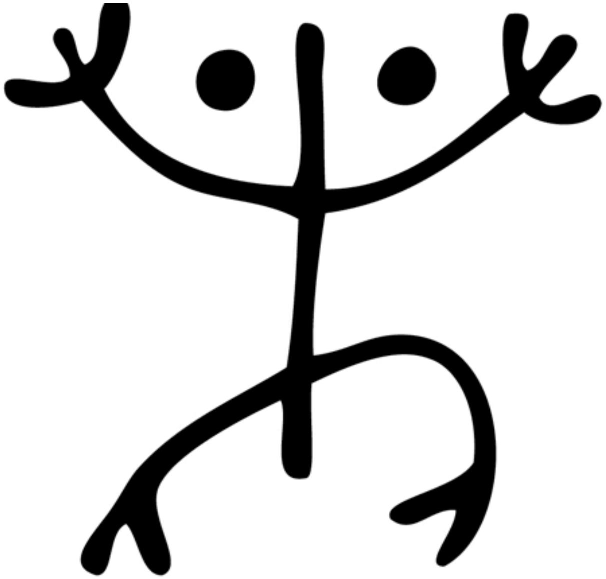
Ancient metagenomics, the paleo diet and competitive exclusion

Steven E Massey
Associate Professor
of Bioinformatics
University of Puerto Rico



A new technology is revolutionizing the Life Sciences –
NEXT GENERATION SEQUENCING !!!!

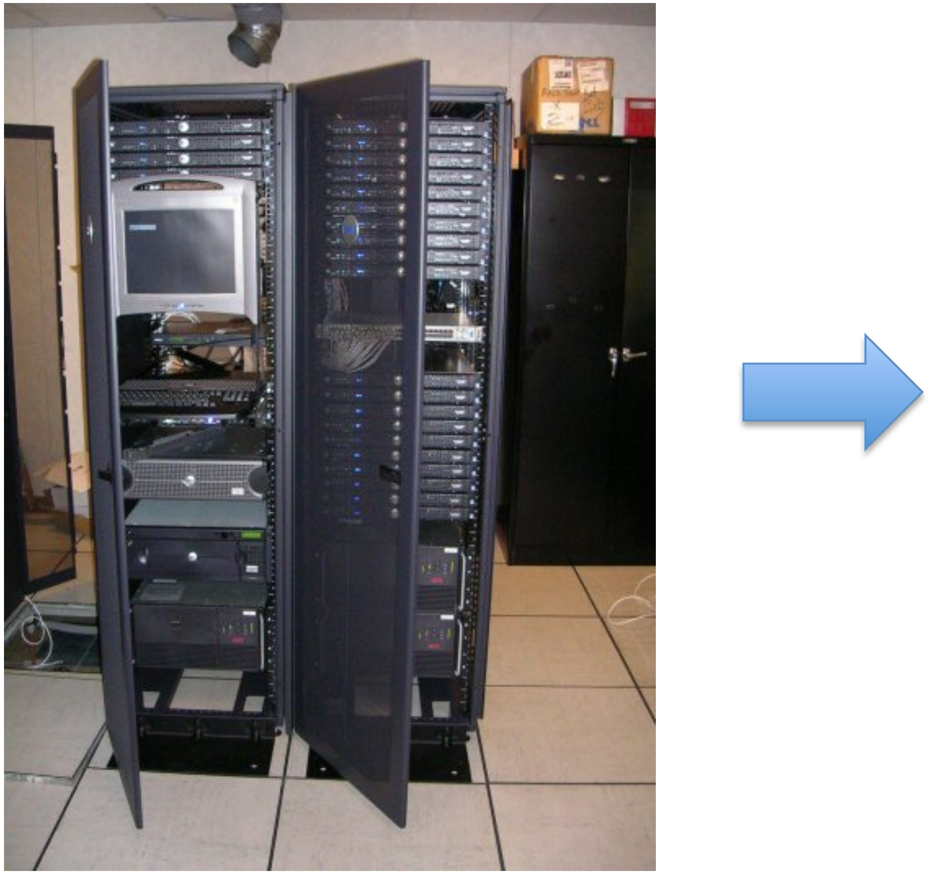
There are enormous potential applications in diverse fields and the pace of technical innovation is breathtaking

As a taster of the diverse applications of this technology today's presentation will explore its impact on archaeology, health and agriculture (agrogenomics)

To: MrDNA

In order to assemble, annotate and analyze these sequences we use bioinformatics

Bioinformatics refers to the computational analysis of biomolecular data, often very large datasets ie 'Big Data'



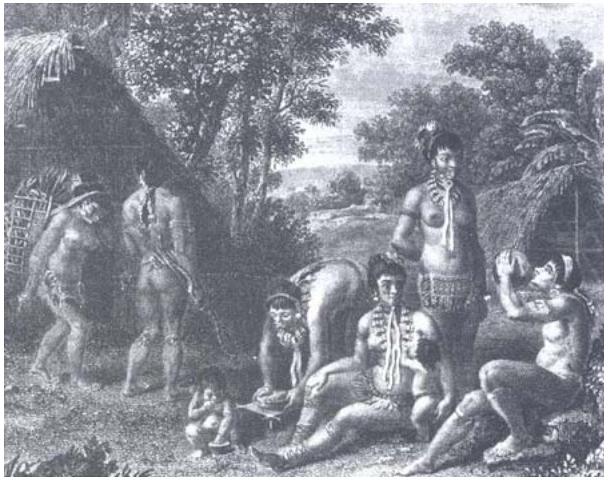
Ancient DNA in the Caribbean

The Caribbean amerindian peoples were the first to be contacted by Columbus

There are no surviving populations, although their genetic component lives on in present day Caribbeans and there are some cultural remnants

This means that archaeology is key to understanding the cultures of the original Caribbean peoples

Due to the Spanish ‘cronistas’ we have a picture of the Tainos and Caribs at the point of contact, however their origins are clouded in mystery



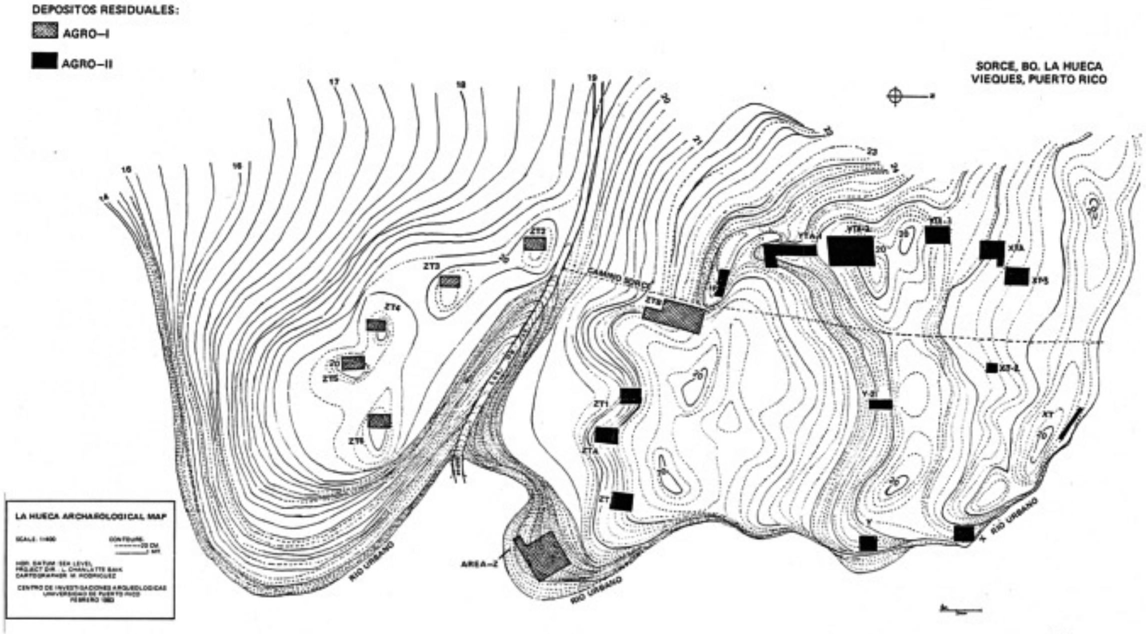
The biggest puzzle in Caribbean archaeology is the ‘Huecoid problem’, which refers to a mysterious group of peoples called the Huecoids

The question is: were they ethnically distinct from the majority Saladoid people (who gave rise to the Tainos), or were they a Saladoid caste of priests or artesans

There are many differences between the two groups

They can be distinguished by their pottery :

They lived in close conjunction with each other, but physically separated



(A)

Most striking were the differences in ornamentation

The Huecoids acted as craftspeople, manufacturing jewellery for the Saladoids

Condor

King vulture

Some evidence of dietary differences : turtles were taboo for the Huecoids

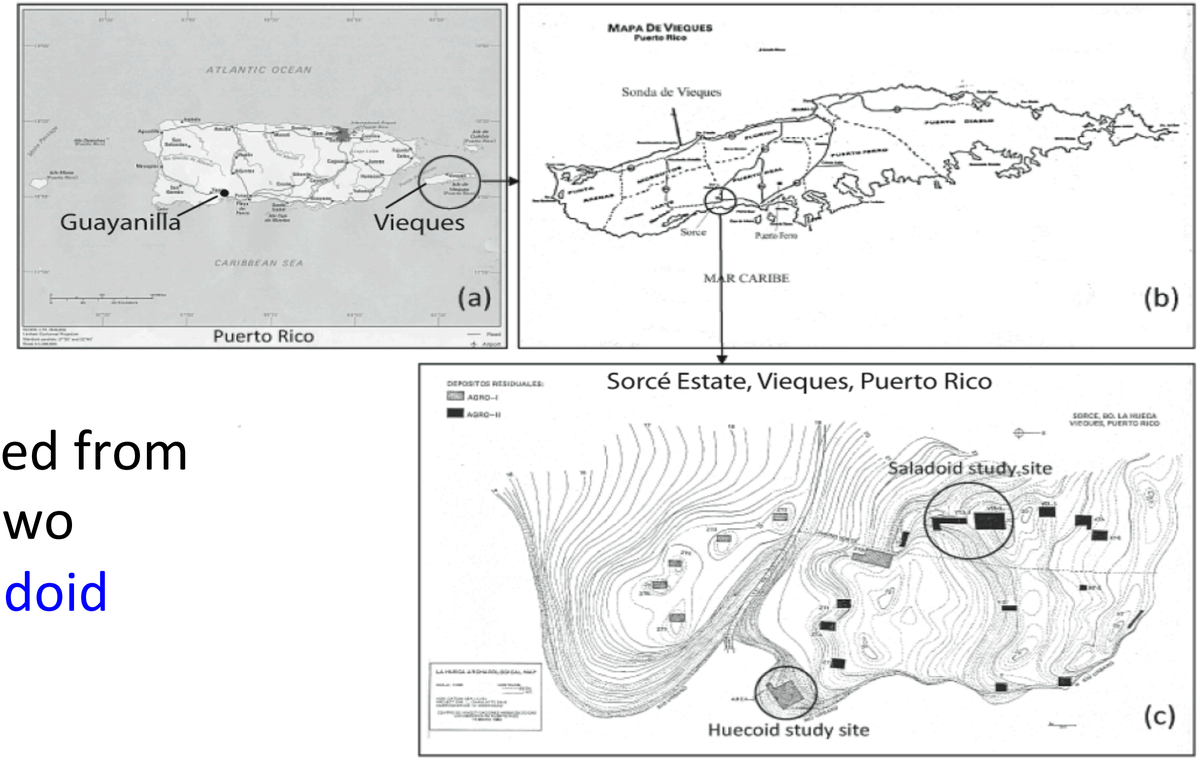
Lastly, there were differences in burial practices. No Huecoid burial has been encountered. This means that no human remains are available to determine if they were genetically (and ethnically) distinct

Apart from :

!

The question was, can we use metagenomics techniques to examine the Huecoid problem ?

Ancient DNA is DNA extracted from ancient specimens ie. specimens that have not been well preserved



DNA was extracted from ancient coprolites recovered from La Hueca, Vieques, from two adjacent settlements, Saladoid and Huecoid

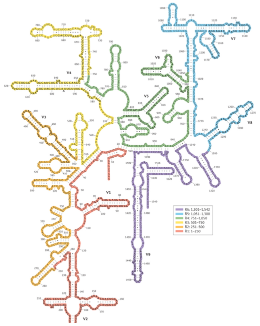
Extraction of ancient DNA is technically challenging due to degradation, fragmentation, chemical modification and contamination

Once it has been extracted with minimal contamination – what next ?

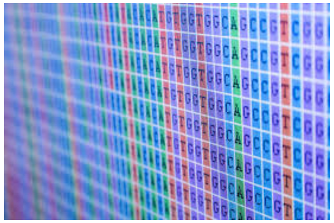
Do community profile metagenomics !

Using NGS this will give us a precise snapshot of the microbes present in ancient DNA

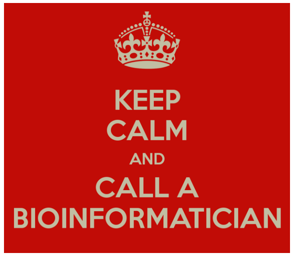
1) Extract environmental DNA



2) Amplify target gene (usually 16S rRNA) using PCR

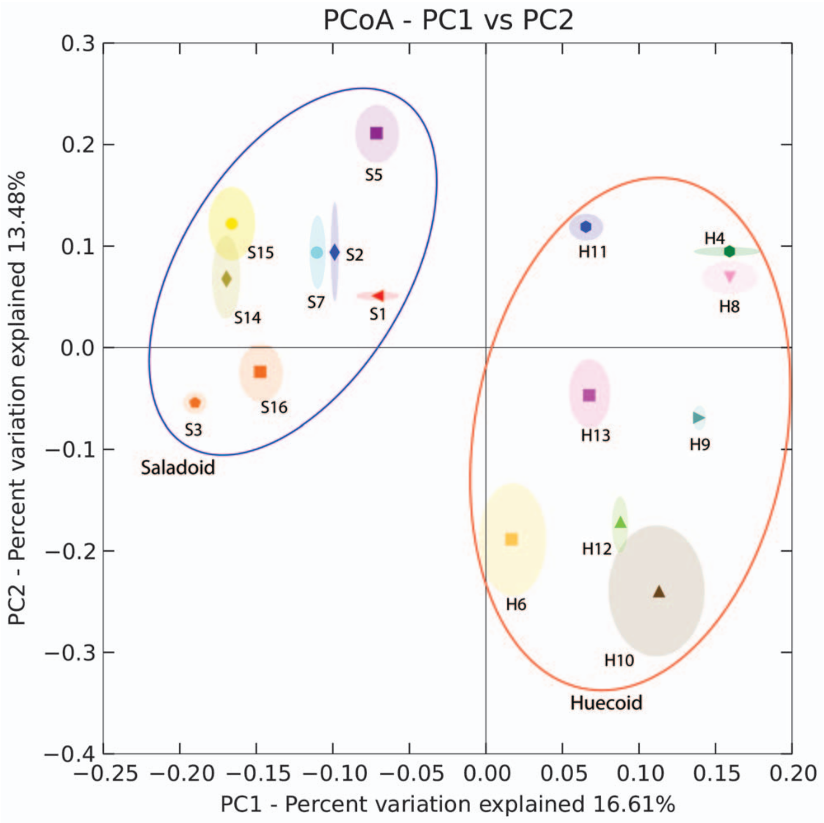


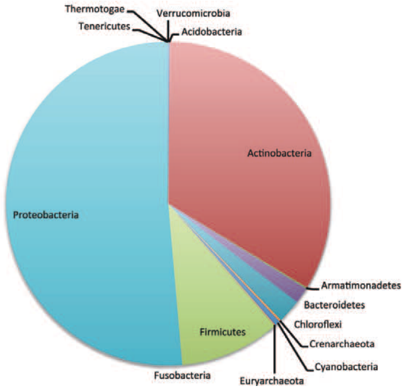
3) Sequence using NGS. Typically generate about 10000 reads per sample. Cost ~ $80



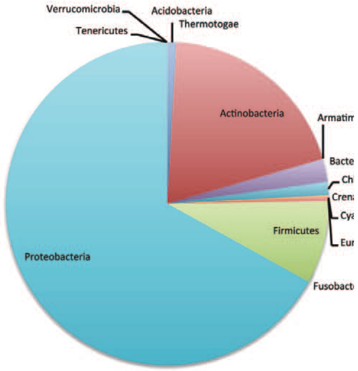
4) Analyze the sequence data. Series of steps that involve quality filtration, phylogenetic assignment of reads, and downstream analysis such as calculation of diversity metrics. Analysis costs typically match those of sequencing.

Coprolites from the two populations were differentiated by their gut microbiomes



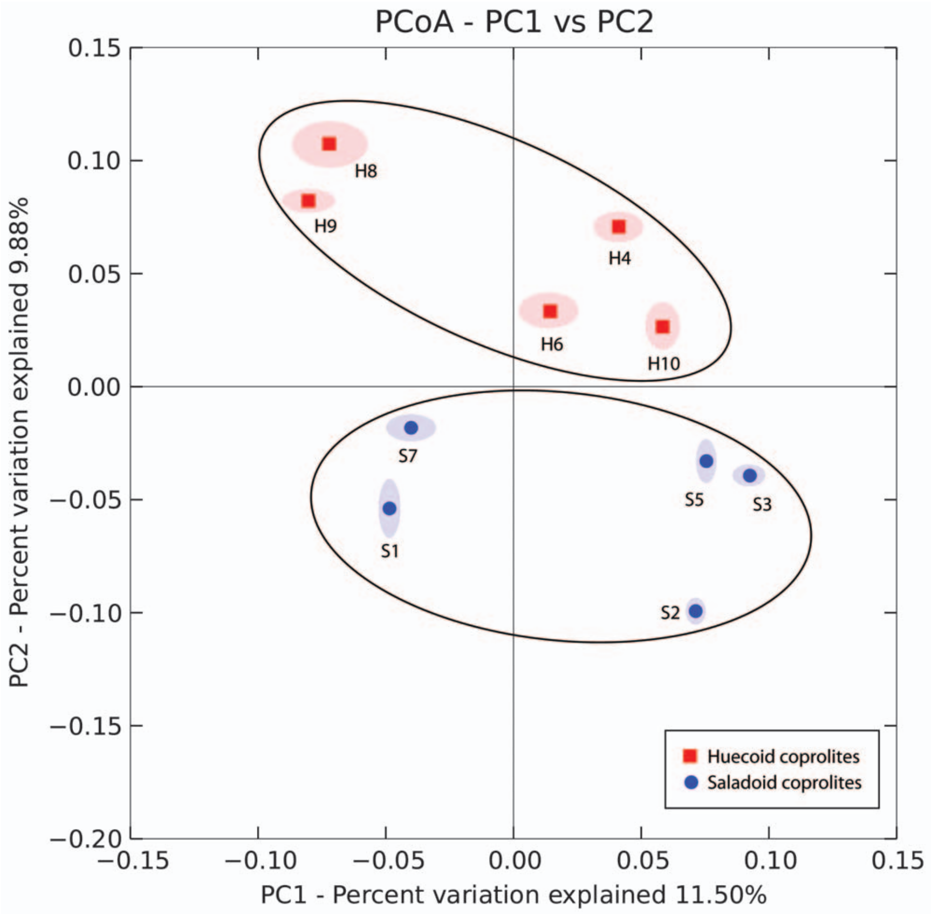


Huecoid



Saladoid

Principal component analysis of
16S rRNA (prokaryotic) PCR products



Principal component analysis of 18S rRNA (fungal) PCR products

Large proportion of basidiomycete sequences

Conclusion: The archaeological and NGS data suggests that Saladoids and Huecoids are distinct peoples

Interestingly, maize was amplified as well, which provides additional support for the early arrival of maize cultivation in the Caribbean

Negrito

Caribe temprano

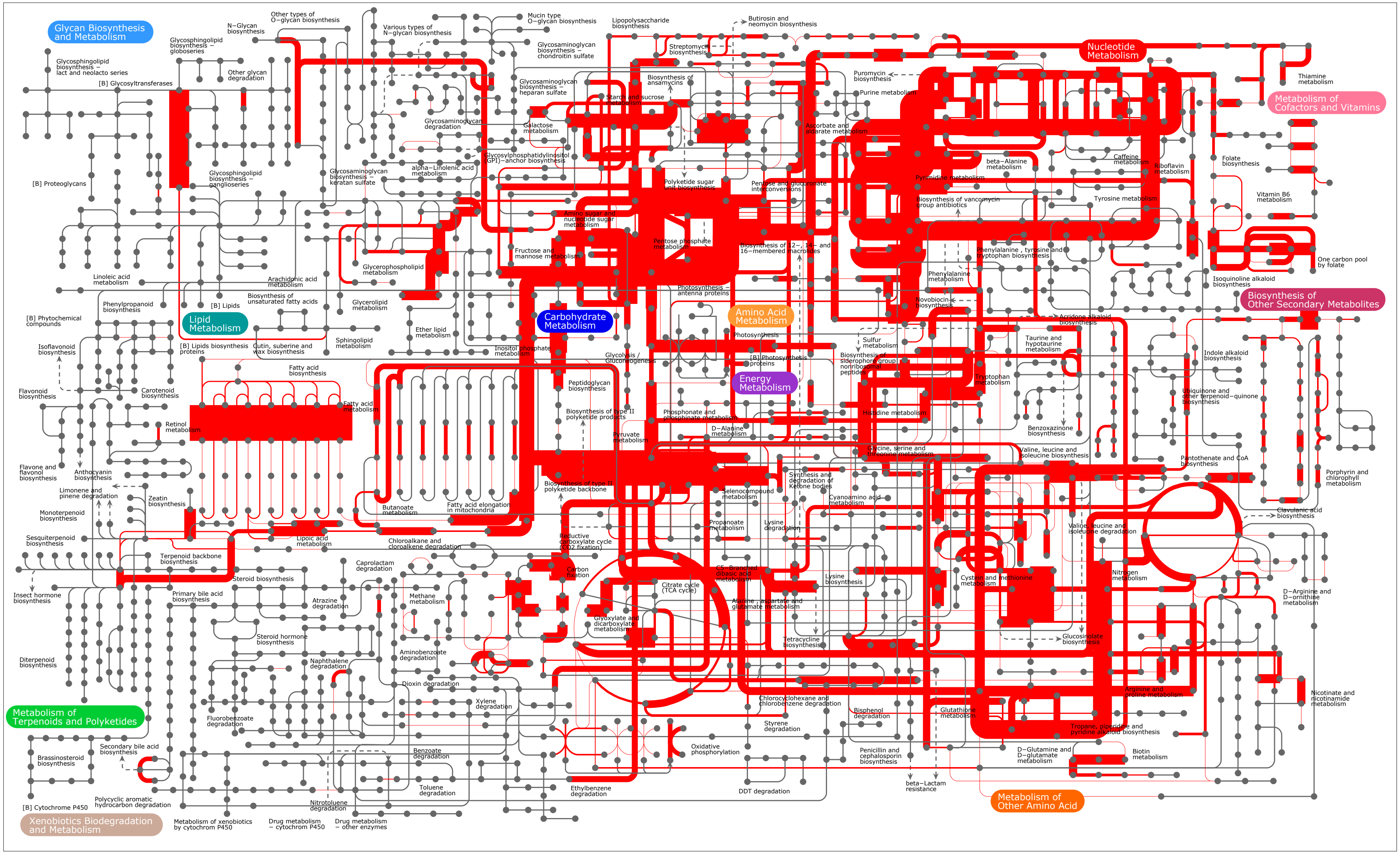
Pollo

Source: Pagan Jimenez 2007 *Paris Monographs in American Archaeology* **18**

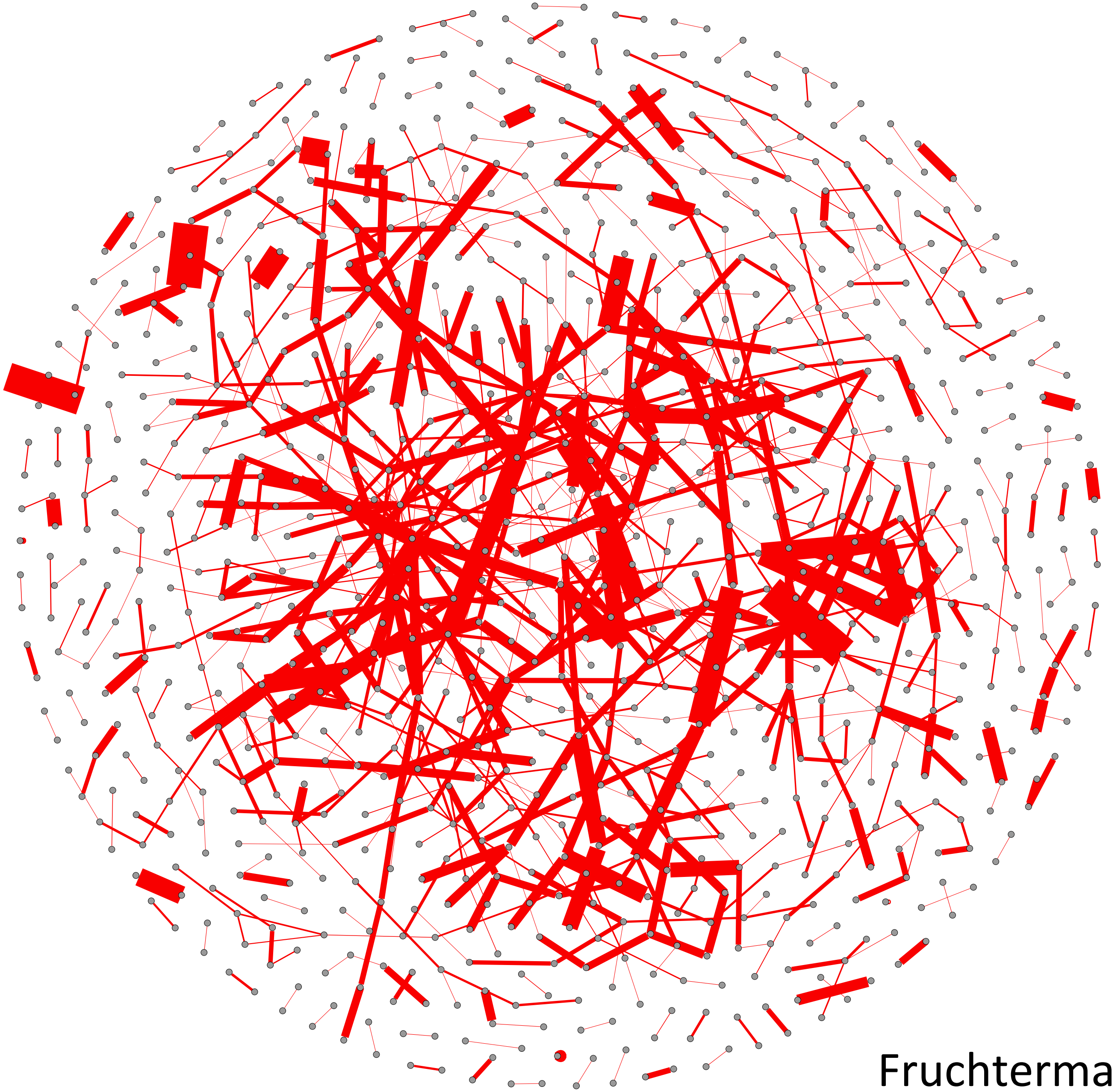
Gut parasites were identified microscopically, but identification can be ambiguous. High parasitic load in these populations

Question – how healthy REALLY was the PALEO diet ?

Shotgun metagenomics can be used to assess the ‘health’ of the gut microbiota, their metabolic interactions with the host and with each other



Meta-metabolomic network from 140 pooled gut samples (Human Microbiome Project) – a universal human meta-metabolomic network ?

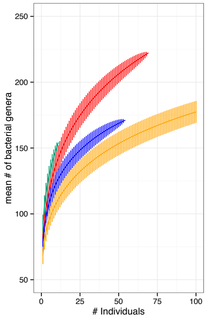


Fruchterman-Reingold representation

What is the true paleo gut microbiome ?

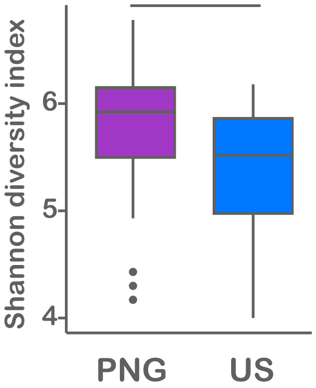
Non-westernized populations typically have diverse gut microbiomes

Yanomami (hunter gatherers)



Clemente et al. 2015
Science Advances 1, e1500183

Asaro (agriculturists)



Martinez et al. 2015
Cell Reports **11**, 527-538.

There is some evidence that high microbial gut diversity is linked to good health

A high fiber diet has been advocated as a way of increasing gut microbial diversity
(Deehan and Walter 2016
Trends in Endocrinology and Metabolism 27, 239-242)

Q. Can sprouts be a good source of microbial diversity ?

Application of metagenomics to competitive exclusion

Competitive exclusion has been proposed as a method of controlling pathogens in an environmentally friendly way

Diversity theory implies that higher microbial diversity is more effective

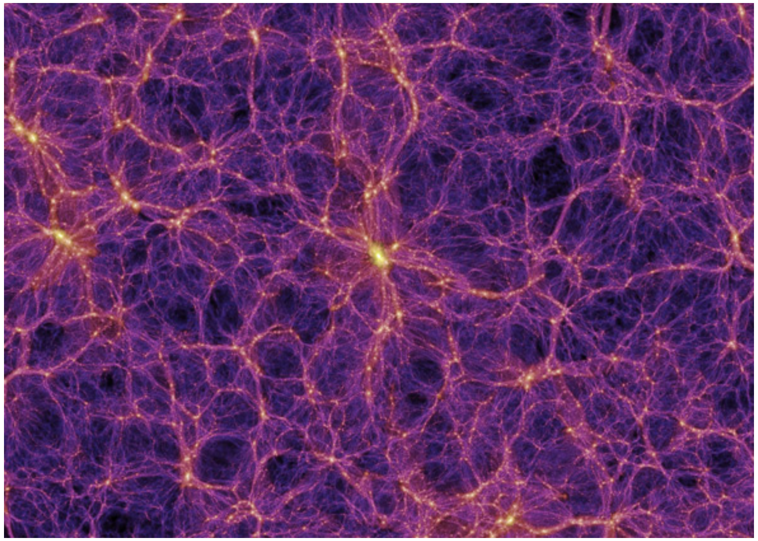
Using community profile metagenomics a precise profile of a microbial community can be generated

This allows precise characterization of:

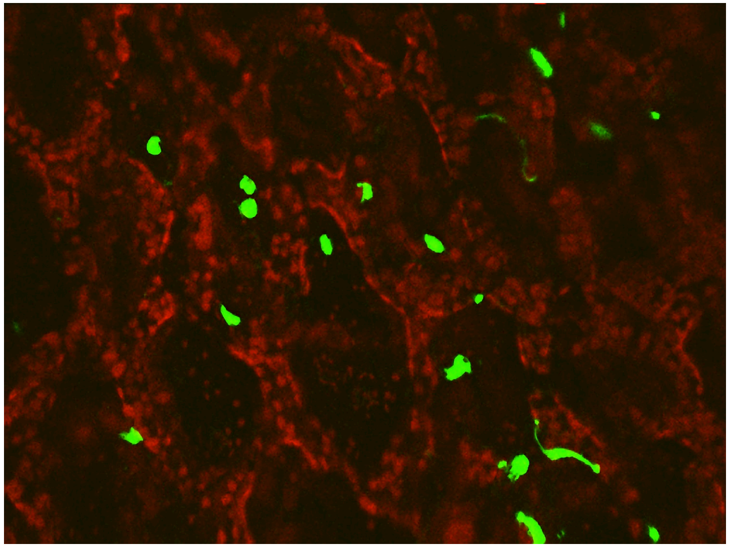
1) A ‘good’ microbial community – its diversity and presence of particular strains
2) Monitoring of plant growth and associated microbial community

This technique circumvents the main barrier preventing the development of competitive exclusion – the non culturability of the majority of microorganisms

(For this reason, environmental microbes have traditionally been described as the ‘dark matter’ of biology)



Credit: technologyreview.com



Credit: W.-L. Deng and A. Collmer, Cornell University

However, the technique is not effective for the identification of trace pathogens 👎

Acknowledgements

Raul Cano (Cal Poly), Gary Toranzos and his lab (UPR – Rio Piedras), Yvonne Narganes and Luis Chanlatte (Dept of Archaeology, UPR – Rio Piedras), Erileen Garcia-Roldan, Lucy Bunkley-Williams (UPR – Mayaguez)

RISE program

RISE
UPR · RIO PIEDRAS