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Insights into human evolution from ancient and contemporary microbiome studies

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Over the past decade, human microbiome research has energized the study of human evolution through a complete shift in our understanding of what it means to be human. The microbiome plays a pivotal role in human biology, performing key functions in digestion, mood and behavior, development and immunity, and a range of acute and chronic diseases. It is therefore critical to understand its evolution and changing ecology through time. Here we review recent findings on the microbiota of diverse human populations, non-human primates, and past human populations and discuss the implications of this research in formulating a deeper evolutionary understanding of the human holobiont.

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Introduction

Over the past decade, it has become increasingly clear that the study of human evolution is not complete without consideration of the human microbiome [1–3]. In addition to our own somatic cells, our bodies are a patchwork landscape that is home to thousands of different microbial species that number in the tens of trillions of cells [4•]. Rather than mere transient germs, these co-resident microbes contain an immense diversity of genes that interact directly with our physiology to carry out vital functions [5,6]. A growing awareness of these roles has resulted in a radical shift from thinking of human-associated microbes solely in terms of pathogenicity to considering them essential members of human biology [7,8] and a key component of the human holobiont [9].

Until very recently, human evolutionary genetics focused almost exclusively on patterns of variation found within our mitochondrial and nuclear genomes. Yet, the microbiome plays important roles in multiple core aspects of human biology, including digestion and energy metabolism, immune development, neurological function, and infectious disease susceptibility. As such, human-associated microbial communities (microbiota) and their microbial ecosystems (microbiomes) [10•] serve as accessory genetic reservoirs that are highly responsive to changes in human environments and lifestyles (Figure 1) and function as a shared target for natural selection. The growing body of knowledge on the relationship between the host genome, the microbiome, and the environment thus helps to answer fundamental questions about the role of microbial evolution and ecology in broader patterns of human evolution.

In this review, we discuss how recent human microbiome research informs work in human evolutionary genetics and how our understanding of human origins stands to benefit from a unification of both fields.

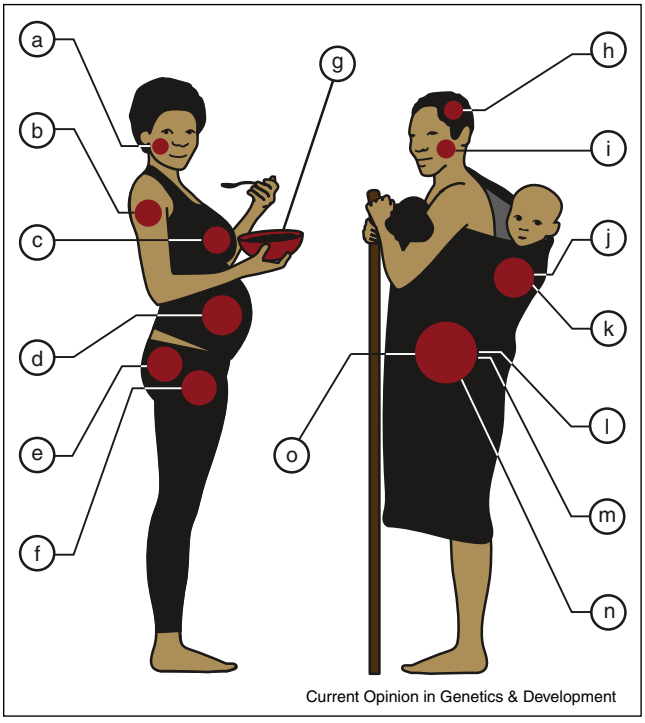
We also highlight how paleogenomics, the study of ancient genomes, is advancing our knowledge of the ancestral human microbiome and propose critical next steps forward.

Human microbiome research in context

With the conclusion of the Human Genome Project in 2003 [11] and successive efforts in whole genome sequencing of modern humans [e.g. [12]], archaic humans (including Neanderthals, Denisovans and the recently discovered Sima de los Huesos hominins) [13,14], and the great apes [15–18], comparative functional genomics has emerged as a leading research front poised to gain crucial insights into human-specific biology [19,20]. Complementing this endeavor, the Human Microbiome Project, initiated in 2007, leveraged advances in high-throughput DNA sequencing technologies to extend this research to the human microbiome [21].

Historically, the human microbiome has been generally overlooked in human genetics research, in large part due to the difficulty and complexity of characterizing microbial ecosystems using conventional molecular tools and

Figure 1



Overview of major functions of the human microbiome with evolutionary significance. **(a)** The oral microbiome a reservoir for numerous pathobionts and opportunistic pathogens [104,105,154]. **(b)** Skin microbiota influence mosquito attraction and may impact transmission of insect-borne diseases, such as malaria [138,139]. **(c)** Bacterial inoculation of the breast assists infants with milk digestion [31]; breastmilk contains human-specific oligosaccharides that promote the growth of beneficial gut bacteria [45–47,48••,49]. **(d)** The placenta harbors an oral-like microbiome [32]; oral and vaginal dysbioses increase risk for preterm labor and stillbirth [33,34]. **(e)** Human microbiota are hotspots for horizontal gene transfer [128•,129–131]; antibiotic resistance genes in oral and gut microbiota predate the use of therapeutic antibiotics [56•,104,127]. **(f)** Ecological structure of the vaginal microbiome influences risk for contracting sexually transmitted infections [137••]. **(g)** External microbial fermentation expands the food resources available to humans [94,99,100]; gut microbes play key roles in milk lactose [89] and wheat gluten [87,88,92] digestion and intolerance. **(h)** Gut microbes produce neurotransmitters and influence stress, anxiety, and mood by communication with the brain via the vagus nerve [106–108,115•]. **(i)** Oral biofilms exhibit extensive evidence for host–microbial and microbial–microbial coevolution in biofilm formation [166–169]. **(j)** Gut microbes are critical for the development of the immune system early in life [31,51,52,117]. **(k)** Infants acquire their microbiota via both vertical and horizontal transmission and are subject to environmental influences [28•,36••,37,44••]. **(l)** Traditional and industrialized societies have distinct gut microbiota [53,54,55•,56•,57]; industrialized microbiota are less diverse and lack specific taxa [55•]. **(m)** Gut microbes convert dietary fiber into short chain fatty acids such as butyrate [86••,170], the primary nutrient for colonocytes [80,81•]. **(n)** Gut microbes synthesize B and K vitamins [82,83], catabolize xenobiotics, drugs, and toxins [65], and play key roles in cholesterol and bile acid metabolism [84]. **(o)** Some microbial strains exhibit patterns of genetic variation that mirror human migration histories [125,155,156].

culture-based techniques [22,23]. The advent of Next-Generation Sequencing (NGS) technologies has made microbiome research feasible for the first time, allowing not only large-scale microbial surveys based on the amplification and massively parallel sequencing of taxonomically informative marker genes (metataxonomics), but also detailed community gene inventories (metagenomics) and functional analyses (metatranscriptomics) [10•]. In addition to these technological achievements, NGS has also enabled a rapid expansion of microbial reference genomes available for comparative analysis, and as of 2016 complete reference genomes were available for 1665 bacterial, 3 archaeal, 111 viral, and 1 eukaryotic human-associated taxa (http://hmpdacc.org/reference_genomes/reference_ genomes.php). Increasingly, these reference genomes are not limited to cultured organisms alone, but can be reconstructed directly from metagenomic data [24•]. This approach has yielded some of the first genomic glimpses at 'dark matter' candidate phyla such as TM7 [25], a clade of epibiotic and parasitic bacteria that includes important members of the human oral microbiome [26], but which has proven largely uncultivable to date. Collectively, these developments have allowed us to advance our understanding of the role of the microbiome in human biology and evolution.

Role of the microbiome in human biology and evolution

Microbiome establishment and dispersal

As with any complex biological system, the initial establishment of the microbiome in infants is controlled by a combination of environmental factors and host genetics. Specifically, mode of birth (vaginal delivery vs C-section) plays a significant role in seeding the infant microbiome, with transference of taxa to the infant from maternal vaginal and gut microbiomes in the case of vaginal birth, and skin and environmental microbes in the case of C-sections [27,28•,29,30]. While this results in a significant difference in early microbiome structure, particularly with reduced species richness among C-section infants, the long-term implications are still unknown [31]. Microbial colonization of the placenta in utero may also play a role in microbial seeding [32], but the impact of prenatal microbial exposure is unclear and largely associated with adverse effects [33–35]. Once established, the infant microbiome undergoes a series of microbial succession events, coinciding with changes in diet, and begins to achieve an adult-like profile with the introduction of solid foods [36••,37].

While environmental factors play a major role in the acquisition and structuring of the human microbiome, several studies have now also identified associations between specific microbial taxa and host genotypes, particularly in the human gut [38,39•,40–43]. One such association is between the expression levels of the maternal Fucosyltransferase-2 gene (FUT2) and the establish-

ment of *Bifidobacterium* in the infant gut, with infants born to non-secretor mothers (FUT2−/−) having a delayed acquisition of this genus [44••]. Members of this genus are a major component of the gut microbiome in breast-fed infants, are uniquely adapted to metabolize human milk oligosaccharides [45–47,48••], and are known to play a critical role in infant health by regulating gut permeability and reducing inflammation [49]. Understanding these differential patterns in the establishment and maturation of the microbiome [50], particularly in relation to the onset of metabolic and inflammatory disorders [51,52], is a major emerging topic of interest in the study of early childhood development.

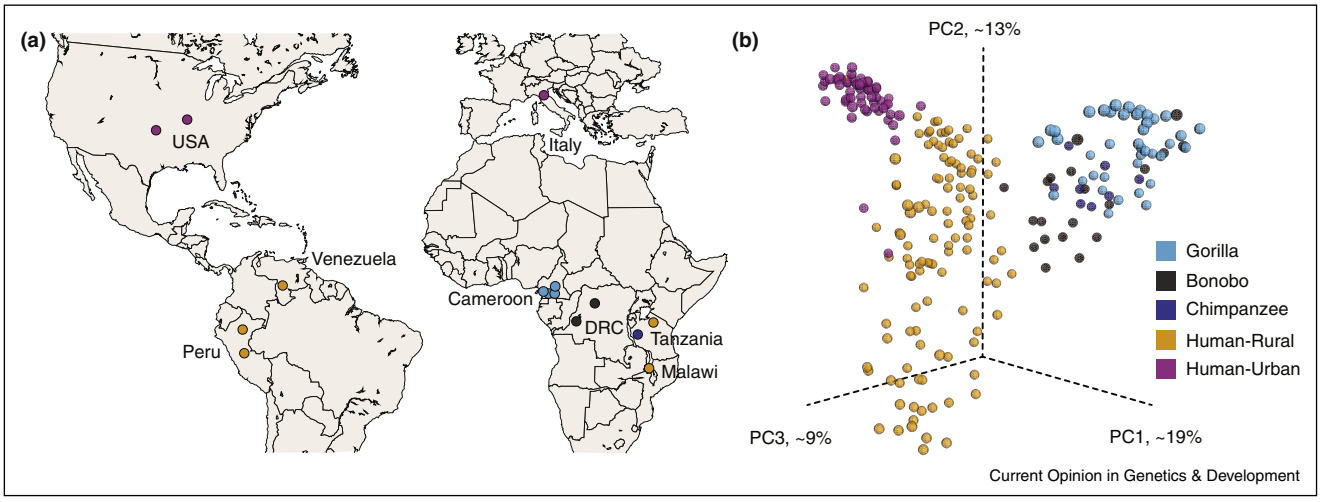
Diet is also a major driver of gut microbiome diversity in adulthood (Figure 2). Several microbial taxa show strong associations with dietary lifestyle, particularly across human populations (Figure 3). This is evident in a higher prevalence and abundance of genera such as *Prevotella*, *Catenibacterium*, *Succinivibrio*, and *Treponema* among both contemporary [53,54,55•,56•,57,58] and ancient [59,60] populations following a traditional subsistence lifestyle (i.e., hunting and gathering or subsistence agriculture), whose diets are often characterized by greater consumption of dietary fiber and other complex carbohydrates. The specific loss of these bacterial taxa during the transition from rural-traditional to urban-industrial lifestyles governs the primary structure observed in global biogeographic analyses of the human gut microbiome [55•]. A similar pattern of generational microbial loss accompanying dietary change has recently been demonstrated in a mouse model [61••]. Importantly, unlike human genomic diversity, this loss of bacterial diversity is independent of physical distance from the birthplace of humanity, Africa.

Among primate species, humans have the largest biogeographic spread, occupying diverse niches ranging from tropical rainforests to polar deserts, with vastly different nutritional sources. When compared to other primates, humans show a greater similarity in gut microbiome composition to omnivorous New World monkeys than our nearest evolutionary neighbors, the great apes [62]. Comparisons of gut microbiome community structure among hominids (great apes and humans) show human populations forming a distinct cluster (Figure 2). Further, comparisons of relative abundance profiles of microbial genera reveal the presence of host specific microbial assemblages (Figure 3), though not all microbial taxa are reflective of host phylogeny. While these broad patterns of association between microbial taxa and host phylogeny and subsistence have been primarily inferred from metataxonomic data, the increasing availability of high throughput shotgun metagenomic datasets and analytical tools [24•,63,64] makes it feasible to expand these analyses to explore genome-wide strain level variation of specific microbial taxa. This is important as we seek to better understand the mechanisms underlying observed microbial-host associations.

Digestion and metabolism

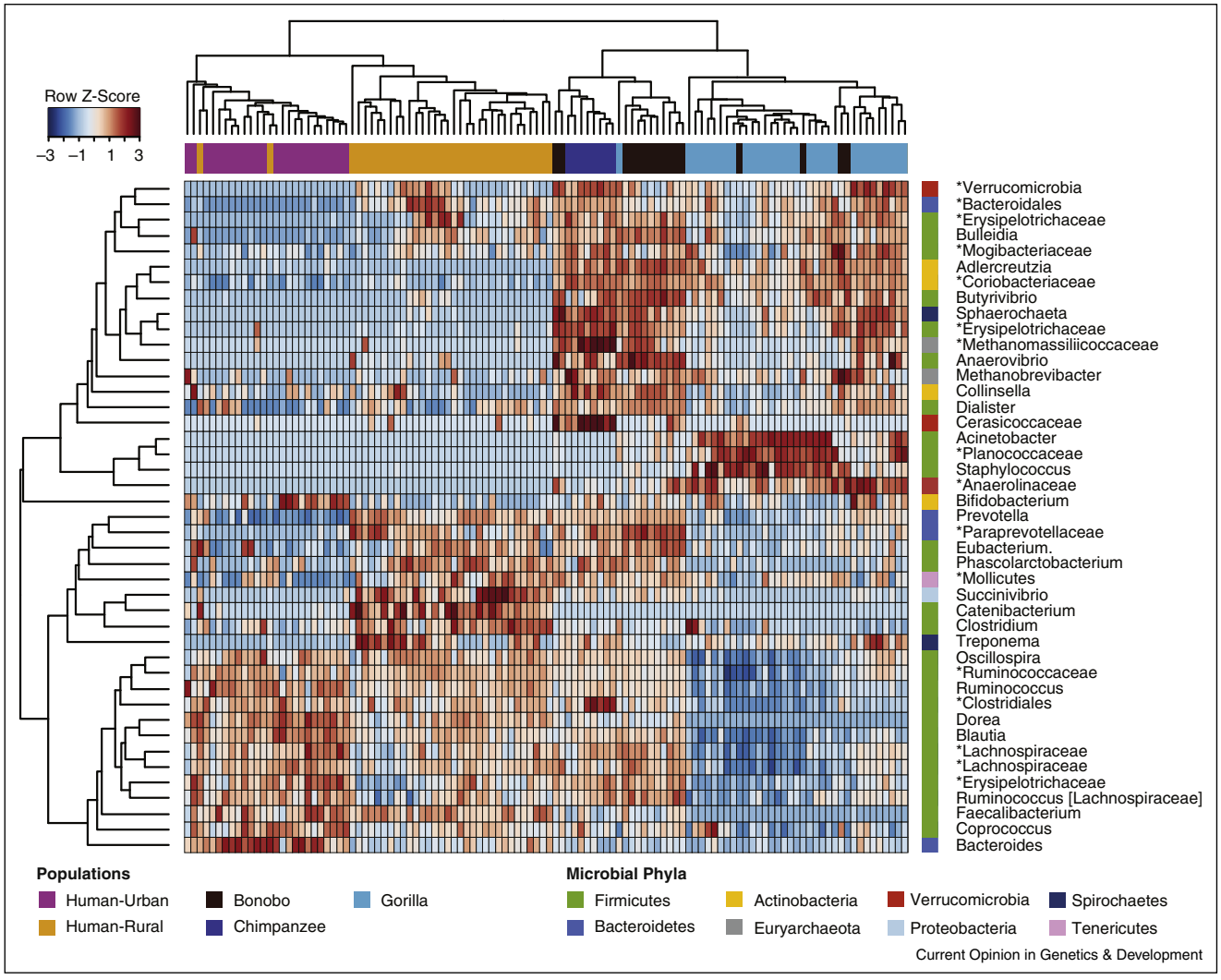
The success of the genus *Homo* was aided by progressive advancements in tool and cooking technologies, which likely contributed to our relatively reduced and unspecialized digestive physiology [65,66]. Yet through evolution, we have acquired an increase in energy throughput

Figure 2



Comparison of hominid gut microbiota. **(a)** Map of human and non-human primate populations for which comparable 16S rRNA V4 datasets are available [55•,57,58,171]. **(b)** Principle Coordinates Analysis (PCoA) representing genus-level community structure observed among hominid gut microbiota. Human gut microbiota are highly distinct from that of other hominids, and the gut microbiota of traditional human societies, including the Hadza of Tanzania, the Matses and Tunapuco of Peru, the Guahibo of Venezuela, and rural Malawians, exhibit the greatest microbial diversity. The proportion of variance explained by each principal coordinate axis is denoted in the corresponding axis label.

Figure 3



Heatmap of genus-level taxa differentiating human, chimpanzee, bonobo, and gorilla gut microbiota. Analyses were performed on 16S rRNA V4 region data, rarefied to a depth of 10 000 reads per sample [55•,57,58,171]. Each column represents an individual, and each row represents a bacterial genus. Taxa for which genus identification is currently unknown are denoted by asterisks. The heatmap is color-coded based on row *z*-scores. Host population is indicated using the same color scheme as in Figure 2, and bacterial genera are color-coded by phylum. Heatmap shows 43 genera with significant differences in abundance between populations (Kruskal–Wallis, FDR-corrected $p < 0.05$). Data are organized by hierarchical clustering, and microbiota form distinct clades broadly corresponding to host taxonomy. Humans and non-human primates form two distinct clades, and within humans, gut microbiota form two subclades based on traditional (Tanzania, Malawi, Peru, Venezuela) vs. industrialized (USA and Italy) subsistence strategies. Among non-human primates, the gut microbiota of chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*) are broadly similar, while gorillas exhibit a high degree of substructure. Similar microbial patterns shared between some chimpanzees and gorillas have been attributed to sympatric microbial transmission [172].

relative to other great apes [67], and simultaneously imposed stringent nutritional demands to support reproduction and brain function [68]. These evolutionary processes did not exclude the microbiome, as anatomical and physiological variations overwhelmingly define its taxonomic and gene composition [62,69–71]. This is consistent with the view that, while guided in part by host genetic factors specific to diet sensing, metabolism, and immunity [40,43,72,73] the gut microbiome is also strongly influenced by diet, gut morphology, and available energy substrates [31,74,75•,76,77]. Long-term residents of the gut microbiome must maintain some degree of genetic stability in order to effectively integrate with the host; however, factors that influence ecological structure, such as taxa proliferation and horizontal gene transfer, are highly dynamic over time. The result of these competing pressures is an ecological system that is highly responsive to short-term [75•,78] and long-term [55•,56•] dietary

influences, but which is ultimately constrained by host anatomy and physiology [62,79], resulting in microbiota that are distinctive for each host species (Figure 2).

Microbiota play an important role in nutritional buffering. Many recent studies lend evidence to oral and gut microbiome function in human nutrition, including key roles in nutrient [80,81•] and vitamin [82,83] synthesis, xenobiotic and secondary bioactive compound metabolism [56•,65,84,85], gluconeogenesis [86••], and digestion of refractory compounds such as polysaccharides, gluten, and lactose [87–93]. In addition, humans are able to take advantage of a wide range of microbially assisted and fermented foods [94], in part because of an enhanced capacity to digest ethanol in the hominid lineage [95]. As such, microbes can be viewed as important players in recent human events, such as the rise of grain-based agriculture and the spread of human genetic variants that facilitate lactose tolerance and dairying. Gluten-degrading microorganisms are found in the human oral and gut microbiomes [87,88], but their role in digesting gluten-containing grains (e.g., wheat, barley and rye) is not well understood. Comparative studies of gluten-degrading microorganisms in diverse populations with and without Triticeae grain-based agriculture may yield valuable information about the role of gluten and gluten-digesting microbes in the human microbiome.

Milk consumption beyond infancy has clear nutritional advantages, as evidenced in part by the widespread and independent prehistoric origins of dairying on three continents [96–98]. However, adult milk consumption is hampered by the fact that humans, like all mammals, lose the ability to produce functional levels of lactase, the enzyme responsible for host milk lactose digestion, after infancy [96,97]. Continued milk consumption in the absence of sufficient lactase production results in extensive microbial fermentation of lactose in the distal colon, the by-products of which produce the symptoms of lactose intolerance [89]. Different human populations have responded to the challenges of milk consumption in different ways, with some populations developing adaptive alleles that enable persistent host lactase expression, while others have come to rely almost exclusively on external microbial fermentation. External fermentation of milk lactose into lactic acid and carbon dioxide facilitates adult consumption of dairy products in the form of yogurts and cheeses [89,98–100]. Because of colder climates, such external paths to fermentation are more challenging in northern latitudes, and the absence of efficient external methods of fermentation may have produced a strong selective environment for the rise of genetic lactase persistence in northern Europe [96].

In addition to microbiota enabling humans to expand their ecological niche, humans have likewise provided evolutionary opportunities for our resident microbes. Phylogenetic analysis of the dental caries-associated pathogen *Streptococcus mutans* indicates that it underwent a rapid population expansion within the last 10 000 years, strongly implicating the adoption and intensification of agriculture in this process [101]. Ancient genetic sequences from *S. mutans* isolated from the dental calculus (calcified dental plaque) of early Bronze Age individuals (ca. 2200–1000 BCE) [102] and subsequent populations [103,104] may yield future insights into the dynamics of this process [105].

Brain growth, development, and behavior

The human brain is our defining species trait, and its developmental underpinnings are key foci of evolutionary genetics research. Recent research on brain development and social interaction in both humans and animal models has revealed that microbes exert a major impact on cognitive function and behavioral patterns [106]. For example, a growing consensus recognizes that cognitive and behavioral pathogenesis are often co-expressed with functional bowel disorders [107]. This hints at a shared communication or effector pathway between the brain and gut, termed the gut-brain-axis (GBA). The enteric environment is considered a third arm of the autonomic nervous system [108], and gut microbes produce more than 90% of the body's serotonin (5-hydroxytryptamine or 5-HT) [109]. Factors critical to learning and plasticity such as serotonin, γ-aminobutryic acid (GABA), short chain fatty acids (SCFAs), and brain derived neurotrophic factor (BDNF), which train amygdalin and hippocampal reactivity, can be mediated through gut-brain chemical signals that cross-activate bacterial and host receptors [86••,106,107]. Probiotic treatment is associated with positive neurological changes in the brain such as increased BDNF, altered expression of GABA receptors, increased circulating glutathione, and a reduction in inflammatory markers. This implicates the gut microbiome in early emotional training as well as in affecting long-term cognitive plasticity.

Critically, gut microbiota can modulate synthesis of metabolites affecting gene expression for myelin production in the prefrontal cortex (PFC), presumably influencing the oligodendrocyte transcriptome [110•,111••]. Prosocial and risk associated behavior in probiotic treated mice, a mild analog for novelty-seeking and risk-seeking behaviors in humans, suggests a potential corollary between entrenched behavioral phenotypes and catecholamines (serotonin and dopamine) produced by the gut microbiota [108,112,113]. Evolutionary acceleration of the human PFC metabolome divergence from chimpanzees, particularly the dopaminergic synapse [114], reifies the notion that an exaggerated risk-reward complex characterizes human cognitive differentiation, which is facilitated by microbiome derived bioactive compounds. Therefore,

quintessentially human behavioral phenotypes in stress, anxiety, and novelty-seeking is additionally reinforced by microbial production of neuroactive compounds. As neurological research expands to include the microbiome, it is increasingly clear that host–microbe interactions have likely played an important role in human brain evolution and development [115•].

Health

The microbiome is essential in immune system development and regulation [51,52,116–122], as well as pathogen protection and environmental interfacing [123•,124], and thus it represents our link to understanding deep evolutionary relationships between human health and ancient ecologies [59,60,102,104,125]. Our endemic microbiome can mitigate pathogens and pathogenicity by ecological niche exclusion and pro-inflammatory or proliferation signal inhibitions [118,126]. In doing so, our microbiome also acts as a cohesive unit by exchanging genetic elements such as antibiotic resistance genes (ARGs) [56•,104,127,128•,129–131] or by metabolic cross-feeding to influence community structure and mitigate ecological collapse. Increasingly, we realize that our modern behaviors have changed the selective environment and transmission of our microbiome through sanitization and a lack of exposure to environmental or infectious agents, a concept embedded in the now classic 'hygiene hypothesis' [132,133]. A revision of this hypothesis focuses less on exposure to infectious agents, and more on exposure to commensal agents [134], building off of observations that reduced microbial biodiversity associates with autoimmune disease [43,135•]. Biodiversity compression also provides opportunities for consortia of less beneficial bacteria to impact inflammation directly, inducing complications of metabolic syndrome via alteration of insulin signaling and intestinal permeability through local and systemic induction of cytokines [118,120]. In addition to bacteria, single and multicellular eukaryotic parasites have also been lost through increased sanitation, but their ecological role within the broader microbiome is less well understood, in part because of the greater difficulty of studying them using molecular methods. Such eukaryotic parasites may influence the host either directly or indirectly by altering microbial ecology. Recently, it was proposed that eukaryotic parasites may positively associate with certain bacterial taxa in the gut [136], but disentangling these patterns from confounding variables such as host residence and subsistence patterns remains a challenge.

Recent research on the vaginal and skin microbiomes has been further instructive on the patterns of immunity and protection conferred by human-associated microbiota. For example, the vaginal microbiota have distinct ecotypes, dominated by different subtypes of lactobacilli, which function to maintain low vaginal pH and high hydrogen peroxide levels. However, functional equivalence is called into question, as different ecotypes confer unequal susceptibility to bacterial vaginosis and sexually transmitted infections (STIs) [137••]. Likewise, volatile compounds produced by skin microbes have been found to influence mosquito attraction, and thus may play a role in malaria transmission [138,139]. These observations have important implications for understanding virulence and disease transmission within a population, as well as strategies for personalized healthcare.

Recent conceptual advancements in understanding the oral microbiome have shifted the conventional view that oral bacteria play a largely neutral or negative role in the oral cavity to one in which the native oral microbiota actively contribute to maintaining oral health [123•], and that disruption of this community is a proximate cause of periodontal disease [140]. We can also witness these disruptions through time in ancient populations by investigating ancient DNA and proteins preserved within semi-fossilized material, such as dental calculus [102,104], and begin to understand the extent to which our microbiome may rapidly adapt to shifts in human lifestyles, even in the absence of host genetic change.

The human microbiome is recognized as a powerful vector for therapeutic interventions because it is easily manipulated and highly responsive [75•,78,141]. Moreover, in addition to playing an important role in maintaining general health, the microbiome also impacts drug efficacy [142] and the effectiveness of chemotherapy treatments [143,144••]. Therefore, in the wake of a new funding announcement by the White House Office of Science and Technology Policy (OSTP) National Microbiome Initiative (NMI) 'to advance understanding of microbiome behavior and enable protection and restoration of healthy microbiome function' [145], future human health research will likely expand to include targeted microbial therapies, such as engineered genetic elements, or probiotic 'seeds', to manipulate community function and structure. This is where the study of human evolution and microbiome variation can have direct impact on biomedical research.

Ancient microbiome research

The origin of humanity is a major focus of genetic inquiry. Questions about how humans evolved from ancestral apes and what traits were maintained or derived in archaic lineages have driven profound accomplishments in paleogenomics. Technological advances in this field are also now enabling direct comparisons between the gut and oral microbiota of contemporary and ancestral human populations through the study of coprolites (paleofeces) and dental calculus, respectively, and such studies have great potential to reveal the microbial impact of specific environmental and lifestyle changes that have occurred throughout human history and prehistory.

Unlike the nuclear and mitochondrial genomes of the host, the microbiome continuously responds to external

pressures [75•,78] as well as internal endocrine and immune signals [107]. Therefore, ancient microbiome data offer snapshots of the health and environmental experience of the host, providing an unprecedented level of detail on the lives of ancient individuals. However, these investigations are still in their infancy. At present, knowledge of ancient human oral and gut microbiota is mainly derived from a relatively small number of specimens dating to the past 8000 years, with the majority of samples originating from Europe and the Americas [3].

The ancient gut microbiome

Current research on contemporary populations primarily focuses on the gut microbiome, in part because it is the largest and arguably most influential bacterial community in the human body. Well-preserved coprolites such as those recovered from the Cueva de los Muertos Chiquitos, a site near Rio Zape, Mexico, have yielded DNA from numerous human gut microbial symbionts [59,60]. Interestingly, these coprolites also exhibit taxonomic profiles similar to those observed in contemporary rural and traditional human communities [55•,57,58,146], suggesting a relatively stable profile of the human gut microbiome within similar subsistence modalities. However, obtaining ancient microbiome data from coprolite material is challenging. As open systems, gastrointestinal contents and excreted feces rarely preserve in the archeological record, and even morphologically intact specimens, such as mummified intestinal contents and latrine refuse, are highly susceptible to post-depositional alteration. Most coprolite samples that have been investigated to date show evidence of contamination by environmental microbes [59,147,148], which, coupled with DNA degradation through time, can result in highly skewed taxonomic reconstructions, especially when using amplicon-based sequencing approaches [149]).

Despite these preservation challenges, however, archaeological coprolite samples are ideally situated to test many hypotheses regarding evolutionary and recent changes in the human gut microbiome. For example, it has been shown that the gut microbiota of Western populations lack several taxa that are commonly found across diverse traditional populations with differing subsistence strategies, [53,54,55•,57], supporting the notion that this loss is recent and occurred due to factors outside of evolutionary processes or early subsistence transitions. It may be possible to clarify the factors driving microbiome changes associated with industrialized society today by analyzing pre-industrial coprolites sourced across different localities, especially from regions such as Europe and Asia, which have an enduring population presence over time and where robust contemporary microbiome data are available. Such a sample set would be critical for addressing whether ancient microbiota from pre-industrialized contexts resemble those observed today among traditional small-scale societies, or whether a temporal shift is present on a global scale, independent of Westernization and lifestyle factors, that would indicate the irreconcilable loss of our ancient microbial selves.

The ancient oral microbiome

Dental calculus is considered a 'living fossil' because dental plaque calcifies during the lifetime of the individual, and preservation of this record upon death is not predicated on random diagenetic effects. All humans, and even archaic hominins, produce dental calculus, and its ubiquity in the fossil record has already yielded important discoveries about diet and environment through analysis of microfossils embedded and preserved within the calcified matrix [150,151]. Thus, in terms of both availability and preservation, dental calculus holds the greatest promise for the large-scale study of ancient microbiota, enabling the investigation of oral microbiome evolution. For example, given the very high prevalence of periodontal disease in human populations today, investigating the temporal changes that have occurred in the oral microbiome would help focus our attention on the likely factors in the etiology of our modern oral dysbiosis [152]. Dental calculus investigation may inform caries research as well. In a recent study of 34 ancient dental calculus samples from multiple locations in Europe, it was argued that both the dietary transition from hunting and gathering to farming and the expansion of refined foods in the post-industrial diet resulted in shifts in microbiome composition and caused a proliferation of cariogenic taxa [102]. However, currently available data lack the resolution to determine whether these changes seen in the oral microbiome through time are a direct result of a change in subsistence versus other confounding factors such as geographic location or host genetics. Further, unlike the gut microbiome, the degree to which the oral microbiome responds to environmental and lifestyle stimuli is relatively unknown. Obtaining time series profiles of the oral microbiome within a single location would enable these factors to be independently evaluated. Finally, a more extensive characterization of the oral microbiome among diverse contemporary populations, similar to that currently available for the gut microbiome, is essential to interpreting ancient oral microbiome findings. As we continue to improve our abilities to extract viable microbiome data from ancient samples, we can apply these techniques to dental calculus specimens from as far back in time as our current projections for the survival of ancient DNA [153] to include archaic humans such as Neanderthals and Denisovans.

Pathobionts

In addition to mutualistic microorganisms, the human microbiome is also home to a large number of pathobionts (endogenous potential pathogens), including *Helicobacter pylori*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, and *Neisseria meningitidis*

[105,154]. These microbes are responsible for a variety of chronic (e.g., gastritis) and acute (e.g., diphtheria) infections that range from mild (e.g., ear infections) to life-threatening (e.g., bacterial meningitis). With the exception of *H. pylori*, most of these pathobionts inhabit the oral cavity, although their carriage rates are highly variable and often age-dependent [105]. Several of these pathobionts have been identified in ancient dental calculus [104], and further research may yield insights into the evolution of their pathogenicity [105]. Studies of pathobionts can also reveal information about past human migration events [155,156]. A recent study of *H. pylori* recovered from the stomach tissue of the Tyrolean Iceman found that Asian strains predate African strains within Europe, indicating that the hybrid population of *H. pylori* found in Europe today is a recent development [125]. These studies complement a growing body of research on ancient epidemic pathogens that include the causative agents of plague [157,158], cholera [159,160], leprosy [161], smallpox [162], and tuberculosis [163,164]. Together, paleogenomic investigation of pathobionts and pathogens promise to yield insights into the origins and spread of many clinically-relevant infectious diseases.

Finally, in addition to bacteria, the human microbiome harbors a number of eukaryotic parasites, ranging from unicellular microeukaryotes to multicellular helminths. These parasites are found primarily in the gut and are more prevalent in traditional societies than in industrialized populations. To date, archaeological investigations of parasite loads in ancient human populations have almost exclusively focused on morphological characterizations of helminth eggs and larvae, but taxonomic analyses are greatly aided by the addition of molecular techniques [165].

Future directions

The integration of genomic data obtained from humans and their associated microbiota has great potential to answer key questions posed by evolutionary geneticists and anthropologists, particularly in light of emerging evidence for the critical role played by the human microbiome in dietary ecology, reproductive ecology, immune regulation and disease management, brain growth and development, behavior, and energetics and metabolism. Even more tantalizing is the possibility that, due to the vastly higher diversity of some microbial genes, it may be possible to trace past human dispersals and the effects of environmental variability on fitness. Finally, the emerging availability of microbiome data from ancient sources has great potential to directly test hypotheses about microbiome evolution and changing ecology throughout human evolutionary history. In redefining what it means to be human, we must expand our queries of human genetic diversity to also encompass the ecological and evolutionary histories of our core microbial members.

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- • of special interest
- •• of outstanding interest

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