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The Interplay between the Gut Microbiota and the Immune System

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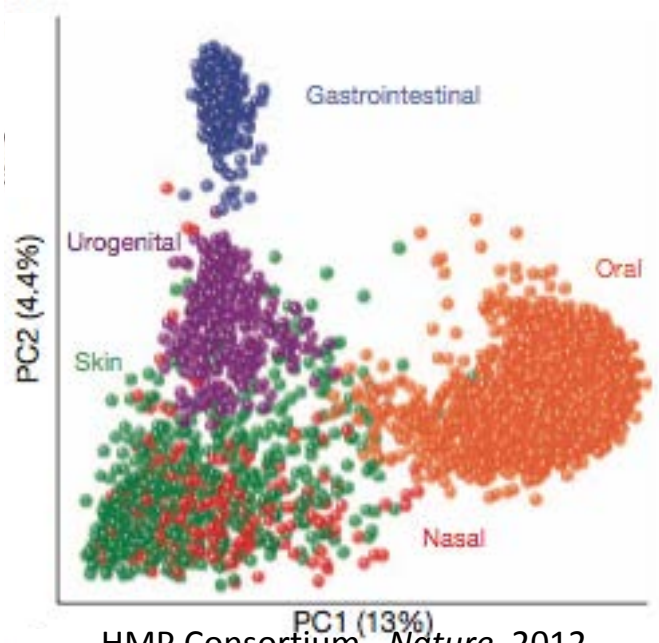
The Microbiota

- 10^{14} microorganisms live in association with humans
  - Contain 100-fold more genes than the human genome
  - Concept of a "super-organism": a combination of human genes and the genes of our microbial partners, the microbiome

- Distinct taxa are found across human environment
  - Main phyla include:
    - Bacteroidetes
    - Firmicutes
    - Actinobacteria
    - Proteobacteria
  - At lower taxonomic levels great variation

  Is seen among individuals



HMP Consortium., *Nature*, 2012

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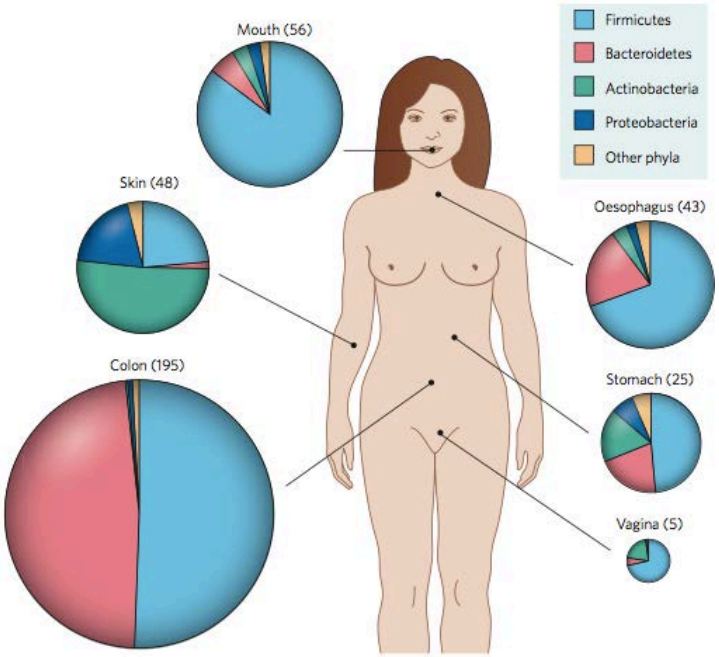
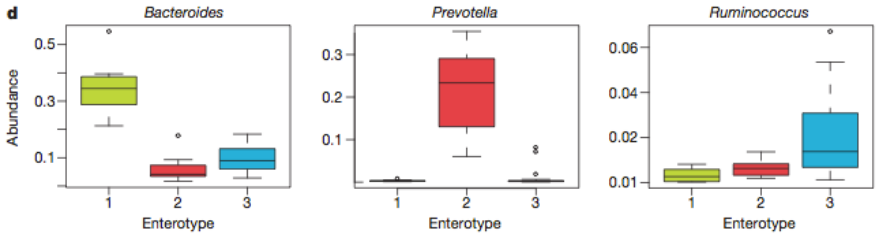
The Gastrointestinal Microbiota

- Diverse community
  - Main phyla: Bacteroidetes, Firmicutes
  - Variation among individuals and over time

- "Core" microbiome
  - What taxa are shared in healthy subjects?
  - Enterotypes: distinct community types present in the human population, each defined by a dominant genus
    - *Bacteroides, Ruminococcus, Prevotella*



Dethlefsen et al., *Nature*, 2007



Arumugam et al., *Nature*, 2011

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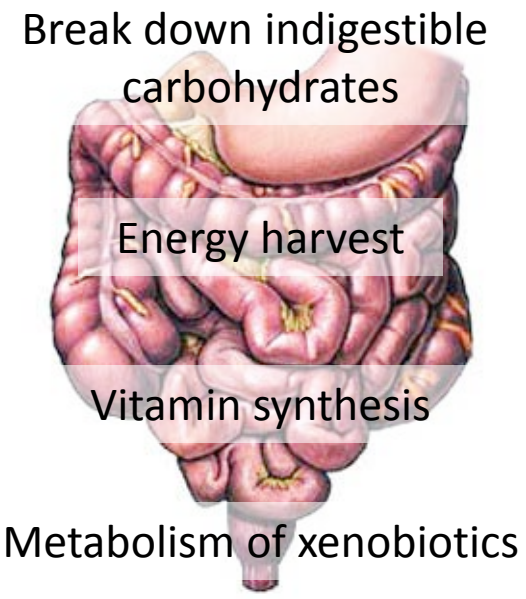
The Gastrointestinal Microbiota: Nutrition and Metabolism

- Host and microbiota have co-evolved
- Microbiome provides essential functions to host
  - Evidence from gnotobiotic mouse models
    (Backhed et al., 2004; Turnbaugh et al., 2009)
- Diet/nutrition can shape the microbiota
  - Microbiome reflection of dietary habits?
    (Wu et al., 2011; De Filippo et al., 2011)



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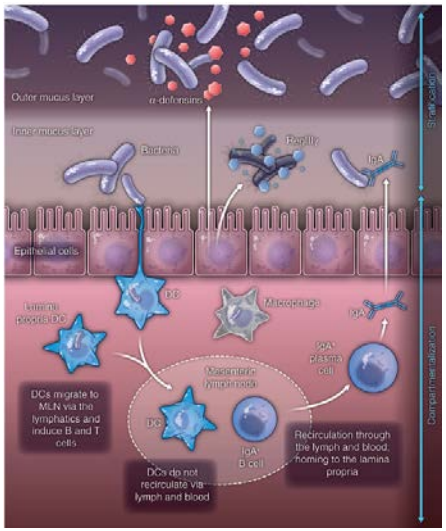
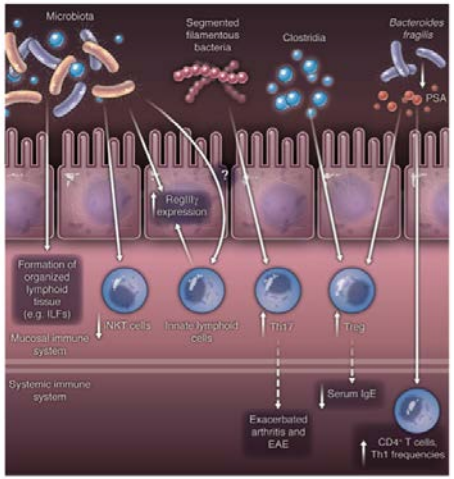
The Gastrointestinal Microbiota and the Immune System

- The immune system shapes the microbiota
  - Mucus layer
  - Antibacterial proteins
  - IgA
- The microbiota impacts immune system development
  - Mucosal immune system:
    - Formation of organized lymphoid tissue
    - Regulation of innate lymphoid cells
  - Systemic immune system:
    - CD4, T_{reg}, T_h17 cells





Hooper et. al., *Science*, 2012

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Shigellosis: An Endemic Disease

- Caused by *Shigella*, a mucosally invasive bacterium
  - Bloody diarrhea, fever, stomach cramps
  - Transmitted fecal-oral route

- Restricted to humans and non-human primates
  - Cynomolgus monkeys can serve as an animal model in vaccine development

- Evidence for effective live-attenuated vaccines administered orally (Levine et al., *Nat Rev Micro*, 2007)
  - No approved vaccine, but current vaccine trials are underway
  - **Variability in vaccine response observed in different global populations**
    - Unknown cause (diet, environment, genetic)
    - Role of gastrointestinal microbiota has not been investigated

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- Oral live-attenuated *Shigella* vaccine trials with cynomolgus monkeys
  - Collect stool samples pre- and post-immunization and after WT challenge
  - Characterize the microbiota post-immunization and post-infection

- How does exposure to an enteric pathogen affect the intestinal microbiota?

HYPOTHESES:

Wild-type and live-attenuated strains of *Shigella* will alter the gastrointestinal microbiota

Composition of the intestinal microbiota may affect the outcome following immunization, challenge

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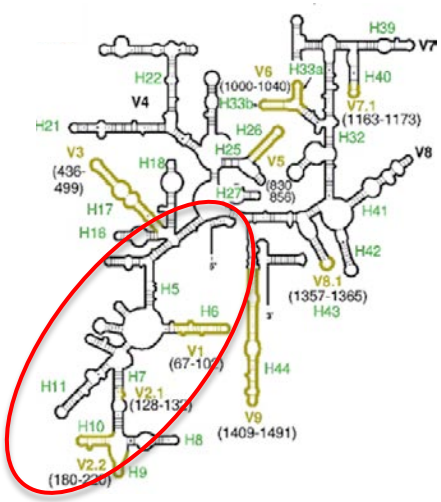
16S rRNA Studies: Molecular census of the microbiota

- Most of the microbiota cannot be cultured
- High-throughput, multiplex, parallel sequencing and analysis technology has enabled characterization of microbiota
- 16S rRNA Surveys: Use 16S rRNA hypervariable region for bacterial identification
  - Universal gene in prokaryotes
  - Variable regions can be used for assigning taxa and phylogenetic relationships
  - Conserved regions can be used to design universal primers to amplify specific regions



Gary Andersen, Todd DeSantis, Lawrence Berkeley Lab

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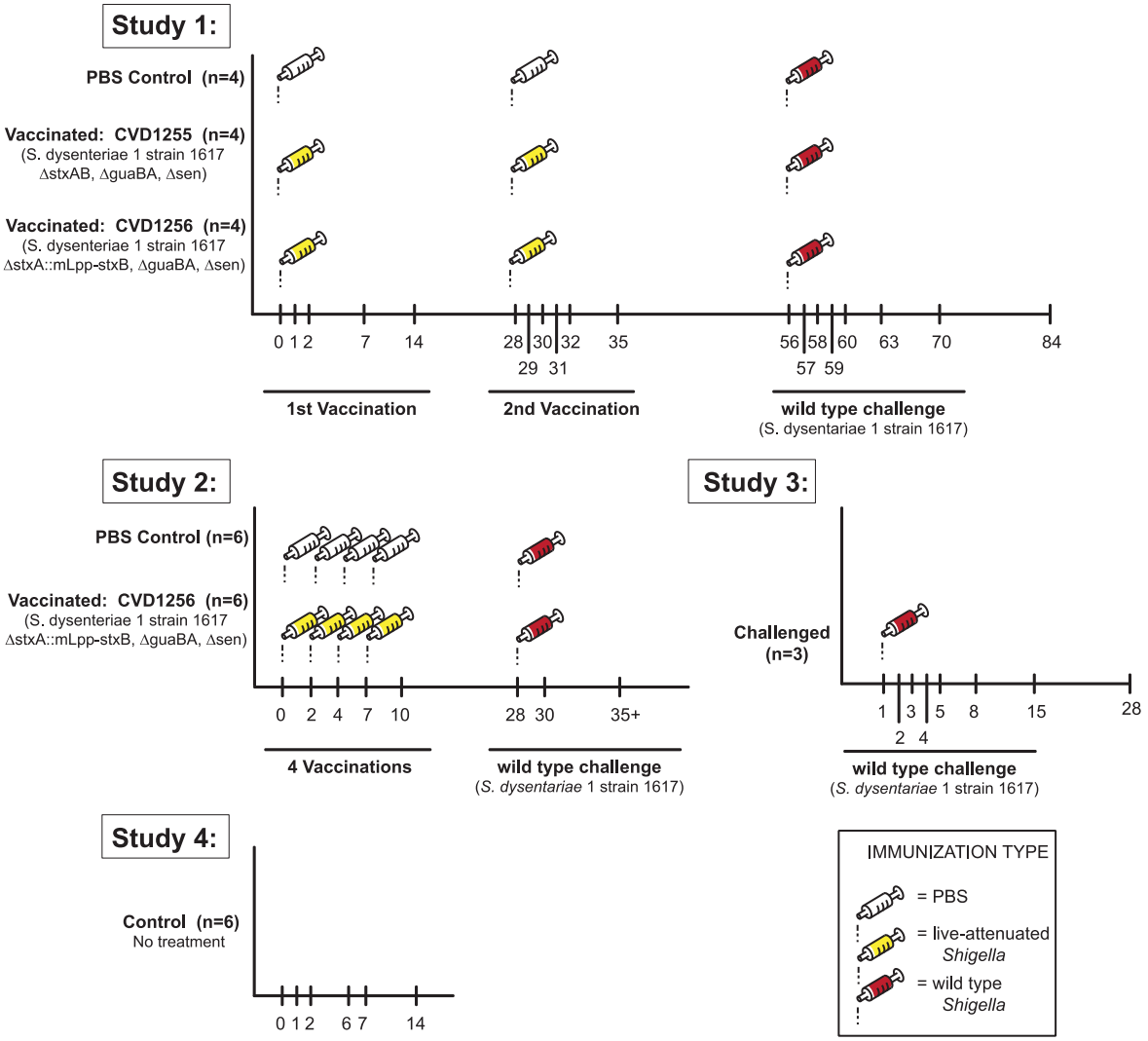

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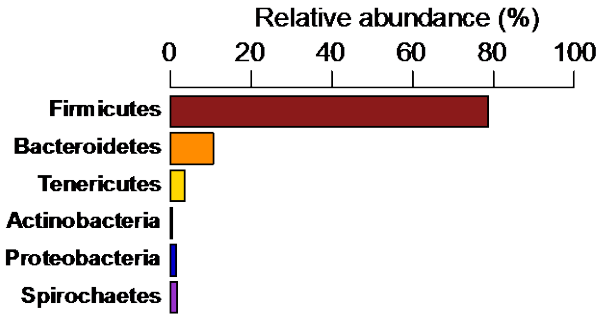

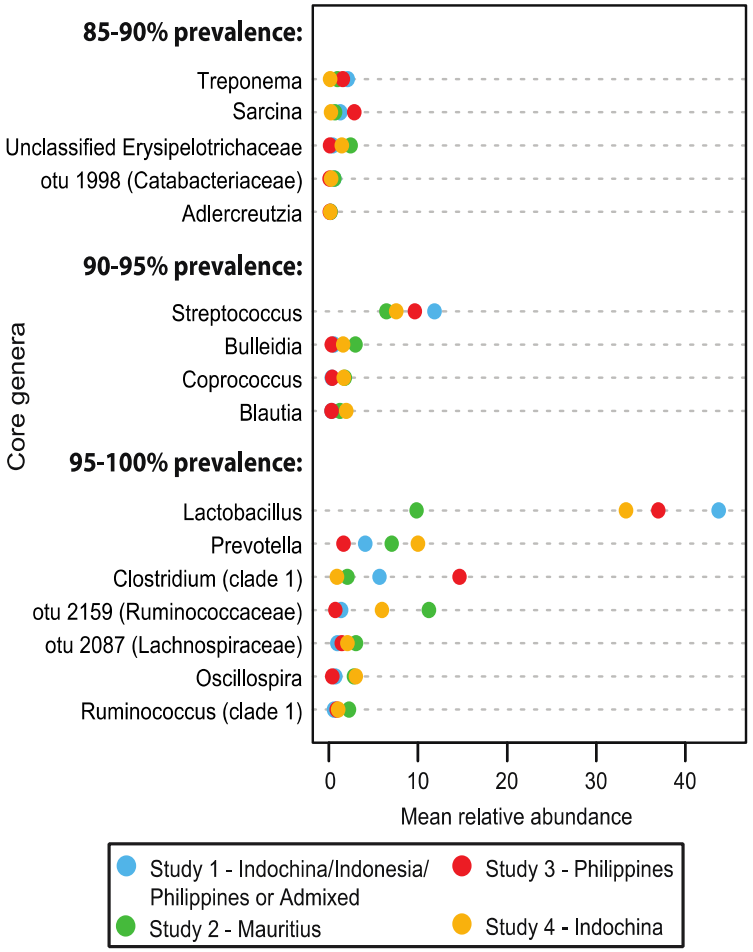

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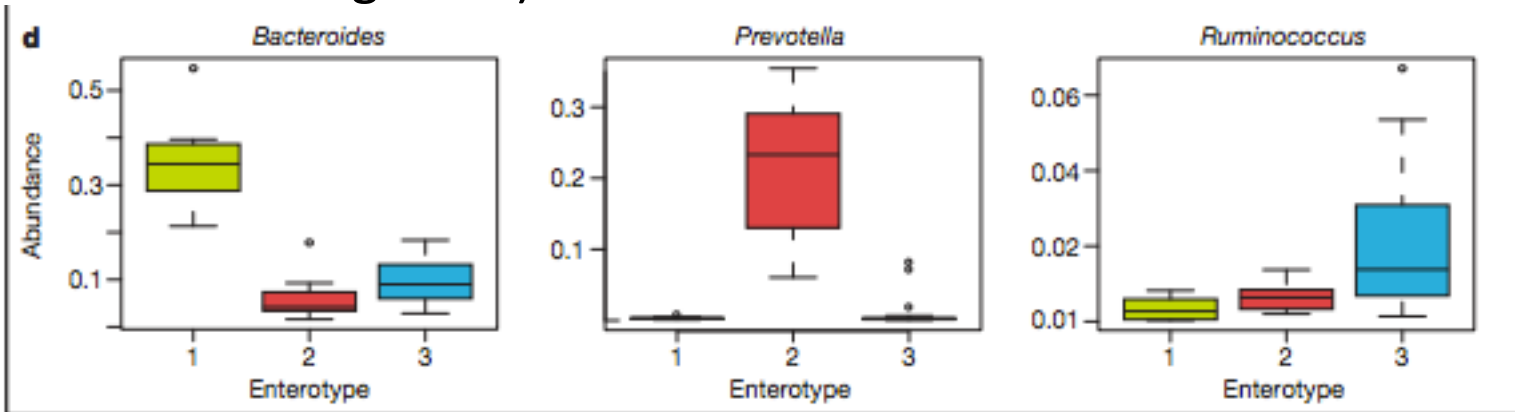
Enterotypes in human GI microbiota

- Identification of enterotypes within humans
  - Limited number of community types within human population, dominated by *Prevotella*, *Bacteroides,* or members of the phylum, Firmicutes
  - Stable over time

- Determined by multidimensional cluster analysis from Jensen-Shannon divergence (measures similarity between probability distributions of genera)



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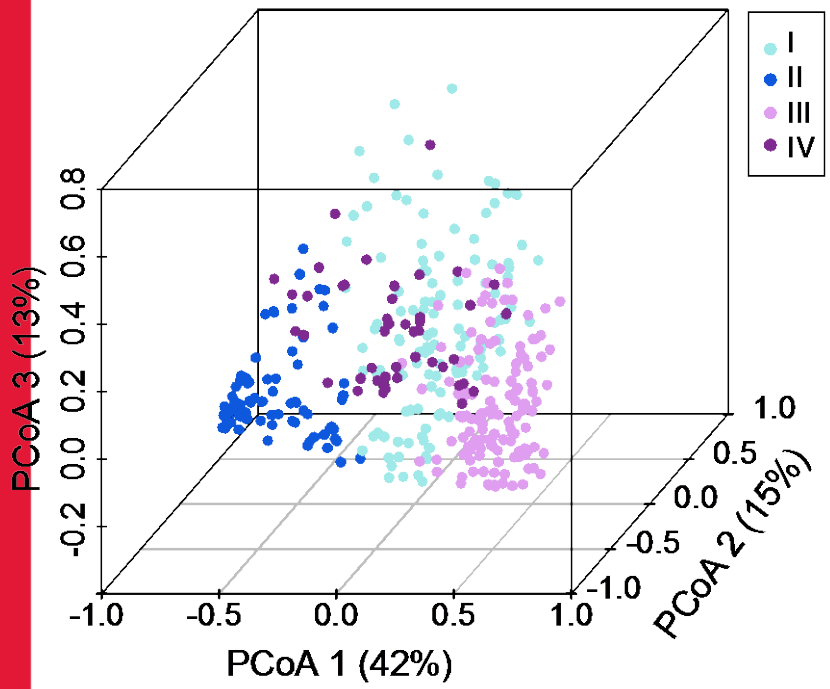
Enterotypes within the cynomolgus monkey microbiota



→ Cluster analysis reveals 4 distinct community profiles

→ Each is characterized by a dominant genera

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Genetic diversity in cynomolgus macaques

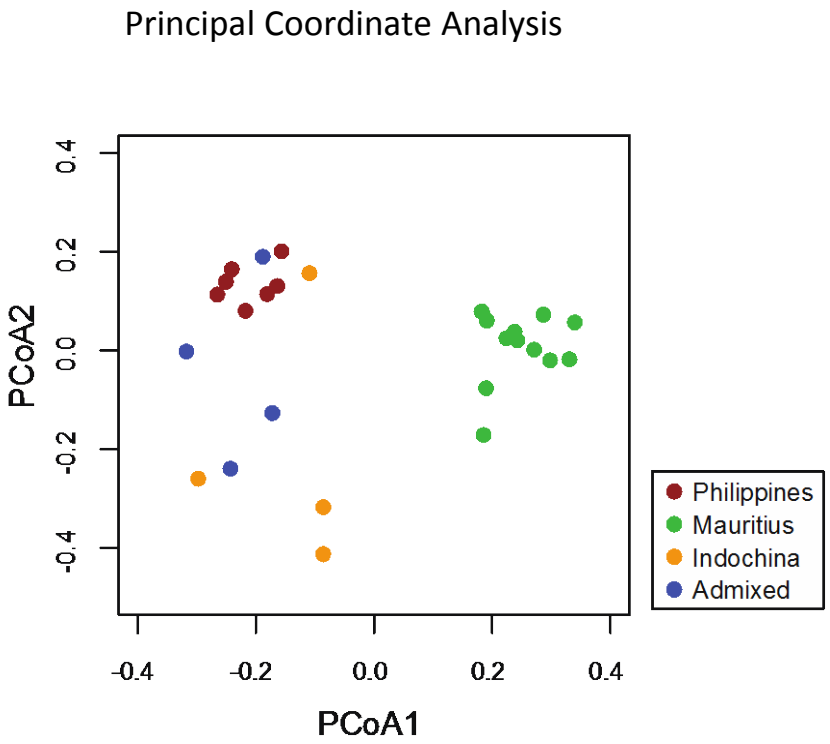
- Previous studies indicate allelic differences within cynomolgus macaques of different geographic origin

  (Krebs et al., 2005; Mee et al., 2009; Florese et al. 2009; Mitchell et al. 2012)
  - Indochinese/Indonesian: high level of diversity within MHC regions I and II
  - Mauritian: geographically isolated, restricted genetic diversity in MHC region

- MHC haplotype has been shown to be important in disease susceptibility in cynomolgus macaques (Wiseman et al. 2007; Florese et al., 2009)

- Analysis of non-MHC and MHC genotypes in our cynomolgus macaque population
  - Analyzed 24 short tandem repeats (STRs) to determine geographic origin
  - Analyzed seven microsatellite regions spanning the MHC
  - Determine whether genetic diversity correlated with differences in the microbiota

Determination of macaque geographic origin

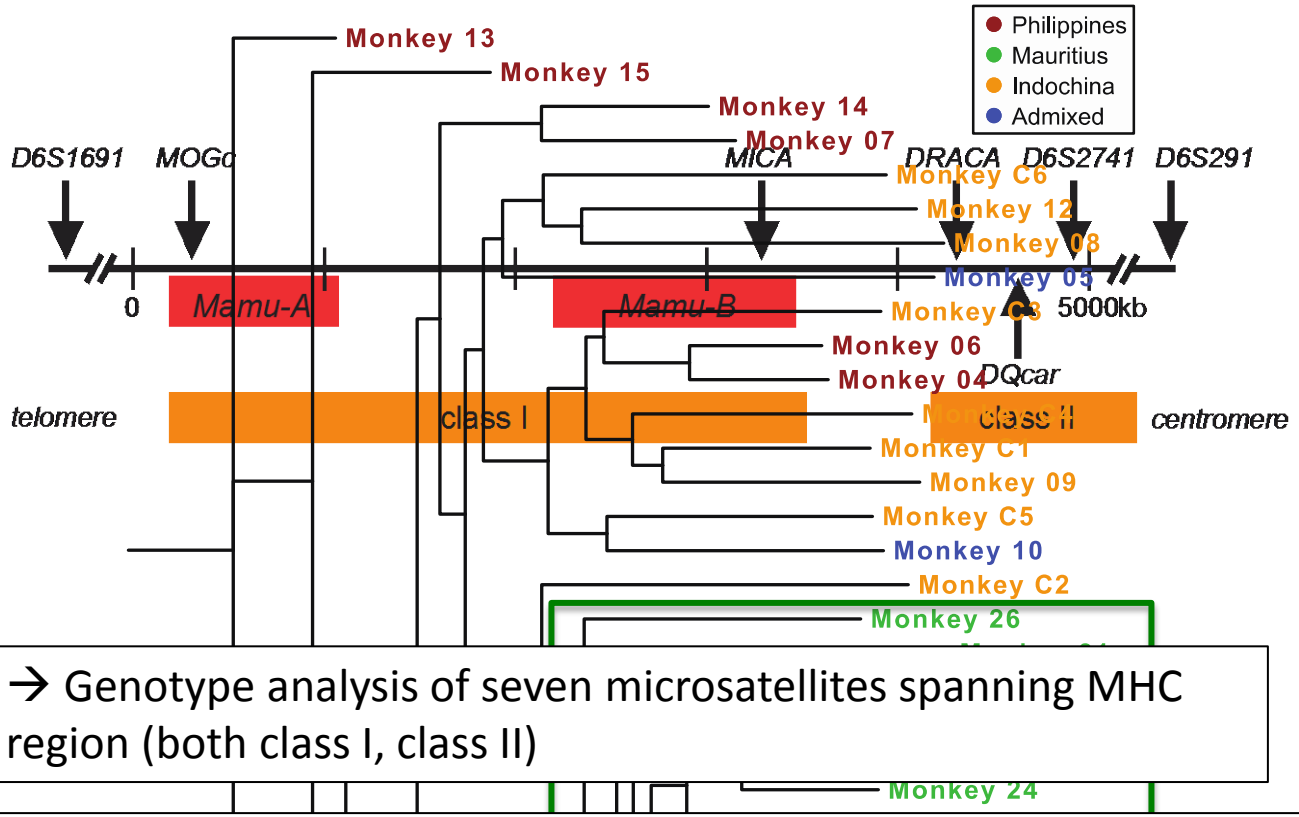


→ Genotype analysis using 24 non-MHC STRs from peripheral blood lymphocytes

→ Macaques from Mauritius cluster together

→ Comparison of STR data to macaques of known geographic origin confirmed macaque origin

MHC allele repertoire in macaques from different geographic origin



→ Genotype analysis of seven microsatellites spanning MHC region (both class I, class II)

→ Broad range of alleles for regions tested

→ As with non-MHC alleles, macaques from Mauritius cluster together

→ **Macaques from Mauritius exhibit unique profile compared to macaques from Indonesia/Indochina/Philippines**

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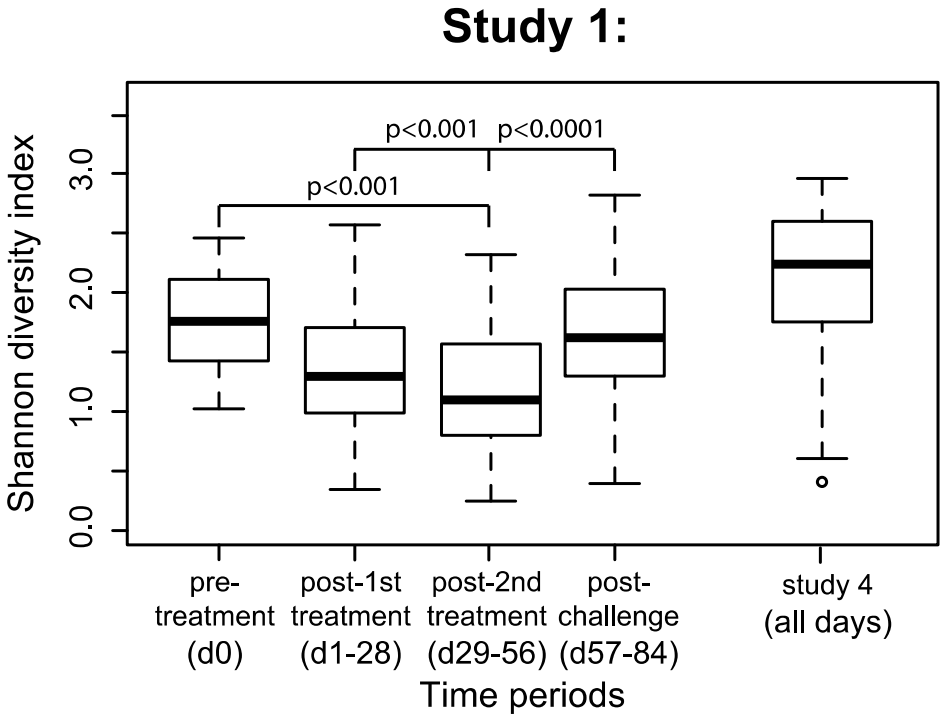
Impact of live-attenuated and wild type *S. dysenteriae* 1 strains on microbiota composition

- Does vaccination and/or WT challenge alter microbiota composition?
  - Changes in diversity
  - Changes in community types
  - Clinical and immunological outcome following these events
  - Are observed changes the same for all populations

Measures of diversity:
Does vaccination, challenge affect microbiota?



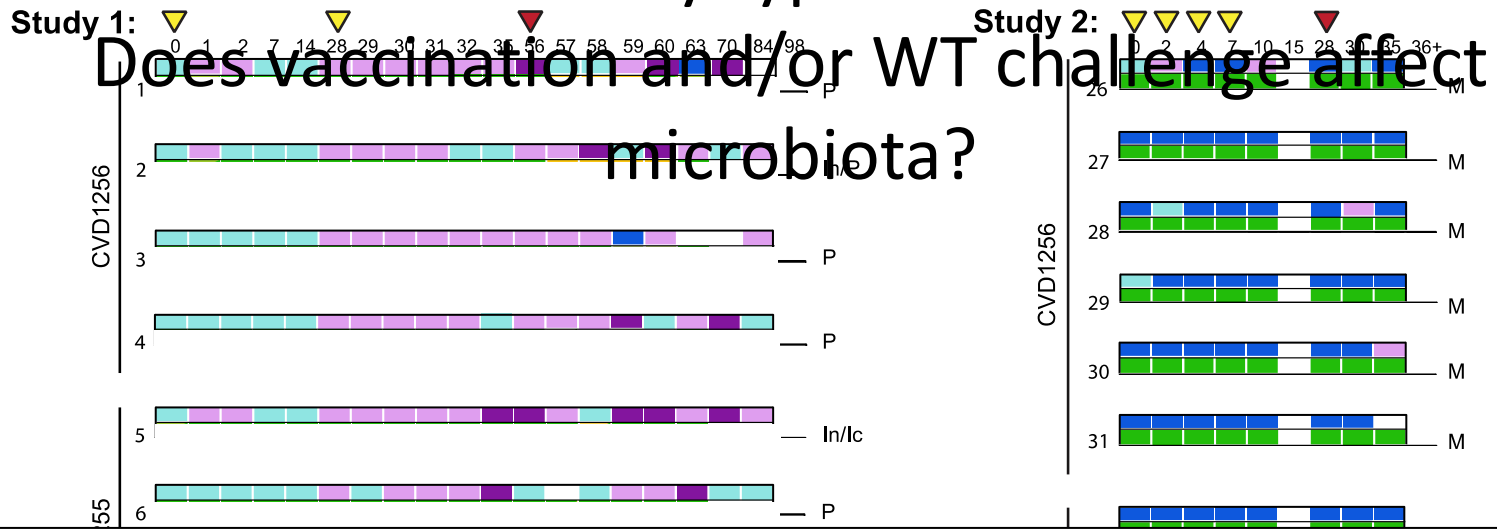
→ Significant change in Shannon diversity index following immunization in study 1 macaques and compared to study 4 macaques (untreated)

→ No change in diversity estimates in study 2 macaques!

→ **Differences observed in macaques from study 1 and 2**

Community types over time:

Does vaccination and/or WT challenge affect microbiota?



→ Both vaccination and challenge induce anti-LPS IgA, IgG antibody production (observed in both studies 1 and 2)

→ Difference in community type, clinical response between study 1 and study 2 macaques:

-- **Study 1:**

-- changes in community type, diversity measures following both vaccination and challenge

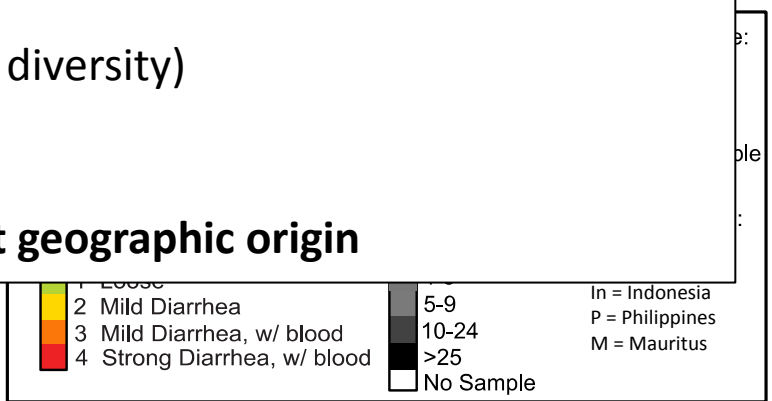
-- Exhibit clinical symptoms

-- **Study 2:**

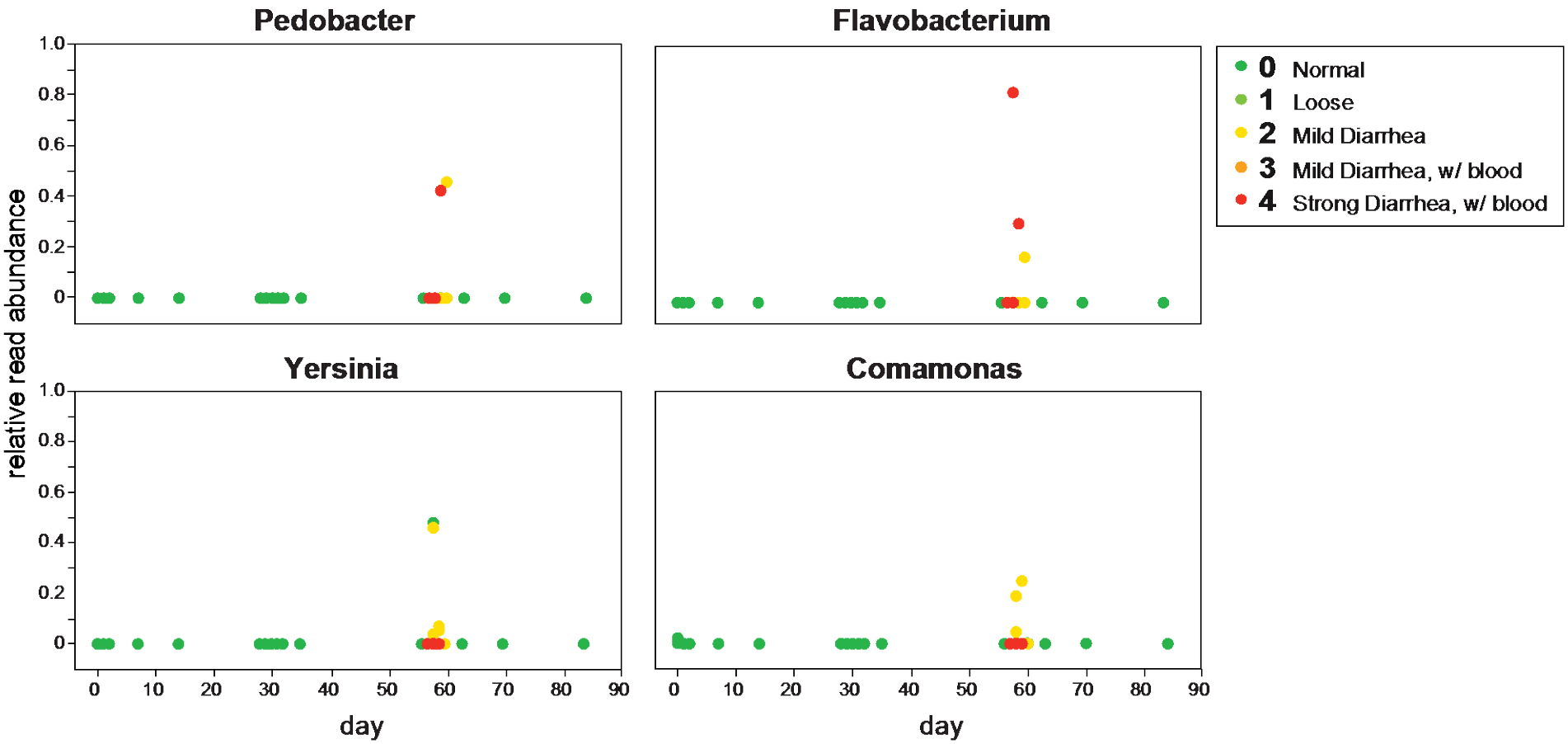
-- persistence of initial community type (high diversity)

--do not exhibit clinical symptoms

→ **Different community types in monkeys of different geographic origin**



Increase in normally rare genera is associated with clinical symptoms following challenge



→ Increased abundance of *Pedobacter, Yersinia, Flavobacterium*, and *Comamonas* reads following challenge

→ **Only in monkeys that exhibit clinical disease symptoms → -- study 1 and 3 macaques (non-Mauritian)**

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Immune response following immunization and WT challenge

- Both immunization and challenge induce an immune response
  - Observed in both study 1 and 2 macaques
  - However, only study 1 macaques exhibited clinical shigellosis
    - Immune response as measured here not a determinant of protection—so what is?
      - Potential role for microbiota

- Are there correlations between strength, type of response and microbiota?
  - Utilized the statistical model, LSA (Local Similarity Alignment)
    - Time-dependent correlations
    - Used for intra-study comparisons

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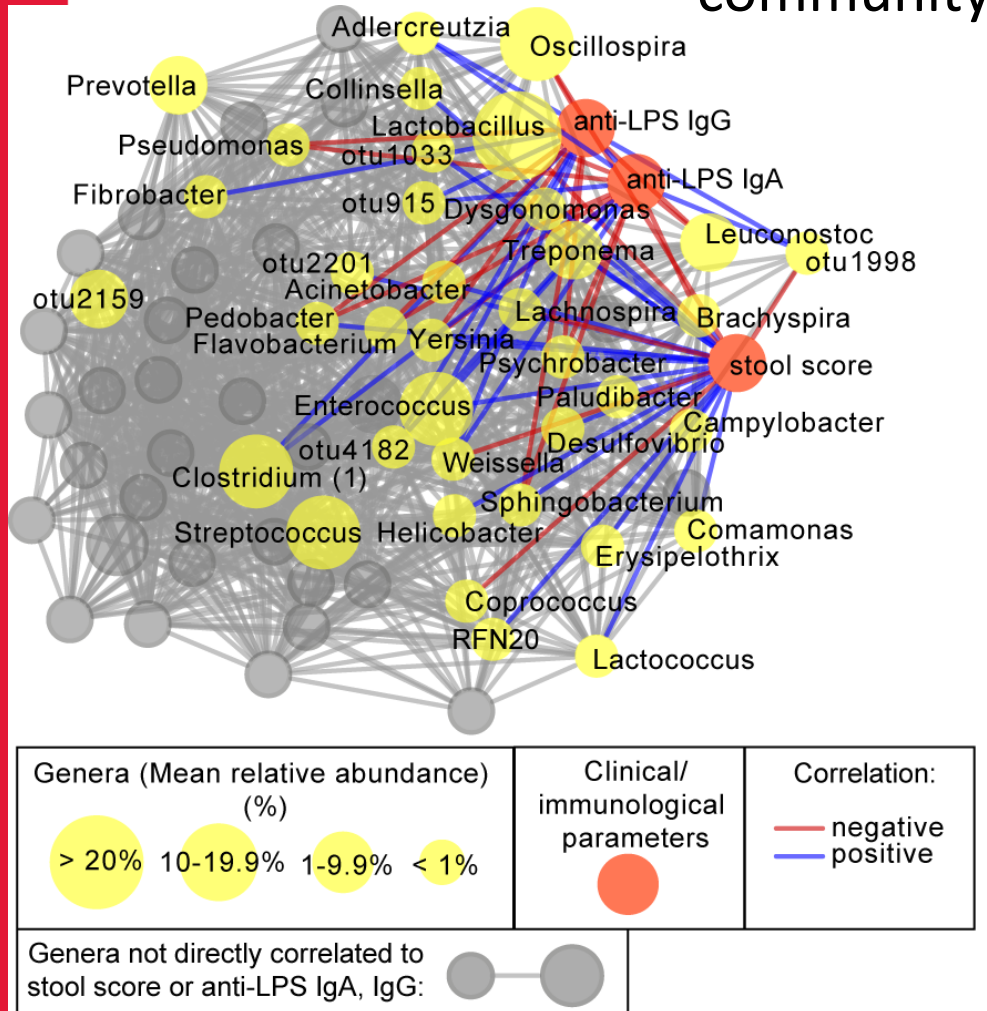
Correlation between immune responses and the microbiota community over time



Study 1:

→ Dense network of several genera related to anti-LPS IgA, IgG and stool score

-- complex relationships between genera

-- stool score correlated with many rare genera

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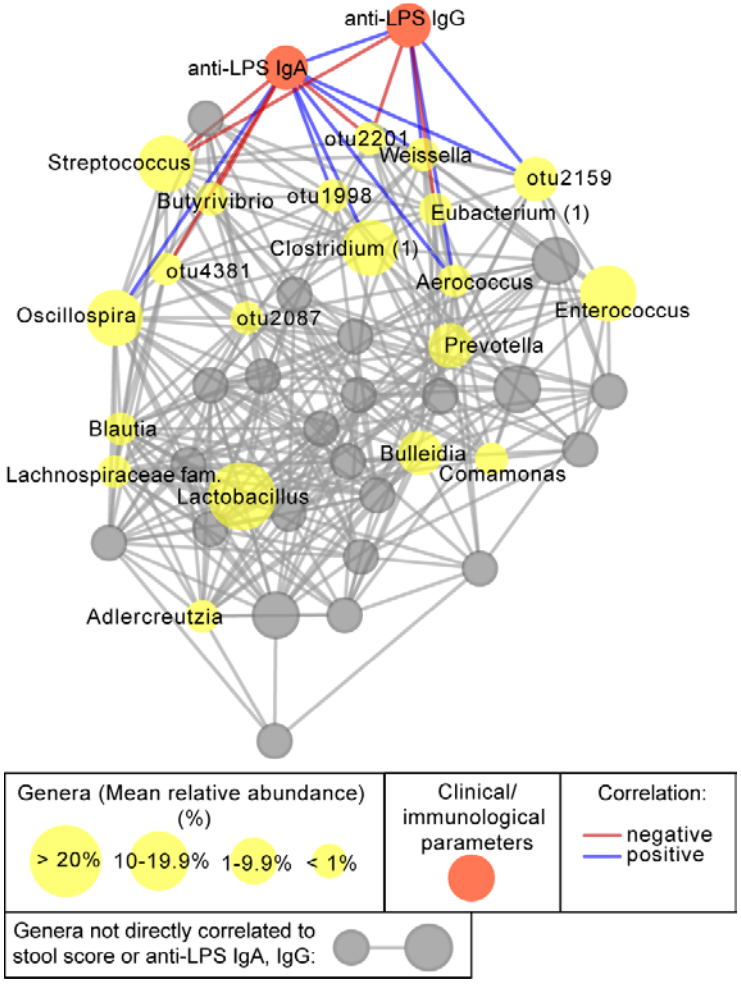
Correlation between immune responses and the microbiota community over time



Study 2:

→ Less dense network

--reflection of high community stability for these macaques

-- many observed correlations among core genera

→ Shared correlations among studies:

--*Weissella*

--otu2998

--*Clostridium* (clade 1)

→ Differences in vaccine regimen, however, make it difficult to determine how these may be related

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- We observe 4 enterotypes within the cynomolgus macaque gastrointestinal microbiota
  - 2 “healthy”, 2 “transient”
  - control macaques (study 4) are stable over time

- Different enterotypes are present in macaques of different geographic origin:
  - study 1:
    - Indochinese/Indonesian/Philippine origin
    - post-vaccination: change in community type, diversity
    - post-challenge: change in community type, increase in normally rare organisms
    - exhibited clinical symptoms
  - study 2:
    - Mauritius origin
    - no changes post-vaccination, post-challenge
    - did not exhibit clinical symptoms
  - STR and microsatellite genotypic analysis suggests a unique genotypic profile in Mauritian macaques

- Vaccination and challenge induced immune response in both studies

- **Role of genotype in shaping microbiota composition**
- **High-diversity community type: protective against *Shigella***
- **Need for characterization of the microbiome in future vaccine studies**

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- **Fraser Laboratory:**
  - Emiley Eloe-Fadrosh
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