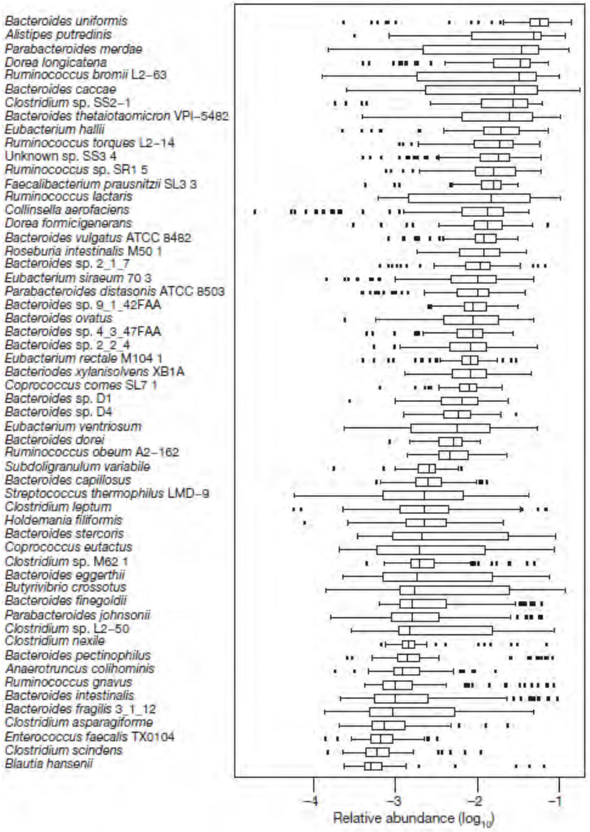
Translational Science and the Microbiome

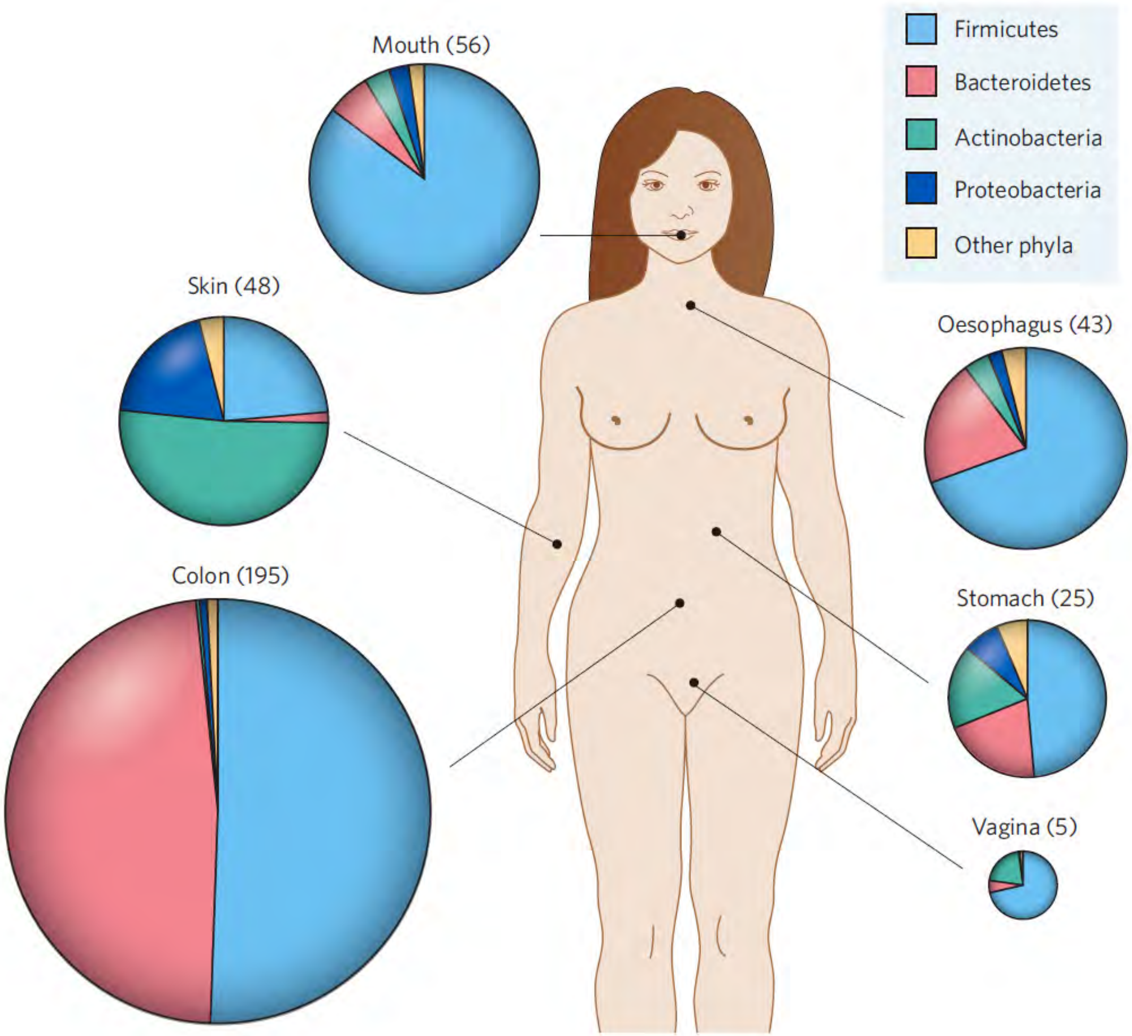
Jonathan Braun, M.D., Ph.D.
David Geffen School of Medicine
University of California, Los Angeles

Eli

May it be known that her
Royal Majesty Effie
of the noble House Braun
was safely delivered of a son,
at 2:03 ~~p.m.~~/a.m., on the 24th
day of July, in the year 2013,
in California, of the
emancipated former colonies
of Great Britain.
The child is and shall be the
Duke of Studio City
and
Protector of the Laurel Pass

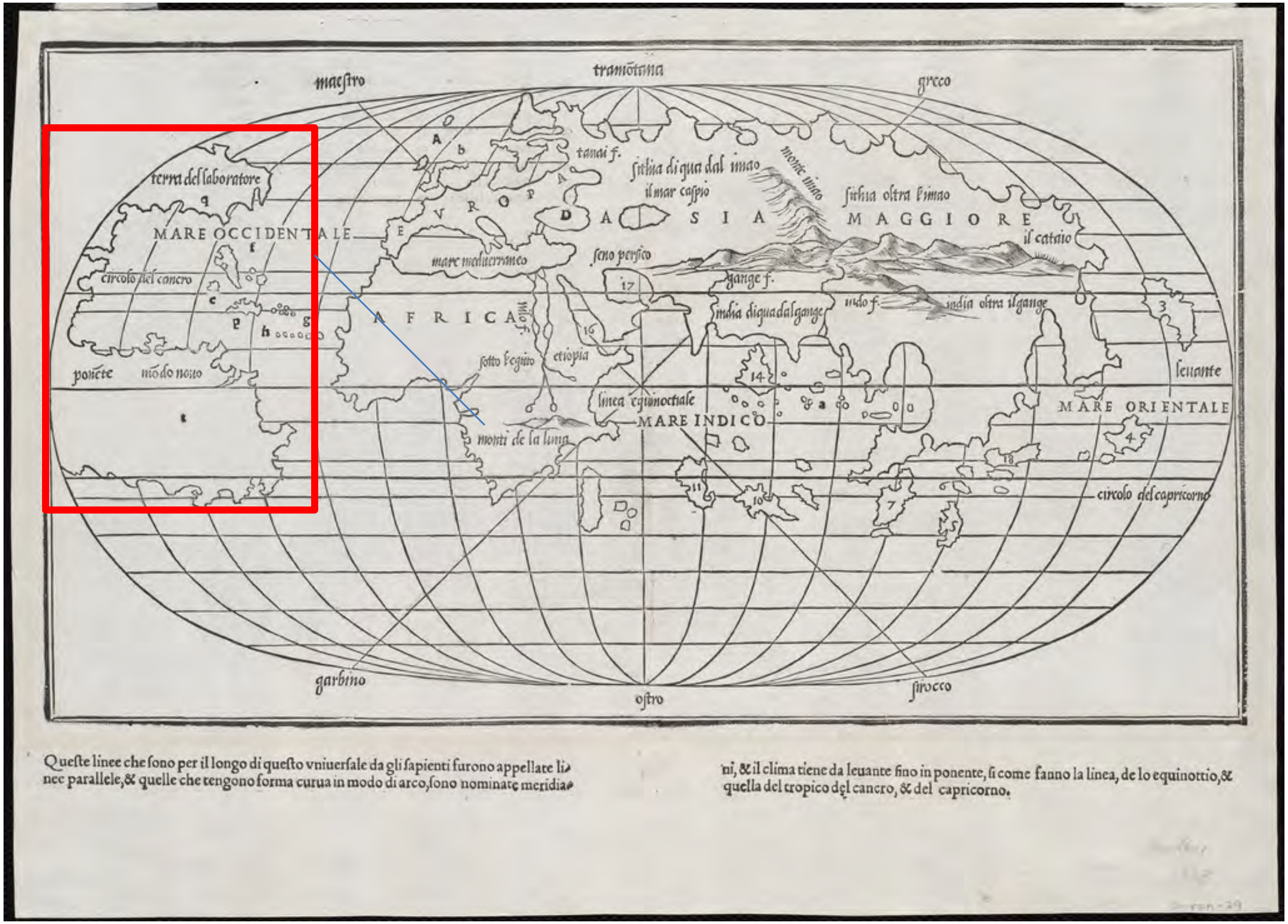
2007: Exceptional Scale and Diversity of Bacterial Phyla in Healthy Humans





Relman, Nature. 2007 449:811

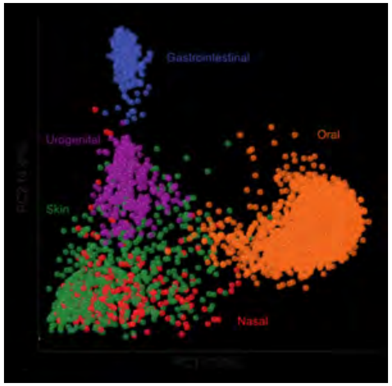
Microbiome, 2007

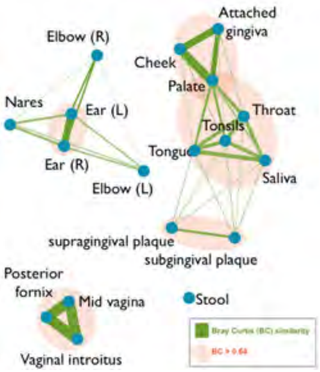


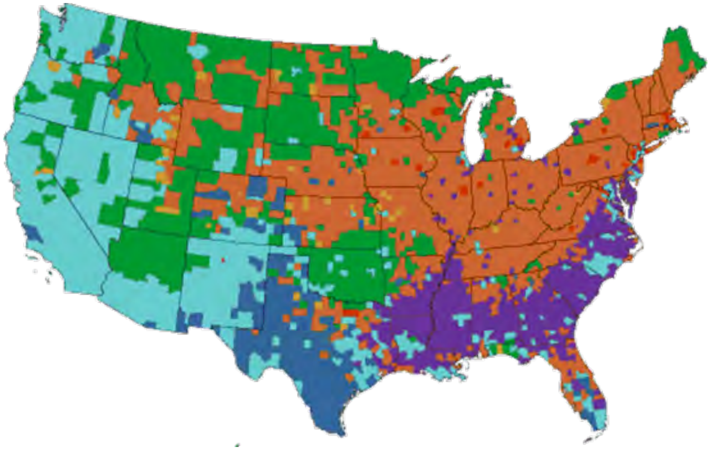
Queſte linee che ſono per il longo di queſto vniuerſale da gli ſapienti furono appellate linee parallele,& quelle che tengono forma curua in modo di arco,ſono nominate meridiani, & il clima tiene da leuante fino in ponente, ſi come fanno la linea, de lo equinottio,& quella del tropico del cancro, & del capricorno.

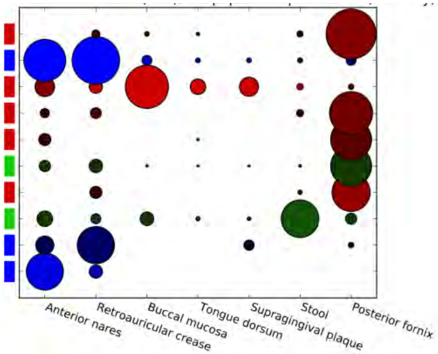
Benedetto Bordon, 1528
http://maps.bpl.org/

2012: The microbiome "aerial view" is complete



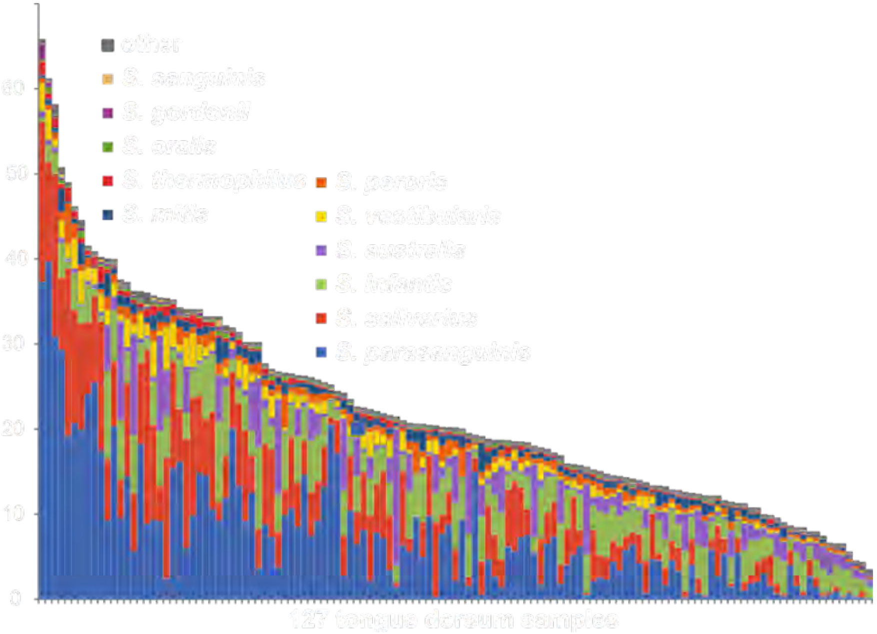




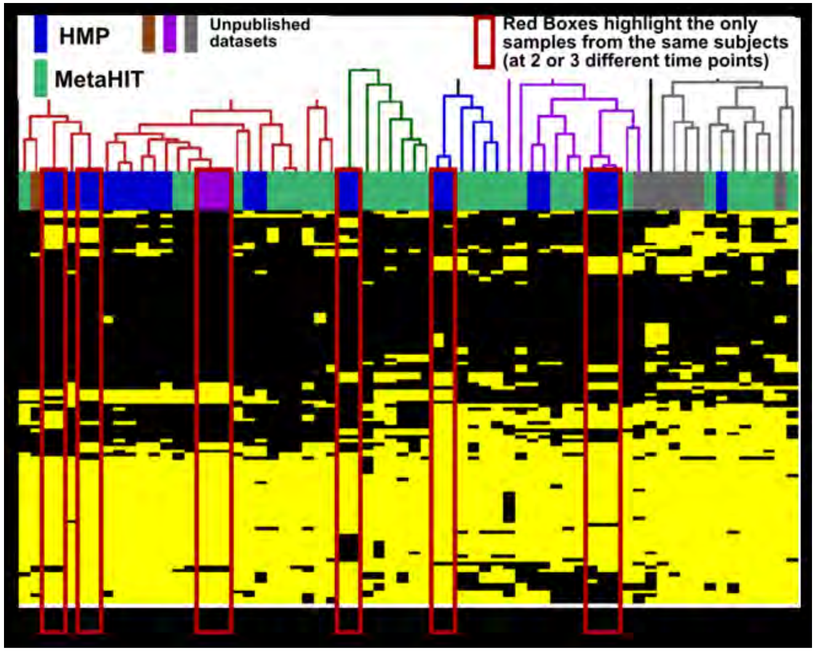


HMP Consortium,
Nature 2012

Like host genetic variants, our microbiomes are unique and (somewhat) stable



Microbiome variants are genetically distinct and persistent up to a period of at least months.

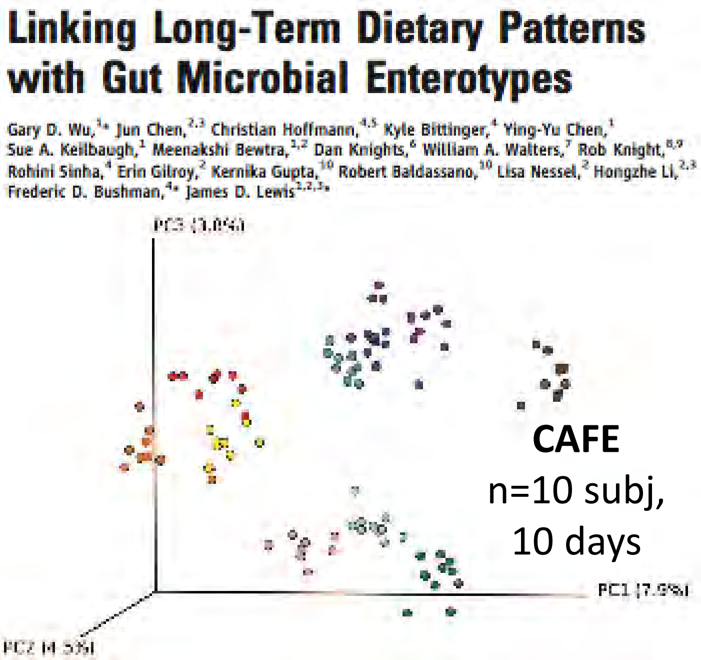


40 genes in the "same" microbe, *Prevotella copri*

🟨 **Present**

☐ **Absent**

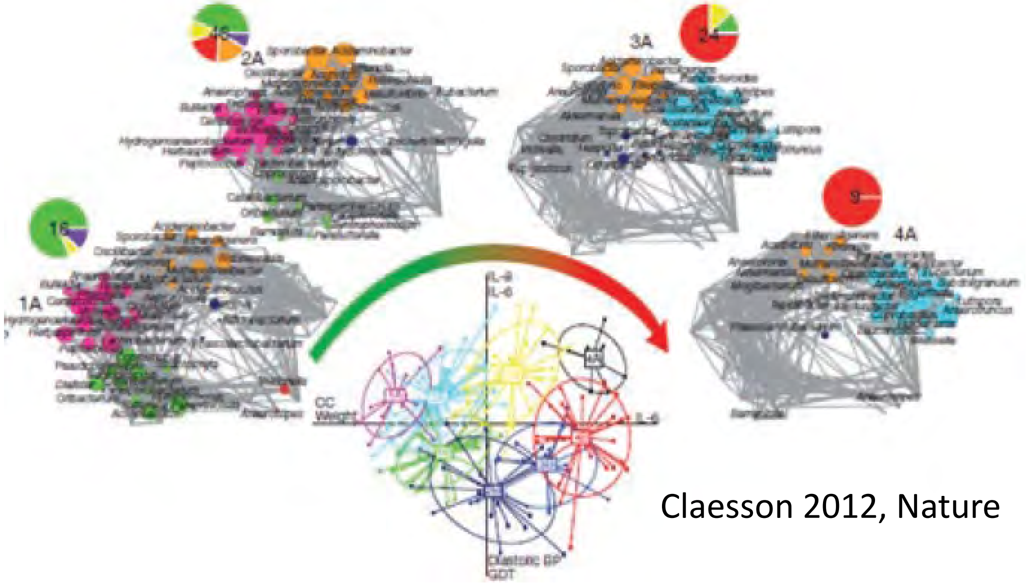
Diet affects composition of the microbiome only in the long-term



Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes

Gary D. Wu,[1*] Jun Chen,[2,3] Christian Hoffmann,[4,5] Kyle Bittinger,[4] Ying-Yu Chen,[1]
Sue A. Keilbaugh,[1] Meenakshi Bewtra,[1,2] Dan Knights,[6] William A. Walters,[7] Rob Knight,[8,9]
Rohini Sinha,[4] Erin Gilroy,[2] Kernika Gupta,[10] Robert Baldassano,[10] Lisa Nessel,[2] Hongzhe Li,[2,3]
Frederic D. Bushman,[4*] James D. Lewis[1,2,3*]

CAFE
n=10 subj,
10 days

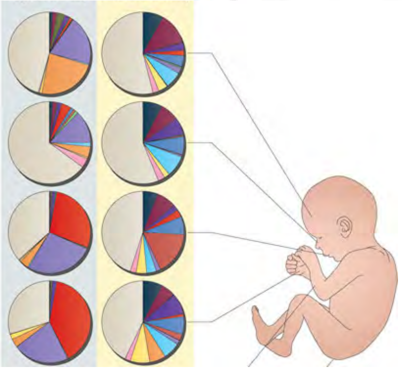


Claesson 2012, Nature

Effects of birth mode, lifetime, and geography on the microbiome

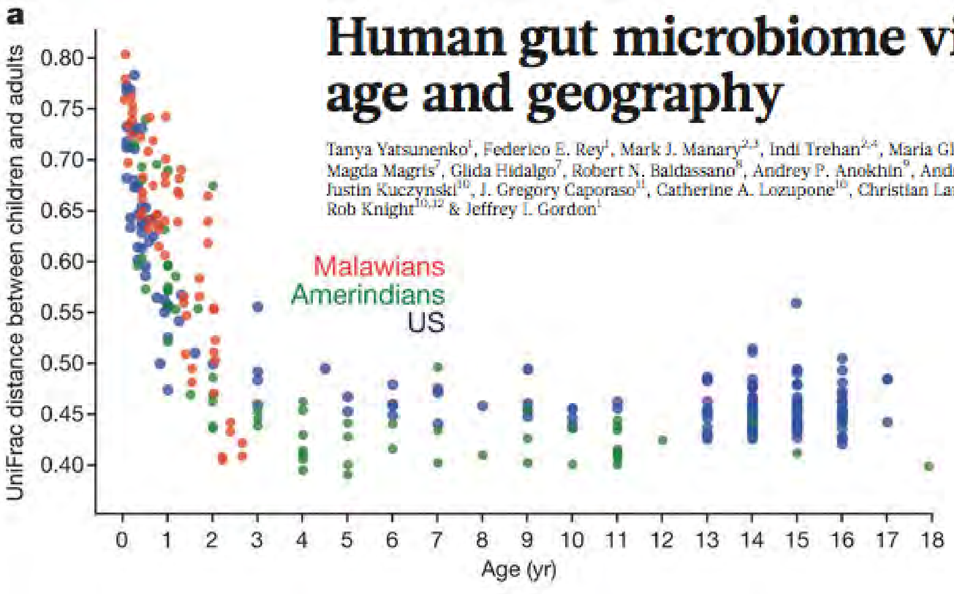
Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

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]



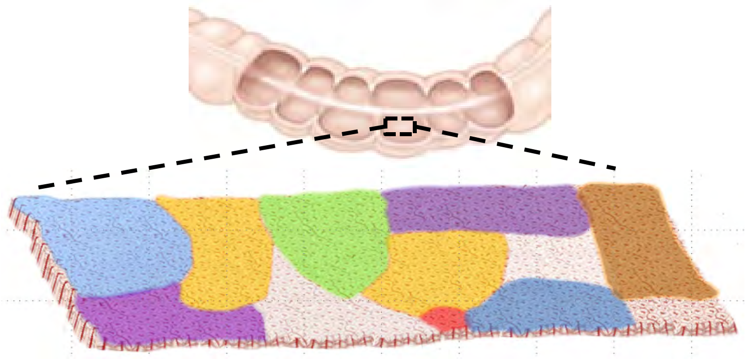
Human gut microbiome viewed across age and geography

Tanya Yatsunenko[1], Federico E. Rey[1], Mark J. Manary[2,3], Indi Trehan[2,4], Maria Gloria Dominguez-Bello[5], Monica Contreras[6], Magda Magris[7], Glida Hidalgo[7], Robert N. Baldassano[8], Andrey P. Anokhin[9], Andrew C. Heath[9], Barbara Warner[2], Jens Reeder[10], Justin Kuczynski[10], J. Gregory Caporaso[11], Catherine A. Lozupone[10], Christian Lauber[10], Jose Carlos Clemente[10], Dan Knights[10], Rob Knight[10,12] & Jeffrey I. Gordon[1]

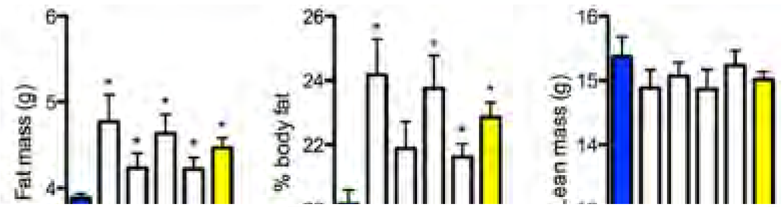


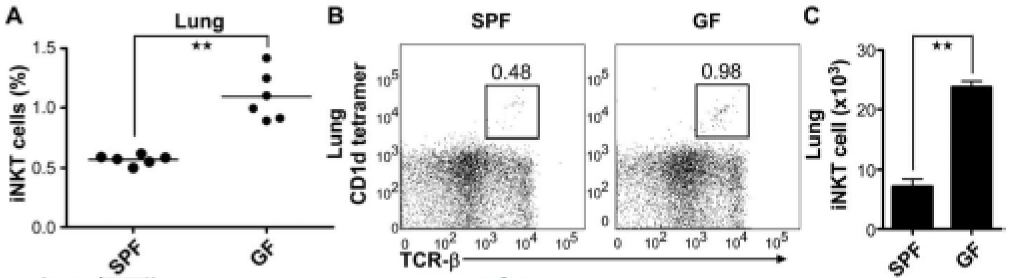
Plan of talk

- **Disease association processes of the microbiome**
  - **Hit and run (the neonatal window)**
  - **Good and bad guys (C. difficile, atherosclerosis)**
  - **Good and bad ecosystems (obesity, behavior, IBD, systemic lymphoma)**
- Functional ecology
  - The phylogeny paradox
  - Co-occurrence networks of bacteria, proteins, and metabolites
  - Functional analysis of ecosystems
- Fixing radios, and the way forward
  - Ecosystems repair
  - Targeting functional features of the microbiome
  - Systems versus pathways



Hit and Run: neonatal microbiome has life-long effect on elements of immune function and obesity





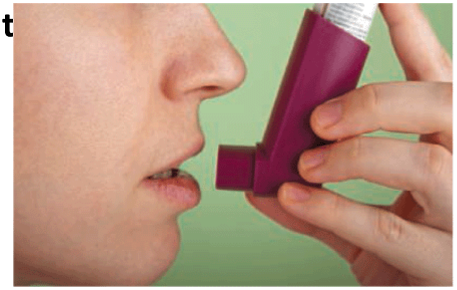
iNKT cells and risk of colitis, asthma

Olszak T, Science 2012

Neonatal antibiotics and body fat

Cho I, Nature 2012

Trassande J, I J Obesit

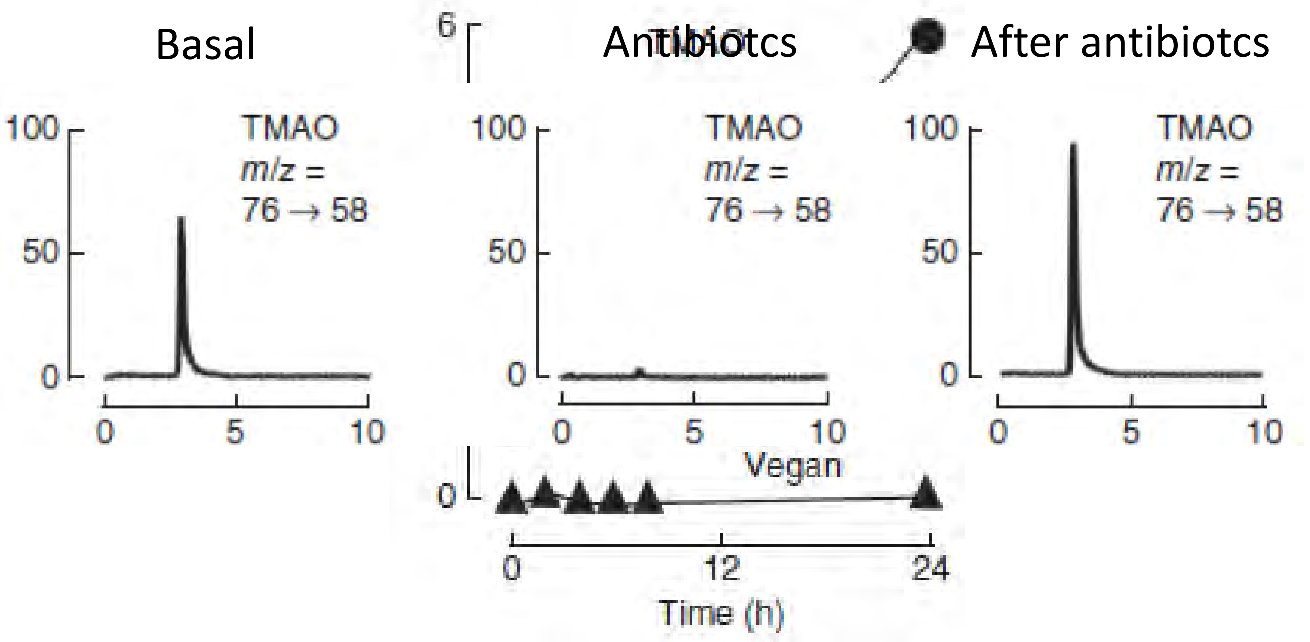


Good and Bad Bugs

nature medicine
VOLUME 19 | NUMBER 5 | MAY 2013

Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis

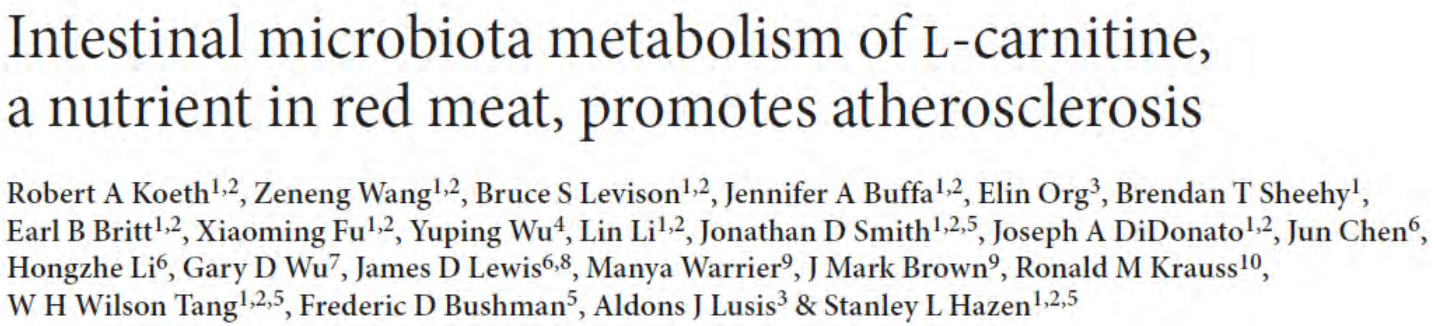
Robert A Koeth[1,2], Zeneng Wang[1,2], Bruce S Levison[1,2], Jennifer A Buffa[1,2], Elin Org[3], Brendan T Sheehy[1], Earl B Britt[1,2], Xiaoming Fu[1,2], Yuping Wu[4], Lin Li[1,2], Jonathan D Smith[1,2,5], Joseph A DiDonato[1,2], Jun Chen[6], Hongzhe Li[6], Gary D Wu[7], James D Lewis[6,8], Manya Warrier[9], J Mark Brown[9], Ronald M Krauss[10], W H Wilson Tang[1,2,5], Frederic D Bushman[5], Aldons J Lusis[3] & Stanley L Hazen[1,2,5]



Tang WHW, N Engl J Med 2013

Good and Bad Bugs

nature medicine
VOLUME 19 | NUMBER 5 | MAY 2013



Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis

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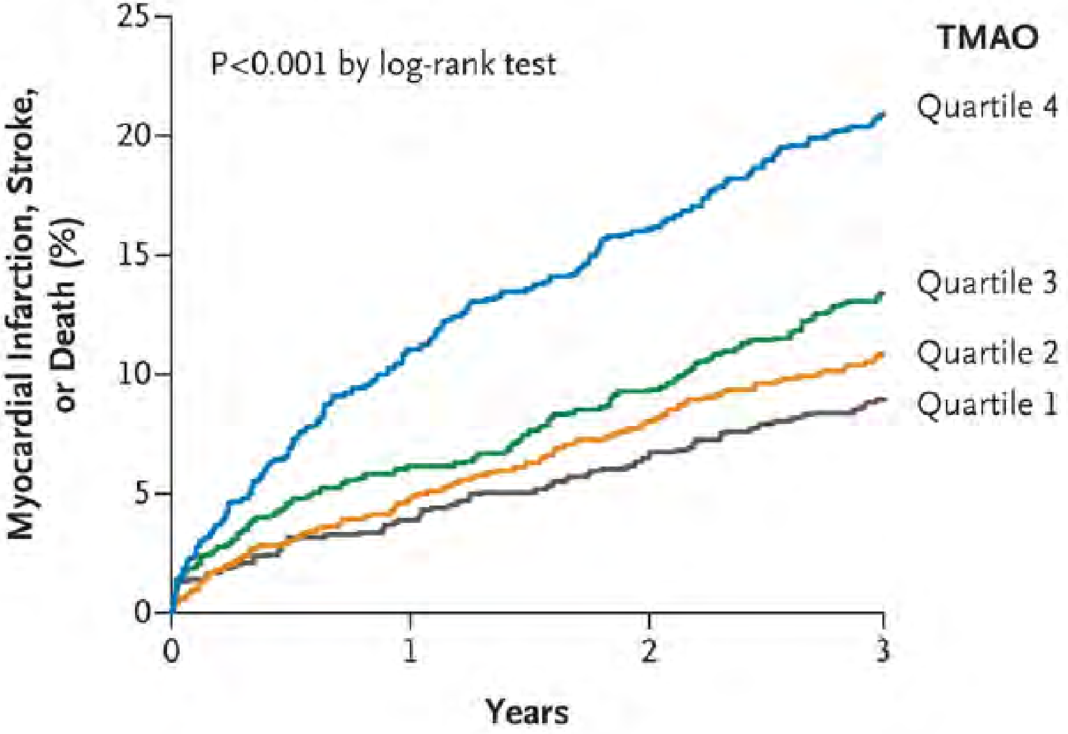
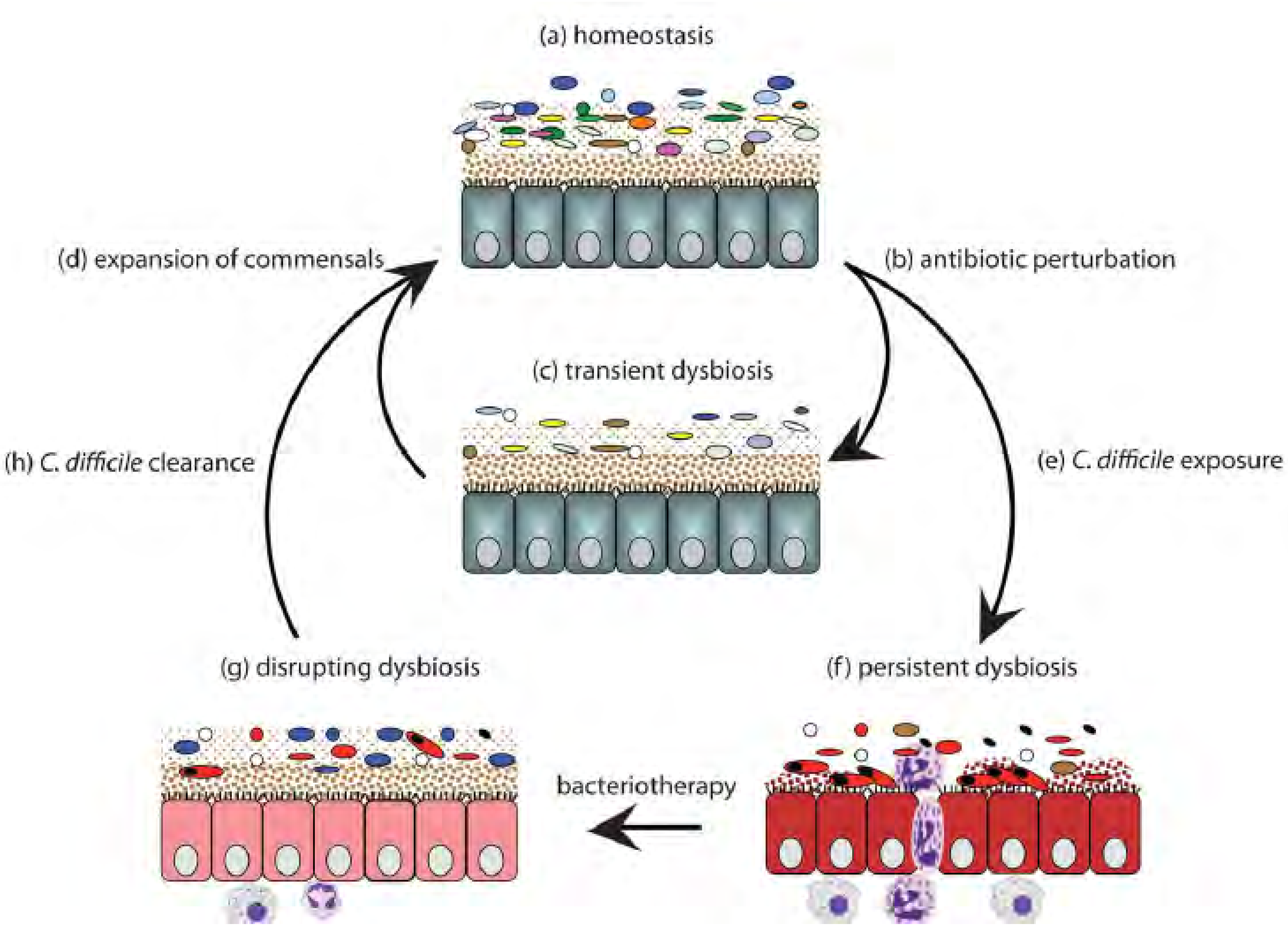


Tang WHW, N Engl J Med 2013

Ecosystem disruption and *C. difficile* colitis

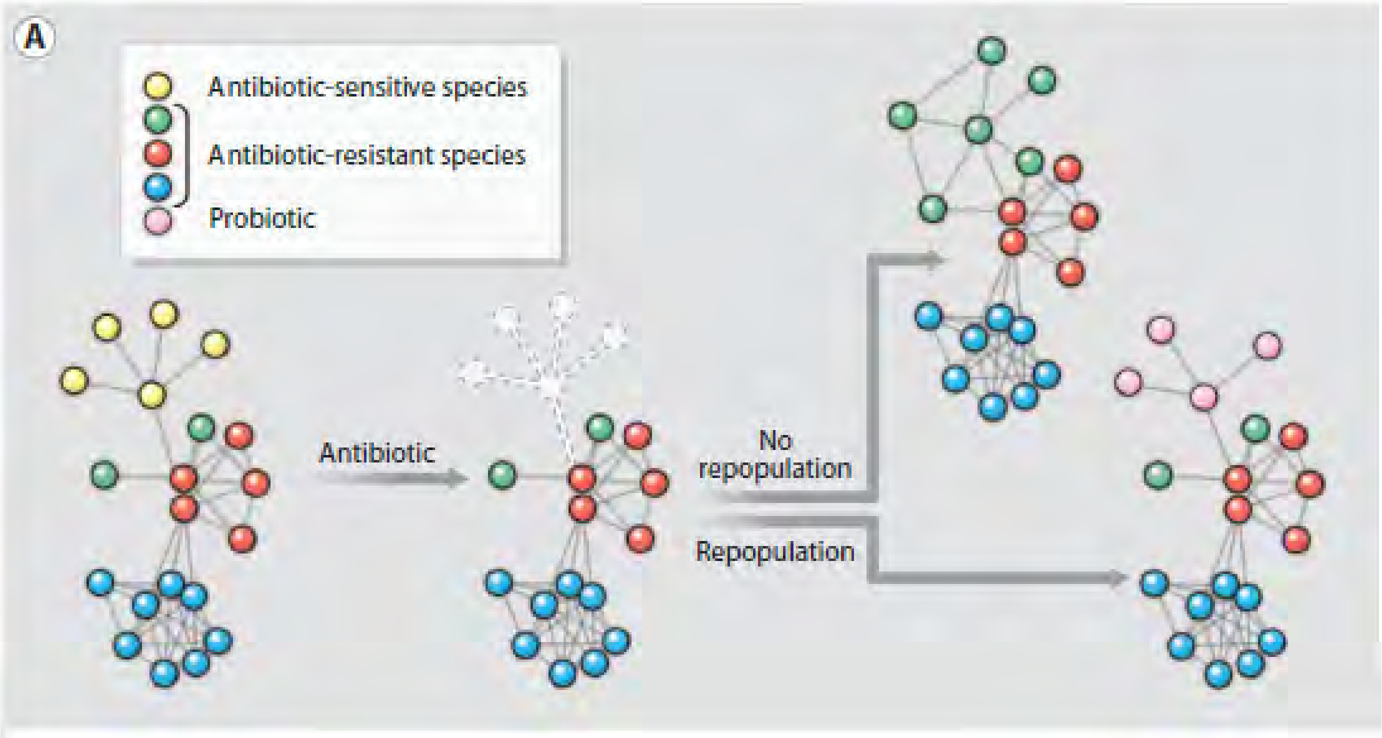


GASTROENTEROLOGY 2012;142:490–496

Fecal Transplantation, Through Colonoscopy, Is Effective Therapy for Recurrent *Clostridium difficile* Infection

EERO MATTILA,[*] RAIJA UUSITALO–SEPPÄLÄ,[‡] MAARIT WUORELA,[§] LAURA LEHTOLA,[||] HEIMO NURMI,[¶] MATTI RISTIKANKARE,[#] VEIKKO MOILANEN,[**] KIMMO SALMINEN,[§,¶] MAARIA SEPPÄLÄ,[§] PETRI S. MATTILA,[‡‡] VELI–JUKKA ANTTILA,[*] and PERTTU ARKKILA[§§]

[*]Department of Infectious Diseases, [‡‡]Department of Otorhinolaryngology, and [§§]Department of Gastroenterology, Helsinki University Central Hospital, Helsinki; [‡]Department of Infectious Diseases and [**]Department of Gastroenterology, Satakunta Central Hospital, Pori; [§]Turku City Hospital, Turku; [||]Maria Hospital, Helsinki City Hospital, Helsinki; [¶]Department of Medicine, Turku University Central Hospital, Turku; [#]Laakso Hospital, Helsinki City Hospital, Helsinki, Finland



Lemon KP, Sci Transl Med 2012

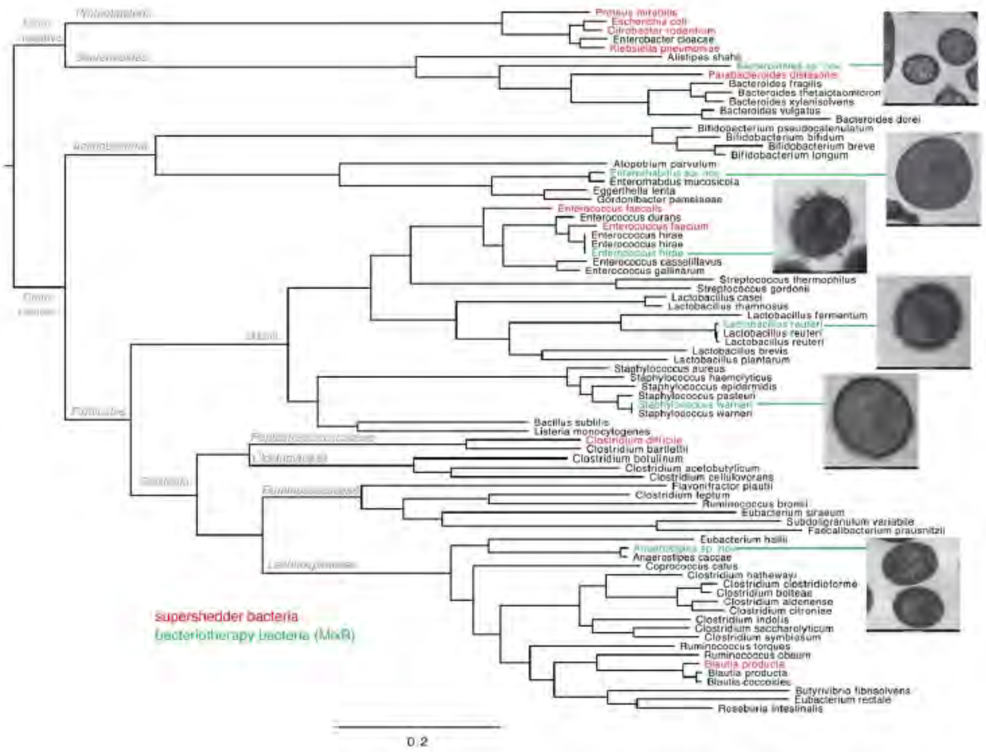
OPEN ACCESS Freely available online

PLOS | PATHOGENS

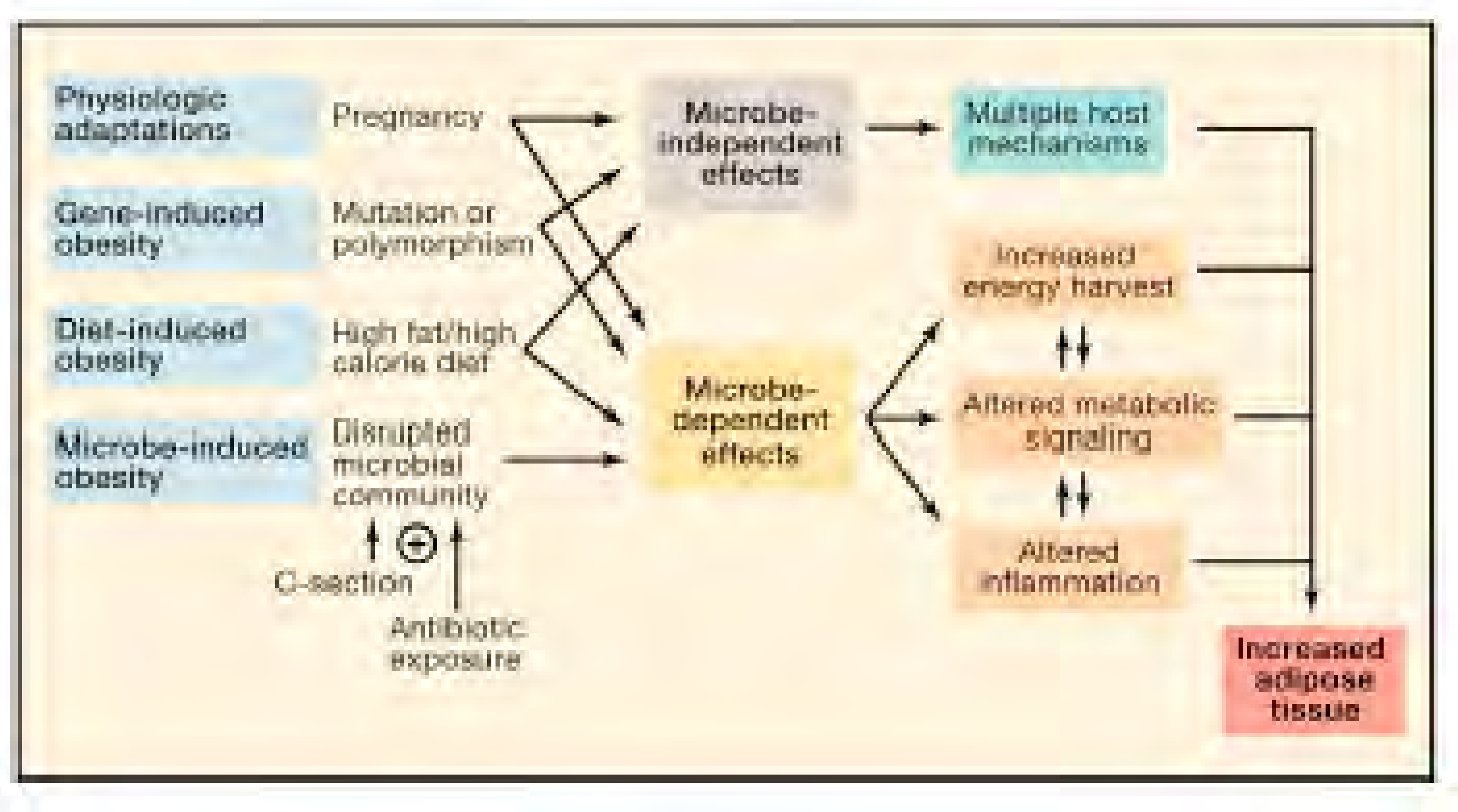
Targeted Restoration of the Intestinal Microbiota with a Simple, Defined Bacteriotherapy Resolves Relapsing *Clostridium difficile* Disease in Mice

Trevor D. Lawley[1*], Simon Clare[1⁹], Alan W. Walker[1⁹], Mark D. Stares[1], Thomas R. Connor[1], Claire Raisen[1], David Goulding[1], Roland Rad[1], Fernanda Schreiber[1], Cordelia Brandt[1], Laura J. Deakin[1], Derek J. Pickard[1], Sylvia H. Duncan[2], Harry J. Flint[2], Taane G. Clark[3], Julian Parkhill[1], Gordon Dougan[1]

1 Wellcome Trust Sanger Institute, Hinxton, United Kingdom, **2** Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom, **3** London School of Hygiene and Tropical Medicine, London, United Kingdom



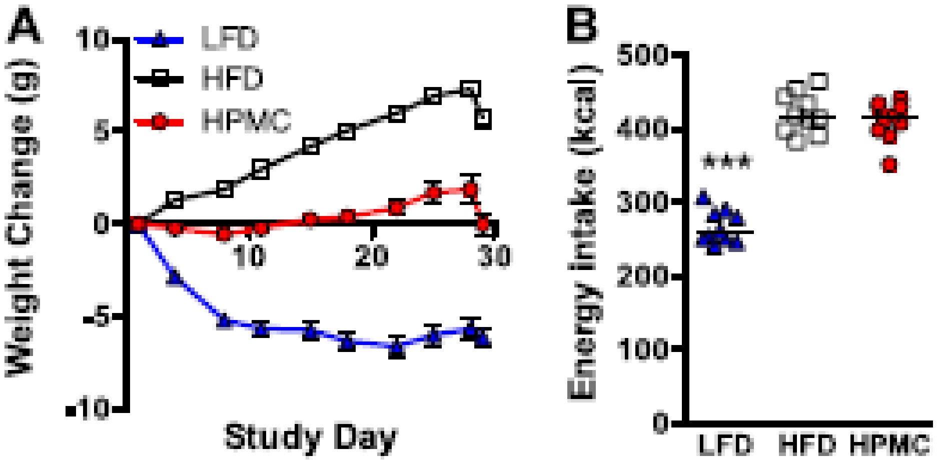
Alternate ecosystems
Microbiome, direct and indirect, in obesity

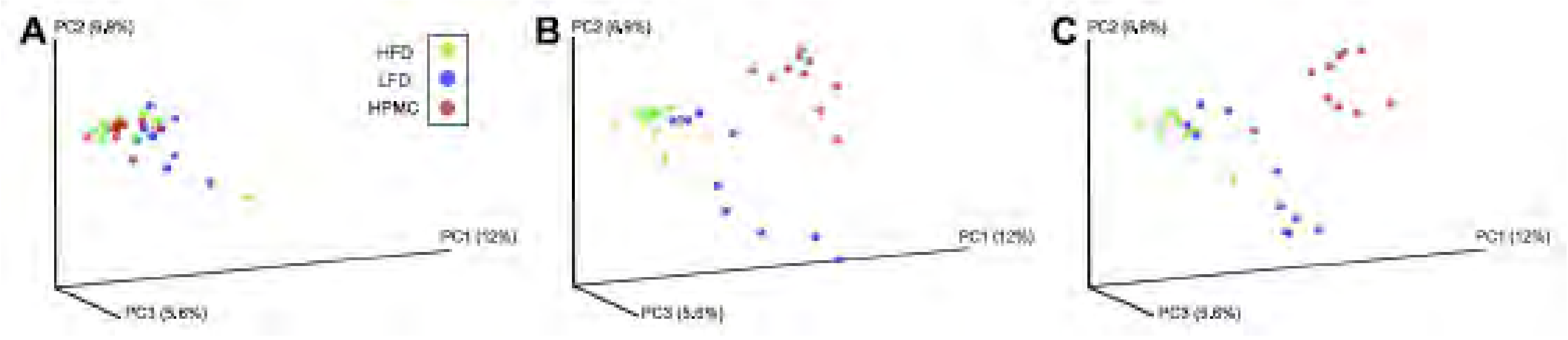


Cox LM, Cell Metabolism 2013

Prebiotic modification of microbiome

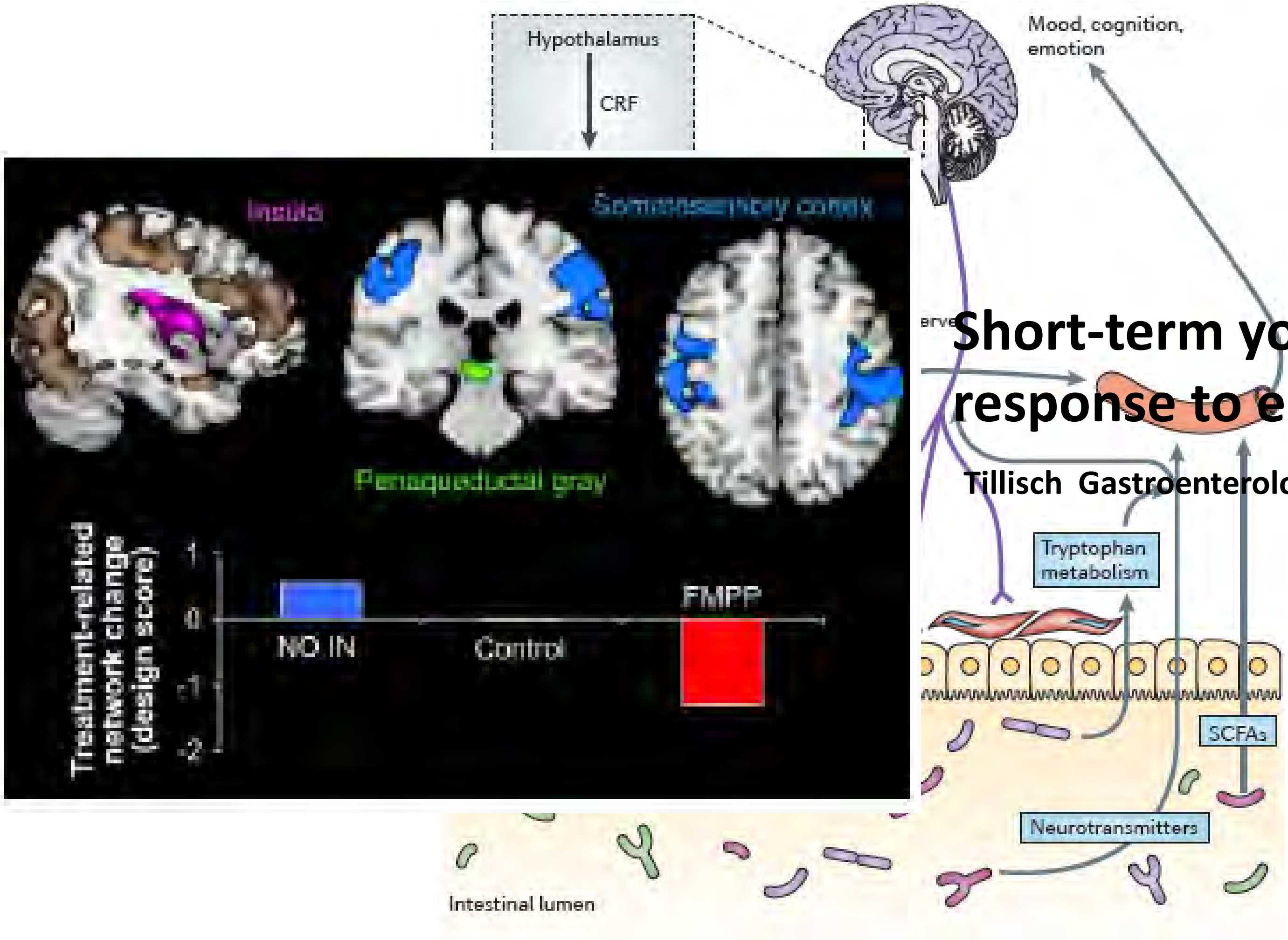
"Neutralization" of high fat-induced obesity with complex carbohydrates





Cox LM, FASEB J 2013

Microbiome and behavior



Short-term yogurt ingestion and response to emotional stimuli

Tillisch Gastroenterology 2013

Cryan JF Nat Rev Neuroscience 2012

Ataxia Telangiectasia (AT)

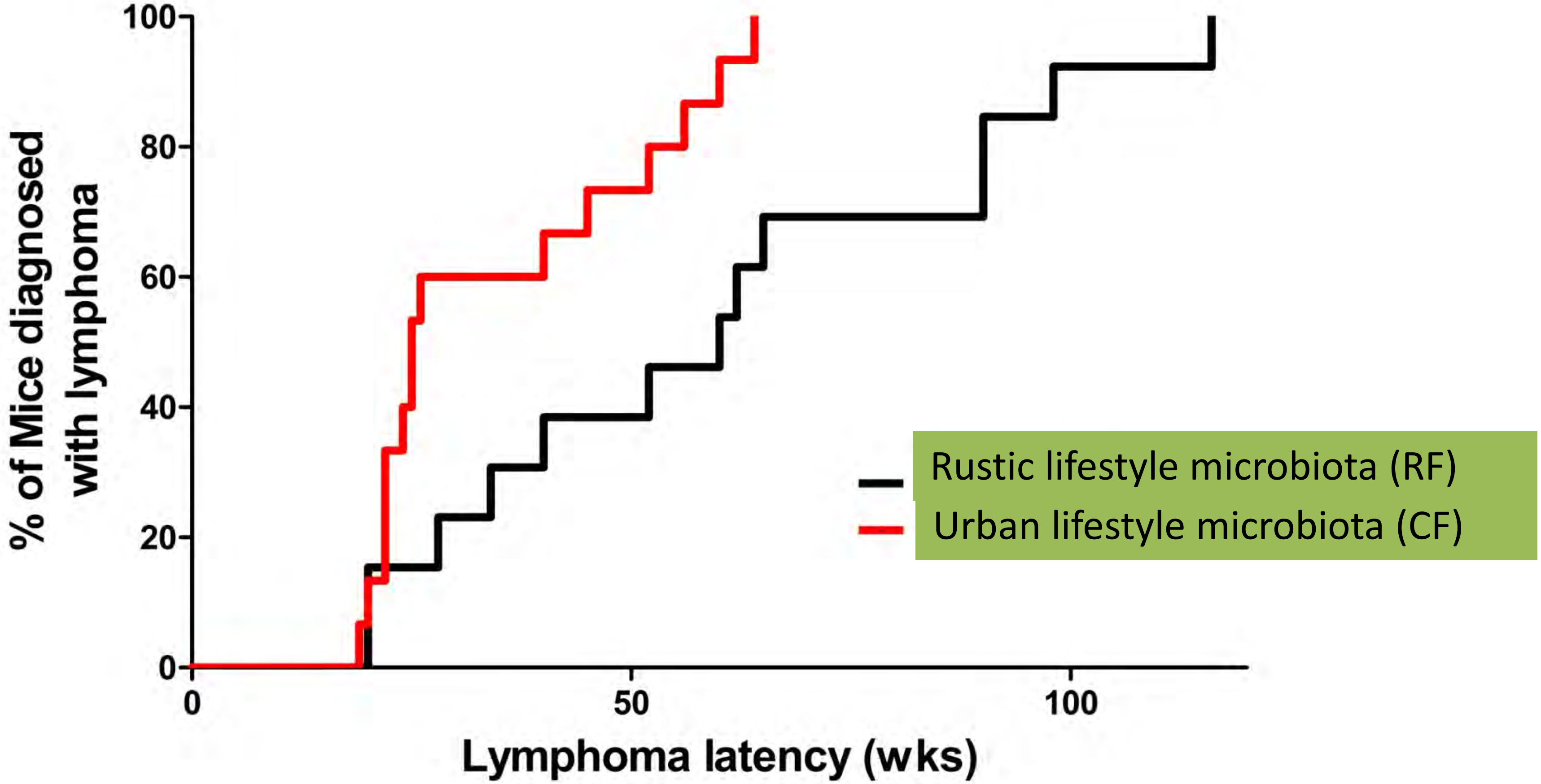
Clinical manifestation:

- Autosomal recessive disease (1 in 40.000-100.000 people affected)
- Early-onset progressive cerebellar ataxia
- High incidence of tumors (1 in10 develop lymphoma or leukemia)
- Growth retardation
- Immunodeficiency

Biological markers:

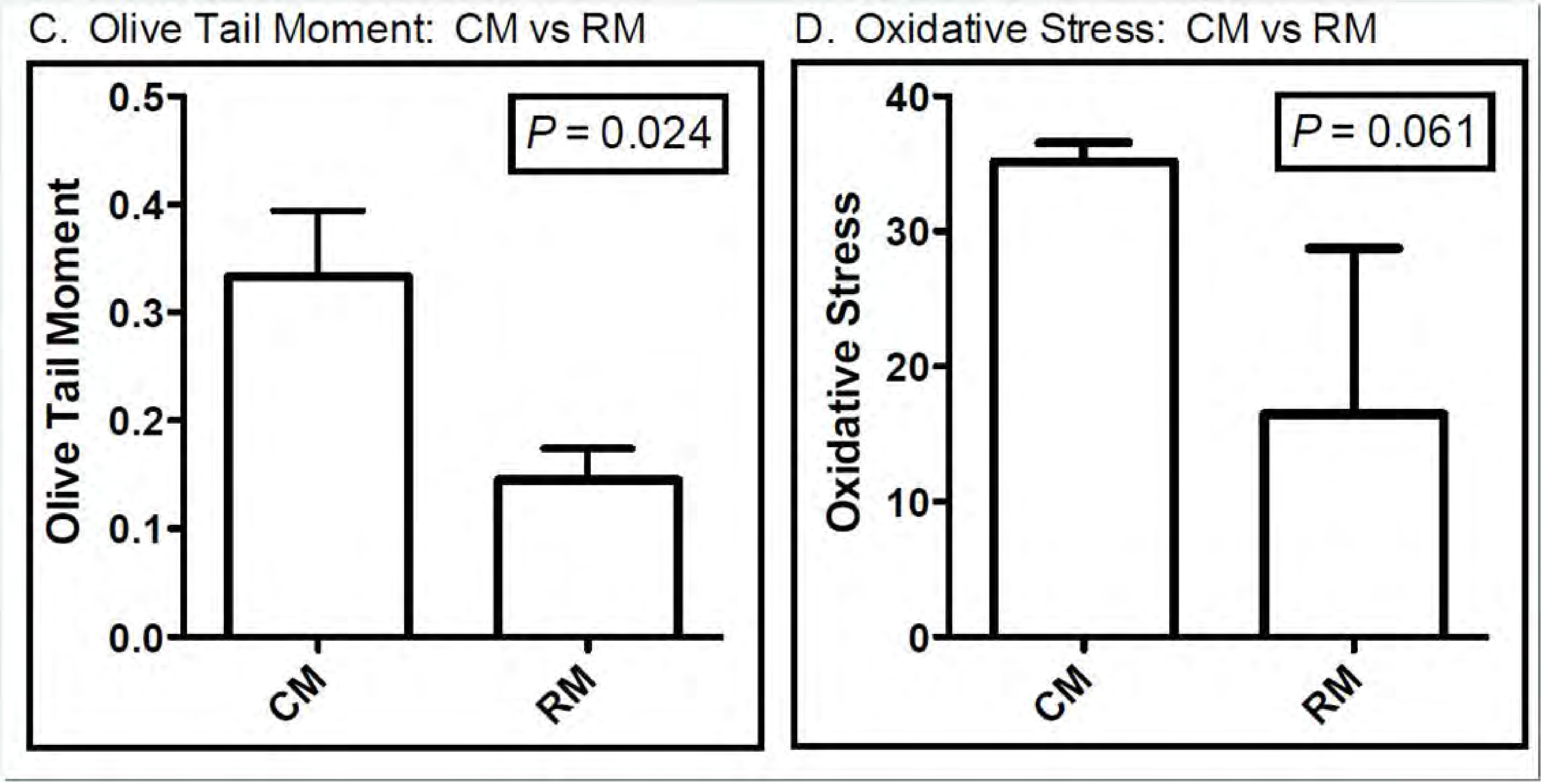
- Chromosomal instability
- Hypersensitivity to radiation
- Imbalance in antioxidant levels and antioxidative enzymes

Lymphoma latency in ATM-/- mice is modified by microbial composition



Wei B, J Immunol 2010
Yamamoto ML. Cancer Res 2013

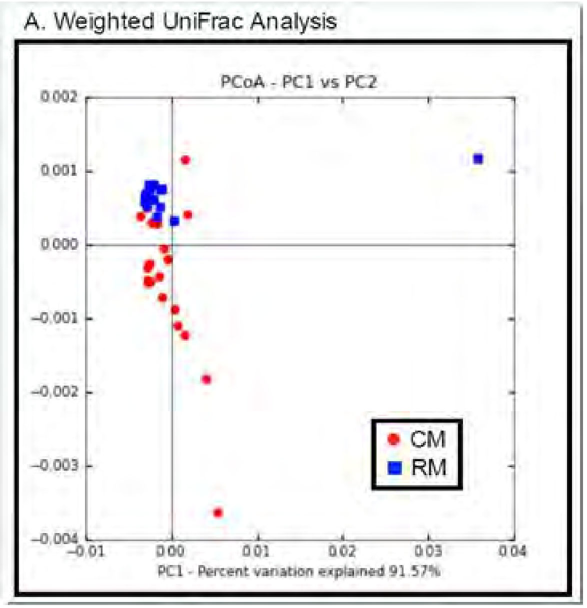
Systemic lymphocyte DNA damage and oxidative stress is modified by microbial composition



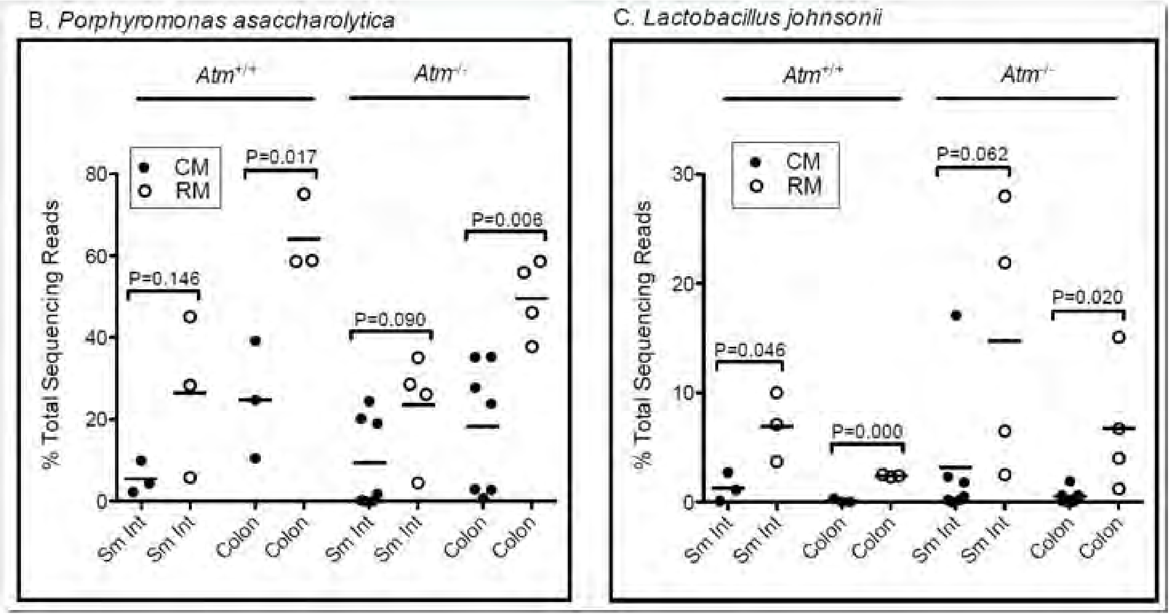
Yamamoto ML. Cancer Res. 2013

Identification of microbiota selectively augmented or depleted in lymphoma resistant RM microbiota

UniFrac (PCA)



Candidate organisms (2 of 8)



Candidate prioritization

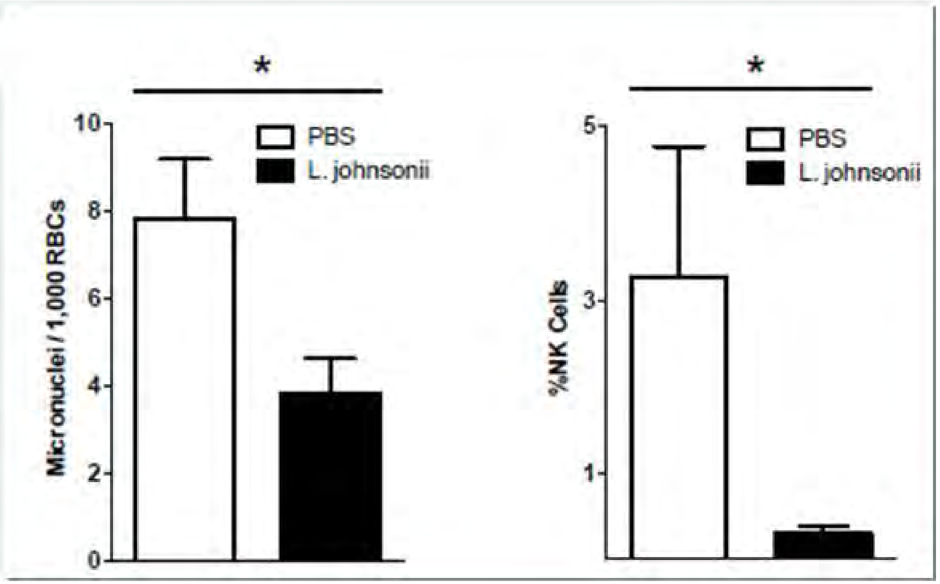
- Contribution to PC1 and 2
- False-detection significance of association
- Consistent presence and correlation in individual mice
- Relevant biologic features
- Culturability

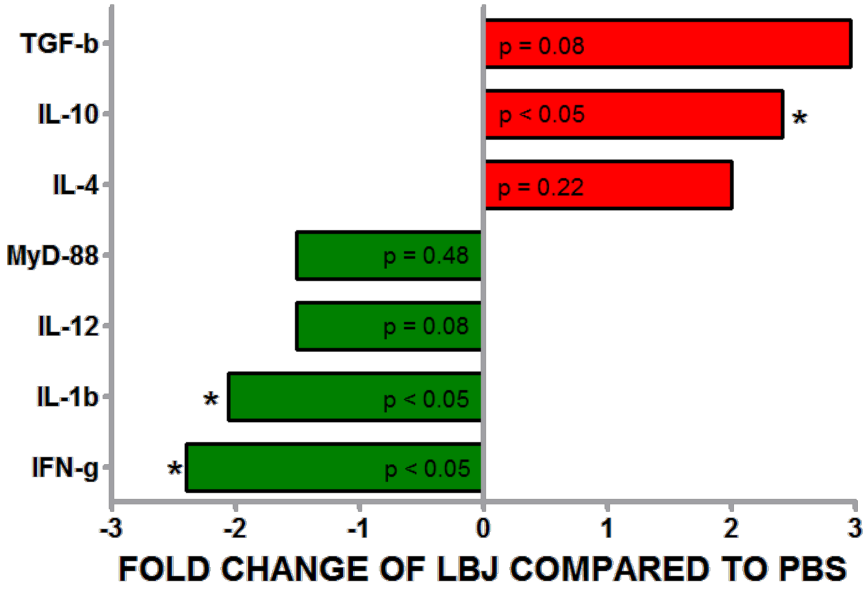
Lactobacillus johnsonii reduces genotoxicity and inflammatory state of lymphoma-prone (CM) ATM$^{-/-}$ mice

Hemopoietic DNA damage

Hepatic innate lymphocyte levels

Systemic inflammatory cytokine levels

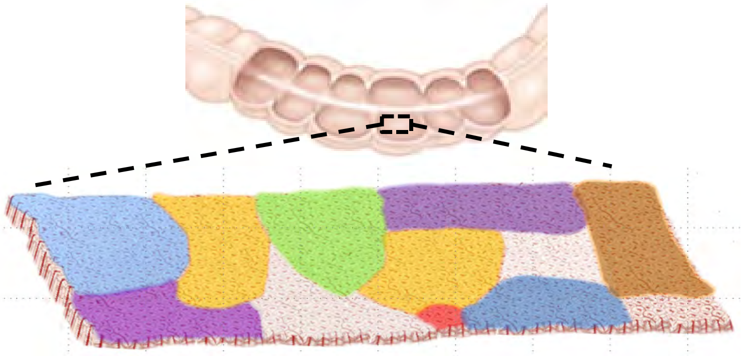




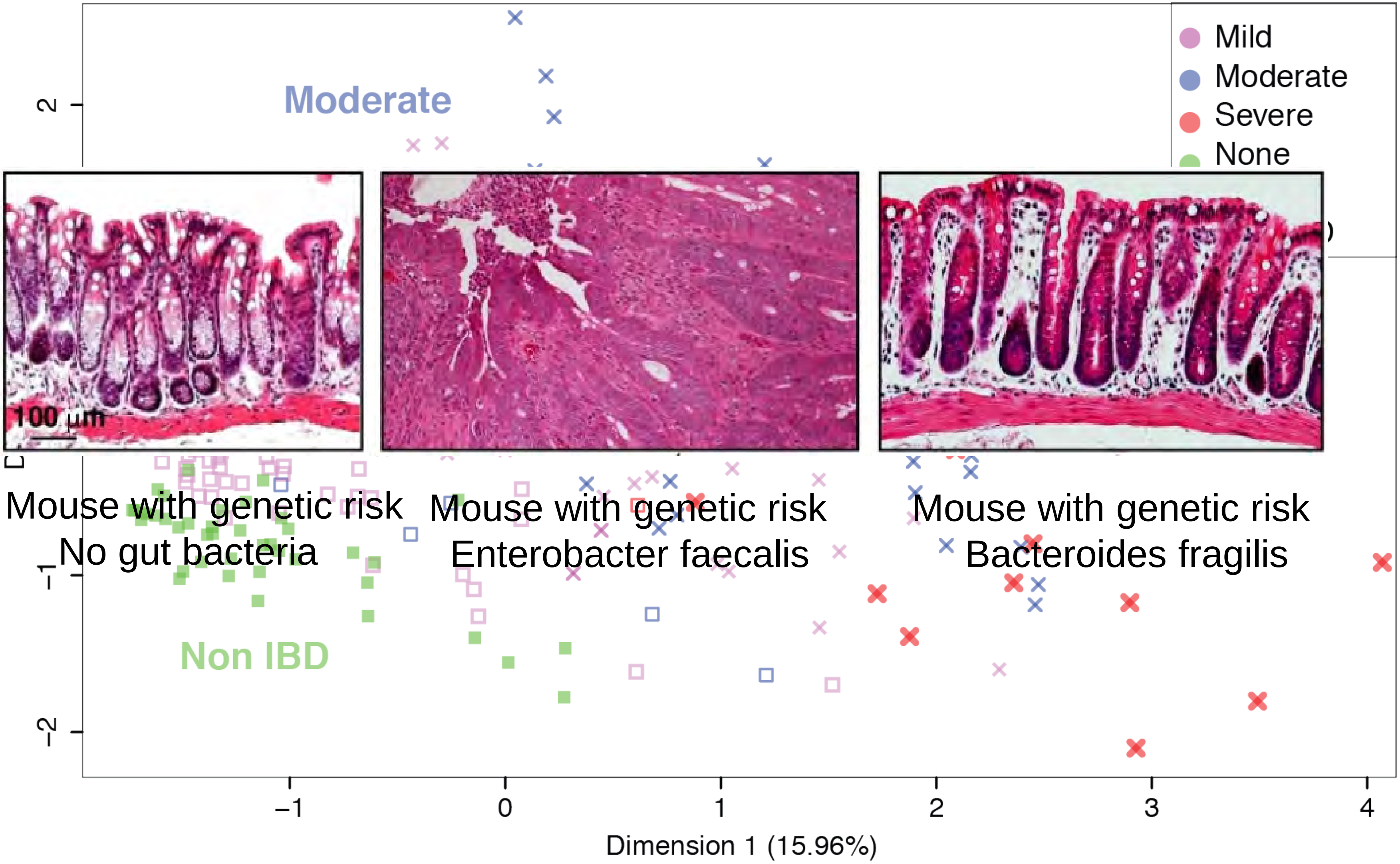
Yamamoto ML. Cancer Res. 2013

Microbial composition and lymphoma

- Ataxia Telangiectasia, a DNA repair disease, results in highly penetrant lymphoma
- Mucosal inflammation induces systemic DNA damage
- Lymphoma and survival in ATM-/- mice is dependent on intestinal microbiota
- Microbiota identified that fulfill Koch’s postulate for correcting mucosa-associated inflammation and systemic genotoxicity
- Microbial composition is a modifiable factor in lymphoma



Microbial composition and IBD

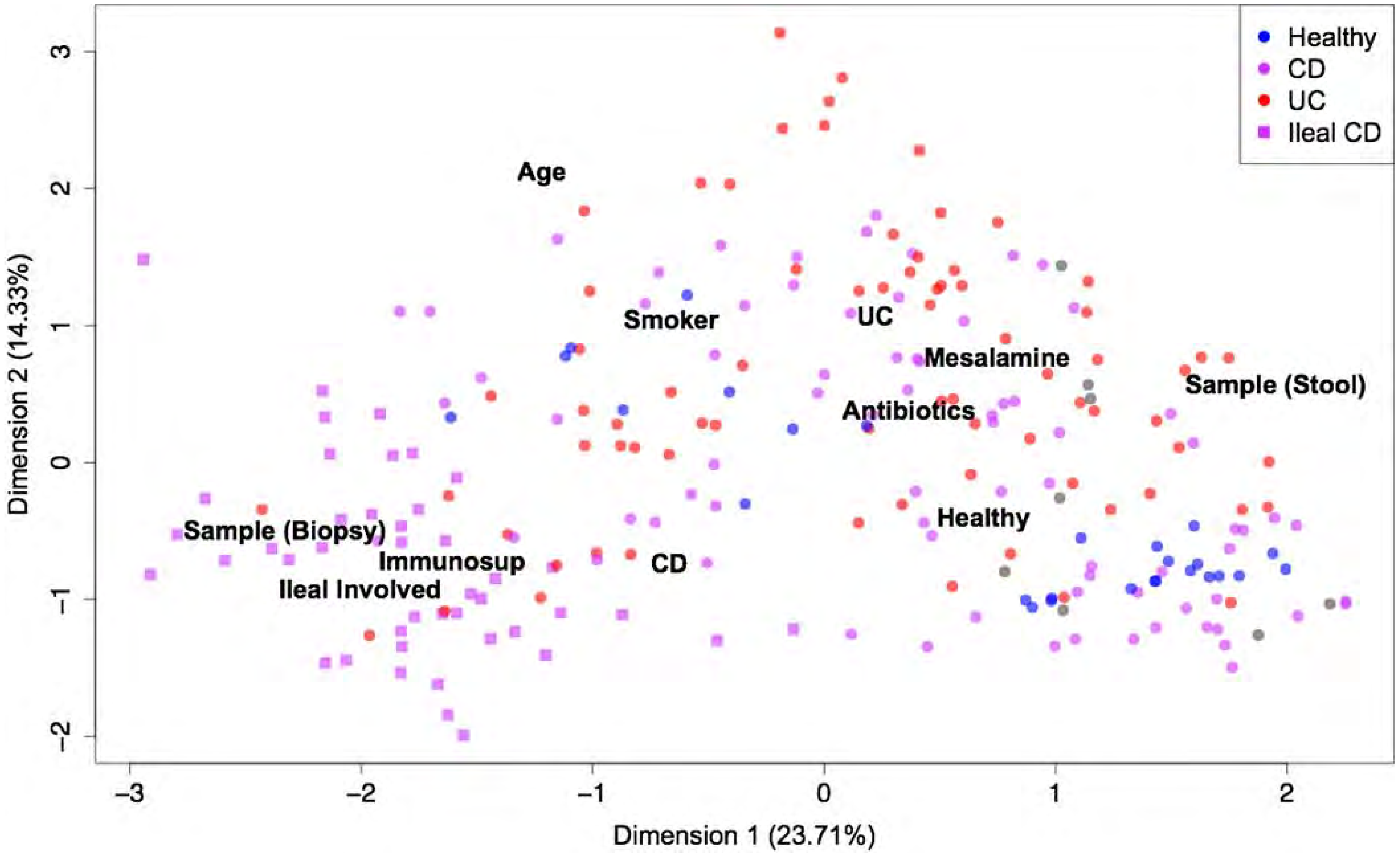


Mouse with genetic risk
No gut bacteria

Mouse with genetic risk
Enterobacter faecalis

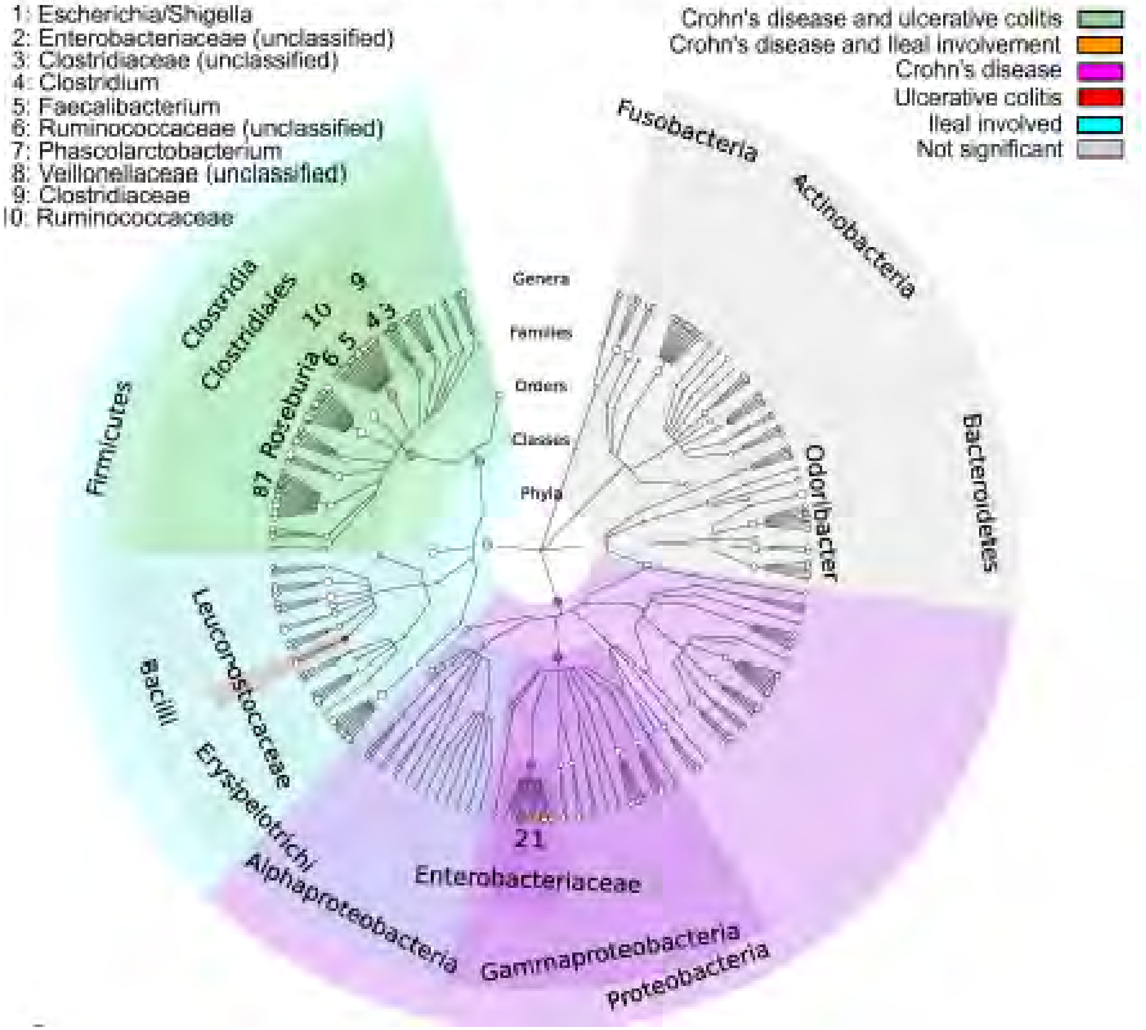
Mouse with genetic risk
Bacteroides fragilis

IBD risk factors and microbiome composition



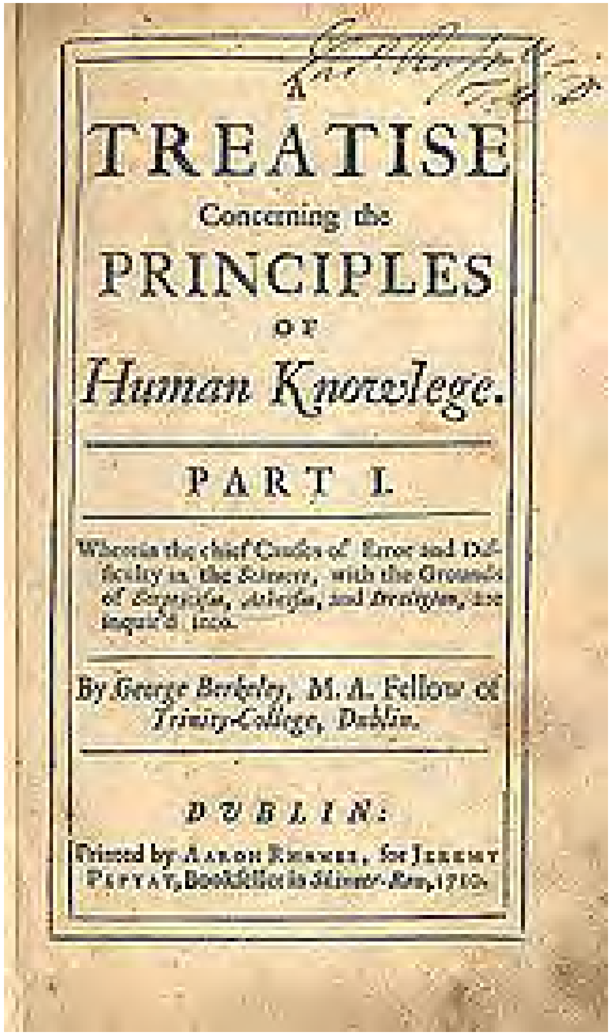
Morgan, Genome Biology 2012

Taxonomic distribution of organisms associated with disease



Morgan X, Genome Biol 2012

Correlation vs. Causation



A
TREATISE
Concerning the
PRINCIPLES
OF
Human Knowlege.

PART I.

Wherein the chief Causes of Error and Difficulty in the Sciences, with the Grounds of *Scepticism*, *Atheism*, and *Irreligion*, are inquir'd into.

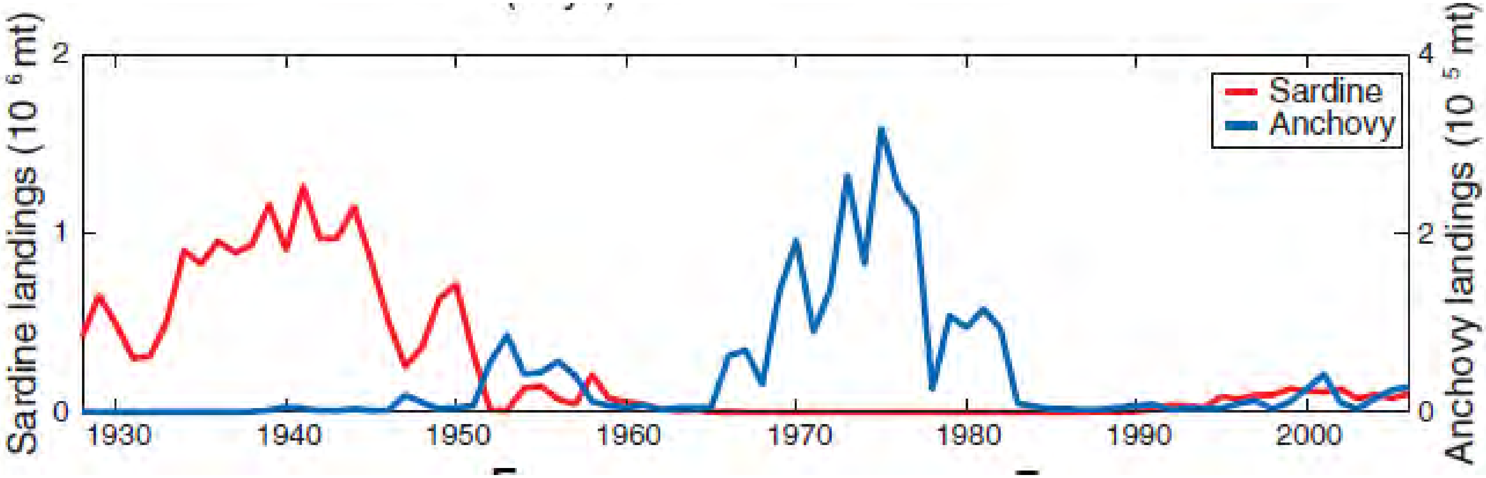
By *George Berkeley*, M. A. Fellow of *Trinity-College*, *Dublin*.

DUBLIN:
Printed by AARON RHAMES, for JEREMY PEPYAT, Bookseller in *Skinner-Row*, 1710.

- Better cohort study design
- Integration of habitat and remote biologic metadata

- <u>Mechanistic definition and validation of microbial function</u>

Detecting Causality in Complex Ecosystems
George Sugihara *et al.*
Science **338**, 496 (2012);
DOI: 10.1126/science.1227079



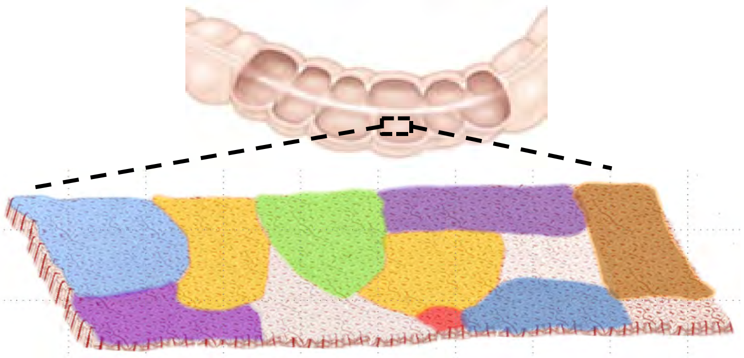
Microbiome, 2013

(Western Hemisphere, 1600)

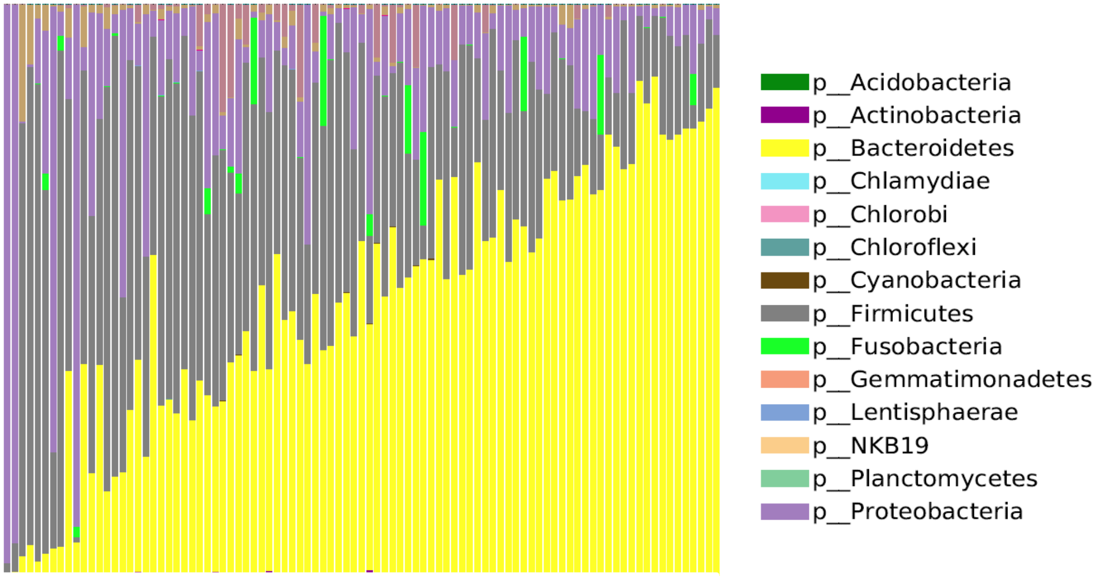
Justus Kanckerts 1600
http://maps.bpl.org/

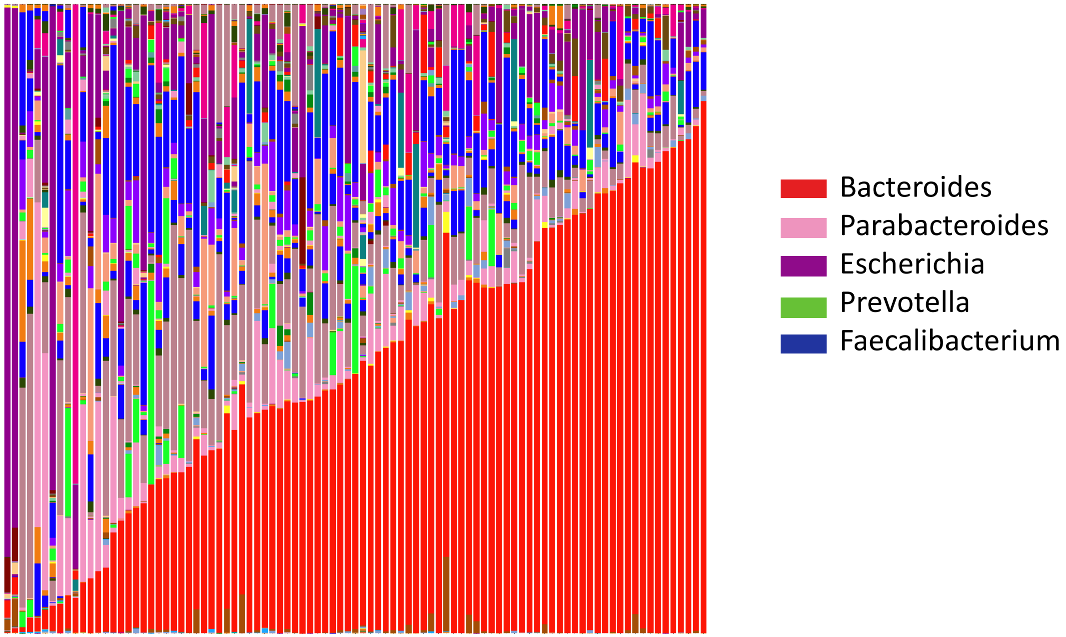
Plan of talk

- Disease association processes of the microbiome
  - Hit and run (the neonatal window)
  - Good and bad guys (C. difficile, atherosclerosis)
  - Good and bad ecosystems (obesity, behavior, IBD, systemic lymphoma)
- Functional ecology
  - **The phylogeny paradox**
  - **Co-occurrence networks of bacteria, proteins, and metabolites**
  - **Functional analysis of ecosystems**
- Fixing radios, and the way forward
  - Ecosystems repair
  - Targeting functional features of the microbiome
  - Systems versus pathways



16S view of individual microbial composition

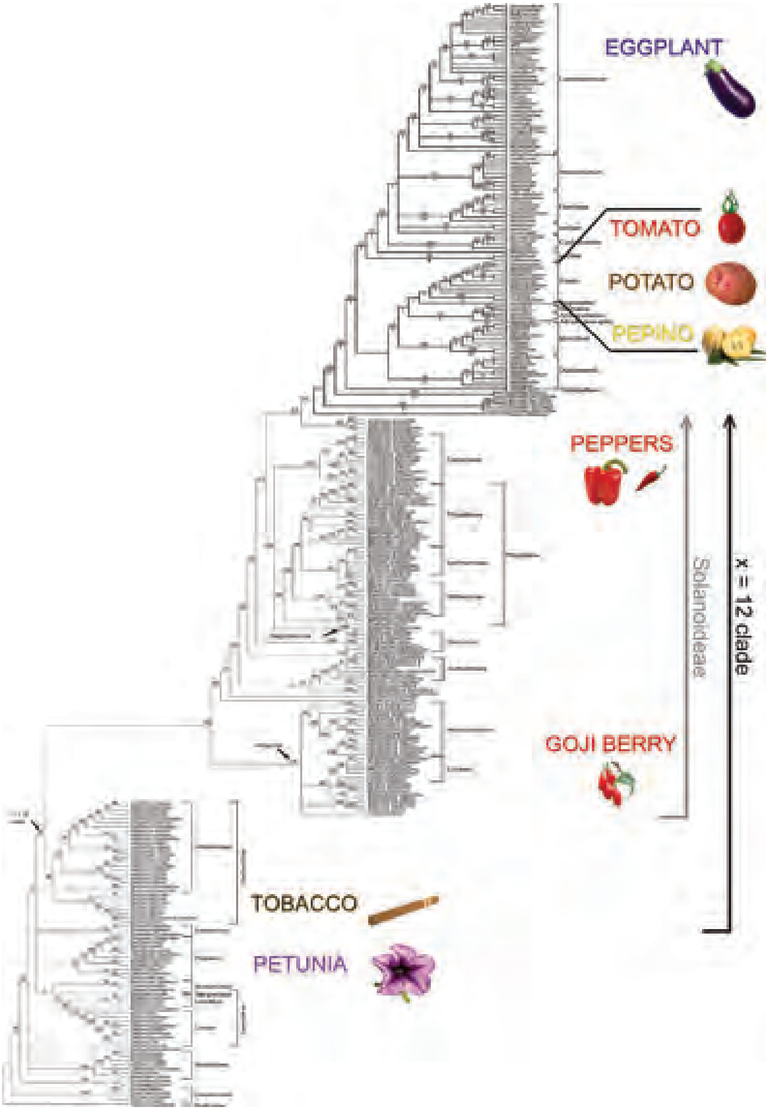




Tong M, in press


SERVICE & PRICE CHECK
Scoop Away
Fresh Step
TIDY CATS
Deli-Cat

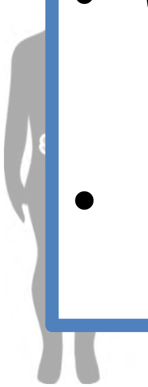
Functional diversity within a single genus (Solanales)

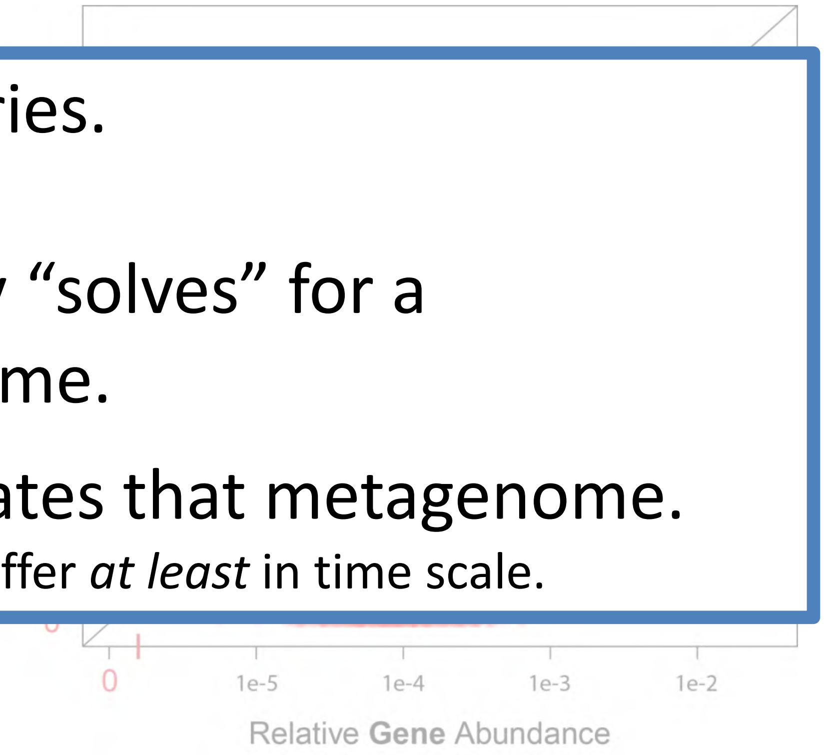


Functional diversity within a single species

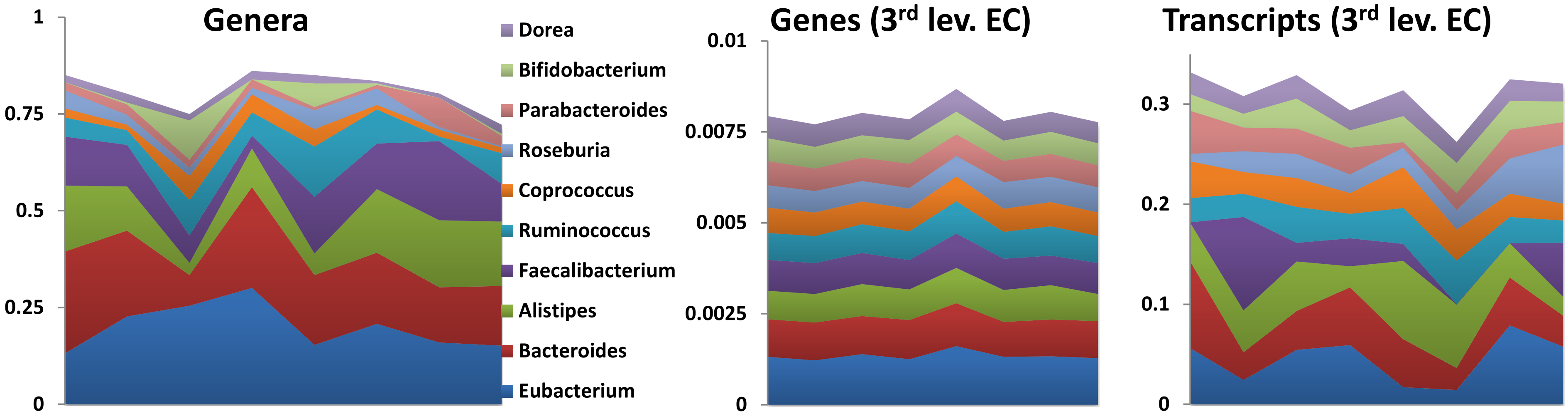
Relating the gut metagenome and metatranscriptome

- Microbial membership varies.
  - Early colonization? Genetics?
- Over time, the community “solves” for a habitat-specific metagenome.
- It then differentially regulates that metagenome.
  - These two types of regulation differ *at least* in time scale.

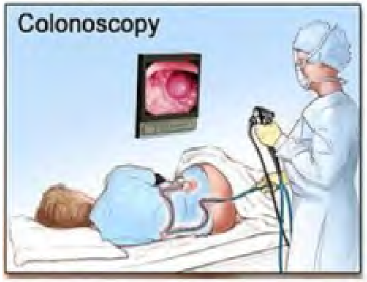




How consistent are the top ten...

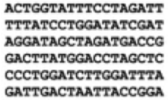


Analytical Pipeline



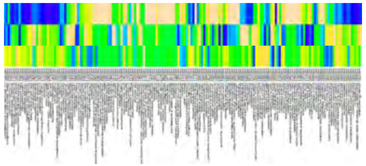
Colonic samples
100s of patients and controls

OTUs


ACTGGTATTTCCTAGATT
TTTATCCTGGATATCGAT
AGGATAGCTAGATGACCG
GACTTATGGACCTAGCTC
CCCTGGATCTTGGATTTA
GATTGACTAATTACCGGA

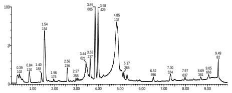
PICRUST
HUMAnN

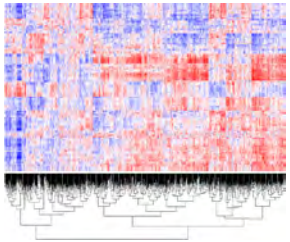
Imputed metagenome



KEGG functional profiling

Metabolites, proteins

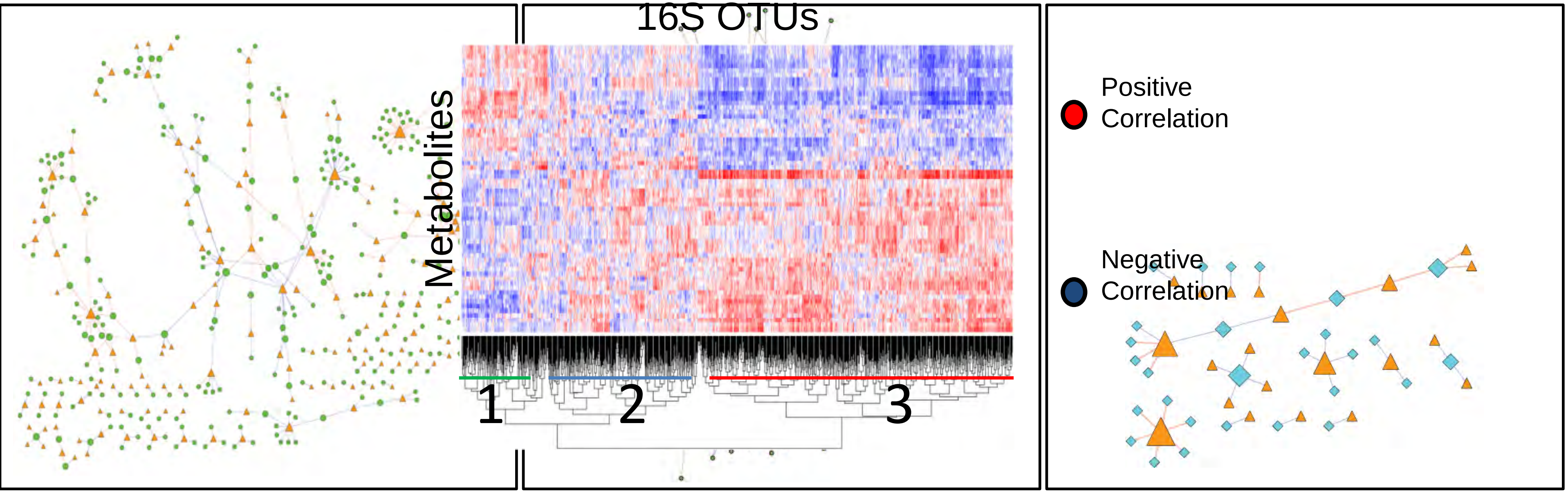




Metabolite comparisons with
bacteria and human proteins

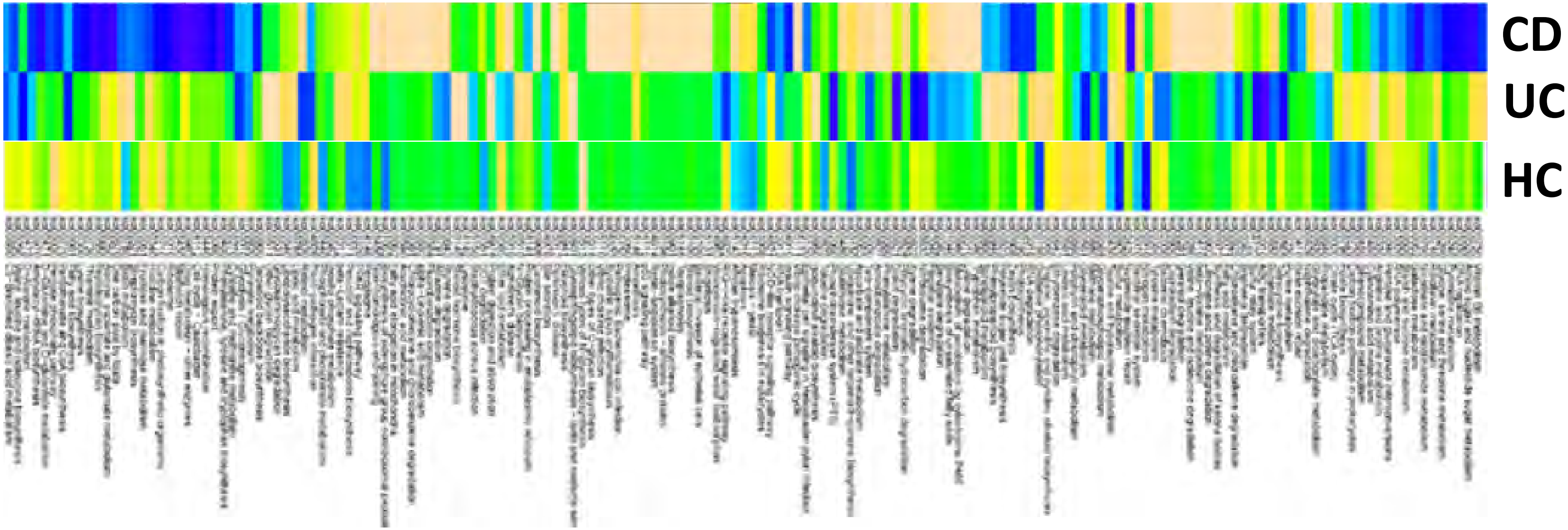
Microbiota are wired to the local metabolic milieu

Bacteria x Human Proteins **Bacteria x Metabolites** **Human Proteins x Metabolites**

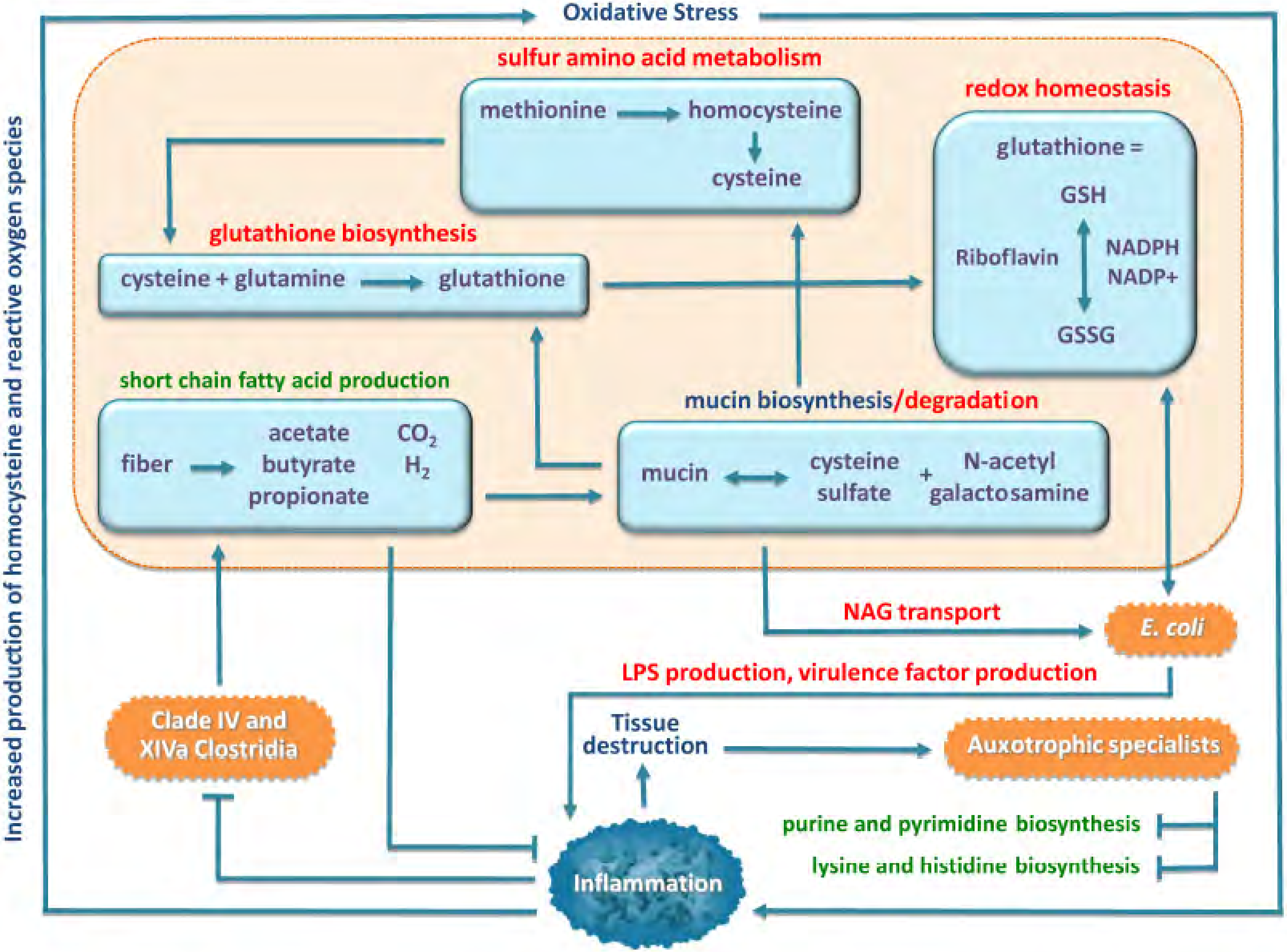


McHardy, Microbiome2013

Functional specialization of microbiota in Crohn’s disease and ulcerative colitis

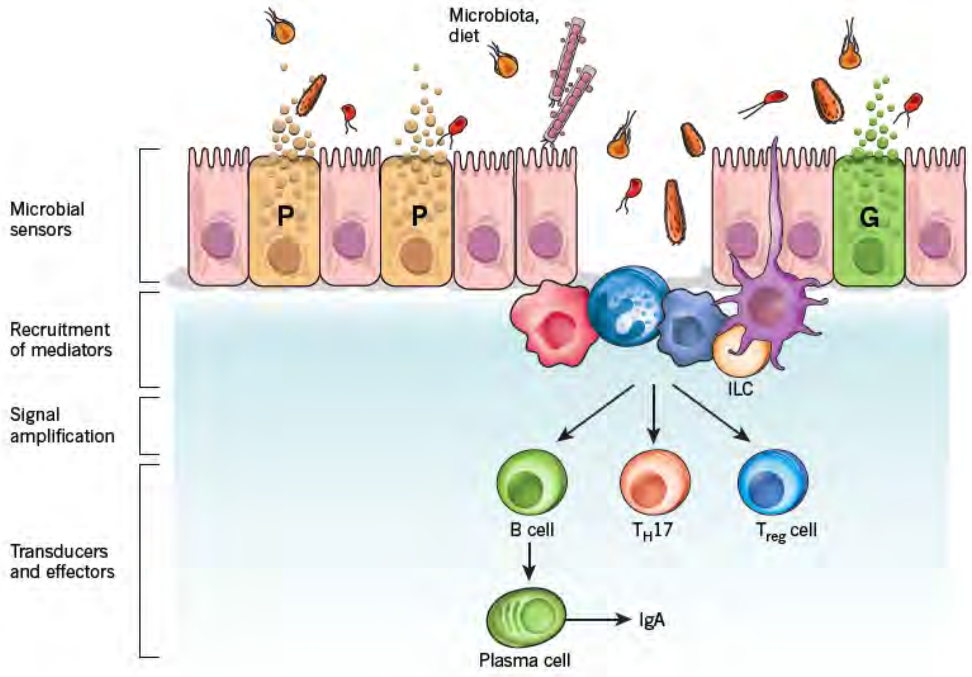


Tong M, in press



Morgan X, Genome Biol 2012

Mucosal inflammation: a genetic and microbial process in intestinal neoplasia and systemic disease



IBD-related processes

Epithelial barrier
GNA12*, HNF4A, CDH1,
ERRFI1, MUC19, ITLN1*

Restitution
REL, PTGER4, NKX2-3, STAT3,
ERRFI1, HNF4A, PLA2G2A/E

Solute transport
SLC9A4, SLC22A5, SLC22A4*,
AQP12A/B, SLC9A3, SLC26A3

Epithelial Barrier

Paneth cells
ITLN1*, NOD2*, ATG16L1*, XBP1*

Innate mucosal defence
NOD2*, ITLN1*, CARD9*,
REL, SLC11A1, FCGR2A*/B

Immune cell recruitment
CCL11/CCL2/CCL7/CCL8,
CCR6, IL8RA/IL8RB, MST1*

Bacterial Control

Antigen presentation
ERAP2*, LNPEP, DENND1B

IL-23/T_H17
IL23R*, JAK2, TYK2*, STAT3,
ICOSLG, IL21, TNFSF15*

T-cell regulation
NDFIP1, TNFSF8, TAGAP, IL2, IL2R
TNFRSF9, PIM3, IL7R*, IL12B, IL23
PRDM1, ICOSLG, TNFSF8, IFNG, IL

B-cell regulation
IL5, IKZF1, BACH2, IL7R*, IRF5

Immune tolerance
IL10, IL27*, SBNO2, CREM,
IL1R1/IL1R2, NOD2*

Immune Regulation

Cellular responses

Autophagy
ATG16L1*, IRGM, NOD2*,
LRRK2, CUL2, PARK7, DAP

ER stress
CPEB4, ORMDL3,
SERINC3, XBP1*

Intracellular logistics
VAMP3, KIF21B, TTLL8,
FGFR1OP, CEP72, TPPP

Cell migration
ARPC2, LSP1, AAMP

Apoptosis/necroptosis
FASLG, THADA*,
DAP, PUS10, MST1*

Carbohydrate metabolism
GCKR*, SLC2A4RG

Oxidative stress
PRDX5, BACH2, ADO, GPX4, GPX1*, SLC22A4, LRRK2,
NOD2*, CARD9*, HSPA6, DLD, PARK7, UTS2*, PEX13

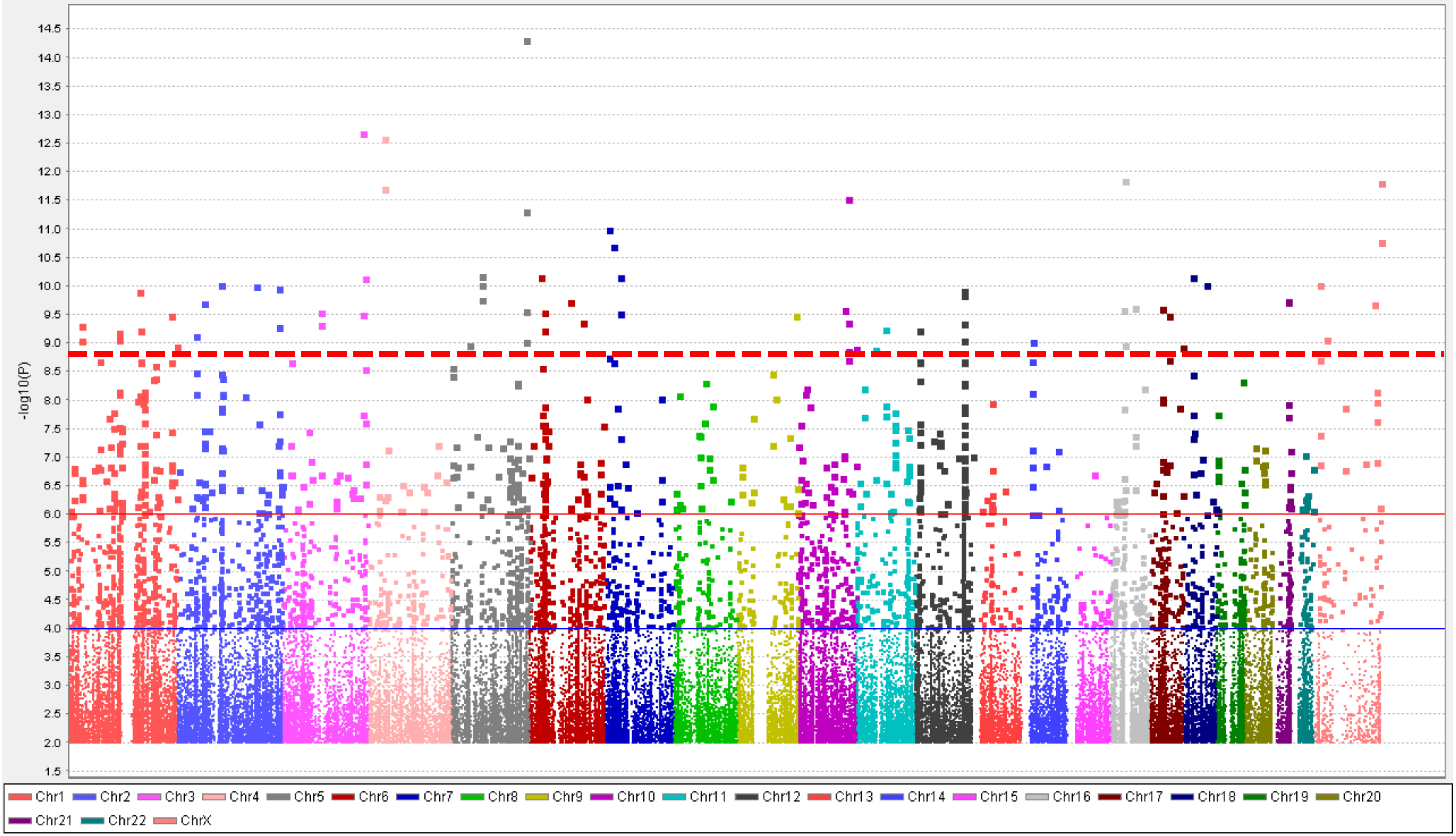
UC
CD
UC/CD
cis-eQTL
*Coding mutation

Cellular Stress

Nature (2011) 474:307

Do we genetically "garden" or microbiome?

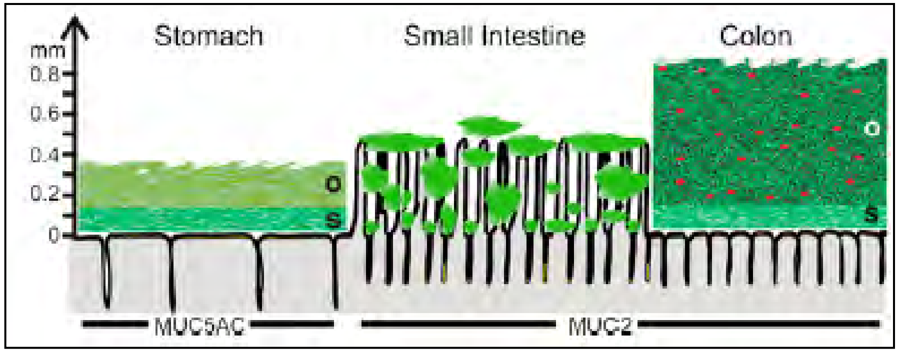
Human QTL analysis for 115 highest abundance bacterial taxa



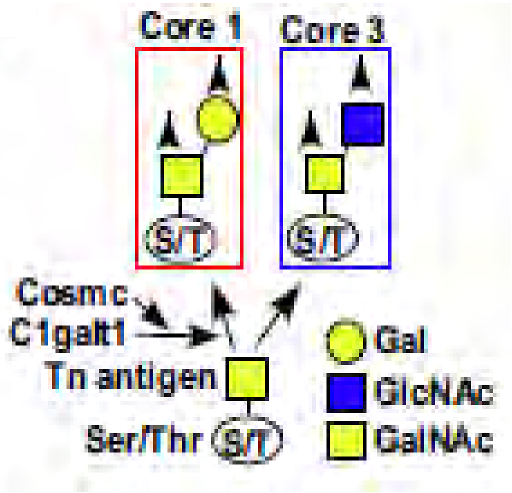
Tong M, in preparation

Mucin O-glycan genetics and IBD

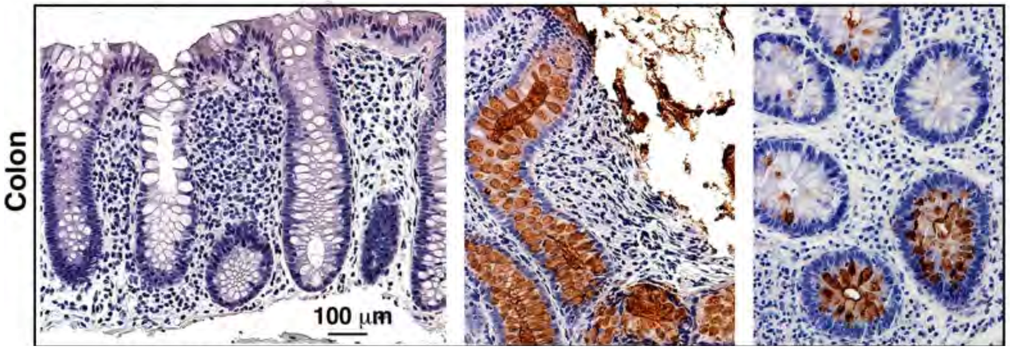
Lumen (food)



Intestinal Wall



- **COSMC**
- **Core 1 O-glycanase**
- **Fut2**

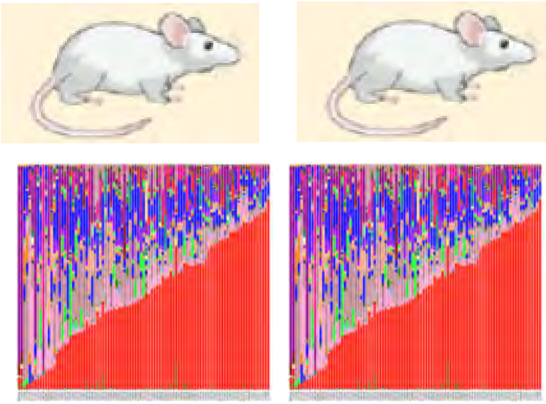


Johansson MV, PNAS 2010

FUT2 risk polymorphism (null) in healthy individuals

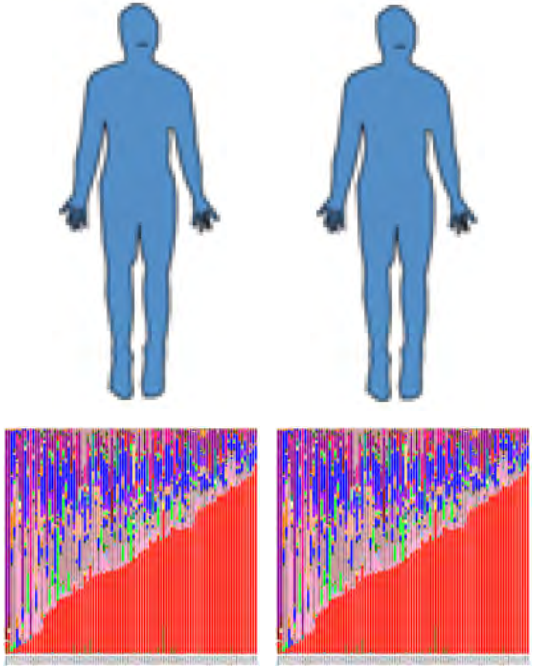
WT

Fut2-/-



SE

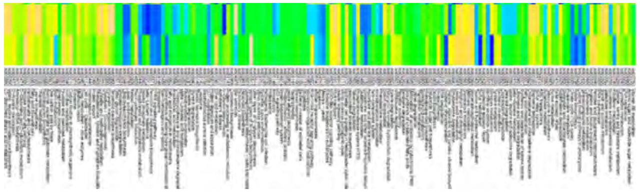
se



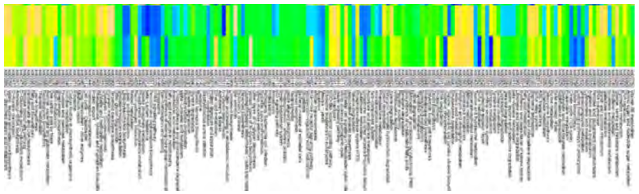
Microbial composition in members of each genetic group

PICRUST
HUMAnN

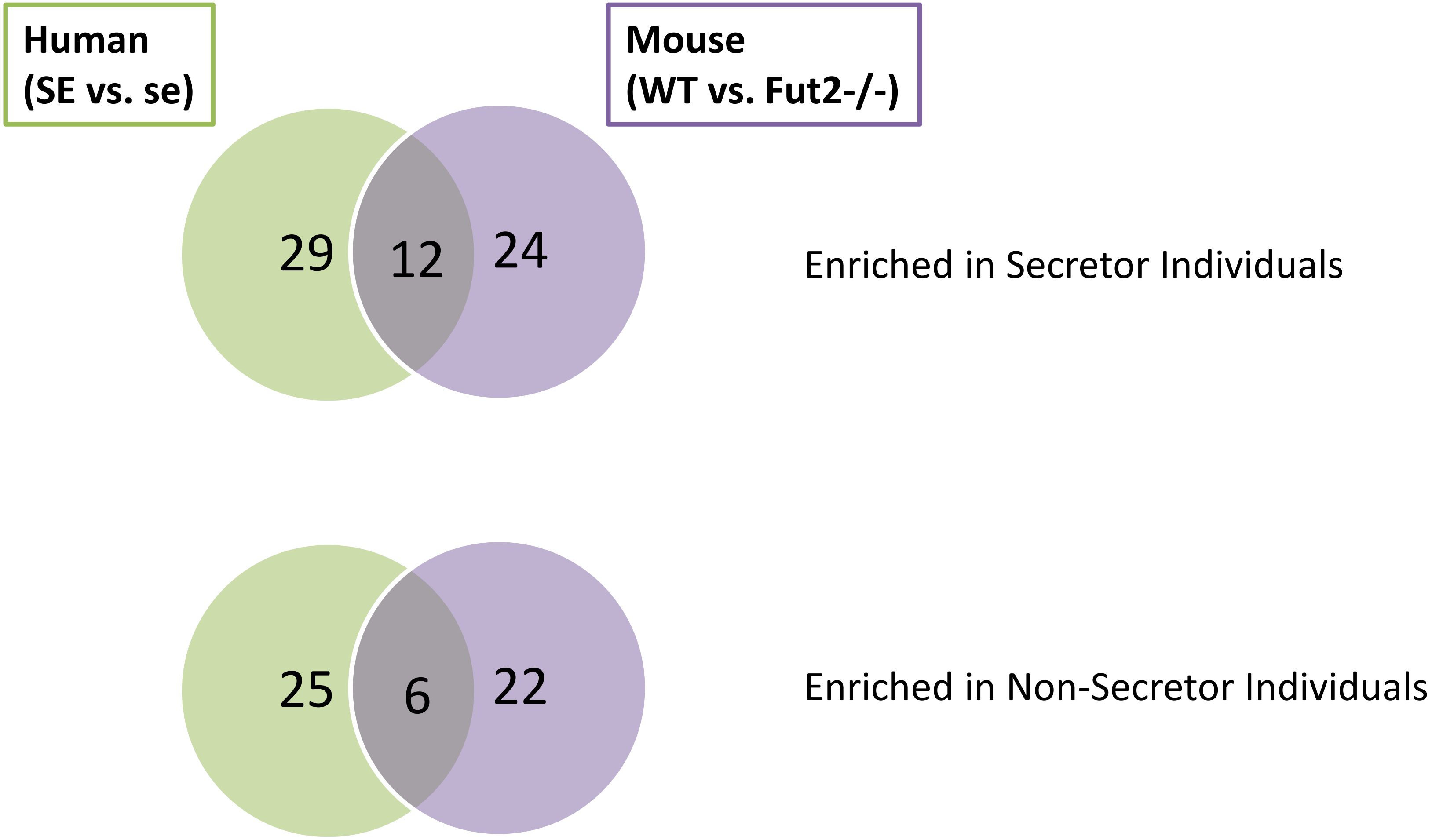
PICRUST
HUMAnN



Differential microbial genes



KEGG pathway genes concordant in human and mouse microbial communities after FUT2 gardening



FUT2 Polymorphism Pervasively Affected the Metabolic Functions Encoded by Gut Microbiome

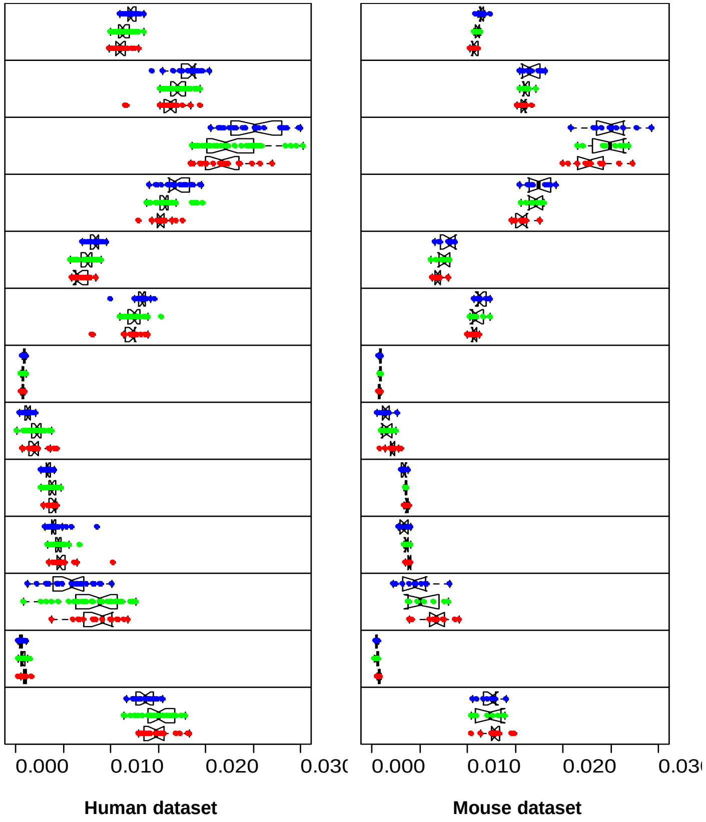
Enriched in *SeSe* individuals

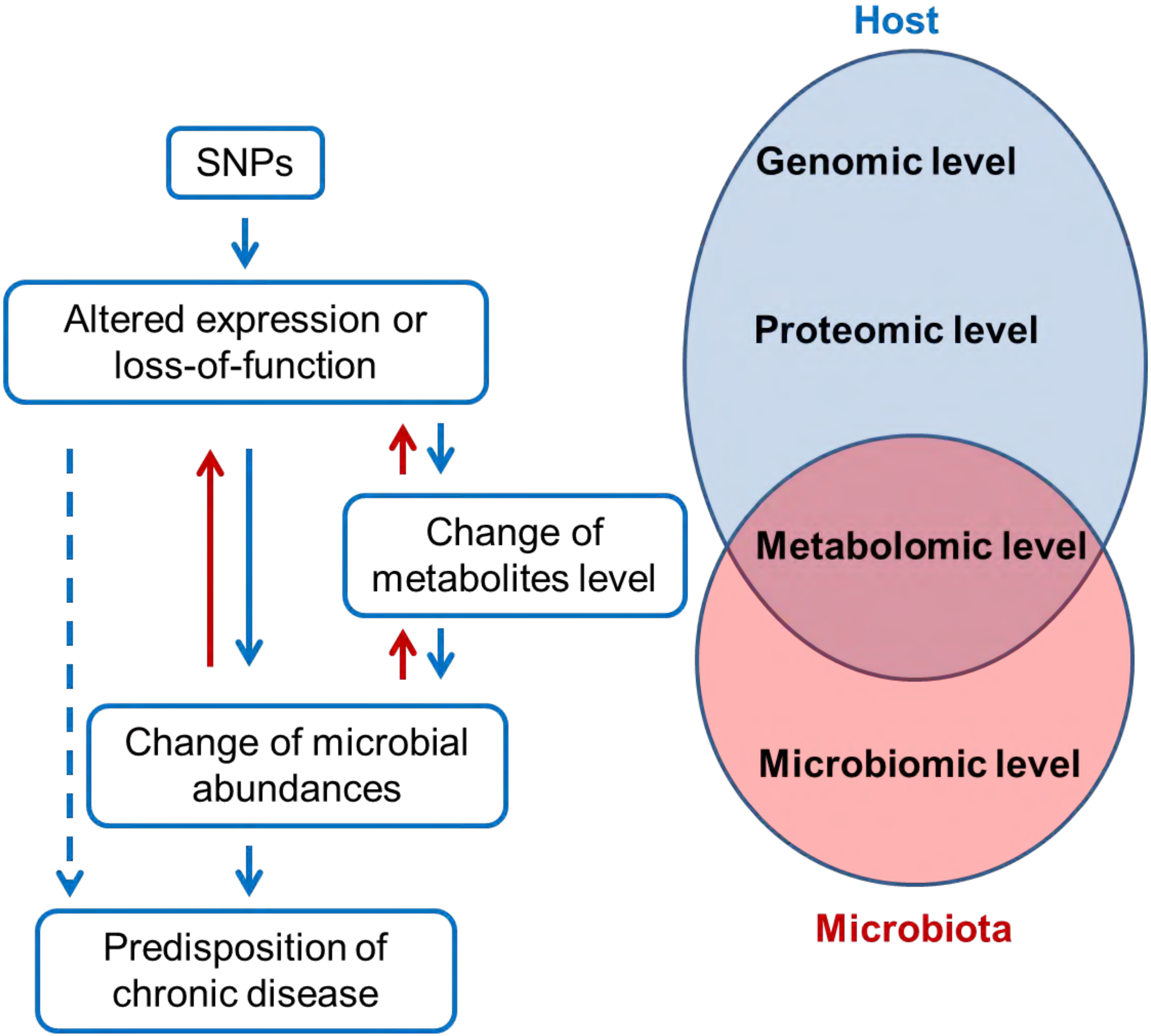
- Cysteine and methionine metabolism
- Lysine biosynthesis
- C5-Branched dibasic acid metabolism
- Pantothenate and CoA biosynthesis
- Porphyrin and chlorophyll metabolism
- Terpenoid backbone biosynthesis
- RNA transport

Enriched in *Sese* and *sese* individuals

- Penicillin and cephalosporin biosynthesis
- Phenylalanine metabolism
- Glutathione metabolism
- Lipopolysaccharide biosynthesis
- Arachidonic acid metabolism
- Biotin metabolism

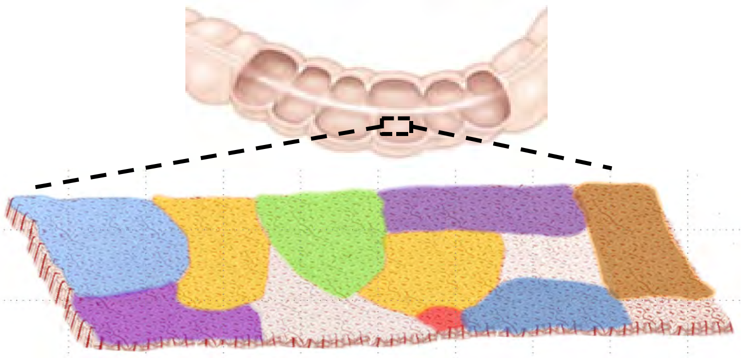
***SeSe*/*FUT2*$^{+/+}$**
***Sese*/*FUT2*$^{+/-}$**
***Sese*/*FUT2*$^{-/-}$**




SNPs
Altered expression or loss-of-function
Change of metabolites level
Change of microbial abundances
Predisposition of chronic disease
Host
Genomic level
Proteomic level
Metabolomic level
Microbiomic level
Microbiota

Plan of talk

- Disease association processes of the microbiome
  - Hit and run (the neonatal window)
  - Good and bad guys (C. difficile, atherosclerosis)
  - Good and bad ecosystems (obesity, behavior, IBD, systemic lymphoma)
- Functional ecology
  - The phylogeny paradox
  - Co-occurrence networks of bacteria, proteins, and metabolites
  - Functional analysis of ecosystems
- **Fixing radios, and the way forward**
  - **Ecosystems repair**
  - **Targeting functional features of the microbiome**
  - **Systems versus pathways**



Repairing the ecosystem

- Experimental observations and ecological principles suggest that disease states are not explained by individual organisms

- The disease-associated microbiome is powerfully described by functional features

- This makes sense mechanistically
  - Microbial products drive disease (or protective) host states
  - Inter-microbial interactions determine composition and function

Repairing the ecosystem

- Selectively deplete pathobionts?
- Add back deficient benefibionts?
- Intervention by targeting functions
  - Mass action: substrate or end-products
  - Pathway inhibitors
  - Alien functionalbionts

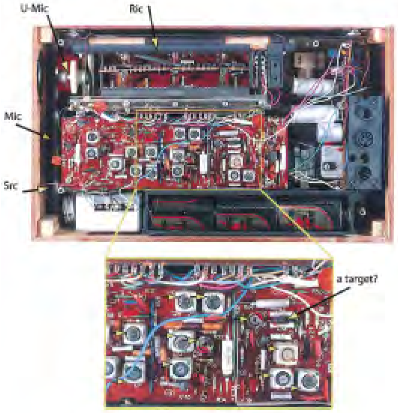
Can a biologist fix a radio?—Or, what I learned while studying apoptosis

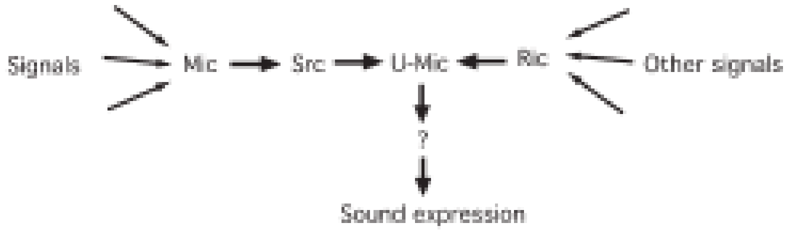
CANCER CELL : SEPTEMBER 2002

Yuri Lazebnik

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724
E-mail: lazebnik@cshl.edu

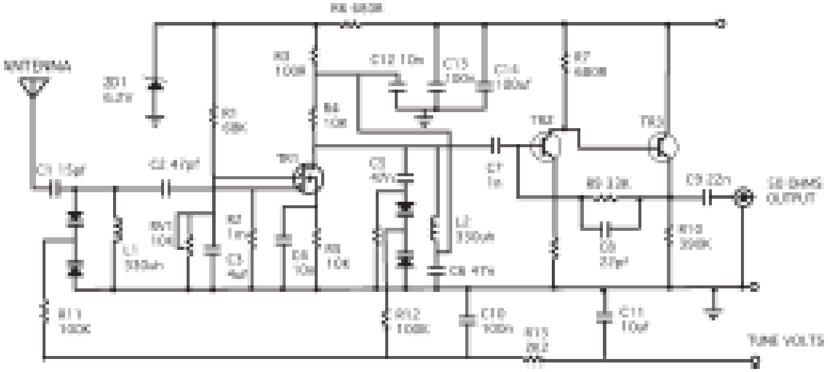
Figure 1. The radio that has been used in this study



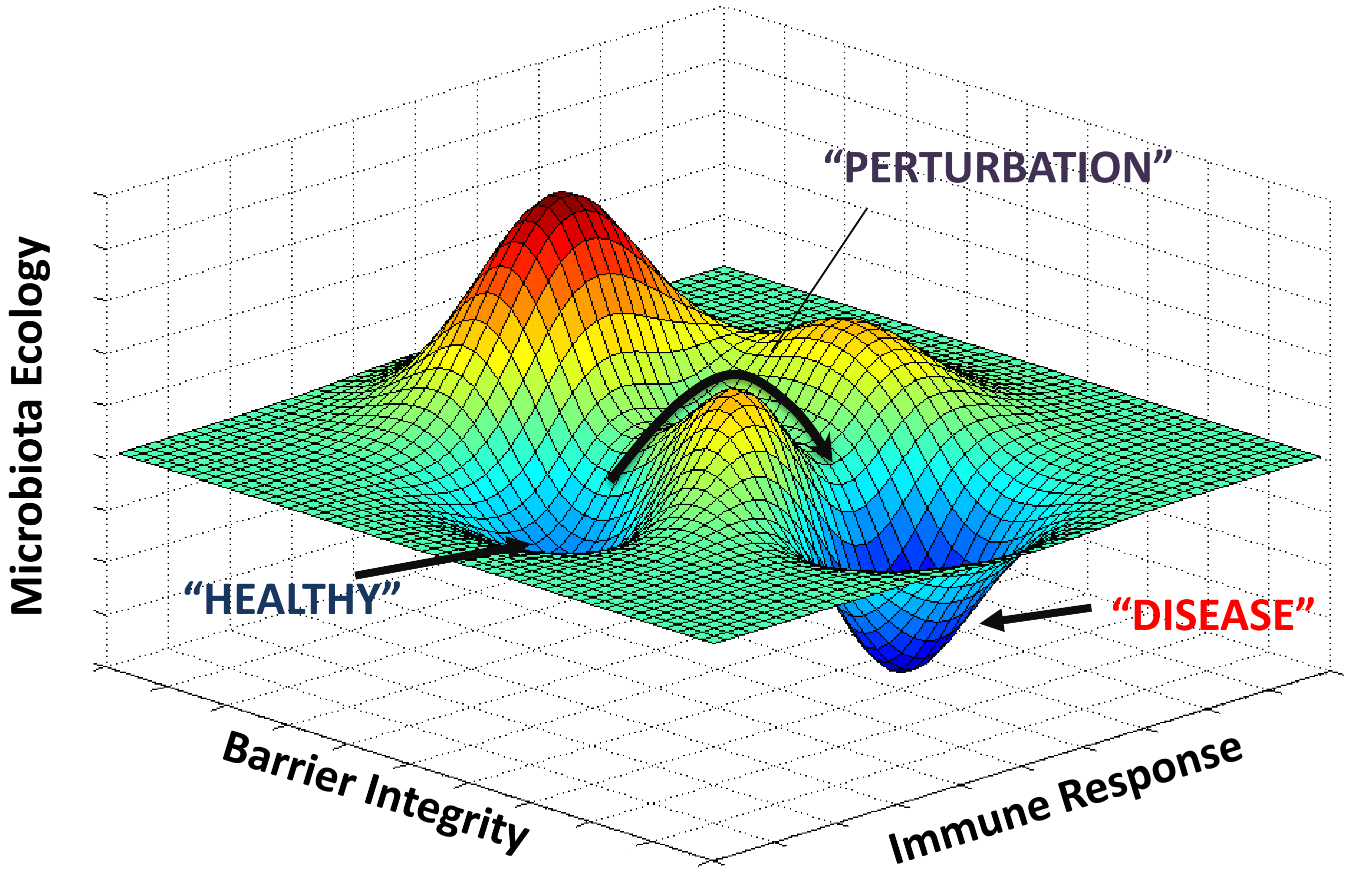


Signals → Mic → Src → U-Mic ← Ric ← Other signals

U-Mic ↓ ? ↓ Sound expression



Alternate ecosystem states
A multi-stable, multi-factorial system



Host genetics and environmental/physiological factors shape the contours of the landscape and determine an individual's ***disease susceptibility or tolerance***

Challenges

- We need robust knowledge of ecosystem networks
  - What are the set of stable ecosystem states?
  - How do we navigate between them?
- What are the microbiome problems in each disease?
  - A hit and run event (obesity, NKT cell immunity)
  - A broken or altered component (C. difficile, atherosclerosis)
  - The wrong ecosystem state (obesity, IBD)
- Will hub or causal targets be more robust for intervention?
- We need the right analytics to define and testt systems
  - Easy, robust sampling and preanalytic platforms
  - Quantitative functional measurements (transcripts, proteins, metabolites)
  - Component testing experimental design

Harnessing the microbiome by microbial product targeting (2016)

- 250 million people
- Maps have vast amounts and types of data
- Map data accessible by everyone
- Useful in amazing ways

Acknowledgements

Braun Lab
- Jonathan Braun
- Michelle Li
- Ian McHardy
- Maomeng Tong
- Bo Wei

Mouse O-glycan biology
- Lijun Xia (OMRF)
- Justin Sonnenberg (Stanford)

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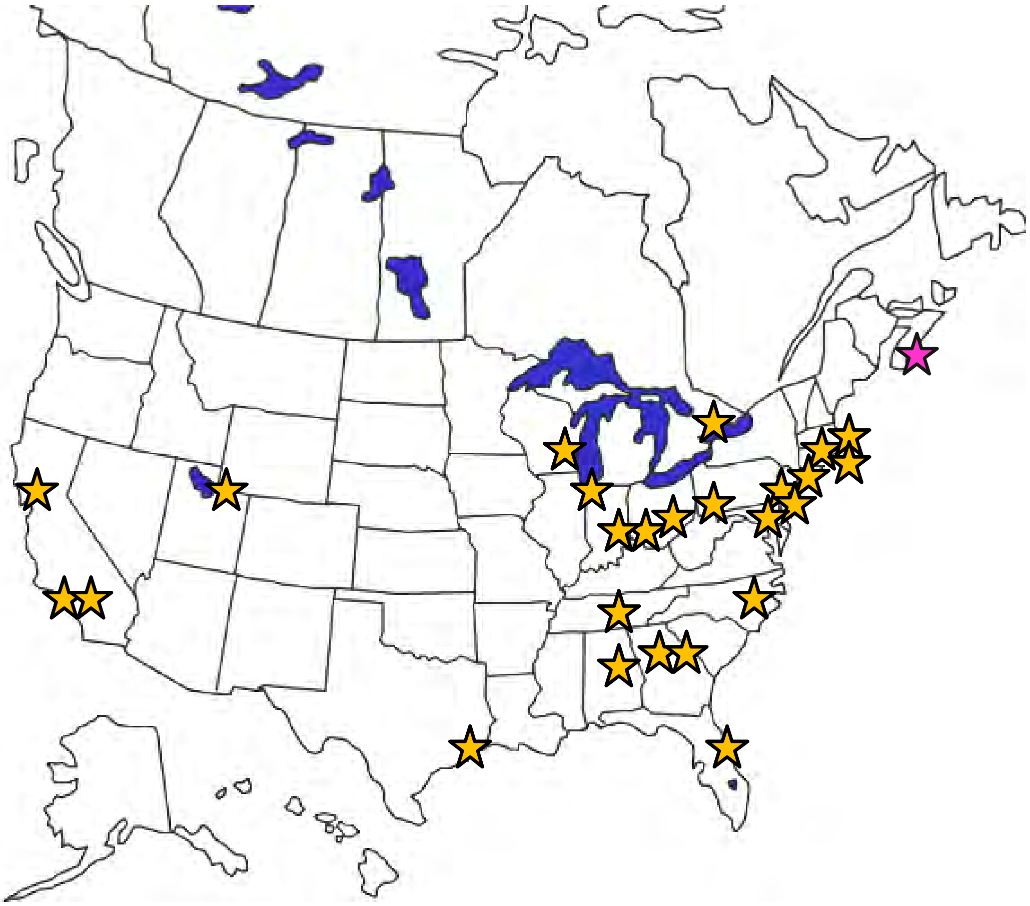
- Dermot McGovern
- Ramnik Xavier
- Curtis Huttenhower
- Balfour Sartor
- Thad Stappenback
- Skip Virgin

MLI consortium
- Dermot McGovern
- James Borneman
- Tom Graeber
- Al Fornace
- Justin Sonnenburg



DNA damage and AT
- Robert Schiestl (UCLA)