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Microbial ecology

Assembly of the human intestinal microbiota

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Complex microbial ecosystems occupy the skin, mucosa and alimentary tract of all mammals, including humans. Recent advances have highlighted the tremendous diversity of these microbial communities and their importance to host physiology, but questions remain about the ecological processes that establish and maintain the microbiota throughout life. The prevailing view, that the gastrointestinal microbiota of adult humans is a climax community comprised of the superior competitors for a stable set of niches, does not account for all of the experimental data. We argue here that the unique history of each community and intrinsic temporal dynamics also influence the structure of human intestinal communities.

Introduction

Like other mammals, humans have been associated with complex microbial communities throughout our evolution, with every birth renewing this intimate web of relationships. The specialized microbes inhabiting the surfaces and alimentary tract of a human adult (Box 1) outnumber the human cells by a factor of ten [1]. The hundreds of microbial species native to the colon, where microbial richness and abundance are maximal, contain 100-fold more genes than does the human genome [1]. This large and diverse microbiota (see Glossary) has long been recognized as contributing to gut maturation, host nutrition and pathogen resistance [2]; more recently, microbes have been shown to regulate intestinal epithelial proliferation [3], host energy metabolism [4] and inflammatory immune responses [5]. Intestinal communities are implicated in diseases ranging from allergies [6] to late-onset autism [7], inflammatory bowel disease [8] and cancer [9]. Hence, our understanding of intestinal microbial ecology has a direct bearing on our ability to manage and maintain human health (Box 2).

The prevailing ecological paradigm for the adult human gut microbiota, now decades old, is that each region of the intestine (Box 1) harbors a stable climax community comprising the superior competitors for a fixed set of niches [2,10]. This perspective has fostered fruitful research into the factors that define microbial niches in the intestine, such as the diet and genotype of the host. However, we argue here that the diverse and personalized gut communities of humans are also shaped by less predictable influences, such as historical contingencies during community assembly and temporal dynamics arising from interactions within the microbiota.

History: new evidence and old perspectives

During the 1970s, epidemiological links between diet and cancer stimulated intensive cultivation-based research in gastrointestinal microbiology. The diversity of human fecal bacteria was unexpectedly high, with only a minority of individuals harboring most species, even among those individuals with similar diets, ethnicity and geographical origin [11,12]. By contrast, the identity and relative abundance of most genera was similar within such social groups and, to some extent, between groups [12]. Interpretations at the time focused on explaining the shared features of these communities, rather than interindividual

Glossary

Archaea: a domain (the highest taxonomic rank) containing prokaryotic microbes that includes the methanogens. Archaea, Bacteria (another domain of prokaryotic microbes), and Eukarya (all organisms with nucleated cells) comprise the three-domain taxonomy of life.
Bacteriocin: a protein released by a microbe with killing activity against other microbes.
Climax: a steady-state community composition attained as the endpoint of succession.
Fermentation: an anaerobic energy-generating pathway involving the release of a partially reduced organic molecule. Primary fermentation consumes monomers released by the hydrolysis of polysaccharides and proteins; secondary fermentation further transforms the products of primary fermentation.
Glycans: polysaccharides, such as those attached to protein to form mucin. Fucosylated glycans terminate in a fucose residue that can be removed by bacterial extracellular enzymes.
Historical contingency: an event that occurred but was not inevitable; in essence, an accident.
Interference: a biological interaction in which one organism either prevents another organism from colonizing, or reduces its abundance by some means other than competition for resources.
Methanogens: anaerobic archaea that obtain energy by oxidizing hydrogen and reducing carbon dioxide to methane. Other methanogenic pathways exist, but are not thought to be important in the human intestine.
Microbiota: a collective term for microbes. (Terms such as 'microflora' and 'normal flora' perpetuate an outdated classification of these organisms as plants.)
Mucin: proteins with abundant, covalently linked polysaccharide chains that are the primary component of mucus.
Phage: a virus that infects bacteria.
Phylotype: a microbial group defined only by 16S rRNA sequence similarity rather than by phenotypic characteristics. A similarity of 97–99% indicates approximately a species-level taxon.
Toxic metabolites: small molecules released by the normal metabolism of some microbes that are toxic or inhibitory towards other microbes (e.g. sulfide from sulfate-reducing bacteria).

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Box 1. Prominent communities of the human microbiota

Our knowledge of the human microbiota is far from complete. Despite more than a century of culture-based investigations, most microbial species have yet to be recovered, and several habitats in human have been investigated either by only a single 16S rRNA-based study, or by none at all. Even for the mouth and colon, which are the most studied, much work remains to establish the causes and consequences of temporal and interindividual variation in microbiota community composition.

Mouth
Approximately 700 species from nine bacterial and one archaeal phyla inhabit the human mouth, the taxonomic richness of which approaches that of the colon, although the abundance is lower. The genus *Streptococcus* in the Firmicutes phylum is numerically dominant. Surface attachment is a key selective feature, with different communities inhabiting different surfaces, such as the teeth, cheek and tongue. Multispecies biofilms are typical [68].

Esophagus
A 16S rRNA survey of the esophageal mucosa detected 95 species from six phyla, most of which are similar or identical to species found in the oral cavity [69].

Stomach
Gastric acidity kills many ingested microbes [12] and *Helicobacter pylori,* associated with the gastric mucosa in some individuals, is the only undisputed resident. A 16S rRNA survey has found sequences of 128 bacterial species from eight phyla, which could represent either ingested or resident strains [63].

Small intestine
Although the short residence time limits microbial growth in the lumen [2,12], mucosal populations might be important for colonizing lumenal material and for pathogen resistance. Bacterial populations increase from $\sim 10^4$ ml^{-1} near the stomach to $\sim 10^7$ ml^{-1} near the colon, where transit slows; their composition also changes, with an increasing proportion of anaerobic species [12,16].

Colon
Rapid microbial growth via polysaccharide hydrolysis and carbohydrate fermentation occurs in the cecum and ascending colon; growth slows and amino acid fermentation becomes more important in the transverse and descending colon [41]. Populations increase from $\sim 10^8$ ml^{-1} in the cecum to 10^{11}–10^{12} ml^{-1} in stools; the species composition changes as facultative anaerobes decrease from ~25% to 1% or less of the community [70]. Approximately 800 species are estimated to be present, representing nine bacterial and one archaeal phyla [24,25], the most numerous being obligate anaerobes of the Bacteroidetes and Firmicutes phyla [15,24,25].

Vagina
Lactobacillus spp. have long been considered to be the predominant resident bacteria of the vaginal mucosa in healthy pre-menopausal women. However, recent 16S rRNA surveys indicate that other genera are abundant or predominant in a minority of healthy women [71].

Box 2. Medical implications of intestinal microbial ecology

There is a role for community ecology in explaining pathogenesis given that Intestinal pathogens must somehow invade or escape the commensal microbiota to become established. Some pathogens, such as *Salmonella* and *Listeria*, succeed by accessing unoccupied niches, escaping the lumen by entering epithelial cells. Certain commensal *Lactobacillus* and *Bifidobacteria* strains appear to block physically pathogen access to host cells [32]; consumption of probiotics (live microbes) or prebiotics (selective microbial substrates) are intended to augment such populations. However, the implicit ecological assumptions of these strategies, that the relevant populations are dispersal or resource limited, are rarely tested. *Clostridium difficile,* the cause of potentially life-threatening pseudomembranous colitis, represents another class of pathogen that can persist in the lumen without becoming abundant or causing disease unless the existing community is perturbed, for example by antibiotics [72].

Perturbations of the microbiota are also associated with chronic health conditions. Allergies and asthma are statistically linked to childhood antibiotic use and altered intestinal communities. One possible mechanism was recently demonstrated in mice [5] whereby antibiotics followed by a gastric dose of the fungus *Candida* caused a permanent increase in intestinal *Candida* and an allergic response following pulmonary exposure to *Aspergillus* fungus [5]; neither treatment alone had these effects. Some cases of late-onset autism develop following childhood antibiotic use and are associated with persistent diarrhea. Treatment with the antibiotic vancomycin has been tried in a few such patients, leading to an improvement in autistic symptoms that vanishes once vancomycin is discontinued [7]. Several *Clostridia* species are found in stool samples of these individuals but not control subjects; the resistant spores of these species could explain their persistence after vancomycin treatment [7]. Effective prevention and treatment of these conditions could amount to ecological restoration of the intestinal microbiota.

Chronic diseases also result from interactions between the microbiota and genetic or dietary factors. Inflammatory bowel diseases appear to result from abnormal host immune responses, with many human genes contributing to susceptibility [8]. The commensal community rather than a specific microbe might act as a pathogen in susceptible individuals, but commensal species vary in their effects on the immune system [8]. Similarly, the elevated risk of colon cancer associated with meat and fat in the diet might be mediated largely by the activity of sulfate-reducing bacteria and bile-transforming bacteria, respectively [9]. Although microbes are not the primary causes of these conditions, ecological interventions to modify the abundance or activity of specific populations could prove beneficial.

differences [2,10]. Postnatal development of microbial communities throughout the gastrointestinal tract was described as a predictable succession driven by internal factors, such as oxygen depletion, and by external factors, such as diet [2]. Succession in each intestinal compartment was believed to culminate in a stable climax shortly after weaning [2]. Given that the communities were presumed to consist of the superior competitors in all available niches, they were expected to persist through life in the absence of perturbation [2,10].

Over the past decade, our knowledge of human microbial diversity has been expanded by molecular techniques using small subunit rRNA (16S rRNA) gene sequences as culture-independent phylogenetically informative biomarkers of microbial taxa. Different 16S rRNA-based techniques are appropriate for different investigations, including identifying microbial species or phylotypes, quantifying microbial taxa, or making broad comparisons of microbial communities [13] (Table 1). These techniques have not only revealed that the majority of intestinal microbes belong to previously unknown (i.e. uncultivated) lineages, but have also clarified the relationships between taxa at all phylogenetic scales and have facilitated investigation into factors affecting community composition [13–23]. At broad phylogenetic scales, the intestinal communities of all humans appear quite similar. Most intestinal microbes belong to the Firmicutes and Bacteroidetes, only two of at least 50 known bacterial phyla [15,24,25]. Even within the two predominant phyla, the human microbiota contains relatively few deep lineages; however, these terminate in broad, shallow radiations comprising hundreds of species and thousands of strains [24,25]. At

Table 1. Methods of analyzing microbial communities

Method	Main use	Advantages	Limitations	Refs
Traditional methods				
Cultivation	Identify and quantify taxa	Provides isolates for further characterization; can focus on recovering strains with desired traits	Incomplete and biased community representation; many types of media needed to maximize species recovery	[11,12]
Microscopy (general stains)	Estimate abundance of all microbes or recognizable types	Quantification without cultivation or broad-range PCR bias[d] can reveal spatial relationships	Cannot distinguish between many taxa with different traits and ecological roles; low throughput	[11]
16S rRNA methods				
Oligonucleotide hybridization[a]	Detect and quantify known phylogenetic groups	Can be high-throughput; can reveal spatial relationships; phylogenetic identification of visible cells	Detects only taxa that hybridize to chosen probes, typically 6–18 genus- or family-level groups detected	[17,23,66]
5′ exonuclease PCR[b]	Detect and quantify known phylogenetic groups	Rapid; high throughput	Detects only taxa that hybridize to chosen probes; typically 6–18 genus- or family-level groups detected	[21]
Community profiling[c]	Compare communities	Rapid, inexpensive assessment of abundant 16S rRNA sequence variants	Broad-range PCR bias[d]; additional work needed to identify groups represented in profiles; hard to compare analyses done at different times	[16,18–20,22]
16S rRNA sequencing	Phylogenetic identification of microbes; generates data for other 16S rRNA-based methods	Identification to strain level; can detect novel taxa; analysis possible at multiple phylogenetic levels	Broad-range PCR bias[d]; expensive; laborious data analysis	[15,16]

[a]Includes membrane hybridization, fluorescent *in situ* hybridization (FISH) with cells detected by microscopy (for spatial information) or flow cytometry (for high throughput and accurate quantification), and microarrays. Microarrays detect many more taxa simultaneously, but quantification is more difficult and broad-range PCR bias can be an issue.
[b]Includes denaturing gradient gel electrophoresis (DGGE) and similar techniques, and terminal restriction fragment length polymorphism (tRFLP).
[c]Also called quantitative PCR, real-time PCR, or TaqMan assay. PCR is a biochemical amplification of particular DNA sequences.
[d]The broad-range PCR used for profiling, 16S rRNA sequencing and microarrays might not include all taxa or accurately represent their relative abundance, but these problems are reduced relative to those of cultivation approaches.

this fine scale, with respect to strain identity and relative abundance, the microbiota of an individual appears to be as personalized as a fingerprint [14,15,17,21,26,27].

New concepts have also entered community ecology since the 1970s. As well as competitive interactions, colonization history is now also recognized as a potential influence on community composition [28,29]. The niche construction perspective emphasizes that organisms alter niches for themselves and other organisms by changing the local environment [30], which can extend the effects of historical contingencies. Development of a stable climax is no longer assumed; even long-standing communities can remain out of equilibrium owing to internal dynamics or changing external conditions [31]. These ideas have barely begun to penetrate discussions of intestinal microbial ecology (e.g. [14]), perhaps owing to some undeniable successes of the more deterministic, competition-centered view. For example, interspecies competition involving resources, attachment sites and interference mechanisms accounts for the colonization resistance of intact gut communities [32], and variation between intestinal compartments in physicochemical factors such as retention time, oxygen availability and pH accounts for their characteristic microbial communities [2,12] (Box 1).

However, microbial interactions are not limited to competitive exclusion in pre-existing niches; they are also crucial factors in generating the diversity of niches within an intestinal compartment. The assembly of the microbiota is partially recursive, as it creates and responds to many of its own selective pressures [30]. Hence, the traditional explanations of community structure need to be evaluated along with newer ecological concepts, such as the importance of historical contingency and the potential for intrinsic population dynamics.

Factors generating a diverse, personalized human microbiota

Although many factors are known to influence intestinal microbiota, we focus here on diet, host genotype and microbial interactions, three categories that seem most able to explain the diversity and the individuality of these communities.

Diet

Diet is an obvious potential source of niche-determining factors in the adult colon, especially given that fact that the fecal microbiota of infants is influenced by transitions between breast milk, formula and solid foods [2,33,34]. However, early studies comparing broadly defined diets (e.g. 'Japanese' versus 'Western') or manipulating the proportion of food categories over several weeks found only moderate effects involving few genera [2,12,35]. By contrast, studies of chemically defined diet components have shown effects on particular taxa. Dietary sulfate favors several genera of sulfate-reducing bacteria over methanogenic archaea [36]; inulin and related fibers increase the abundance of *Bifidobacteria* when this genus is rare [37,38] (Box 3).

Additional selective effects of specific dietary compounds could remain undiscovered, but the influence of diet on the composition of the microbiota is probably not pervasive at fine phylogenetic scales. The strains of many intestinal genera are generalists with comparable growth abilities on overlapping ranges of substrates [36,37,39]; particular metabolic traits (especially among Firmicutes) can also be distributed discontinuously among lineages [36,39,40] (Box 3). In either case, the partitioning of resources among microbial strains is incomplete. Furthermore, for the secondary fermenters and hydrogen consumers that use

Box 3. Niches of prominent microbial taxa in the colon

- *Bacteroides*, of the Bacteroidetes phylum, are prominent starch degraders and many strains are also capable of degrading some types of structural polysaccharides. The ability of *Bacteroides* spp. to import oligosaccharides into their periplasmic space for further hydrolysis (thus monopolizing the hydrolysis products) might contribute to their abundance. *Bacteroides* are also primarily responsible for removing the sulfate ester-linked substituents of mucin [39–41].
- Clostridium Group XIVa is a family-level taxon within the Firmicutes phylum containing many butyrate-producing strains, including *Roseburia* and relatives that can degrade starch and inulin. A second abundant cluster related to *Eubacterium halii* ferments lactate and acetate to butyrate and hydrogen. Important non-butyrate producing members of Group XIVa are *Ruminococcus torques* and *R. gnavus*, which are among the primary mucin-degrading organisms [39,40].
- Clostridium Group IV is another prominent family-level taxon containing *Faecalibacter*, abundant fermenters of starch and inulin to butyrate and lactate. Traits of most other human-associated members of Group IV are not known, owing to the lack of cultured representatives [39].
- Species of Firmicutes given the genus names *Peptococcus*, *Peptostreptococcus* and *Clostridia* are the predominant proteolytic and amino acid-fermenting organisms in the colon; some also ferment sugars [41]. The taxonomy is not consistent with their 16S rRNA phylogeny, which shows them to be scattered and interspersed with other lineages.
- *Bifidobacteria* in the Actinobacteria phylum are inulin and starch degraders, the genus also includes strains in several species that are prominent mucin degraders. Lactate is the primary fermentation product of *Bifidobacteria*, much of which is converted to butyrate by secondary fermenters [39–41].
- *Methanobrevibacter smithii* is the predominant if not the only species of methanogen in the human intestine. The lack of species diversity in intestinal Archaea, in comparison to the diversity among Bacteria, is striking but as yet unexplained [15].
- Sulfate-reducing bacteria are found in five distinct genera in the Delta subdivision of the Proteobacteria phylum. Hydrogen-consuming sulfate-reducing bacteria are found in two of these genera; the remaining genera consume partially reduced fermentation products (e.g. lactate) while reducing sulfate to sulfide [36].

fermentation products released by other species, any influence of dietary resources is indirect [39,41]. Finally, mucin and other host-derived resources are sufficient to support a diverse microbial community [40,41].

Genotype

Host genotypes are thought to influence interindividual variation in the intestinal microbiota, given that some immune-related genes are highly polymorphic. The mucosal barrier does not prevent interactions between the microbiota and the immune system as was once thought; in fact, this communication is routine and essential [3,6]. In healthy humans, however, these interactions do not determine the composition of the community in the intestinal lumen, but instead promote immune tolerance by restricting microbial penetration of host tissue [6,42,43]. The major histocompatibility complex genotype has been reported to influence the murine fecal microbiota [44], but this study did not distinguish genetic effects from preexisting differences in the native intestinal microbiota of the source mouse colonies.

The host genotype could also influence the intestinal microbiota via the availability of attachment sites and host-derived resources. For example, *Bacteroides thetaiotaomicron*, an abundant commensal species of humans and mice, regulates the production of fucosylated glycans by the murine gut epithelium to match bacterial demand for fucose as a growth resource [45]. If similar interactions occur in humans, they might involve the human polymorphisms in fucosyltransferase enzymes that are responsible for different blood types [46]; some blood type-related differences in intestinal communities have been reported [40,47]. Polymorphism in such genes is not surprising given that human glycoproteins probably evolve continually owing to selective pressures exerted by commensal and pathogenic microbes, and vice versa [48].

A correlation between overall genetic relatedness and fecal community similarity in adults was found by Zoetendal *et al.* [19] using a technique that displays a profile of abundant 16S rRNA sequence variants (Table 1). Unfortunately, the study could not differentiate effects of genetic relatedness from those of early cohabitation, because the correlation is driven by the lower average profile similarity among unrelated individuals than among family members that presumably lived together at least until children were weaned [19]. The fecal community profiles of identical adult twins appeared no more similar than those of less-related family members in this study [19], although just such a genetic effect has now been reported among infants and children by a study using a similar profiling technique [22]. According to the latter study, the fecal communities of cohabiting monozygotic twins before weaning differed in 10–25% of the detected 16S rRNA sequence variants [22]; a technique with greater sensitivity and phylogenetic resolution is likely to reveal at least as much variation. Thus, although host genotype certainly influences the intestinal microbiota, genetically identical individuals in very similar environments can still show differences in their intestinal microbiota.

Microbial interactions

As suggested by niche construction theory, the microbiota itself accounts for some of its diversity and interindividual variability [30]. Microbial interactions in addition to resource competition have long been recognized as important determinants of intestinal niches [2,10]. Interspecies cooperation occurs during the hydrolysis of complex carbohydrates and sequential fermentation of the resulting sugars, as well as by the exchange of growth factors [39–41] (Box 3). Common interference mechanisms include the production of toxic metabolites and specific antimicrobial compounds, such as bacteriocins [32,39]. Bacteriocins are microbicidal proteins once thought to be effective only among closely related strains, but broader activity and selective action against distant relatives also occur [49,50]. Similarly, phages are abundant and diverse in human feces [51] and vary in host range breadth [52]. Both bacteriocins [53–55] and phages [52] can promote population diversity and induce complex dynamics owing to evolutionary tradeoffs between resistance and sensitivity, but their effects on the human microbiota remain unexplored. At the least, interactions among gut microbes complicate the community responses to external factors, even when isolated populations respond deterministically. In some cases, such as evolution in response to bacteriocin or phage attack, the fates of individual strains include elements of chance.

The importance of unpredictable events

Colonization history

The effects of diet, genotype and microbial interactions on the composition of the intestinal microbiota are productive areas of research, but 'accidents' also influence the microbiota of every individual. The thousands of microbial strains capable of inhabiting the gut are distributed unevenly between hosts and differ in transmissibility [12]; thus, the colonization history of a particular human can only be described in probabilistic terms, rather than being predicted in detail [29,56]. The numerical advantage of established over novel strains, and the retention of cells in the colon via attachment to mucus [10] or particles in the lumen [57], confer some degree of founder control on community composition, which can perpetuate accidents of colonization order. Stochastic niche theory shows that interactions amplify the role of chance during community assembly, because for a novel strain to persist, it must grow in the lumen or mucus fast enough to avoid washout on resources left unused by the existing community [28]. Recent results suggest that the complexity of the fecal microbiota increases gradually through childhood, rather than attaining adult levels soon after weaning [58], which is more consistent with stochastic niche theory than with fixed niches. The similarity of the intestinal microbiota at higher taxonomic levels across individuals is likely to reflect the smaller degree of niche overlap between more distantly related taxa, while the unique combination of species and strains comprising each genus in an individual could be a reflection of history (Box 3).

Selection via host fitness for stable and beneficial functioning of the microbiota [25] adds a level of complexity not considered in stochastic niche theory, but it does not imply selection for a single community structure. Complex communities of quite different composition can have similar functional characteristics, as observed in identical anaerobic bioreactors (simple colon analogs) inoculated from different sources [59].

Temporal dynamics

Although the fecal microbiota of healthy adults is often claimed to be stable in the absence of perturbation [2,12,17–21], this description warrants closer scrutiny. To the extent that diet influences the microbiota, community stability could reflect only dietary consistency when averaged over timescales that depend on the microbial populations and resources involved. A rare strain with a slight growth advantage on a new shared resource can increase only slowly in competition with abundant populations, whereas specialized populations could grow quickly following the introduction of a selective resource. Thus, prediction of diet-driven changes in microbial populations depends on knowledge of the existing community composition. Bacteriocins or phages could cause rapid community changes by decimating established strains; the outcome of such dynamics might be unpredictable in practice because evolutionary changes in multiple populations could be involved [52,54]. Community stability has typically been examined with several samples spread over weeks or months or, at most, a year [12,17–21]; whether this adequately represents the variability over shorter or longer timescales is not yet known.

The phylogenetic scale of community stability is also uncertain. Temporal studies have generally examined the abundance of microbial groups at the genus or family level, using culture methods [12], hybridization of 16S rRNA probes or primers [17,21], or profiling of 16S rRNA sequence variants [18–20]. Communities have been called stable because the temporal variability is smaller than interindividual differences; however, quantitative comparisons find that they are within an order of magnitude [17–21]. Studies with greater phylogenetic resolution, using either culture or molecular techniques, have found variation in the identity and abundance of taxa in some cases, and in stability in others. For example, in three individuals on a consistent diet, the already-abundant species *B. thetaiotaomicron* increased approximately tenfold then declined over several months, whereas other species changed little [60]. Strains of *Escherichia coli* [10,12] and species or strains of *Bifidobacteria* [27] and *Lactobacillus* [20,26] have been found to be stable for up to a year in some humans and highly variable in others.

Simultaneous deterministic and stochastic effects at different phylogenetic levels

Some factors affecting intestinal communities are intrinsically probabilistic, such as colonization history and evolutionary responses to bacteriocins and phages. Other factors are more deterministic, such as the effect of particular diet- or host-derived compounds on genera specialized to exploit them. Phenomena of both types can extend their influence through a web of microbial interactions. Although there is some merit in the traditional ecological paradigm that emphasizes deterministic factors, it remains unclear to what extent the composition of an individual's microbiota is a product of chance (i.e. is stochastic).

Remarkably, a recent study of inbred mouse families with genetic variation at only a single locus found both stochastic and deterministic effects operating simultaneously at different taxonomic levels [61]. Individual mice kept in microbiological isolation after weaning, with a uniform diet and environment and nearly identical genomes, had differences in the species comprising their intestinal microbiota. In second-generation mice, the similarity was greatest among littermates, intermediate among cousins, and least between separate families, indicating that stochastic differences in the microbiota that developed over time between genetically identical sisters were inherited by their offspring. However, a powerful deterministic effect of genotype was also demonstrated: homozygosity of the *obese* allele (associated with dramatically increased food consumption) caused the same broad shift in the relative abundance of the two dominant bacterial phyla in second generation mice of all litters. The increase of Firmicutes and decline of Bacteroidetes affected all species within each phylum approximately equally, suggesting that factors related to the host genotype interacted deterministically with common, ancestral traits of these phyla.

Conclusions and future directions

Recent culture-independent investigations have greatly expanded our knowledge of the human gastrointestinal

Box 4. Outstanding questions for human microbial ecology

- At which phylogenetic depths, and over what lengths of time, does the microbiota appear stable or variable? Does apparent stability represent the consistency of external conditions or a property of the community? The timescales of community change can suggest which factors influence community composition, and which approaches might be necessary to alter the community intentionally. The answers need not be the same for all taxa or all individuals.
- What is the effect of significant perturbations of the human microbiota, such as those resulting from treatment with broad-spectrum antibiotics or bowel cleansing before colonoscopy? If the community returns to its prior composition, is this due to the regrowth of remnant populations, or to selection by diet, genotype and other factors? Is the response similar after repeated perturbation? At least occasionally, perturbations lead to adverse long-term changes in the community. Preventing or reversing these events depends on understanding the community response to disturbance; perturbations might also provide an opportunity to introduce beneficial changes.
- What is the importance of diversity in the human intestinal microbiota? Do the hundreds of rare species contribute any functions beyond those found in the few dozen most abundant species? Management of the microbiota could be simplified if only a subset of species is important to intestinal health and function. Alternatively, if the insurance hypothesis for the benefit of biodiversity is relevant to the gut, what conditions lead to a shift in the identity of the organisms providing important functions?
- Is the apparently cooperative nature of some metabolic interactions among gut microbes maintained only by selection on individual populations? If some behaviors are altruistic, are there mechanisms more immediate than host-level selection to sanction cheaters and enforce cooperation? Learning how the human body and the commensal microbiota maintain a stable symbiosis might help us reduce the opportunity for pathogens to invade the system.

microbiota, emphasizing its diversity and uniqueness in every individual. Several external factors have predictable effects on the composition of colonic communities, but interactions within the microbiota determine the existence and extent of many microbial niches. The uneven distribution, overlapping resource-use profiles and strong interactions that are characteristic of human-associated microbes enable historical contingencies of colonization, in addition to deterministic phenomena, to influence these communities. Furthermore, the composition of gut communities varies over time, particularly at fine phylogenetic scales, for reasons that remain as yet unexplained but are likely to include both stochastic and deterministic factors. The traditional view of the human microbiota as a stable community composed of superior competitors for all available niches might prove to be an approximation more appropriate for higher taxonomic levels.

The approaches of community ecology, designed to reveal relationships between the structure of complex communities and multiple, heterogeneous environmental parameters, can be coupled with powerful molecular techniques to help clarify the scope and relative impact of deterministic and stochastic factors. For example, an ordination technique using a continuous measure of species dissimilarity to reveal relationships among communities [62] has been used to help interpret 16S rRNA clone libraries from the colon and stomach [15,63]. Stochastic community assembly models [28,56] could be compared with actual abundance distributions within phylogenetic or functional subsets of the human microbiota to explore the extent of stochastic influences. Distance–decay relationships of community similarity [64,65] could be used with interindividual 'distances' measured by diet similarity, genetic relatedness, or shared history to assess the impact of these factors. Fundamental ecological investigations in this arena are immediately relevant to human health (Box 4). As even more powerful molecular tools become available, such as 16S rRNA microarrays to survey community composition [66] and metagenomic sequence analysis to compare functional capabilities between communities [67] ecological approaches will be essential for transforming the resulting data into an improved understanding of the complex, intimate ecosystems of the human microbiota.

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