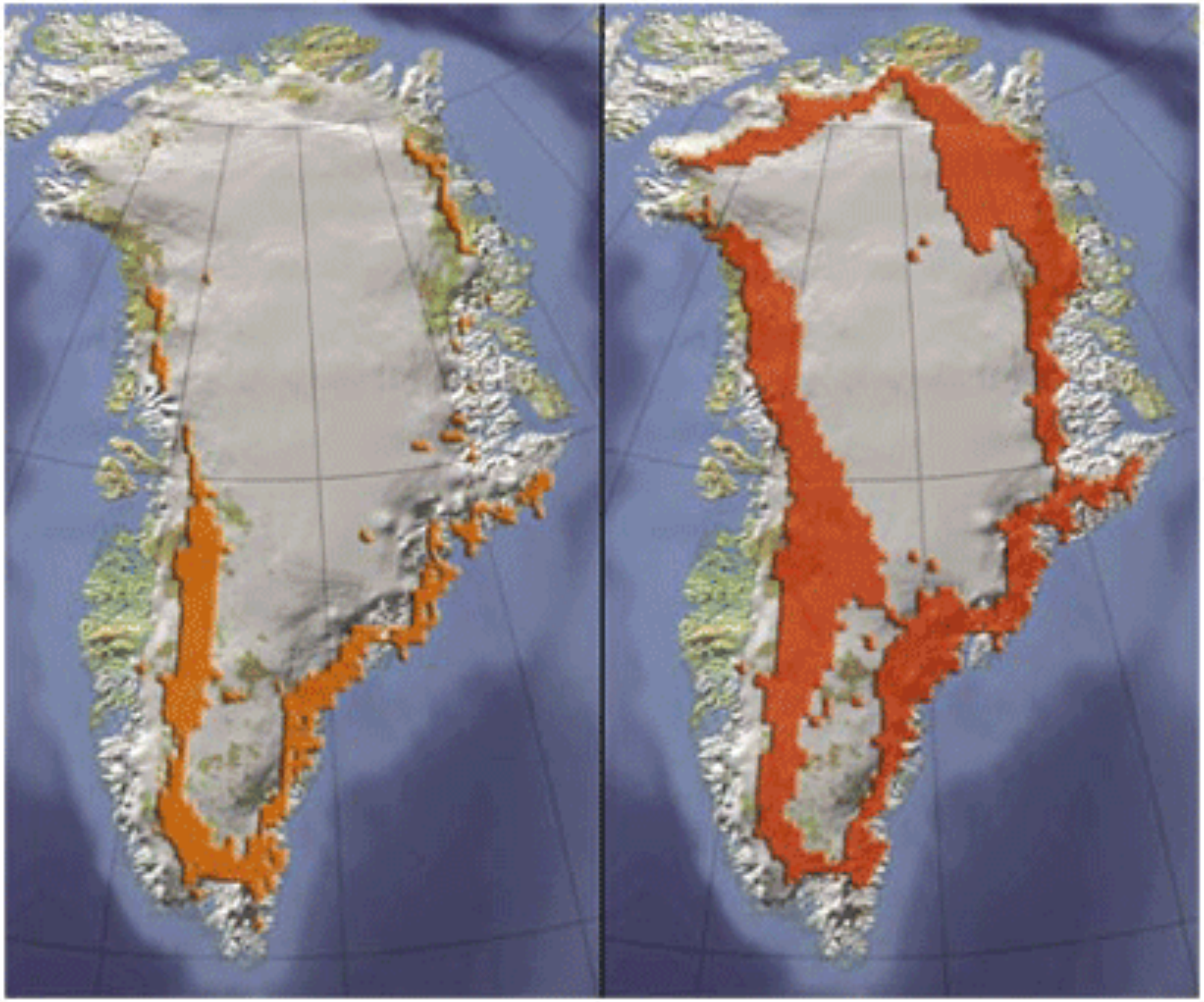
What happens when our early life microbes are perturbed?

Martin J Blaser

Departments of Medicine and Microbiology
New York University School of Medicine
Department of Biology, NYU
New York Harbor VA Medical Center

Greenland Ice Sheet

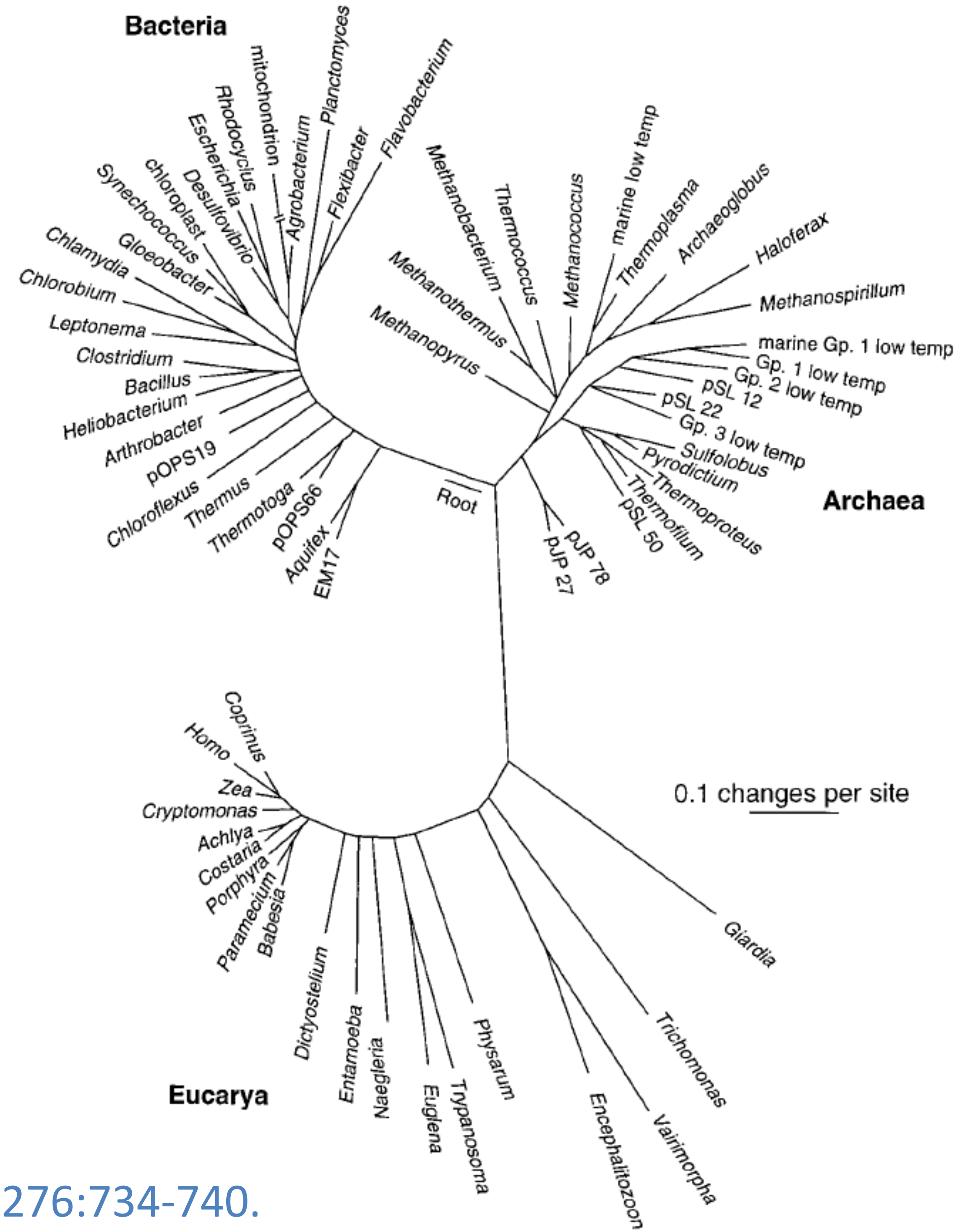


1992 2002

Map by Clifford Grabhorn, from the Arctic Climate Impact Assessment. NEJM 2005; 353:14.

ANTIBIOTICS

The tree of life:
3 kingdoms

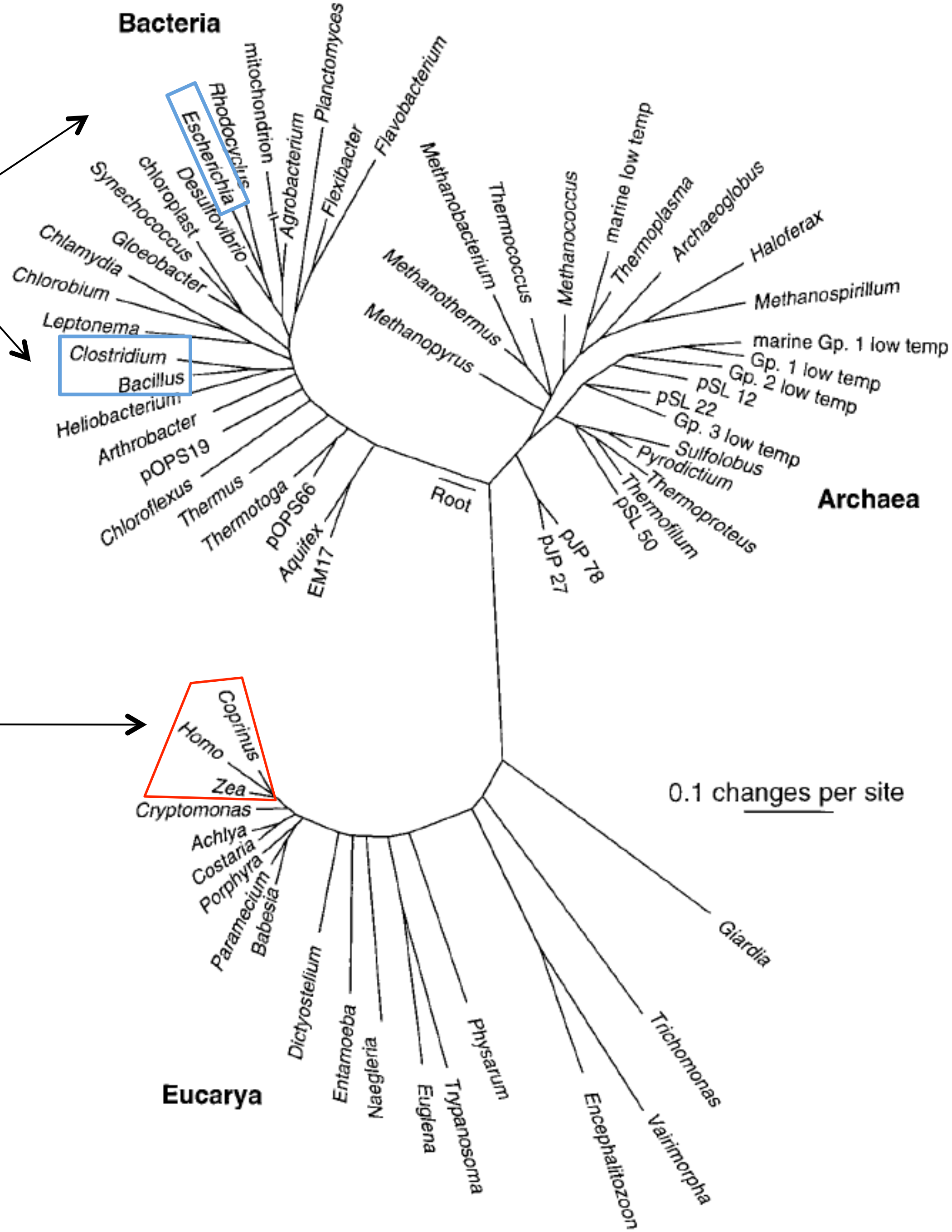


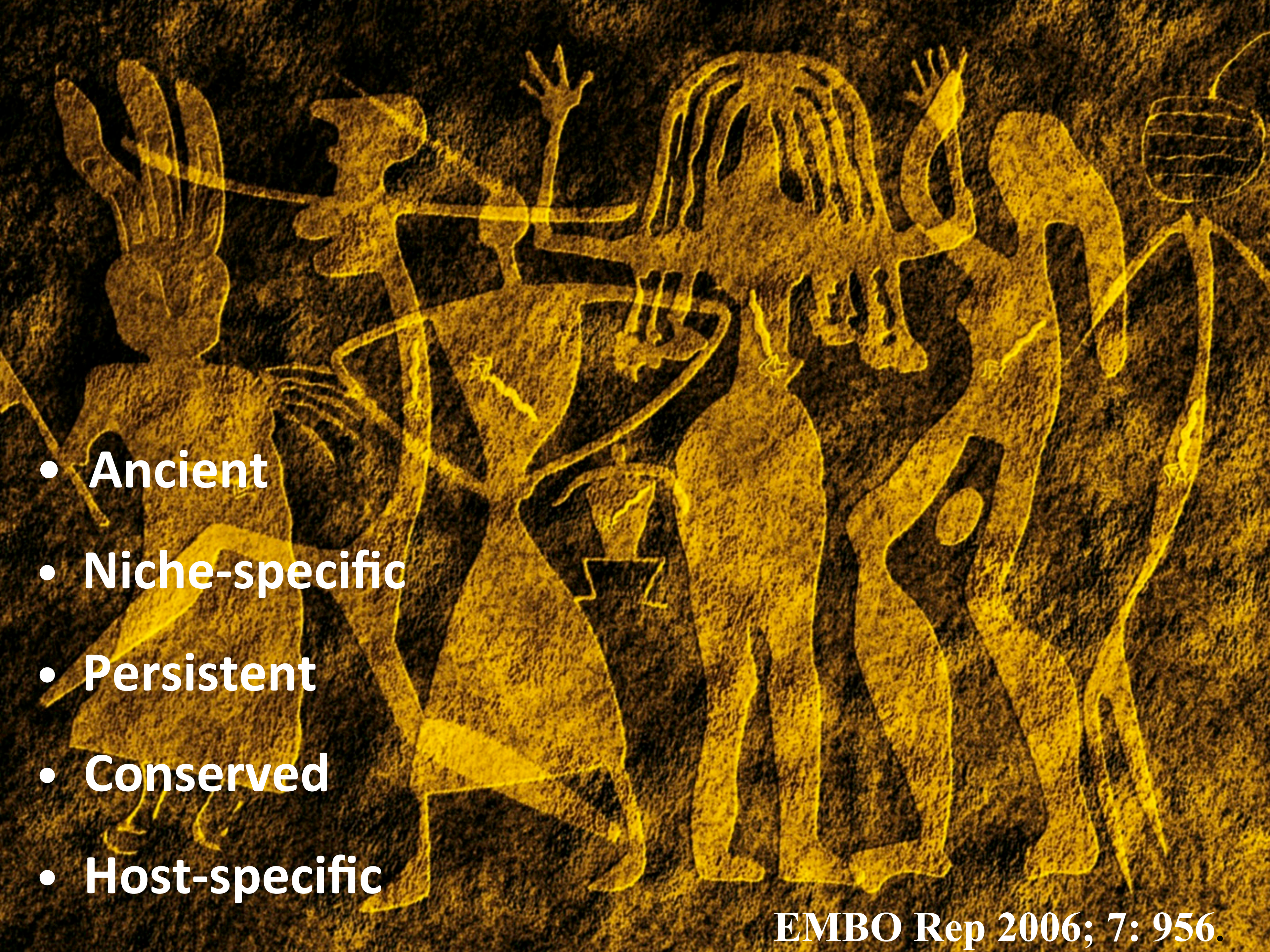
NR Pace. Science 1997;276:734-740.

The tree of life

Distant relatives

Close cousins

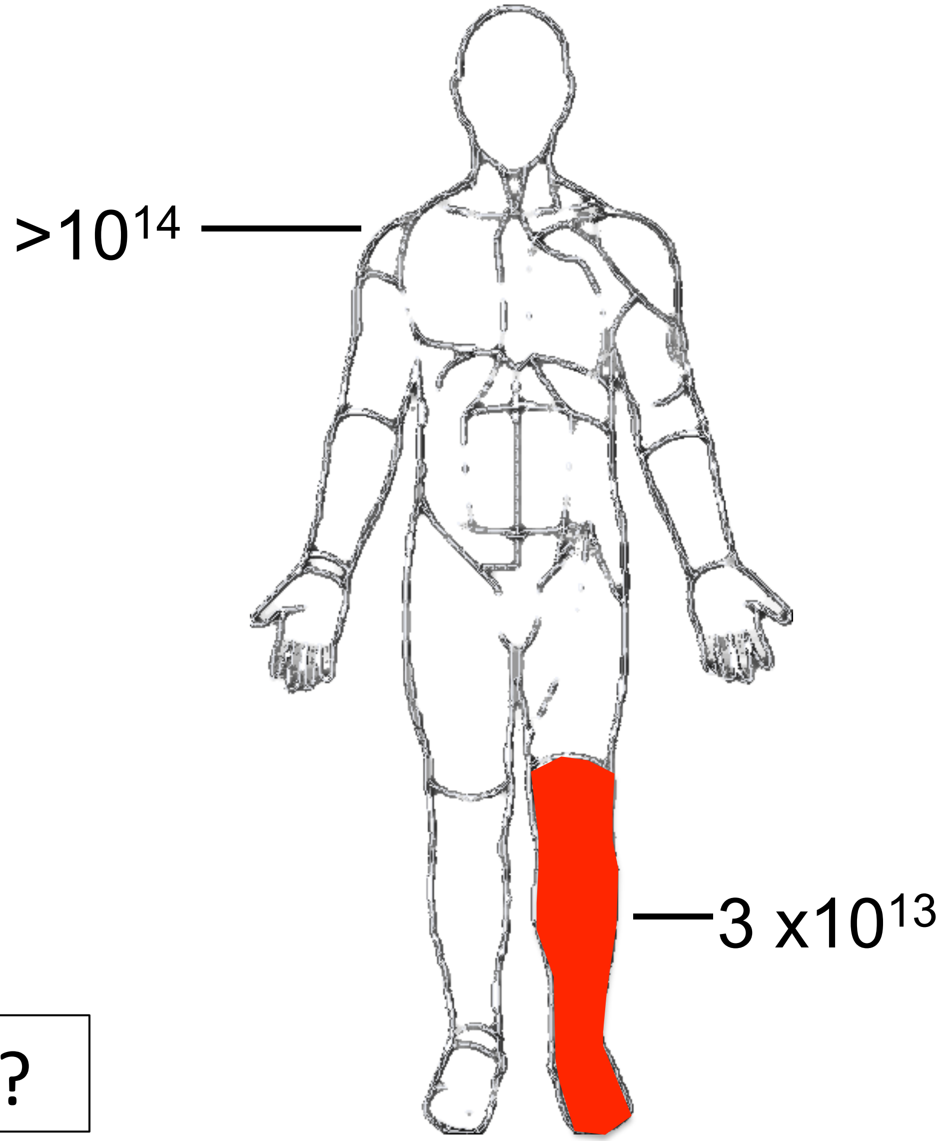




- Ancient
- Niche-specific
- Persistent
- Conserved
- Host-specific

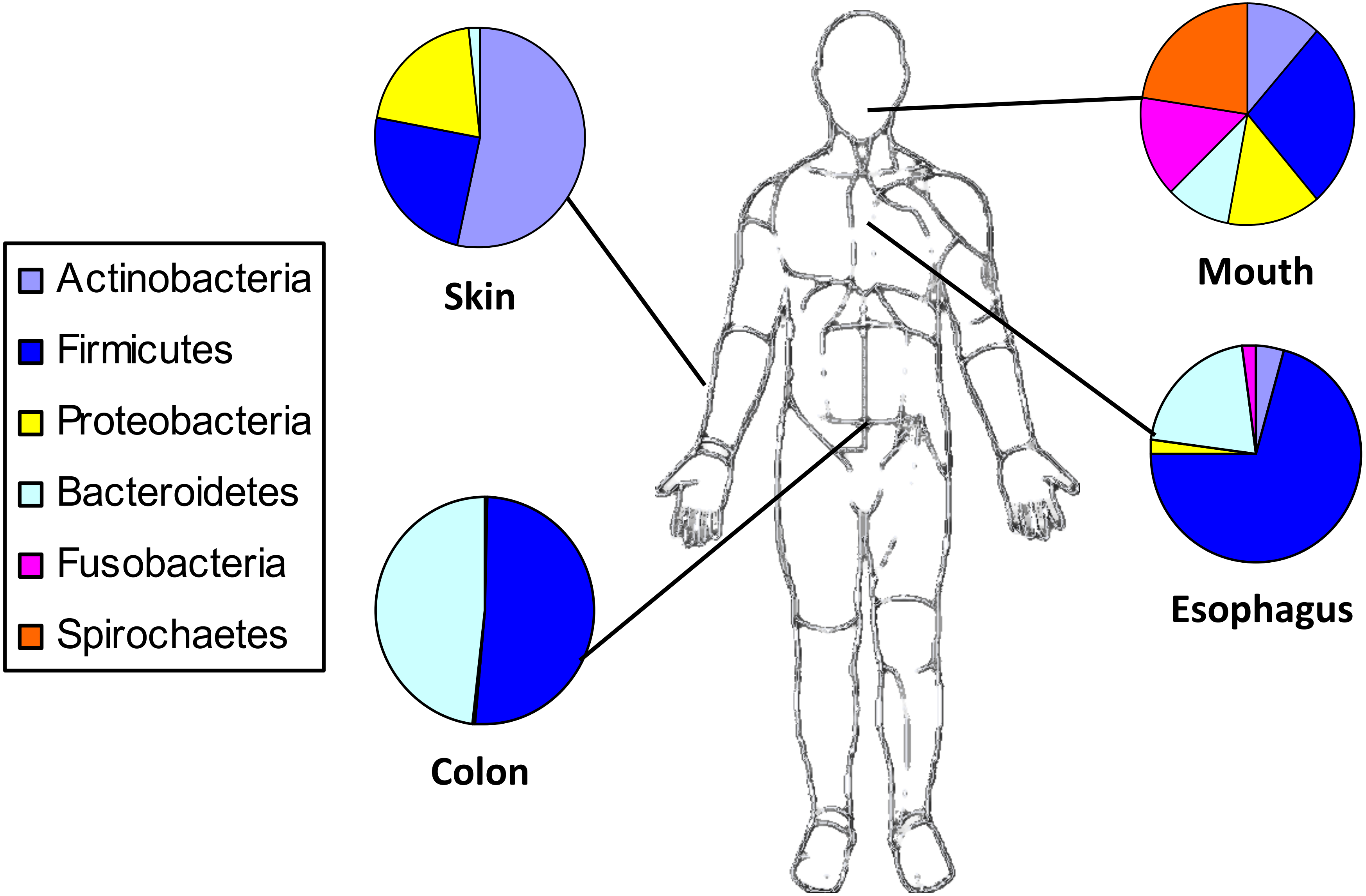
EMBO Rep 2006; 7: 956.

Proportion of cells in the human body

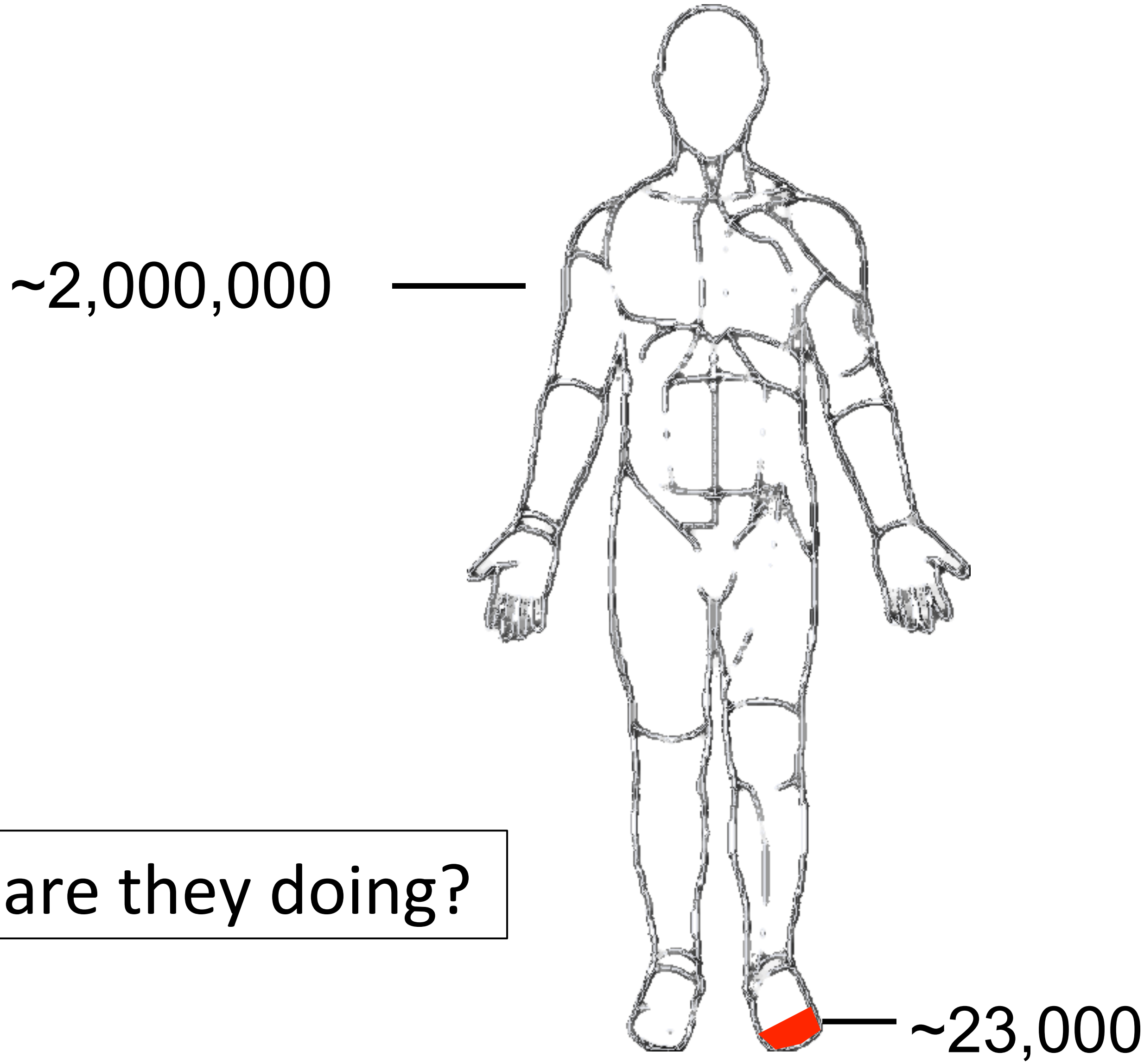


Who are we?

Who they are: bacterial phyla at 4 anatomical sites



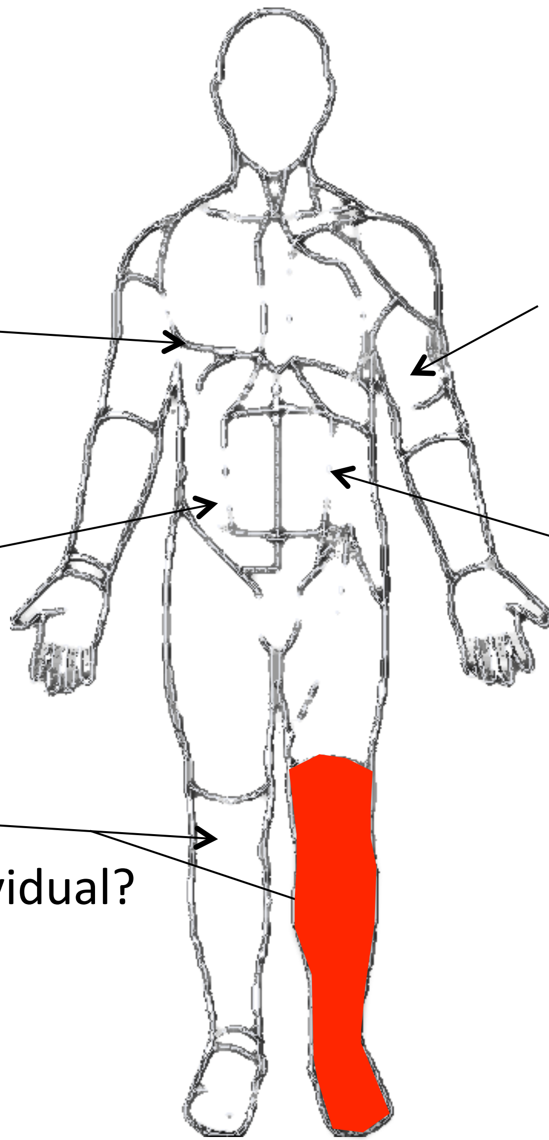
Proportion of unique genes in the human body



What are they doing?

9

Biological questions related to the human microbiome



1. What is the identity of the microbes that populate their host?

2. What are these microbes doing?

3. How is the host responding to them?

4. What are the forces that maintain equilibrium among the populations?

5. What are the unique characteristics of each individual?

PNAS 2010;107:6125.

Biological questions related to the human microbiome

1. What is the identity of the microbes that populate their host?

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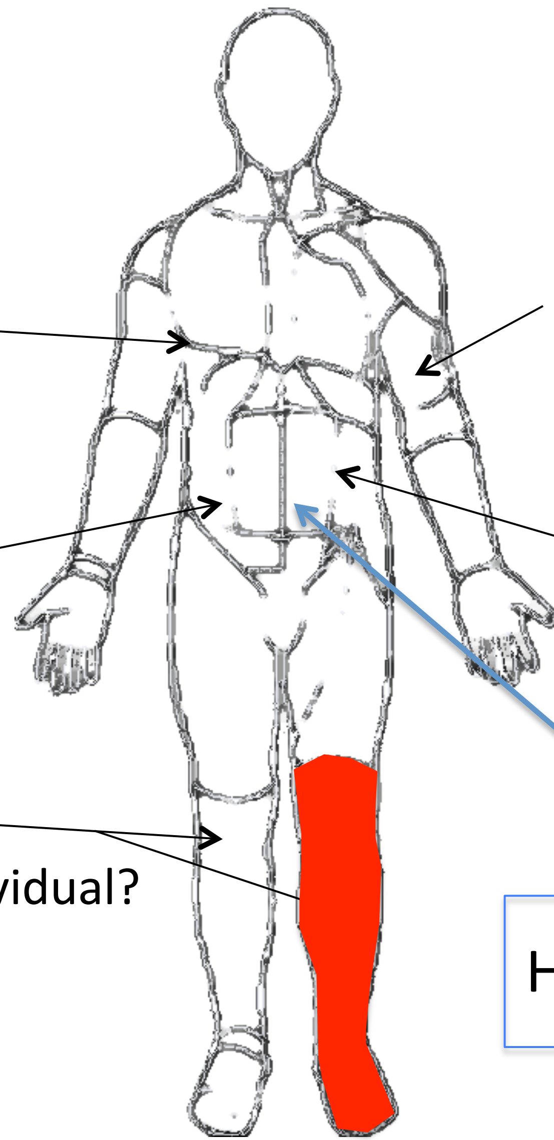
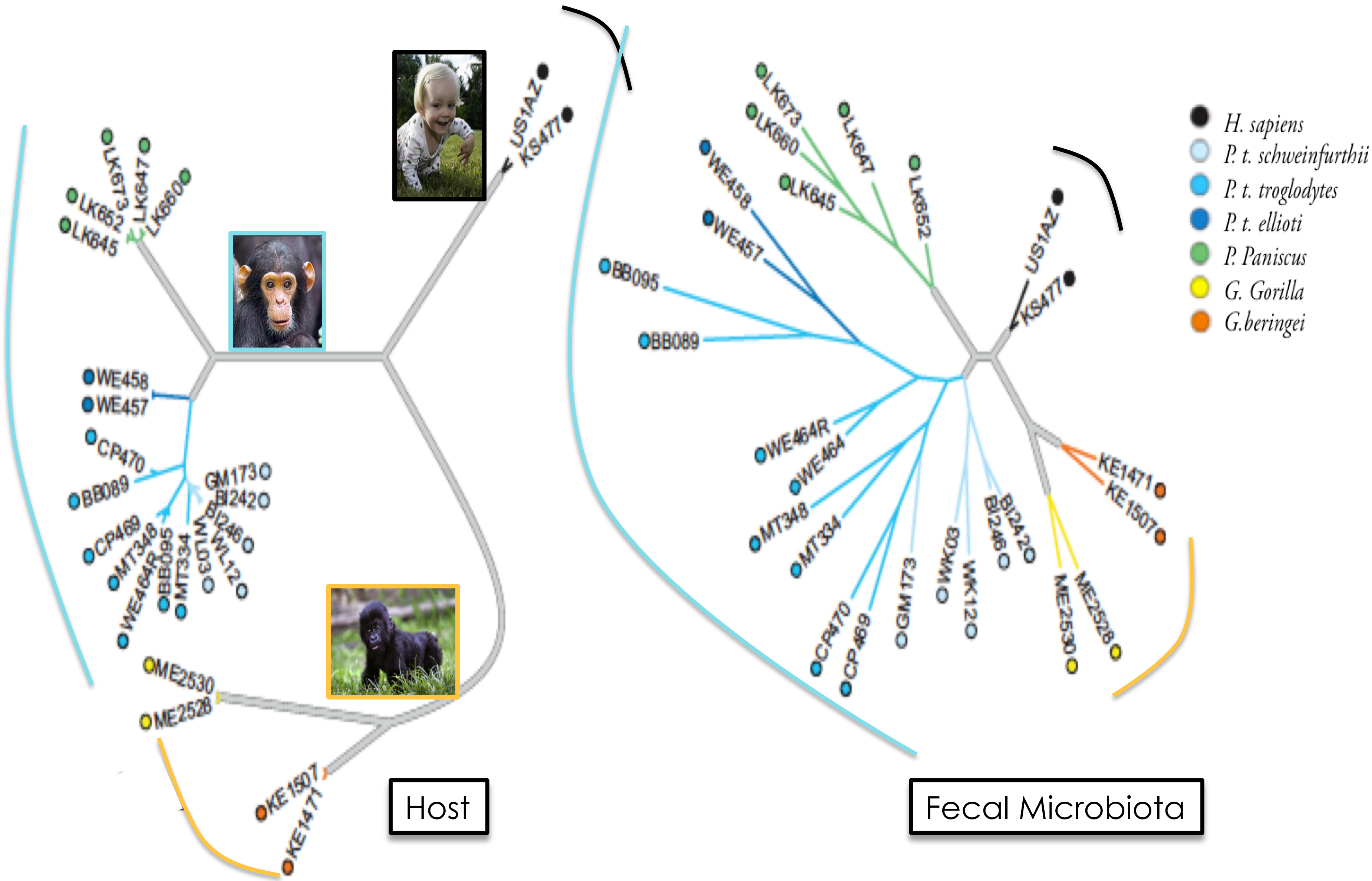


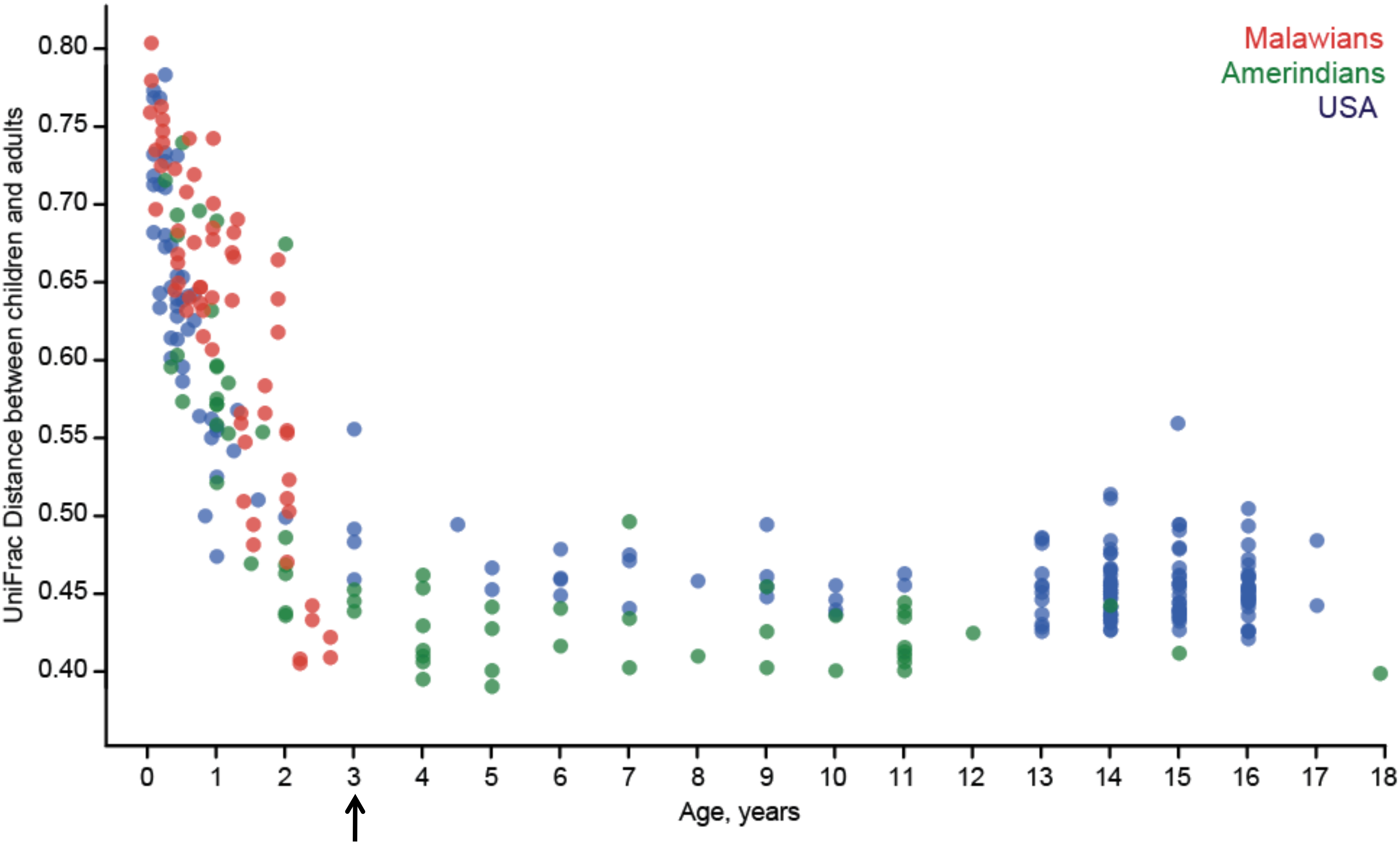
How can we manipulate it?



Evolutionary relationships of wild hominids

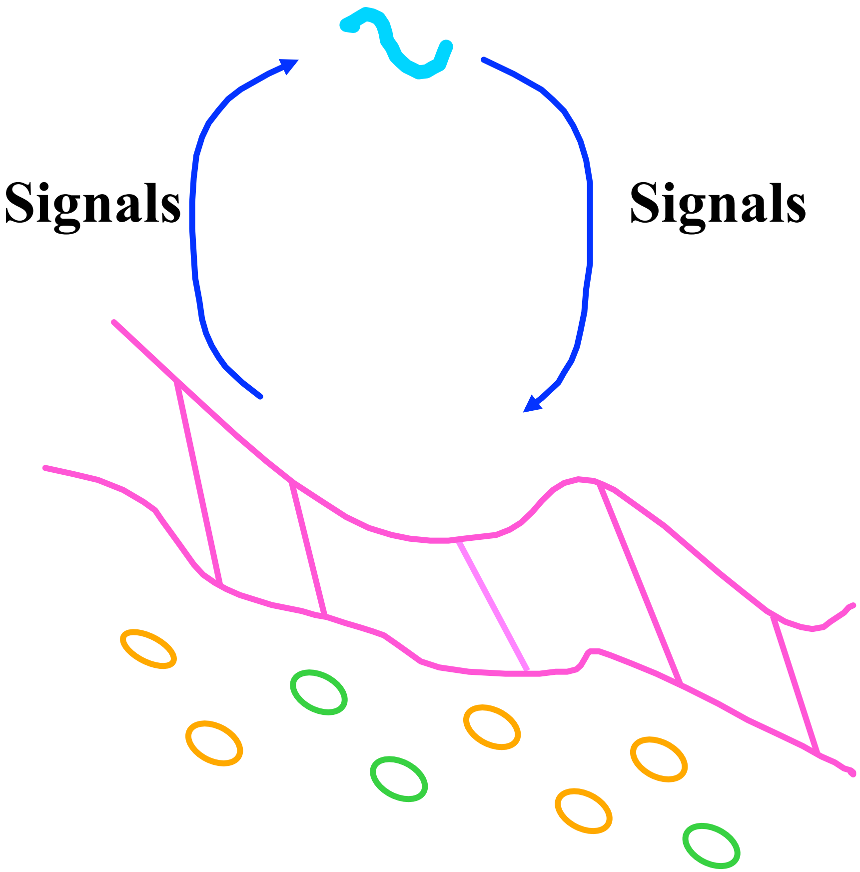
H. Ochman et al. *PLoS Biology* 2010

When does the adult gut microbiome become established?



T Yatsunenko et al. Nature 486, 222-227 (2012)

Schematic of interaction between a co-evolved colonizing microbe and host



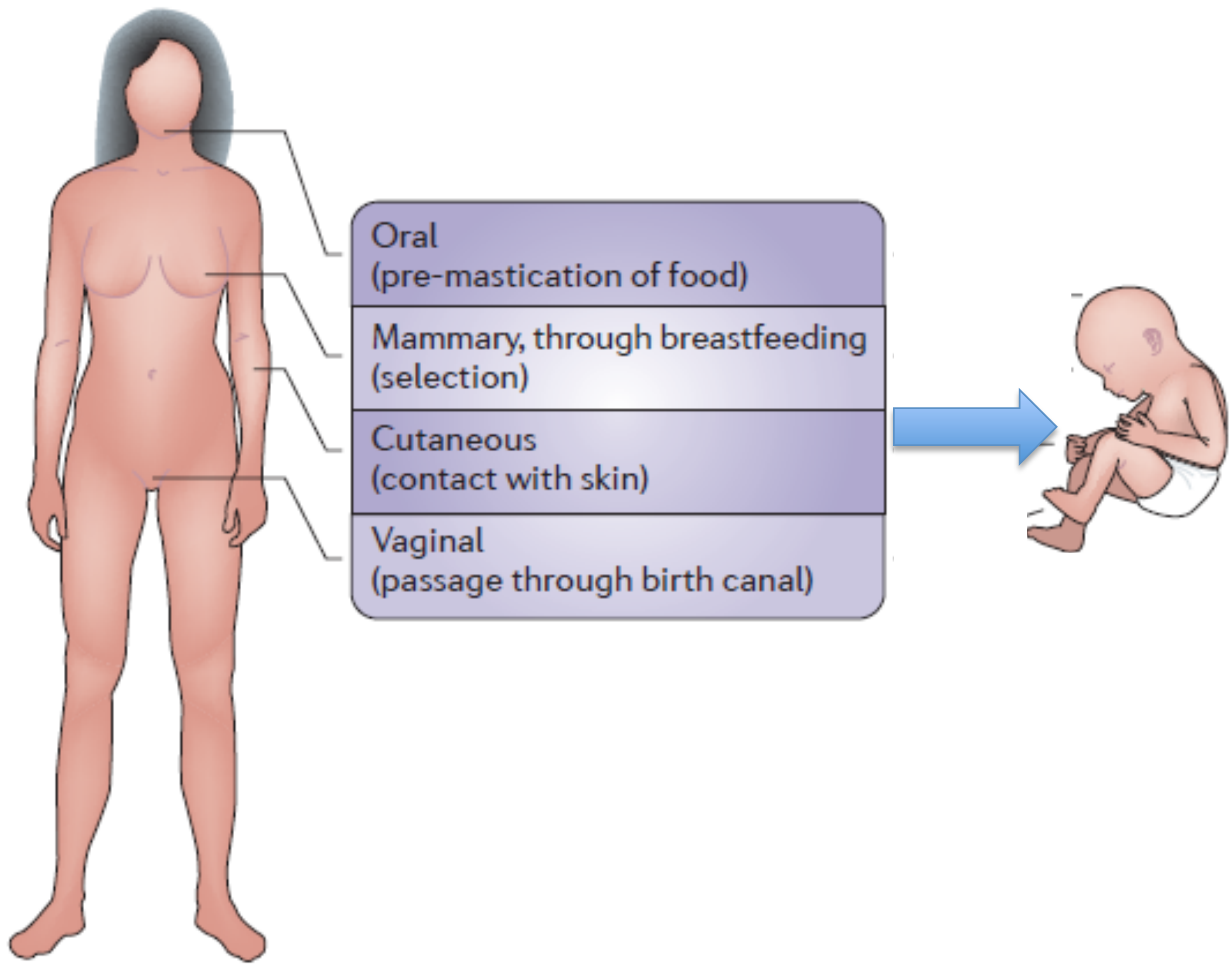
- **Robust**
- **Resilient**

↓

Perturbation?

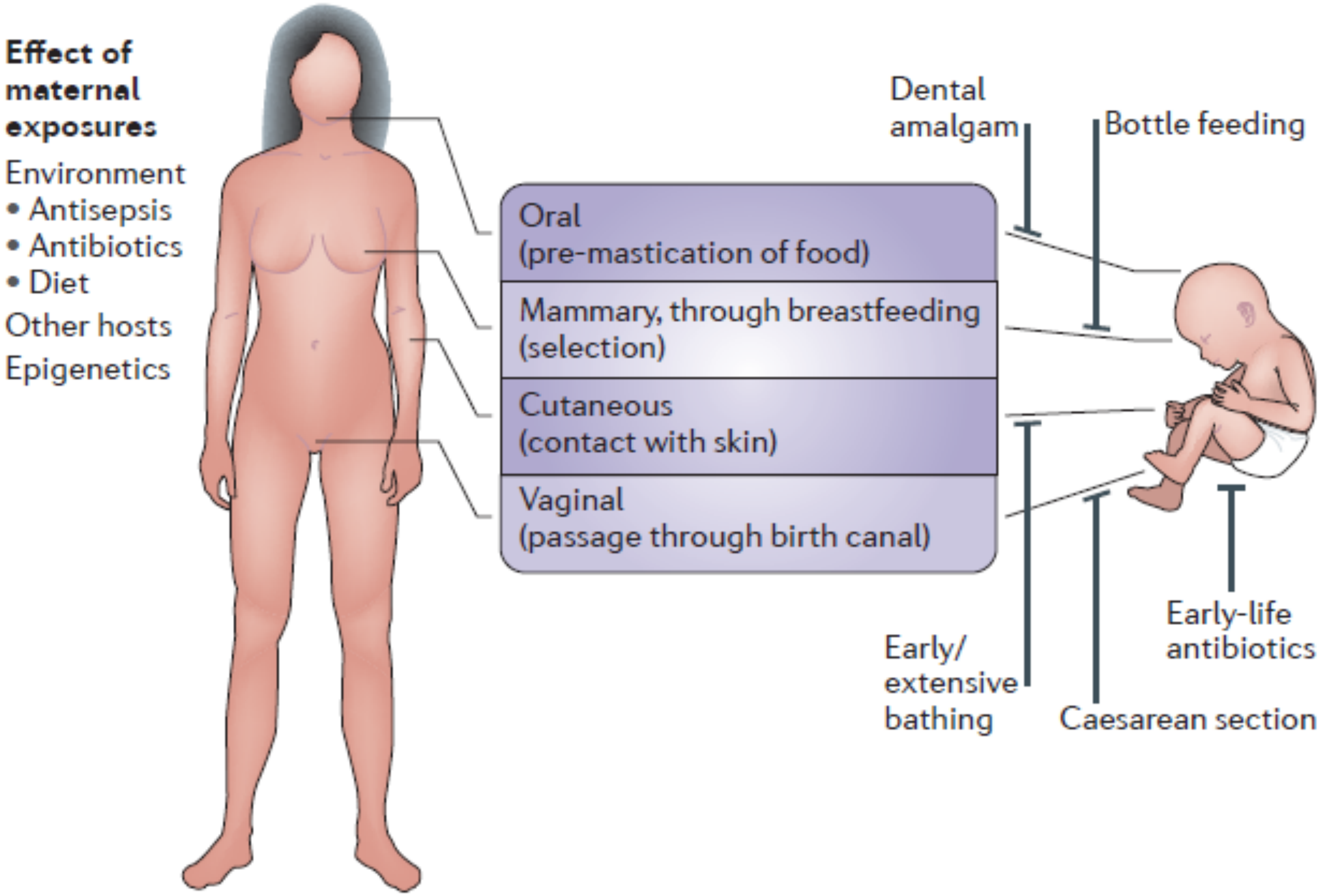
Nature 2007; 449:843.

Mother→ Child Transfer of Microbes (Ancient)



Nature Reviews Genetics 2012;13:260-70

Mother→ Child Transfer of Microbes (Modern)



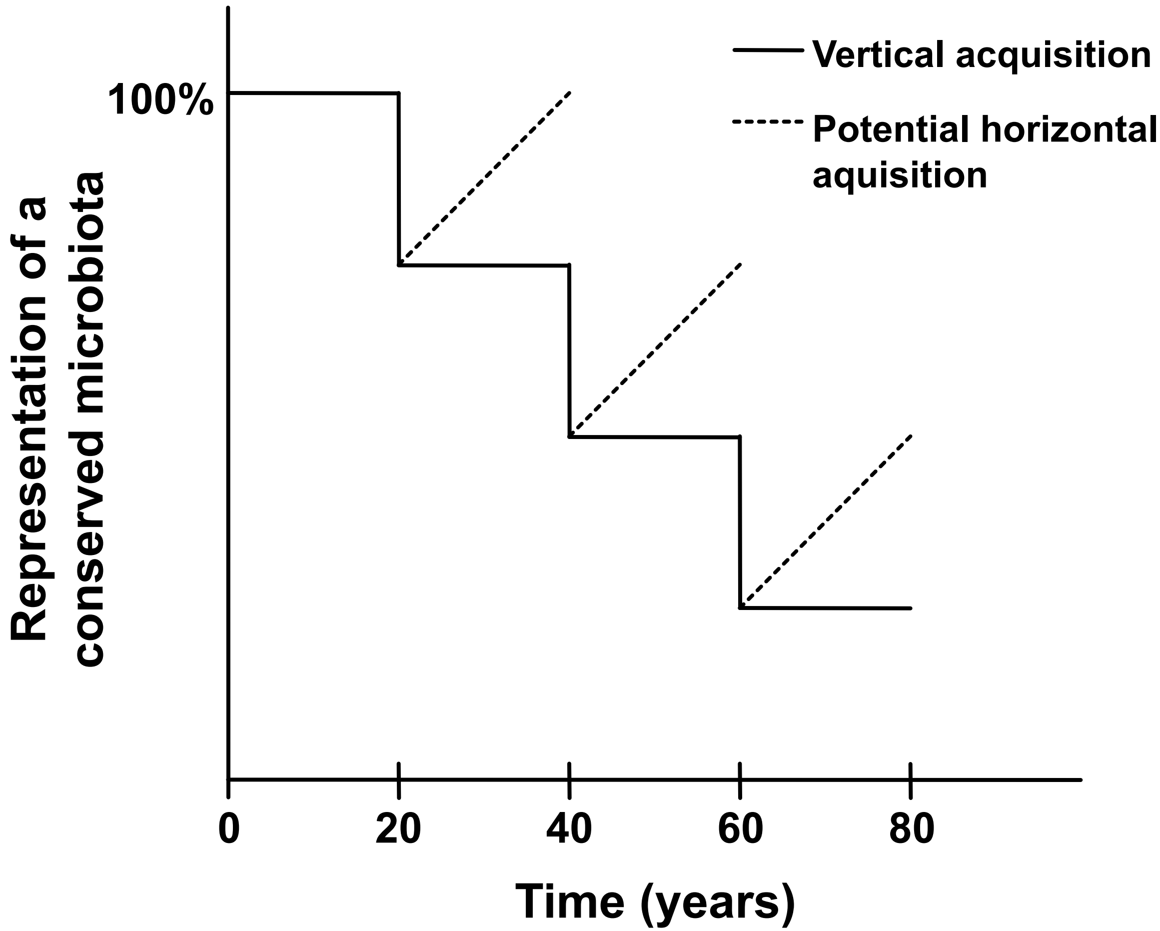
Nature Reviews Genetics 2012;13:260-70

The "Disappearing Microbiota" hypothesis

- Beginning in the late 19th century, changing human ecology has dramatically altered the transmission and maintenance of our indigenous microbiota.
- These changes have affected its composition.
- This altered composition affects human physiology, and thus disease risk.
- Loss of ancestral bacteria, usually acquired **early in life,** is especially important, because it affects a developmentally critical stage.

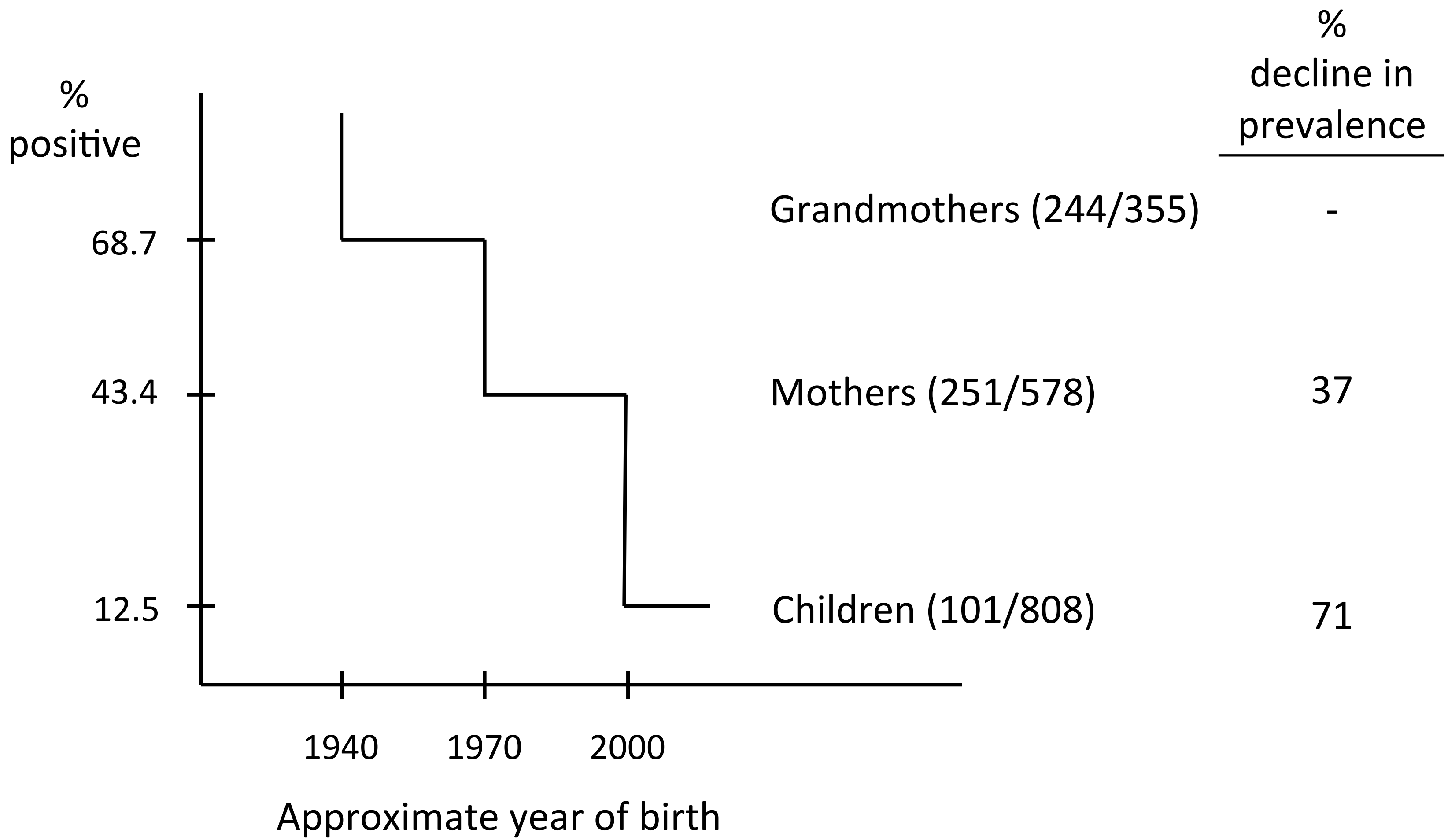
Lancet 1997;349: 1020
Gut 1998; 453: 721
Perspect Biol Med 2002;45:475.
Scientific American 2005; 292: 38.
EMBO Reports 2006; 7:956.
Cancer Prev Res 2008; 1:308.
Nature Reviews Microbiol 2009; 7:887.
Nature 2011; 476:393.

The effect of maternal status on the resident microbiota of the next generation



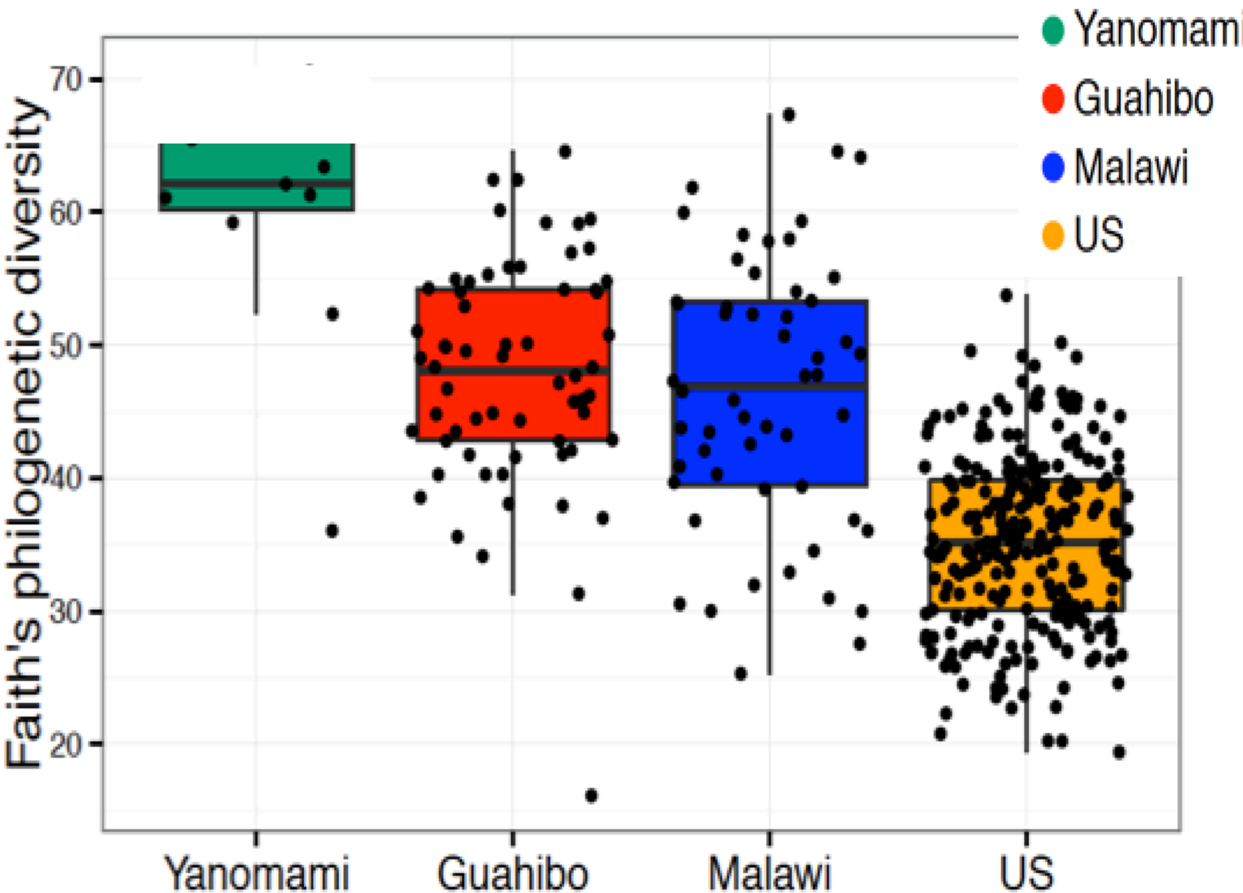
Nature Rev Microbiol 2009;7:887.

Disappearance of *Helicobacter pylori* in Japanese families

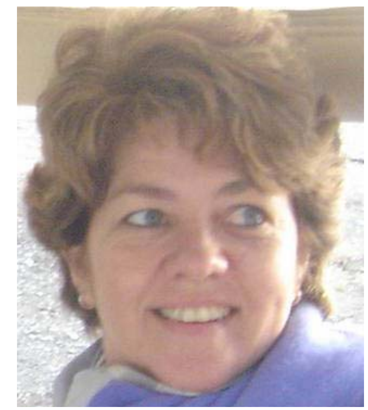


Adapted from Y. Urita et al. *J Ped Child Health* 2013; 49:394-8

Fecal diversity in four human groups



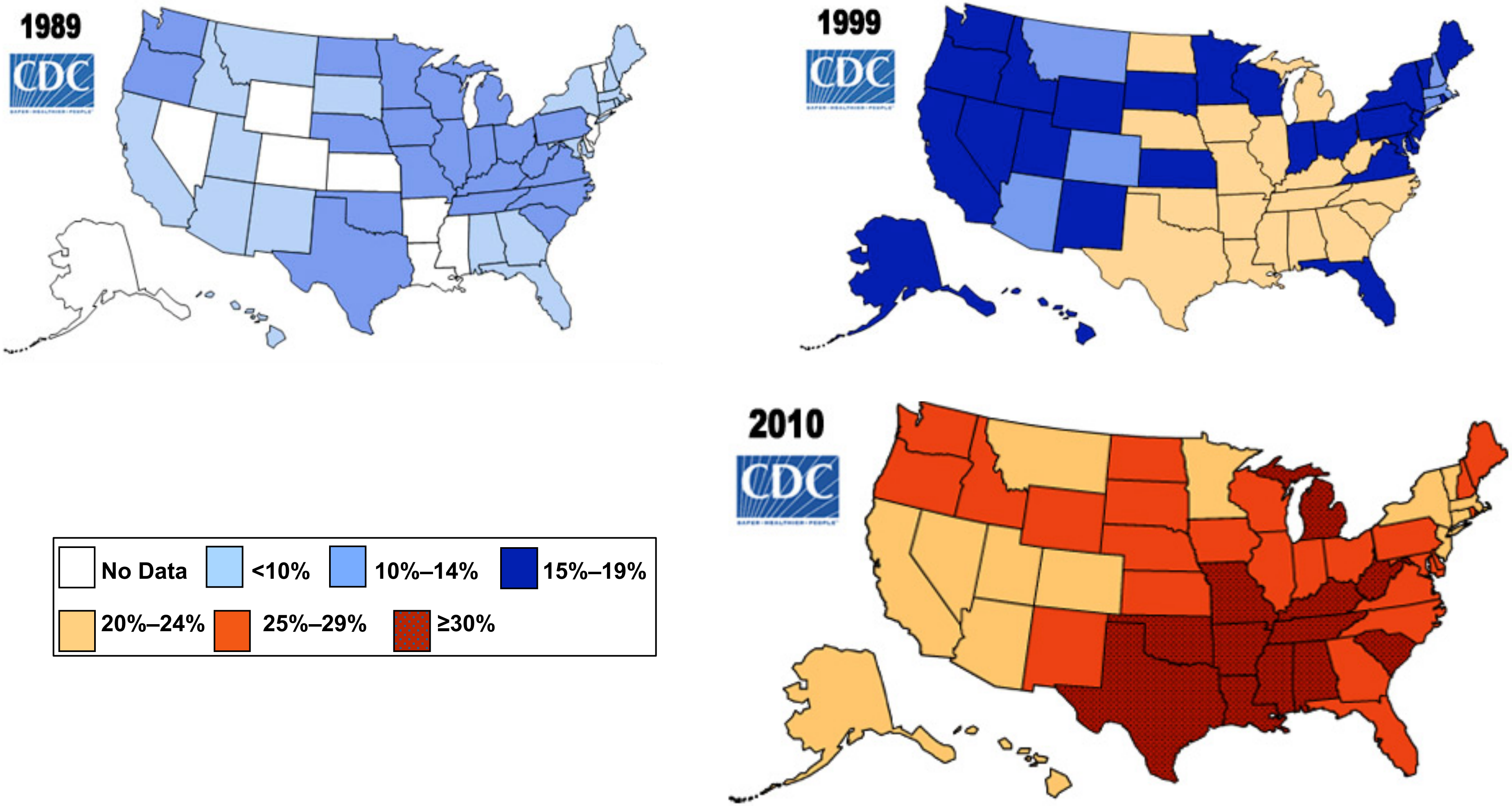
Westernization



Maria Gloria Dominguez Bello

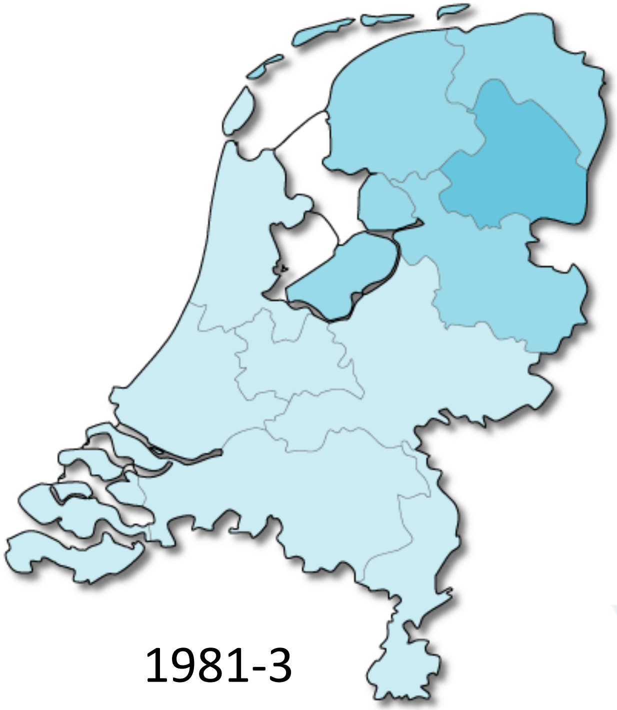
J Clemente et al.
Science Advances 2015

Obesity trends in US adults: changing physiology

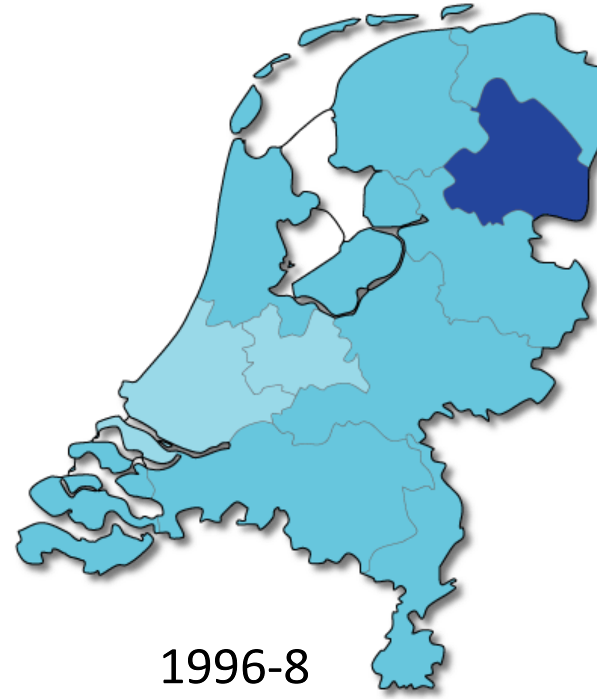


Source: CDC Behavioral Risk Factor Surveillance System.

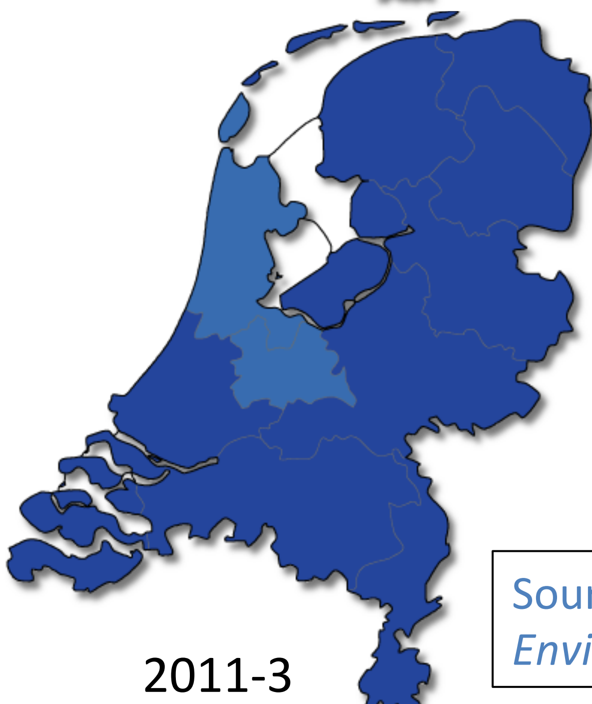
Proportion of overweight adults in the Netherlands, 1981-2013



1981-3



1996-8



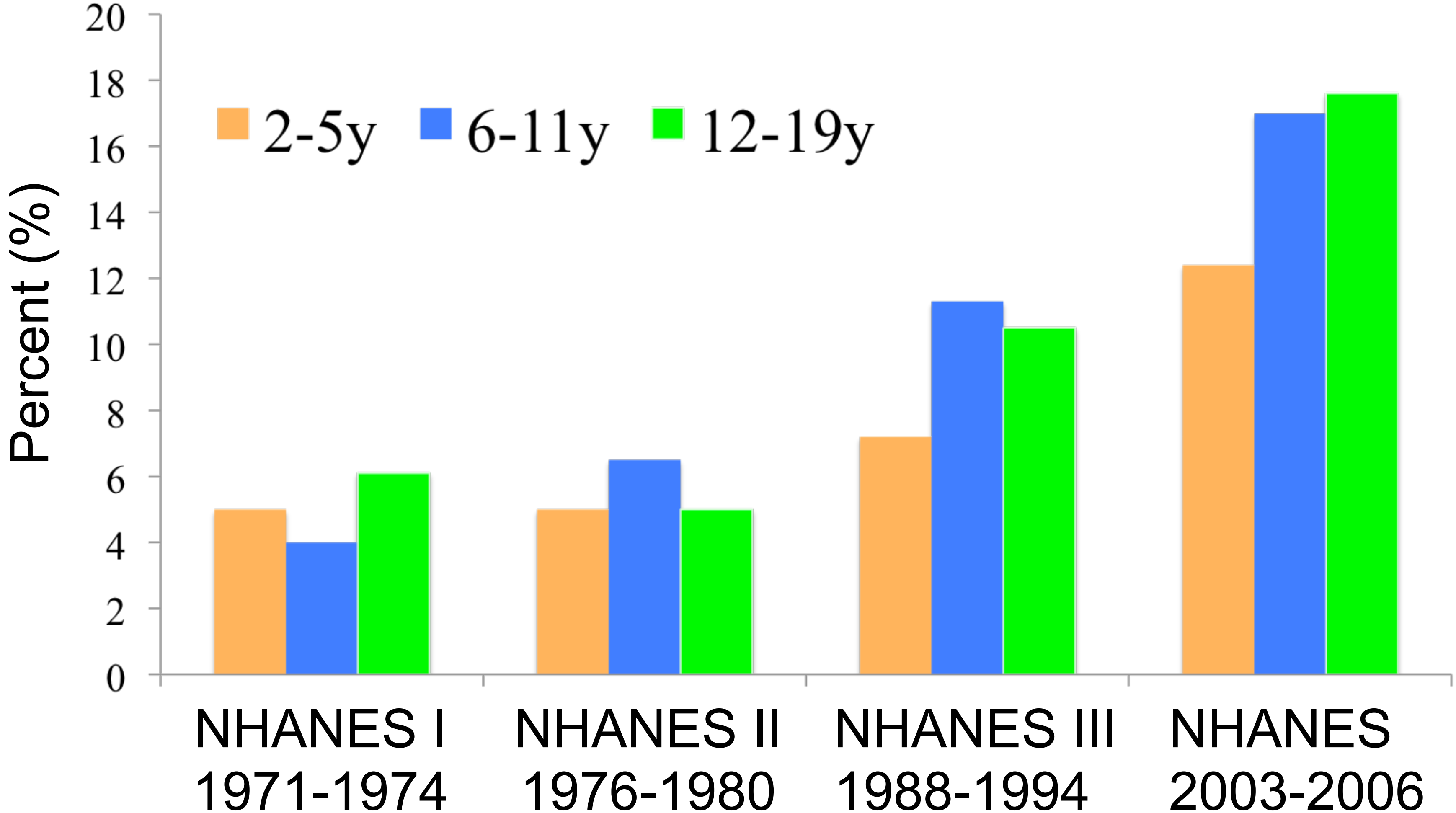
2011-3

Percent
- 29 - 35
- 35 - 39
- 39 - 43
- 43 - 47
- 47 - 56

Overweight = BMI > 25

Source: *Dutch National Institute for Public Health and the Environment: Ministry of Health, Welfare and Sport (RIVM)*

Obesity trends among U.S. children and adolescents*



**Sex- and age-specific BMI > 95th percentile based on CDC growth charts*

THE TREATMENT OF LOBAR PNEUMONIA WITH PENICILLIN[1]

By WILLIAM S. TILLETT, JAMES E. McCORMACK, AND MARGARET J. CAMBIER

(From the Department of Medicine of New York University College of Medicine and the Third Medical Division of Bellevue Hospital, New York City)

(Received for publication January 18, 1945)

This communication, which deals with the use of penicillin in the treatment of lobar pneumonia, and an accompanying one (1) describing the local treatment of pneumococcal empyema with penicillin, are extensions and elaborations of an earlier report (2) on the same subjects. The material embodied in the earlier article consisted of 46 cases of pneumonia and 8 cases of pneumococcal empyema. In the present report, 64 additional cases of pneumonia are included, and, in the accompanying article, 13 additional cases of empyema have been added, making total numbers of 110 pneumonias and 21 empyemas, respectively, that have been assembled for presentation.

of 46 cases were due to the particular pneumococcal types mentioned and in the 1943–44 series 41 out of 64 cases were caused by the same types, the number in each instance representing approximately two-thirds of the total. The most definite difference in the type incidence during the two separate years is to be noted in the occurrence of 17 cases of Type II pneumococcus pneumonia during 1943–44 as contrasted with 6 similar cases in the preceding year.

The number of cases listed bacteriologically as "unclassified" refers to instances in which the sputum contained pneumococci but the organisms were not identified either by quellung reaction or

J Clin Invest 1945;24:589.

Top 8 prescriptions in US children, 2010

Prescription	Pediatric Patients (*N*)
• Amoxicillin	18 292 768
• Azithromycin	10 171 046
Albuterol	7 343 063
• Amoxicillin/clavulanate	4 454 926
• Cefdinir	4 308 857
• Cephalexin	4 009 275
Fluticasone	3 144 844
Prednisolone sodium phosphate	2 932 124

Age: 0-17 years
Criteria: Unique patients
Source: Retail pharmacies

G Chai et al. *Pediatrics* 2012;130:23-31.


Ibuprofeno
Genfar
CEFUROXIMA
500 mg
DURACELL
5.60
4.90
60.00
20.00

Outpatient antibiotic use, by age, 2010

Patient age group (years)	Number of prescriptions (millions)	Prescriptions /1000 people
0 - 1	16.6	**1365**
2 - 9	29.0	1021
10 - 19	28.9	677
20 - 39	55.4	669
40 - 64	81.6	797
≥ 65	41.1	1020
Total	258.0	833

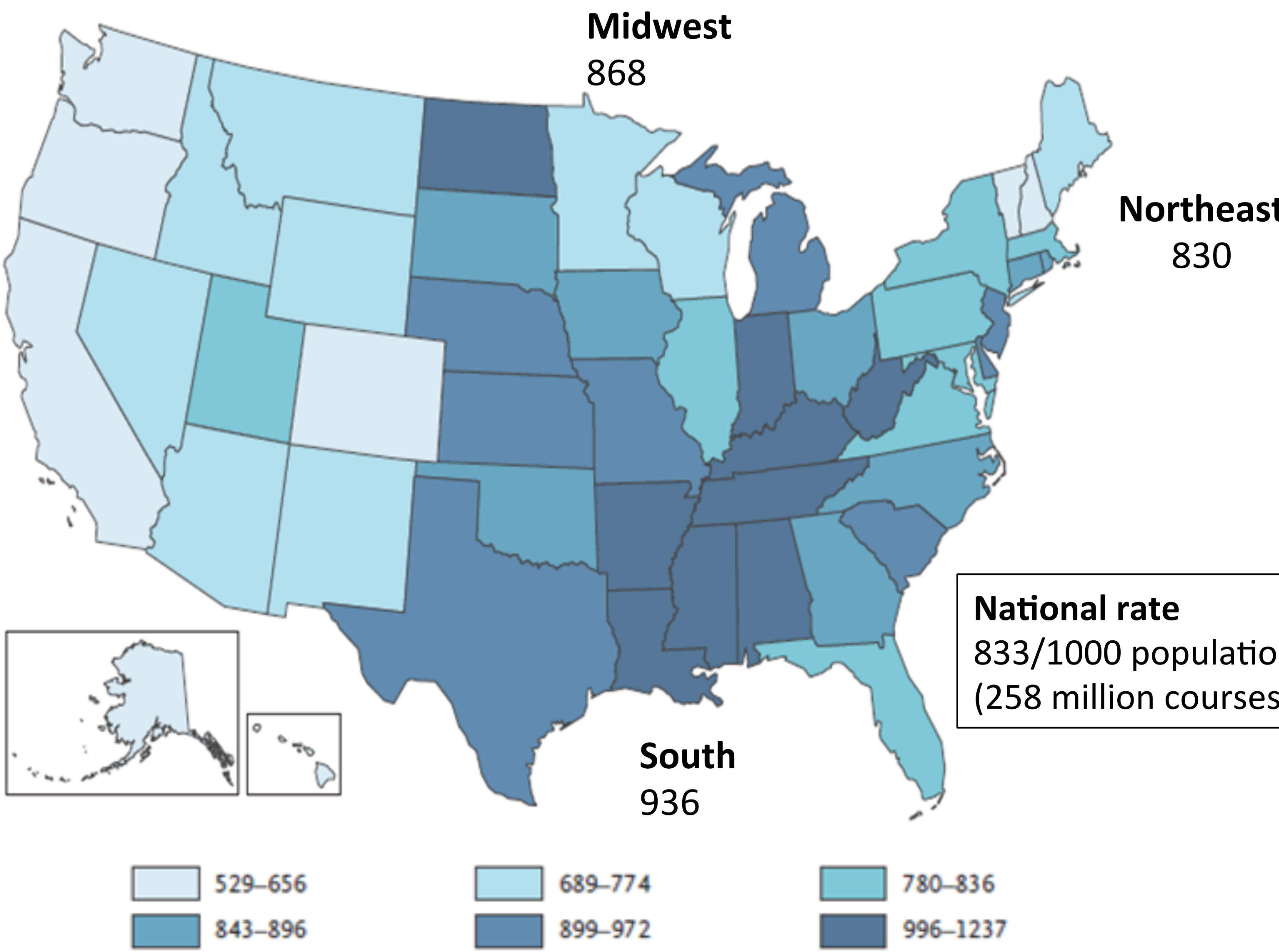
Source: L Hicks et al. *N Engl J Med* 2013, 368:1461.

Cumulative outpatient antibiotic use, by age

Patient age group (years)	Number of prescriptions (millions)	Prescriptions /1000 people	Average number of courses	
			During period	Cumulative
0 - 1	16.6	**1365**	**2.73**	**2.73**
2 - 9	29.0	1021	8.17	10.90
10 - 19	28.9	677	6.78	17.68
20 - 39	55.4	669	13.38	31.06
40 - 64	81.6	797	19.93	50.98
≥ 65	41.1	1020	-	-
Total	258.0	833		

L Segal & MJ Blaser. *Ann Am Thor Soc* 2014
Adapted from L Hicks et al. *N Engl J Med* 2013, 368:1461.

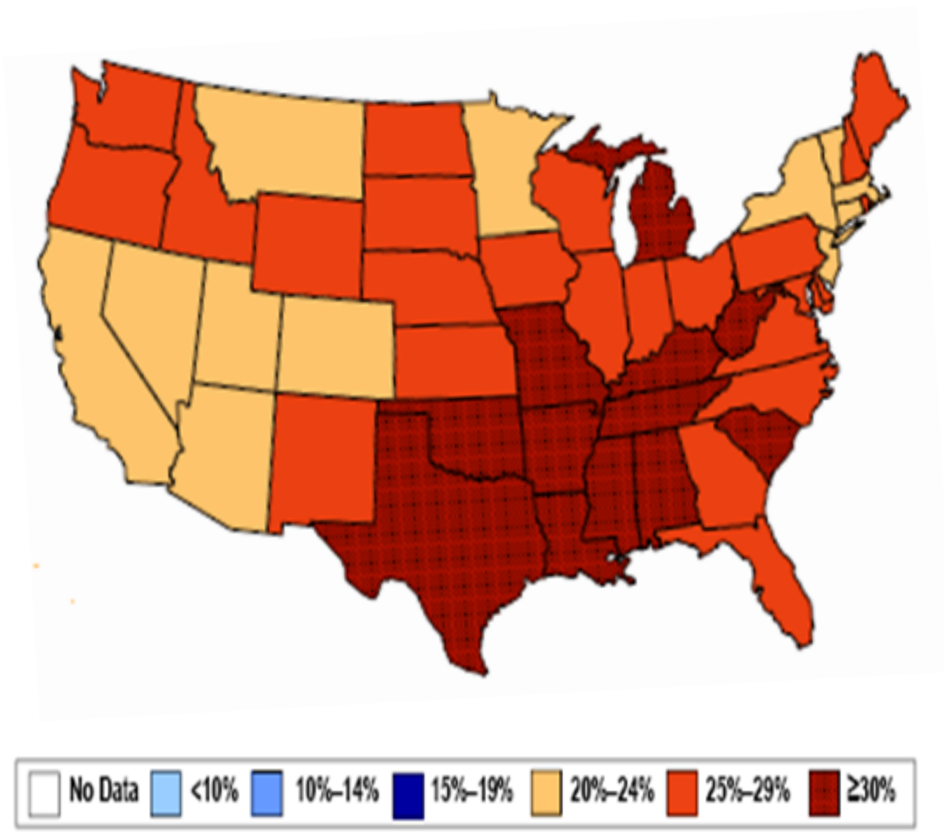
Outpatient antibiotic usage rates by region, 2010

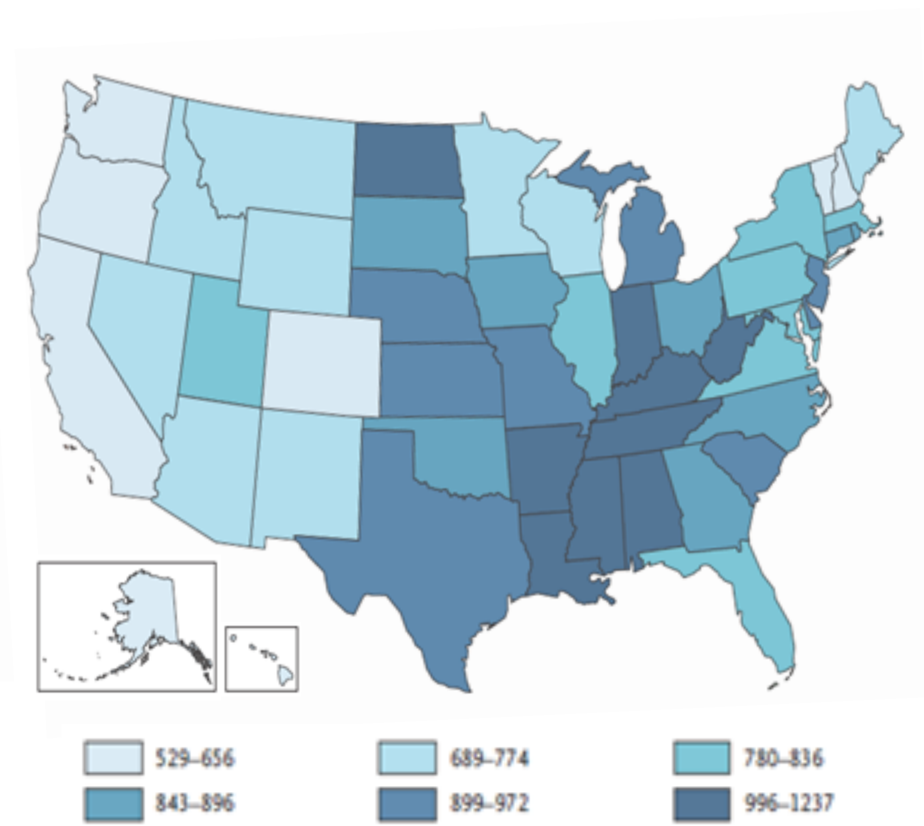


National rate
833/1000 population
(258 million courses)

Source: L Hicks et al. *N Engl J Med* 2013, 368:1461.

Comparisons between the geography of obesity and antibiotic use, 2010



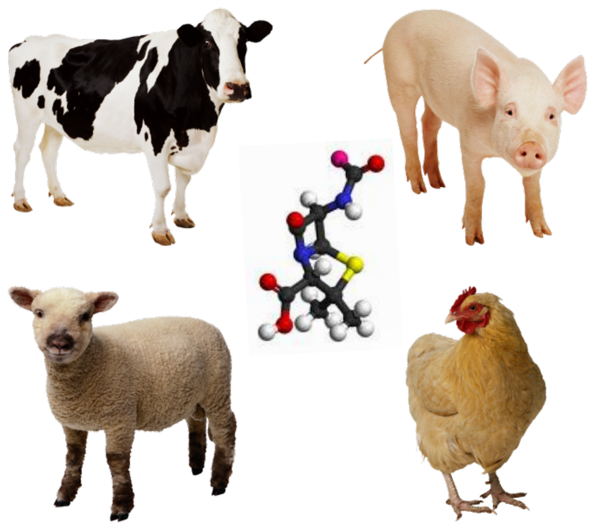


Antibiotic prescriptions per 1000 persons, 2010

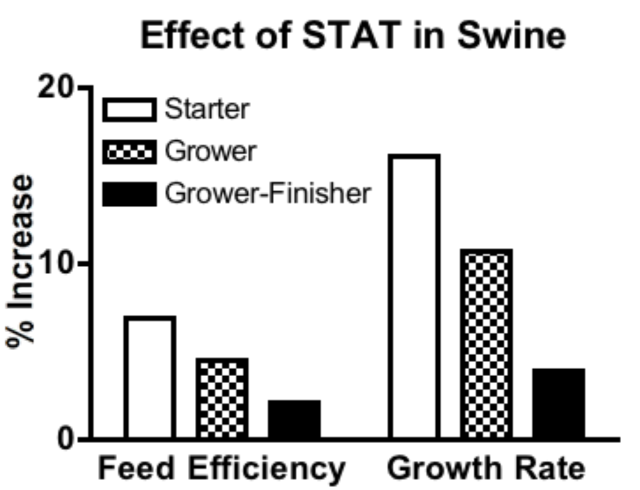
L Segal & MJ Blaser, *Ann Am Thor Soc* 2014

Observational data

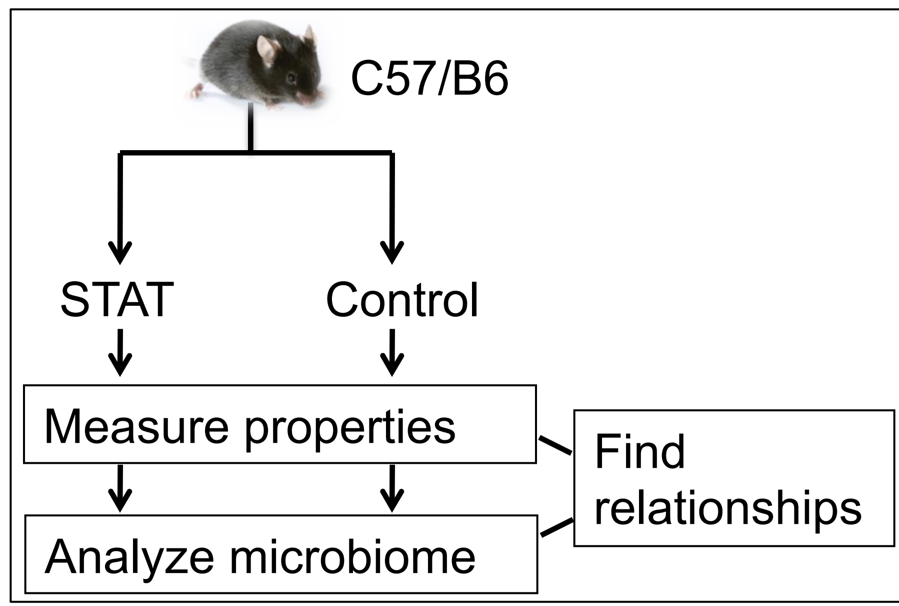
Sub-therapeutic antibiotic treatment (STAT) used for growth promotion of livestock



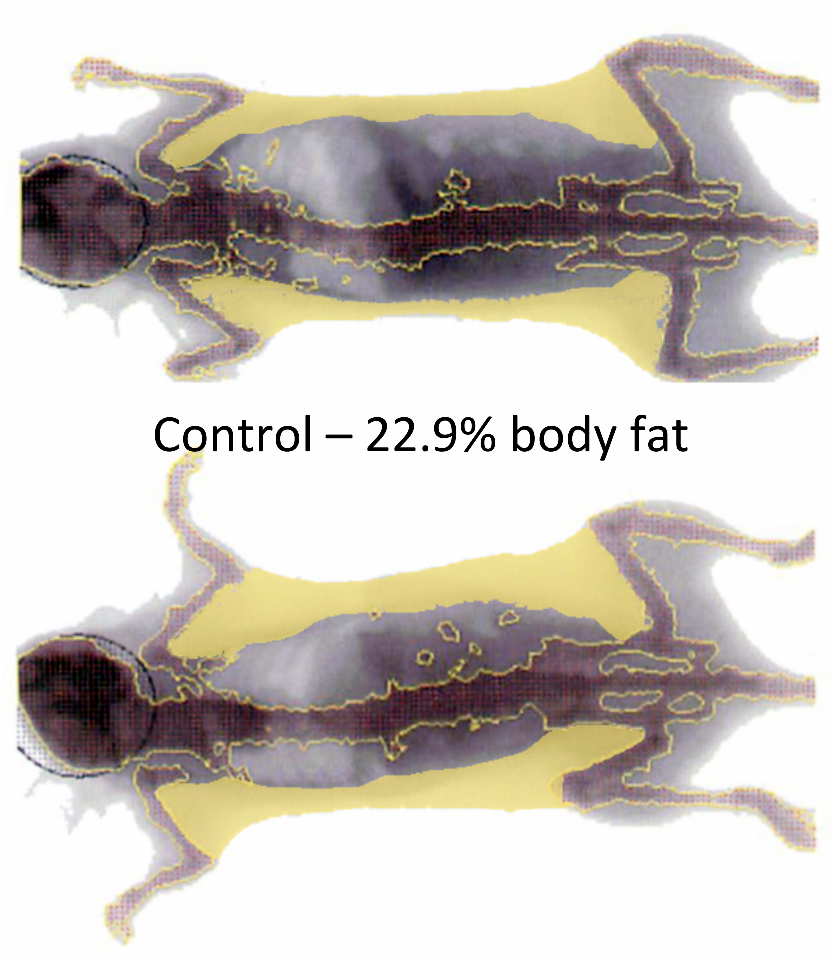
Antibiotic	Class	Target
Bambermycin	Glycolipid	Cell wall
Virginiamycin	Streptogrammin	Protein synthesis
Avilamycin	Orthosomycin	Protein synthesis
Bacitracin	Cyclic peptide	Cell wall synthesis
Monensin	Ionophore	Cell membrane
Carbadox	Quinoxaline	DNA Synthesis



(Adapted from Zimmerman, J Animal Sci, 1986)

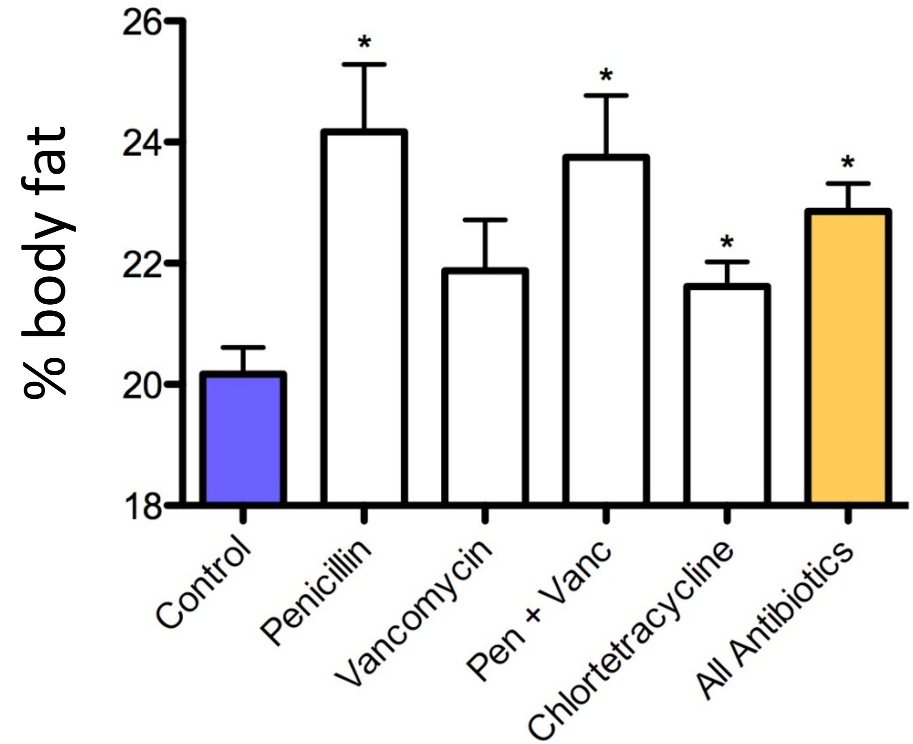


Chronic, low-dose exposure: Body composition in STAT and control mice at age 10 weeks

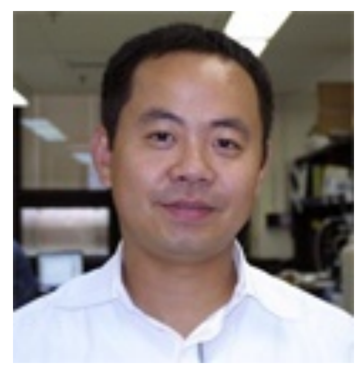


Control – 22.9% body fat

STAT – 32.0% body fat



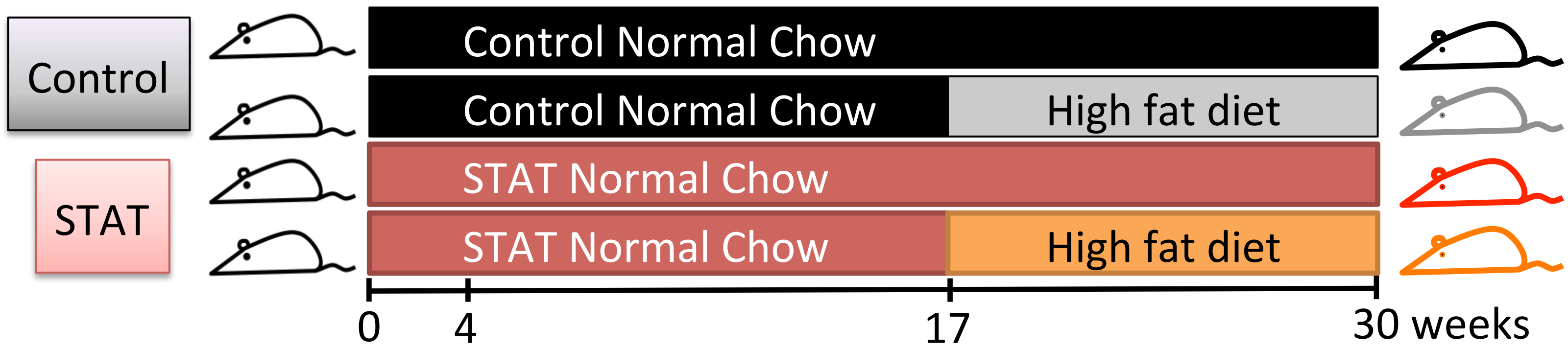
*p<0.05



Ilseung Cho

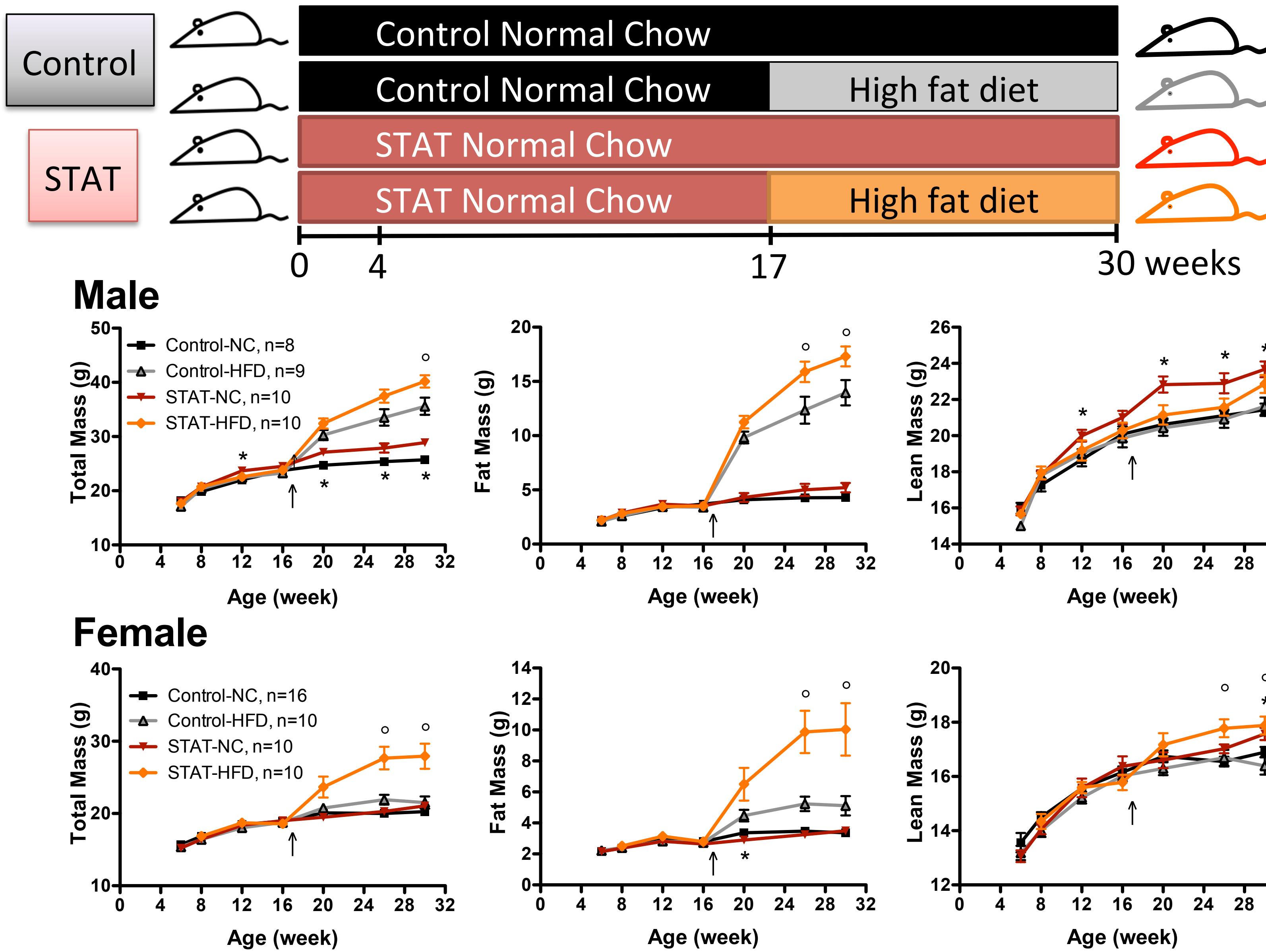
I Cho et al. *Nature* 2012; 488:621-6

FatSTAT: the effects of HFD and STAT on body composition



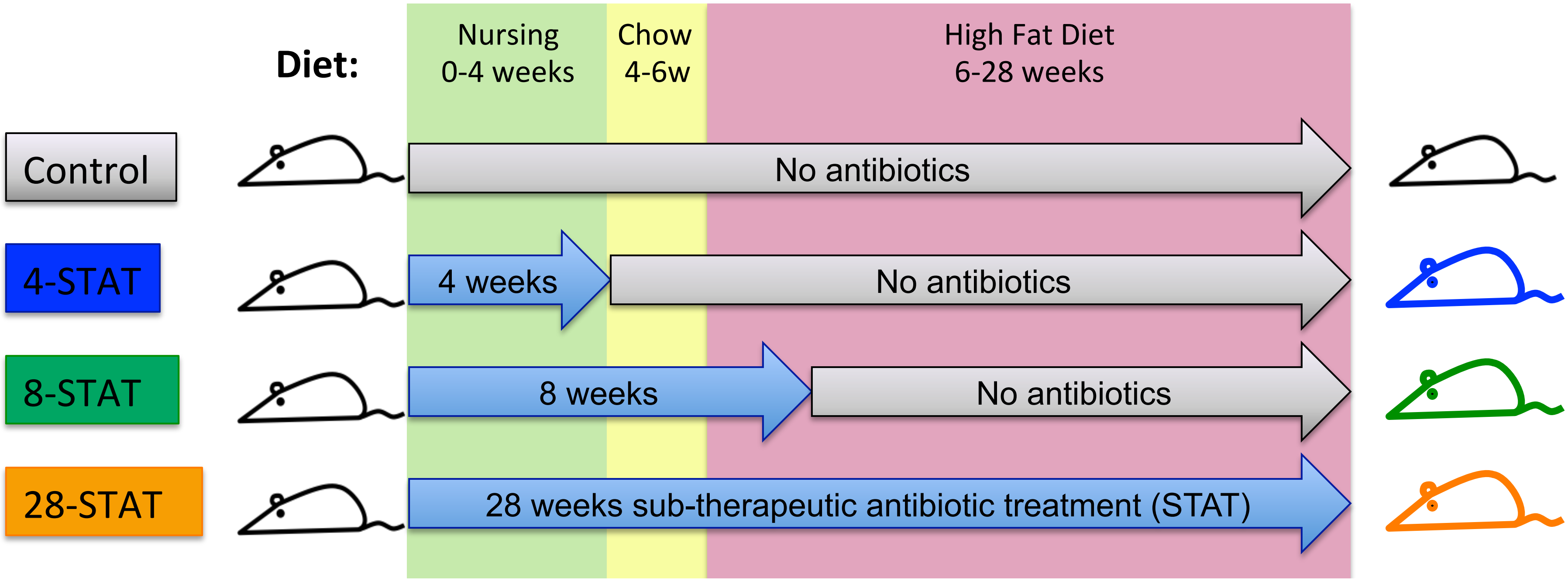
Laurie Cox
Cell 2014;158:705-21

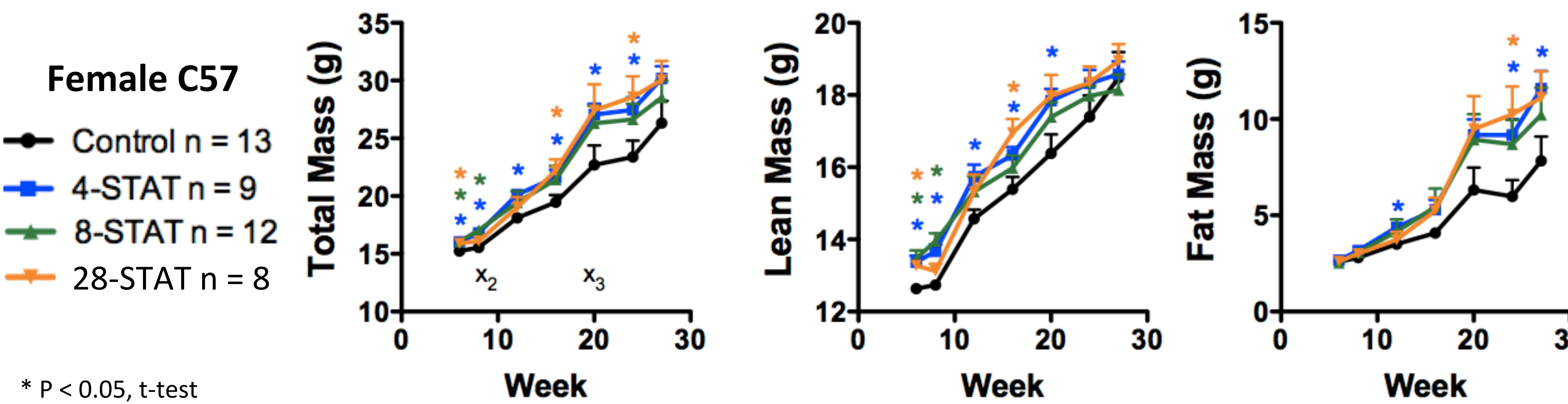
FatSTAT: the effects of HFD and STAT on body composition



↑ High fat diet introduced   * $p < 0.05$ NC   ° $p < 0.05$ HFD

DuraSTAT: Are the changes **durable** with limited antibiotic exposure?





Body Composition

* $P < 0.05$, t-test

X_2: sacrificed 4 control and 4 8-STAT

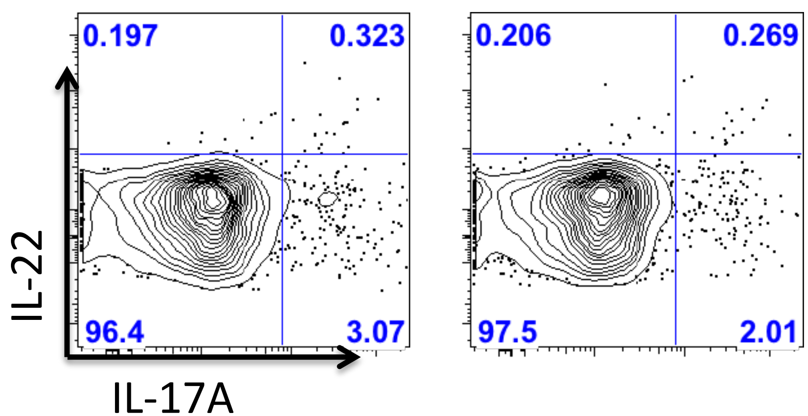
X_3: sacrificed 3 control and 3 28-STAT

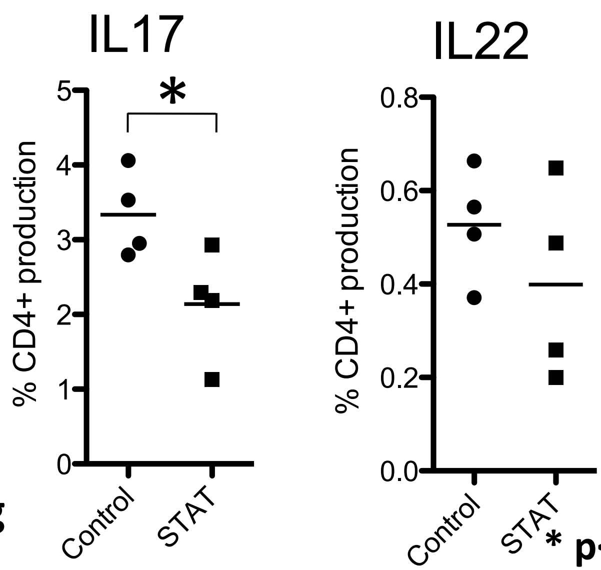
Effects of STAT on intestinal Th17 populations

8 week males

Small intestine

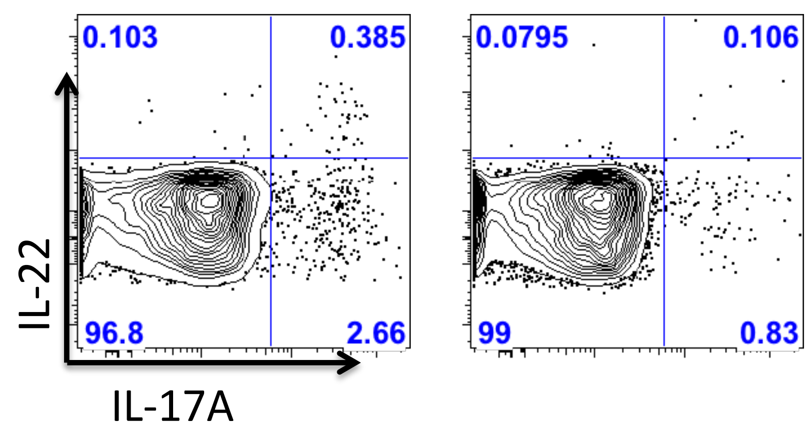
Control | STAT

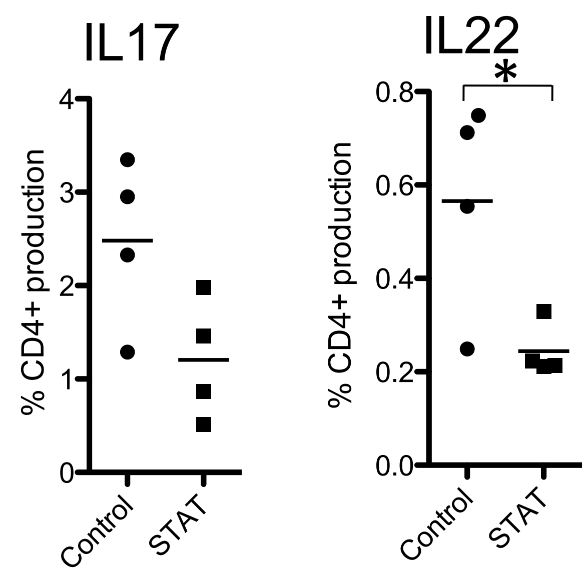




Large intestine

Control | STAT

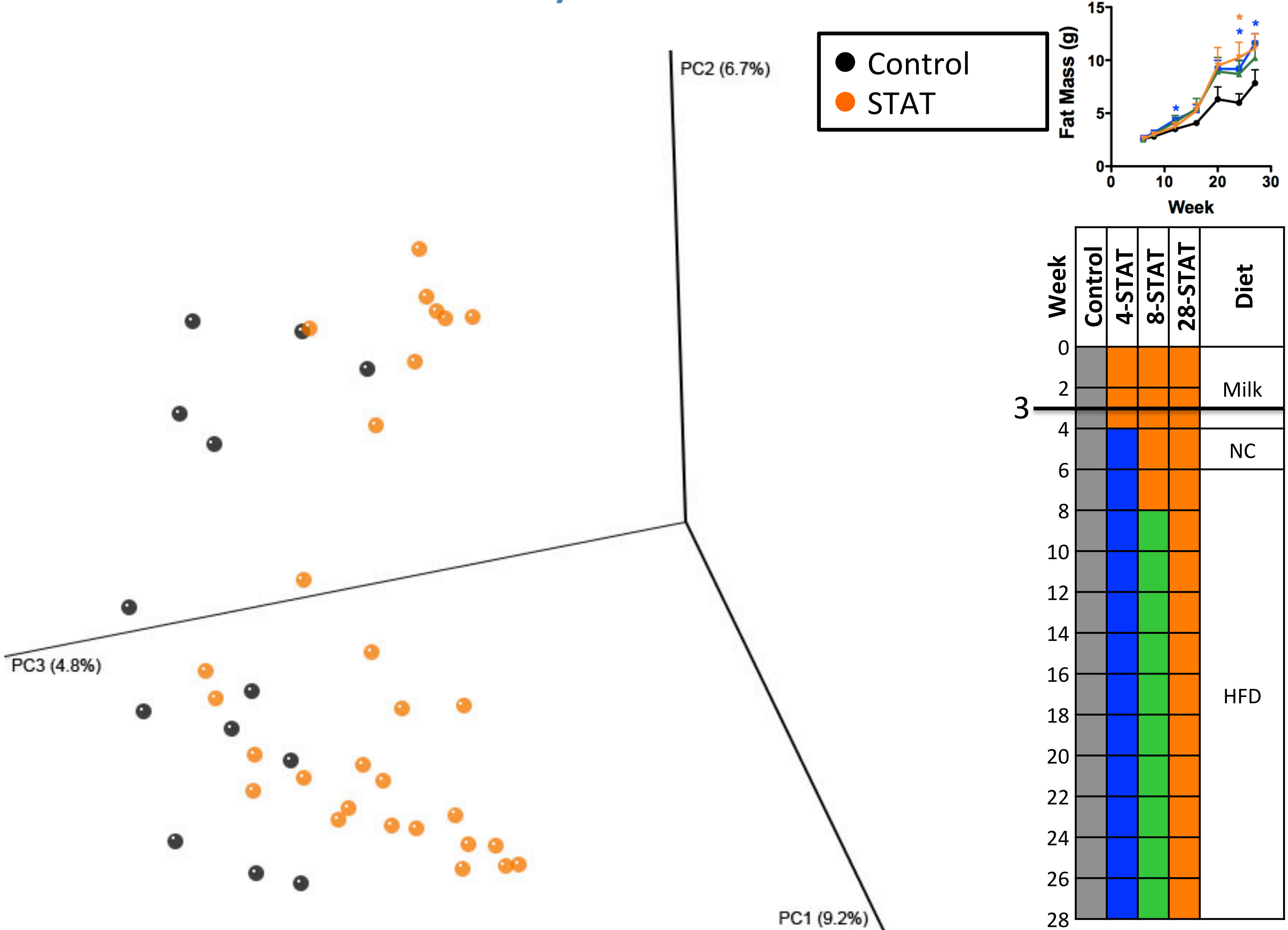




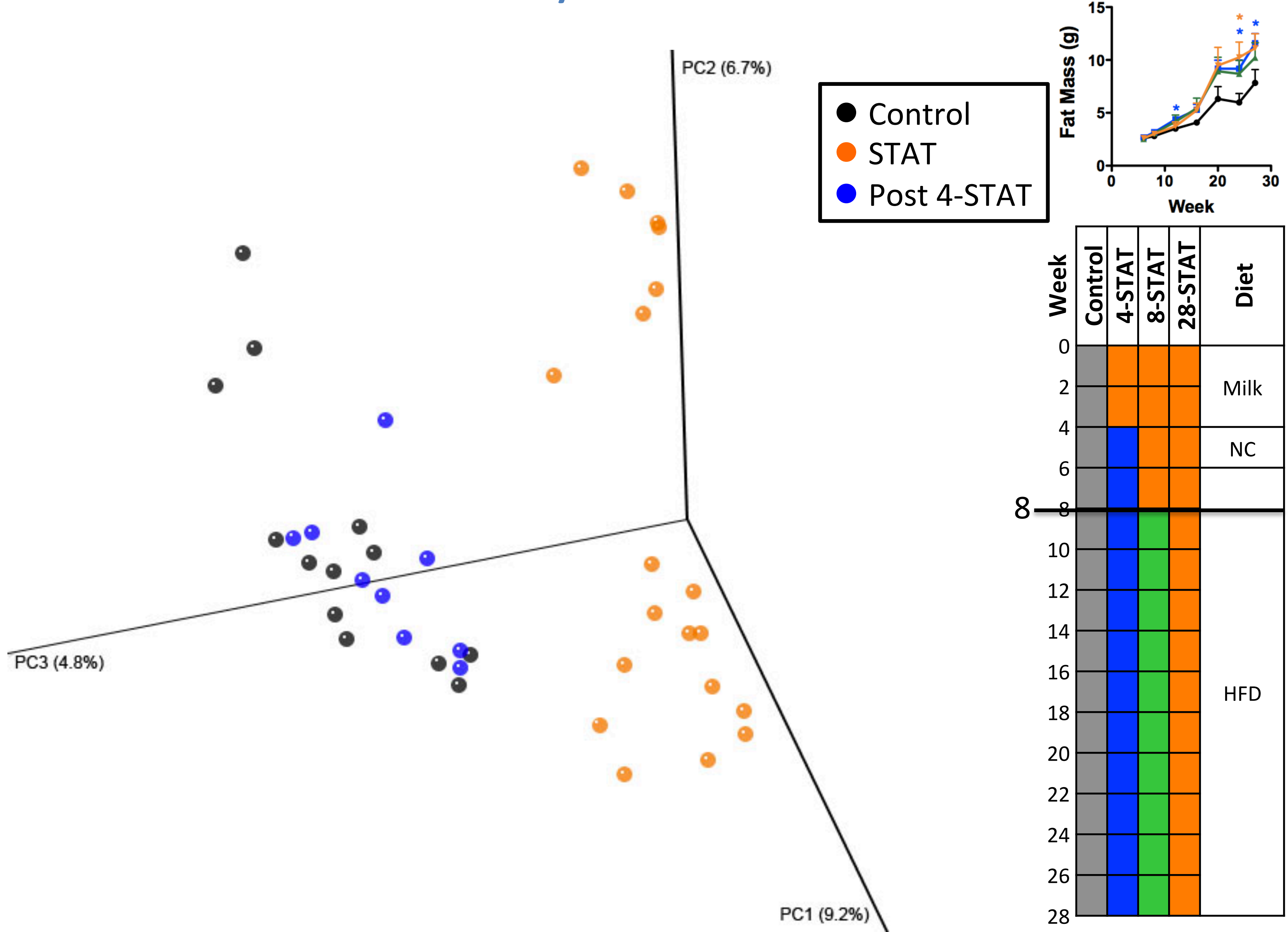
* p<0.05 (t-test)

Jacqueline Leung
P'ng Loke Lab

Fecal community structure at 3 weeks

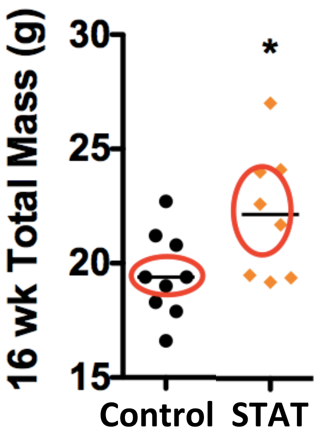


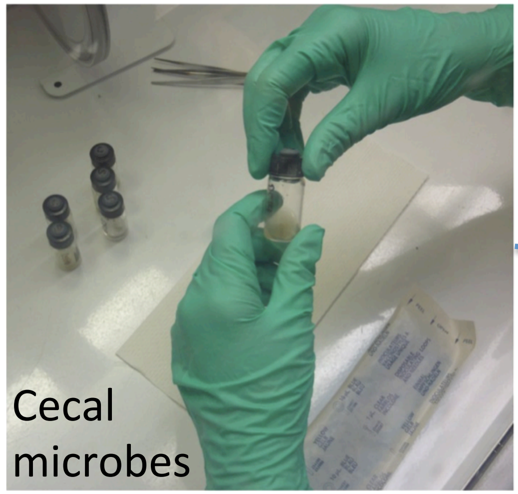
Fecal community structure at 8 weeks



TranSTAT: is the growth phenotype **transferable** by microbiota alone?

Donors 18-week female C57BL/6J



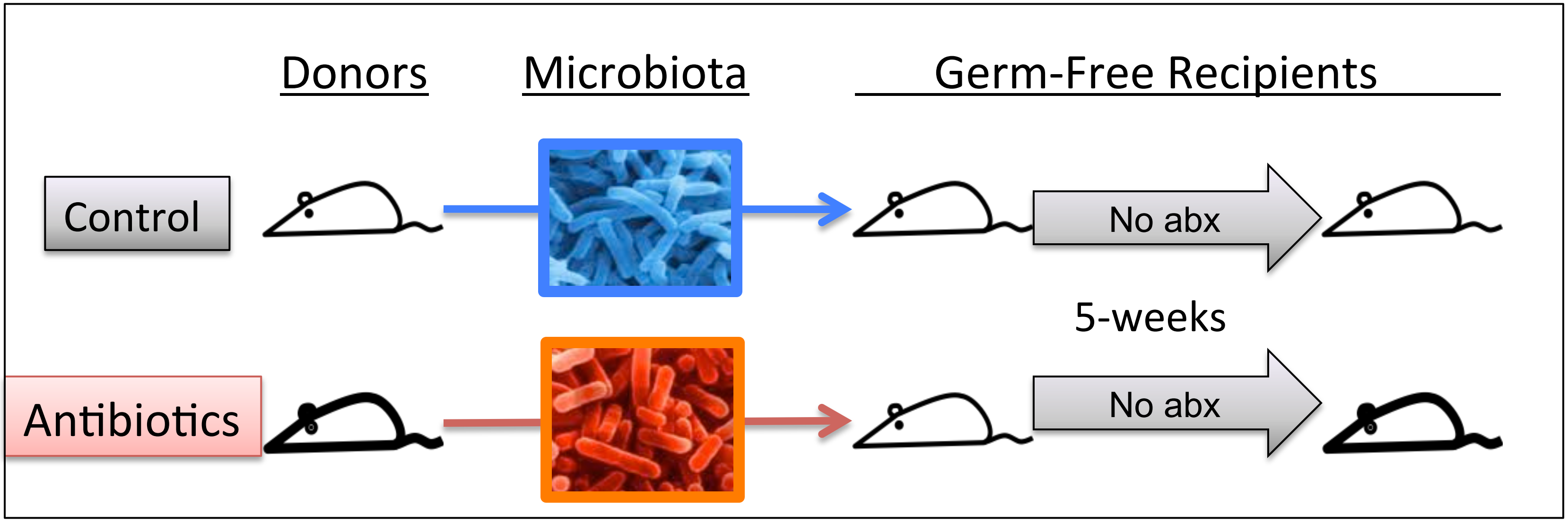


Oral Gavage

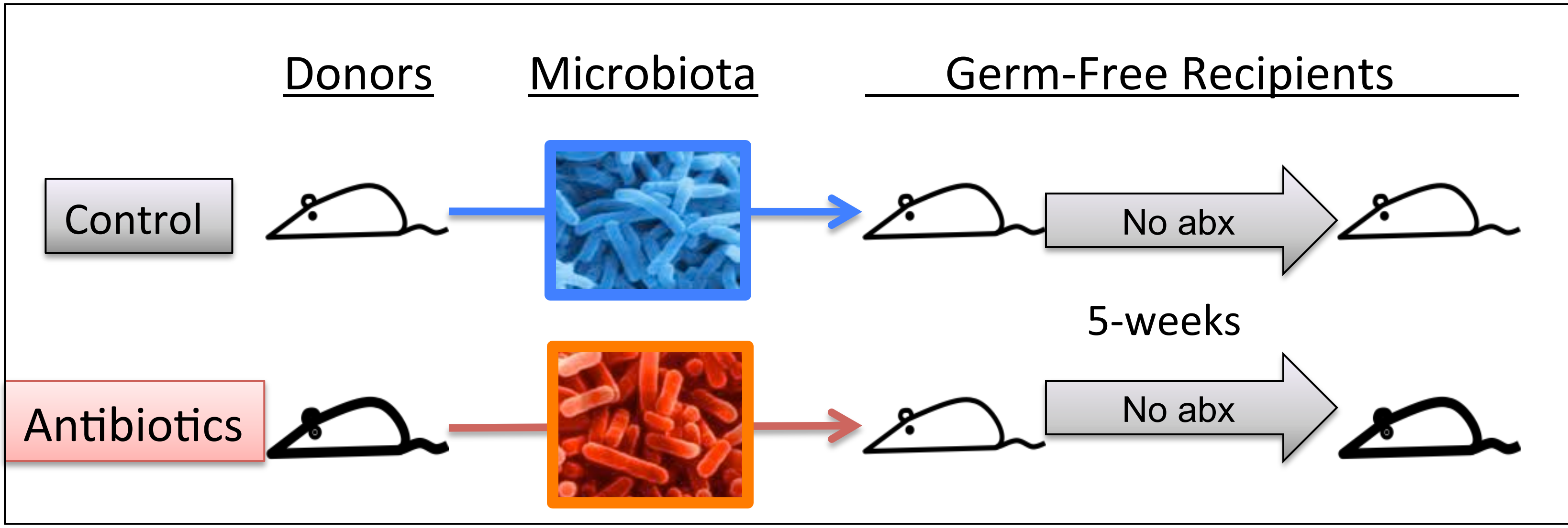
Recipients 3-week female germ-free Swiss-Webster

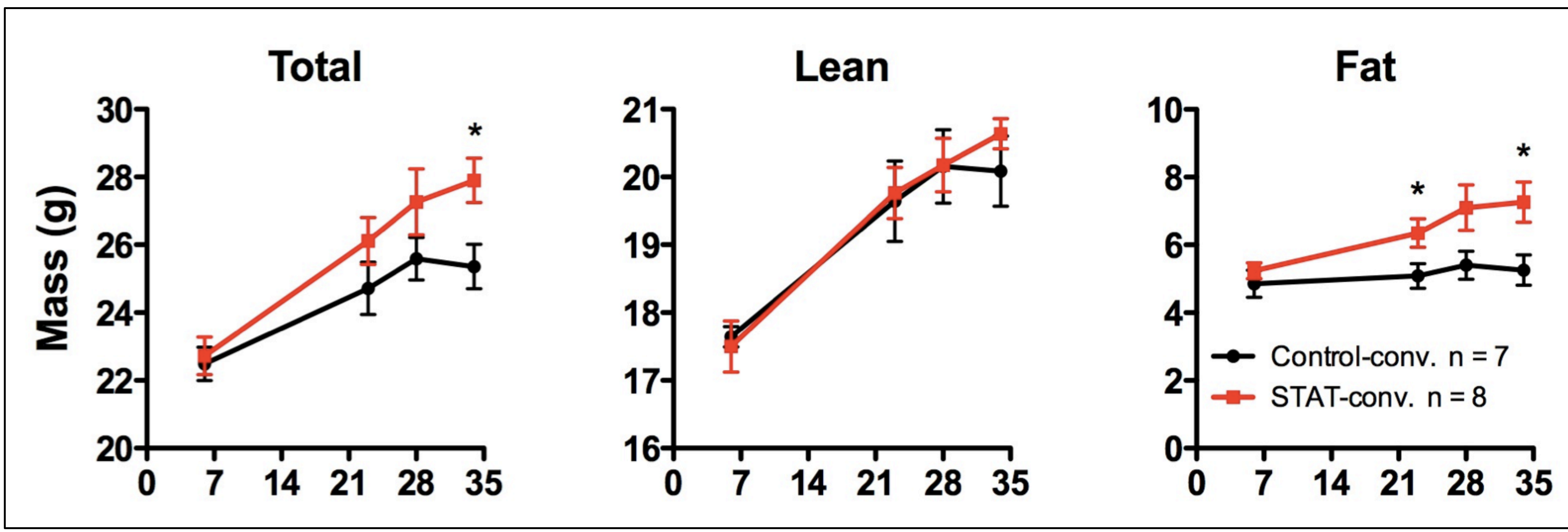
Recipients housed for 35 days in specific pathogen-free (SPF) conditions

Is microbe-induced obesity transferable?



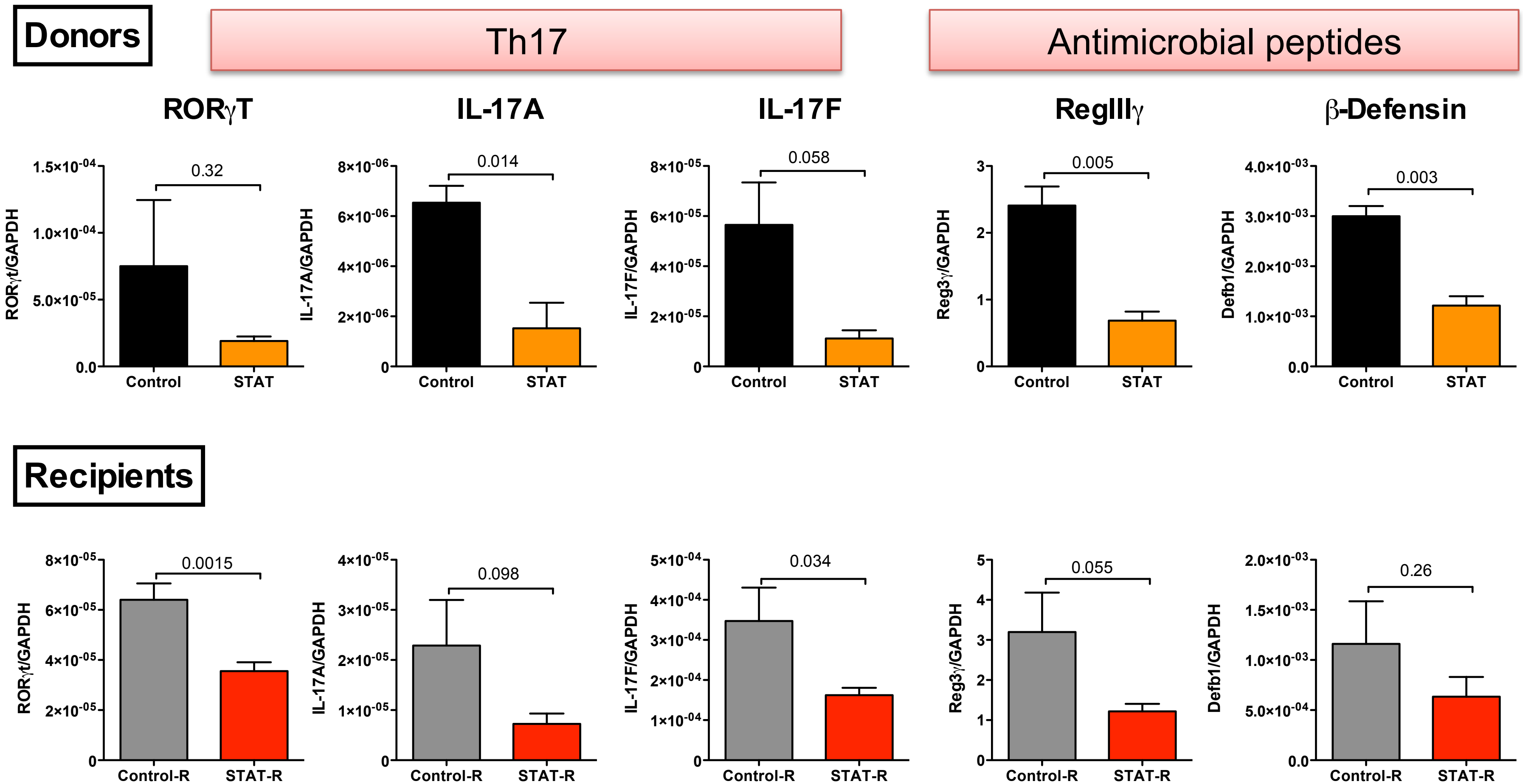
Is microbe-induced obesity transferable?





Body Composition - Days post-transfer

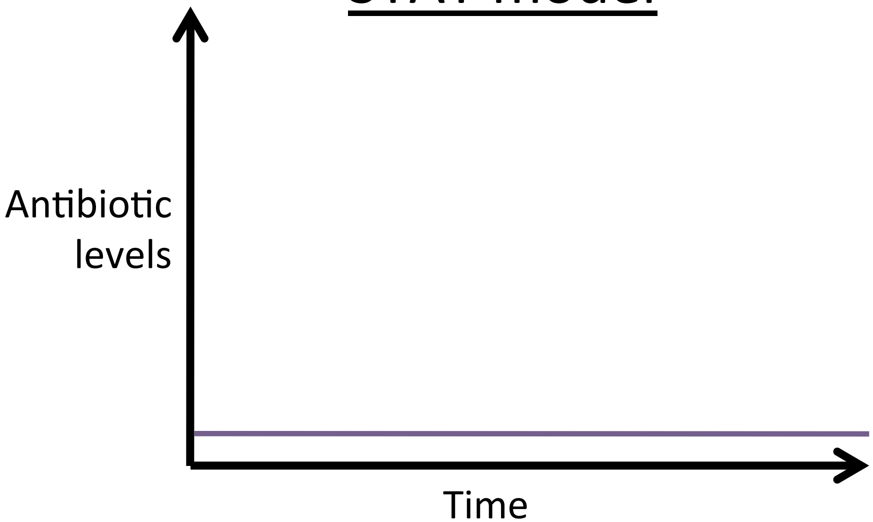
Expression of genes involved in intestinal defenses in the microbiota donor and recipient mice



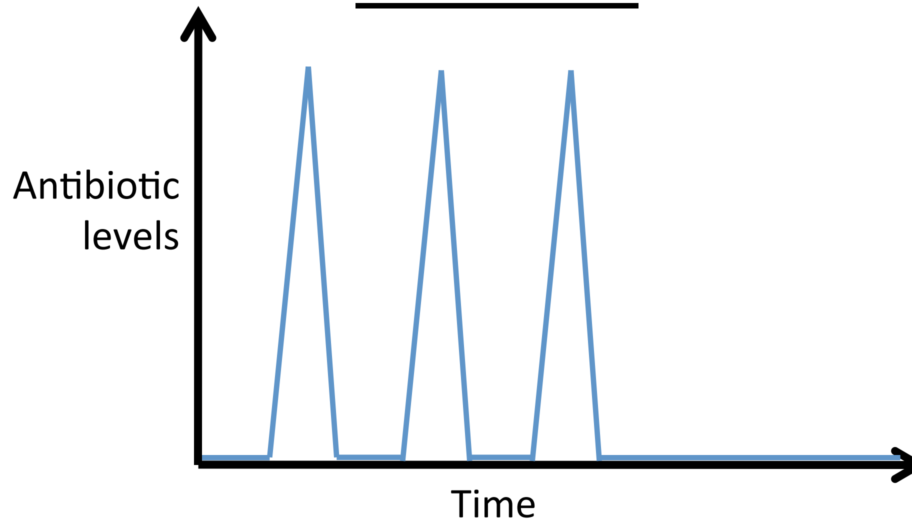
p-values, by t-test

Model 2: Pulsed Antibiotic Therapy

STAT model



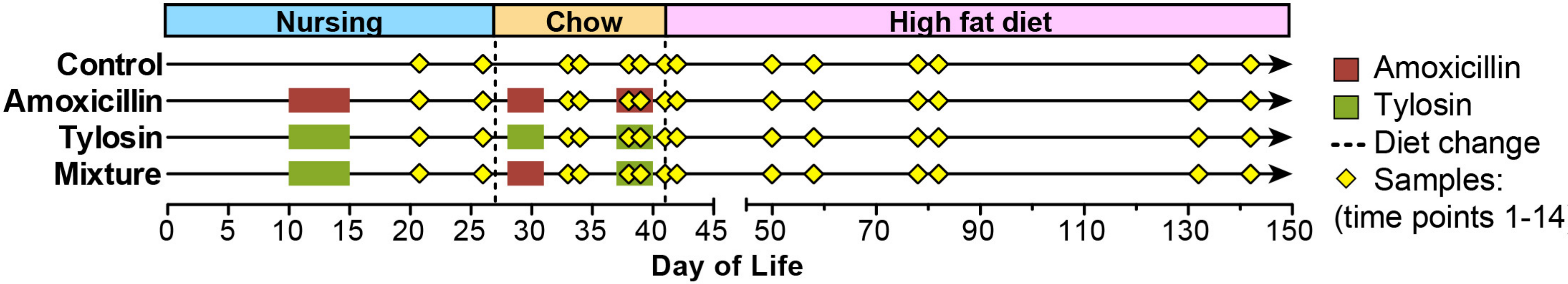
PAT model



HYPOTHESIS: A series of short, therapeutic-dose pulses of antibiotic administered early in life will sufficiently change the gut microbiome to alter body composition.

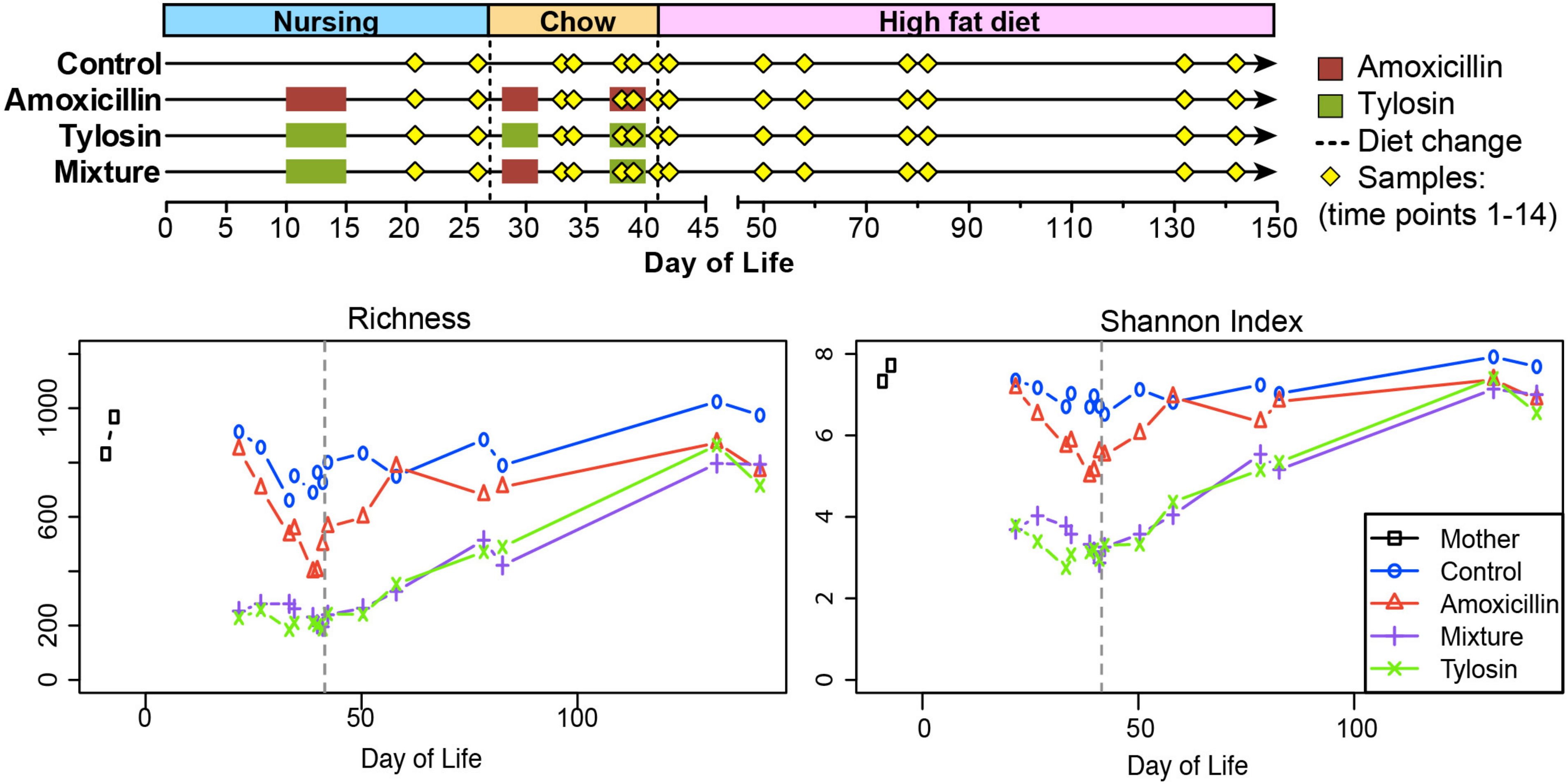
Yael Nobel

PAT study design and sampling



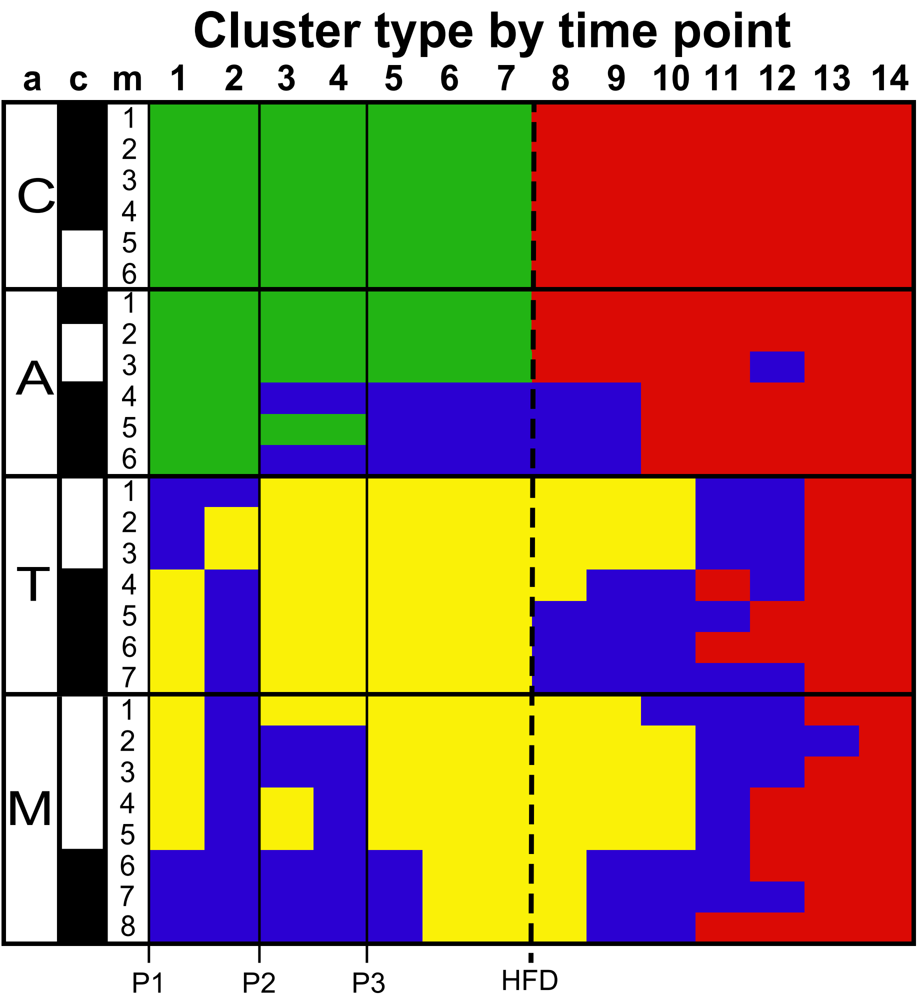
Y Nobel, L Cox et al. *Nature Communications* 2015.

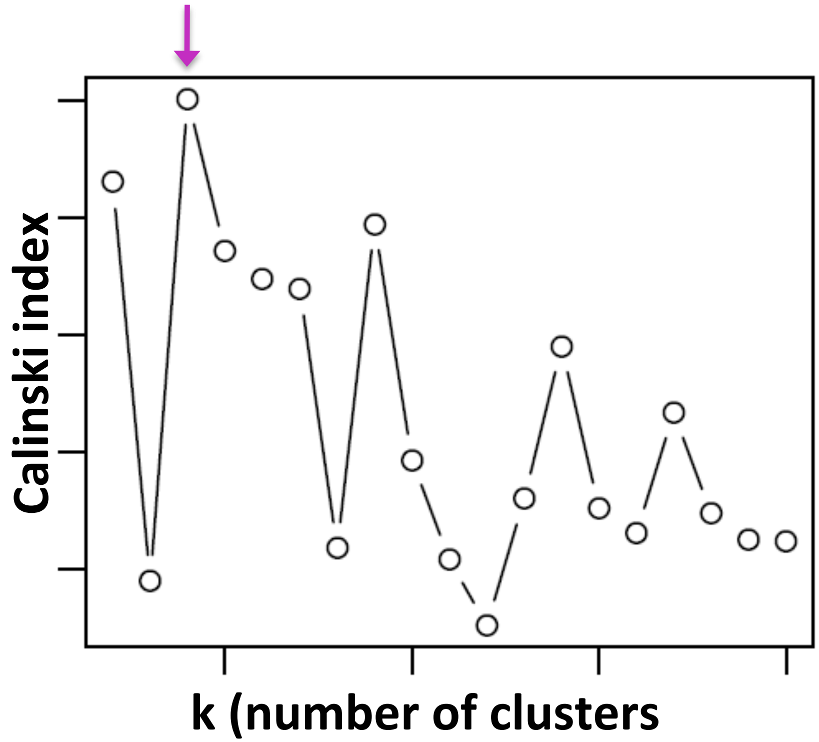
PAT effects on alpha diversity



Y Nobel, L Cox et al. *Nature Communications* 2015.

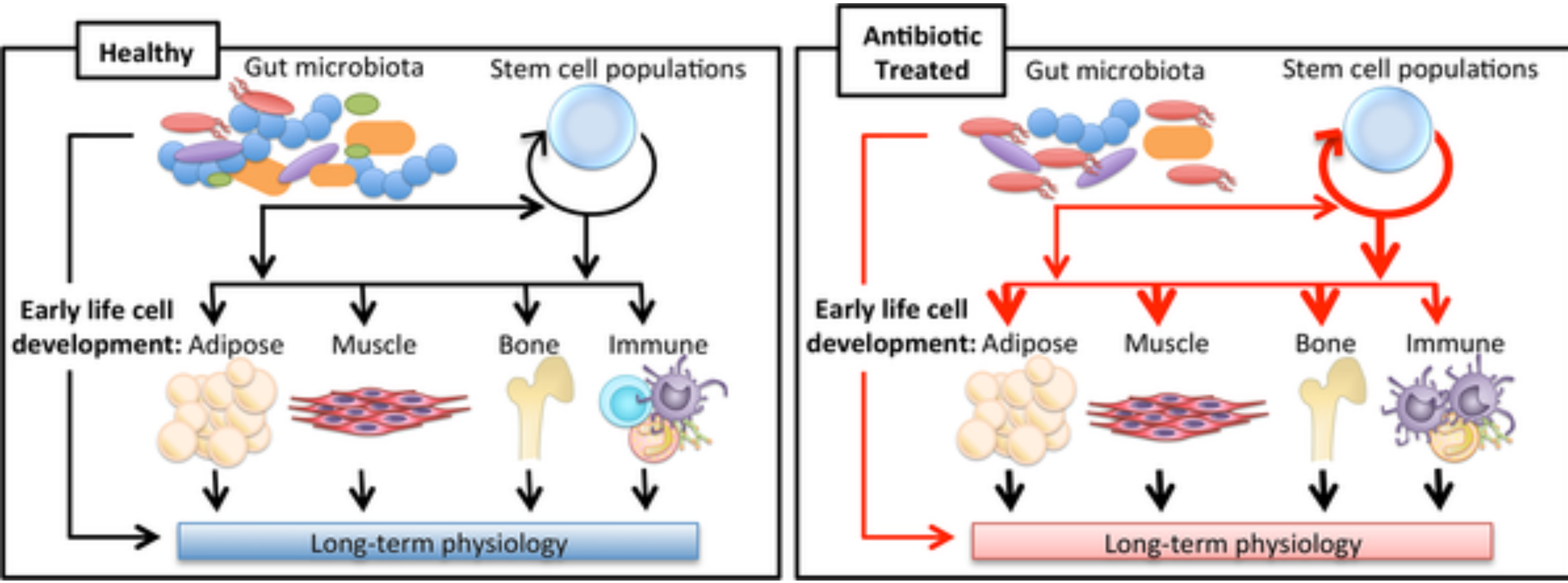
Cluster types in control and PAT mice over time





Alexander Alekseyenko

Antibiotic impact on long-term physiology through microbiota changes



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ARTICLE

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Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children

Katri Korpela[1], Anne Salonen[1], Lauri J. Virta[2], Riina A. Kekkonen[3], Kristoffer Forslund[4], Peer Bork[4] & Willem M. de Vos[1,5,6]

Early-life antibiotic use is associated with increased risk for metabolic and immunological diseases, and mouse studies indicate a causal role of the disrupted microbiome. However, little is known about the impacts of antibiotics on the developing microbiome of children. Here we use phylogenetics, metagenomics and individual antibiotic purchase records to show that macrolide use in 2–7 year-old Finnish children ($N = 142$; sampled at two time points) is associated with a long-lasting shift in microbiota composition and metabolism. The shift includes depletion of Actinobacteria, increase in Bacteroidetes and Proteobacteria, decrease in bile-salt hydrolase and increase in macrolide resistance. Furthermore, macrolide use in early life is associated with increased risk of asthma and predisposes to antibiotic-associated weight gain. Overweight and asthmatic children have distinct microbiota compositions. Penicillins leave a weaker mark on the microbiota than macrolides. Our results support the idea that, without compromising clinical practice, the impact on the intestinal microbiota should be considered when prescribing antibiotics.

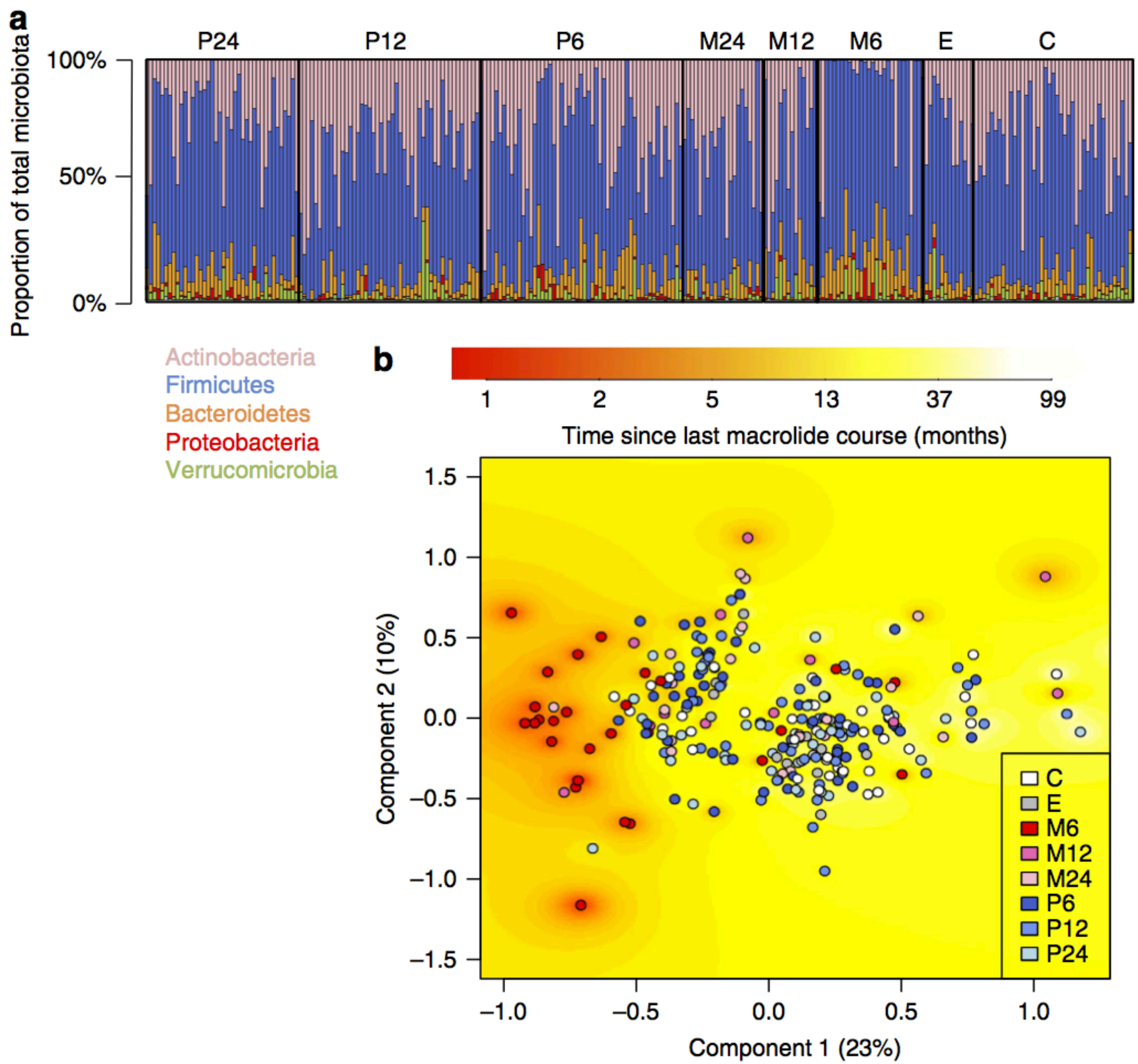


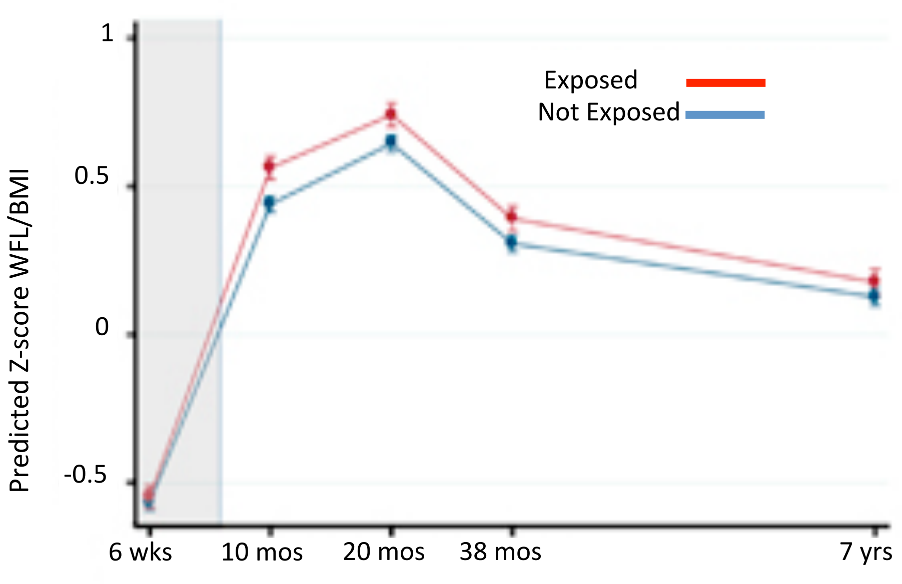
Figure 1 | Microbiota composition in 257 fecal samples as arranged per group. C denotes control group, no antibiotics for the past 2 years and in total <1 course per year of life on average. E denotes early-life exposure group, no antibiotics for the past 2 years and >1 course per year of life on average. M6 denotes macrolide course within 6 months; M12 denotes macrolide course within 6–12 months; M24 denotes macrolide course within 12–24 months. P6, P12 and P24 denote penicillin courses within 6, 6–12 and 12–24 months, respectively. (**a**) Phyla composition. (**b**) Genus-level microbiota composition according to PCoA analysis. The background colour indicates interpolated time since the last macrolide course.

Early life microbiome disruption is associated with weight gain in humans

International Journal of Obesity 2012

Infant antibiotic exposures and early life body mass

L Trasande, J Blustein, M Liu, E Corwin, LM Cox, MJ Blaser

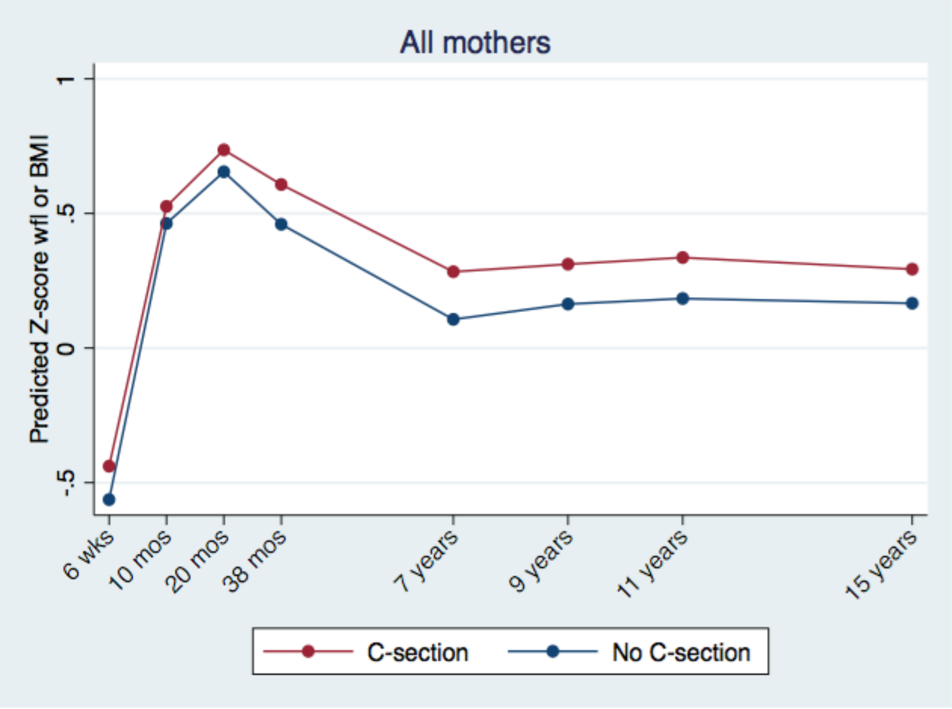


Exposed to antibiotics during the first 6 months of life

International Journal of Obesity 2013

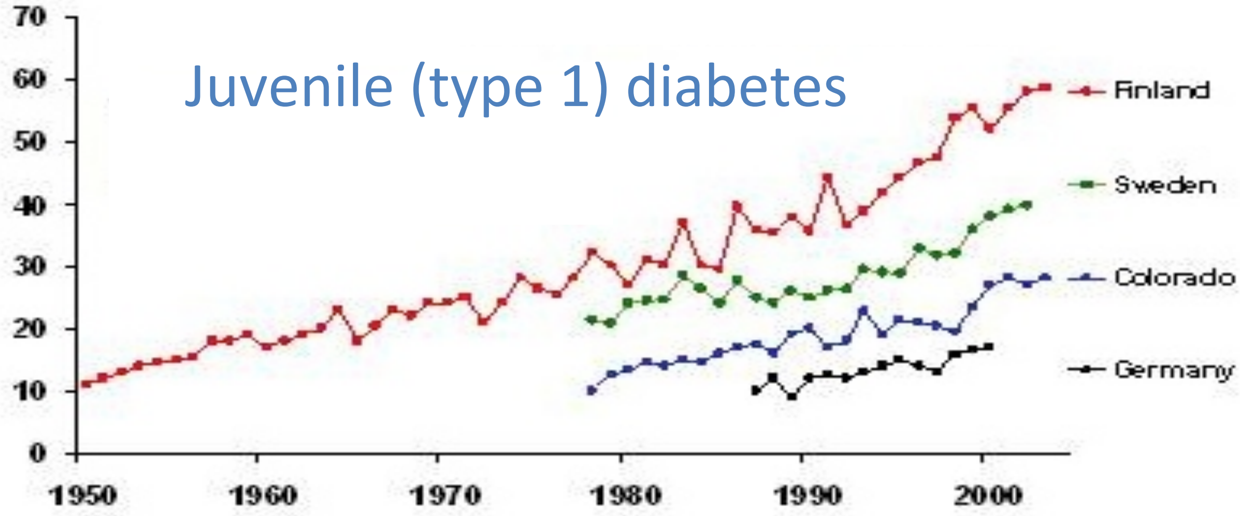
Association of caesarean delivery with child adiposity from age 6-weeks to 15 years

J Blustein, T Attina, M Liu, AM Ryan, LM Cox, MJ Blaser, L Trasande

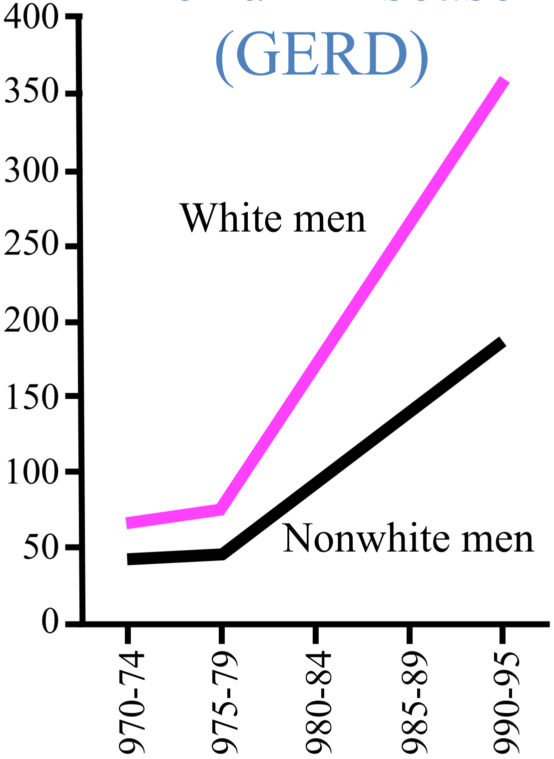


Diseases increasing in recent decades

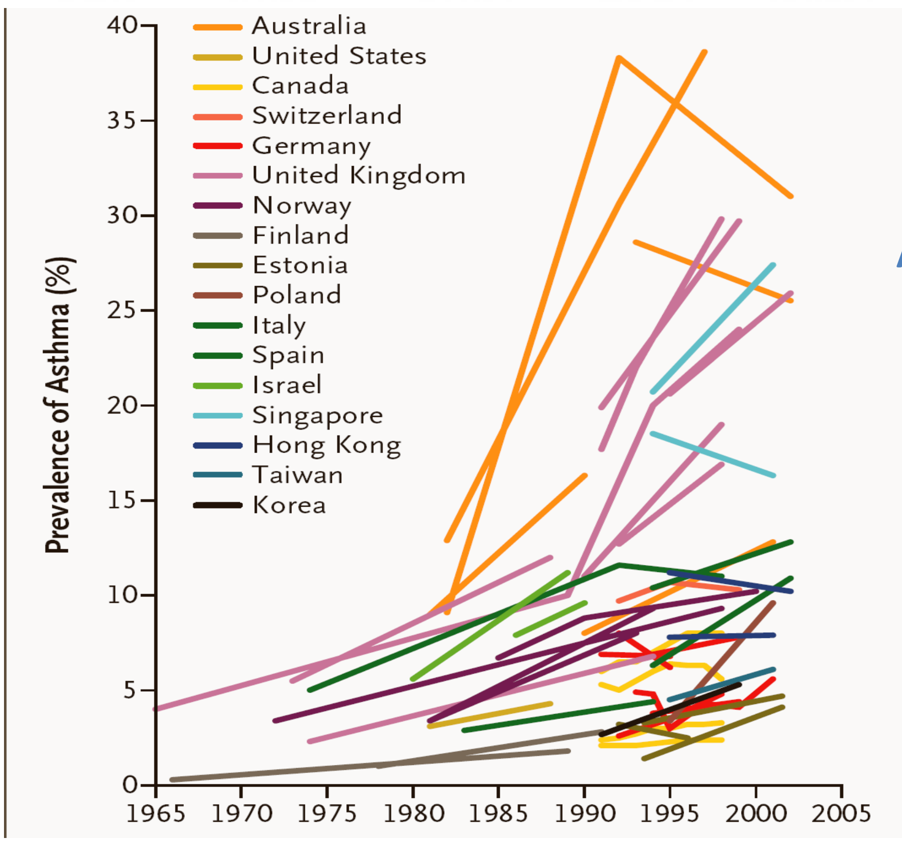
Juvenile (type 1) diabetes



Reflux Disease (GERD)



Asthma



Sources:

Ann NY Acad Sci 2008 12:1150

N Engl J Med 2006;355:2226

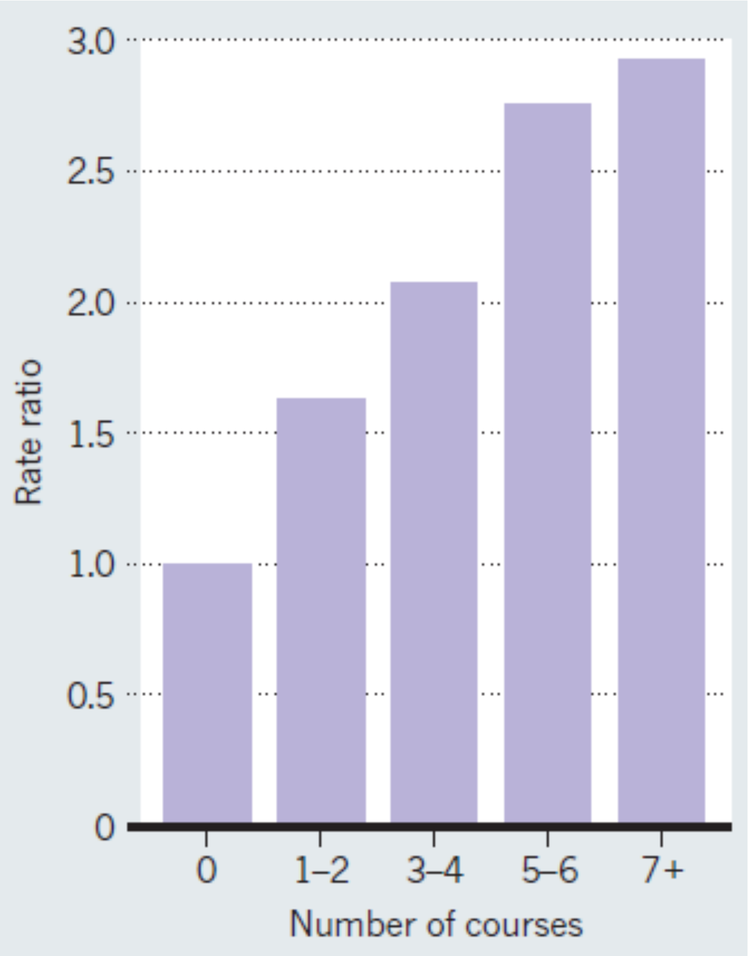
Gut 1997;41:594

Asthma risk at 7 years in 13,116 Manitoba children, according to antibiotic use in their first year of life

Courses of antibiotics	**OR (95% CI)**
0	1.0
1-2	1.21 (1.01-1.46)
3-4	1.30 (1.04-1.63)
>4	1.46 (1.14-1.88)
Non-respiratory infection	1.86 (1.02-3.37)

AL Kozyrskyj et al. Chest 2007; 131:1753-9.

Likelihood of IBD in Danish children, by early life antibiotic exposure



A. Hviid et al. Gut 2011; 60:49–54.

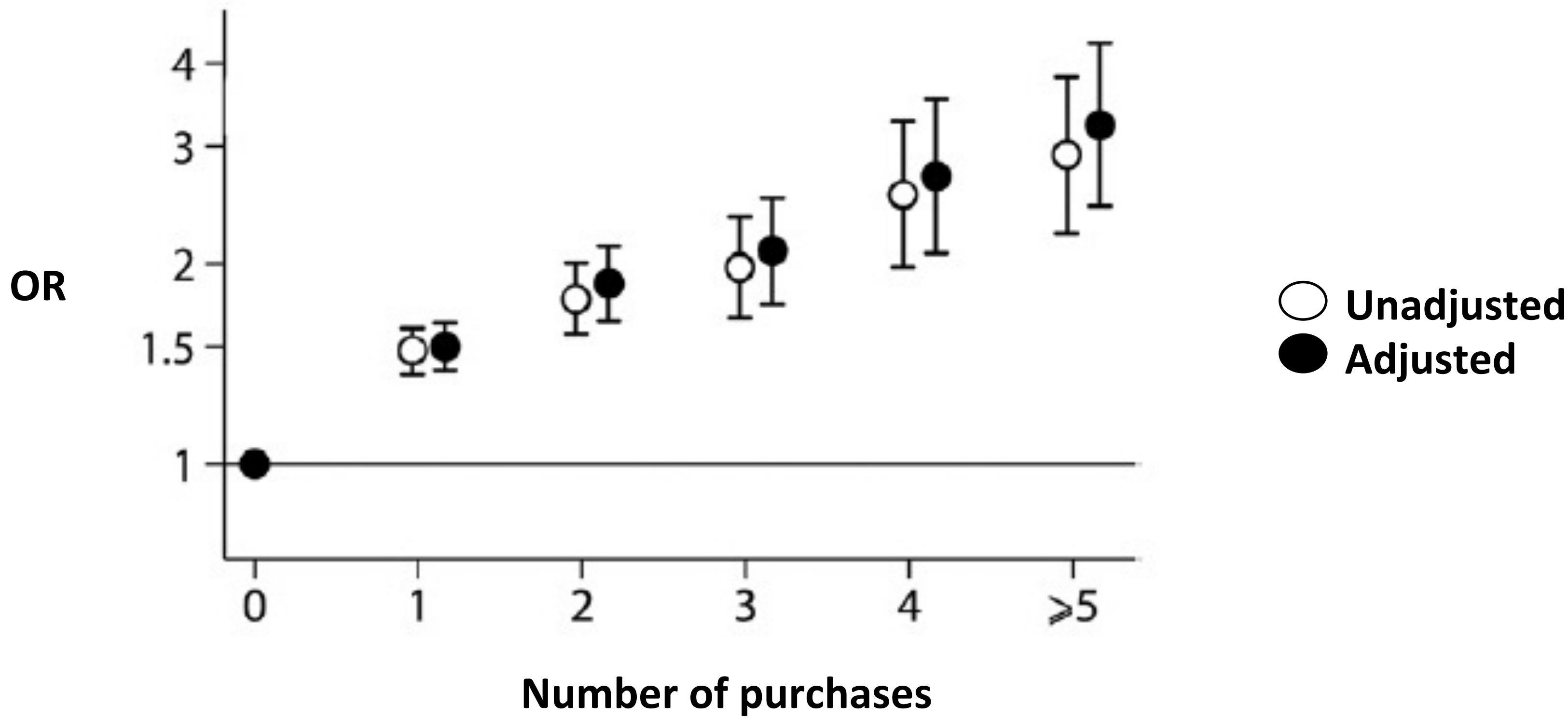
Child's use of prior antibiotics and risk of allergy to cow's milk

Type and number of antibiotic purchases	Percent		Adjusted model[a] OR (95% CI)
	Cases (n=15,672)	Controls (n=15,672)	
Any	21	15	1.71 (1.59-1.84)
Amoxicillin	14	11	1.39 (1.29-1.51)
Macrolides	8	6	1.65 (1.49-1.82)
Cephalosporins	6	3	2.43 (2.14-2.77)
Sulfas/TMP	1	1	1.60 (1.27-2.02)
Pen VK	1	1	1.97 (1.50-2.58)

[a] Reference group in each case is no use of that antibiotic. Model adjusted for maternal age, smoking, prior deliveries, mode of birth, child's birth weight.

J Metsala et al. Epidemiology 2013; 24:303-9.

Association between number of antibiotic courses from birth to diagnosis of allergy to cow's milk



Adjusted model includes maternal age, smoking, prior deliveries, mode of delivery, and child's birth weight.

J Metsala et al. Epidemiology 2013; 24:303-9.

Maternal use of antibiotics during pregnancy and risk of allergy to cow's milk in offspring

Type of antibiotic	Percent: Cases (n=15,672)	Percent: Controls (n=15,672)	Adjusted model[a] OR (95% CI)
Any	28	24	1.21 (1.14-1.28)
Cephalosporin	13	10	1.27 (1.17-1.38)
Extended spectrum penicillin	14	13	1.14 (1.06-1.23)
Macrolides	4	3	1.32 (1.15-1.51)
Tetracyclines	1	1	0.97 (0.72-1.29
Pen VK	2	2	1.04 (0.86-1.27)
Sulfas/TMP	<1	<1	0.87 (0.51-1.47)
Fluoroquinolones	<1	<1	1.66 (0.92-3.01)

[a] Adjusted for maternal age, SES, smoking, parity, multiple pregnancies, and child's use of antibiotics.

J Metsala et al. Epidemiology 2013; 24:303-9.

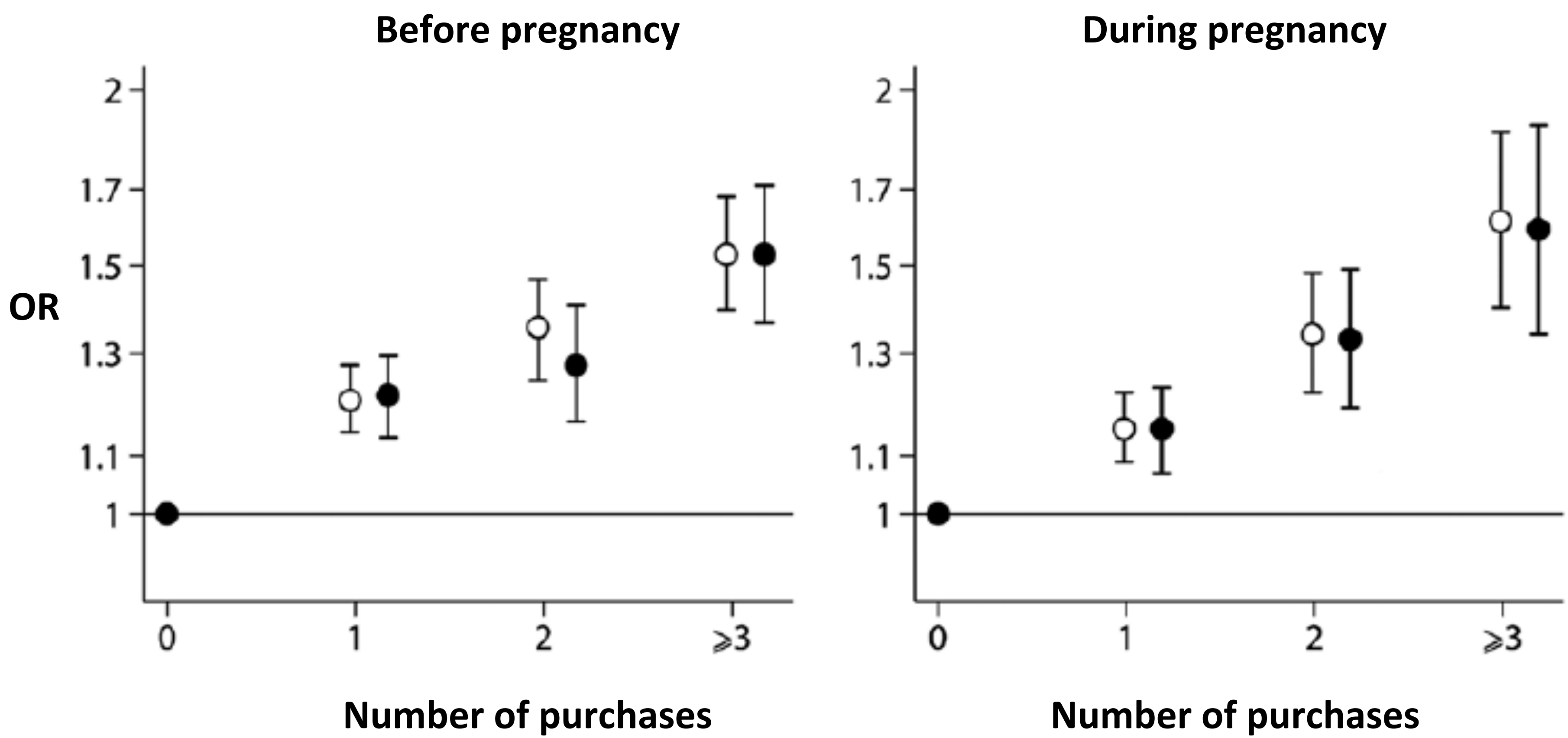
Maternal use of antibiotics before pregnancy and risk of allergy to cow's milk in offspring

Type of antibiotic	Percent: Cases (n=15,672)	Percent: Controls (n=15,672)	Adjusted model[a] OR (95% CI)
Any	41	35	1.26 (1.20-1.33)
Cephalosporin	16	13	1.29 (1.20-1.39)
Extended spectrum penicillin	12	10	1.16 (1.06-1.26)
Macrolides	11	8	1.26 (1.15-1.38)
Tetracyclines	10	8	1.25 (1.14-1.37)
Pen VK	4	3	1.14 (0.99-1.31)
Sulfas/TMP	3	2	1.00 (0.84-1.18)
Fluoroquinolones	2	2	1.09 (0.91-1.32)

[a] Adjusted for maternal age, SES, smoking, parity, multiple pregnancies, and child's use of antibiotics.

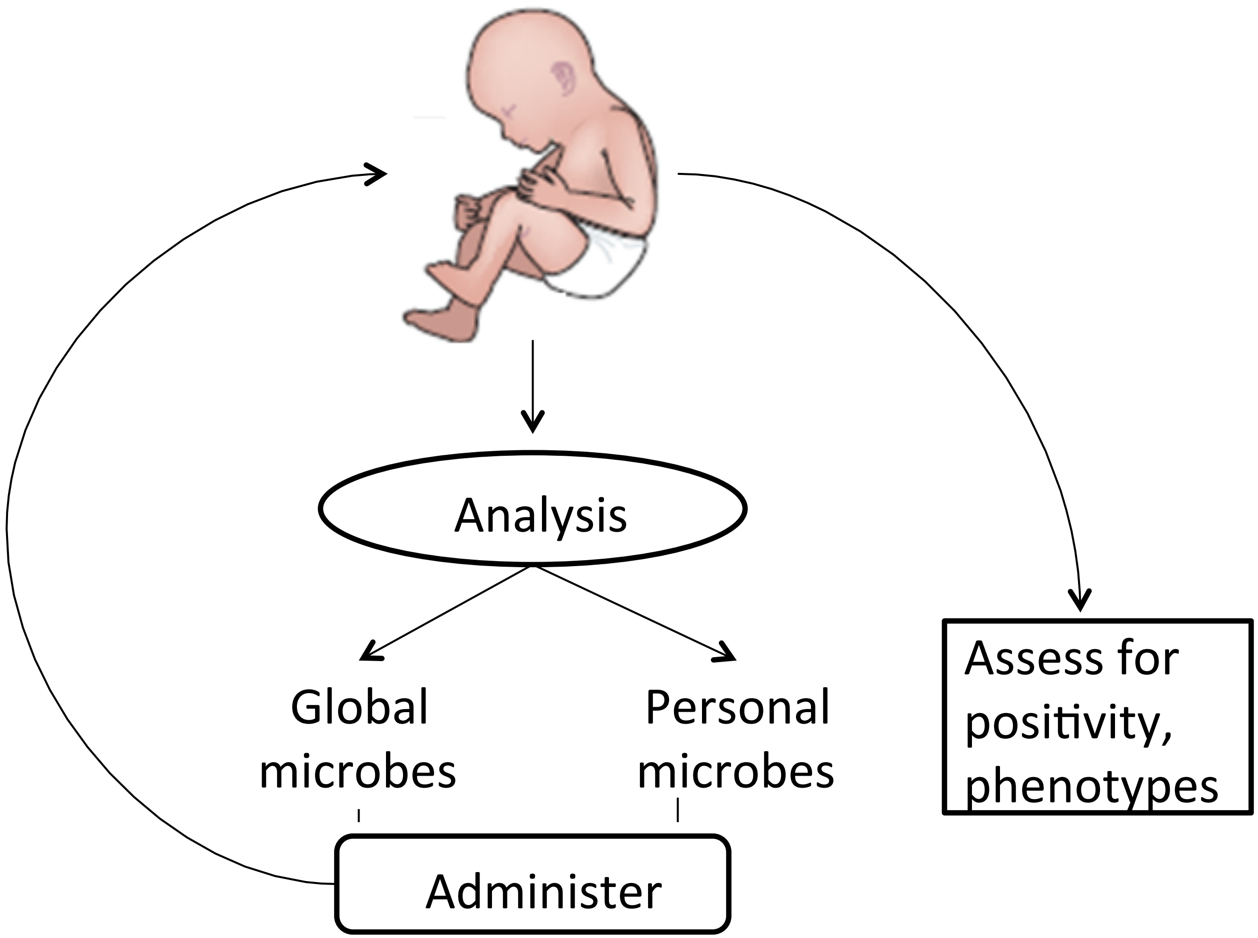
J Metsala et al. Epidemiology 2013; 24:303-9.

Association between number of maternal antibiotic courses and risk of allergy to cow's milk in offspring



J Metsala et al. Epidemiology 2013; 24:303-9.

New algorithm for child health



Jakob Feist, citizen of Graz, Austria

Human microbiome labmates at NYU