

american Preliminary Characterization of the gut American Gut Population

We have, as of September 5, 2013, completed sequencing and quality control of the gut bacteria from the first 1080 samples from 844 participants in the American Gut study. This document gives a first look at results for the whole population. Your individual results (if your sample is among these first 1080 samples) will be available shortly.

Here we compare the American Gut population to other populations who have had their gut bacteria characterized, describe the participants in the study, show the major kinds of bacteria in the gut microbiota of the American Gut population, and provide some information about what affects the gut bacteria (as well as show that some variables such as sex have surprisingly little effect).

Technical note: “microbiota” refers to a particular community of microbes, including bacteria (e.g. the human gut microbiota); “microbiome” refers to the genes those microbes contain (e.g. the human gut microbiome). Most participants in American Gut have signed up for characterization of the microbiota.

A Map of Your Microbes

A useful way to compare thousands of microbiota samples at a glance is by putting them on a map where more similar microbiota are placed together, more dissimilar ones further apart.

Figure 1A shows how the microbes from different parts of the body relate to one another: as you can see, the fecal, oral and skin microbiota form three points of a triangle, reflecting the major differences between these body locations (amazingly, even the same person has essentially none of the same bacteria in their skin, their mouth, and their gut). In these figures, we compare the data from American Gut to the data from the Human Microbiome Project, a \$173 million initiative funded by the National Institute of Health (NIH) to characterize both the microbiota and the microbiome of a total of 300 healthy subjects. Broadly the American Gut data are very comparable to the Human Microbiome Project data, and to several other projects.

In **Figure 1B**, we highlight the data from participants in the Personal Genome Project, who have agreed to make their genome data publicly available and who have also had their microbes characterized in the mouth, feces and skin through American Gut.

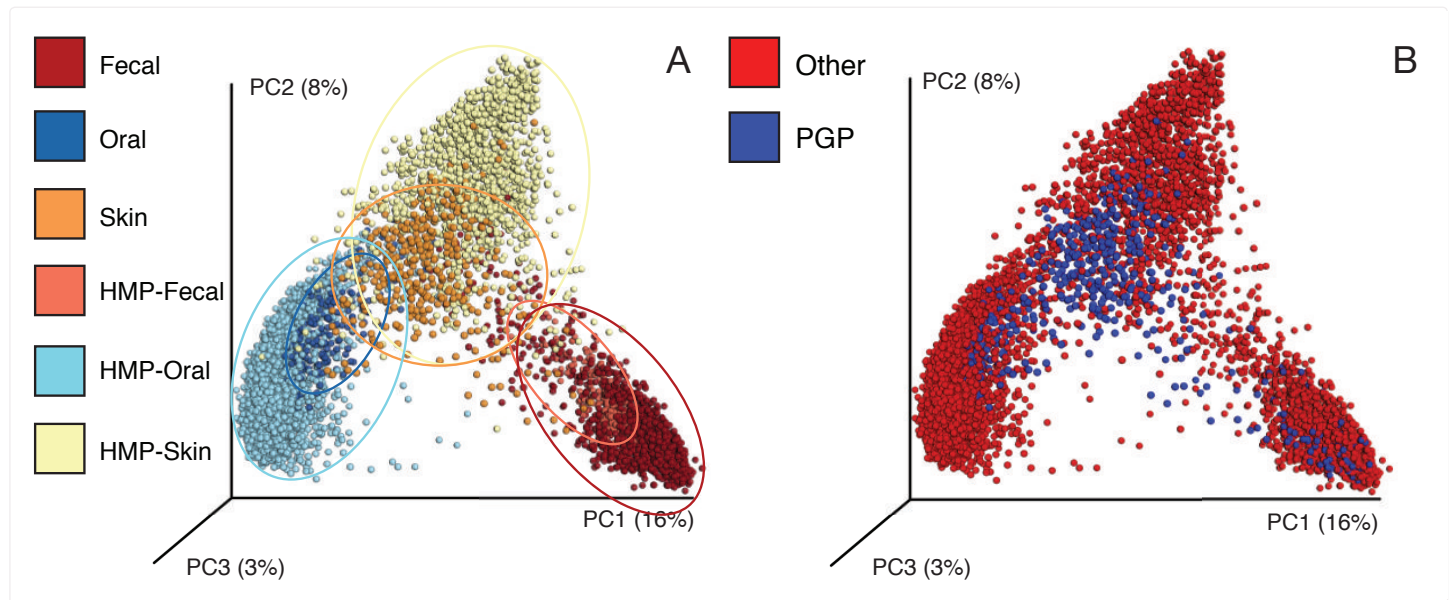


Figure 1: Comparison of microbiota in different studies and body sites. **Figure 1A** is colored by body habitat, showing how distinct the skin, oral and fecal samples are, and that American Gut and the Human Microbiome Project (HMP) agree about these differences with each other and with other studies. **Figure 1B** highlights one subgroup of participants, those of the Personal Genome Project (PGP).

Who is Participating in the AGP?

Our Participants

We have sequenced 1080 microbiota samples from 844 people, including 86 participants in the Personal Genome Project. We are just getting started but this number of participants substantially exceeds the numbers in other recent high-profile studies, including the Human Microbiome Project and the Global Gut study, which were published in the same issue of the scientific journal *Nature* last year.

Table 1

Samples and participants in the American Gut project (AG) compared to the Human Microbiome Project (HMP), Global Gut project (GG), and the Personal Genome Project microbiota component (PGP).

	HMP	GG	PGP	AG
Study Subjects	Adults USA	Adults, Children Venezuela, Malawi, USA	Adults	Adults, Children USA
Total Samples	4,788 ^a	531 ^b	439*	1,080*
Total Participants	242	531	86	844
Sequences	36,797,226	1,093,740,274	9,509,776	45,389,415

^aHMP = The Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome. 2012. *Nature* 486: 202-214.

^bGG = Global Gut, Yatsunenko et al. 2012. Human gut microbiome viewed across age and geography. *Nature* 486: 222-228

*PGP = Personal Genome Project, participants and samples being continuously added (unpublished data)

*AG = American Gut, participants and samples being continuously added

Figure 2

Comparison of gender, age and BMI across different projects that characterize the human microbiota. Relative to the HMP, AG contains more females, far more older and younger people, and a greater number of obese people. The concentration of older people is important because many health issues, including those that affect the gut, only appear later in life.

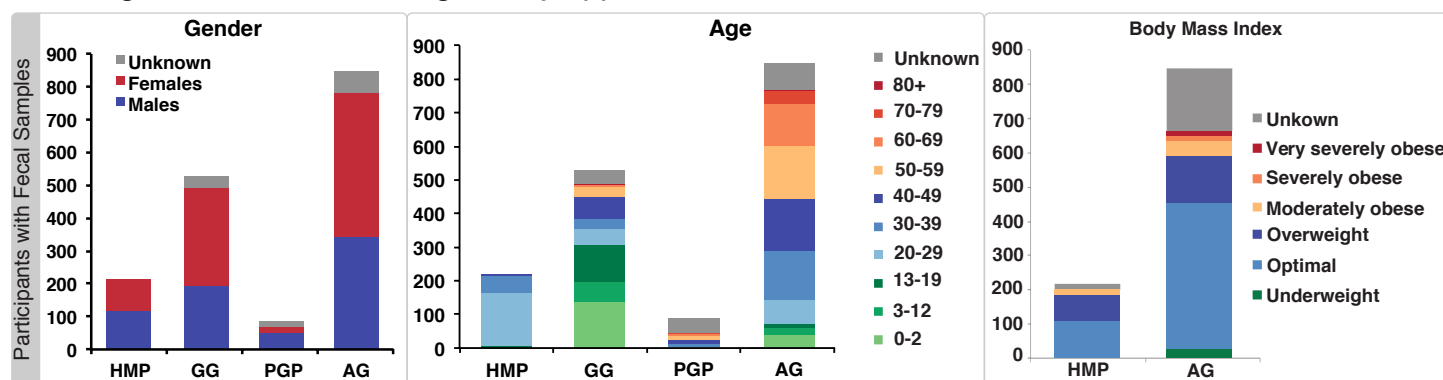
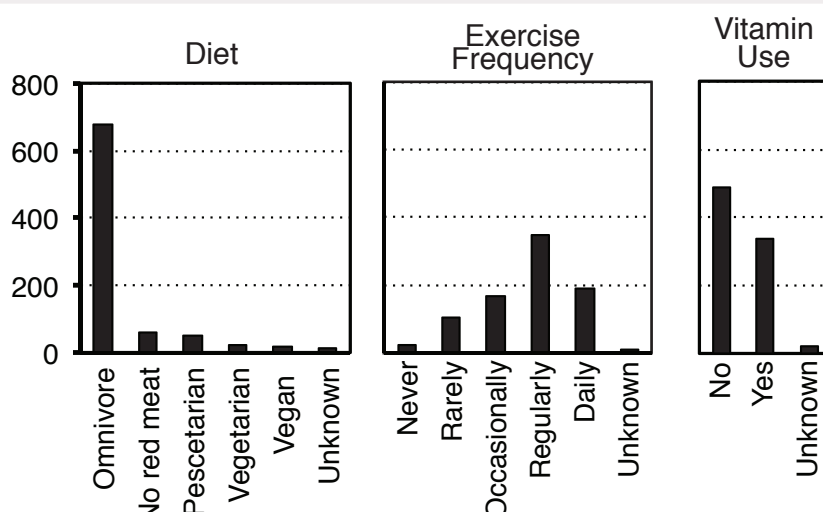


Figure 3

Diet and exercise characteristics of the AG population. According to self-reported data, the majority of our participants are omnivores who exercise frequently. Roughly half use vitamins regularly. As additional participants with unusual diet and exercise characteristics join the study (e.g. more vegans, or more people who never exercise), we will be able to say more about how these unusual lifestyles affect your gut microbes, and perhaps provide recommendations about what lifestyle factors are associated with healthier gut bacteria.





Who is Living in Your Gut?

Microbes in Your Gut

This section provides an overview of the kinds of microbes living in the gut of our study participants and an overview of some of the major players in the gut ecosystem. Just as seeing the major types of plants and animals (say, oaks versus pine trees) can give you a broad idea of what a traditional ecosystem looks like, knowing who is in your gut provides a useful guide to what is going on in there. And it is a very complex ecosystem, far more like a rainforest than like a desert.

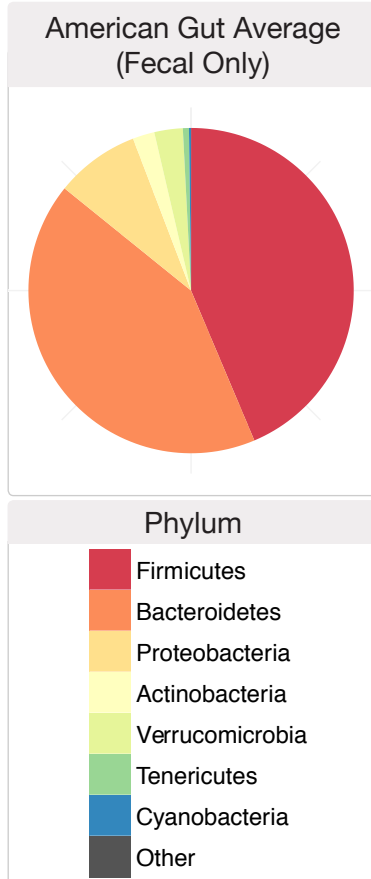


Figure 4

Average gut bacterial communities in the American Gut participants. This figure shows results at the level of bacterial phyla: a phylum is a big branch on the evolutionary tree of bacteria. (For context, all arthropods, from shrimp to spiders, are a single phylum). Many bacterial phyla can be present in guts, not all those that are present are common. For example, although *E. coli* is often thought of as a classic gut bacterium, we think this because it is good at growing on a petri dish, not because it is really the main component of the gut, and most healthy adults have only a few percent of the Proteobacteria phylum that contains it. At the taxonomic level of phyla, the major players are Firmicutes, which include *Clostridium* and *Lactobacillus*, among others, (including many producers of the short-chain fatty acid butyrate), and Bacteroidetes, which includes *Bacteroides* and *Prevotella*, both of which break down polysaccharides (big sugars).

What Type of Sample Did **You** Take?

Most participants in American Gut have been interested in their gut, but some have had their microbes characterized from other sites in the body, such as the skin (hands and/or forehead) and the mouth. Different parts of the body have very different microbes, as also shown in **Figure 1**, but additionally different people have very different microbes even at the same site in the body: for example, some people have over 90% Firmicutes in the gut, whereas other people have less than 1%. Understanding the reasons for these differences is a major goal of the project.

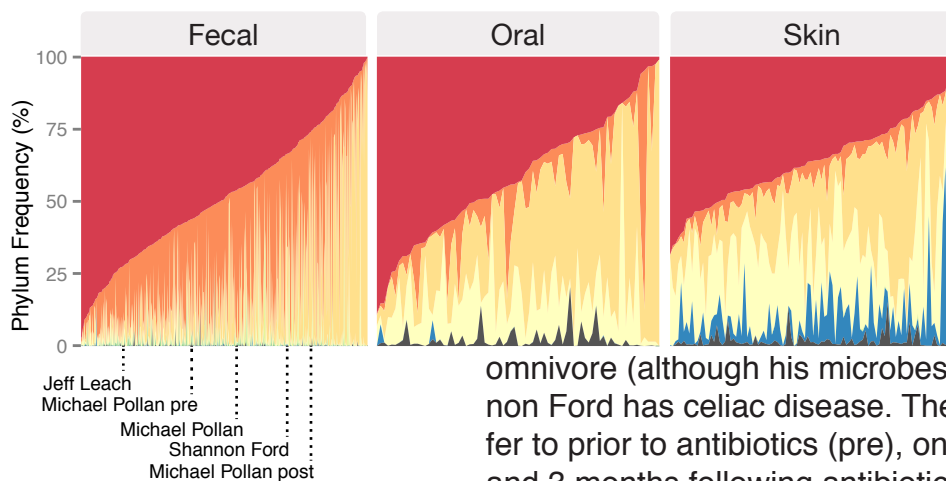


Figure 5

Microbial communities in fecal, oral and skin samples of AG participants (same colors as in **Figure 4**). A few of our participants have agreed to have their samples identified for comparison. Jeff Leach is a paleo dieter; Michael Pollan is famously an omnivore (although his microbes changed post-antibiotics); Shannon Ford has celiac disease. The three Michael Pollan samples refer to prior to antibiotics (pre), one week following antibiotics (post) and 3 months following antibiotics (without suffix).

What Affects Your Gut Microbes?

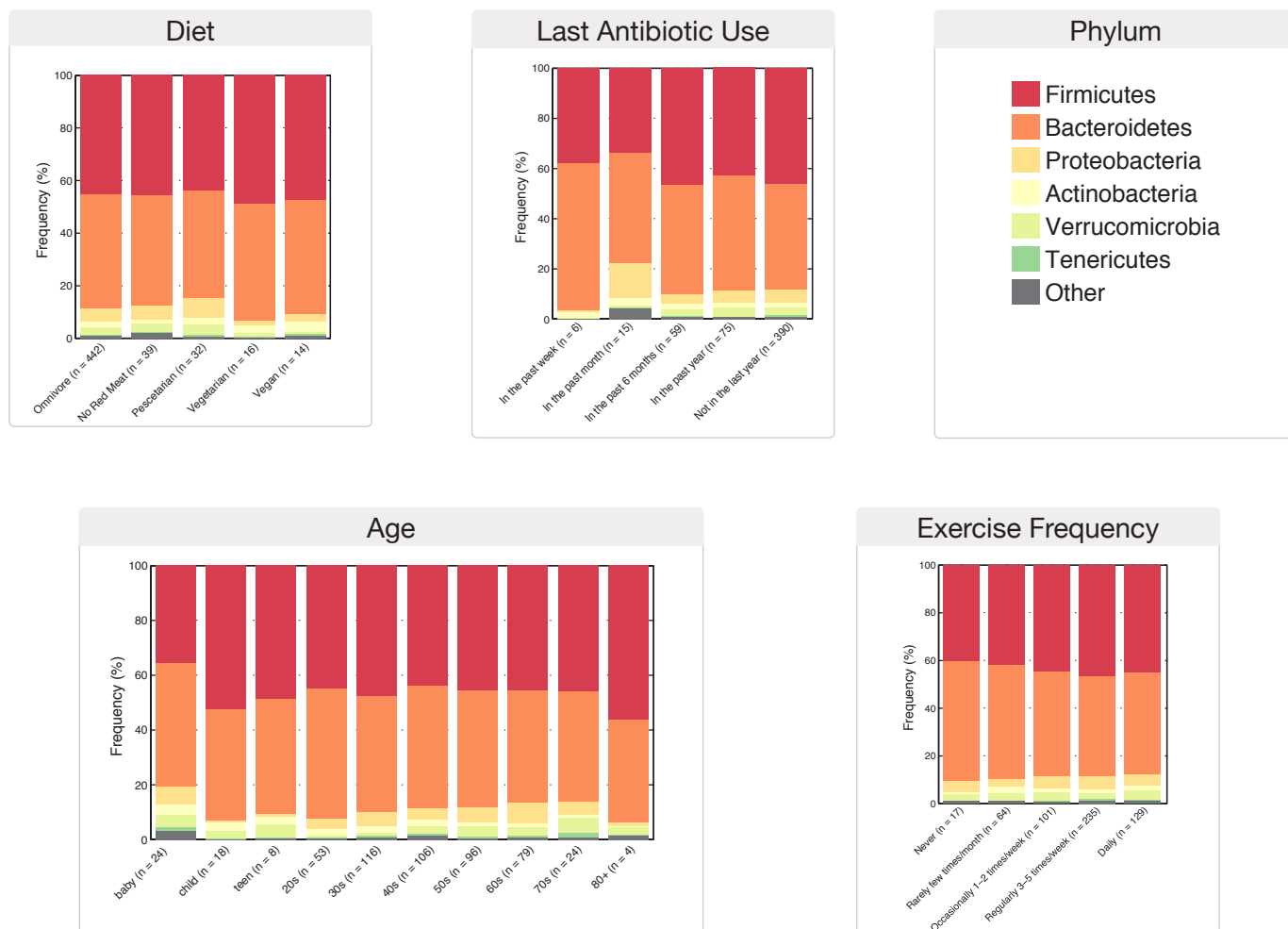


Factors That Affect Your Gut Habitat

As we saw in the last section, different people differ dramatically in what lives in their gut. Many factors, including antibiotic use, have some effect on the composition of species in the gut. But which of the many possible factors has the largest effect? Here we examine the effects of several obvious features that might affect your gut bacteria.

Figure 6

This figure shows the effects of diet, age, exercise frequency, and last antibiotic use on the average phylum-level variation in the gut bacteria of the American Gut participants. As you can see, these average compositions across many people are far less variable than the individual-level results shown in **Figure 5**, reflecting that a lot of the variability is individual and explained by other factors. However, we do see some statistically significant differences: for example, younger people, especially babies, have more Proteobacteria (as expected from other published results), and vegetarians and vegans have fewer Proteobacteria (which technically means vegans are less like babies than are meat eaters. Go figure). Antibiotic use within the past month or the past week also has a substantial effect at this level, although other research has shown that the results depend on which antibiotic it is and what was in that person's gut to begin with.



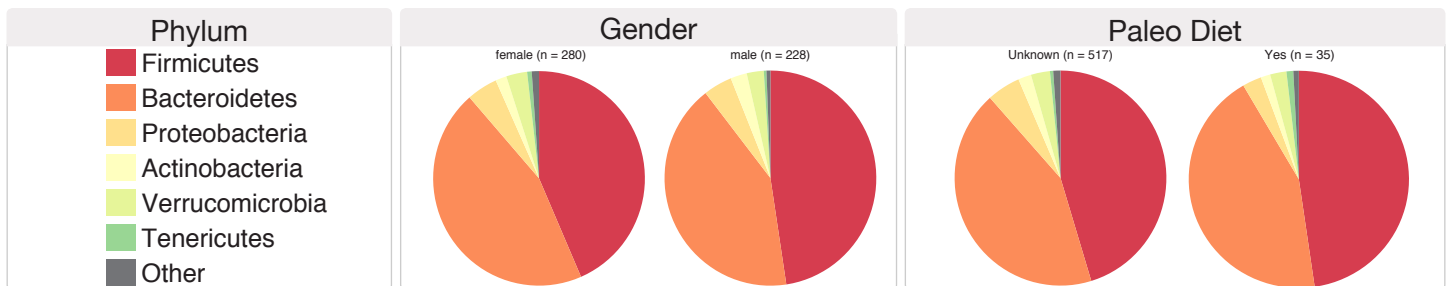


Diversity and Taxonomy

Where Do You Fit In?

Figure 7

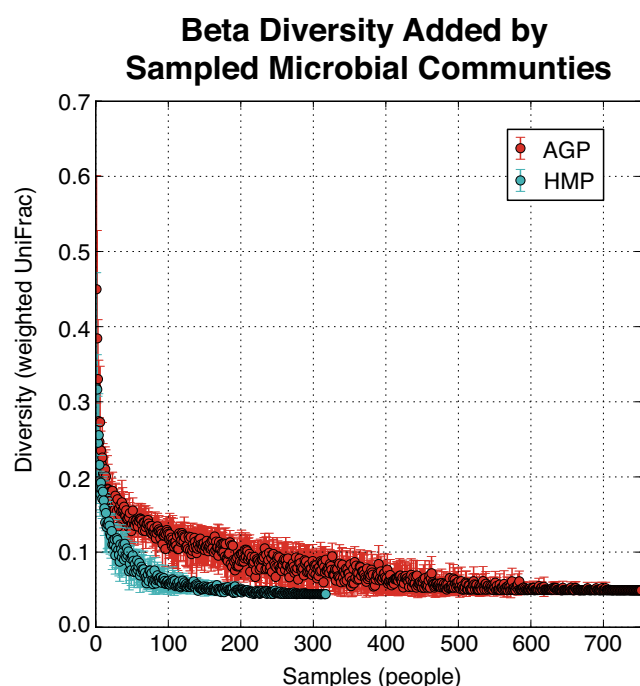
The effects of gender (small) and of the Paleo diet (relatively large) on the overall kinds of bacteria in samples from American Gut participants are shown below. Paleo dieters tend to have fewer Proteobacteria, which have been linked to inflammation. On the other hand, they tend to have more Firmicutes which have been linked to obesity. Additional work needs to be done to test whether the same kinds of Firmicutes are important in both cases.



How Diverse Are Your Microbes?

Just as knowing which microbes are in your gut is important, understanding how many different kinds of microbes are in there may be just as critical. In this section, we ask how much novelty we see with each additional person we sequence. Less diverse microbial populations have been associated with a range of diseases, just as less diverse ecosystems often arise from pollution or disturbance (think of a lake choked with algae).

Figure 8



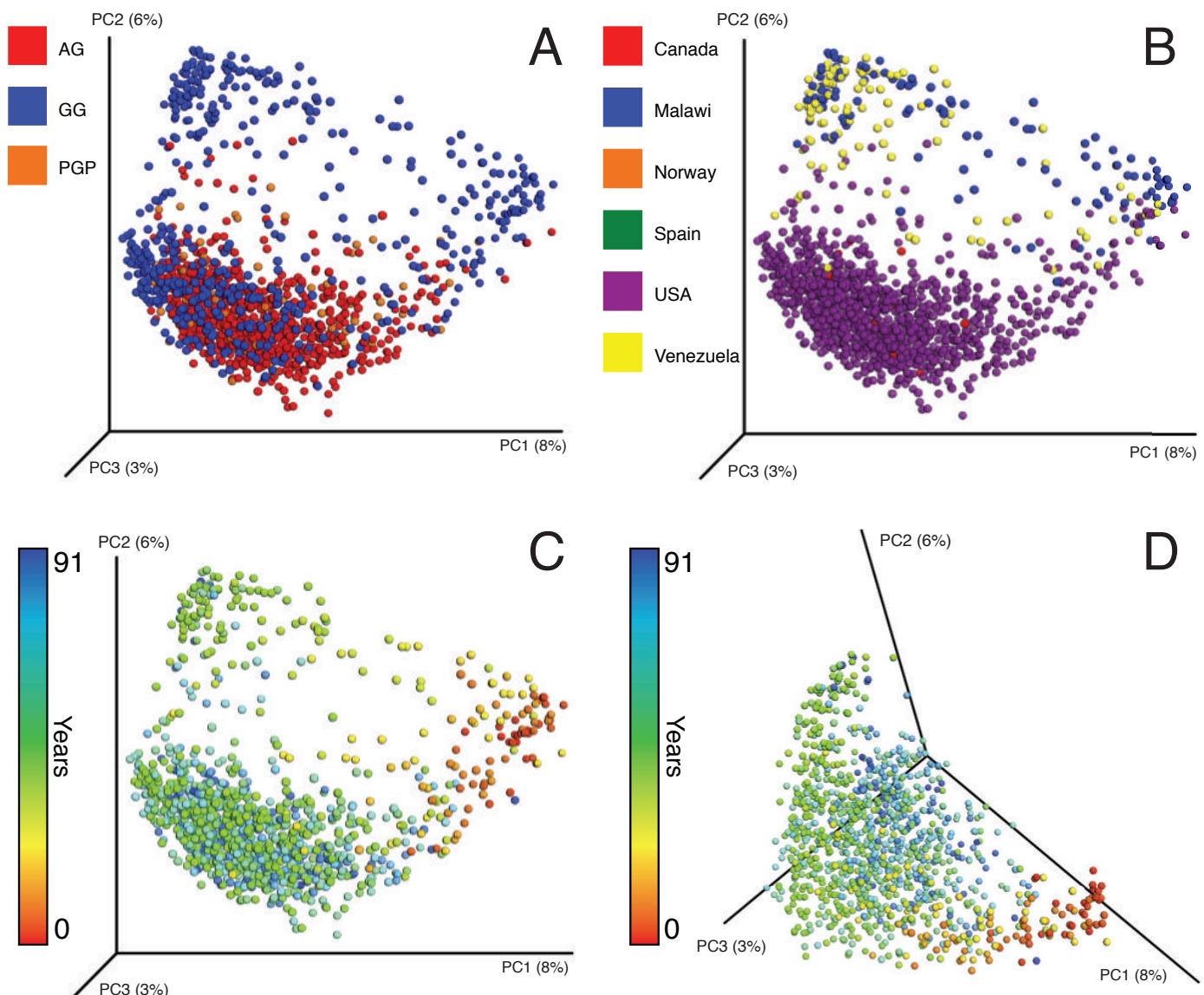
Diversity of the American Gut population relative to the population in the Human Microbiome Project. The x axis shows the number of people examined; the y axis shows how close each additional sample is to the closest sample already in the dataset. Essentially, now that we have sampled more than 1000 people adding more samples does not increase the number of new mixes of gut bacteria we see. We understand, in other words, the range of kinds of gut microbiotas that exist. Now we just need to understand why.

What Affects Gut Bacteria in Different Studies?



Figure 9

This figure compares the fecal samples in the American Gut population to those in other studies, especially the Global Gut study covering people from the USA, Malawi and Venezuela. **Figure 9A** shows that the studies generally overlap (at least for American participants). **Figure 9B** shows that people from different countries, especially non-Western countries such as Venezuela and Malawi, are very different from those in the US. **Figure 9C** shows that age has a large effect, and especially infants (in red on the right-hand side of the diagram) are very different from adults. **Figure 9D** looks at the age effect just within the American population, showing that the age effect is clearly seen in this population alone.



The project would like to thank:

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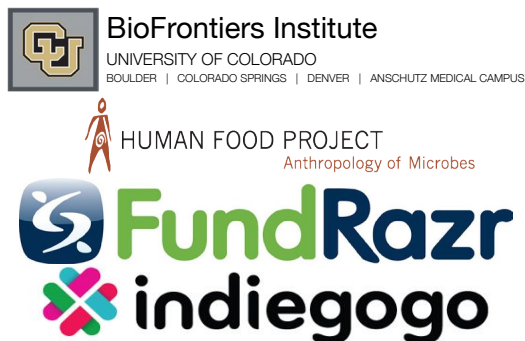
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