Microbiology

Ancient human faeces and gut microbes of the past

Matthew R. Olm & Justin L. Sonnenburg

Appreciation is growing of how our gut microbes shape health and disease. Now, a study of ancient human faeces sheds light on how microbial populations in the gut have changed during the past 2,000 years. See p.234

The microbial cells that inhabit the human gut, collectively called the gut microbiota or microbiome, have key influences on our metabolic and immune-system biology[1,2]. Many microorganisms are passed down over the generations[3,4]. However, the gut microbiota (tracked by analysing the microbial DNA in faeces) can be radically reshaped within days to months of certain events, such as immigration into a different country[5] or antibiotic treatment[6]. Defining which microbes were once part of our evolutionary history and have since been lost might provide a key to understanding the relationship between microbes and human health. On page 234, Wibowo *et al.*[7] address this issue by turning to a microbial 'time machine': palaeofaeces. By using DNA sequencing to study the microbiomes of human stool samples that are 1,000–2,000 years old, this study provides valuable insights into gut microbes from a time before industrialization.

The human microbiome is a malleable component of our biology that adapts to specific circumstances, for example displaying seasonal variation corresponding to food availability[8]. Although this malleability offers a potential avenue for the treatment of human diseases linked to microbiota, it is also a vulnerability. Many aspects of industrialized life, such as antibiotic use and a fibre-deficient Western diet[9,10], have a negative effect on gut microbes.

Which core microbes and microbial functions from the pre-industrial microbiota were lost as societies became industrialized? Certain broad bacterial groups (referred to as 'volatile and/or associated negatively with industrialized societies of humans' (VANISH) taxa) are highly prevalent in present-day Indigenous populations living traditional lifestyles, but are rare or absent in industrialized populations[10]. There are also numerous bacterial taxa (referred to as 'bloom or selected in societies of urbanization/modernization' (BloSSUM) taxa) that have the opposite pattern[10]. Whether present-day non-industrialized populations have microbiotas that are similar to those of humans who lived thousands of years ago has remained an open question, until now.

Wibowo *et al.* report DNA-sequencing analysis of 15 samples of palaeofaeces collected from the southwestern United States and Mexico. Seven of these samples were excluded for further study because of poor-quality DNA or evidence of soil contamination, or because the sample was found to come from a canine host. The age of the eight remaining samples was determined using carbon dating, and analysis of DNA damage revealed hallmarks that confirmed the antiquity of the material (ancient DNA has specific characteristics of degradation). The human origin of these samples was validated by microscopic analysis of dietary remains present in the palaeofaeces and by evidence of human mitochondrial DNA.

The high quality of the data generated enabled the authors to detect known microbial species and to discover previously unknown microbes through the reconstruction of microbial genomes. A total of 181 of the 498 reconstructed microbial genomes were classified as gut derived and had extensive DNA damage, consistent with an ancient origin, and 39% of the ancient genomes offered evidence of being newly discovered species.

Wibowo and colleagues compared their data from the ancient gut samples with data from a collection of previously sequenced stool samples from present-day populations with industrialized and non-industrialized lifestyles. The species *Treponema succinifaciens*, a microbe in the Spirochaetaceae family shown to be lost from industrial populations[8], was present in palaeofaeces, as were other VANISH taxa that were absent in industrialized samples and prevalent in non-industrialized samples. BloSSUM taxa, including the species *Akkermansia muciniphila* (which degrades human mucus), were more abundant in the industrialized samples than in the non-industrialized samples and the palaeofaeces. Together, these results support the idea that features of non-industrialized microbiomes are similar to the microbiomes of our human ancestors, and that industrialized

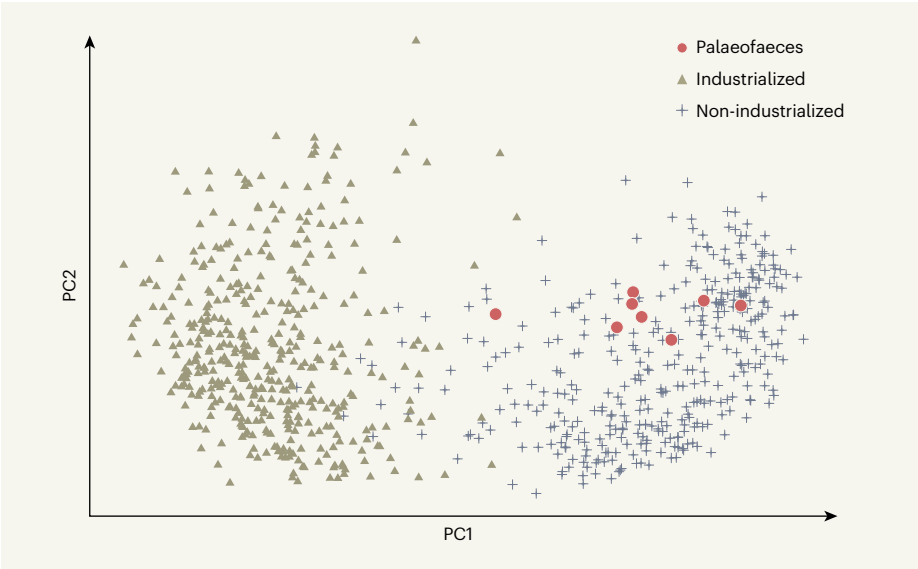

Figure 1 | A comparison of ancient and modern human gut microbes. Wibowo *et al.*[7] analysed the DNA of gut microbes found in 1,000–2,000-year-old human palaeofaeces, and compared this with the DNA of gut microbes in faecal samples from present-day individuals from industrialized and non-industrialized societies. The authors used a statistical method called principal component analysis to compare the patterns of bacterial species present in the samples from each individual. This approach distributes data points corresponding to each individual's sample along two axes, termed PC1 and PC2. Samples that are more similar to each other are grouped more closely together on the graph. This analysis reveals that the samples of palaeofaeces are distributed among those from individuals living in non-industrialized societies, indicating a similarity of gut-microbe profiles between ancient humans and modern humans living traditional lifestyles – both of which are distinct from the microbial profile of people in industrialized societies. (Figure based on Fig. 1b of ref. 7.)

populations have diverged from this microbial signature (Fig. 1).

The authors moved beyond focusing on species identity: they compared the genes, and the predicted functions of the proteins encoded by those genes, for the microbes in palaeofaeces with those found in present-day samples. Both the industrialized and the non-industrialized present-day samples had a greater prevalence of antibiotic-resistance genes than did the palaeofaeces, a finding consistent with the ancient microbes being from before the era of antibiotic use. Palaeofaeces had a high prevalence of genes encoding proteins that can degrade the molecule chitin, a component of insect exoskeletons. This finding is consistent with human consumption of insects, known to be a component of ancestral diets. Insect ingestion was confirmed by the authors' microscopy analysis of material in the palaeofaeces. The authors report many genes that were particularly prevalent in industrialized samples, including those involved in the degradation of mucus in the human gut.

Wibowo and colleagues' study is a remarkable technical achievement. They were able to recover high-quality DNA from microbial organisms that lived thousands of years ago, probably because of the good preservation possible in the dry desert environment in which the samples were located. Multiple independent lines of evidence authenticated the sample age and the human origin of the faeces. Having these ancient DNA sequences available in the public domain will undoubtedly benefit scientists for years to come.

However, DNA-sequence-based analyses do have limitations when the results are not paired with validation by other types of laboratory experiment. Using computational tools to predict information about proteins encoded by DNA is an imperfect method under ideal conditions, and is particularly tricky when analysing gene functions for previously unknown organisms, such as those discovered in this study. Moreover, microbiomes are highly variable between individuals and between populations. Analyses of more palaeofaeces from a wider range of timescales and locations will be needed to better understand general and population-specific features of ancient human gut microbiomes.

The authors found notable differences in the composition and function of microbes in palaeofaeces compared with those of microbes in present-day faeces. The higher prevalence of mucus-degrading species and genes in industrialized microbiomes than in ancient and non-industrialized ones is probably driven by Western diets, which often lack sufficient dietary fibre to support once-numerous fibre-degrading microbial species[11,12]. Given the links between the microbiome and the immune system, these differences might be connected to the rising rates of autoimmune, inflammatory and metabolic disorders in industrialized populations[9,10].

Wibowo and colleagues' work indicates that there are now two viable alternatives to time travel for understanding the composition of ancient microbiomes. Palaeofaeces enable the direct investigation of ancient microbiomes, but the sample age limits the further measurements and experiments that can be performed. Importantly, this study validates that present-day Indigenous populations living traditional lifestyles have similar microbiome compositions to those of ancient humans. It is essential to acknowledge that most of these present-day populations are marginalized, lead a vulnerable existence, and require exceptional protections to ensure they are not exploited. With ethically conducted research, these modern populations might open a window on our microbial past.

Matthew R. Olm and **Justin L. Sonnenburg** are in the Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305, USA. **J.L.S.** is also at the Center for Human Microbiome Studies, Stanford University School of Medicine.
e-mails: mattolm@stanford.edu; jsonnenburg@stanford.edu

1. Hooper, L. V., Littman, D. R. & Macpherson, A. J. *Science* **336**, 1268–1273 (2012).
2. Karlsson, F., Tremaroli, V., Nielsen, J. & Bäckhed, F. *Diabetes* **62**, 3341–3349 (2013).
3. Asnicar, F. *et al. mSystems* **2**, e00164-16 (2017).
4. Moeller, A. H. *et al. Science* **353**, 380–382 (2016).
5. Vangay, P. *et al. Cell* **175**, 962–972 (2018).
6. Dethlefsen, L. & Relman, D. A. *Proc. Natl Acad. Sci. USA* **108**, 4554–4561 (2011).
7. Wibowo, M. C. *et al. Nature* **594**, 234–239 (2021).
8. Smits, S. A. *et al. Science* **357**, 802–806 (2017).
9. Blaser, M. J. *Cell* **172**, 1173–1177 (2018).
10. Sonnenburg, J. L. & Sonnenburg, E. D. *Science* **366**, eaaw9255 (2019).
11. Makki, K., Deehan, E. C., Walter, J. & Bäckhed, F. *Cell Host Microbe* **23**, 705–715 (2018).
12. Desai, M. S. *et al. Cell* **167**, 1339–1353 (2016).

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Computer engineering

Superhuman floorplans for microchips

Andrew B. Kahng

A machine-learning system has been trained to place memory blocks in microchip designs. The system beats human experts at the task, and offers the promise of better, more-rapidly produced chip designs than are currently possible. **See p.207**

Success or failure in designing microchips depends heavily on steps known as floorplanning and placement. These steps determine where memory and logic elements are located on a chip. The locations, in turn, strongly affect whether the completed chip design can satisfy operational requirements such as processing speed and power efficiency. So far, the floorplanning task, in particular, has defied all attempts at automation. It is therefore performed iteratively and painstakingly, over weeks or months, by expert human engineers. But on page 207, researchers from Google (Mirhoseini *et al.*[1]) report a machine-learning approach that achieves superior chip floorplanning in hours.

Modern chips are a miracle of technology and economics, with billions of transistors laid out and interconnected on a piece of silicon the size of a fingernail. Each chip can contain tens of millions of logic gates, called standard cells, along with thousands of memory blocks, known as macro blocks, or macros. The cells and macro blocks are interconnected by tens of kilometres of wiring to achieve the designed functionality. Given this staggering complexity, the chip-design process itself is another miracle — in which the efforts of engineers, aided by specialized software tools, keep the complexity in check.

The locations of cells and macro blocks in the chip are crucial to the design outcome. Their placement determines the distances that wires must span, and thus affects whether the wiring can be successfully routed between components and how quickly signals can be transmitted between logic gates. Optimization of chip placement has been extensively studied for at least six decades[2,3]. Seminal innovations in the mathematical field of applied optimization, such as a method known as simulated annealing[4], have been motivated by the challenge of chip placement.

Because macro blocks can be thousands or even millions of times larger than standard cells, placing cells and blocks simultaneously is extremely challenging. Modern chip-design methods therefore place the macro blocks