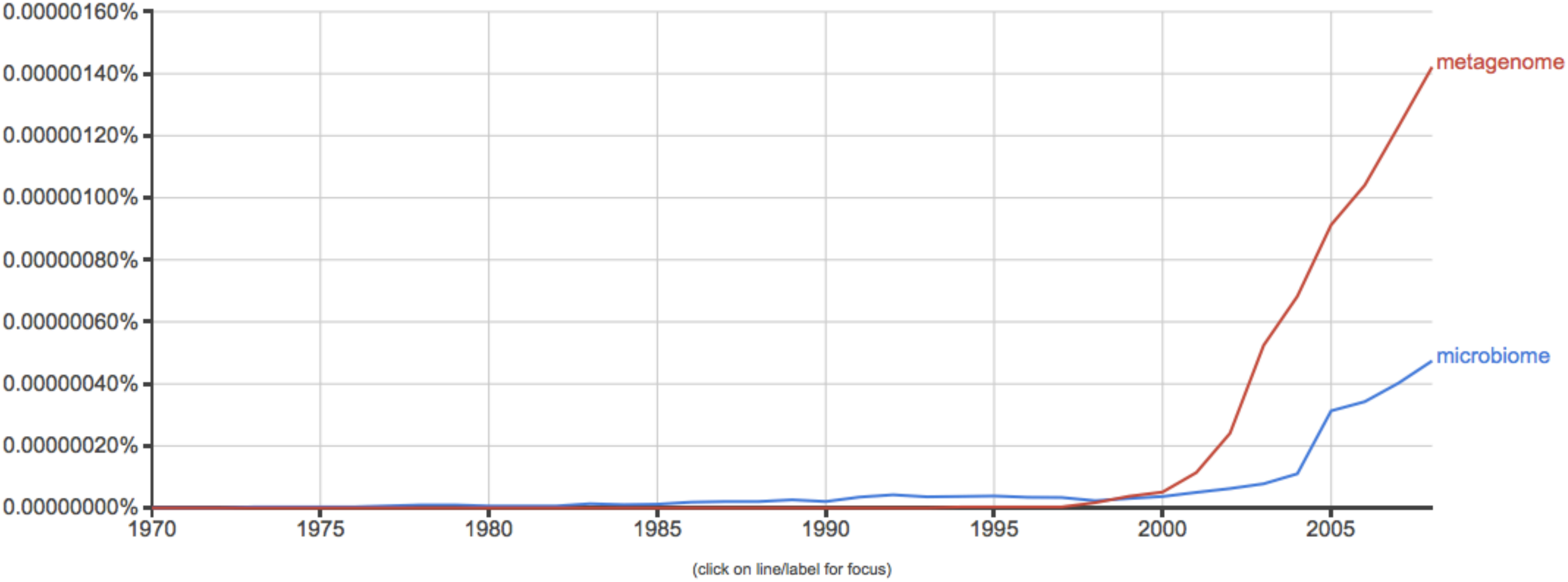
The Microbiome in Health and Disease

Paul J. Planet MD PhD
University of Pennsylvania
Children's Hospital of Pjiladelphia
American Museum of Natural History

Outline of the talk

1. The Human Microbiome
2. The origins Microbiomics
3. Disease and Dysbiosis
4. The Naturalistic Fallacy
5. Some Examples you should know about.
6. The Future.



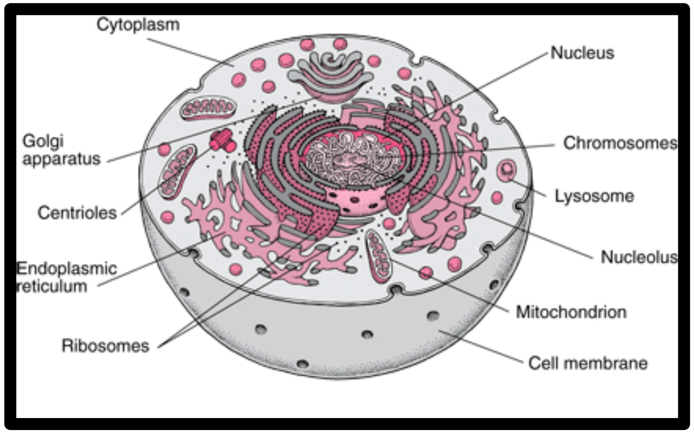
Microbiome: The collection of all microbial organisms in a given place

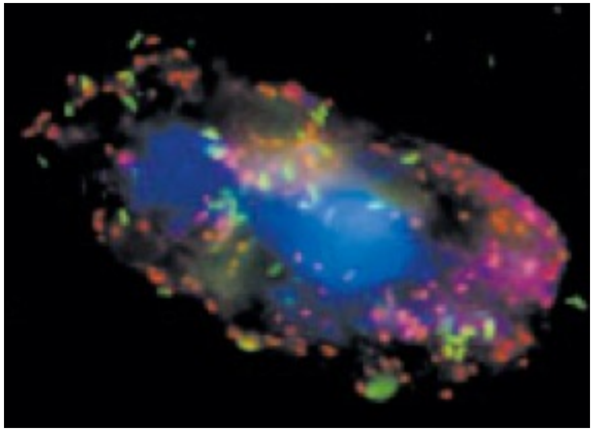
Metagenome: The collection of all genomes in a given place

From Hype to Hope?

We are about 90% bacteria.

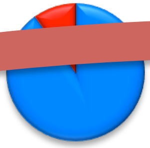
10 trillion human cells
100 trillion bacterial cells



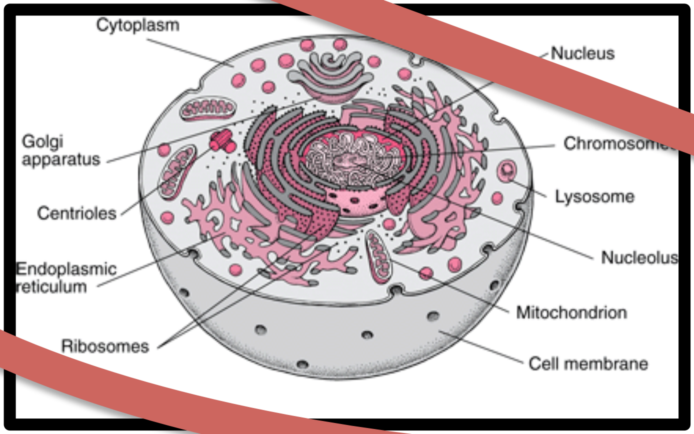


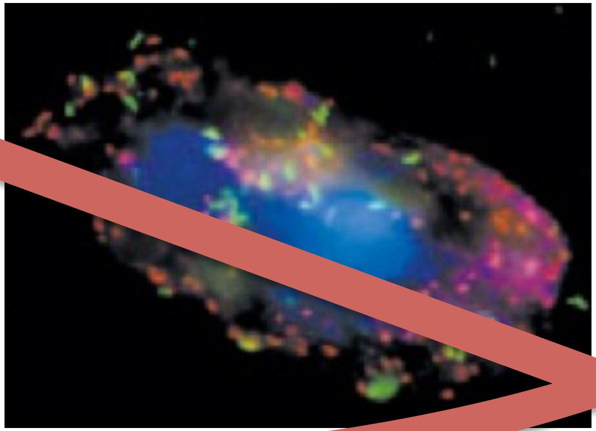
From Hype to Hope:

We are about 90% bac



10 trillion hu[illegible]lls
100 trillion bacterial cells





Bacteria to Human cells ratio (B/H) is based on one historical back-of-the-envelope estimate

PNAS, 2010: "With the greatest quantity and diversity found in the lower gastrointestinal tract (Nature, 2007). **One-hundred trillion bacteria** representing hundreds of species"

Nature, 2007: "The microbes that live inside and on us (the microbiota) **outnumber our somatic and germ cells by an estimated 10-fold...**(Science, 2006)"

Science, 2006: "Our body surfaces are home to microbial communities whose aggregate membership outnumbers our human somatic and germ cells by **at least an order of magnitude…** (Science, 2005)"

Science, 2005: "The adult human intestine is home to an almost inconceivable number of microorganisms. The size of the population - up to 100 trillion... and is **~10 times greater than the total number of our somatic and germ cells** (Savage,1977)"

Trends Microbiol., 1996: "In summary, there are **ten viable indigenous bacteria in the GI tract for every cell in the human body:** 10^{14} total GI bacteria compared with 10^{13} total cells making up the human body (Savage,1977)"

Wikipedia (Human microbiome): "…Bacterial cells are much smaller than human cells, **and there are at least ten times as many bacteria as human cells in the body** (approximately 10^{14} versus 10^{13}) (Savage,1977, Berg, 1996)"

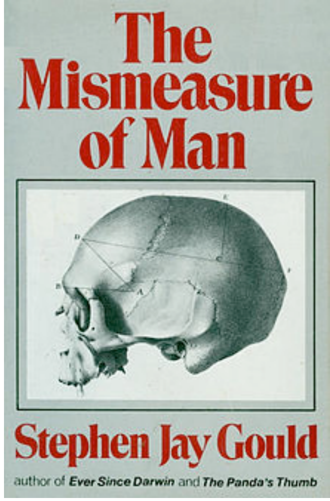
Savage, Annu. Rev. Microbiol., 1977

"The adult human organism is said to be composed of approximately 10^{13} eukaryotic animal cells (Dobzhansky, 1971)... The various body surfaces and the gastro intestinal canals of human may be colonized by as many as 10^{14} indigenous prokaryotic and eukaryotic microbial cells (Luckey,1972)… From another point of view, therefore, **the normal human organism can be said to be composed of over 10^{14} cells, of which only about 10% are animal cells.**"

Luckey, Am. J. Clin. Nutr., 1972

"Adult man carries 10^{12} microbes associated with his epidermis and **10^{14} microbes** in his alimentary tract (Fig.1). The latter number is **based upon 10^{11} microbes/g contents of an alimentary tract with a capacity of approximately 1 liter.**"

The Mismeasure of Man (and Microbes)

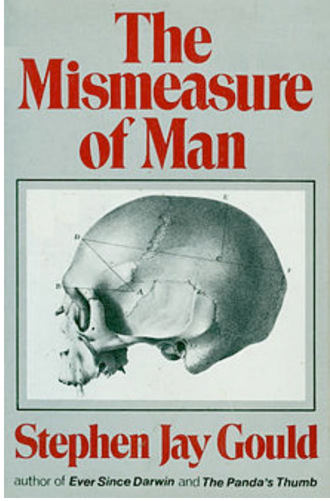


January 6 2016 Revised estimates for the number of human and bacteria cells in the body Ron Sender, Shai Fuchs & Ron Milo1 (*BioRxiv*)

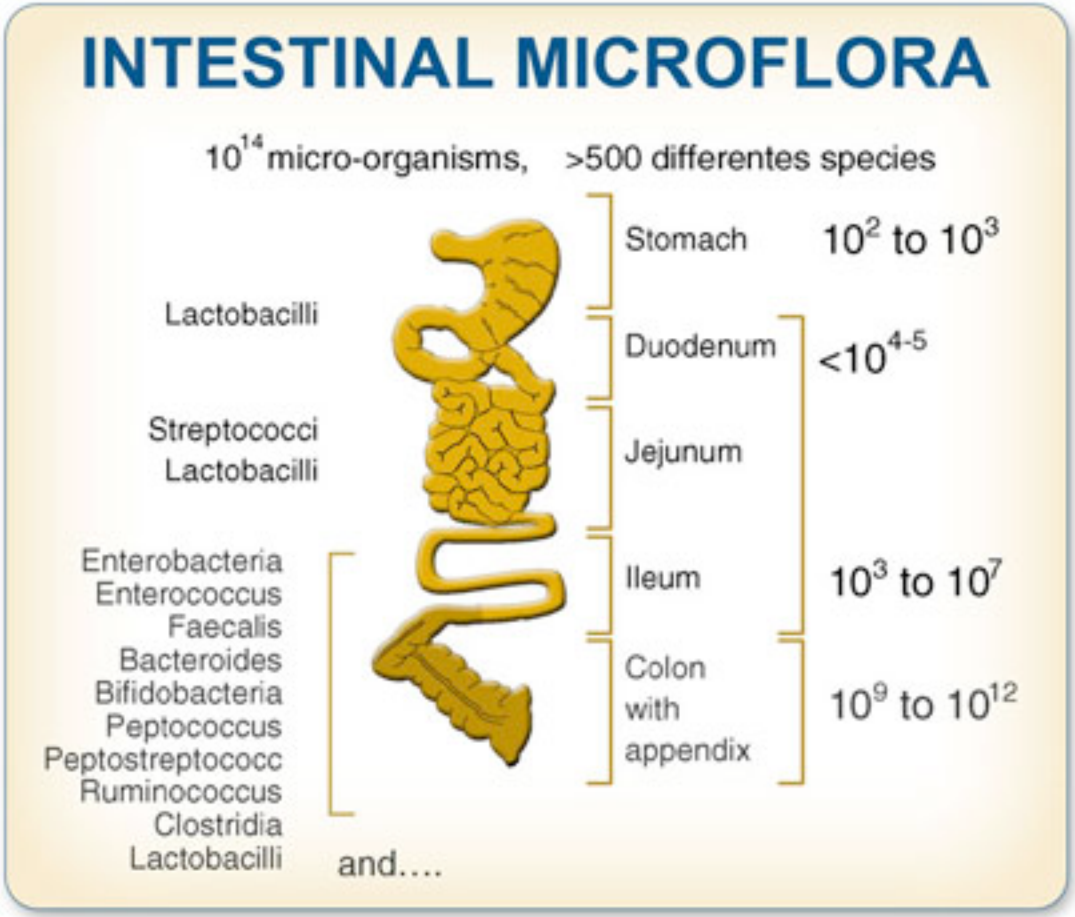
Original "back of the envelope calculation was"

10^{11} bacteria/gram x 1 Liter = 10^{14}
(assuming that 1 g = 1 ml)

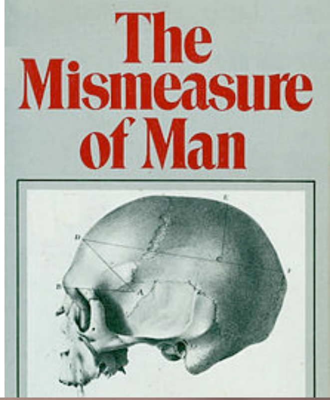
The Mismeasure of Man (and Microbes)



January 6 2016 Revised estimates for the number of human and bacteria cells in the body Ron Sender, Shai Fuchs & Ron Milo1 (*BioRxiv*)

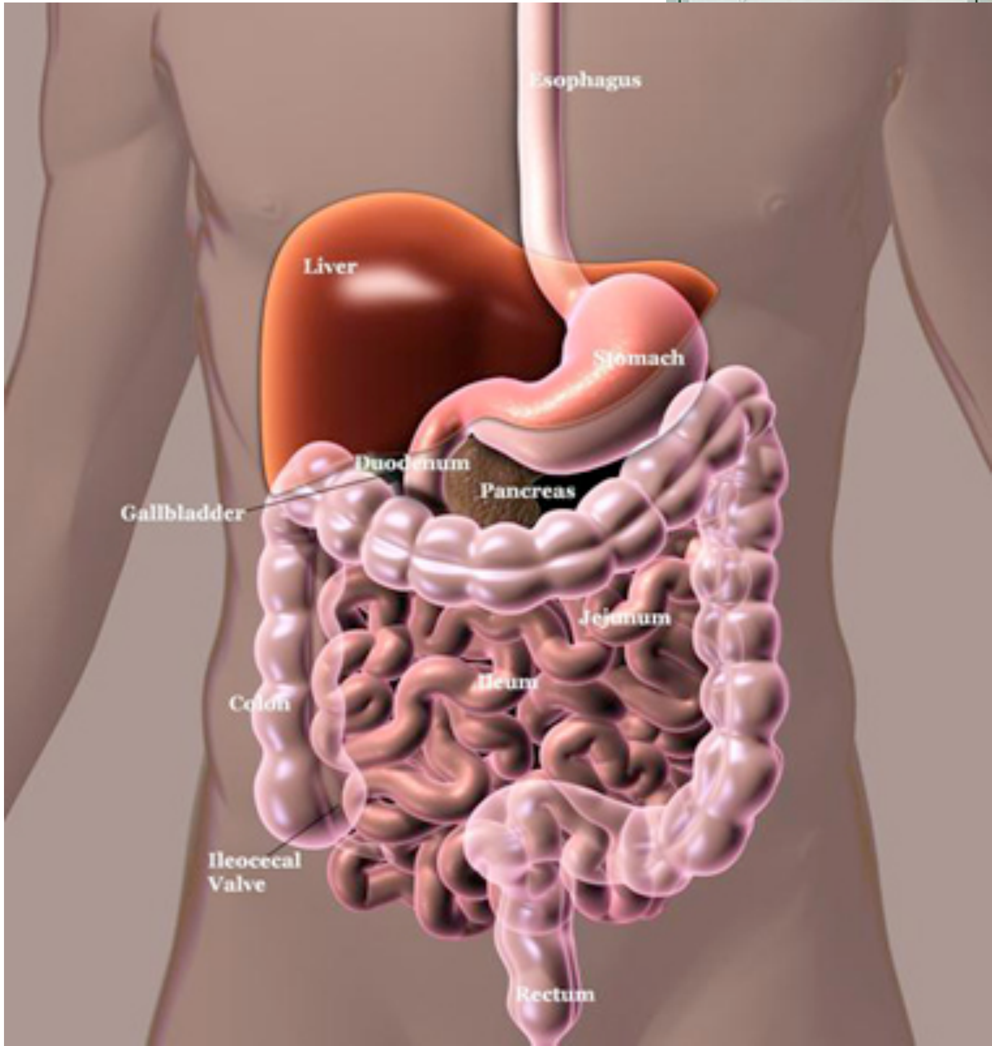


The Mismeasure of Man (and Microbes)

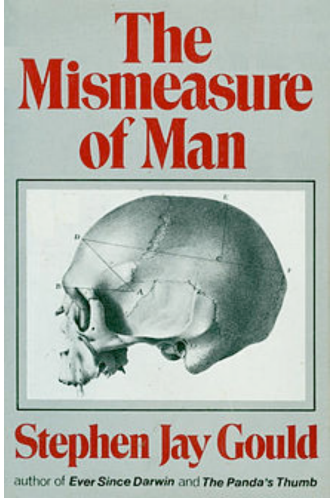


January 6 2016 Revised estimates for the number of human and bacteria cells in the body Ron Sender, Shai Fuchs & Ron Milo1 (*BioRxiv*)

Colon which has vastly more bacteria has 350-450 ml of volume.



The Mismeasure of Man (and Microbes)



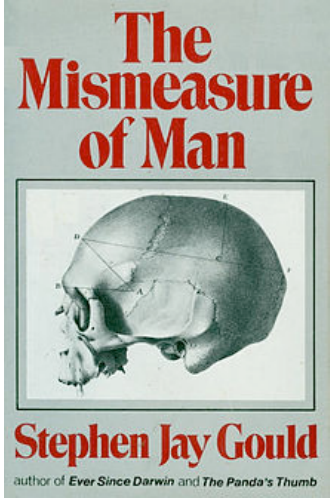
January 6 2016 Revised estimates for the number of human and bacteria cells in the body Ron Sender, Shai Fuchs & Ron Milo1 (*BioRxiv*)

70 kg "reference man" :

Bacteria: 3.9×10^{13} (25% uncertainty and 52% variation over the population)

Human: 3.9×10^{13} (2% uncertainty and 14% CV)

The Mismeasure of Man (and Microbes)



January 6 2016 Revised estimates for the number of human and bacteria cells in the body Ron Sender, Shai Fuchs & Ron Milo1 (*BioRxiv*)

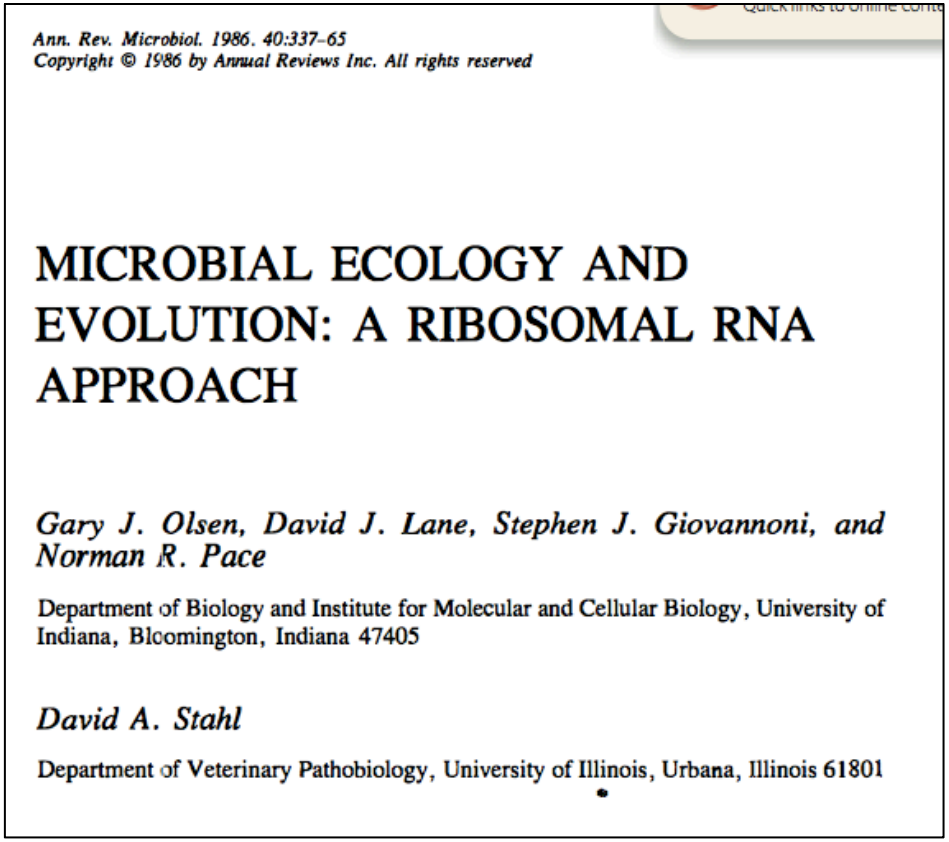
"Indeed, the numbers are similar enough that each defecation event may flip the ratio to favor human cells over bacteria."

More to you than you think:

1. In a human body the ratio of Human cells:Microbial cells is about 1:1.
2. 10^{11}-10^{12} microbes/ml in GI tract
3. Bacteria constitute about 50% of the content of feces by weight
4. Estimates of 400-600 different species of bacteria in mouth and colon.

High Hopes

- Infectious disease risk
- Development of gut
- Development of healthy immunity
- Responses to vaccines
- Obesity
- Nutrient metabolism
- Drug toxicity/ metabolism
- Brain development
- Drug discovery



Ann. Rev. Microbiol. 1986. 40:337–65

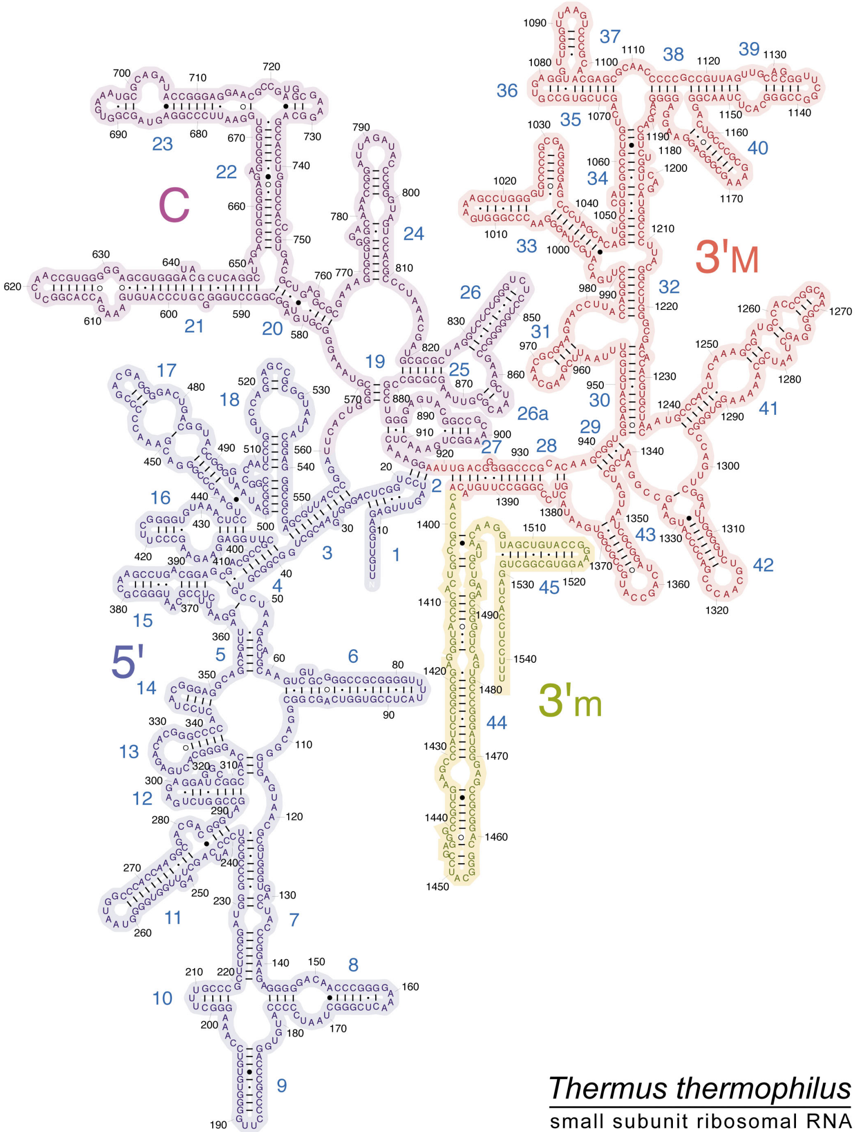
MICROBIAL ECOLOGY AND EVOLUTION: A RIBOSOMAL RNA APPROACH

Gary J. Olsen, David J. Lane, Stephen J. Giovannoni, and Norman R. Pace

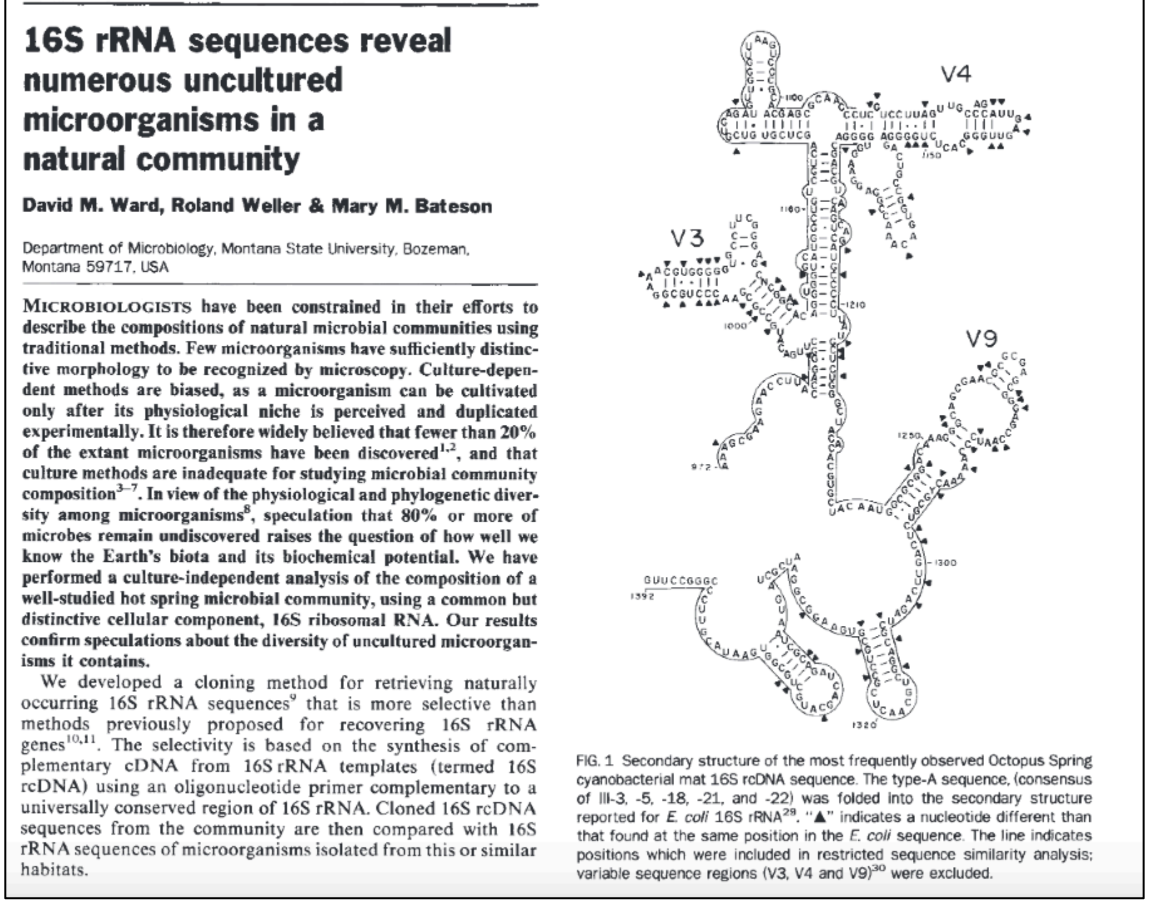
Department of Biology and Institute for Molecular and Cellular Biology, University of Indiana, Bloomington, Indiana 47405

David A. Stahl

Department of Veterinary Pathobiology, University of Illinois, Urbana, Illinois 61801



Originally proposed by Pace NR 1986 and Olsen et al 1986



16S rRNA sequences reveal numerous uncultured microorganisms in a natural community

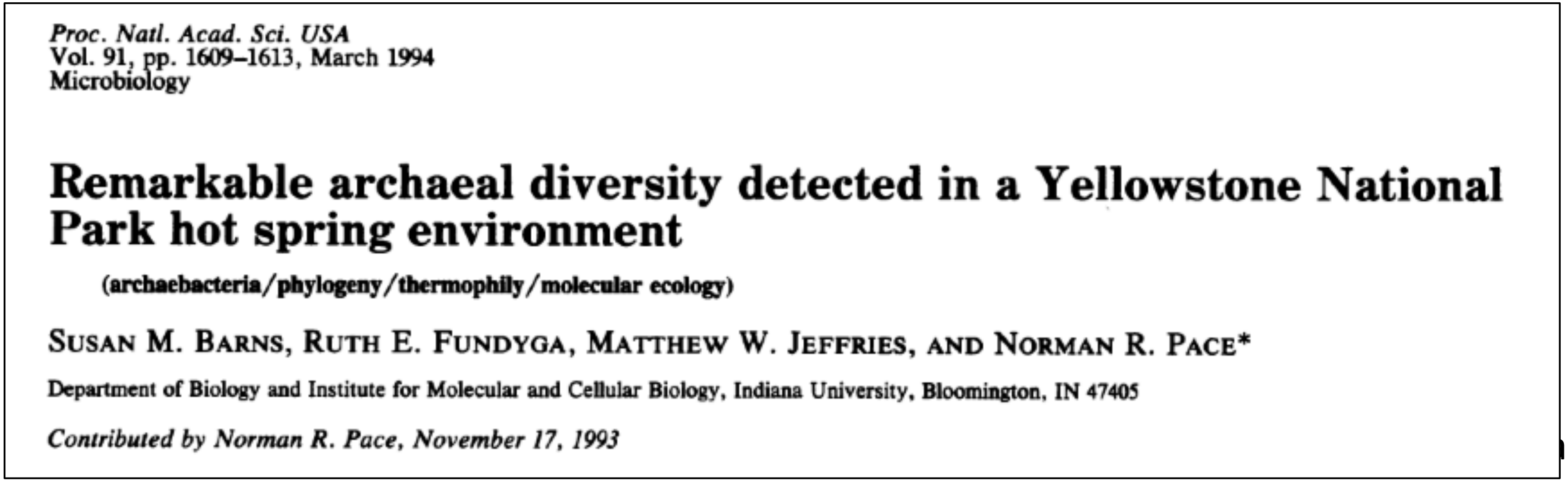
David M. Ward, Roland Weller & Mary M. Bateson

Department of Microbiology, Montana State University, Bozeman, Montana 59717, USA

MICROBIOLOGISTS have been constrained in their efforts to describe the compositions of natural microbial communities using traditional methods. Few microorganisms have sufficiently distinctive morphology to be recognized by microscopy. Culture-dependent methods are biased, as a microorganism can be cultivated only after its physiological niche is perceived and duplicated experimentally. It is therefore widely believed that fewer than 20% of the extant microorganisms have been discovered[1,2], and that culture methods are inadequate for studying microbial community composition[3–7]. In view of the physiological and phylogenetic diversity among microorganisms[8], speculation that 80% or more of microbes remain undiscovered raises the question of how well we know the Earth's biota and its biochemical potential. We have performed a culture-independent analysis of the composition of a well-studied hot spring microbial community, using a common but distinctive cellular component, 16S ribosomal RNA. Our results confirm speculations about the diversity of uncultured microorganisms it contains.

We developed a cloning method for retrieving naturally occurring 16S rRNA sequences[9] that is more selective than methods previously proposed for recovering 16S rRNA genes[10,11]. The selectivity is based on the synthesis of complementary cDNA from 16S rRNA templates (termed 16S rcDNA) using an oligonucleotide primer complementary to a universally conserved region of 16S rRNA. Cloned 16S rcDNA sequences from the community are then compared with 16S rRNA sequences of microorganisms isolated from this or similar habitats.

FIG. 1 Secondary structure of the most frequently observed Octopus Spring cyanobacterial mat 16S rcDNA sequence. The type-A sequence, (consensus of III-3, -5, -18, -21, and -22) was folded into the secondary structure reported for *E. coli* 16S rRNA[29]. "▲" indicates a nucleotide different than that found at the same position in the *E. coli* sequence. The line indicates positions which were included in restricted sequence similarity analysis; variable sequence regions (V3, V4 and V9)[30] were excluded.



Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1609–1613, March 1994
Microbiology

Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment

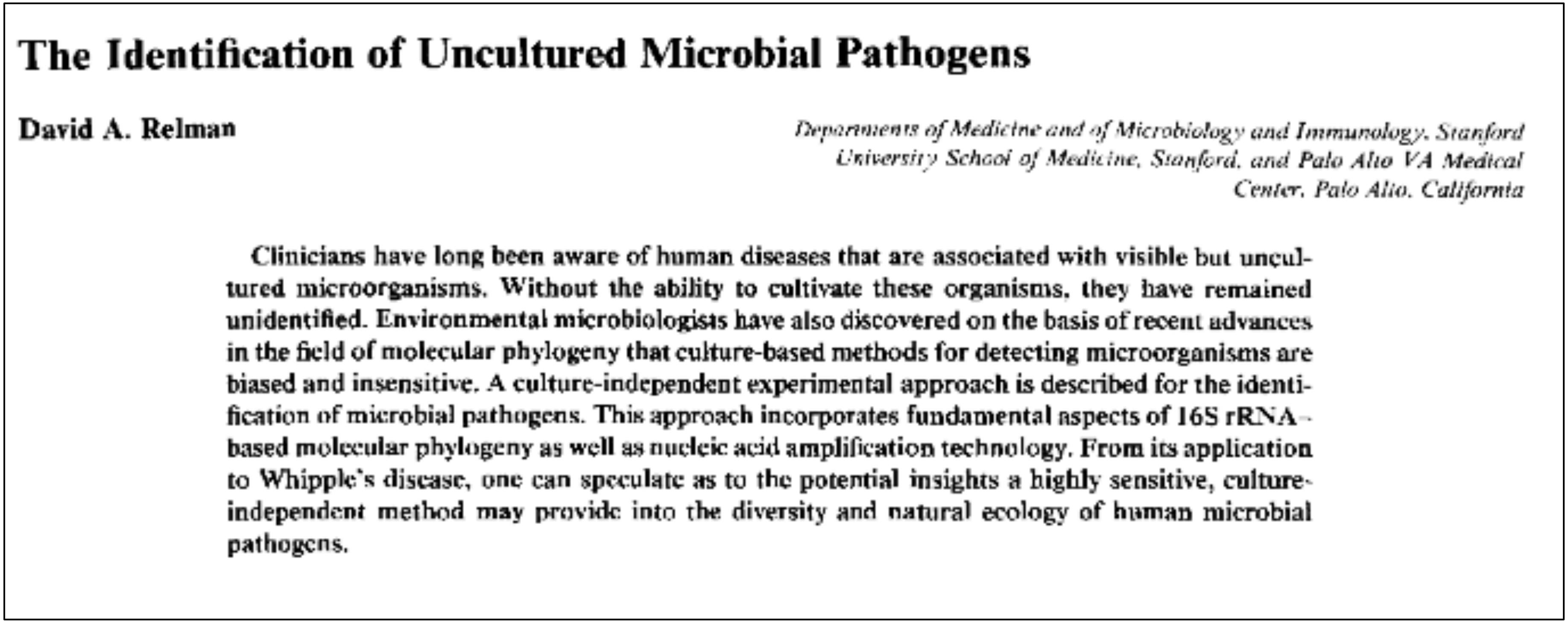
(archaebacteria/phylogeny/thermophily/molecular ecology)

SUSAN M. BARNS, RUTH E. FUNDYGA, MATTHEW W. JEFFRIES, AND NORMAN R. PACE*

Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405

Contributed by Norman R. Pace, November 17, 1993

l 1986



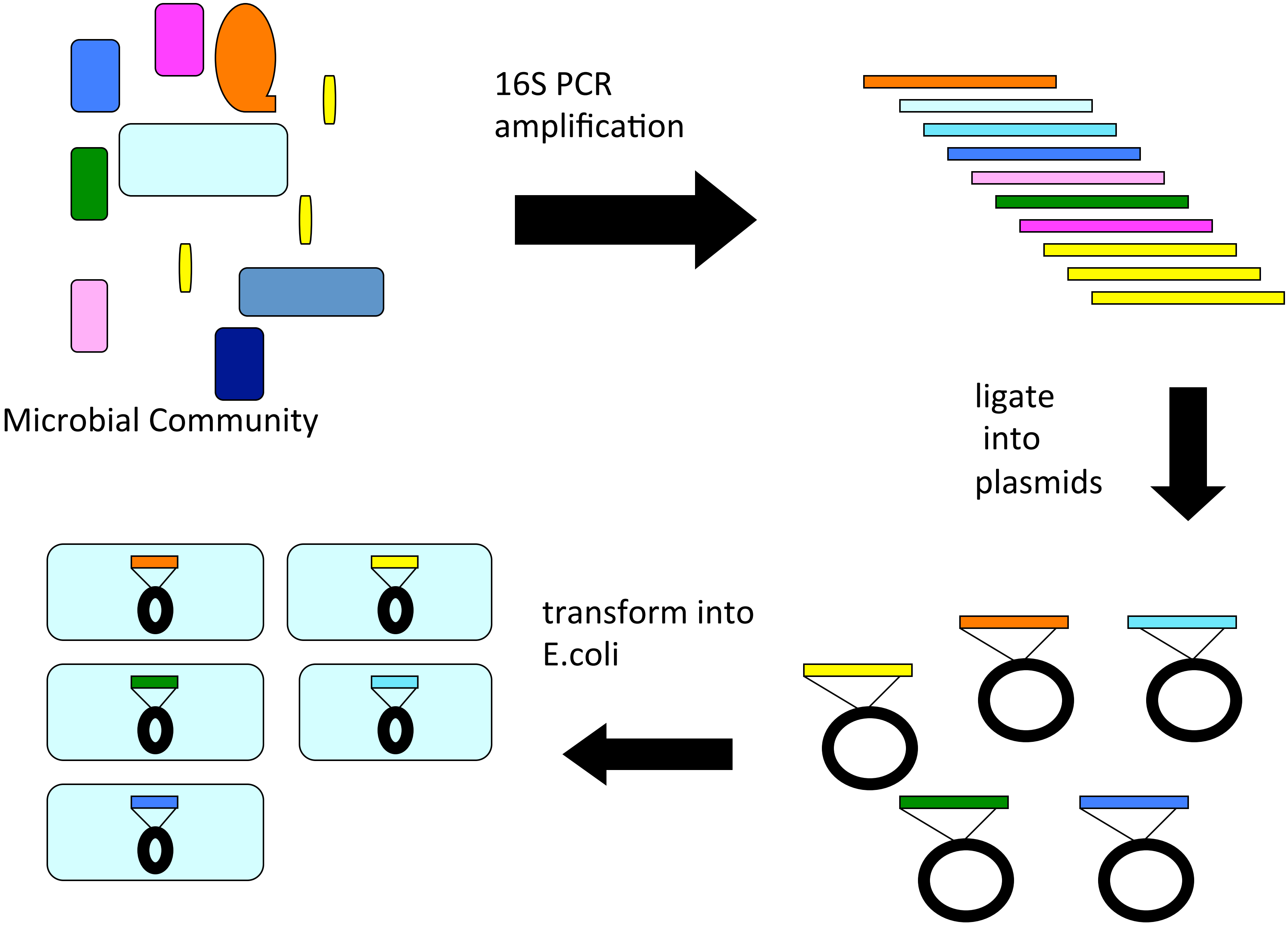
The Identification of Uncultured Microbial Pathogens

David A. Relman

Departments of Medicine and of Microbiology and Immunology, Stanford University School of Medicine, Stanford, and Palo Alto VA Medical Center, Palo Alto, California

Clinicians have long been aware of human diseases that are associated with visible but uncultured microorganisms. Without the ability to cultivate these organisms, they have remained unidentified. Environmental microbiologists have also discovered on the basis of recent advances in the field of molecular phylogeny that culture-based methods for detecting microorganisms are biased and insensitive. A culture-independent experimental approach is described for the identification of microbial pathogens. This approach incorporates fundamental aspects of 16S rRNA–based molecular phylogeny as well as nucleic acid amplification technology. From its application to Whipple's disease, one can speculate as to the potential insights a highly sensitive, culture-independent method may provide into the diversity and natural ecology of human microbial pathogens.

1993

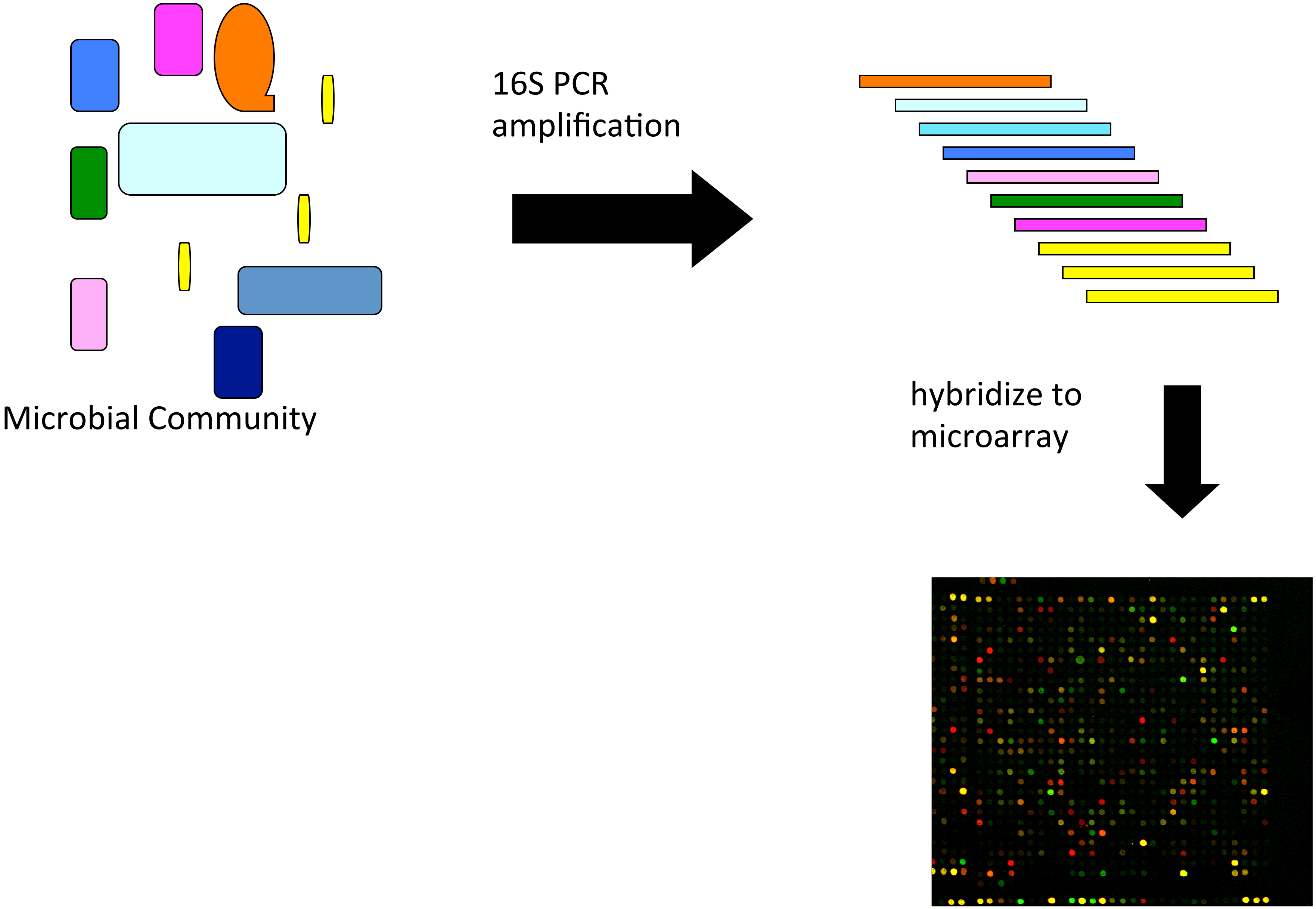


Microbial Community

16S PCR
amplification

ligate
into
plasmids

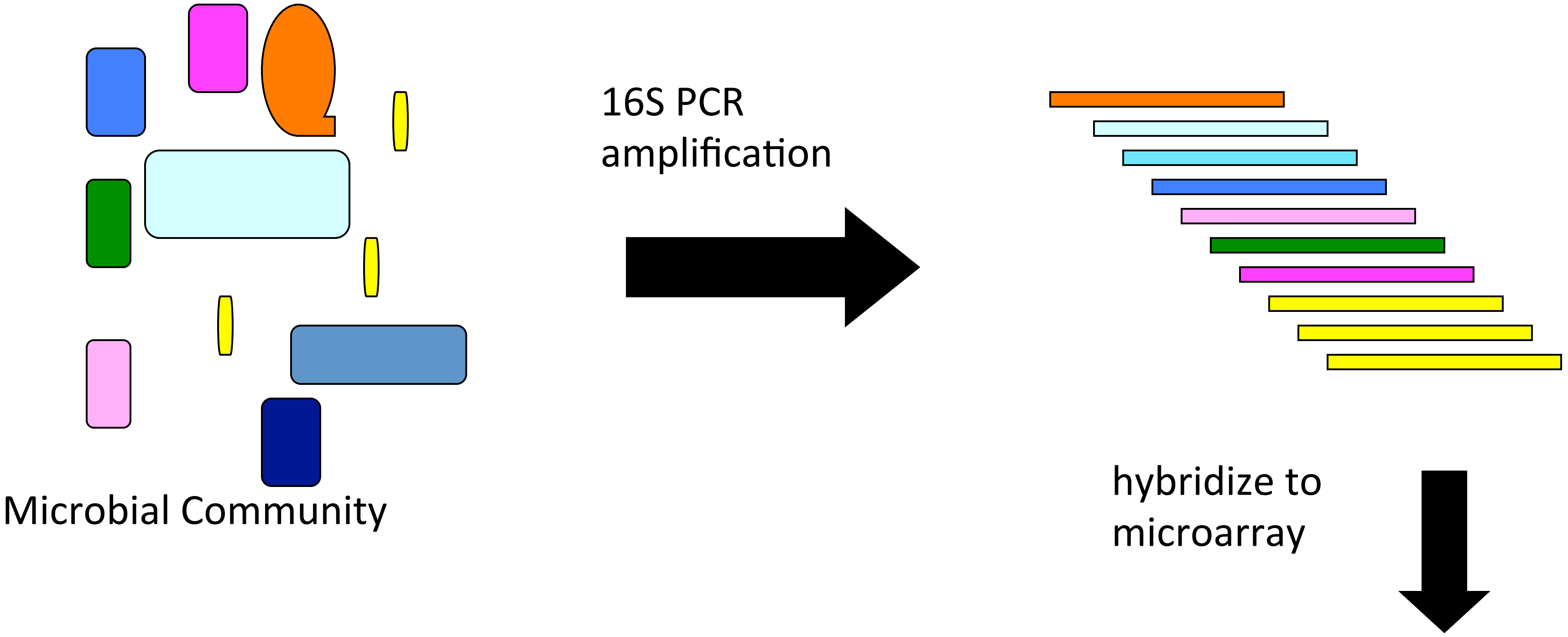
transform into
E.coli



Microbial Community

16S PCR amplification

hybridize to microarray

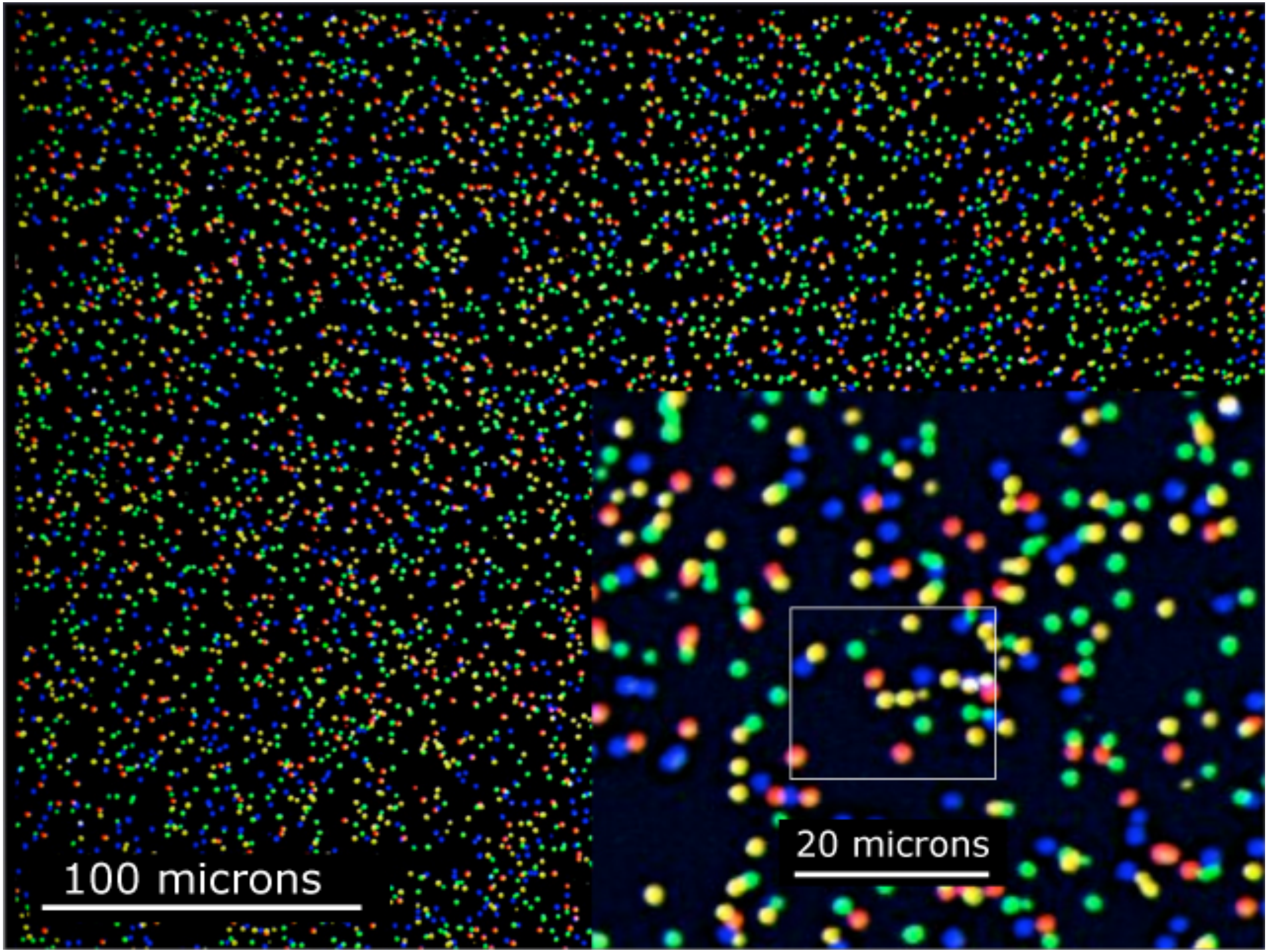


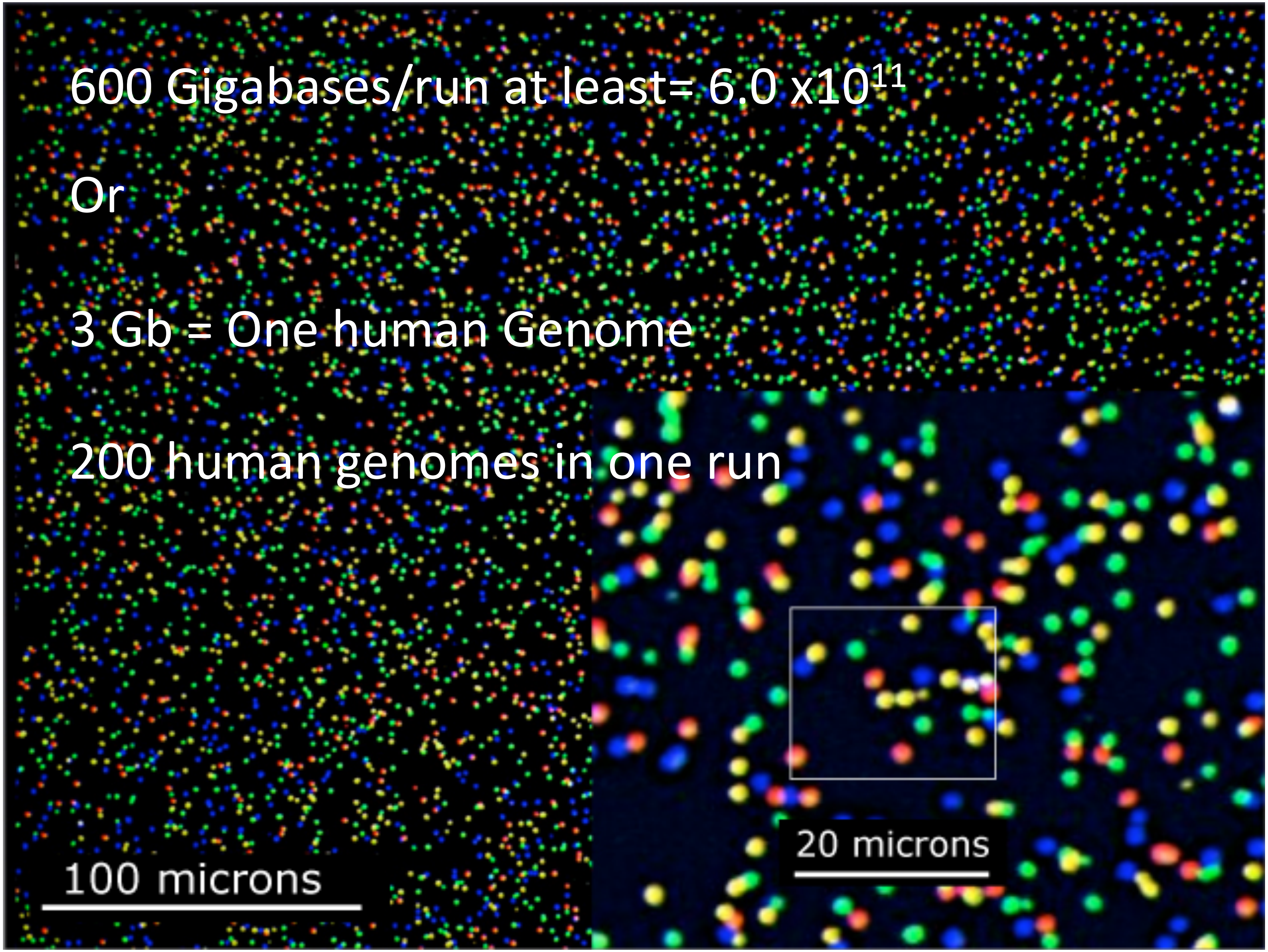
Microbial Community

16S PCR
amplification

hybridize to
microarray

Illumina/Solexa


100 microns
20 microns



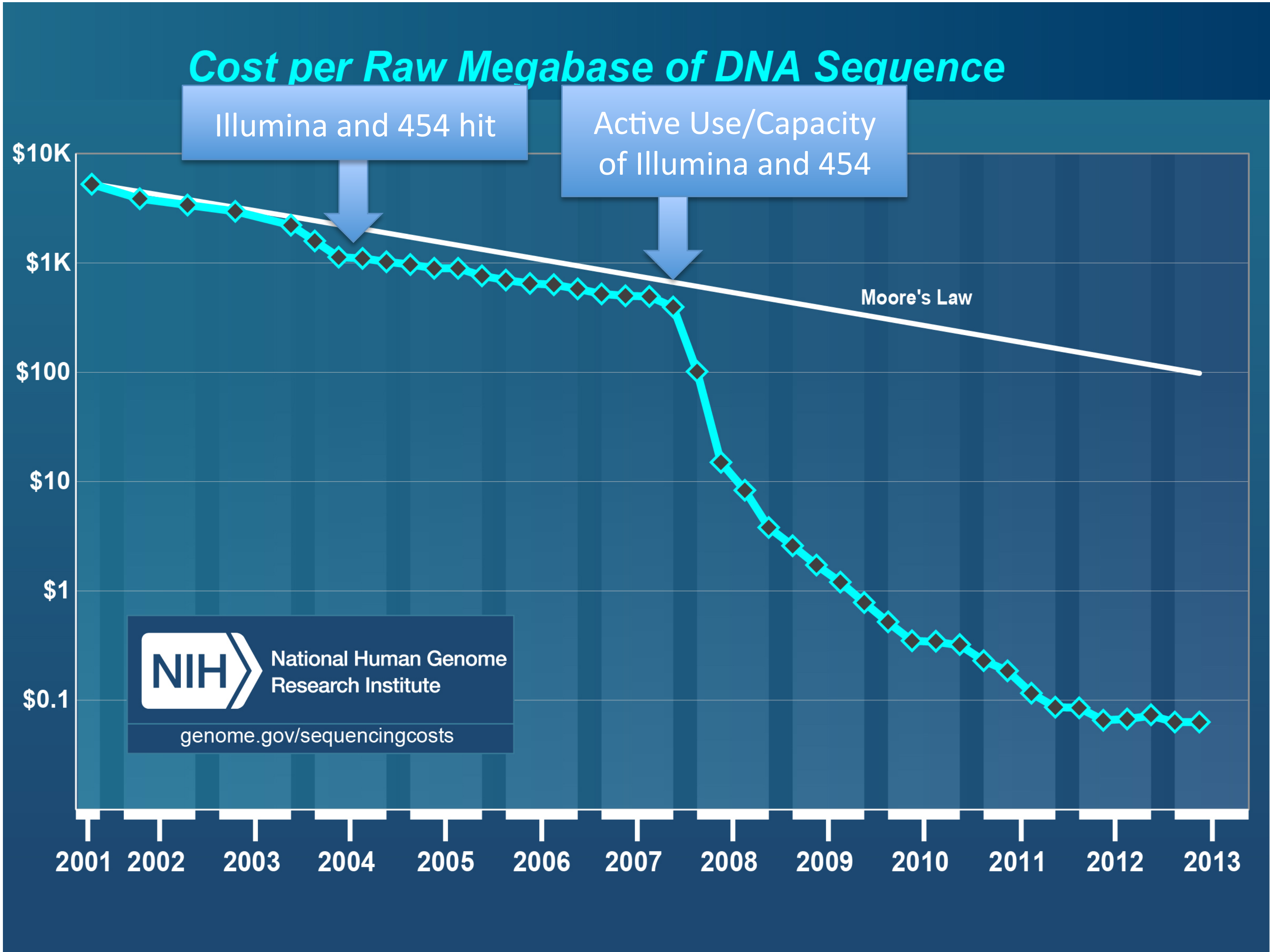
600 Gigabases/run at least= 6.0 x10^{11}

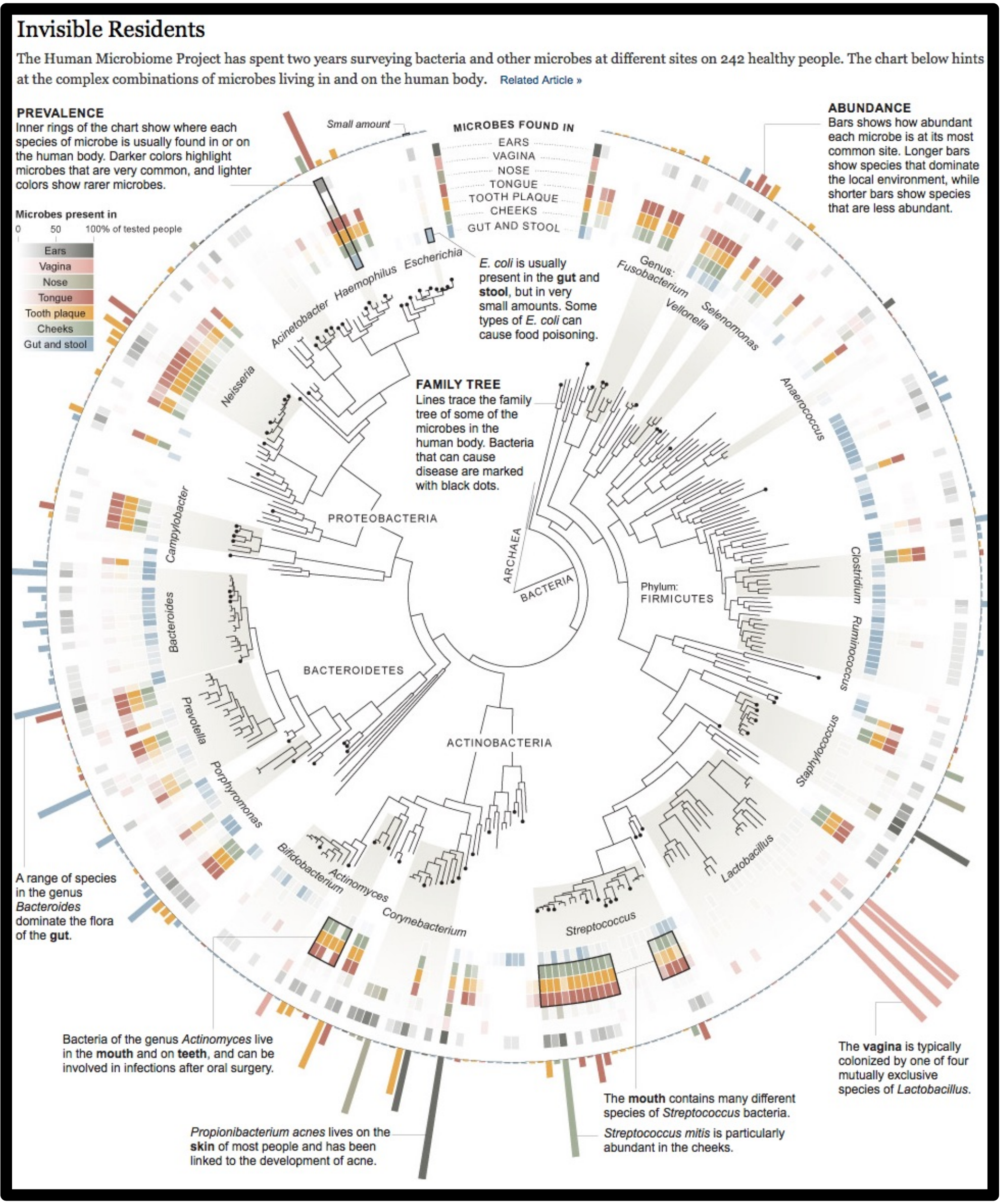
Or

3 Gb = One human Genome

200 human genomes in one run

Sequencing capability has been doubling every 6–9 months





Invisible Residents

The Human Microbiome Project has spent two years surveying bacteria and other microbes at different sites on 242 healthy people. The chart below hints at the complex combinations of microbes living in and on the human body. Related Article »

PREVALENCE
Inner rings of the chart show where each species of microbe is usually found in or on the human body. Darker colors highlight microbes that are very common, and lighter colors show rarer microbes.

ABUNDANCE
Bars shows how abundant each microbe is at its most common site. Longer bars show species that dominate the local environment, while shorter bars show species that are less abundant.

FAMILY TREE
Lines trace the family tree of some of the microbes in the human body. Bacteria that can cause disease are marked with black dots.

E. coli is usually present in the **gut** and **stool**, but in very small amounts. Some types of *E. coli* can cause food poisoning.

A range of species in the genus *Bacteroides* dominate the flora of the **gut**.

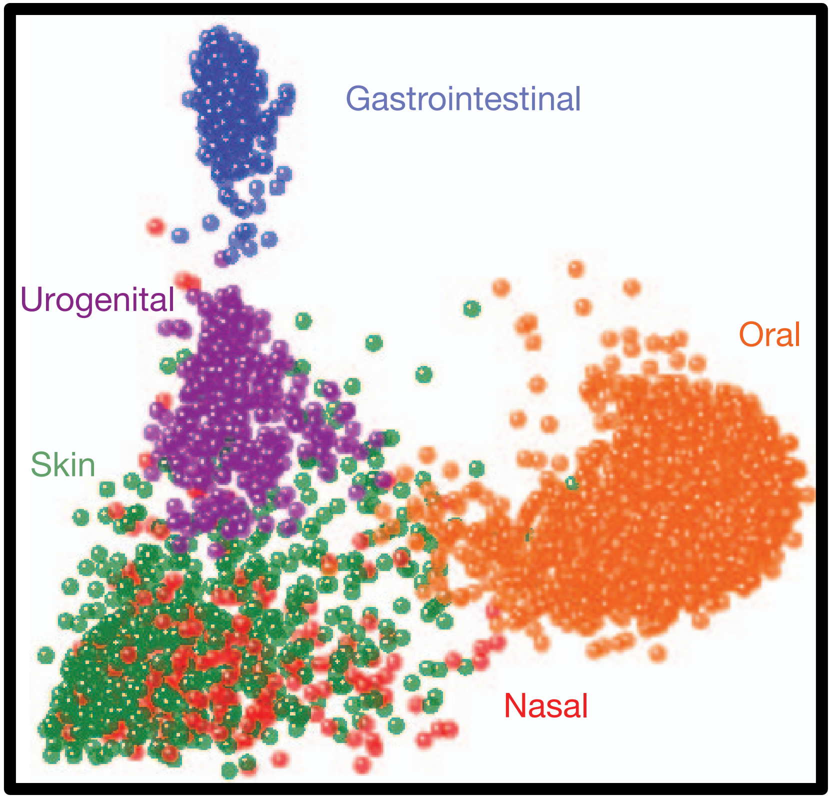
Bacteria of the genus *Actinomyces* live in the **mouth** and on **teeth**, and can be involved in infections after oral surgery.

Propionibacterium acnes lives on the **skin** of most people and has been linked to the development of acne.

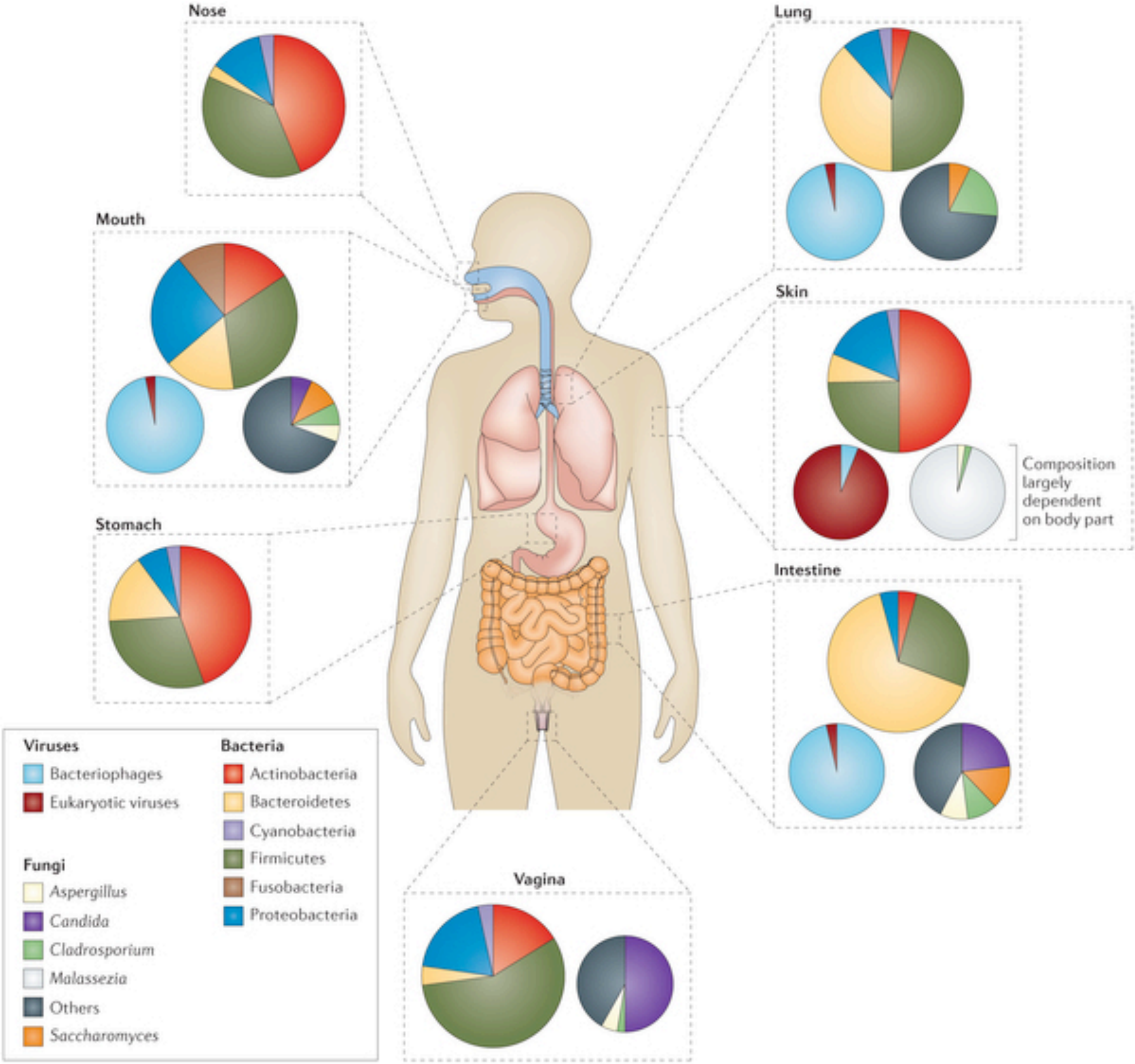
The **mouth** contains many different species of *Streptococcus* bacteria.

Streptococcus mitis is particularly abundant in the cheeks.

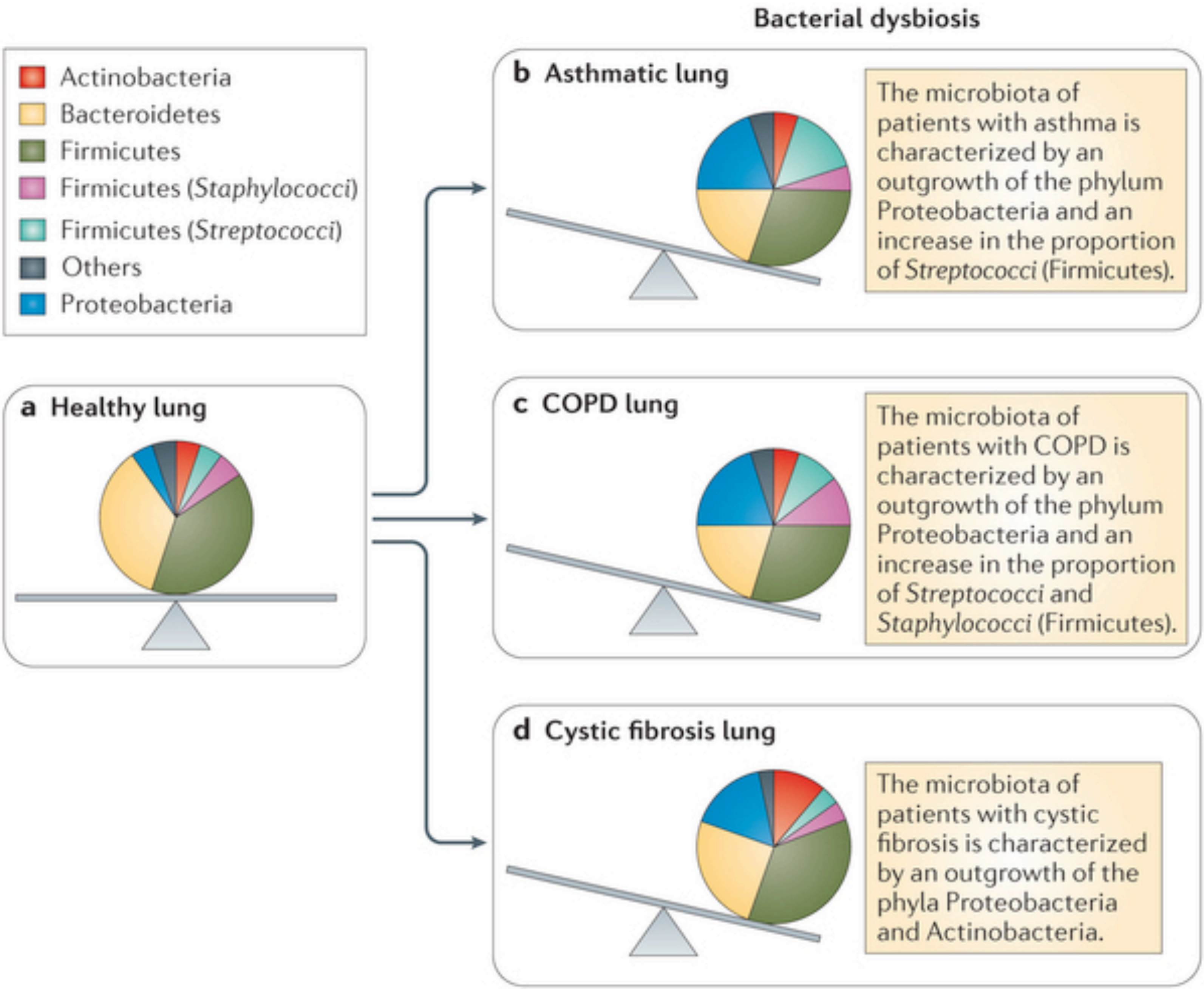
The **vagina** is typically colonized by one of four mutually exclusive species of *Lactobacillus*.



HMP


Nose
Lung
Mouth
Skin
Composition largely dependent on body part
Stomach
Intestine
Vagina
Viruses
Bacteriophages
Eukaryotic viruses
Fungi
Aspergillus
Candida
Cladrosporium
Malassezia
Others
Saccharomyces
Bacteria
Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Fusobacteria
Proteobacteria

DYSBIOSIS → DISEASE



The microbiota of patients with asthma is characterized by an outgrowth of the phylum Proteobacteria and an increase in the proportion of *Streptococci* (Firmicutes).

The microbiota of patients with COPD is characterized by an outgrowth of the phylum Proteobacteria and an increase in the proportion of *Streptococci* and *Staphylococci* (Firmicutes).

The microbiota of patients with cystic fibrosis is characterized by an outgrowth of the phyla Proteobacteria and Actinobacteria.

Nature Reviews | Immunology

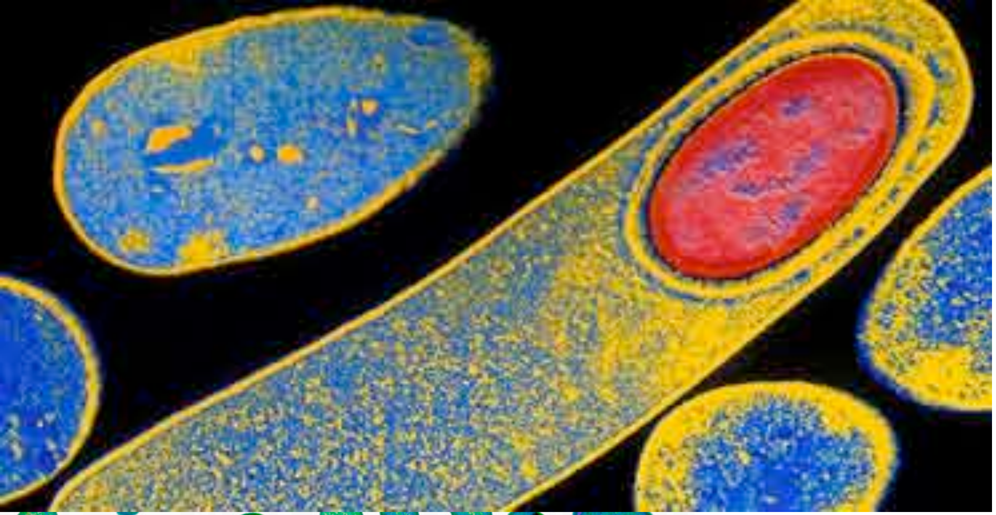
The Gut Microbiome

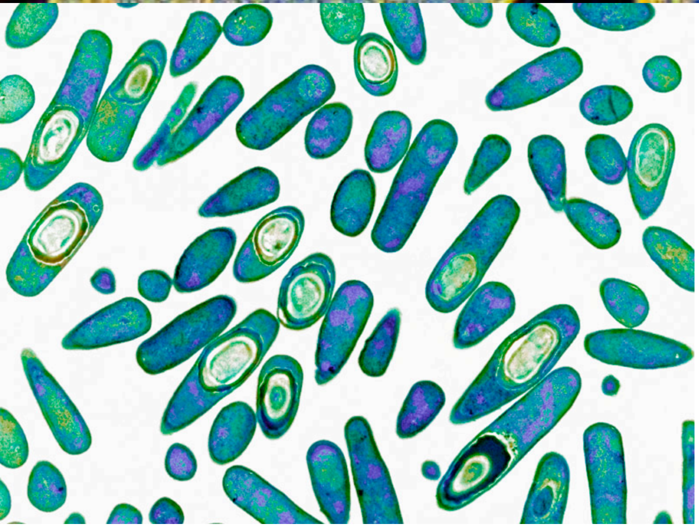
1. Primary Focus of Microbiomics to date
2. Linked to: autoimmune disorders, obesity, type-I diabetes, food sensitivities, colorectal cancer, gastic cancer, prostate cancer
3. Age, living situation, antibiotic exposure
4. Diet is key!
5. Huge variation between individuals/relative stability within individuals over time
6. Mechanisms:
   1. "training the immune system"
   2. Production of toxins (inflammation, carcinogens)
7. Prebiotics and Probiotics may influence and show some promise
8. Fecal transplant: works for recurrent C. diff [being considered for VRE, IBD]

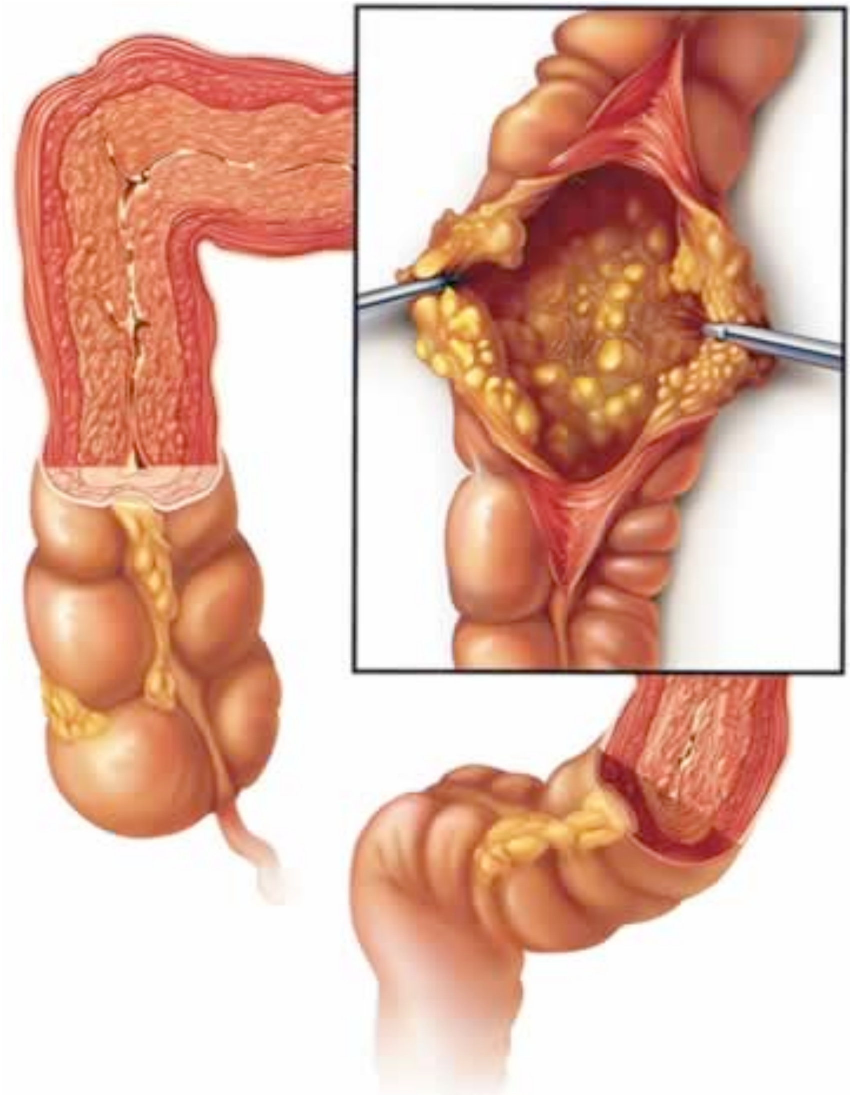
Parsing the Microbiome: Reducing the Complexity

1. Focus on Phyla (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria)
2. Focus on Ecological Patterns (Diversity, Richness, Evenness)
3. Focus on specific organisms (Bifidobacterium, Lactobacillus, Staphylococcus)

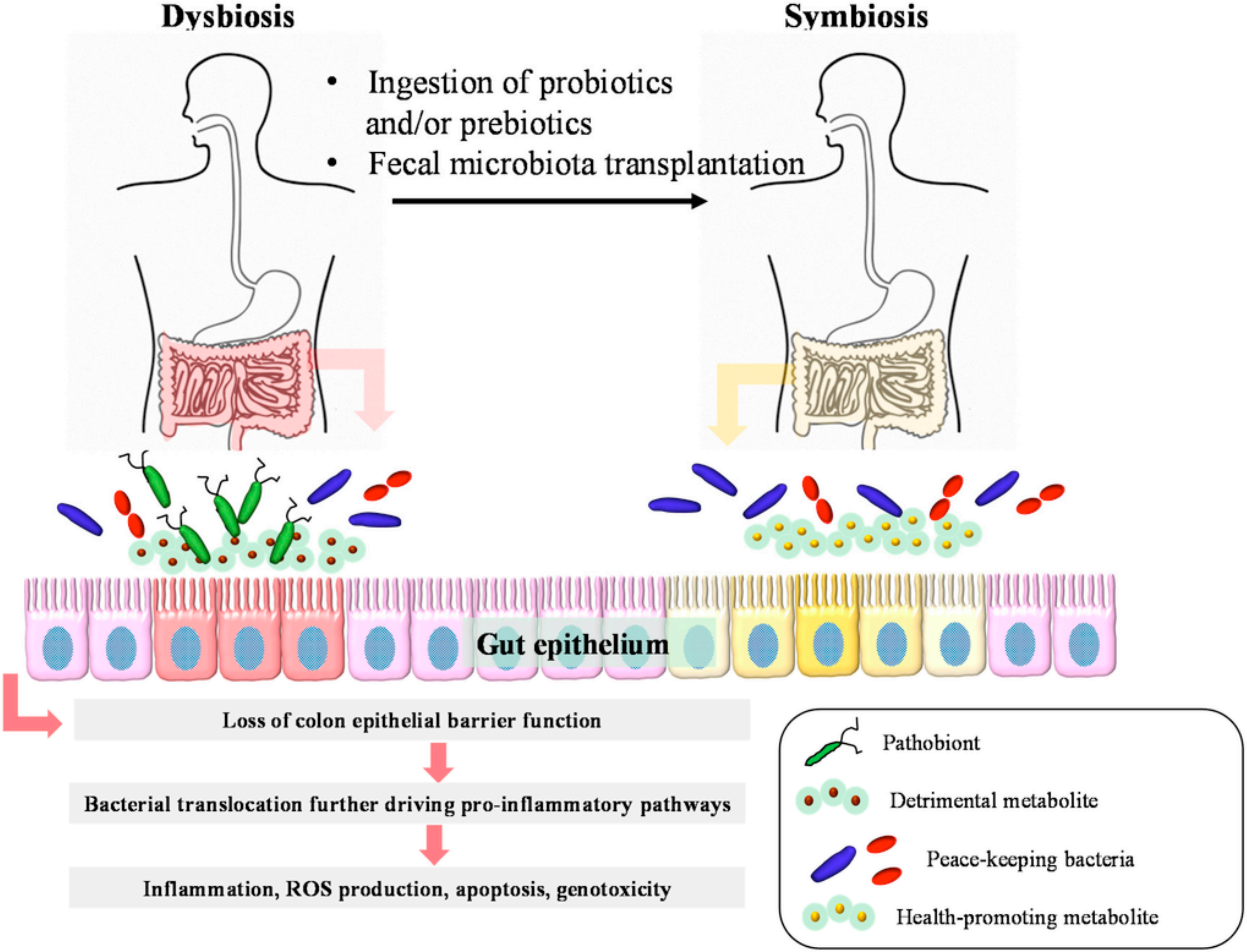
DYSBIOSIS → DISEASE







Clostridium difficile


Dysbiosis
Symbiosis
• Ingestion of probiotics and/or prebiotics
• Fecal microbiota transplantation
Gut epithelium
Loss of colon epithelial barrier function
Bacterial translocation further driving pro-inflammatory pathways
Inflammation, ROS production, apoptosis, genotoxicity
Pathobiont
Detrimental metabolite
Peace-keeping bacteria
Health-promoting metabolite

FECAL MICROBIAL TRANSPLANT (FMT)

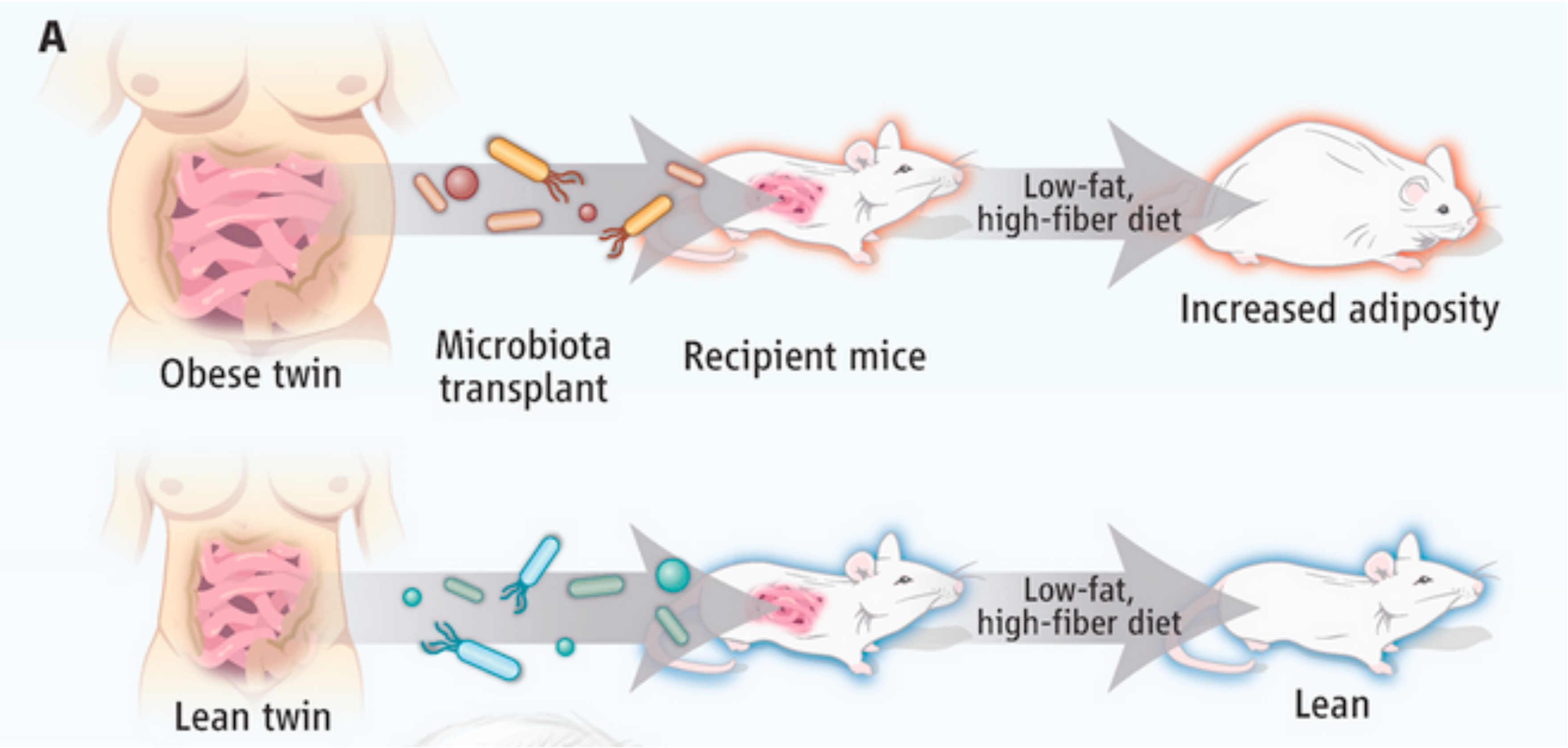
Lee et al 2016 JAMA

Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Infection A Randomized Clinical Trial

80-90% cure

Germ free mice: completely free of any miroorganisms

Gnotobiotics: known microorganisms



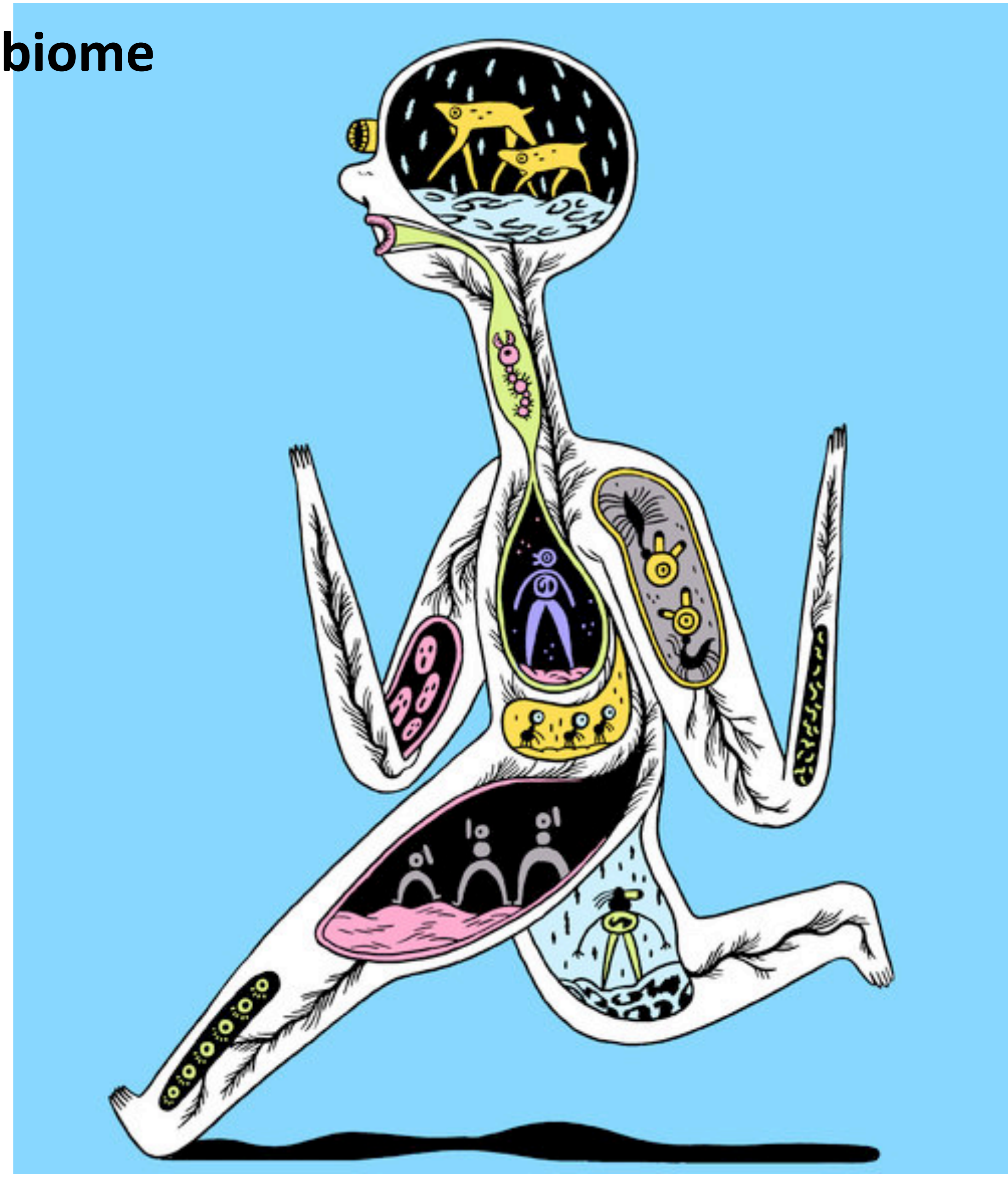
Depiction of Ridaura 2013 **Gut microbiota from twins discordant for obesity modulate metabolism in mice.**
Science

What does health mean to you?

Health may mean:
Being athletic
Living a long time
Not having intrusive thoughts
Being slim
Being the right weight
Being happy
Not having pain
Reproducing beautiful children
Being able to care for children
Being able to work
Being able to make love
Being able to relax
Being you!

There Is No 'Healthy' Microbiome

By ED YONG
NOV. 1, 2014
New York Times



http://www.nytimes.com/2014/11/02/opinion/sunday/there-is-no-healthy-microbiome.html?_r=0

Naturalistic fallacy: a concept like “good” cannot be defined by some natural properties it sometimes evokes or represents (happiness, normal, average, pleasure etc.,) (GE Moore, *Principia Ethica*, 1873-1958).

“appeal to nature”

Naturalistic fallacy: a concept like "good" cannot be defined by some natural properties it sometimes evokes or represents (happiness, normal, average, pleasure etc.,) (GE Moore, *Principia Ethica*, 1873-1958).

"appeal to nature"

Good things are sometimes natural.

Natural things must therefore be good.

Naturalistic fallacy: a concept like “good” cannot be defined by some natural properties it sometimes evokes or represents (happiness, normal, average, pleasure etc.,) (GE Moore, *Principia Ethica*, 1873-1958).

Some other appeals to nature

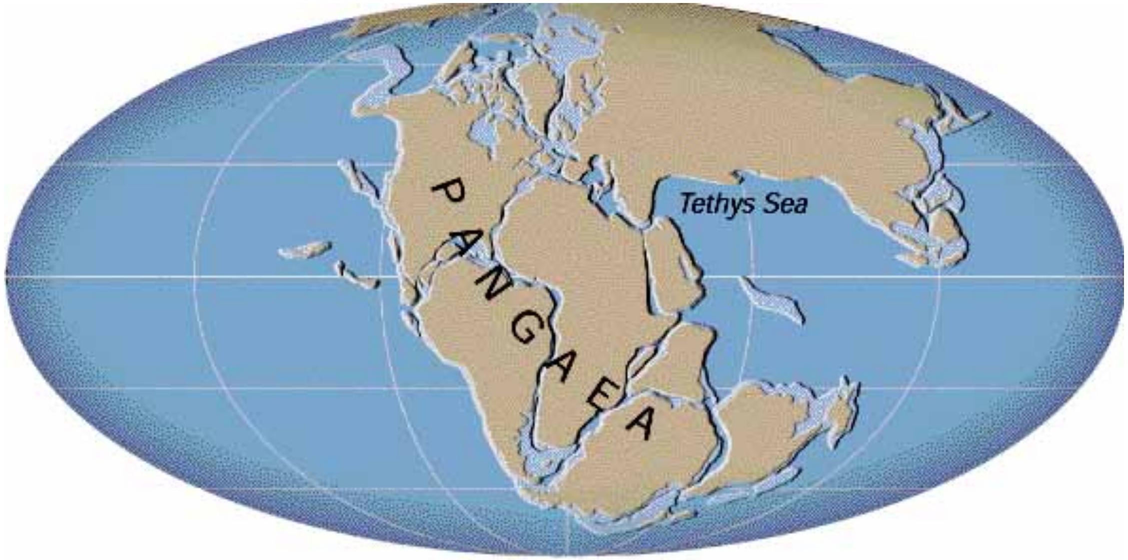
Meat eating
Vegetarianism
Sexuality (homosexuality, promiscuity etc.,)
Home birth
Vaccines
Warfare

OPEN vs. CLOSED/MEANINGLESS Questions

I know he is a vegetarian, but does he eat meat? Closed/Meaningless Question

I know it is pleasurable, but is it good? Open Question

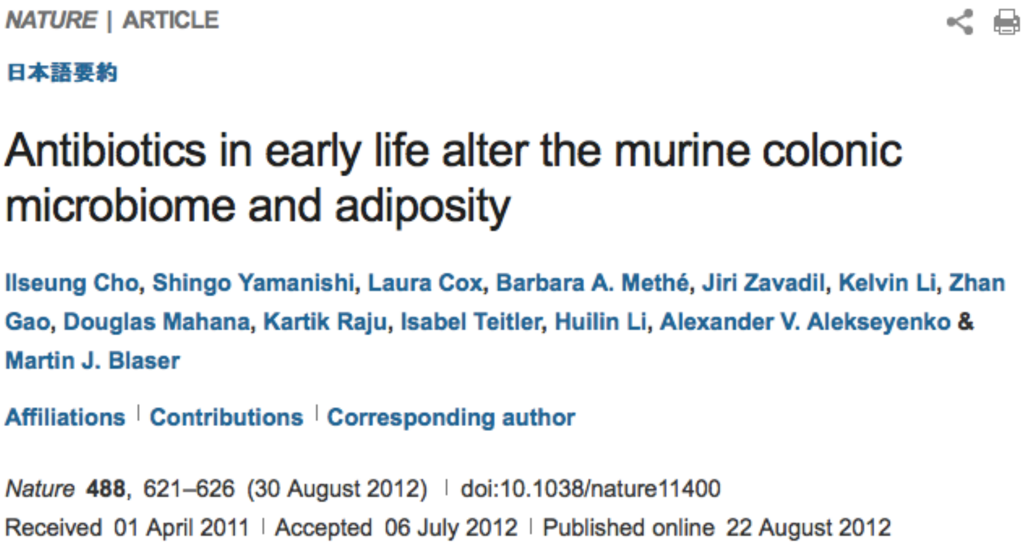
"Almost immediately after a human being is born,
so too is a new microbial ecosystem…" Palmer et al 2007 PLoS Biology



0-9 Months (Newborn)		9-18 Months (Infant-Pre-Toddler)	18-36 Months (Toddler)
Breast-Fed Characteristics (BF) • Low Species Diversity • Bacterial Composition Flux • Major Phyla: *Actinobacteria & Firmicutes*	**Formula-Fed Characteristics (FF)** • Low Species Diversity • Bacterial Composition Flux • Major Phyla: *Actinobacteria & Bacteriodetes*	**Introduction of Weaning & Solid Food** • Increased Species Diversity • Bacterial Composition Flux Persists • Increasing Butyrate Producing Bacteria • Major Phyla: *Bacteriodetes & Firmicutes*	**Diet-Influenced Microbiome Profile** • Stable Gut Microbiome Formation • Increased Species Diversity • Breast-Feeding History Ceases To Impact Gut Microbiome Profile • Increasing Butyrate Producing Bacteria Abundance • Dietary Intake Strongly Influences Abundances (*Prevotella* vs *Firmicutes)* • Major Phyla: *Bacteriodetes & Firmicutes*

Vooreades et al 2014

Microbial exposure has a huge impact on health



NATURE | ARTICLE

日本語要約

Antibiotics in early life alter the murine colonic microbiome and adiposity

Ilseung Cho, Shingo Yamanishi, Laura Cox, Barbara A. Methé, Jiri Zavadil, Kelvin Li, Zhan Gao, Douglas Mahana, Kartik Raju, Isabel Teitler, Huilin Li, Alexander V. Alekseyenko & Martin J. Blaser

Affiliations | **Contributions** | **Corresponding author**

Nature **488**, 621–626 (30 August 2012) | doi:10.1038/nature11400

Received 01 April 2011 | Accepted 06 July 2012 | Published online 22 August 2012

REPORT

Microbial Exposure During Early Life Has Persistent Effects on Natural Killer T Cell Function

Torsten Olszak[1,*], Dingding An[2,*], Sebastian Zeissig[3], Miguel Pinilla Vera[4], Julia Richter[5], Andre Franke[6], Jonathan N. Glickman[7], Reiner Siebert[5], Rebecca M. Baron[4], Dennis L. Kasper[2,†‡], Richard S. Blumberg[1,†‡]

+ Author Affiliations

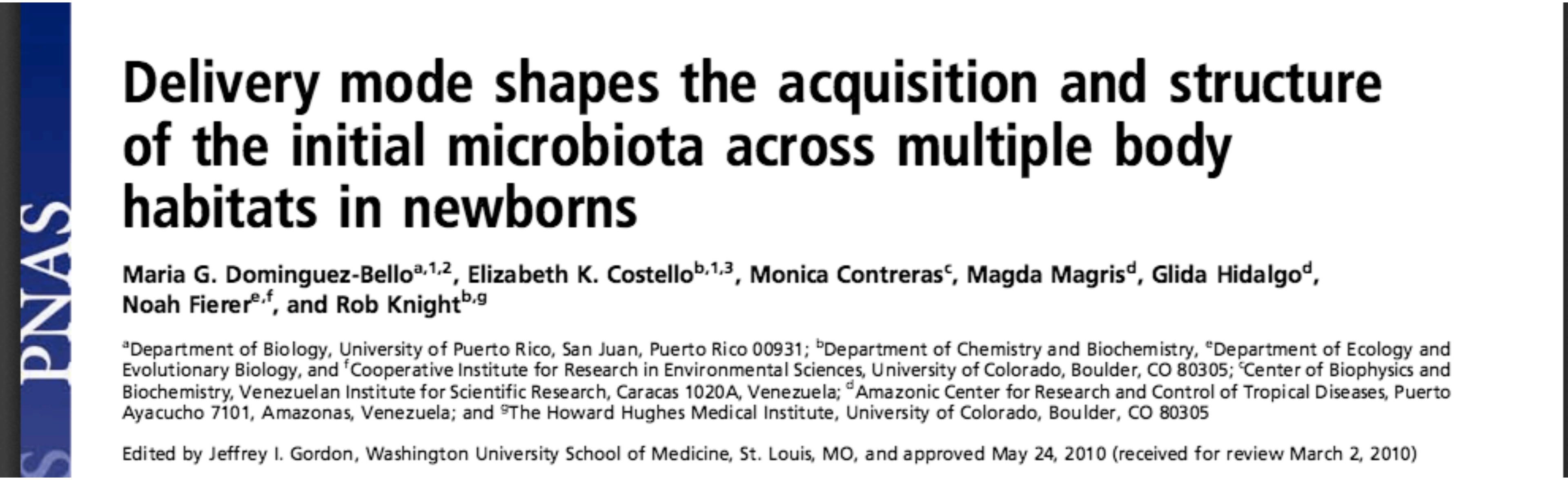
[‡] To whom correspondence should be addressed. E-mail: rblumberg@partners.org (R.S.B.); dennis_kasper@hms.harvard.edu (D.L.K.)

[*] These authors contributed equally to this work.

[†] These authors contributed equally to this work.

Science 27 Apr 2012:
Vol. 336, Issue 6080, pp. 489-493
DOI: 10.1126/science.1219328

Microbial exposure has a huge impact on health

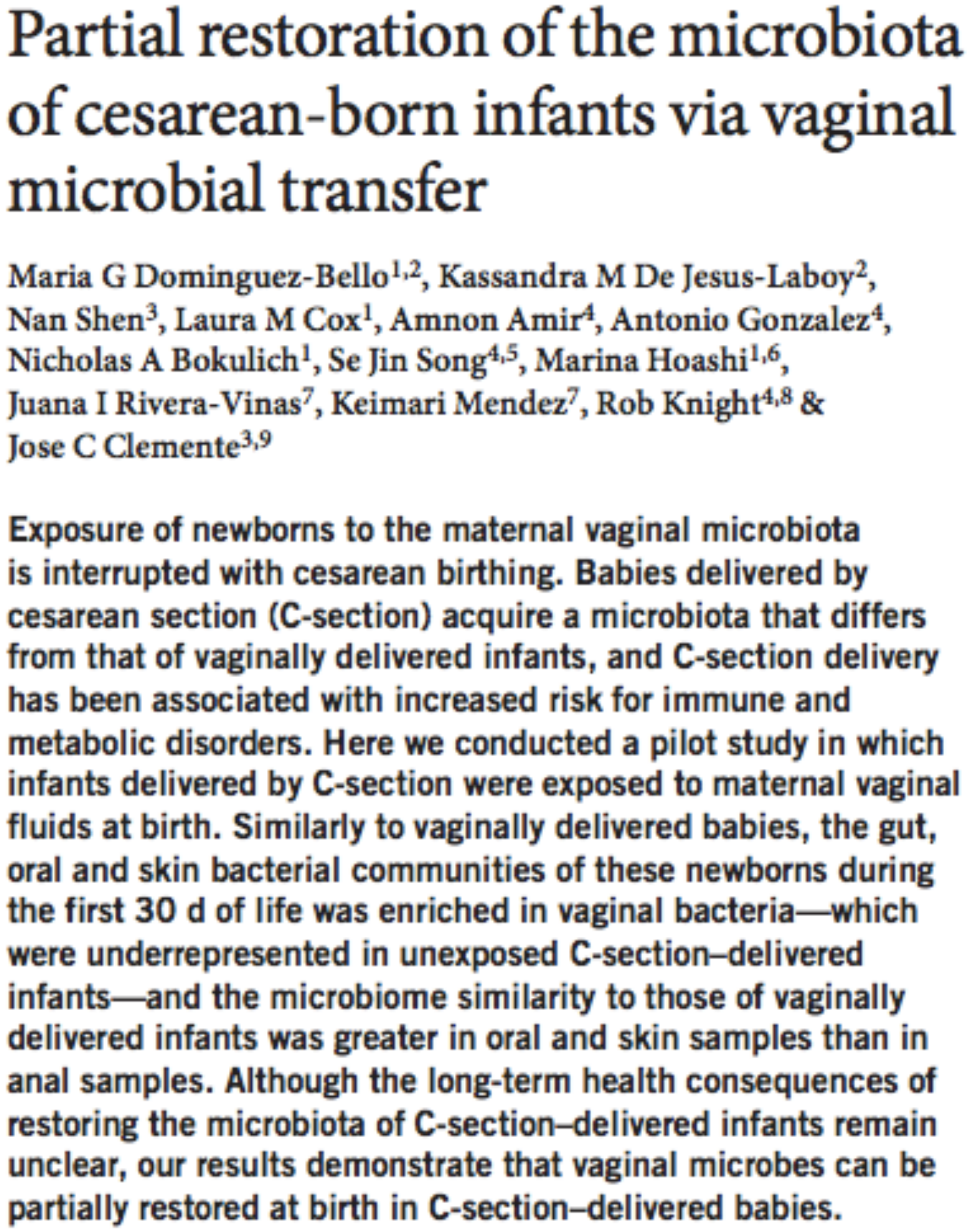


Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

Maria G. Dominguez-Bello[a,1,2], Elizabeth K. Costello[b,1,3], Monica Contreras[c], Magda Magris[d], Glida Hidalgo[d], Noah Fierer[e,f], and Rob Knight[b,g]

[a]Department of Biology, University of Puerto Rico, San Juan, Puerto Rico 00931; [b]Department of Chemistry and Biochemistry, [e]Department of Ecology and Evolutionary Biology, and [f]Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80305; [c]Center of Biophysics and Biochemistry, Venezuelan Institute for Scientific Research, Caracas 1020A, Venezuela; [d]Amazonic Center for Research and Control of Tropical Diseases, Puerto Ayacucho 7101, Amazonas, Venezuela; and [g]The Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80305

Edited by Jeffrey I. Gordon, Washington University School of Medicine, St. Louis, MO, and approved May 24, 2010 (received for review March 2, 2010)



Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G Dominguez-Bello[1,2], Kassandra M De Jesus-Laboy[2], Nan Shen[3], Laura M Cox[1], Amnon Amir[4], Antonio Gonzalez[4], Nicholas A Bokulich[1], Se Jin Song[4,5], Marina Hoashi[1,6], Juana I Rivera-Vinas[7], Keimari Mendez[7], Rob Knight[4,8] & Jose C Clemente[3,9]

Exposure of newborns to the maternal vaginal microbiota is interrupted with cesarean birthing. Babies delivered by cesarean section (C-section) acquire a microbiota that differs from that of vaginally delivered infants, and C-section delivery has been associated with increased risk for immune and metabolic disorders. Here we conducted a pilot study in which infants delivered by C-section were exposed to maternal vaginal fluids at birth. Similarly to vaginally delivered babies, the gut, oral and skin bacterial communities of these newborns during the first 30 d of life was enriched in vaginal bacteria—which were underrepresented in unexposed C-section–delivered infants—and the microbiome similarity to those of vaginally delivered infants was greater in oral and skin samples than in anal samples. Although the long-term health consequences of restoring the microbiota of C-section–delivered infants remain unclear, our results demonstrate that vaginal microbes can be partially restored at birth in C-section–delivered babies.

2016 Nature Medicine

BMJ 2016;352:i227 doi: 10.1136/bmj.i227 (Published 23 February 2016)

Page 1 of 2

EDITORIALS

“Vaginal seeding” of infants born by caesarean section

How should health professionals engage with this increasingly popular but unproved practice?

Aubrey J Cunnington *clinical senior lecturer*[1], Kathleen Sim *clinical research fellow*[1], Aniko Deierl *consultant neonatologist*[2], J Simon Kroll *professor of paediatrics and molecular infectious diseases*[1], Eimear Brannigan *consultant in infectious diseases and infection prevention and control*[3], Jonathan Darby *infectious diseases physician*[4]

[1]Section of Paediatrics, Department of Medicine, Imperial College London W2 1PG, UK; [2]St Mary's Hospital, London, UK; [3]Charing Cross Hospital, London, UK; [4]St Vincent's Hospital, Fitzroy, VIC 3065, Australia

---

Vaginal 'seeding': Could this new birth trend be putting babies at risk?

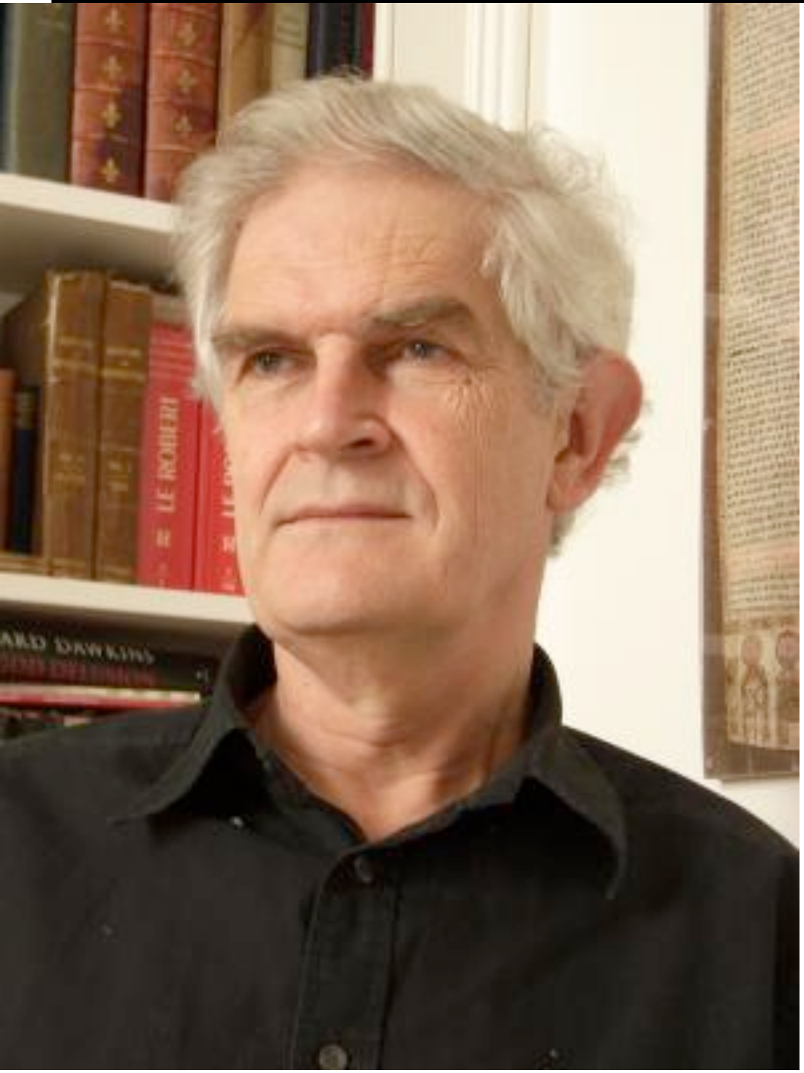
So what is the solution to this debate?

DATA and long term follow up!!

Much more important to avoid unnecessary antibiotics and breast feed.

Do not do it at home!

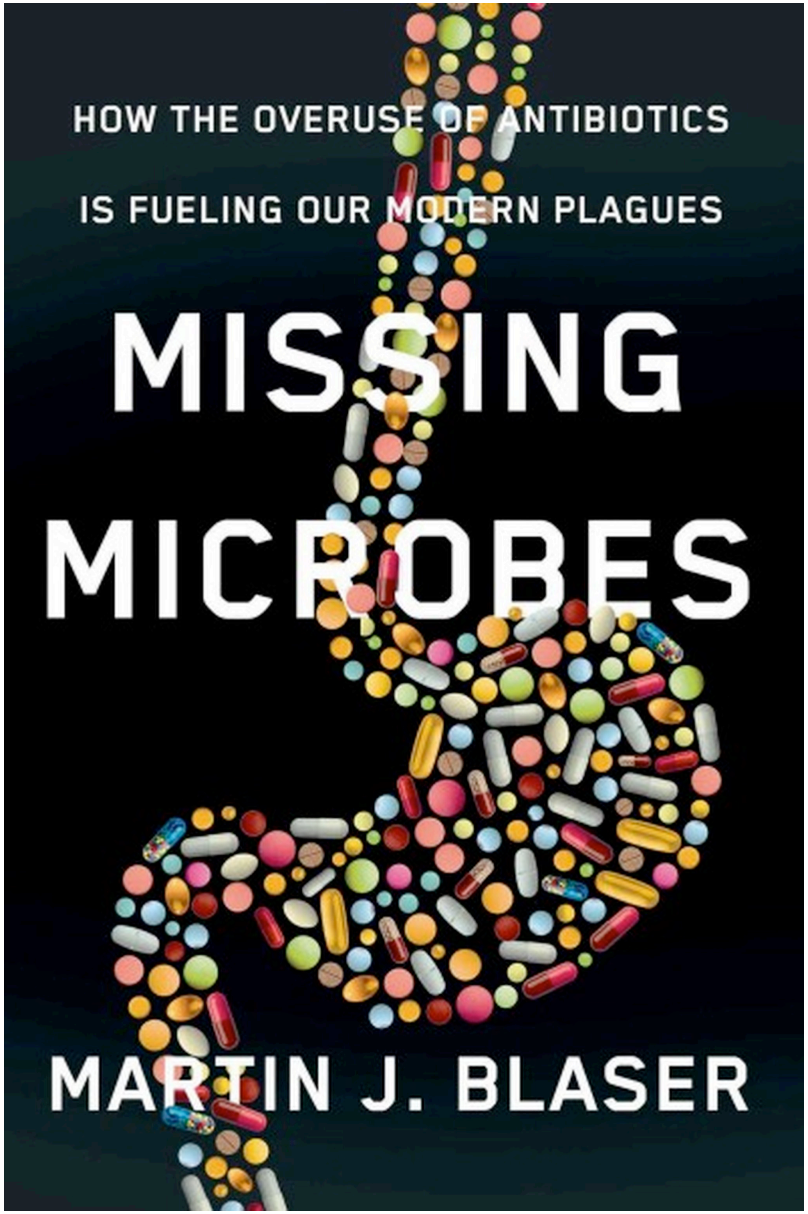
The Hygiene Hypothesis, old friends hypothesis: Education by our “friends”



Graham Rook, Royal Free and University Medical School in London, UK

David Strachan, Population Health Research Institute Research Institute, UK

Marty Blaser MD



Summary: The Naturalist's Dilemma

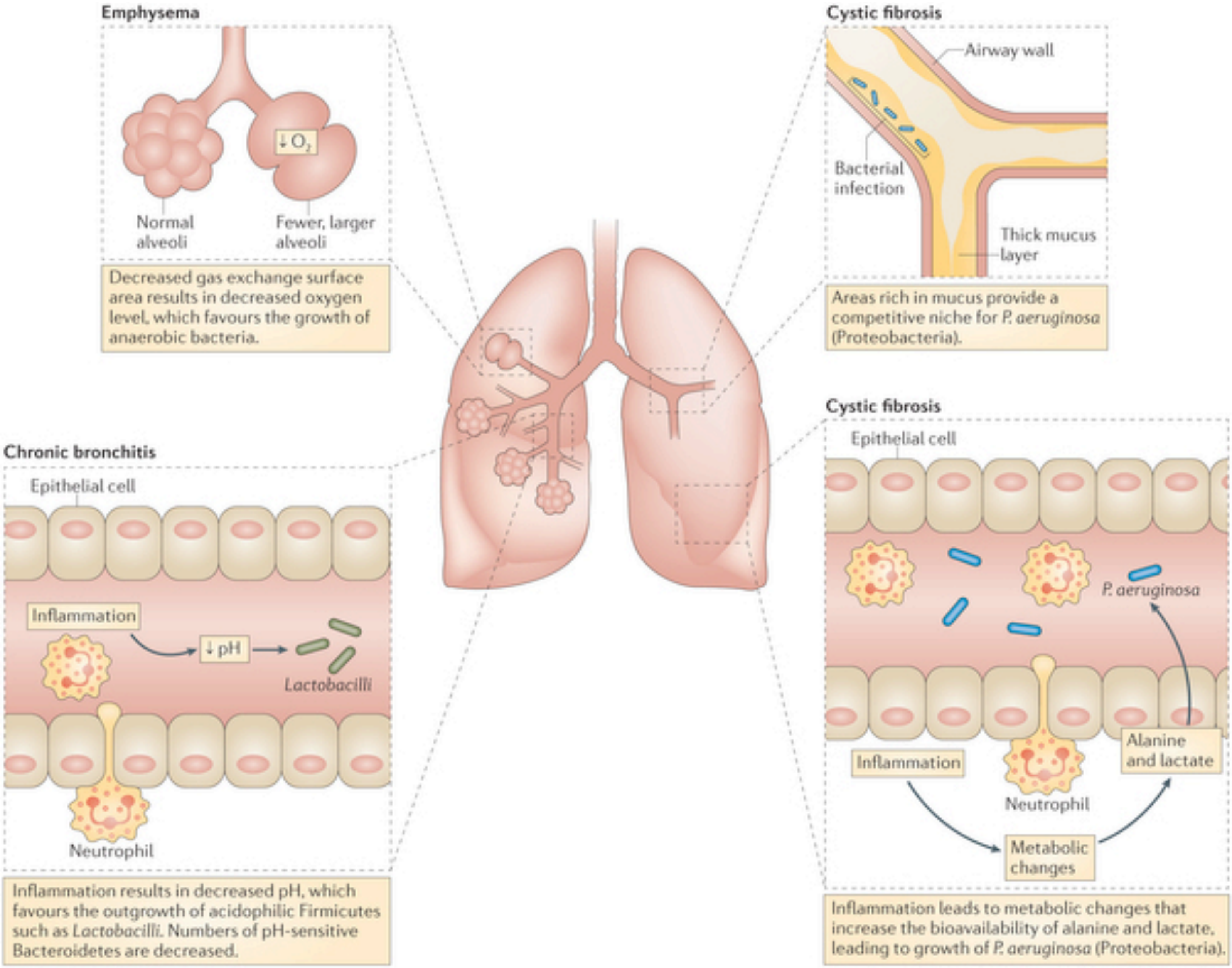
1. The human microbiome has a complex relationship to human health—**Can we study it without changing it or do we have to change it to study it?**

2. Do not commit the naturalistic fallacy!--**Health is not a reasonable scientific outcome. But specific outcomes might be.**

3. Watch out for mushy thinking.

The Respiratory Microbiome

- 75 m^2 surface area
- Biomass is relatively low (controls, techniques are important)
- 10 to 100 bacterial cells per 1000 human cells
- Major genera: *Pseudomonas, Streptococcus, Staphylococcus, Prevotella, Haemophilus, Neisseria, Veillonella, Haemophilus, Porphyromonas*
- Don't forget viruses and fungi! (major cause of exacerbation in COPD and Asthma)
- Heterogeneous changes in different locations (microenviroments).



Decreased gas exchange surface area results in decreased oxygen level, which favours the growth of anaerobic bacteria.

Areas rich in mucus provide a competitive niche for *P. aeruginosa* (Proteobacteria).

Inflammation results in decreased pH, which favours the outgrowth of acidophilic Firmicutes such as *Lactobacilli*. Numbers of pH-sensitive Bacteroidetes are decreased.

Inflammation leads to metabolic changes that increase the bioavailability of alanine and lactate, leading to growth of *P. aeruginosa* (Proteobacteria).

Nature Reviews | Immunology

The Respiratory Microbiome: What do we know so far?

1. The microbiome of the **healthy airway** is distinct from asthma, COPD, CF
2. Origin: **Rapid accumulation after birth** (Madan et al 2012 mBio, Gollwitzer et al 2014 Nat Med)
3. Core Stabilizes after **first month of life**

The Respiratory Microbiome: What do we know so far?

1. The microbiome of the **healthy airway** is distinct from asthma, COPD, CF
2. Origin: **Rapid accumulation after birth** (Madan et al 2012 mBio, Gollwitzer et al 2014 Nat Med)
3. Core Stabilizes after **first month of life**
4. **Diet** also affects respiratory flora (Madan et al 2012 mBio)

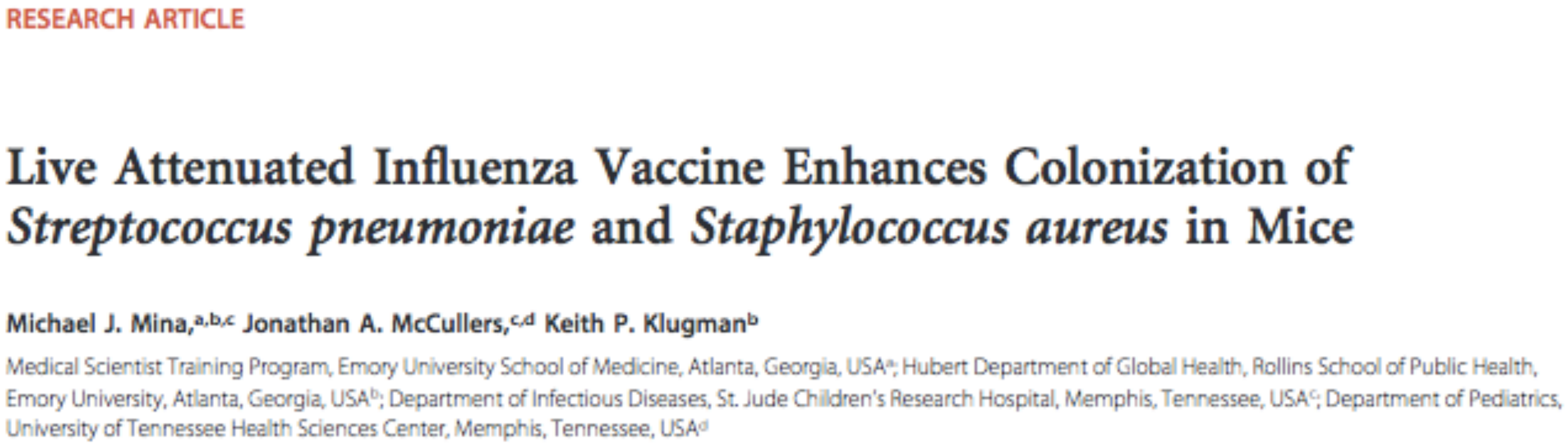
The Respiratory Microbiome: What do we know so far?

1. The microbiome of the **healthy airway** is distinct from asthma, COPD, CF
2. Origin: **Rapid accumulation after birth** (Madan et al 2012 mBio, Gollwitzer et al 2014 Nat Med)
3. Core Stabilizes after **first month of life**
4. **Diet** also affects respiratory flora (Madan et al 2012 mBio)
5. Interaction between Gut Microbiome and **Respiratory/Allergic disease** (Trompette et al 2014 Nat Med, Madan et al 2012 mBio, , Gollwitzer et al 2014 Nat Med, Ege et al *NEJM* 2011, Abrahamsson 2014 et al Clin Ex Allergy, Russell et al 2012 EMBO, Herbst et al 2011, Nembrini et al 2011)

The Respiratory Microbiome: What do we know so far?

1. The microbiome of the **healthy airway** is distinct from asthma, COPD, CF
2. Origin: **Rapid accumulation after birth** (Madan et al 2012 mBio, Gollwitzer et al 2014 Nat Med)
3. Core Stabilizes after **first month of life**
4. **Diet** also affects respiratory flora (Madan et al 2012 mBio)
5. Interaction between Gut Microbiome and **Respiratory/Allergic disease** (Trompette et al 2014 Nat Med, Madan et al 2012 mBio, , Gollwitzer et al 2014 Nat Med, Ege et al *NEJM* 2011, Abrahamsson 2014 et al Clin Ex Allergy, Russell et al 2012 EMBO, Herbst et al 2011, Nembrini et al 2011)
6. Perhaps a role **for prebiotics/probiotics** (Gollwitzer & Marsland 2014 Pharm Ther)
7. Certain Organisms are associated with steroid nonresponsiveness (H. parainfluenzae [Goleva et al 2013])
8. COPD→**viruses** and outgrowth of specific pathogens (Mallia et al 2012, Molyneaux et al 2013, Goulding et al 2011)

IMPACT OF VIRUSES



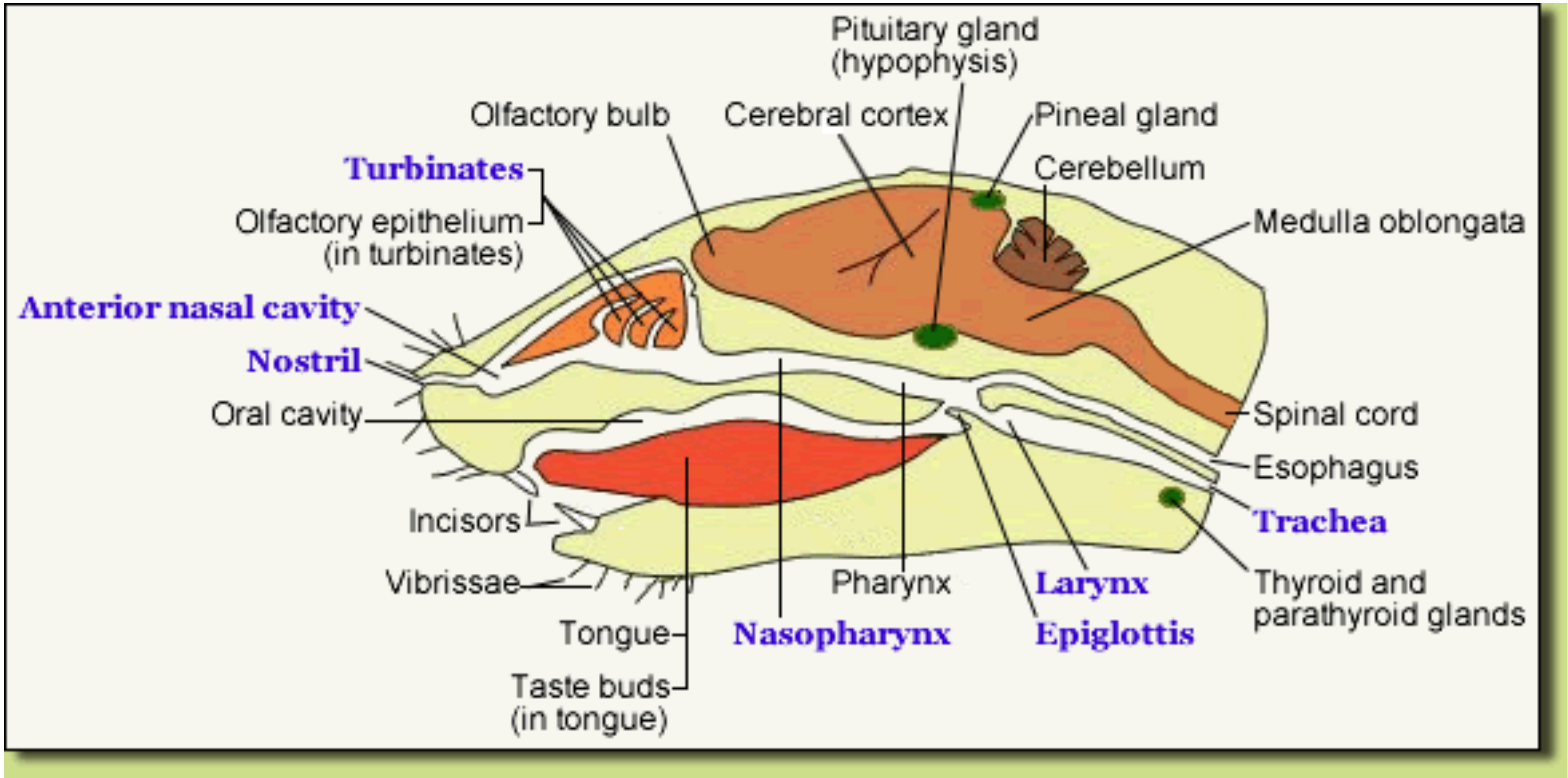
RESEARCH ARTICLE

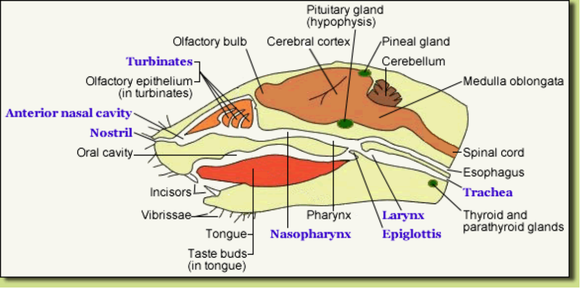
Live Attenuated Influenza Vaccine Enhances Colonization of *Streptococcus pneumoniae* and *Staphylococcus aureus* in Mice

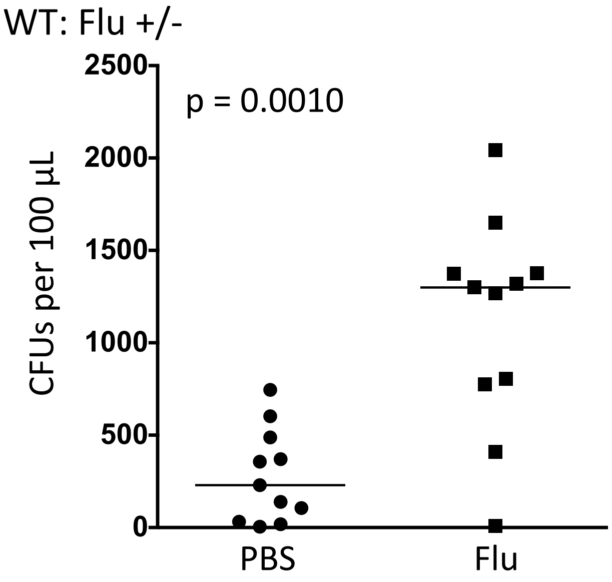
Michael J. Mina,[a,b,c] **Jonathan A. McCullers,**[c,d] **Keith P. Klugman**[b]

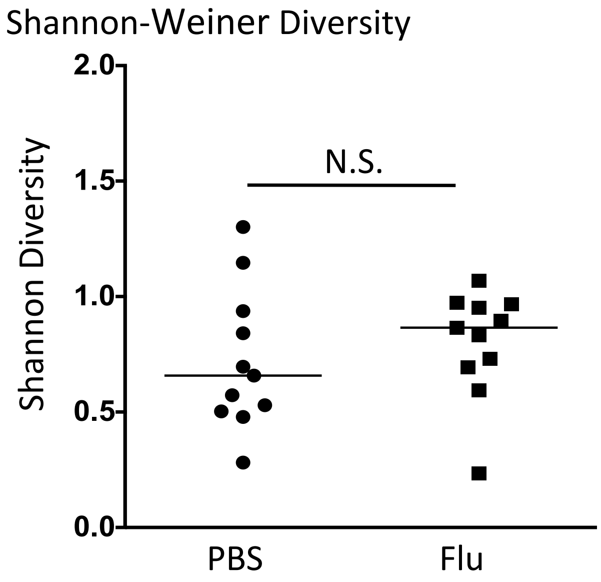
Medical Scientist Training Program, Emory University School of Medicine, Atlanta, Georgia, USA[a]; Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA[b]; Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA[c]; Department of Pediatrics, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA[d]

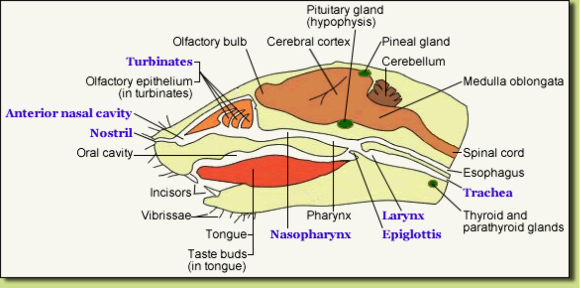
IMPACT OF VIRUSES

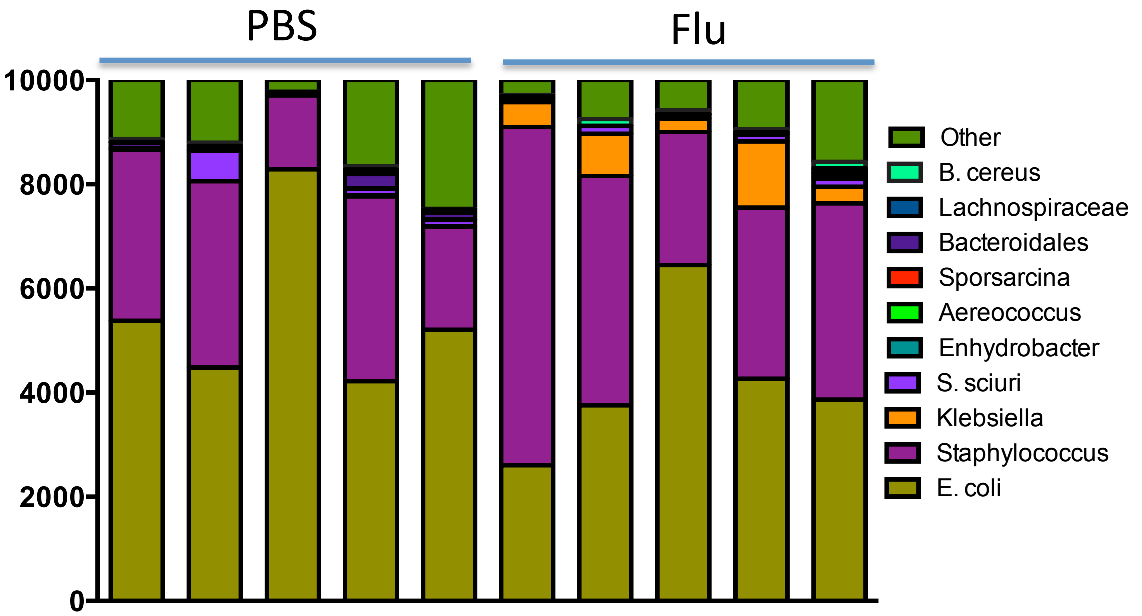



Pituitary gland
(hypophysis)
Olfactory bulb
Cerebral cortex
Pineal gland
Turbinates
Cerebellum
Olfactory epithelium
(in turbinates)
Medulla oblongata
Anterior nasal cavity
Nostril
Oral cavity
Spinal cord
Esophagus
Trachea
Incisors
Vibrissae
Pharynx
Larynx
Thyroid and
parathyroid glands
Tongue
Nasopharynx
Epiglottis
Taste buds
(in tongue)

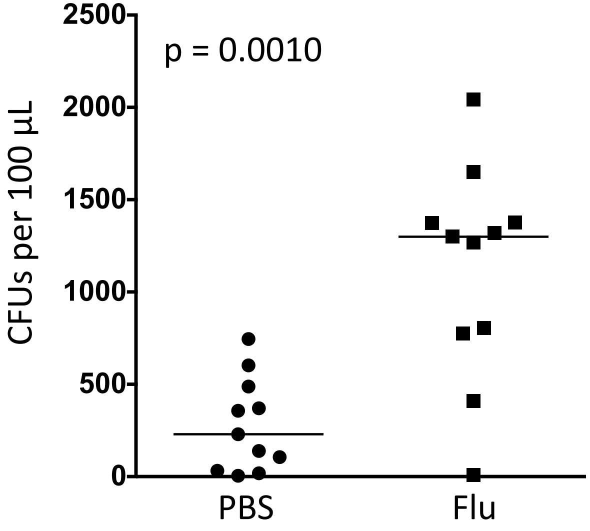

WT: Flu +/-
p = 0.0010
CFUs per 100 µL
2500
2000
1500
1000
500
0
PBS
Flu


Shannon-Weiner Diversity
N.S.
Shannon Diversity
2.0
1.5
1.0
0.5
0.0
PBS
Flu

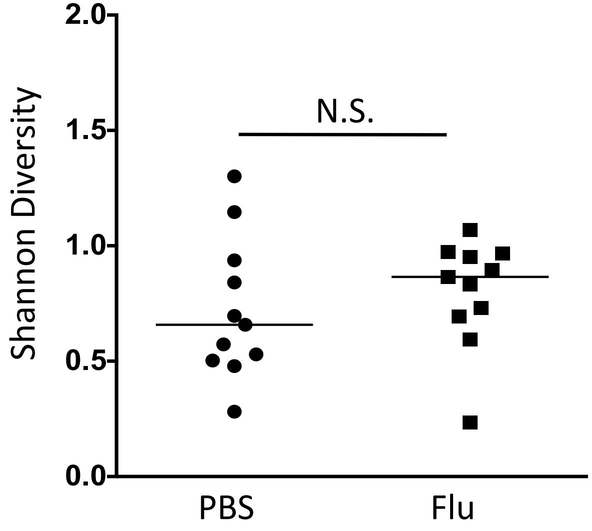




WT: Flu +/-

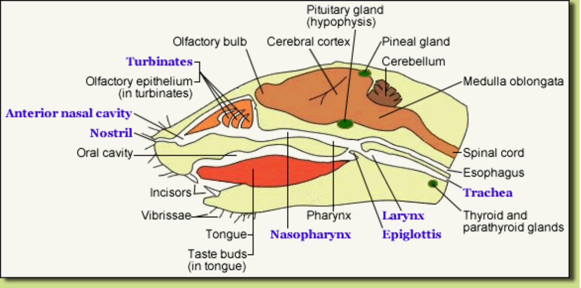


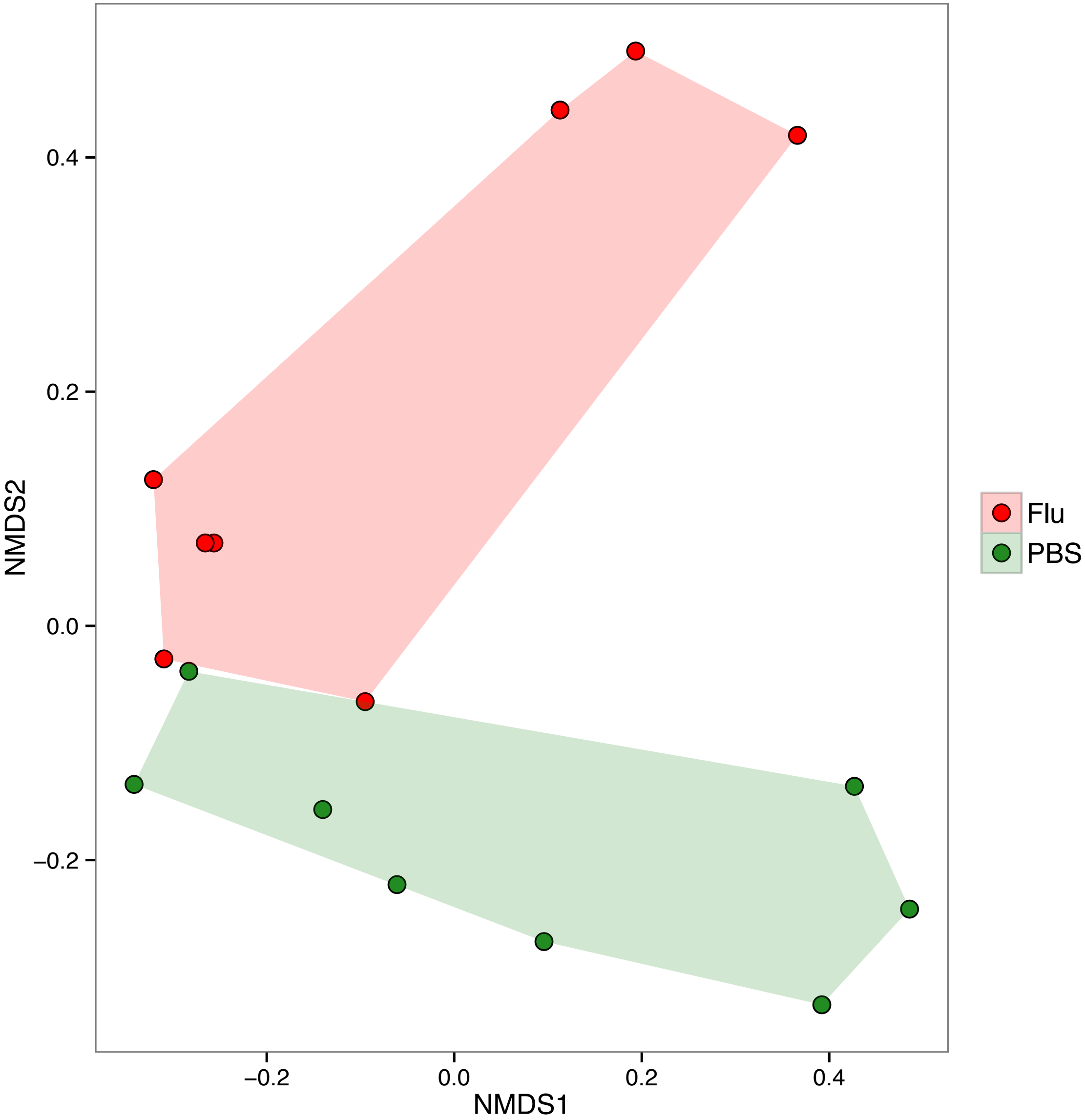
Shannon-Weiner Diversity

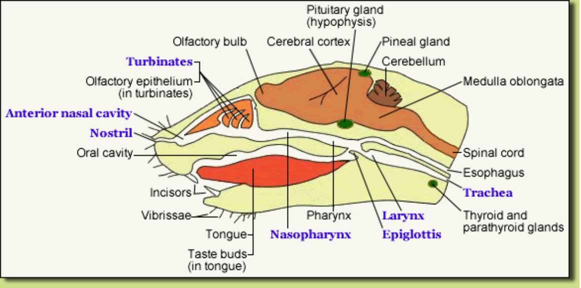


Prevalence and Colony Counts by Species

	Species	**Prevalence**				**Mean Colony Counts**		
		Flu -	Flu +	Total	P	Flu -	Flu +	P
Gram +								
	Staphylococcus lentus	9	10	19	1.0000	268	483	0.3910
	Staphylococcus xylosus	9	9	18	1.0000	19	192	0.0264*
	Staphylococcus nepalensis	8	7	15	1.0000	24	123	0.4698
	Enterococcus faecalis	3	4	7	1.0000	21	21.25	0.3545
	Bacillus thuringiensis	4	1	5	0.3108	8	1	0.2556
	Enterococcus gallinarum	2	1	3	1.0000	3	4	> 0.9999
	Staphylococcus cohnii	2	1	3	1.0000	11	106	> 0.9999
	Aerococcus urinaeequi	0	2	2	0.4762	0	13	0.4762
	Jeotgalicoccus halotolerans	0	2	2	0.4762	0	58	0.4762
Gram -								
	Klebsiella oxytoca	3	5	8	0.6594	2	43	0.1883
	Enterobacter hormaechei	4	2	6	0.6351	32	1224	0.6351
	Enterobacter absurie	1	4	5	0.3108	7	464	0.1454

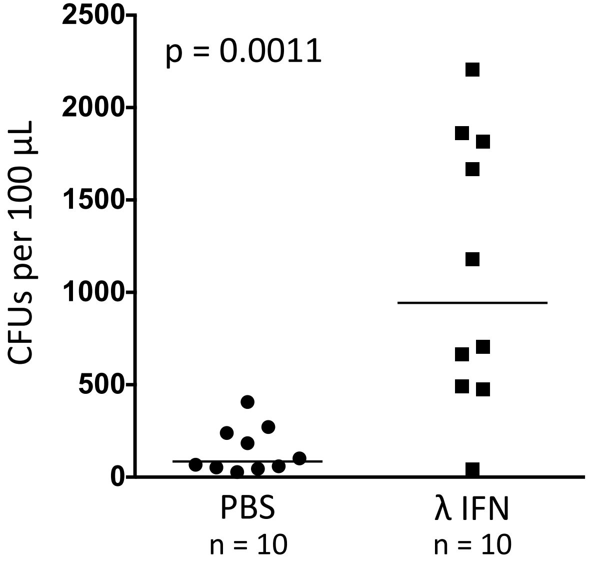

Pituitary gland (hypophysis)
Olfactory bulb
Cerebral cortex
Pineal gland
Turbinates
Cerebellum
Olfactory epithelium (in turbinates)
Medulla oblongata
Anterior nasal cavity
Nostril
Oral cavity
Spinal cord
Esophagus
Trachea
Incisors
Vibrissae
Pharynx
Larynx
Thyroid and parathyroid glands
Tongue
Nasopharynx
Epiglottis
Taste buds (in tongue)


NMDS2
0.4
0.2
0.0
−0.2
NMDS1
−0.2
0.0
0.2
0.4
Flu
PBS

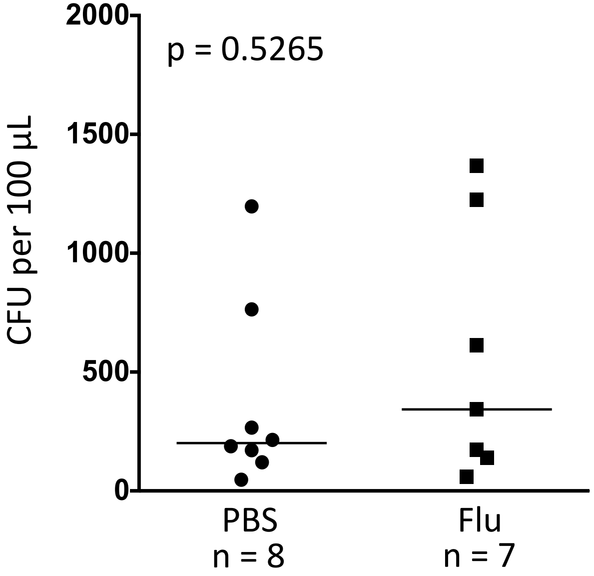


Interferon lambda (IL28) Dependent

WT: λ IFN +/-

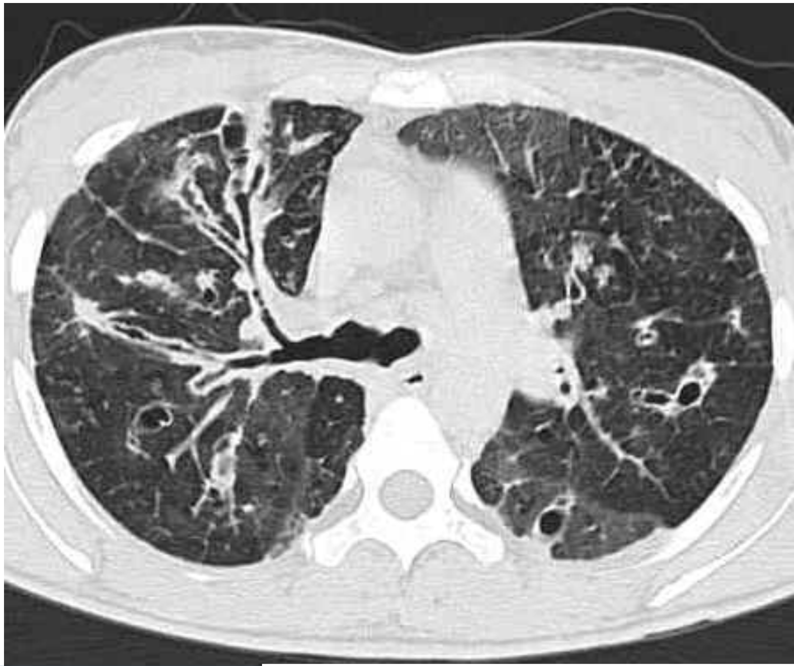


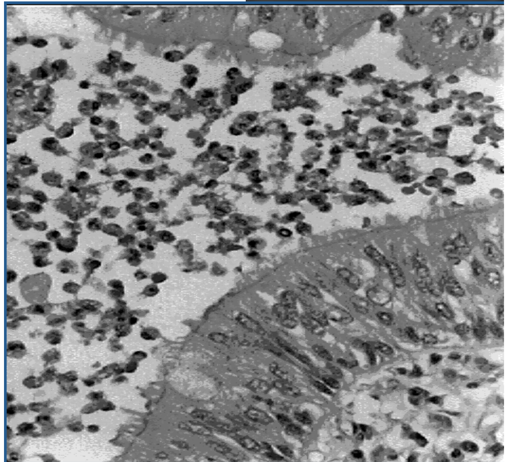
IL28R -/-

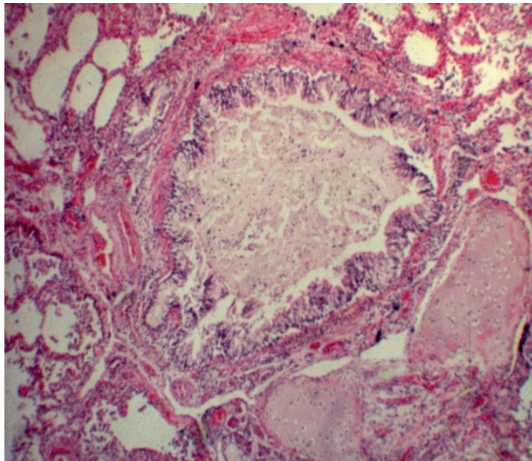


Progressive airway obstruction is the leading cause of morbidity and mortality for patients with CF.

- 30,000 people in the US
- Predicted median age of survival, early 40's
- Defect in CFTR gene
- Abnormal surface airway fluid
- Mucus plugging
- Infection
- Neutrophil-dominated inflammation

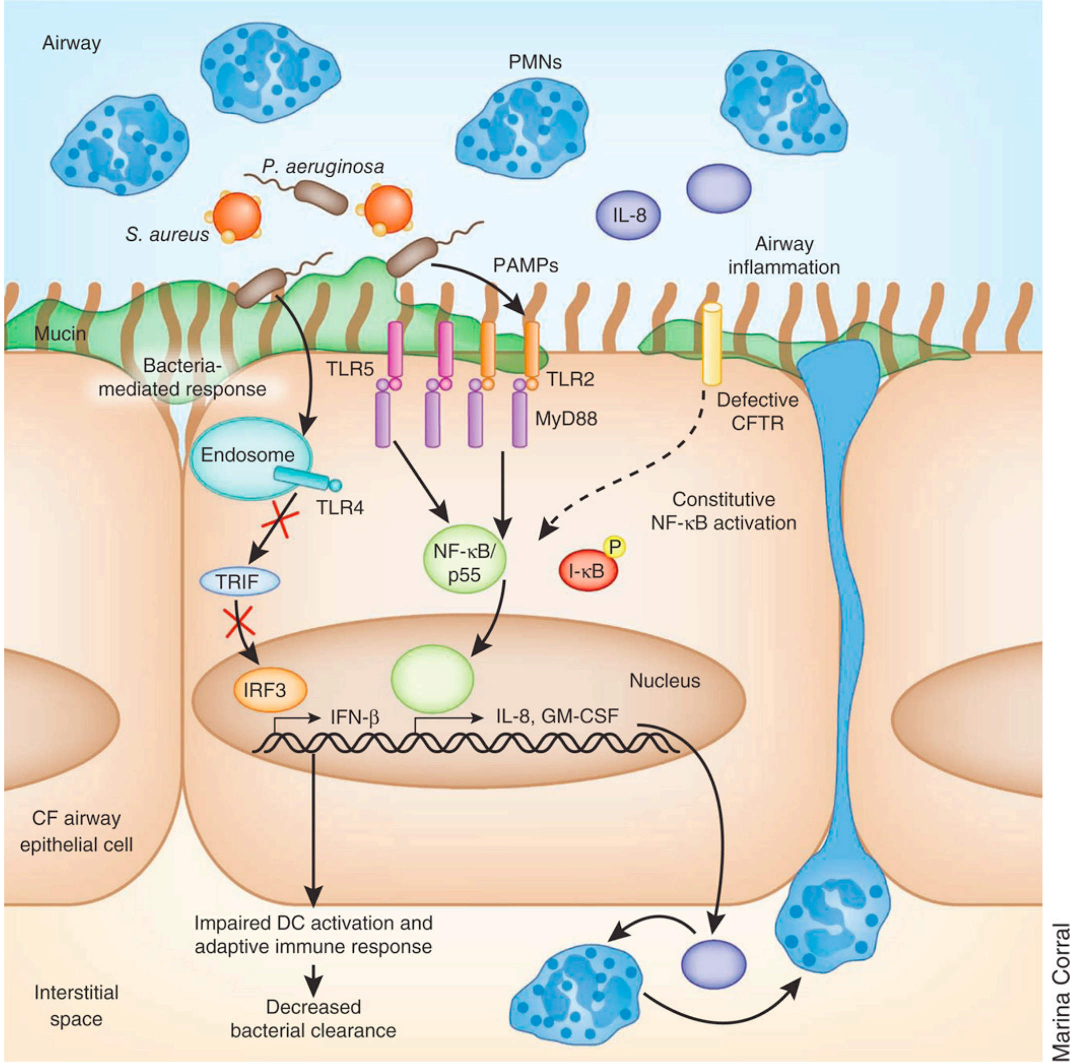






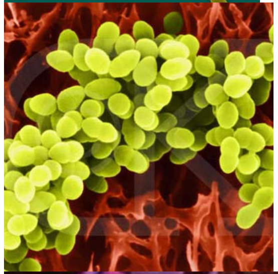
CF is an inflammatory disease and an immunodeficiency

- CF epithelial cells have an exaggerated inflammatory response to bacteria.
- Increase NFkB
- Aberrant TLR receptor localization
- Oxidative stress

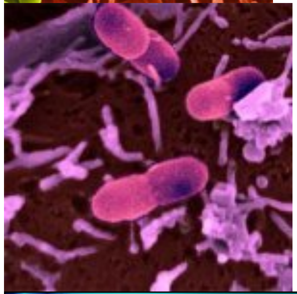


Cohen & Prince 2012

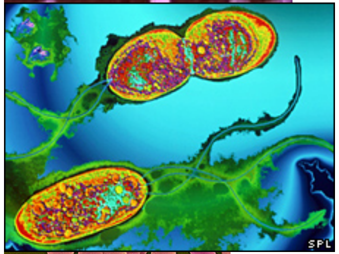
Pseudomonas aeruginosa



Staphylococcus aureus



Burkholderia spp.,

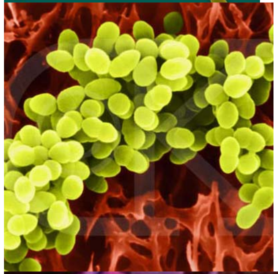


Stenotrophomonas maltophilia

Haemophilus influenzae

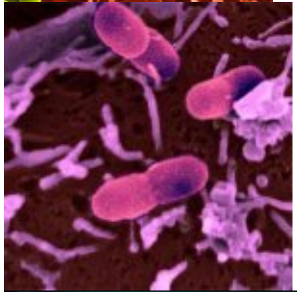
Pseudomonas aeruginosa

Achromobacter xylosoxidans

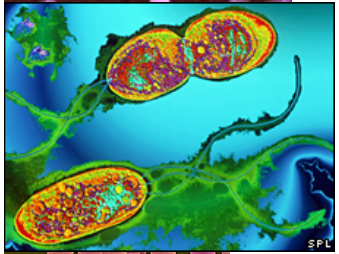


Staphylococcus aureus

Non-tuberculous mycobacteria



Burkholderia spp.,

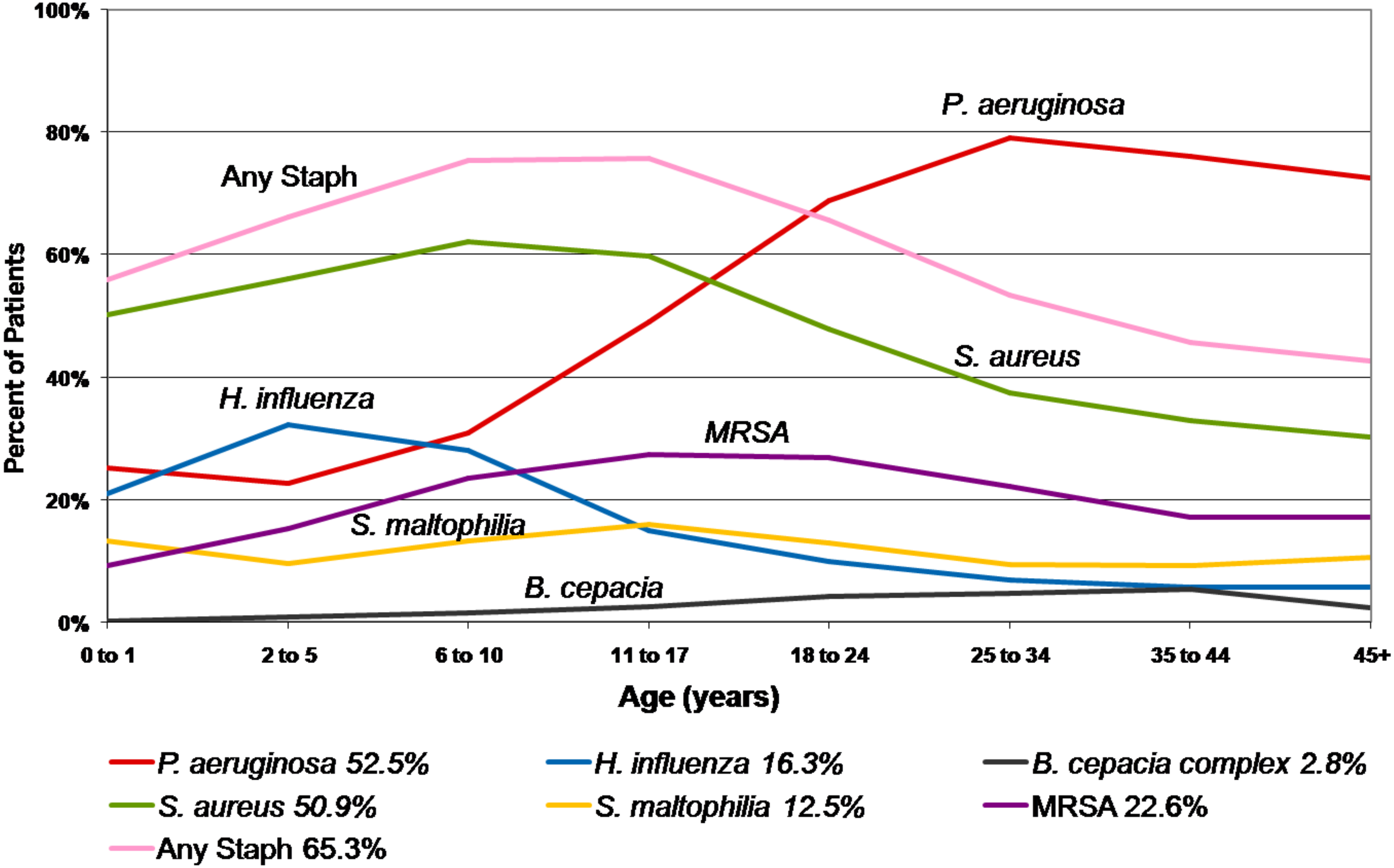


Stenotrophomonas maltophilia

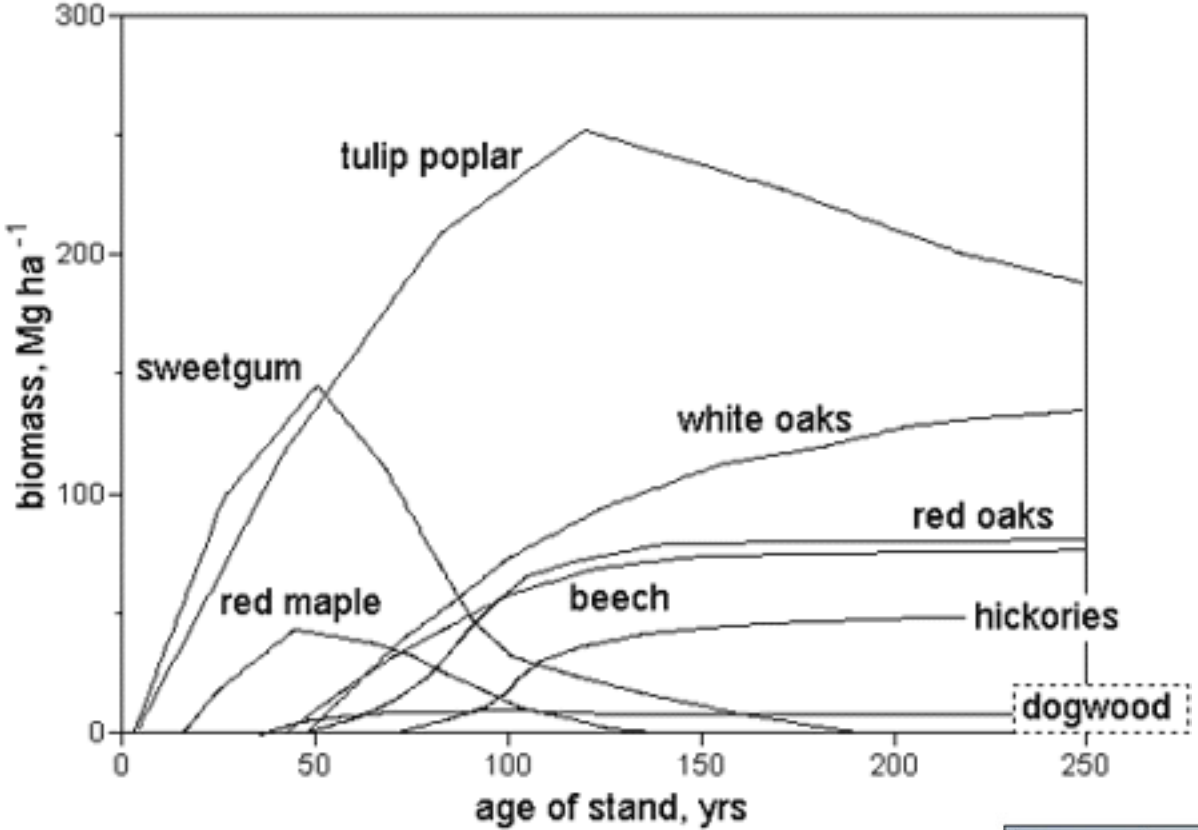
Haemophilus influenzae

Fungi (Genera: *Aspergillus, Scedosporium, Exophiala, Mucor, Penicillium*)

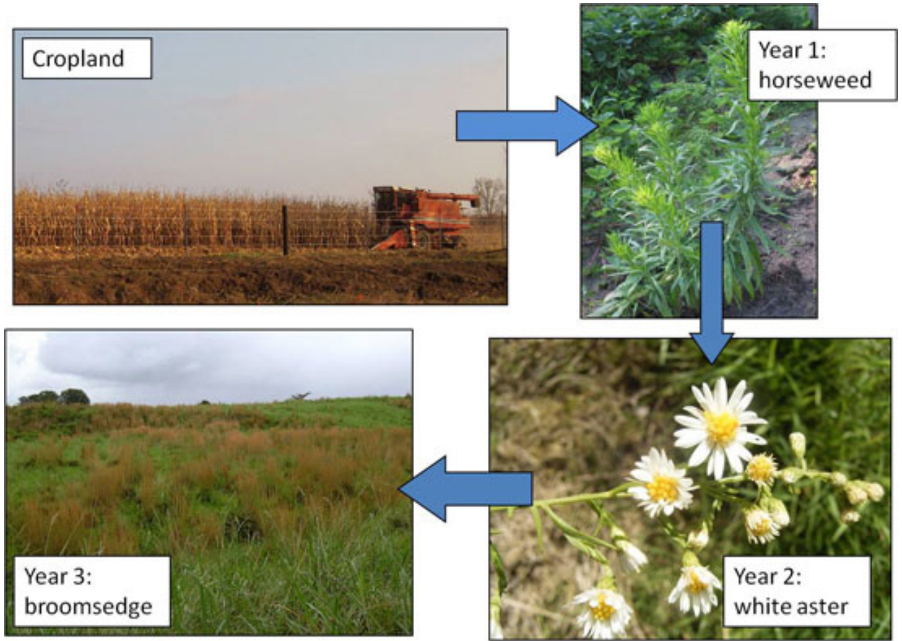
Age-Specific Prevalence of Respiratory Infections in CF Patients



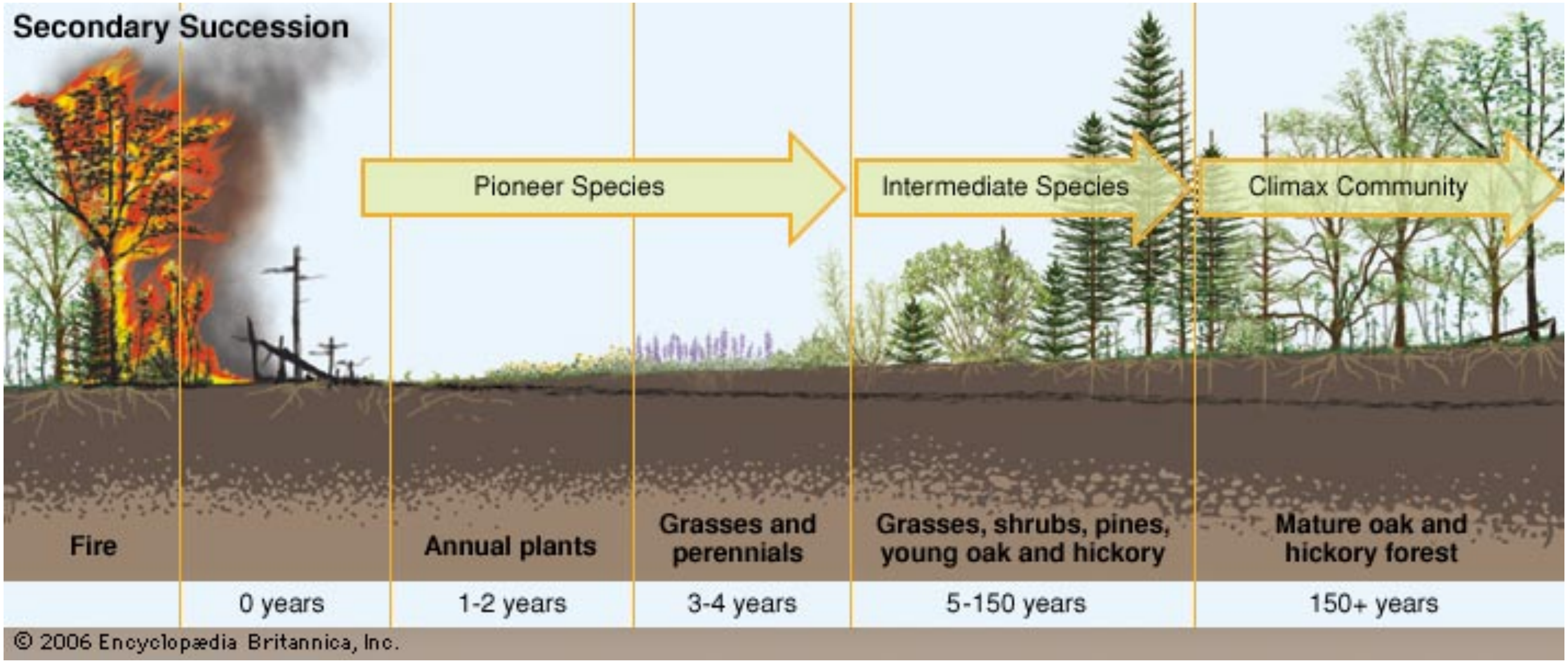
Source: Cystic Fibrosis Foundation Patient Registry, Annual Data Report



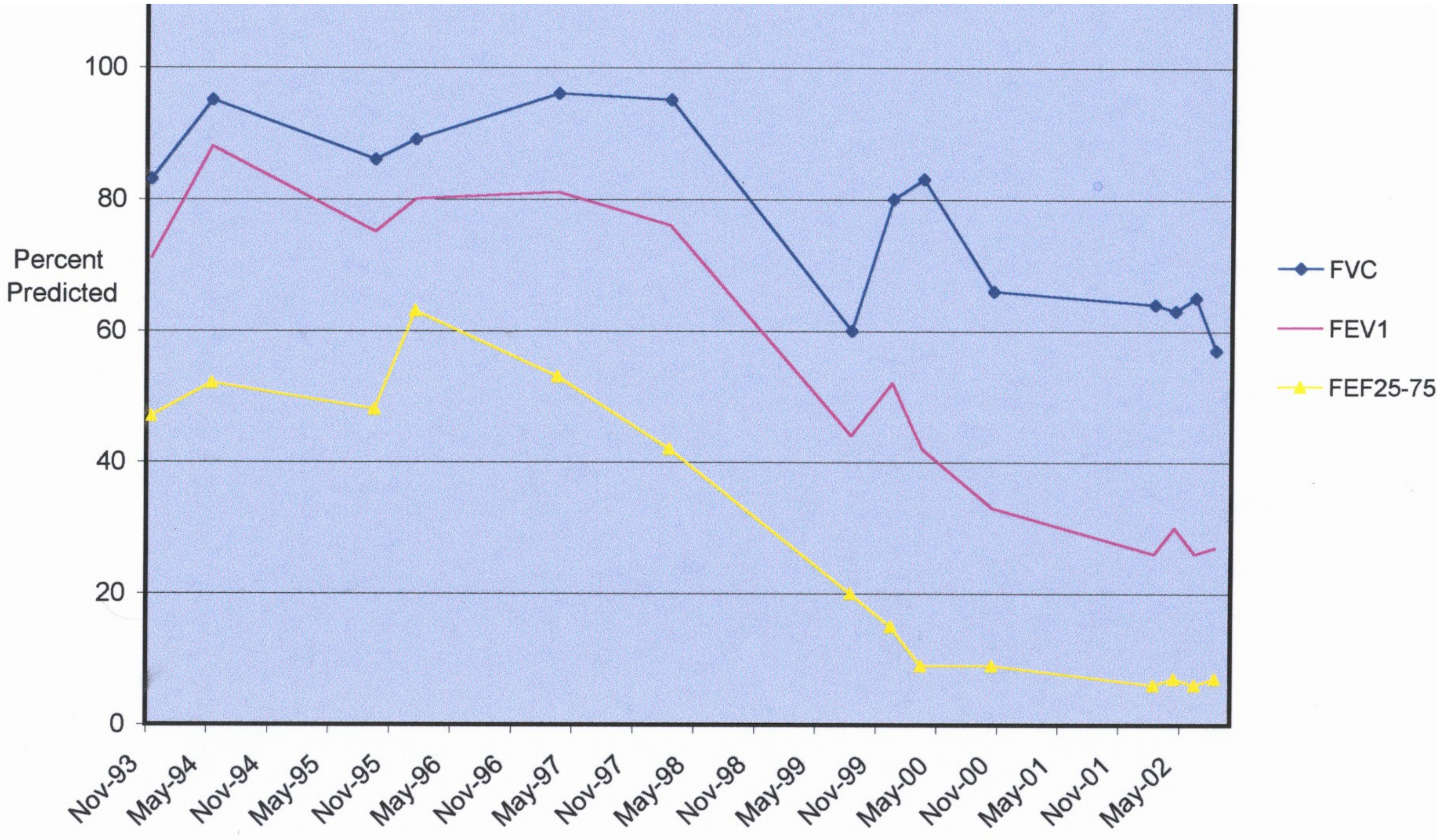
From Smithsonian Environmental Research Center http://www.serc.si.edu/labs/forest_ecology/succession.aspx



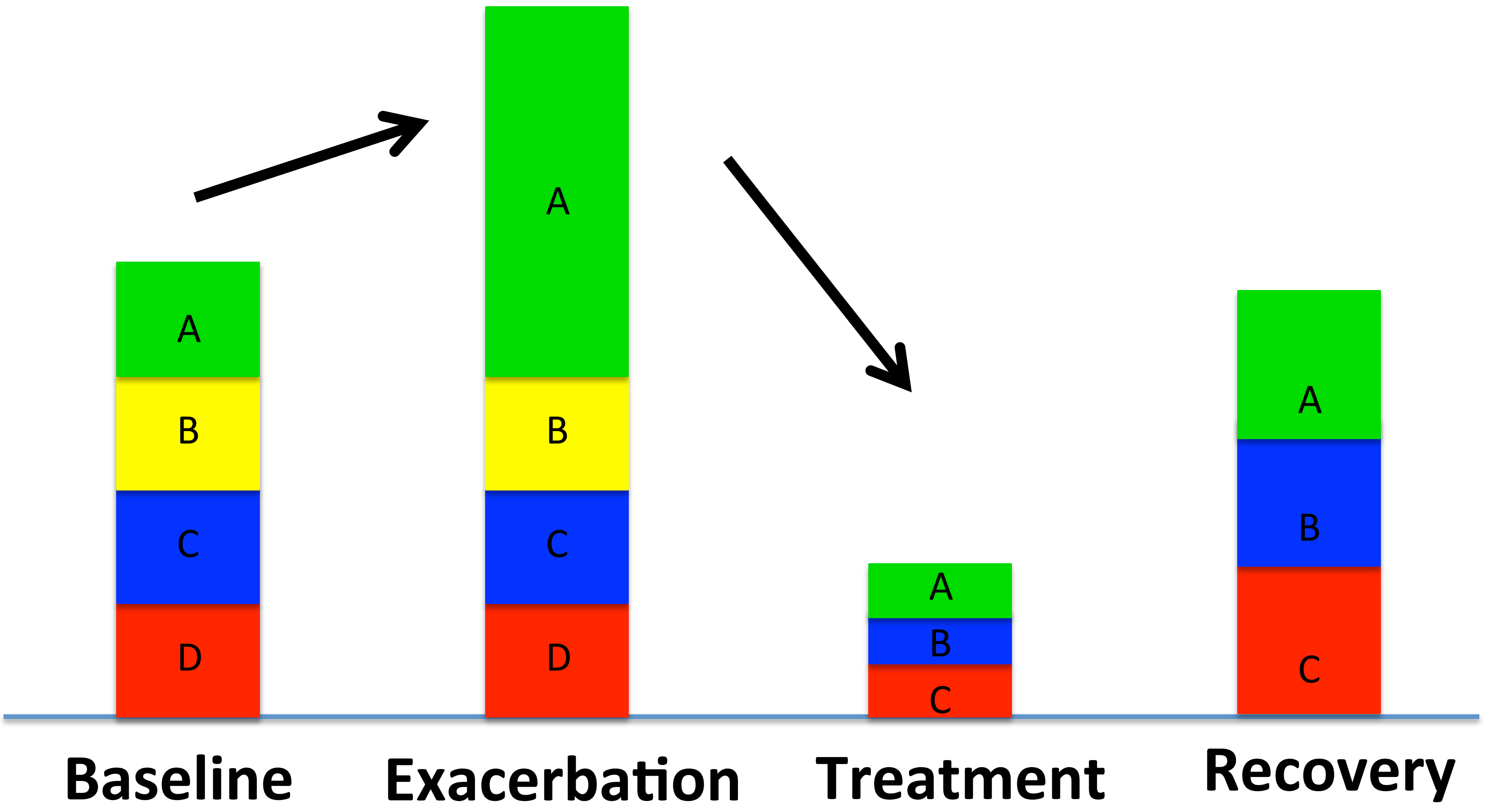
http://www.nature.com/scitable/knowledge/library/succession-a-closer-look-13256638


Secondary Succession
Pioneer Species
Intermediate Species
Climax Community
Fire
Annual plants
Grasses and perennials
Grasses, shrubs, pines, young oak and hickory
Mature oak and hickory forest
0 years
1-2 years
3-4 years
5-150 years
150+ years
© 2006 Encyclopædia Britannica, Inc.

CF Patient Born 1980

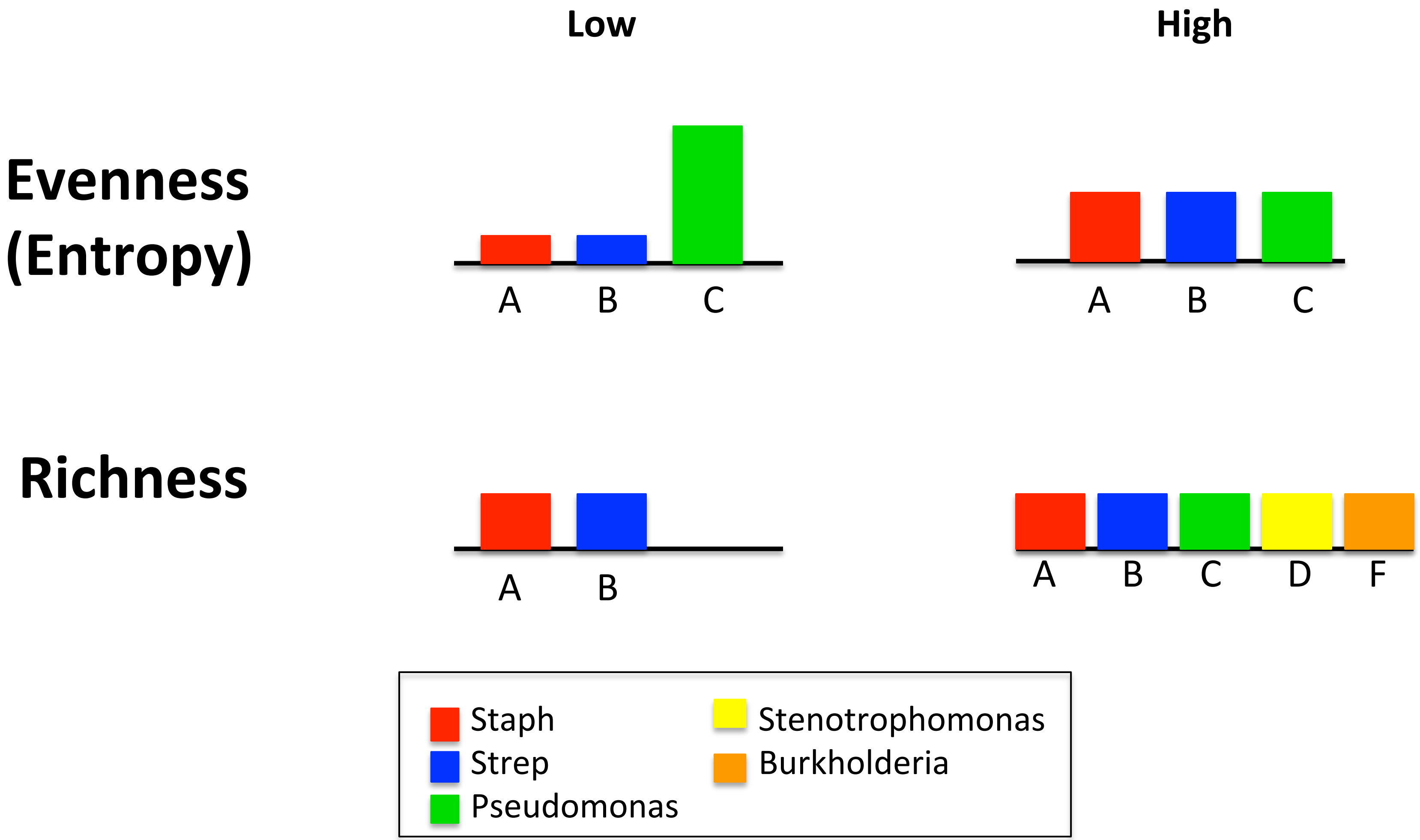


Pulmonary Exacerbation Paradigm
one bad bug ruins the airway



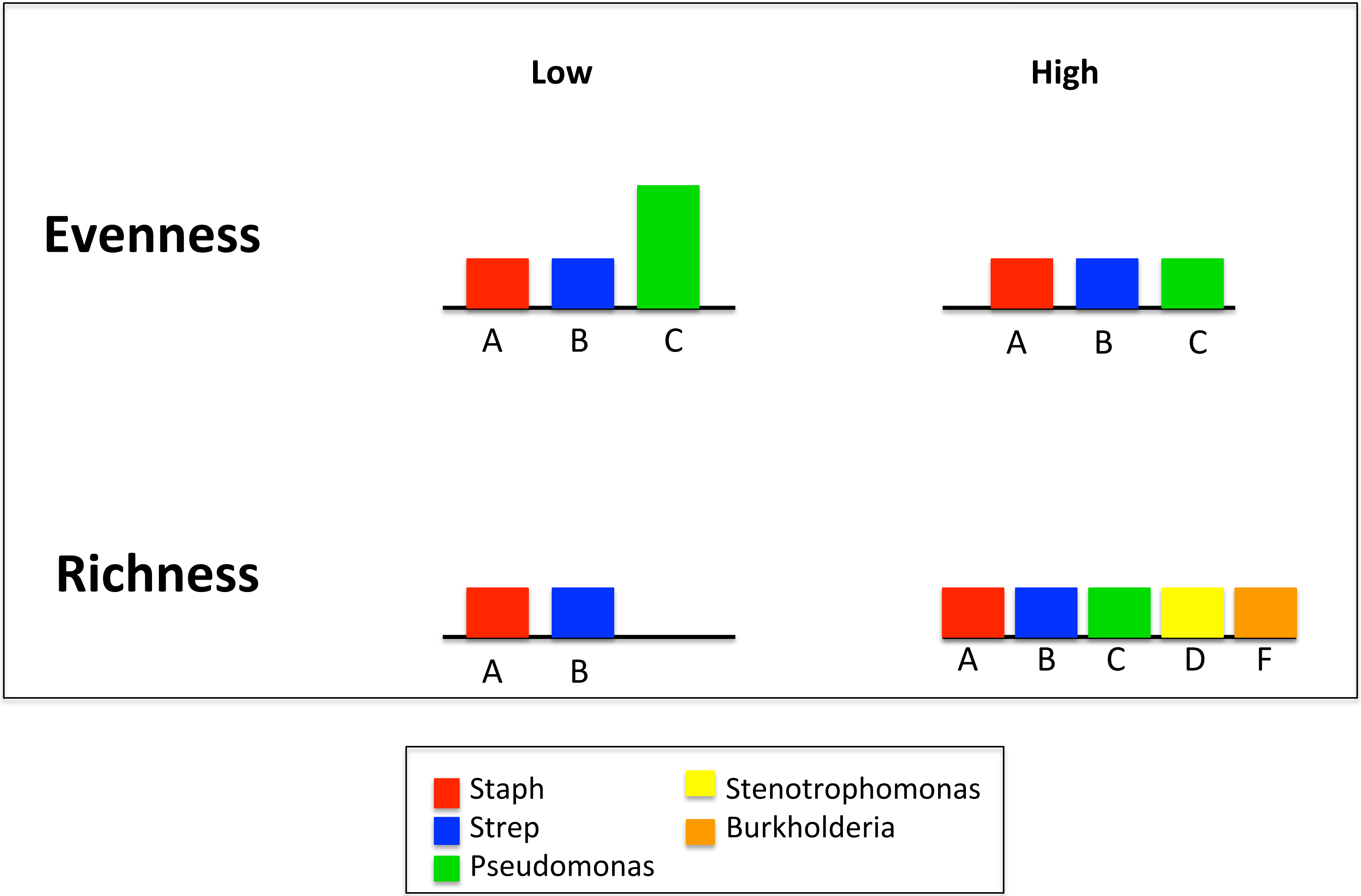
α-Diversity

Intra-ecosystem diversity



α-Diversity

↗ **SHANNON DIVERSITY INDEX**



Low **High**

Evenness

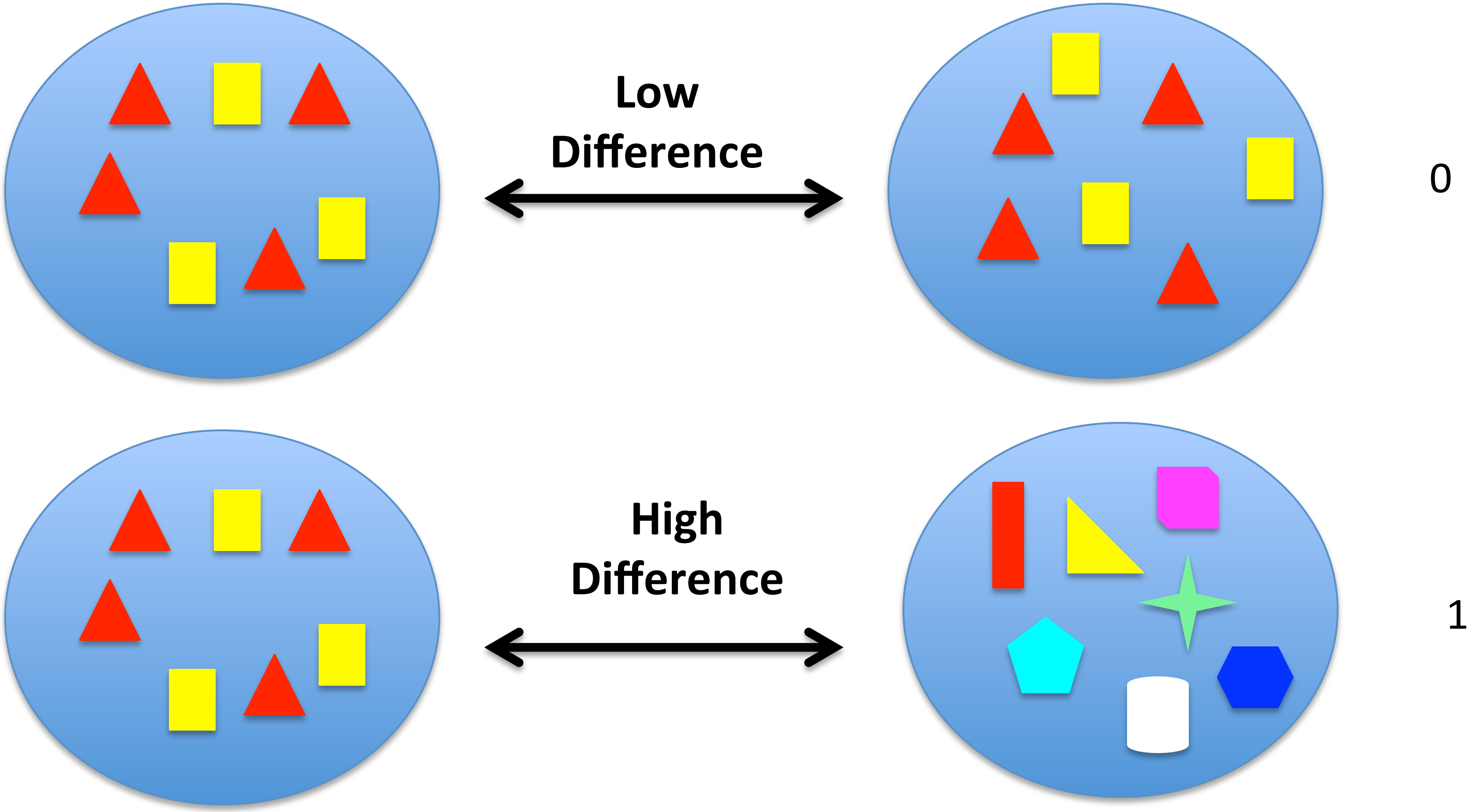
Richness

🟥 Staph
🟦 Strep
🟩 Pseudomonas
🟨 Stenotrophomonas
🟧 Burkholderia

β-Diversity →

- Bray-Curtis
- UNIFRAC

- Inter-ecosystem diversity



Low Difference

0

High Difference

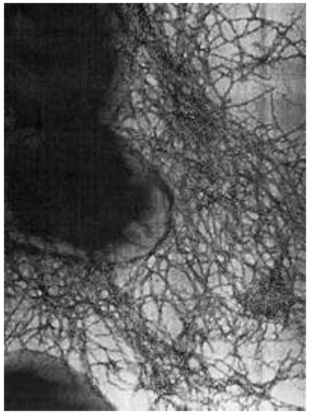
1

Microbial Ecology of the CF Lung

Rationale: Some reasonably large percentage of cystic fibrosis exacerbations cannot be clearly attributed to a single organism (if any!)

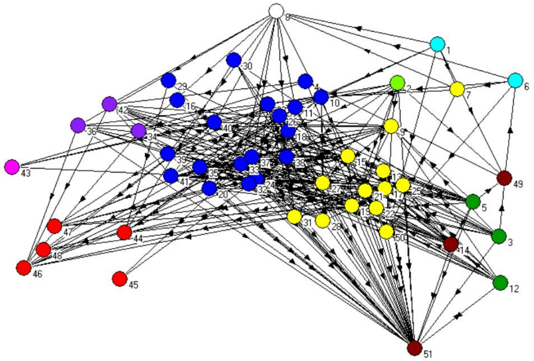
1. Traditional culture might obscure the “true” ecology.
2. Culture-independent studies have been mostly cross-sectional.
3. Few studies in children

Microbial Ecology of the CF Lung



Hypothesis 1: “Unknown” organisms are associated with exacerbations in CF.

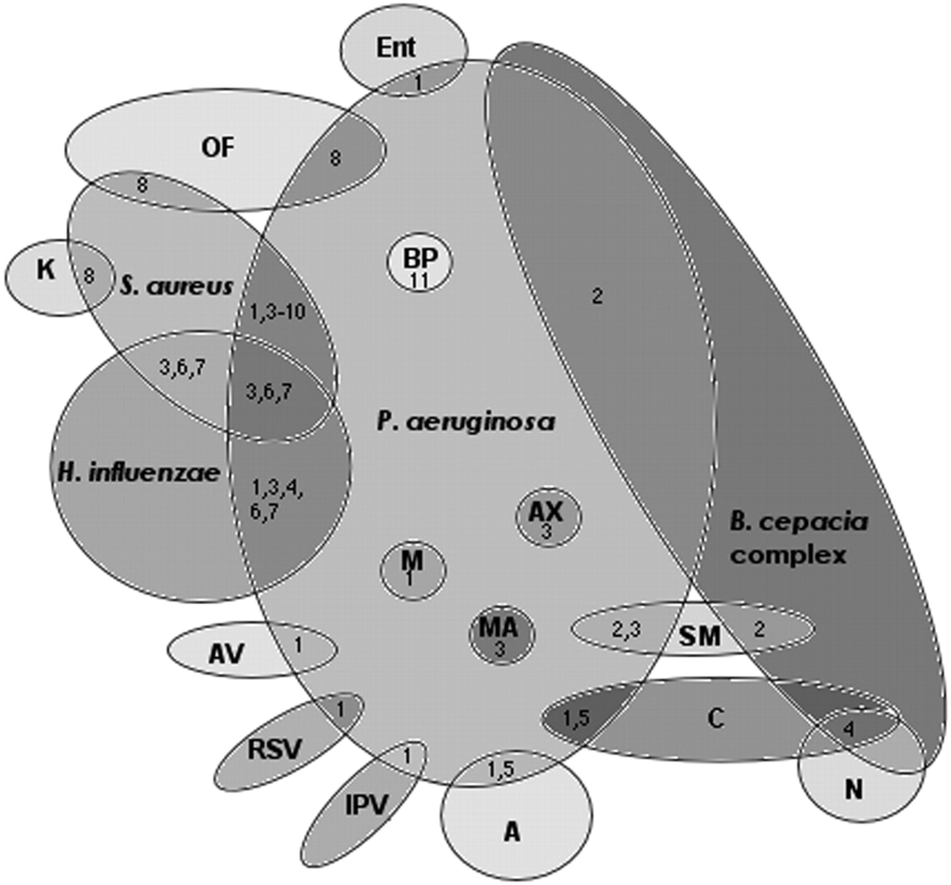
75% (140-186) of organisms are found in only one sample.



Hypothesis 2: **Ecological patterns** (rather than individual pathogens) are associated with exacerbations in CF.(evenness, richness, abundance)

Decreased diversity in exacerbation (cross sectional)

A, *Aspergillus spp.;*
***AV**, adenovirus;*
***AX**, A. xylosoxidans;*
***BP**, bacteriophage;*
***C**, Candida spp.;*
***Ent**, enterobacteria;*
***IPV**, influenza and/or parainfluenza virus;*
***K**, Klebsiella spp.;*
***M**, mycoplasma;*
***MA**, Mycobacterium abscessus;*
***N**, Neisseria spp.;*
***OF**, oropharyngeal flora;*
***RSV**, respiratory syncytial virus;*
***SM**, S. maltophilia.*



Venn diagram showing reported coinfections of the CF airways. (NB: coinfection does not necessarily imply direct interaction between species. *Numbers refer to references: 1, Petersen et al. (1981); 2, Lambiase et al.(2006); 3, Wahab et al. (2004); 4, Moore et al. (2005); 5, Burns et al. (1998); 6, Hoiby (1974); 7, Lording et al. (2006); 8, Santana et al. (2003); 9, Alvarez et al. (2004); 10, Anzaudo et al. (2005); 11, Ojeniyi et al. (1991) From:* *"***Microbial ecology of the cystic fibrosis lung" Freya Harrison 2007 *Microbiology*.**

Microbial Ecology of the CF Lung

STUDY PLAN

- Longitudinal, prospective
- Target of **200** subjects
- **Ages 0-23**
- clinical variables (eg., FEV1)
- 16S-based approach
  - Sanger (8F and 1391R) n=21
  - 454 (515F and 806R; V4 hypervariable) n=84
  - MiSeq (515F and 806R; V4 hypervariable) n=532 (out of 600)
- Samples collected in **regular care (3-6 month intervals)**
- **Case report form** based on prescribing antibiotics (to catch exacerbations)
- **Sputum**, **OP Swab**, and B**AL**

Patient & Sample Demographics

	Illumina (MiSeq)	454 sequencing
Number of Subjects	154	45
Males	80 (52%)	19 (42%)
Females	74 (48%)	26 (58%)
Mean Age (years)	9.4 (+/-6.7)	13.2 (=/-6.7)
Genotype		
-Hom ΔF508	37 (24%)	9 (20%)
-Het ΔF508	67 (44%)	24 (54%)
-Other	39 (25%)	5 (11%)
-Unknown	11 (7%)	7 (6%)
Sample Number		
Total	618	82
-Sputum	109 (18%)	25 (30%)
-Swab	502 (81%)	54 (67%)
-BAL	7 (1%)	3 (4%)

“Species” 4973 unique operational taxonomic units (OTUs)

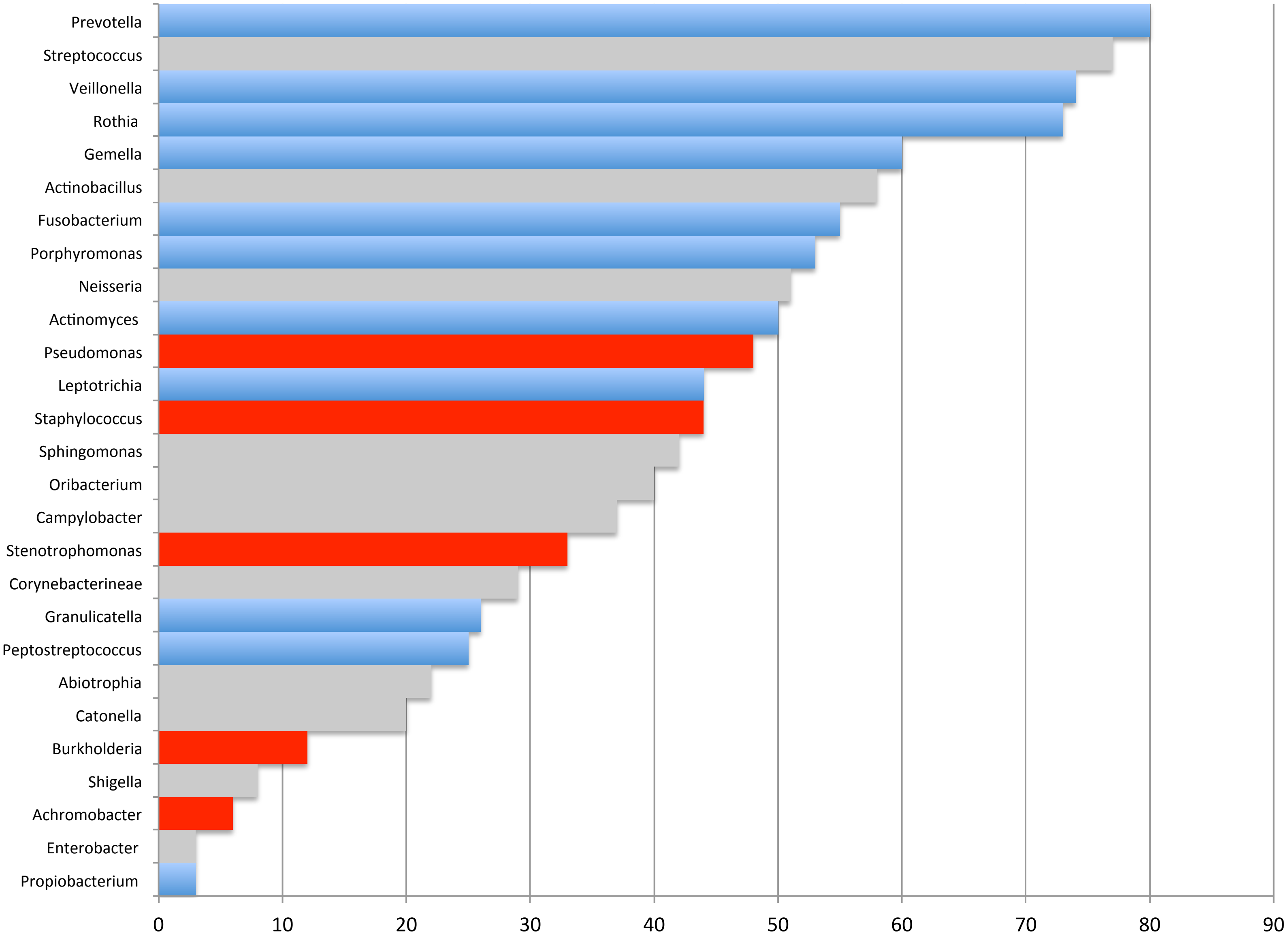
Median **72** (range of 11-137) **per sample**

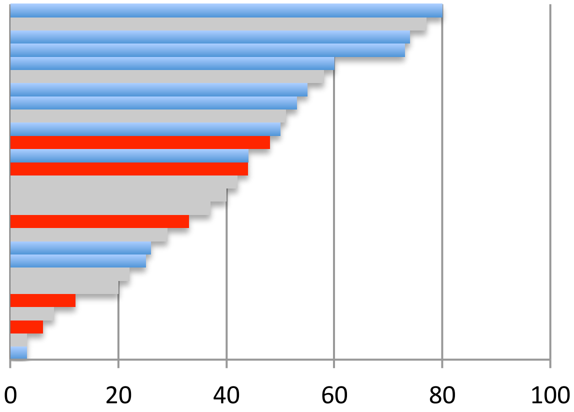
Median **97** (range of 49-175) **per patient**.

Phyla: **23** unique

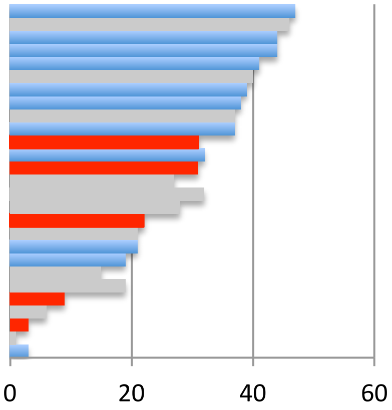
THE AIRWAY IS NOT THE GUT

Number of Samples

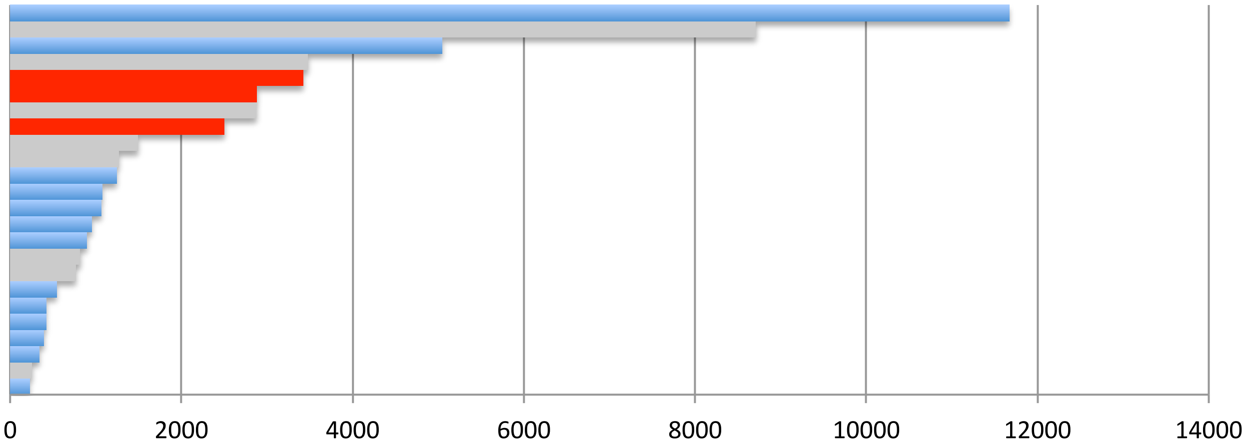




Number of Samples

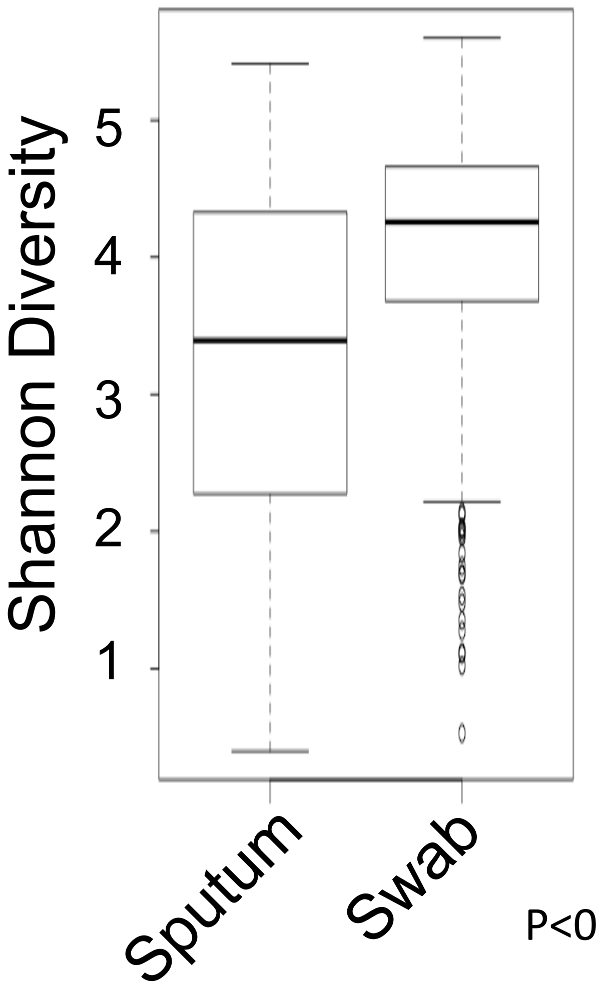


Number of Patients

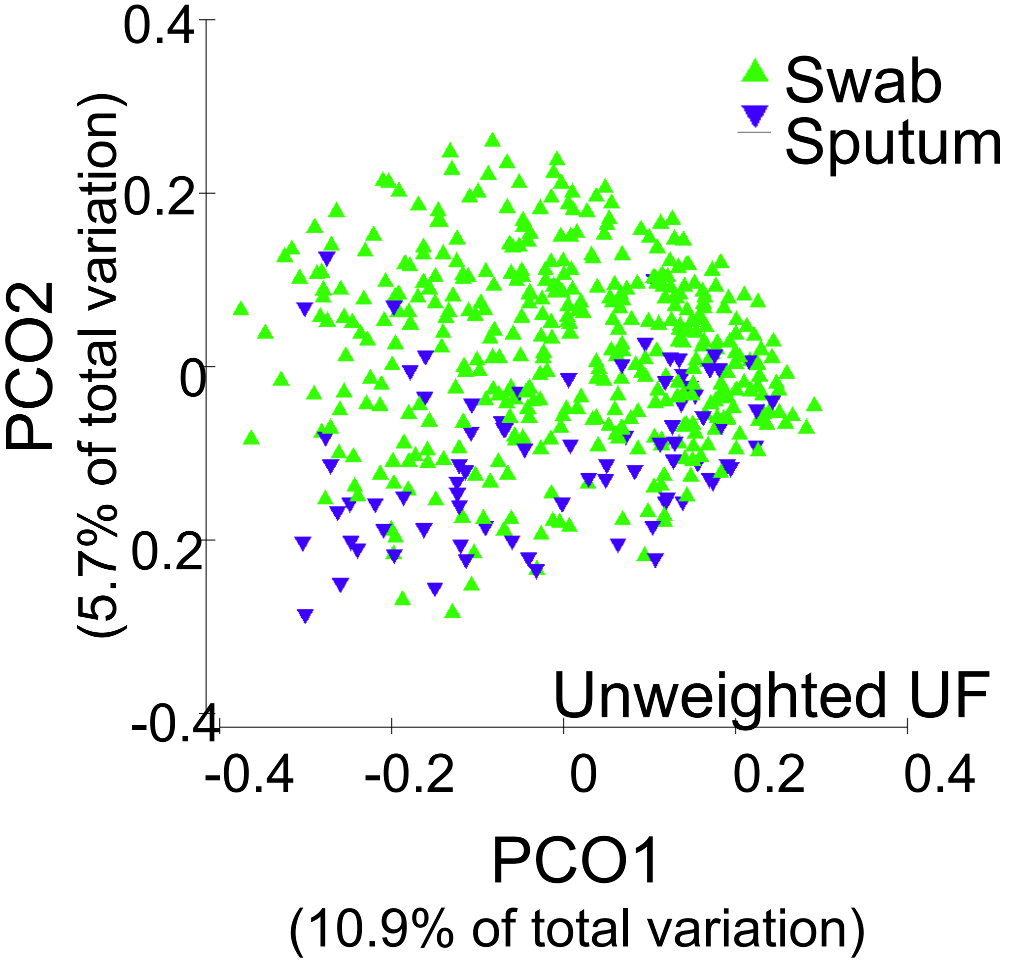


Number of Reads

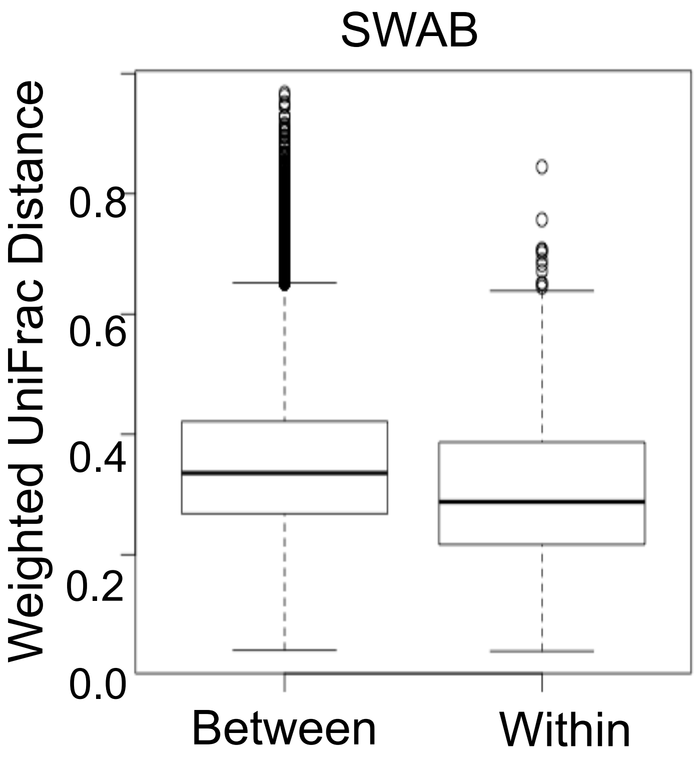
Does body site/sample type matter?



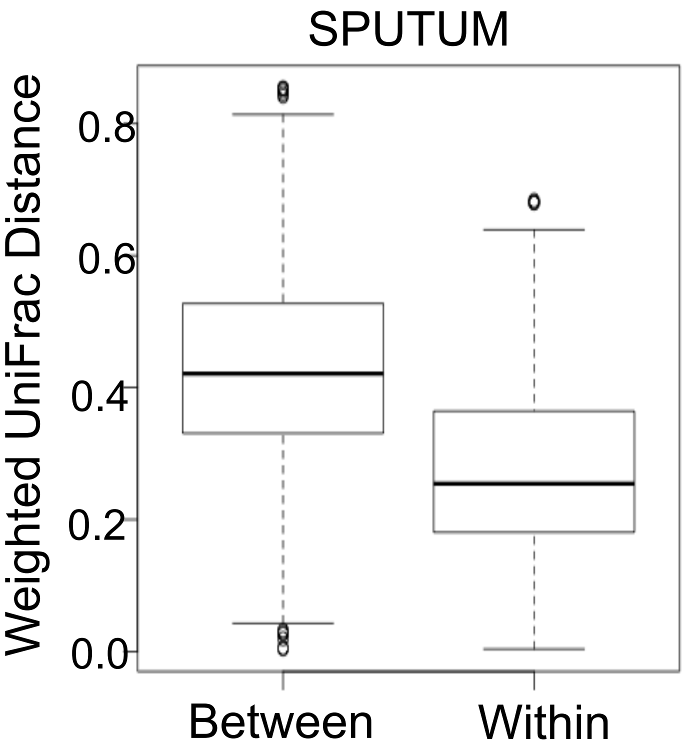
P<0.001



Interpatient diversity > Intrapatient diversity

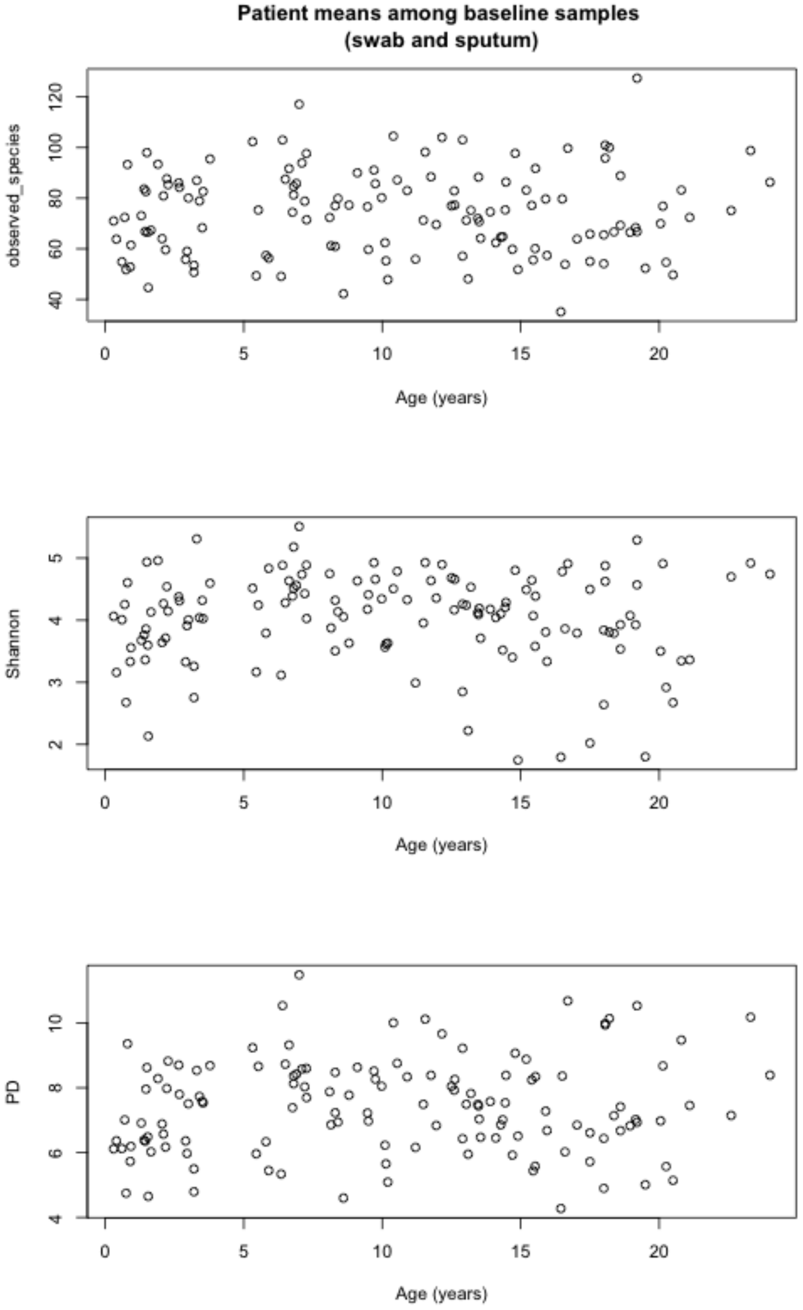


P=0.001



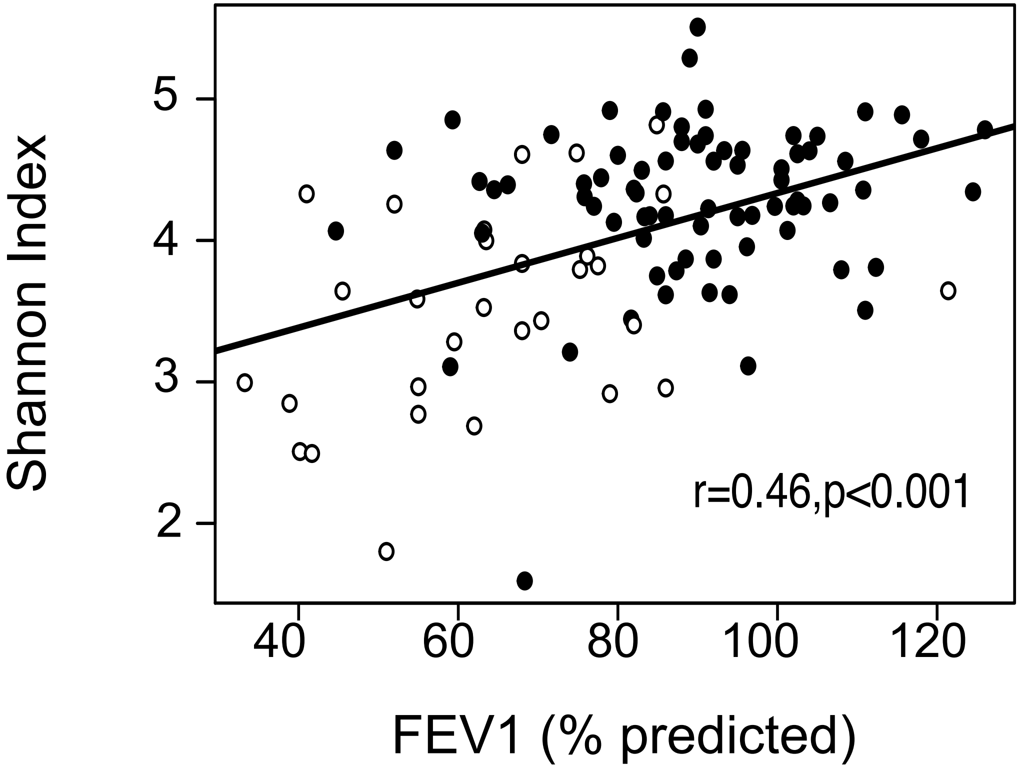
P=0.001

Diversity is not higher or lower in different age groups (adults decrease over time)



Patient means among baseline samples (swab and sputum)

Decreased lung function is associated with decreased diversity



Clinical Categories (BETR)

- BETR Classification
  - Baseline
  - Exacerbation
  - Treatment
  - Recovery (1 month)

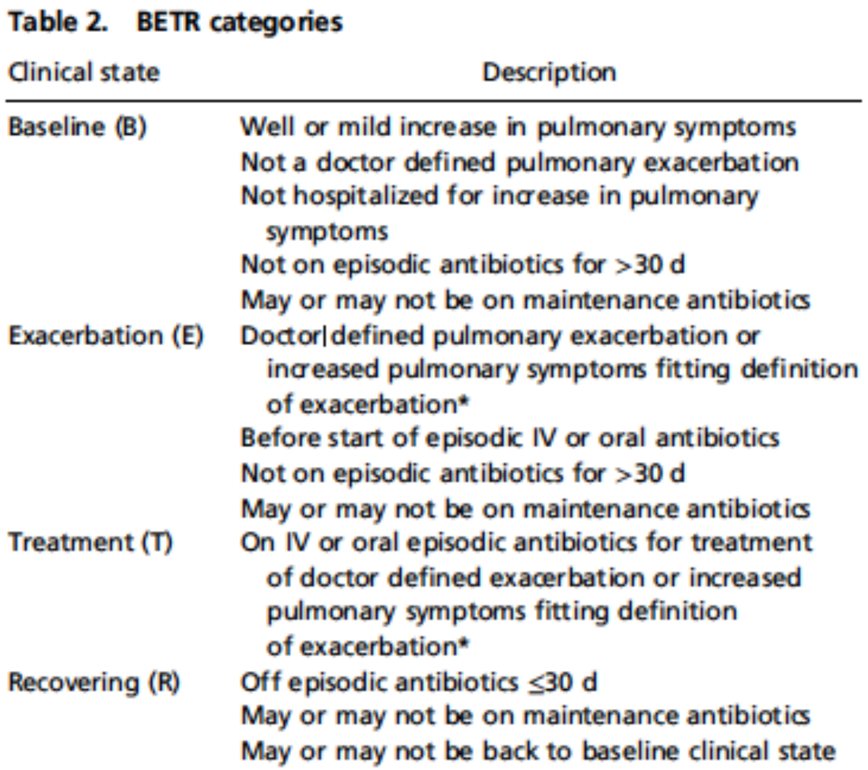
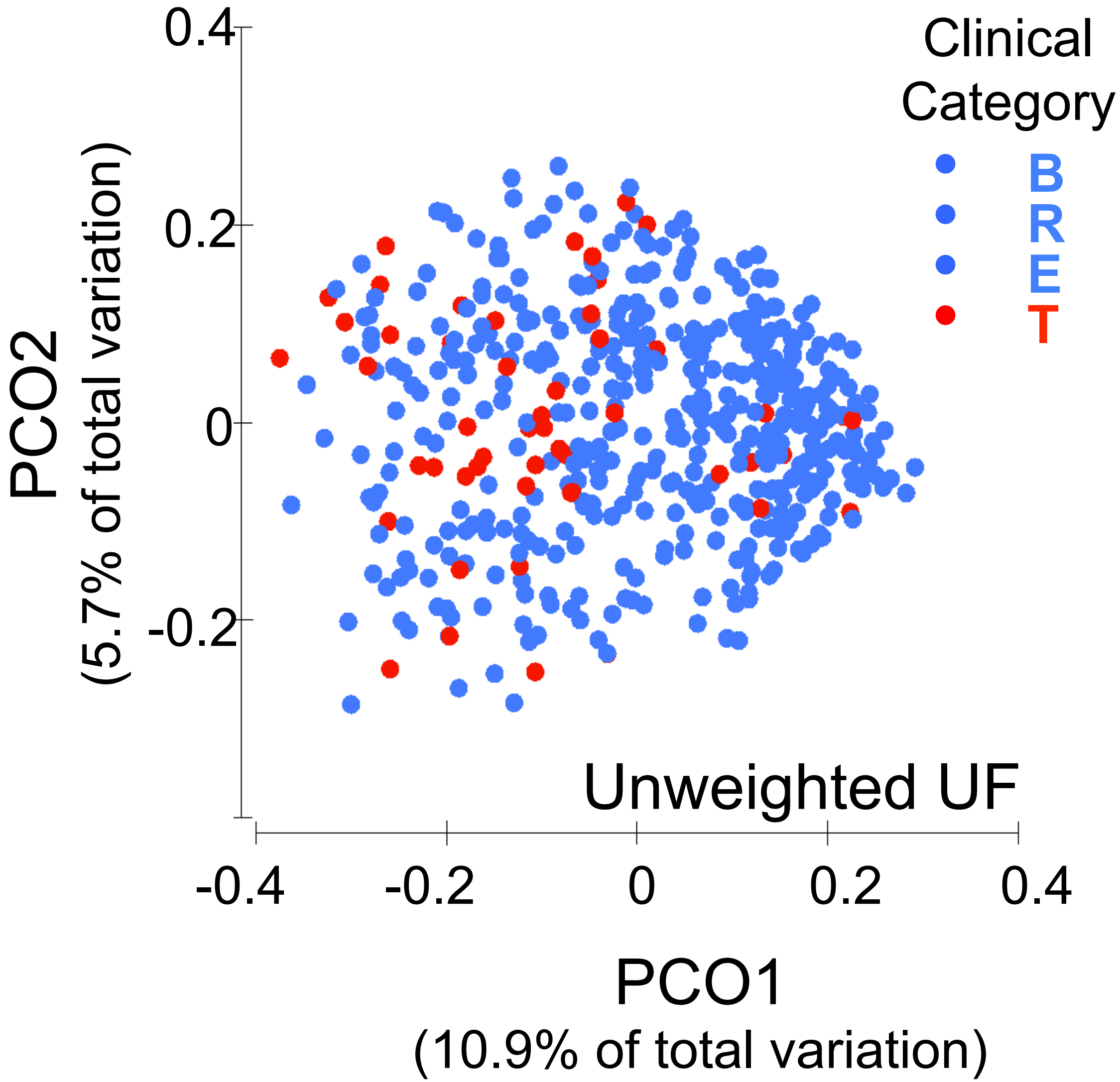


Table 2. BETR categories

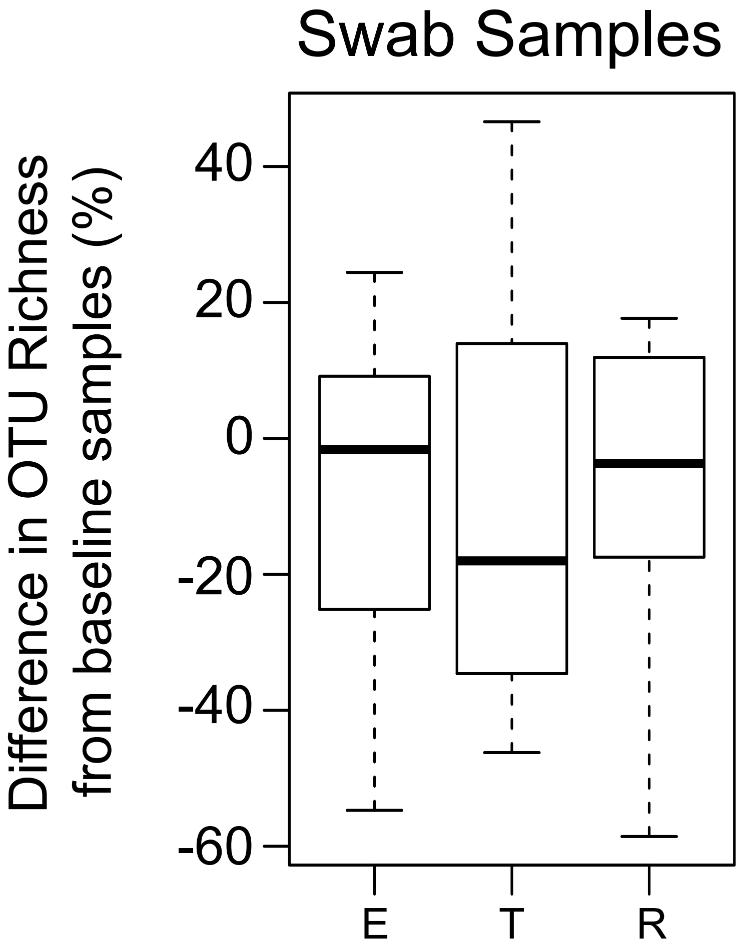
Clinical state	Description
Baseline (B)	Well or mild increase in pulmonary symptoms Not a doctor defined pulmonary exacerbation Not hospitalized for increase in pulmonary symptoms Not on episodic antibiotics for >30 d May or may not be on maintenance antibiotics
Exacerbation (E)	Doctor-defined pulmonary exacerbation or increased pulmonary symptoms fitting definition of exacerbation* Before start of episodic IV or oral antibiotics Not on episodic antibiotics for >30 d May or may not be on maintenance antibiotics
Treatment (T)	On IV or oral episodic antibiotics for treatment of doctor defined exacerbation or increased pulmonary symptoms fitting definition of exacerbation*
Recovering (R)	Off episodic antibiotics ≤30 d May or may not be on maintenance antibiotics May or may not be back to baseline clinical state

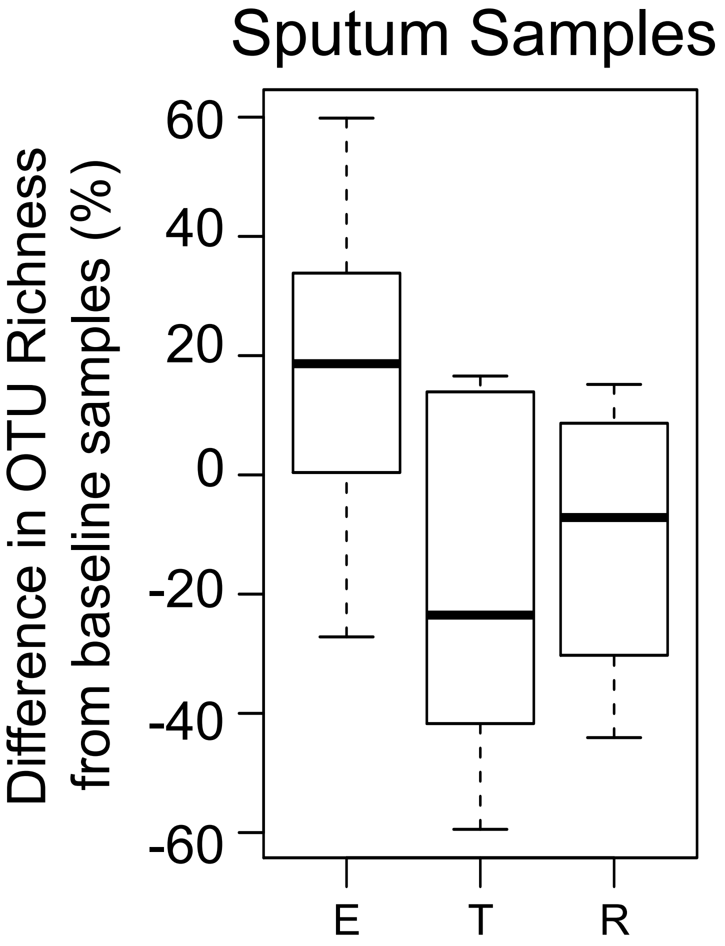
Zhao et al., 2012

Treatment samples stand out

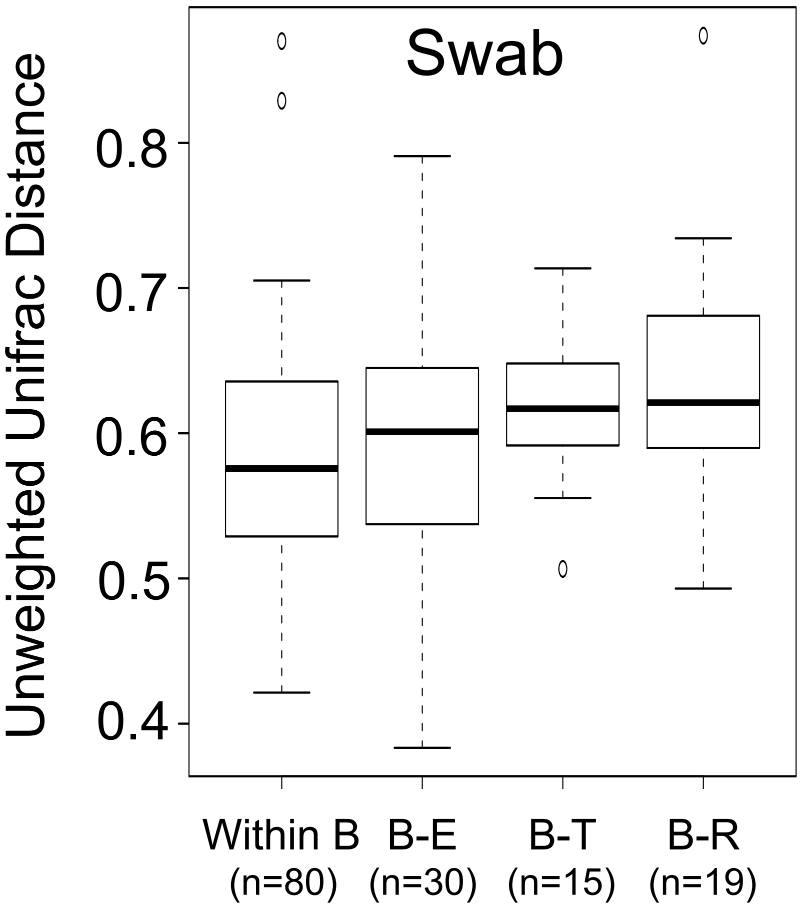


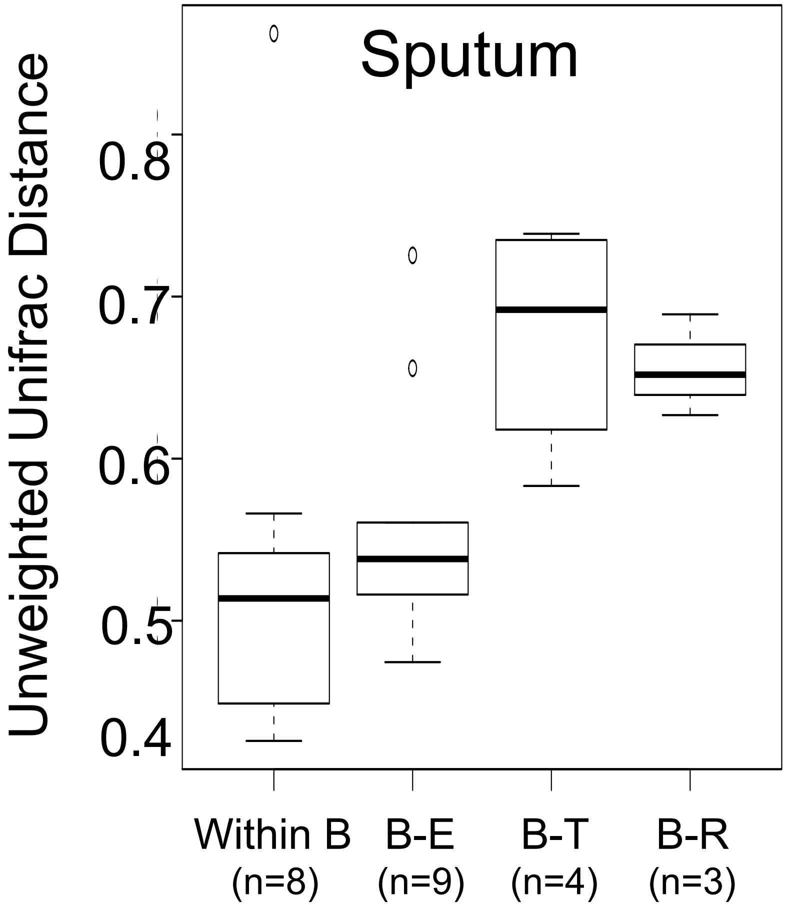
Decrease in richness (α) with treatment



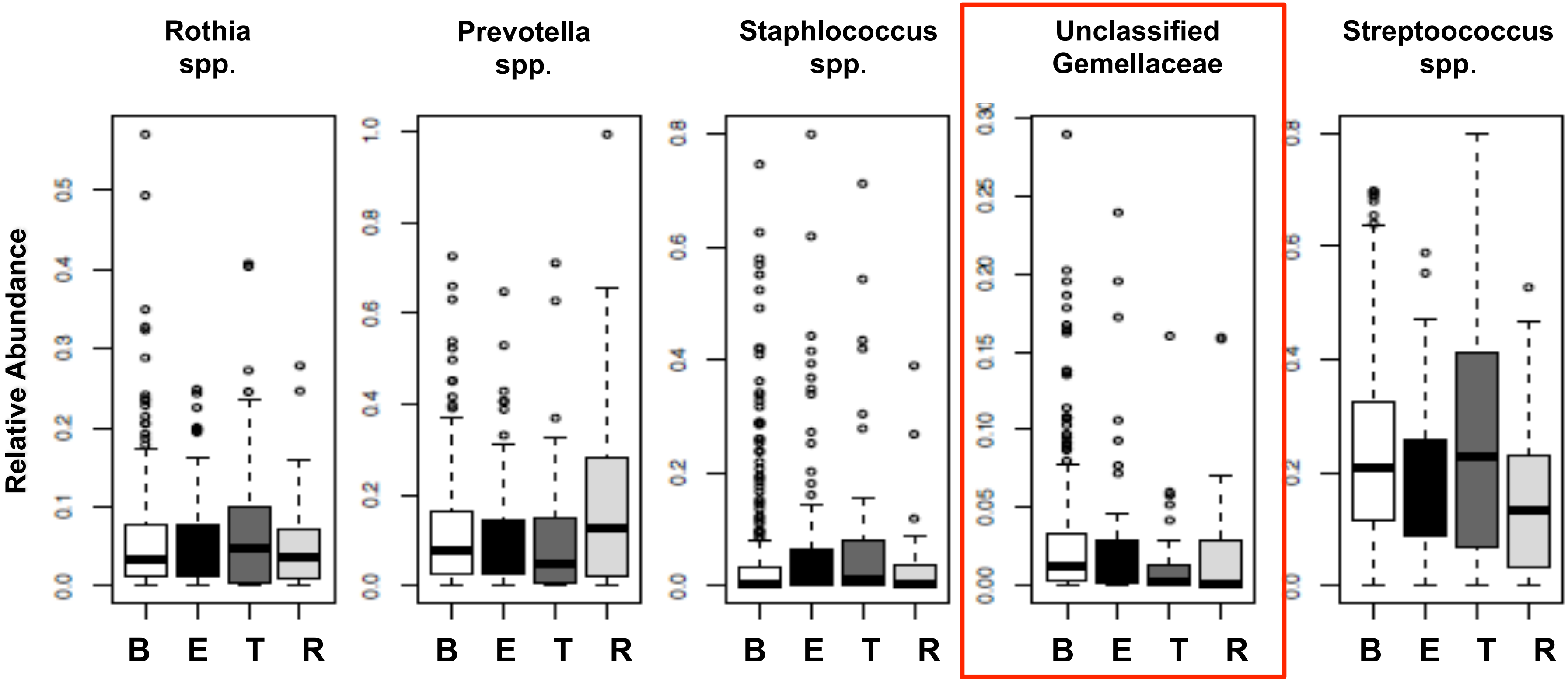


β diversity with treatment

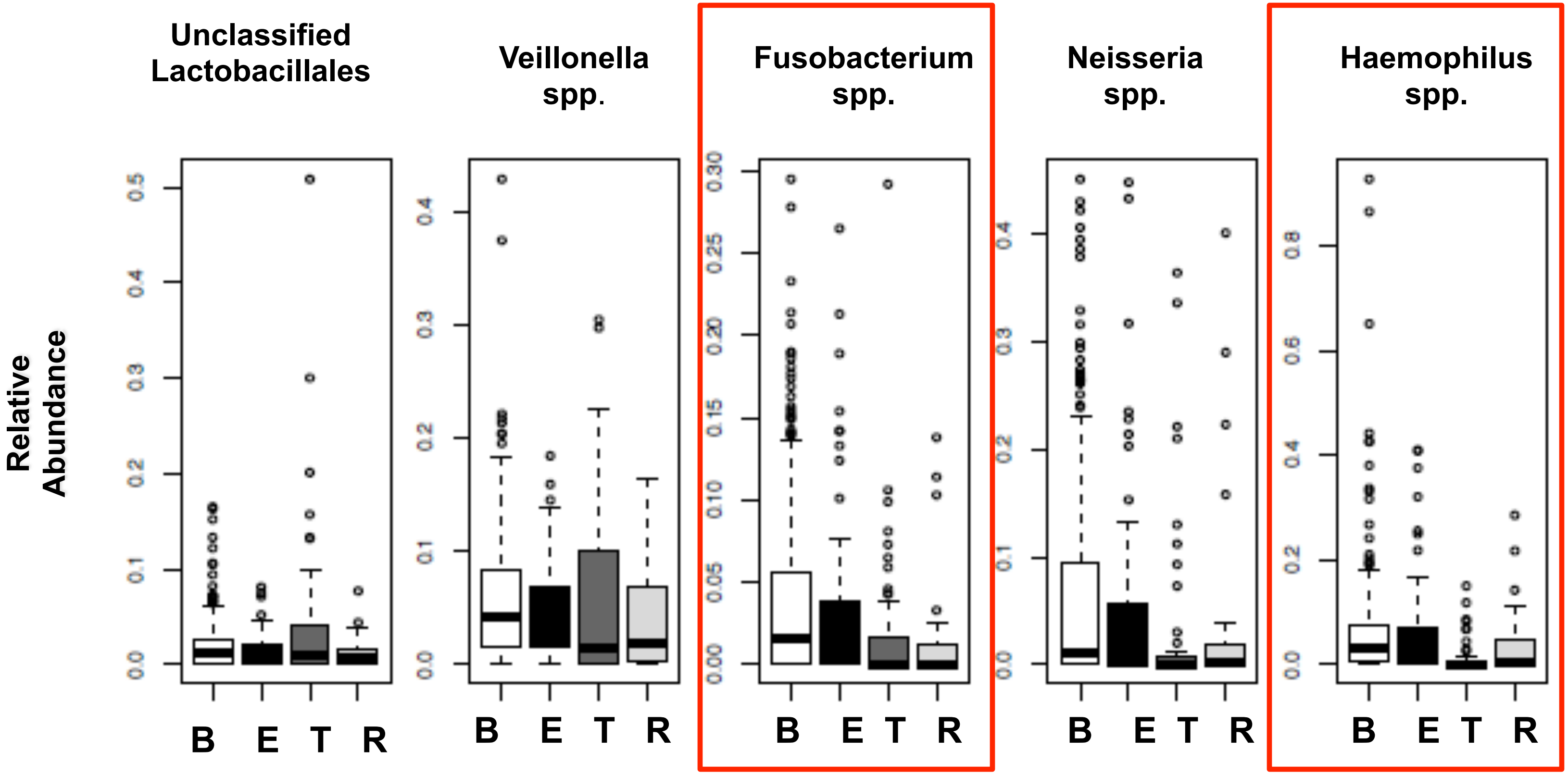




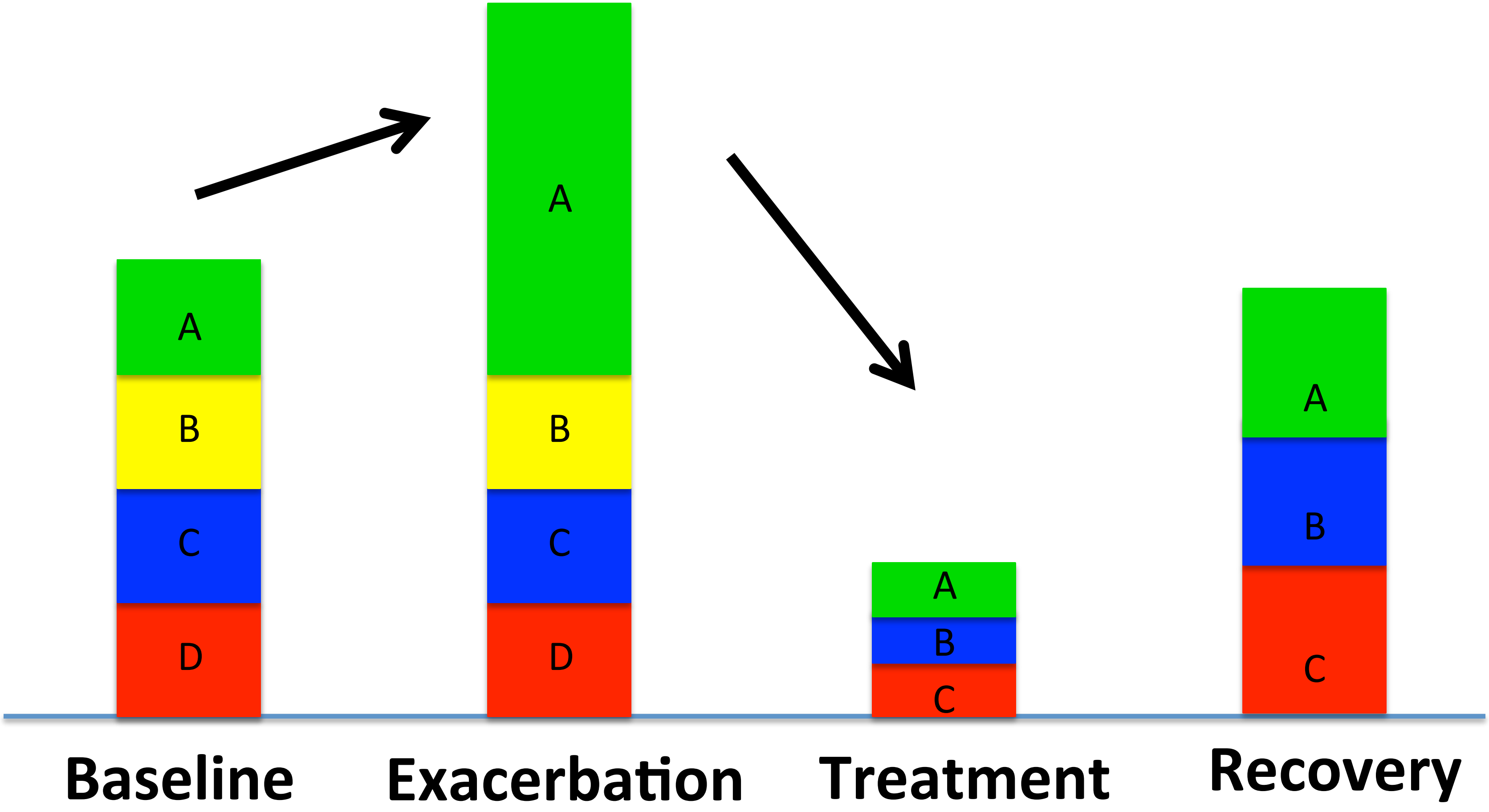
Relative Abundance of Species by Clinical Category



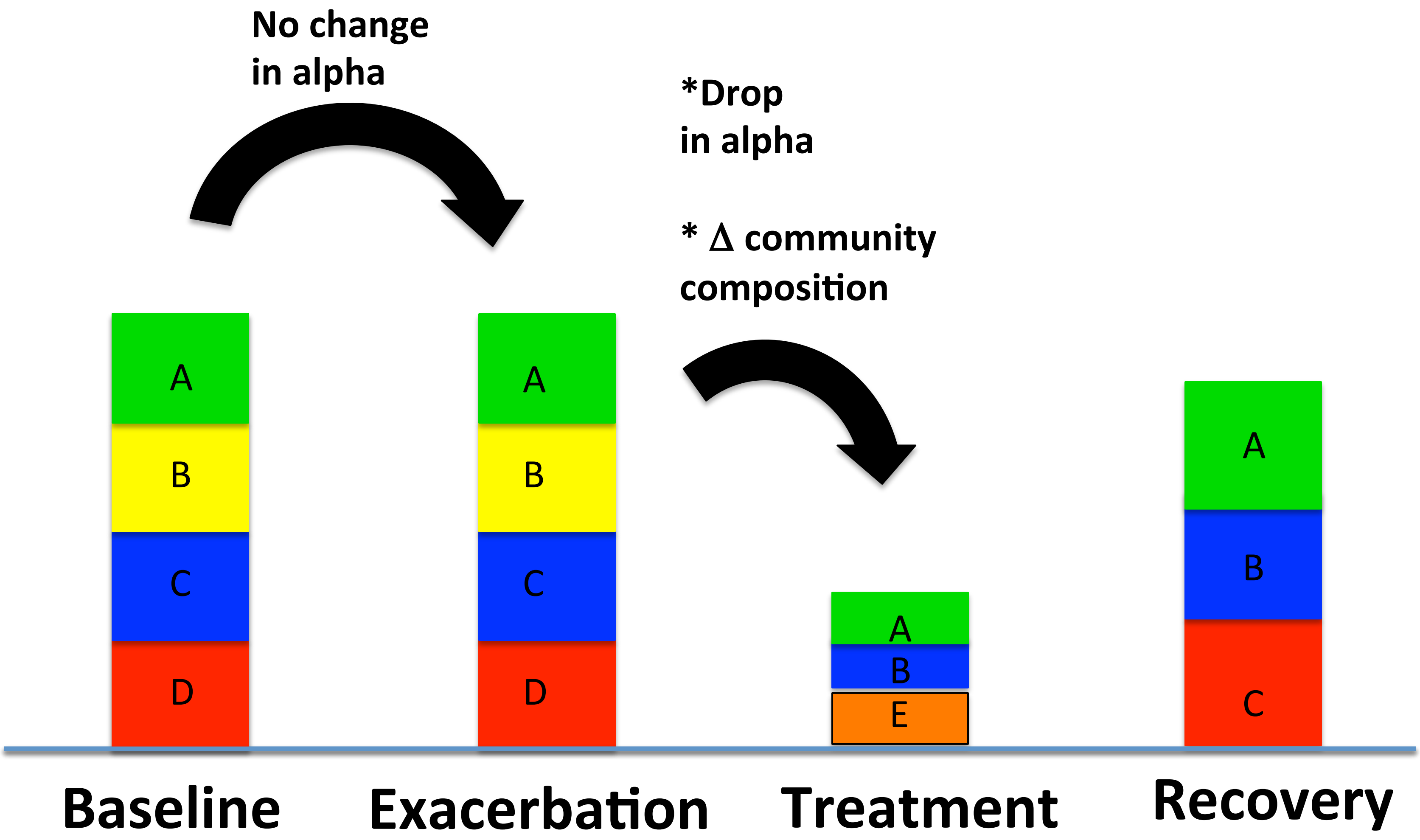
Relative Abundance of Species by Clinical Category



Pulmonary Exacerbation Paradigm
one bad bug ruins the airway



Paradigm shift in Pulmonary Exacerbation



No change in alpha

***Drop in alpha**

*** Δ community composition**

Summary

The bacterial community is associated with different health states in CF, but in a complex way.

α **Diversity--no change** during exacerbation→ NOT OVERGROWTH OF ONE BAD BUG

β **Diversity** is subtle→there is likely no single common exacerbation community but there may be a baseline community.

Changes are mostly associated with **treatment**.

Columbia

Lynne Quittell MD
Lisa Saiman MD
Alice Prince MD
CF clinical team

Planet Lab

Gina Coscia MD
Melanie Harasym PhD
J. Dayanna Orjuela
Tammer Yammany
Nicholas Caruso
Evan Cohen
Sam LaRussa
Chanelle Ryan
Hannah Smith

University of Colorado

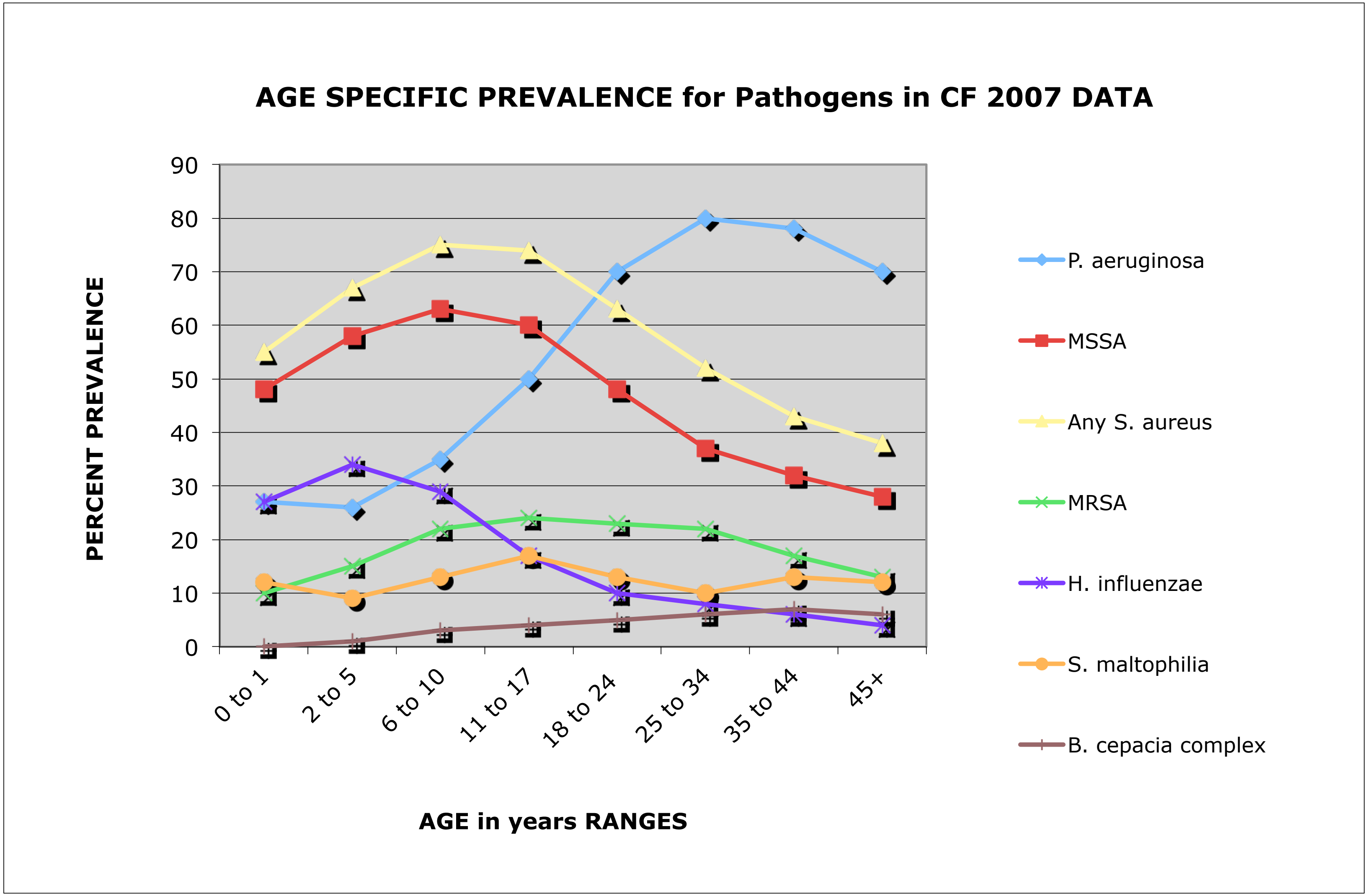
Noah Fierer PhD
Jon Leff
Toby Hammer

American Museum of Natural History/Fordham

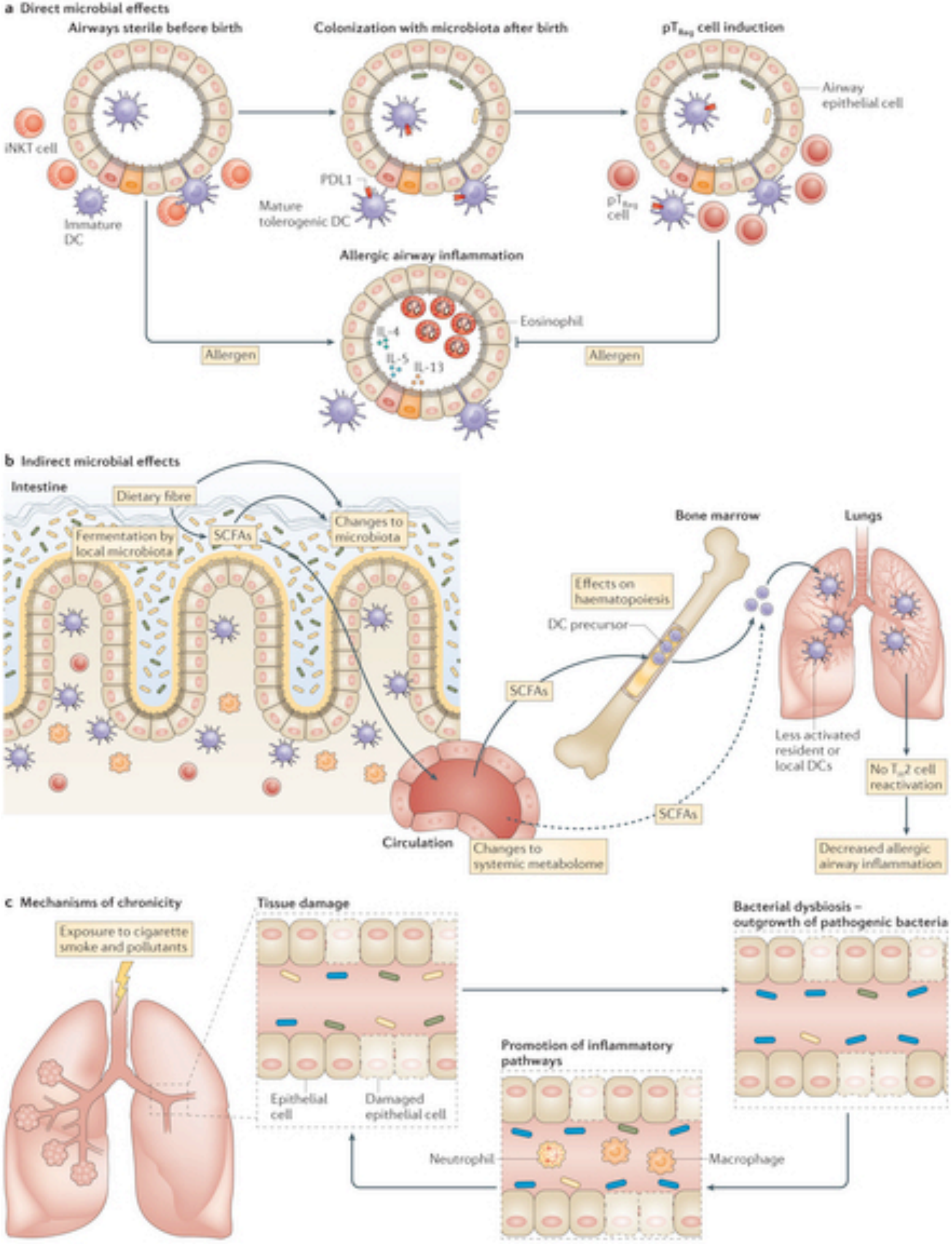
Apurva Narechania
Rob DeSalle

Funding:
K08AI101005
Doris Duke Clinical Scientist Development Award
St. Jude's Pediatric Infectious Disease Society Basic Science Award
Louis V. Gerstner Scholars Award
Thrasher Early Investigator Award

A hints that ecological patterns may play a role in disease:

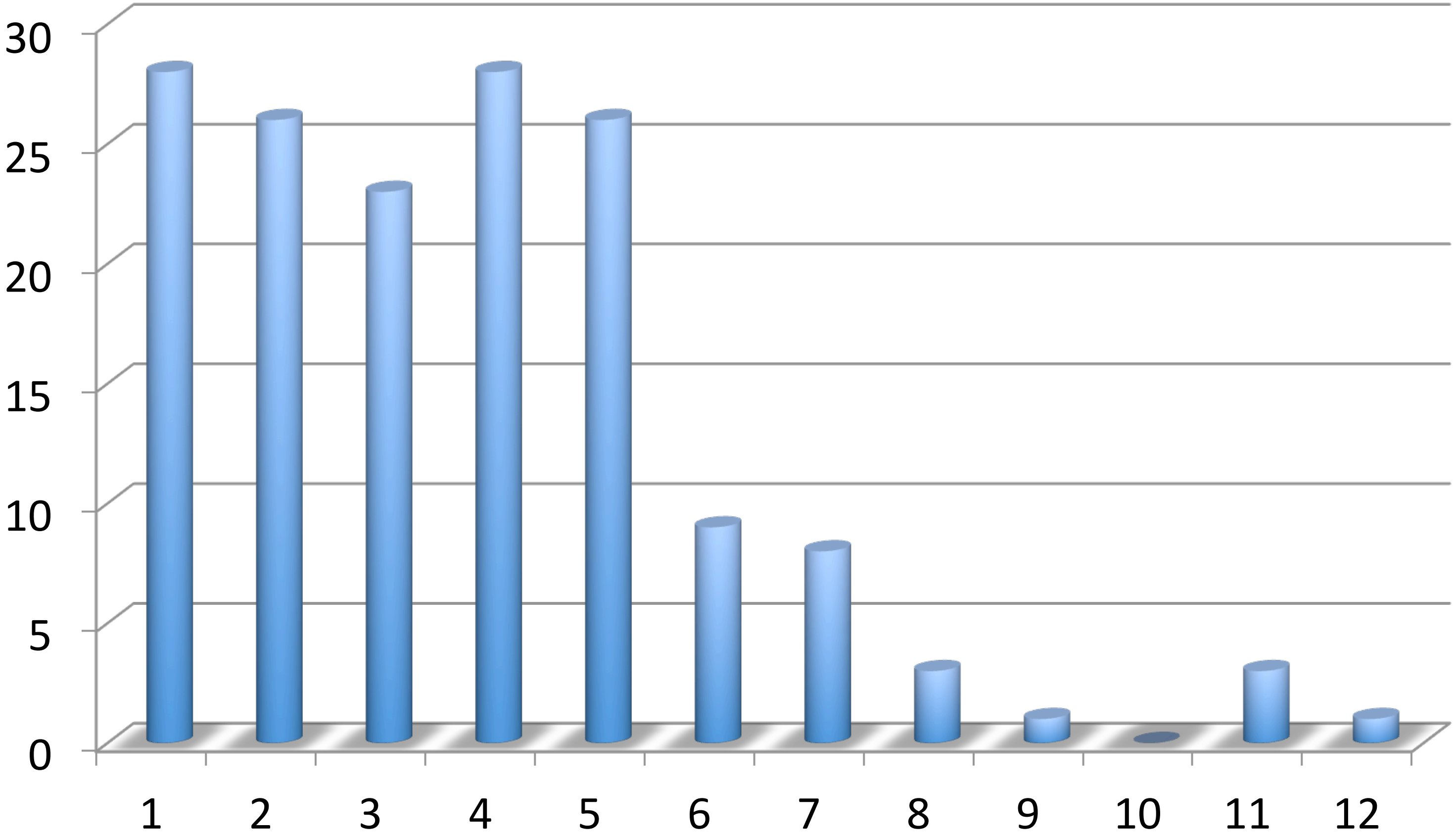


2007 CF Registry

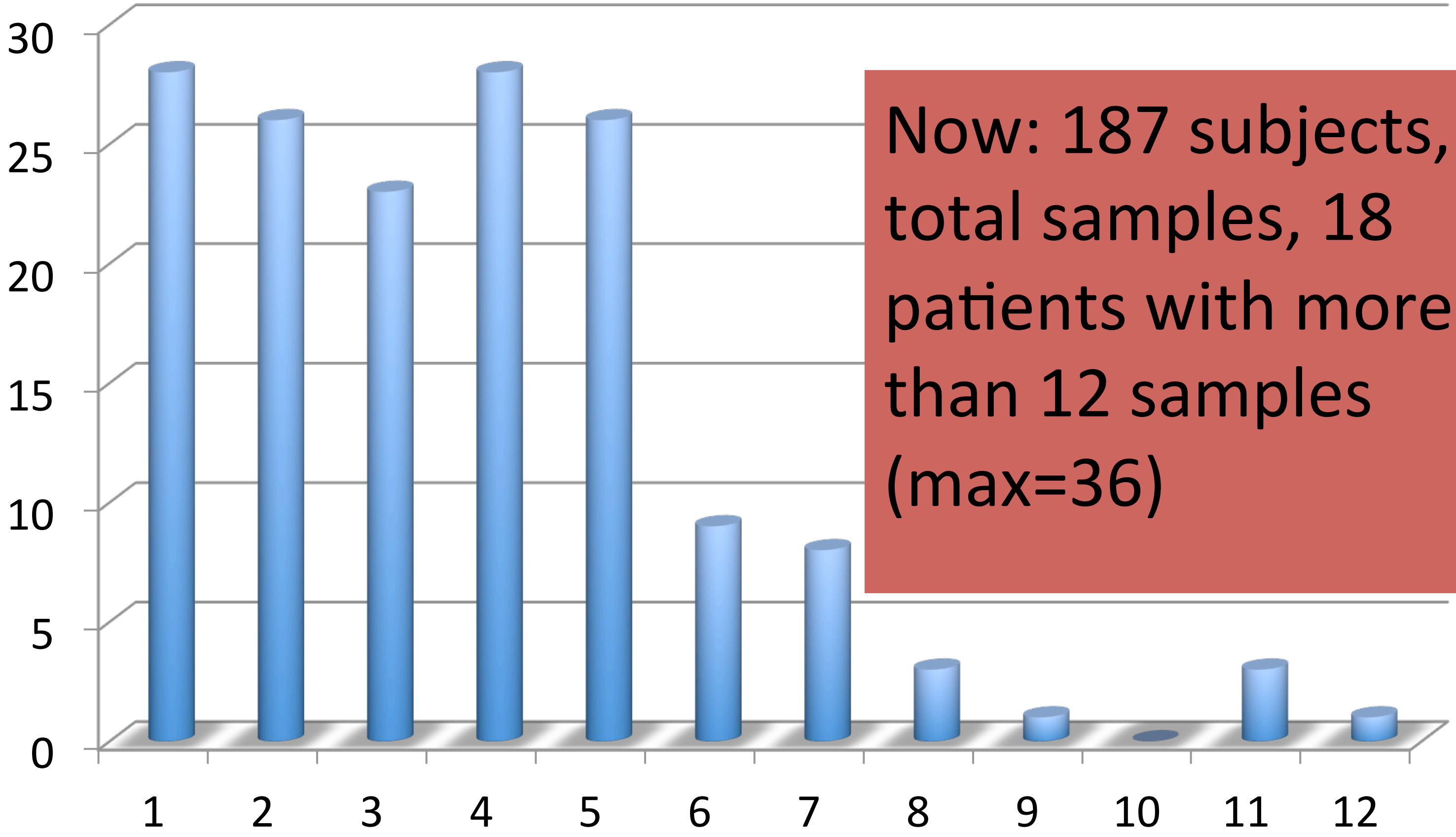


Nature Reviews | Immunology

Longitudinality of 154 subjects and 534 samples.



Longitudinality of 154 subjects and 534 samples.



Now: 187 subjects, 972 total samples, 18 patients with more than 12 samples (max=36)