

Review

Population Genetics in the Human Microbiome

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While the human microbiome's structure and function have been extensively studied, its within-species genetic diversity is less well understood. However, genetic mutations in the microbiome can confer biomedically relevant traits, such as the ability to extract nutrients from food, metabolize drugs, evade antibiotics, and communicate with the host immune system. The population genetic processes by which these traits evolve are complex, in part due to interacting ecological and evolutionary forces in the microbiome. Advances in metagenomic sequencing, coupled with bioinformatics tools and population genetic models, facilitate quantification of microbiome genetic variation and inferences about how this diversity arises, evolves, and correlates with traits of both microbes and hosts. In this review, we explore the population genetic forces (mutation, recombination, drift, and selection) that shape microbiome genetic diversity within and between hosts, as well as efforts towards predictive models that leverage microbiome genetics.

A Population Genetic View of the Dynamic Microbiome

The human microbiome comprises bacteria, archaea, viruses, and microbial eukaryotes living in our bodies. The taxonomic composition of these communities has been extensively studied and is significantly associated with a variety of diseases and traits [1]. However, each species in the microbiome is genetically heterogeneous, comprising individual cells whose genomes contain different mutations [2]. Widespread deployment of sequencing technologies (Box 1) has revealed that most microbiota harbor extensive genetic variation between hosts, within a host over time, and even within a host at a given time [2,3]. As in other species, this variation comprises single-nucleotide variants (SNVs) [2], short insertions and deletions (indels) [4], and larger structural variants (SVs) [5], which include duplications, deletions, insertions, and inversions and can generate gene copy-number variants (CNVs) [6]. There has been substantial progress towards quantifying genetic diversity in the human microbiome [2,6–8].

By contrast, our knowledge of population genetic processes that shape the human microbiome is nascent. Population genetics is a discipline that makes statistical inferences about the evolutionary events that gave rise to patterns of genetic variation across individuals of the same species. The main processes that determine the fate of a new mutation are **drift** (see Glossary), **selection**, migration (or **transmission**), and **recombination** [9]. These processes govern how new traits arise and spread among microbes, such as antibiotic resistance [10], drug side effects [11], pathogenic biofilm formation [12], and responses to diet, sanitation, and health status [13]. However, the relative contributions of these forces to microbiome diversity – as well as how they fluctuate over time and across microbiota, genes, and hosts with different phenotypes – remain to be fully understood.

A population genetic view of the microbiome provides the opportunity to discover the genetic contribution to traits within microbial populations and to infer the processes creating and maintaining trait-associated genotypes. In this review, we focus on the population genetics of human-associated species, but note lessons learned from other environments. Much of the data discussed are from observational studies of natural populations, but we also discuss experimental methods (Box 2) and the use of model systems to establish causality and to interpret inferences from observational studies of human-associated communities.

Evolution versus Ecology

To what extent do microbiota respond to their environment by evolutionary versus ecological processes? **Ecology** is defined here as the presence or abundance of species or strains plus their interactions with each other and their environment (e.g., strain replacements or shifts in species composition over time). **Evolution**, by contrast, refers to genetic modifications that accumulate on the same

Highlights

Genetic variation in host-associated microbiomes can be assayed in a high-throughput manner with a variety of technologies.

Many bacterial species recombine extensively, although they asexually reproduce.

The genetic diversity of many species within and across hosts is spatially structured.

Evidence for rapid adaptation within hosts is starting to emerge.

Modeling efforts are connecting microbiome genetic variants with host phenotypes, highlighting the biomedical importance of genetic variation in the microbiome.

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Box 1. How Do We Obtain the Necessary Genomic Information to Study Population Genetics in the Microbiome?

Genetic variants in the microbiome are assayed using a variety of approaches that vary on several dimensions including whether they require culturing, the number of species sampled concurrently, the length of the resulting haplotypes and reads, and cost.

Cultured Isolate Sequencing

Much of microbiome population genetics relies on identifying SNVs or SVs among sequenced and assembled genomes from cultured isolates. Since variants are linked in assembled genomes, studies of recombination, HGT, and nucleotide changes associated with evolution are feasible [34,35,76,117,127,149,152]. Throughput and difficult-to-culture species are limitations, but targeted culturing efforts [58,153–156], multiplexed sequencing [34], and microfluidics [157] are helping [158].

Metagenotyping

Shotgun metagenomics is the sequencing of pooled DNA from microbial communities. By aligning reads to genomes (or ‘pangenomes’ of all genes observed in a species) and applying statistical models, both SNVs and SVs can be called [2,6,7,46,151,159]. Metagenotyping is relatively unbiased, does not require culturing, and can be used to study hundreds of species simultaneously for relatively low cost. However, alignment to a database misses novel genes and species, is sensitive to reads that align to multiple species or genes [5], and does not provide long-range linkage information, although probabilistic models can be used to computationally phase variants and resolve short-range haplotypes for abundant species [33]. Metagenotyping is distinct from methods that quantify gene or pathway abundance across all species from metagenomes [160] or use genes with covarying abundance to define metagenomic linkage groups as estimates of species [120,161,162].

Metagenome-Assembled Genomes (MAGs)

Genomes can be recovered from metagenomes *de novo* using binning and assembly [120,161,162], which allows the characterization of new species and variant discovery in both known and novel species [163–165]. MAGs can rapidly expand the number of reference genomes and observed genetic diversity of a species, as for *Prevotella copri*, which recently went from having one genome to >1000 genomes from four distinct clades [54,133]. Chimeric and incomplete assemblies are limitations, especially for lower-abundance species. Assembly is improving with chromatin capture scaffolding [166] and longer reads. Improved methods for assessing MAG quality and completeness will be helpful.

Single-Cell and Read-Cloud Sequencing

Flow cytometry [167] and microfluidics [157,168] can be used to capture and barcode single cells, small numbers of cells, or small numbers of DNA fragments [169] from a sample without culturing. Coupled with deep sequencing and specialized bioinformatics methods, these techniques assemble genome segments from which genetic variants, including mobile genes [63], can be identified. This strategy may soon produce genomes comparable with isolates with high throughput and no culture bias.

genetic background via mutation, recombination, selection, and drift. Both processes can produce within-species genetic changes in a microbiome (Figure 1A, Key Figure), but only evolutionary changes produce a succession of changes on the same genetic background over time.

It is commonly believed that evolutionary timescales are longer than ecological time scales [14–17]. If this is true, ecological processes, such as changes in species and strain composition, could be the dominant force shaping diversity in the microbiome within a host’s lifetime. However, short microbial generation times [18], large population sizes [19], and high mutation rates [20] mean that microbes have the potential to accumulate genetic modifications so rapidly that they could impact simultaneous ecological processes. Mounting empirical evidence shows that microbes can evolve rapidly in experiments [20–28], in marine and soil populations [29–32], and within hosts [33–35]. Thus, both evolutionary and ecological processes are likely to play an important role in shaping diversity in the microbiome over a host’s lifetime.

Glossary

μ : mutation rate; measured in units of number of mutations per generation per locus. In bacteria, μ is estimated to be $\sim 10^{-9}$ [20].

Biogeography: spatial structure in allele frequencies either along the gut or across the Earth’s surface.

Clonal interference: when two genomes with identical fitness compete with each other until a clear fitness difference arises via either a subsequent mutation or a recombination event.

Dispersal limitation: when the range of migration or dispersal of a species is smaller than the overall global area in which the species resides.

dN/dS: ratio of nonsynonymous to synonymous fixations between two lineages; dN/dS > 1 indicates positive selection, < 1 indicates purifying selection, and ~ 1 indicates neutrality.

Drift: change in population allele frequencies due to random sampling.

Ecology: interactions between species and strains and their environment.

Evolution: a change in allele frequency in a population driven by a population genetic force such as mutation, drift, migration, recombination, or selection.

Fixation: the process by which an allele reaches 100% frequency in a population.

Four-gamete test: test for recombination in which all four combinations of pairs of alleles at two loci are strong evidence for recombination.

F_{ST} : fixation index measuring the difference in allele frequencies in two populations; F_{ST} values close to 1 indicate that the populations are very differentiated and values close to 0 indicate similar populations.

Gene-specific sweep: a selective sweep in which only the gene or immediate locus bearing an adaptive allele rises to high frequency. This is common when $r \gg s$.

Genetically cohesive population: a population that lacks barriers to gene flow.

Genome-wide sweep: a selective sweep in which the entire genome sweeps to high frequency because it is linked to an adaptive allele. This is common when $r \ll s$.

Box 2. *In Vitro* Experimental Tools for Microbiome Population Genetics**Experimental Evolution**

Observing microbes evolve in the laboratory is a useful method to test hypotheses about microbial population genetics in controlled settings. Longitudinal studies of cultured microbes under different conditions, typically starting from one or a few strains, have shown rapid adaptation from both *de novo* and existing variation. They enabled mutation and homologous recombination rates and patterns to be estimated. Experimental evolution also revealed that population genetic dynamics and targets of selection are often consistent across replicate experiments [20–28,117,170,171].

Reverse Genetics

Genetic manipulation of microbes (e.g., gene knockout, mutation, or overexpression) helps to establish causal links between variants and phenotypes. This powerful approach is widely used in several genetically tractable model human microbiome species [172]. The establishment of tools to edit the genomes of human microbiome species beyond the ~10% that can be engineered currently is a high priority. For difficult-to-culture species, future approaches to edit microbial genomes *in situ* in a human host could be transformative.

Strengths and Limitations

Knowledge from these experimental systems needs to be tested in the human ecosystem to determine whether conclusions about evolutionary mechanisms hold there. Also, the complexity of the human microbiome is difficult to model *in vitro*, although recent experimental evolution studies cocultured different species to understand evolutionary and ecological interactions in controlled complex communities [173,174]. Experiments clarify what is possible in microbial evolution as well as the mechanisms of evolution, whereas observational data in human hosts shed light on what evolved but in a context where establishing mechanisms and causality is harder.

However, it is unclear to what degree microbiomes respond to selective pressures by ecological versus evolutionary processes. Interestingly, the answer may not be either/or: ecological and evolutionary processes can interact and shape each other, especially when evolution is rapid and occurring on timescales similar to ecological processes [14–17]. For example, over time new ecological niches can form in an evolving clonal population [22] and species sharing an environmental niche have higher rates of **horizontal gene transfer (HGT)** [30,36]. This review emphasizes evolutionary processes, but we highlight interactions between ecological and evolutionary processes and note that the two may be interdependent in the context of the human microbiome.

Population Structure

The concept of a population is central to evolutionary biology [37]. Evolutionary changes are defined as modifications within a reproductively isolated, **genetically cohesive population** [37]. Within this defined group of individuals (i.e., cells or **lineages**), population genetic processes of drift, recombination, and selection have the potential to impact all members of the population. For example, an adaptive mutation conferring a beneficial trait that fixes within a population may homogenize genetic diversity, thereby contributing to the genetic cohesion of the population [38,39]. Thus, delineation of a population could reveal the evolutionary history and ecological preferences of a species. Simultaneously, quantification of population structure, in which genetic variation is not randomly distributed, reveals physical or molecular barriers to gene flow. Population structure also provides an important control for genotype–phenotype analyses, such as genome-wide association studies (GWASs) (see later), since they can be confounded by cryptic relatedness among individuals [40].

Unfortunately, there is no consensus on how to define a population [37], and this is particularly challenging in the human microbiome given the pool of interacting microbes that may be exchanging DNA. Even standard microbial species definitions based on the sequence identity of the 16S rRNA gene, panels of protein-coding genes [41], or 95% genome-wide average nucleotide identity

Haplotype: alleles and genes that are inherited together because they are linked on the same chromosome.

Hard sweep: a selective sweep in which a single adaptive allele rises to high frequency, likely to be because the population is mutation limited.

Heritability: degree to which the variance in a phenotype can be attributed to genetics versus environmental factors.

Hitchhiking: when an allele changes frequency not because it is under selection, but rather because it is linked with another allele that changes frequency due to selection.

Homozygosity: probability of randomly observing identical alleles.

Horizontal gene transfer (HGT): transfer of genes from one lineage or species to another, notably not by vertical transfer from parent to offspring.

Lineage: a descendant from a common ancestor. A lineage can harbor as few as just one point mutation difference from the ancestor. In this review, operationally, strains comprise lineages and species comprise strains.

Linkage disequilibrium (LD): nonrandom association of alleles due to nonneutral forces such as selection. Recombination can, over time, break up correlations of alleles.

Oligocolonization: colonization of a host by a small number of genetically distinct lineages.

Pangenome: the union of all genes found in any lineage of a given species.

Partial sweep: a selective sweep that has not reached 100% frequency in the population.

Purifying selection: removal of deleterious alleles from a population.

r: recombination rate; measured in units of number of crossovers per generation per locus.

Recombination: the exchange of DNA between two genomes.

Selection: a change in frequency of an allele due to fitness effects conferred by the allele.

Selective sweep: rise in frequency of an adaptive allele and resulting loss in diversity in the vicinity of the selected allele due to hitchhiking.

[42,43] suffer from incorrect classification of species [44] and lack resolution to detect structure within species. However, fine-scale patterns of genetic diversity within and between hosts can help to identify population structure within species.

Structure within Hosts

Assuming the null hypothesis that ‘everything is everywhere’ [45], if the human body is colonized by a random sample of microbes from the global pool, within-host genetic variation should resemble genetic variation among the broader population of microbes found across hosts. On average, bacterial genomes of the same species from different host stool samples have a difference every 100 base pairs (or, are 10^{-2} /bp diverged) [2,33,34,46]. However, within-host diversity patterns range from $\sim 10^{-6}$ /bp to $\sim 10^{-2}$ /bp [33], suggesting that hosts are not colonized by a random sample. Instead, this wide range of diversity, coupled with peaks in intermediate allele frequencies within hosts [33,46] (Figure 1), reflects that people are rarely colonized by a single strain (with the possible exception of *Bacteroides fragilis* [47]) and instead are oligocolonized [33,34,46–49] by one, two, three, or a few detectable strains that are diverged from each other by $\sim 10^{-2}$ /bp for most species (Figure 1B). Operationally, for the purposes of this review, strains are defined as genetically distinct collections of lineages that have not had sufficient time to recombine extensively to form a genetically cohesive population.

The **oligocolonization** of hosts indicates that there may be some underlying ecological rules governing the host colonization process. For example, Verster *et al.* [47] showed that the type VI secretion system, which plays a role in interbacterial competition, may explain why hosts are colonized by one dominant strain of *B. fragilis*. As we discuss later, intrahost population structure is important to control for when performing population genetic analyses within hosts, because both shifts in strain frequencies and evolutionary modifications can generate allele frequency changes within a species over time inside a host.

Structure across Hosts

Several well-studied host-associated bacteria show evidence for population structure across hosts from different parts of the world (or **biogeography**). For example, genetic diversity in *Helicobacter pylori* [50,51] and *Mycobacterium tuberculosis* [52,53] faithfully mirror human migration routes. However, until recently it was not known how well gut commensal demographic patterns match our own. Recent work leveraging public metagenomic data from around the world showed that some gut commensals, such as *Eubacterium rectale* and *Prevotella copri*, have geographic structures mirroring that of humans [7,46,54]. However, many other species (e.g., *Bacteroides vulgatus*, *Bacteroides uniformis*) do not have diversity patterns that correlate with any one geographic region [7,46], although many of these species cospeciated with primate hosts on longer timescales [55]. Similar strains of many species are found on different continents [33,46] and, simultaneously, very divergent strains of the same species are found in the same host [2,33].

Why do some species have geographic structures that mirror our own while others do not? One possibility is that some property of human hosts (e.g., antibiotic usage, travel, diet, host genetics [56,57] as discussed below) could impose selective pressures on microbiota, resulting in signals of biogeography. However, while a pair of hosts can harbor closely related strains of any given species, this is not consistently true across all species shared by two hosts [33], suggesting that host factors are not the major drivers of microbiome biogeography or that they exert different selective pressures across microbial species. Instead, a more likely explanation for biogeography patterns are properties of the microbes themselves that contribute to their distributions across hosts via differential transmission, colonization, and competition for niches. Traits that could influence transmission rates, for example, include limited dispersal, ability to survive oxygen [58], and mode of transmission (e.g., vertical transmission versus horizontal transmission). Interestingly, vertical transmission of strains from mothers to infants [7,48,49,59,60] and within families [61] may not be the reason why some species show biogeography, since over our lifetimes resident strains of the most prevalent bacteria are replaced [33]. Instead, horizontal transmission of microbes along broader social networks [62] coupled with limited dispersal is a likely mechanism by which signals of biogeography are generated. To test this, detailed

Site frequency spectrum (SFS): histogram of allele frequencies in a sample. This summary statistic is used in population genetics to infer demographic and selection parameters.

Soft sweep: a selective sweep in which multiple adaptive alleles at the same locus, but on distinct haplotypes, rise to high frequency simultaneously.

Transmission: the transfer of a microorganism from one host to another.

Key Figure

Ecological versus Evolutionary Processes in the Microbiome

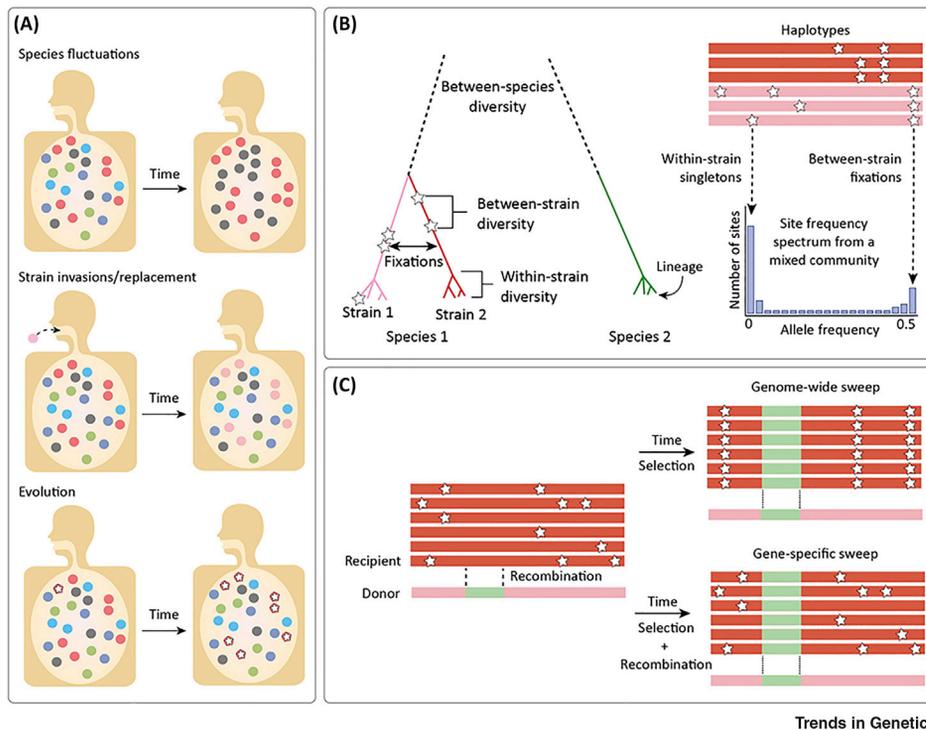


Figure 1. (A) Microbiota may respond to their environment by ecological shifts in species abundances (top; each species represented by a different-colored circle) or invasions of genetically distinct lineages (middle). Alternatively, existing lineages may evolve genetic changes (yellow star) via mutation, recombination, selection, and drift (bottom). (B) An illustration of the genetic diversity of two genetically distinct lineages of one microbiome species colonizing a host at high frequency. In reality, there is a range of colonization scenarios across different hosts and species. On average, lineages from different individuals differ by 1 mutation per 100 bp genome-wide (a typical number; yellow stars). These lineages are likely to have accumulated fixed genetic differences for millions of generations prior to colonization. Recombination and selection will have local effects on divergence. In a host that is oligocolonized by these two lineages at equal abundance, this genome-wide divergence manifests as mutations at 50% in a mixed sample. These mutations populate the 0.5 frequency bin in a **site frequency spectrum (SFS)**. By contrast, mutations that arose since colonization of a host are typically less frequent (e.g., ~ 1 per 100 000 bp) since they have had less time to accumulate. These constitute the low-frequency bins of the SFS. Recently diverged lineages will have greater haplotype **homozygosity** than those of more diverged lineages. (C) Signatures of selective sweeps. In both scenarios, the sweeps are seeded by an adaptive recombinant fragment. Top: In a genome-wide sweep, the haplotype bearing the adaptive allele quickly rises to high frequency, resulting in low genome-wide diversity due to hitchhiking of linked variants. Bottom: In a gene-specific sweep, recombination events introduce the adaptive fragment onto multiple haplotypes, resulting in low diversity only at the adaptive locus.

analyses of the dispersal abilities of human-associated microbes and local transmission rates within and between communities are needed. Rare genetic variants and unique **haplotypes** are a promising means to track strains for such studies.

Recombination

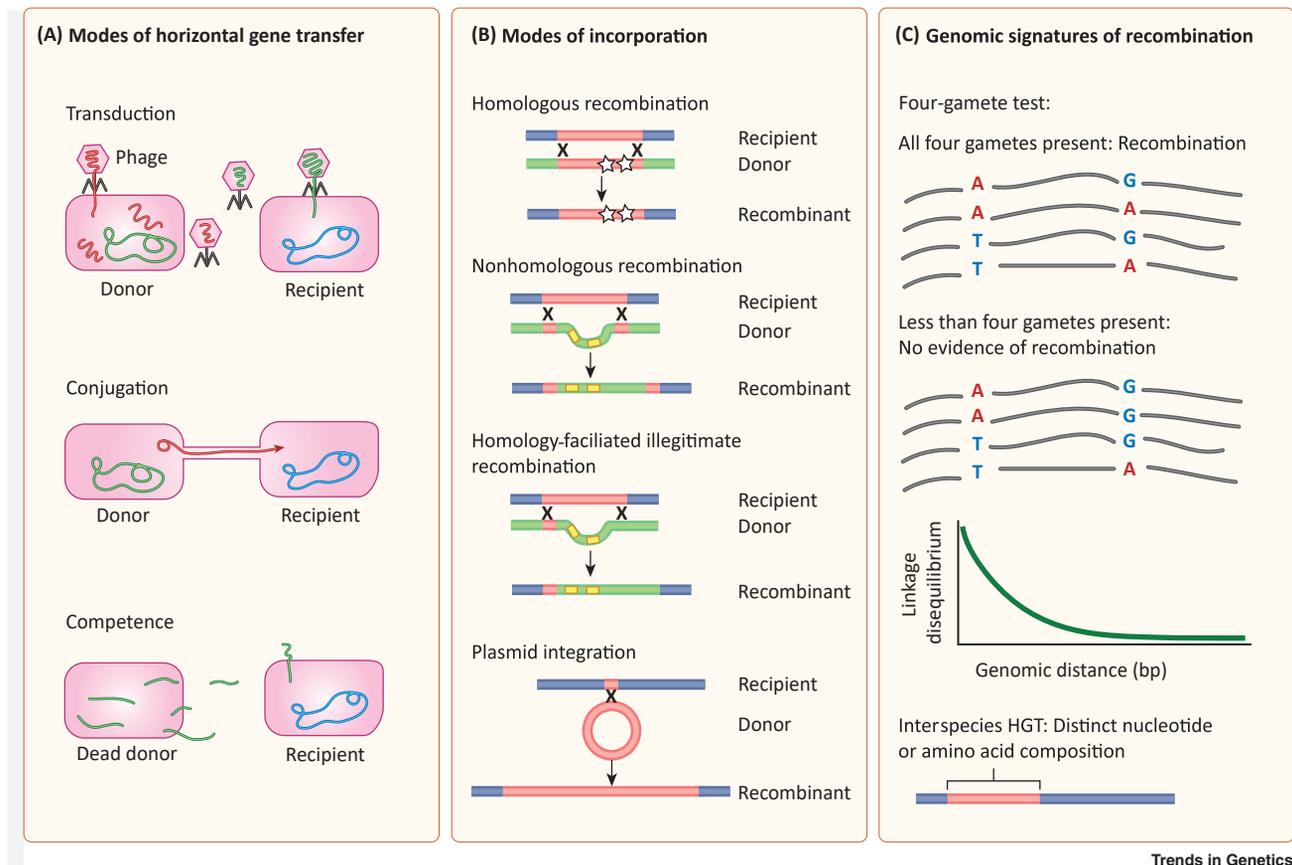
Recombination in asexual prokaryotes is a process whereby a close homolog or distantly related DNA is transferred from a donor to a recipient (also known as HGT) (Box 3). This unlinks loci from one another, allowing them to evolve independently and also results in the gain and/or loss of genetic material on different genomic backbones.

Quantifying recombination in the human microbiome is challenging. Initial work focused on detecting HGT across isolate genomes [36,63–66], leveraging the fact that transferred genes typically have distinct genomic signatures (Box 3). Within-species recombination events are more difficult to detect since the donor and recipient genomes are similar. However, recombination can be quantified by identifying phylogenetic inconsistencies across loci. For example, assuming each nucleotide mutates at most once in the whole population, if different phylogenetic trees parsimoniously describe the evolutionary history at different nucleotides, it is likely that a recombination event occurred. These phylogenetic inconsistencies due to recombination can be captured by the **four-gamete test**, which looks for at least four haplotypes harboring all four combinations of alleles at pairs of polymorphic nucleotides [67] (Box 3). The probability of such recombination events occurring increases with genomic distance between polymorphic sites. Thus, the decay in **linkage disequilibrium (LD)** over genomic distance, which quantifies correlations of alleles (nucleotides or gene presence), is also a signal of recombination (Box 3). Application of LD to microbiomes works best with long-range sequences from isolate genomes, single-cell genomes, or long-read data [34,63,68–70], but it has also been applied to genotypes inferred from shotgun metagenomes [33,71,72].

It is generally thought that certain genomic regions (mobile elements) recombine more than others [73]. However, recent analyses of patterns of LD in several human commensal microbiota and environmental microbes suggest that homologous recombination may affect the majority of loci in several species [33,70–72,74]. This highlights that rates and patterns of recombination vary across microbial species and across the genome [70,71,74–77]. Several examples of the range of recombination rates that bacteria can experience come from environmental samples. For instance, *Myxococcus xanthus* from soil is a highly clonal species, indicating low recombination rates [76,78], whereas *Vibrio* from the ocean experience high recombination, although some loci have high LD indicative of recent selection. Other bacteria have

Box 3. Mechanisms and Genomic Signatures of Recombination

- (i) Bacteria take up DNA by three processes: conjugation (direct transfer of DNA from one cell to another via cell-to-cell contact), competence (uptake of genetic material from the surrounding environment), and transduction (phage-mediated transfer of DNA) (Figure 1; adapted from [90]).
- (ii) Once inside the cell, the donor's DNA can be incorporated into the recipient's DNA by a variety of modes. Homologous recombination is a process where nearly identical strands of DNA are exchanged by crossovers (indicated with 'X') mediated by the *rec* protein [90]. However, in nonhomologous recombination, where only the flanking regions match, or homology-facilitated illegitimate recombination, where only one anchor locus matches, new genes may be introduced in the region in between the crossover points [175], resulting in HGT. HGT can also occur by transfer of plasmids and transposable elements and can occur across species boundaries but also within species [90]. Stars indicate mutations; rectangles indicate genes.
- (iii) HGT across species boundaries can be detected by identifying orthologous genes in multiple species with lower sequence divergence, distinct GC content, tetranucleotide composition, or codon usage [176] compared with the rest of the genome. However, within-species recombination may not leave the same signatures since the recombining genomes may be less diverged. Instead, gene gains and a decay in correlations of allelic states between loci are telltale signs of recombination. For example, the four-gamete test detects recombination by identifying all combinations of a pair of biallelic sites, which, assuming an infinite sites model, could not have arisen by multiple *de novo* mutations. LD captures correlations between sites, which decays over time and genomic distance due to recombination. There are many measures of LD, such as $D_{AB} = p_{AB} - p_A p_B$, where p_A measures the frequency of allele A occurring at one locus, p_B measures the frequency of allele B occurring at another locus, and p_{AB} measures the frequency of the A and B alleles co-occurring on the same haplotype. The rate of decay in LD [33,71], as well as genealogical simulations [75], can be used to infer the ratio (r/μ), which describes the relative contributions of recombination (r) versus mutation (μ) towards genetic diversity.



Trends in Genetics

Figure 1. Mechanisms and Signatures of Recombination

Abbreviation: HGT, horizontal gene transfer.

extreme levels of recombination, such as cyanobacteria from a hot spring where virtually all loci are unlinked [31,79]. While we are starting to get a sense of the range of recombination experienced by human commensals [33,70,71], much work remains to fully characterize this.

The relationship between recombination rate and genetic diversity is complex [77]. HGT frequently diversifies the gene content, or **pangenome**, within a species [80]. It also lowers divergence between species and can reduce diversity within species if horizontally transferred DNA overwrites existing variation or is recently derived from a common low-diversity source. In this manner, homologous recombination helps to create a genetically cohesive population [39,81]. By contrast, when recombination rates are sufficiently low, genomes can diversify into novel species [81]. For example, *B. vulgatus* and *Bacteroides dorei*, which were once defined as one species, are now considered two species that have recently diverged [82].

The ability to incorporate novel genetic material from the broader community and create new combinations of alleles may be particularly important for rapid adaptation to fluctuating environments in humans. The mode by which this happens is a topic of great interest [32,39]. Assuming an adaptive allele with selection coefficient s and a recombination rate r per generation per base pair, if $r \gg s$, an adaptive genetic variant could propagate through the population via a **gene-specific sweep** whereby the trait recombines onto multiple genomic backgrounds or sweeps through the population

on a plasmid [30,31,83–86]. Alternatively, if $r \ll s$, the entire linked genome will rise to high frequency in a **genome-wide sweep**, spreading potentially deleterious alleles along with the beneficial allele [32,87] (Figure 1C). The relative frequencies of these two modes are yet to be fully quantified across human commensals.

Since recombination can facilitate adaptation by incorporating new beneficial genomic material, it is easy to make the mistake of concluding that recombination itself was positively selected. There are costs to recombination too: combinations of beneficial alleles can become unlinked [88] and deleterious material can become incorporated, as observed with plasmids, which may impose costs despite also playing an important role in bringing in public goods from the broader community [89]. However, via the process of **purifying selection**, deleterious variants are purged from the population, thus making it seem retrospectively that everything incorporated by recombination is beneficial [90]. Thus, it is interesting to note that the original purpose of the enzymes involved in recombination is to repair and replicate DNA and not to facilitate gene transfer [90–92].

With growing interest in recombination in the human microbiome, many open questions remain. Although it is common practice to build phylogenetic trees to describe the evolutionary relationship between lineages [46,54,93], branch lengths in trees of highly recombining lineages may reflect the frequency with which lineages recombined with each other in the past rather than divergence time [70,76]. Detecting when this is the case is important for the elucidation of evolutionary mechanisms and determining whether a tree is a useful representation of population history. It is also critical to determine how host-associated bacteria have the opportunity to recombine even when hosts are oligocolonized by just a few strains [33,34,46–49]. Determining why some species like *H. pylori* have high rates of recombination and simultaneously a lot of geographic structure [94] is another focus of ongoing research.

Adaptation

Adaptation is a process whereby a population becomes better able to survive in its environment through changes in allele frequencies. Extensive laboratory experiments, such as the Long Term Experimental Evolution experiment [22,23,25,27], studies of host-associated pathogens [95–99], and natural populations of environmentally associated microbes [30,31,83,85] have shown that adaptation in microbial populations is common and rapid. However, until recently it was unknown how broadly this picture applies to the human microbiome.

In theory, commensal microbes in the human microbiome could be adapting rapidly because their large census sizes [100] and short generation times [18] result in a large daily mutational input [34,101]. However, ecological forces, including shifts in the abundance of species in the ecosystem or invasions of better-fit strains, could be more important for the response to selective pressures in the human microbiome than in experimental evolution. It is also possible that there is less adaptation because microbiota have coexisted with their hosts for millions of years [55,102] and thus could have already evolved to be optimally adapted to the human body.

A picture of evolution on human-relevant timescales is starting to emerge in the microbiome. To identify evolutionary events, it is important to distinguish ecological scenarios, such as strain fluctuations within a host that could drive allele frequency changes, from true evolutionary changes on the background of a lineage. A few recent studies were able to identify rapid allele frequency changes associated with evolution on 6-month to 2-year timescales in the human gut microbiome by either computationally resolving the lineage structure within a host or sequencing isolates [33–35,103].

However, nonadaptive evolutionary forces can also change allele frequencies. For example, neutral and mildly deleterious alleles may increase under drift [35], potentially during population bottlenecks, but alleles that rise to high frequency rapidly in a sufficiently large population are more consistent with adaptation than neutral scenarios [9]. Still, linked nonadaptive alleles may also hitchhike to high frequencies with adaptive alleles [9].

One powerful strategy to identify allele frequency changes associated with adaptation versus neutral processes has been to look for an excess of nonsynonymous versus synonymous **fixations** (dN/dS). This is an indication that a functional change may have been positively selected, the reason being that nonsynonymous mutations change the amino acids of a protein and synonymous mutations are presumed to be neutral. This approach has been applied to many host-associated microbes, including fixed differences between lineages or strains (e.g., [104,105]) and polymorphism within a host (pN/pS) [2].

Genome-wide estimates of pN/pS and dN/dS are significantly less than 1 in the human microbiome [2,33,34], suggesting that purifying selection impacts the majority of the genome over long time-scales. However, $dN/dS > 1$ within specific genes, genomic regions, or pathways has identified specific loci in the human microbiome experiencing adaptation. Demonstrating this approach, Zhao and Lieberman *et al.* [34] sequenced hundreds of isolates of *B. fragilis* from hosts over 2 years and identified 17 genes enriched for nonsynonymous mutations. Further supporting adaptation, these genes involved in cell-envelope biosynthesis and polysaccharide utilization were undergoing parallel evolution in multiple hosts. Interestingly, the same loci show signals of purifying selection across hosts, suggesting that different selective forces act on the same loci on different timescales, potentially due to changing selective pressures.

Several other selection detection methods have been applied to microbes, with a focus on isolate genomes. For example, the fixation index (F_{ST}) quantifies differences in allele frequencies due to population structure by comparing allele frequencies in the joint population with allele frequencies in individual populations. F_{ST} can be driven both by nonadaptive population genetic processes like migration and drift and differential selection pressures. F_{ST} applied to human metagenomic samples has shown that, over time, samples from the same individual are genetically stable and have a much lower F_{ST} than samples from different individuals [2]. Local regions of the genome with a high F_{ST} are indicative of positive selection, as observed in soil bacteria [72] and *Plasmodium falciparum* [106,107].

The dN/dS , pN/pS , and F_{ST} statistics are powerful for detecting selection that has driven alleles to fixation. However, LD or haplotype statistics that account for correlations between pairs or many loci are useful for detecting more recent **selective sweeps** that have not fixed [108]. LD- and haplotype-based statistics have been successfully applied to recombining eukaryotes to detect adaptation [108–110] and could be successful in bacteria with high rates of recombination. For example, Rosen *et al.* showed that although a population of cyanobacteria has low genome-wide LD, individual loci showed elevated LD, consistent with selection and **hitchhiking** [31].

Although we are gaining evidence that human commensal microbiota can evolve, the tempo and mode of adaptation in the microbiome remains to be fully characterized [111]. Theoretical work in population genetics has started to uncover multiple ways by which adaptation proceeds in multiple populations [101,112], such as **hard sweeps**, **soft sweeps**, polygenic adaptation, **partial sweeps**, and more. These different mechanisms of adaptation encode important features of population biology, such as the rate of adaptation, the mode of adaptation (e.g., from *de novo* mutations or pre-existing genetic variants segregating in the population), temporal fluctuations or spatial distribution in selective pressures, and the strength and timing of selection. These properties of selection are relevant to the study of adaptation in virtually any organism.

However, our current understanding of the dynamics of positive selection in the microbiome is nascent. For example, it is unknown how commonly adaptation occurs through recombination-seeded adaptive events from distantly related members of the microbiome versus adaptation from *de novo* mutations. It is also unknown how common partial sweeps are. Such sweeps may be common: (i) if environment and selective pressures fluctuate (e.g., due to medications, diet, urbanization, or climate change); (ii) if there is a lot of spatial structure in the microbiome [113,114]; or (iii) if lineages evolve different niche specializations [22,34]. Additionally, it is unknown whether **clonal interference** is common in the microbiome, whereby multiple lineages with the same fitness cannot outcompete each other until there is a clear fitness difference or drift takes over, as has been commonly observed in experimental evolution of bacteria

[115,116]. Alternatively, it is unknown how common soft sweeps are in the microbiome, in which multiple lineages bearing independent instances of the same adaptive mutations rise in frequency simultaneously, as observed in gut microbiota of mice colonized with *Escherichia coli* [117].

Studying adaptation in the human microbiome is an exciting frontier for both the microbiome and population genetics fields. Since population genetics has typically studied one species at a time, there remains much to learn about adaptation in complex communities and **ecology**–evolution feedbacks. For example, how commonly does the evolution of a focal species result in permanent shifts in the ecological structure of the community [22]? Also, does the ecological structure of the community accelerate or constrain adaptation [14,22,118]? Given the tight connection to many diseases [119–123], the human microbiome affords us a unique system to analyze complex evolutionary and ecological dynamics and their impact on human health.

Connecting Genotypes to Phenotypes

Discovering the functional consequences of within-species genetic variation in the microbiome is an important goal, both for interpreting the forces driving selective sweeps and for engineering the microbiome to improve human health.

Genetic variants in the microbiome have been causally linked to a range of microbial and host phenotypes including drug resistance [124–127], regulation of biofilm formation [12], conversion of commensals into pathogens [128], modulation of host immune responses [129,130], the generation of disease-associated compounds from food [131], and the metabolism of drugs [13]. The primary approach used to identify these SNVs and SVs involves performing isolate genome sequencing [132] or metagenomic analysis (e.g., [133]) of strains that harbor phenotypic variation and then using comparative genomics or phylogenetic regression methods to test for genetic variants that correlate with the phenotype. With abundant metagenomic data, we now can perform higher-throughput genotype–phenotype associations at an unprecedented scale beyond isolate genomes to link genetic variation within hosts to their health. This opportunity is akin to how human genetics was transformed by GWASs spurred by the development of genotyping arrays.

As with other forms of GWAS, tests for association between microbiome variants and traits of communities or hosts have limitations. First, various confounding variables need to be accounted for to make accurate inferences. For example, population structure can create false genotype–phenotype associations if genetically distinct lineages are not evenly distributed across phenotype values [40]. To avoid confounding effects of population structure, it is critical to adjust for or explicitly model relatedness of microbes across samples [134–137]. Another confounder of microbiome GWASs is the potential pervasiveness of selection, since linked variants that have hitchhiked to high frequency make it difficult to identify the causal variant [138]. Another challenge is the huge sample sizes that may be required to detect statistical associations in the face of technical and biological variation. High-throughput assays for genotyping the microbiome may help to make large studies feasible, as in human genetics, but in the near future GWASs will be most useful for variants with big effect sizes (e.g., a large SV deleting a pathway). Finally, the utility of GWASs for improving our mechanistic understanding of host–microbe interactions is currently severely limited by how little we know about the functions of most microbiome genes and variants.

GWAS techniques have been applied to microbiome data in a variety of other ways. The plethora of case-control microbiome studies, for example, typically test for associations between microbial abundance and a host trait (e.g., [119,120,139]). Given that the presence of some microbial species is a **heritable** trait [140–143], there is also a case for using GWASs to identify links between host genetic variants and microbial abundances [57,144], which most notably has identified an association between variants near the *LCT* locus and the abundance of *Bifidobacterium* [56,141,145,146]. However, it

remains to be seen whether this association is due to milk being available in the diets of individuals that are lactase persistent or driven by genotypes at the *LCT* locus itself. While these approaches associate the taxonomic composition of microbiomes to differences – genetic or phenotypic – between hosts, they do not leverage microbial population genetic variation and its ability to map such associations to genes and functions of microbes. An exciting direction is to explore host–microbe interactions through GWASs using both microbial and host genotypes [147]; for example, to test the hypothesis that microbes carrying particular alleles preferentially associate with particular host genetic backgrounds or to explore how microbial genotypes buffer or amplify human genotype–phenotype connections.

Concluding Remarks

The emerging picture of microbiome evolution suggests that each of us harbors populations of microbes whose within-species genetic diversity is limited at any one time point but highly dynamic on timescales of days [34] to a few years [2,3]. These population genetic changes are both an opportunity (e.g., enabling digestion of new foods [148]) and a challenge (e.g., the development of drug resistance [127,149], maintaining stable colonization of therapeutic strains [150,151]). Understanding how, when, and why microbes evolve is essential to leveraging them in medicine, industry, and agriculture.

To paint a clearer picture of the evolutionary dynamics in the microbiome, we need new models that incorporate pervasive recombination, ecology–evolution interactions, and complex relationships between host genetics, highly dynamic microbiome genetics, and the human body's environment. Longitudinal modeling across host lifespan will help to resolve open questions about recombination rates and patterns, as well as the mode, tempo, strength, and functional targets of selection.

We also must continue to develop bioinformatics methods to assay genetic variation from different types of sequencing data, especially as the data become more abundant, higher resolution, and more complex. For example, longer reads with low error rates – or in combination with low-error short reads – will help to resolve long-range linkage and guide assembly. Methods to estimate allele frequencies from metatranscriptomes will shed light on the functionality of different genotypes.

As microbiome GWASs become common, tools for editing microbiome genomes and testing hypotheses through experimental evolution (Box 2) will be essential in establishing causal relationships. The interpretation of genetic variation and the results of GWASs also requires a massive effort to improve functional annotation of genes in the human microbiome, many of which are completely uncharacterized. Newly sequenced genomes are particularly unannotated.

Human genetics has progressed by producing a high-quality genome, assaying common variation, developing and deploying genotyping arrays at scale, and sequencing rare and complex variants. Microbiome genetics is just beginning this process (see Outstanding Questions) but has the advantage of leveraging existing methods, study designs, and theories. As these are adapted and expanded to study the complex populations of microbes living in our bodies, it will be exciting to see microbiome population genetics move towards a predictive science and an important component of precision medicine.

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Outstanding Questions

What factors (e.g., dispersal limitation, transmission, population bottlenecks, recombination rates) contribute to genetic structure within microbiome species across hosts and geographic regions? Why does structure differ so greatly across different microbiome species?

What is the tempo and mode of adaptation in the human microbiome within hosts and across hosts on different timescales? What are the forces driving adaptation and the targets of selection? Longitudinal data and more-complete reference genomes with annotations may be important in answering these questions.

How common are various mechanisms of recombination in the microbiome (e.g., plasmid transfer versus homologous recombination)? Does variability in recombination rates correlate with rates of adaptation or biogeography across microbiota?

Given that evolution in the human microbiome can occur rapidly on short timescales, does this evolution influence ecological processes and vice versa?

Which host and microbial traits are associated with genetic variation in the microbiome?

How large must sample sizes be for a well-powered microbiome GWAS? Answering this question requires estimation of the strength of associations between microbiome genetic variants and phenotypes (effect size) as well as how variable these associations are across hosts.

Microbiome genomics has largely focused on protein-coding genes. Is there evidence of adaptation on mutations in gene-regulatory elements and RNA genes?

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