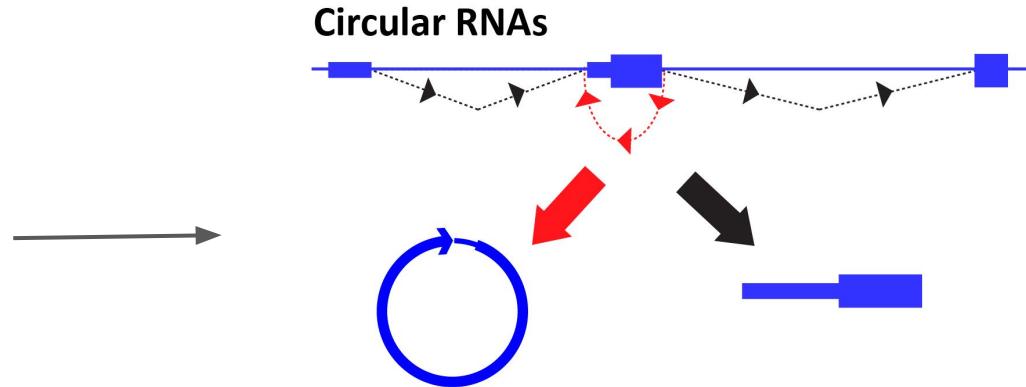
- Schloss • Jackson • Taconic
- Young • Charles River • Envigo

Cleared by Day 7 × no • yes ° no data

Questions?

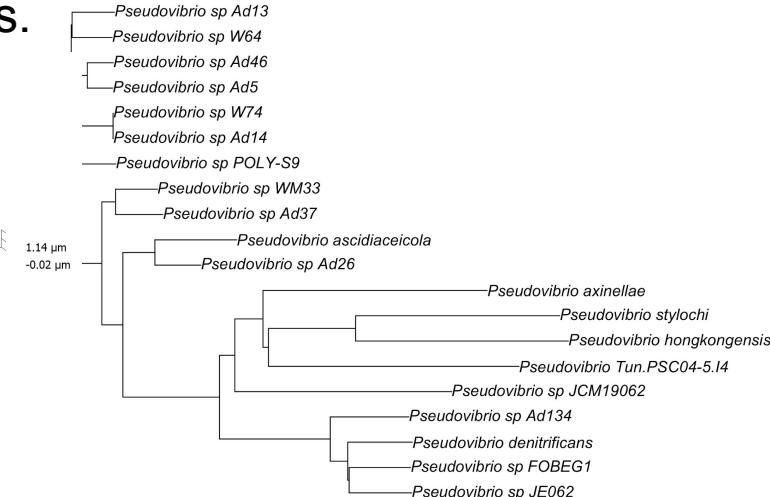
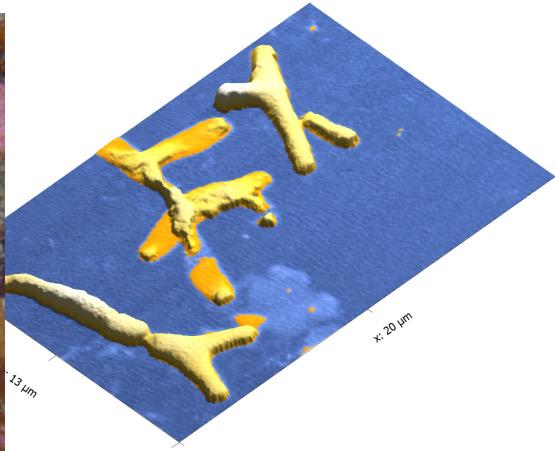
My Story - Wet lab to computer lab

- Always knew I liked science, DNA in particular really interested me.
- Cryptozoologist - interesting field, not practical career choice.
- Undergrad at UNR: started working in fly genetics lab. The experiments were slowww. 3 month time trial experiment for 1 figure?
- Some of the wet lab work was fun, but analyzing the qPCR data was one of my favorite parts.

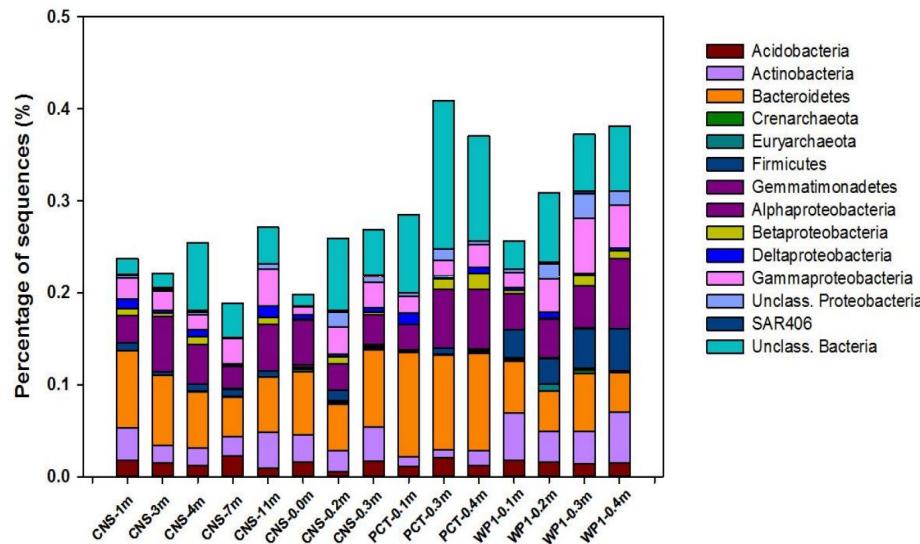


My story - wet lab to computer lab

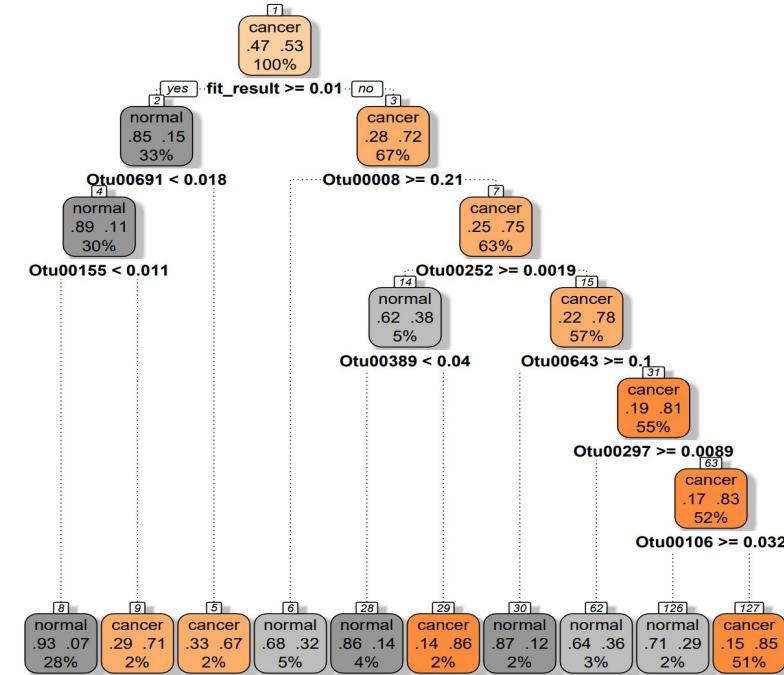
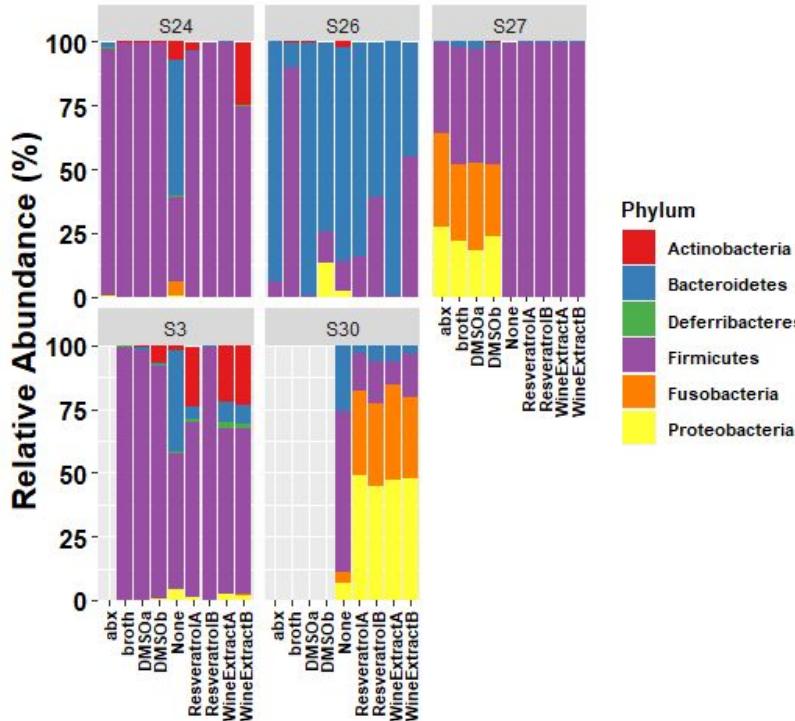
- Left fly genetics lab, started work in immunology lab running ELISA's. Again, data analysis was the highlight.
- Internship in flow cytometry lab. Found out what I don't want to do.
- M.S. at DRI: worked on variety of bacterial ecology projects with Antarctic/cold environment samples.
- Took first bioinformatics class, research emphasis on sequence analysis, started writing code to visualize some results.



- After graduation, continued work at DRI, more computationally focused.
- Field work, samples, sequence, analysis.
- Continued to practice analysis from command line, less reliance online GUI.
- Still made crappy figures.



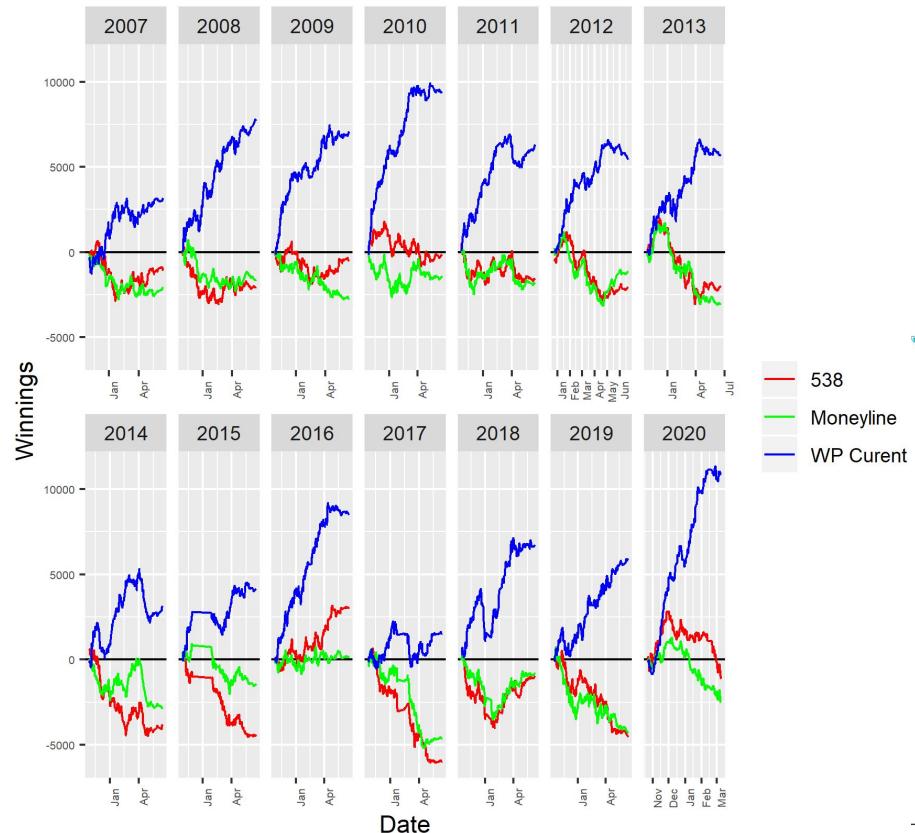
- Got technician job in Microbiome lab @ UM generating very large (>1000 samples) NGS datasets.
 - Took workshops, audited classes, took on side projects
 - Coding skills, interest increase.



Data analysis can be fun!

Gambling loses money - regardless of model

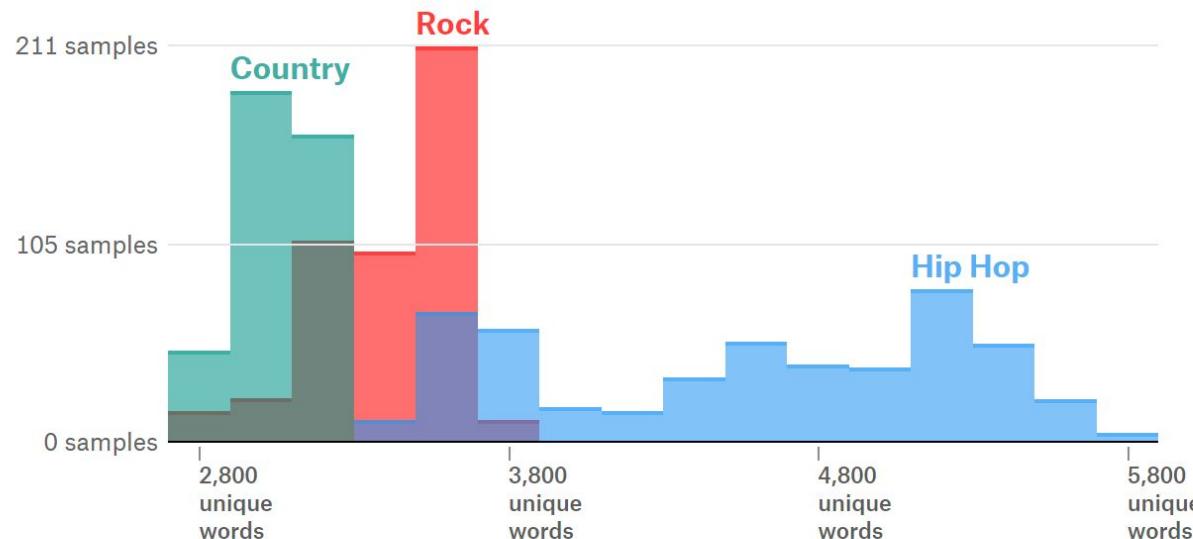
All data since 2007 season when moneyline data became available



Data analysis can be fun!

- <https://pudding.cool/> (like below)
- <https://www.kaggle.com/datasets> (competitions, tutorials, datasets)
- <https://fivethirtyeight.com/>

of Unique Words Used in 500 Random Samples of 35,000 Lyrics from Country, Rock, Hip Hop



Data analysis - many good resources

- <https://training.galaxyproject.org/training-material/>
 - Galaxy - updated and maintained regularly, little of everything
- <http://www.riffomonas.org/>
 - The riffomonas project - introductions to R and microbiome analysis
- <http://evomics.org/workshops/2016-workshop-on-microbial-genomics-harvard-university/>
 - Harvard workshops: many top U's are really good about posting free workshops
- <https://mgm.jgi.doe.gov/img-webinar-series/>
 - Joint Genome Institute hosts webinars and workshops regularly
- <https://meetings.cshl.edu/courses.html>
 - CSHL another great place for online/ in person workshops and courses