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Review

Altered gut ecosystems plus the microbiota's potential for rapid evolution: A recipe for inevitable change with unknown consequences

Celina You [a,b], Milan Jirků [c], David L. Corcoran [d], William Parker [a,b,*], Kateřina Jirků-Pomajbíková [c,e,*]

[a] Department of Surgery, Duke University School of Medicine, Durham, NC, USA
[b] Duke Global Health Institute, Duke University and Duke University School of Medicine, Durham, NC, USA
[c] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, 370 05 České Budějovice, Czech Republic
[d] Genomic Analysis and Bioinformatics Shared Resource, Duke Center for Genomic and Computational Biology, Duke University School of Medicine, Durham, NC, USA
[e] Faculty of Science, University of South Bohemia, 370 05 České Budějovice, Czech Republic

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ABSTRACT

In a single human gut, which is estimated to produce 1000-times more bacteria in a single day than the entire human population on Earth as of 2020, the potential for evolution is vast. In addition to the sheer volume of reproductive events, prokaryotes can transfer most genes horizontally, greatly accelerating their potential to evolve. In the face of this evolutionary potential, Westernization has led to profound changes in the ecosystem of the gut, including increased chronic inflammation in many individuals and dramatically reduced fiber consumption and decreased seasonal variation in the diet of most individuals. Experimental work using a variety of model systems has shown that bacteria will evolve within days to weeks when faced with substantial environmental changes. However, studies evaluating the effects of inflammation of the gut on the microbiota are still in their infancy and generally confounded by the effects of the microbiota on the immune system. At the same time, experimental data indicate that complete loss of fiber from the diet constitutes an extinction-level event for the gut microbiota. However, these studies evaluating diet may not apply to Westernized humans who typically have reduced but not absent levels of fiber in their diet. Thus, while it is expected that the microbiota will evolve rapidly in the face of Westernization, experimental studies that address the magnitude of that evolution are generally lacking, and it remains unknown to what extent this evolutionary process affects disease and the ability to treat the disease state.

Contents

* Corresponding authors at: WPLab, Inc., 1023 Wells St. Durham, NC, USA (W. Parker). Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic (K. Jirků-Pomajbíková).
E-mail addresses: William.Parker@WilliamParkerLab.org (W. Parker), pomajbikova@paru.cas.cz (K. Jirků-Pomajbíková).

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1. Introduction: Evolution of the gut microbiota and health

The gut microbiota in a single human is estimated to contain a host of fungi, viruses, and approximately 3.8×10^{13} bacterial cells [1], about 1000 times the human population on Earth in 2020. Since the discovery of microbes by Robert Hooke and Antoni van Leeuwenhoek less than 400 years ago, understanding of the microbiota has evolved rapidly. However, work today examining the human microbiota evolved only within the last century from the field of infectious disease, and that history profoundly influences our perspective in sometimes not-so-subtle ways. For example, it was only within the past 20 years that scientists realized that the human immune system strongly supports the growth of the microbiota [2,3]. The prior perspective, that of an ongoing battle of containment against a necessary but potentially deadly microbiota, was a universally accepted paradigm consistent with observations made by scientists studying infectious disease. This long-accepted paradigm broke down, however, when considering the biology of symbiotic systems from an ecological and evolutionary perspective [4]. Another view of the microbiota that has strong support from the field of infectious disease is the concept that the nature of a microorganism is a parameter that can be defined and is, for practical intents and purposes, constant. The observation that probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium lactis* are beneficial while pathogens such as *Salmonella enterica* and *Clostridium tetani* are detrimental certainly fits this paradigm. However, as infectious disease decreases due to vaccination and sanitation, chronic inflammatory diseases tend to emerge [5]. These diseases include a range of allergic, autoimmune, digestive, and neurological conditions, with the role of the microbiota garnering significant scientific interest across that range. Importantly, the nature of the interaction between the microbiota and host in the face of chronic disease is fundamentally different than interactions that occur during an infectious disease process. In the face of chronic disease processes, alteration of interactions between host and microbe by the disease process itself can profoundly affect the microbiota, and evolutionary changes in the host-associated microbiota during the pathogenesis of disease can potentially play a major role in patient outcomes as well as the potential to treat disease. In this review, we will consider evolutionary processes in the gut microbiota and the implications of rapid, evolutionary changes for the field of medicine.

In this assessment of the gut microbiota, we will consider classic evolutionary processes involving the appearance of new traits through genetic mutation followed by selection of beneficial mutations. Others have considered changes in microbial community composition within the context of evolution [6,7]. Changes in community composition are primarily driven by diet [8,9], and it is expected that profound changes in diet will not only drive rapid changes in community composition, but may also drive evolution of species within the community. Thus, here we consider dramatic changes in community composition as a potential indicator that evolution of species within the community may be occurring, but do not consider the actual changes in community composition as an evolutionary process, per se.

2. Vast evolutionary potential in the human gut.

The sheer number of bacterial reproductive events in the human gut, previously estimated to be approximately 1.1×10^{13} per person per day [10], provides a potential for Darwinian evolution via vertical gene transfer from parent to offspring that far exceeds the evolutionary potential of their hosts. Given the number of bacteria in the human gut, estimated at 3.8×10^{13} per person at any given time [1], this rate of reproduction corresponds to one bacterial division about every 3.5 days, and suggests that about 29% of the microbiota is lost each day via death within the gut or expulsion from the gut. Although combining independently derived estimates such as these will propagate errors in ways that are impossible to predict accurately, such efforts are nonetheless worthwhile in that they serve as initial best guesses regarding important biological phenomena.

In addition to sheer numbers and high turnover/reproduction rates, bacteria hold another advantage over their hosts in the race to evolve: Bacteria are capable of horizontal gene transfer via transformation, conjugation, and transduction [11,12], three process which entail the acquisition of genetic material without the need for reproduction. These three processes differ in the source of the genetic material: Transformation involves cells incorporating DNA from their surrounding environment, whereas conjugation is the process by which genetic material is transferred from a donor bacterium to a recipient through cell-to-cell contact. Transduction, on the other hand, involves transfer of genetic material via a viral vector.

It is estimated that horizontal gene transfers that have affected roughly 4 out of every 5 of the genes in a typical bacteria at some point in the history of the gene [13]. Further, Hyeonsoo Jeong and colleagues estimate that more than half of all genes in the microbiota of humans were acquired by horizontal gene transfer [14]. Given the close association of a great diversity of species in the gut, the ability of bacteria to shuffle genes by recombination is likely to be higher in the gut than in many other environments, including laboratory environments [3,15]. In this manner, bacteria in the human body can rapidly acquire genes that impart new function without having to undergo the gene sweeps necessary for population-wide genetic changes in their hosts. An example of such evolution is the apparently extensive transfer of genes involved in seaweed digestion from marine bacteria to the human microbiota [16].

3. Changes in the ecosystem accelerate evolutionary processes.

Numerous experimental designs have been utilized to probe the speed at which bacteria can evolve. As shown in Fig. 1, gain of function via Darwinian evolution can occur within days, and dramatic effects can often be seen within months. For example, Richard Lenski's lab saw the evolution of metabolism of glucose by *Escherichia coli* in minimal media within 44 days, and the evolution of the ability to metabolize citrate, a major step forward in evolution, within 12 to 13 years [17,18]. Our lab saw the evolution of glucose metabolism by laboratory *E. coli* in the mouse gut within 114 days, and dramatically improved colonization of the mouse gut under those conditions after 2 to 3 years [19]. Rainey and Travisano saw the evolution of biofilm formation of *Pseudomonas fluorescens* in static culture (structured environments) within only 2 days [20], and Sommer's group found the evolution of resistance to antibiotics by *E. coli* within only 14 days [21]. Bell's group saw the evolution of resistance to antimicrobial peptides by *E. coli* and *P. fluorescens* within 100 days [22], and Rosch's group saw improved colonization of nasal cavities in mice by *Streptococcus pneumoniae* within 30 days [23]. These examples (Fig. 1) represent only a very small fraction

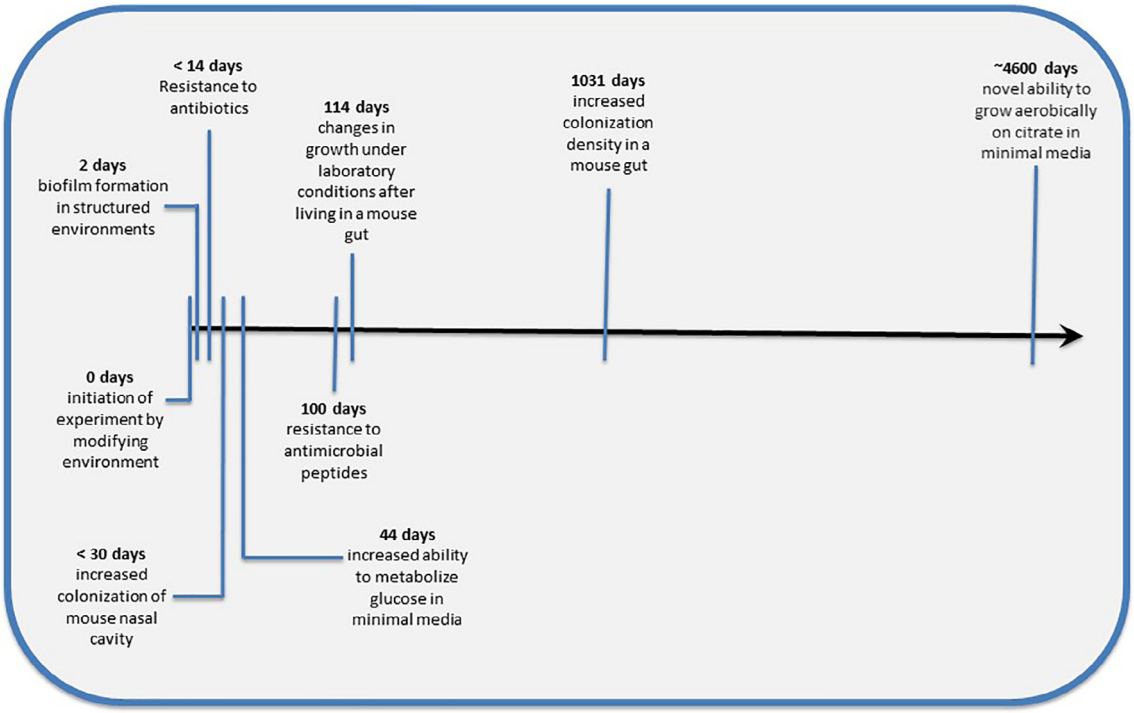

Fig. 1. Speed of adaptation of microbial communities in experimental evolution. This illustrative diagram shows representative times to gain of function via Darwinian evolution (mutation and selection) achieved by experimental evolution. The duration of each experiment is shown in days rather than in replication events in order to facilitate comparison and because the actual number of replication events is difficult to access, depending on the time elapsed, the size of the reaction vessel, and the replication rate. The axis is not labeled in a linear fashion, and is compressed at longer times. Citations for the experiments performed are provided in the text.

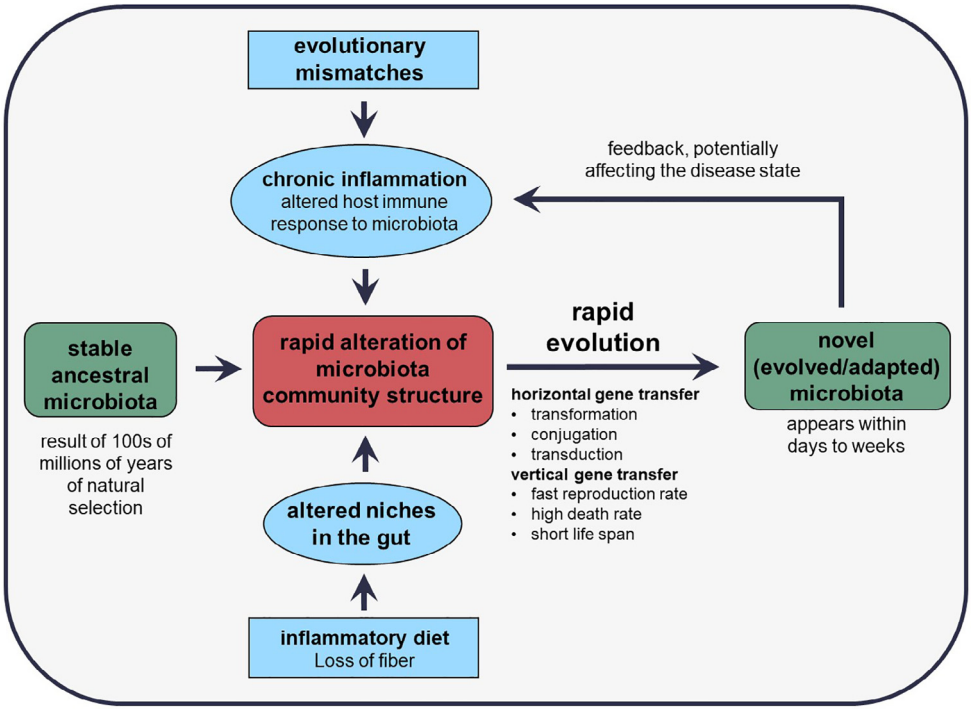

Fig. 2. Schematic diagram showing evolutionary process affecting the microbiota following changes to the ecological niches of the gut. In this model, evolutionary changes feed-back on inflammatory processes, creating a self-sustaining, disease propagating cycle.

of the experiments that have been conducted, but nonetheless demonstrate the ability of microbes to rapidly evolve novel function.

The key to success in the gain of function experiments described above is simply to impose a significant change in the environment [24]. Environmental changes such as those imposed by the impact of the Chicxulub asteroid and several cases of extensive volcanic activity have famously affected vertebrate life on Earth. Such environmental changes result in extinction followed by "adaptive radiation", an array of relatively rapid evolutionary changes in multiple species. In the same manner, rapid environmental changes within the human gut are expected to induce profound changes in the microbial life that resides there, including an acceleration of evolutionary processes. With this in mind, we will consider two of the primary changes imposed on the ecosystem of the human gut by modern lifestyle: increases in chronic inflammation and dietary alteration.

In general, rapid changes to the environment as a result of a modern lifestyle can result in "evolutionary mismatches", or changes in the environment which create disease. In this context, disease is caused by a disconnect or mismatch between the genetics of the organism and the environment. This "fish-out-of-water" model of disease entails a rapid shift in the environment which results in an organism living under conditions in which it is prone to sickness. Some evolutionary mismatches, for example a highly processed diet, might directly drive evolution of the microbiota. In general, however, most modern evolutionary mismatches drive chronic inflammatory processes that, in turn, may drive evolution of the gut microbiota via altered interactions of the immune system with its symbiotic microbiota. An evolving gut microbiota, in turn, may exert a profound influence on human health, creating a complex and as yet poorly understood feedback system that promotes further chronic inflammation [10,25]. A schematic diagram of this complex system is shown in Fig. 2.

4. Altered immune function as a potential driver for evolutionary changes in the microbiota.

Just as rapid changes to an ecosystem lead to evolutionary changes in many of the species inhabiting that ecosystem, rapid changes to the ecosystem of the gut are expected to lead to evolutionary changes to the microbes as they adapt to the new environment [10]. Given the importance of the immune system in maintaining the gut microbiota, the increasing prevalence of a wide range of chronic inflammatory disease [5] raises the specter that chronic inflammation itself may be a major driving force for evolution of the gut microbiota [25]. The effects of alteration of the immune system on the gut microbiota are evident. For example, in individuals who are deficient in IgA production, the microbiota has decreased expression of the IgA receptor [26]. As another example, the microbiota of immunodeficient mice show numerous alterations compared to that in immunosufficient mice [27]. However, it remains unknown whether these alterations to the microbiota are due in any part to evolutionary process leading to gain or loss of function, or whether other factors such as changes in community composition and transient changes in gene expression patterns can fully account for the results. Further, the extent to which the microbiota can adapted to the chronically inflamed state remains almost completely unknown. For example, despite the important role of host-derived mucus in maintaining the gut environment, microbial adaptations to inflammation-associated changes in the gut mucus layer have not been explored.

It might be hoped that studies of the microbiota in patients with inflammatory bowel disease would shed light on the evolution of the microbiota in response to chronic inflammation. However, the system has proven sufficiently complex to elude a clear understanding at the present time. For example, numerous studies have found altered microbiota in patients with inflammatory bowel disease (IBD), but whether such alterations are cause or effect of disease remains unknown. The beneficial effects of "fecal therapy", the repeated infusion of fecal material from healthy donors, in some patients with IBD [28–30] might suggest that the resident microbiota in at least some patients can cause disease, and thus the presence of these bacteria is not a response to the chronic inflammation that characterizes IBD. However, IBD is not infectious, many patients cannot be treated with fecal therapy, and no core microbiota has been identified for IBD, making it difficult to pinpoint how the microbiota could be a causal factor in pathogenesis [31,32]. In addition, in studies of patients with multiple sclerosis, another chronic inflammatory disease, a core microbiota has proven difficult to identify [33]. Further, although many alterations in the microbiota have been associated with a variety of inflammatory conditions, including obesity, it remains unknown whether Darwinian evolutionary process in the patient's gut contributed to any of those alterations.

At the present time, the idea that the presence of chronic inflammation causes evolutionary changes in the gut microbiota remains plausible and even expected, but lacks experimental proof and remains unexplored. Given the inherent complexity in the microbiota and its relationship to disease, it seems likely that experiments using laboratory animals under highly controlled conditions, including association with a defined microbiota, might be the most appropriate study model to shed light on these issues in the future.

5. Evolutionary changes in the microbiota potentially triggered by consumption of processed foods.

Changes in diet as a consequence of modern society are profound, and constitute a major shift in the ecosystem of the gut. Diet is the single most influential factor in determining the microbial community composition of the gut [34]. For example, Gordon and colleagues found that diet accounted for differences between the microbiota of North Americans and pre-industrial agrarian groups in South America and in Africa [8,35]. As another example, work by Chenhong Zhang and colleagues in laboratory mice fed a high-fat diet or normal chow for 25 weeks showed that 57% of genetic diversity in the gut microbiota could be attributed to diet [36].

Loss of fiber from the diet due to food processing is of substantial concern, with studies in animal models demonstrating the magnitude of the effect of the microbiota. For example, studies in laboratory mice showed that total elimination of fiber from the diet results in disruption of more than 85% of the gut microbial community structure [10]. Importantly, Alverdy's group showed that the remaining community structure of the microbiota in mice fed the fiber-free diet failed to recover after treatment with antibiotics, whereas the community structure of mice fed a normal diet recovered within one week [37]. In a more detailed analysis of the effects of fiber on the microbiota,

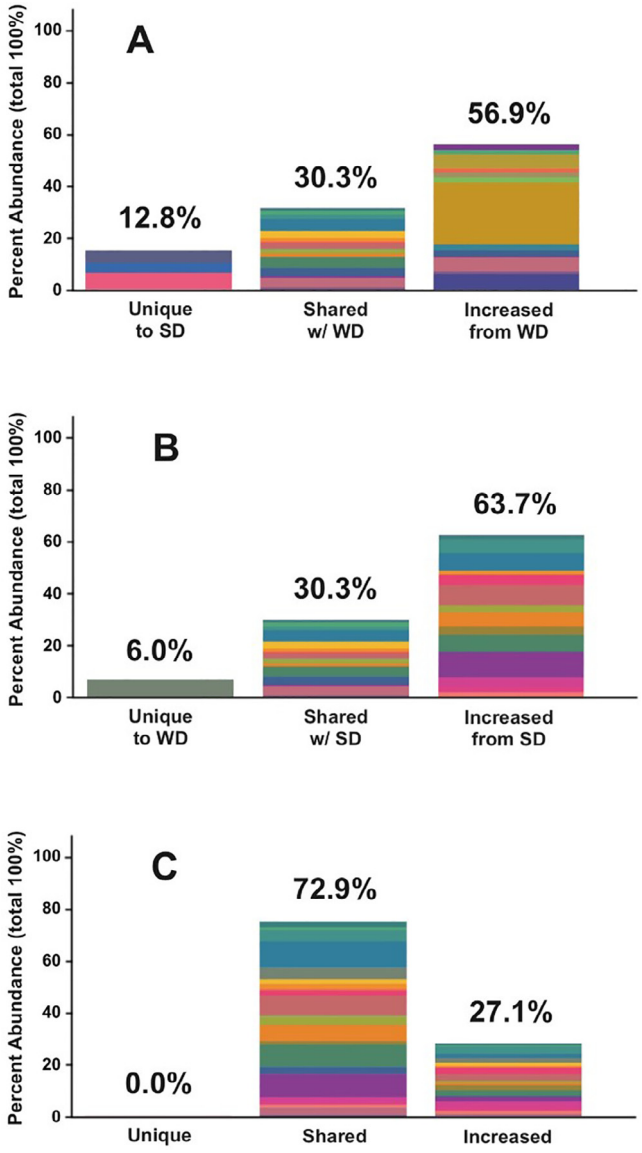

Fig. 3. Comparison of microbial communities between rats fed a standard diet with fiber for 30 days (SD, n = 16) and rats fed a Western diet without fiber (WD, n = 12) for 30 days. Panel A illustrates the microbiota in animals with a standard diet, assessing what that microbiota has in common with the microbiota of animals with a Western diet. Panel B illustrates the microbiota in animals with a Western diet, assessing what that microbiota has in common with the microbiota of animals with a standard diet Each color represents a specific amplicon sequence variant (ASV). In this semi-qualitative assessment of microbial community composition generated for illustrative purposes, ASVs in a given microbial community fall into three categories which add up to a total of 100%: ASVs that are unique to a given group, ASVs that are shared with the other group, and ASVs that are increased compared to the other group. For comparison, rats receiving a standard diet were divided arbitrarily into two groups, and the microbiota of one group receiving a standard diet was compared with the microbiota of another group receiving a standard diet (Panel **C**) This "control" illustrates how this method microbial microbial variation in the absence of dietary differences between groups..

we found that about 70% of the gut microbial community structure was disrupted by the elimination of fiber from the diet of laboratory rats (Fig. 3A and B). In a control study, using the same methods as those used in Figure 3A and 3B, a comparison of two groups that have the same diet showed that more than 70% of their microbiota is shared (Fig. 3C). Consistent with previous studies in laboratory animals [35], alpha diversity, a measure of the complexity of the microbial community within a given individual, was less on average in animals fed a zero fiber diet than in animals fed a normal diet (Fig. 4). Differences in alpha diversity as a function of fiber in the diet were consistent regardless of the diversity index used, although differences were only statistically significant ($p < 0.05$) for the number of amplicon sequence variants (ASVs; $p = 0.01$) and for the Faith's phylogenetic diversity index [38] ($p = 0.015$). In addition, the Bray-Curtis measure of Beta diversity, or differences between the microbial communities of different hosts, was dramatically less ($p < 0.001$) when fiber was absent from the diet (Fig. 5, left panel), indicating that the number of predominant microbial species in animals fed a fiber-free diets is less variable than in fiber-fed controls. These observations suggest that the number of predominant species drops substantially when fiber is eliminated from the diet. However, the Jaccard measure of Beta diversity (Fig. 5, right panel), which is more heavily influenced by low abundant species, was somewhat less ($p < 0.001$) in the group of animals with fiber in their diet compared to their fiber-free counterparts. Thus, loss of fiber in the diet was associated with a loss beta diversity among prominent gut microbial species and, at the same time, an increase in beta diversity among less abundant species.

An elegant study by Sonnenburg's group using a mouse model [39] demonstrated what can be described as a mass extinction event in the microbiome by eliminating fiber from the diet of the mice. The microbiota partially recovered when returned to a normal diet after 7 weeks on a fiber free diet, but mice fed a low fiber diet failed to transfer their microbiota to subsequent generations; permanent alterations to the microbiota accumulated for three generations and was maintained in the fourth generation of animals fed a low fiber diet. Although this study required multiple generations to induce substantial loss to the microbiota, it should be considered that, in humans, common use of broad-spectrum antibiotics may provide a "restart" to the microbiota in a manner that resembles giving birth in the rodent model. The study by John Alverdy's lab, described above, demonstrating profound, long-term antibiotic-induced destabilization of the microbiota in animals with a low fiber but not a high fiber diet [37] is consistent with this view.

Another dietary change associated with modern lifestyles that impacts the microbiota is a lack of seasonal variation in food intake. Prior to the advent of agriculture some 10,000 years ago, diets were seasonal, and current work demonstrates that such seasonal variation in diet causes seasonal variation in the microbiota. For example, Sonnenburg and colleagues evaluated Hadza hunter-gatherers from Tanzania and found that seasonal variation in diet is associated with seasonal variation in the gut microbial community composition [40]. Sharma and colleagues found similar seasonal variations in the fecal microbiota of BaAka hunter-gatherers from the Central African Republic [41]. Further, Sharma showed that the microbiota of gorillas, similar to that of human hunter-gatherers such as the Hadza and BaAka, also experiences seasonal variation with diet [41]. Although some seasonal variation in the diet of agricultural communities does exist and is apparently reflected in some seasonal variation in the microbiota [42], seasonal variation in these populations is reduced compared to hunter gatherers, and what seasonal variation does exist is expected to be lost in individuals

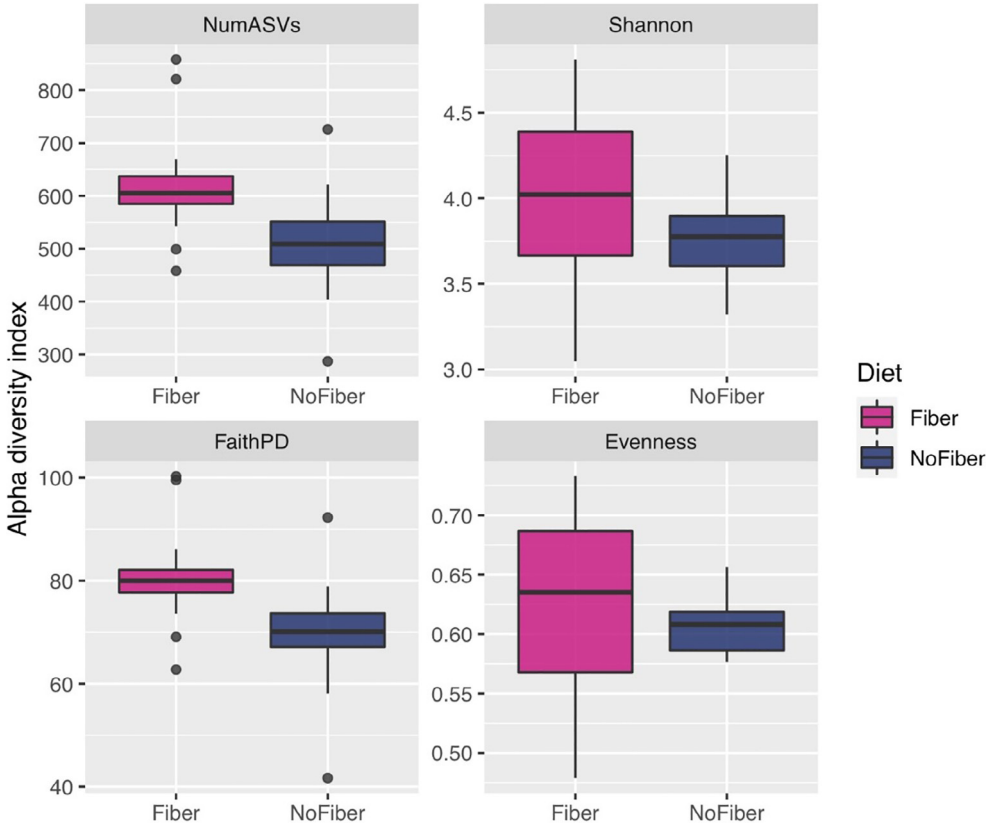

Fig. 4. Alpha diversity as a function of high and zero fiber diets in laboratory rats. In general, consistent with previous reports, Alpha diversity was reduced in animals fed a zero fiber diet (n = 12) compared to controls fed a normal diet (n = 16). The experiment was conducted as described in the caption for Fig. 3. Alpha diversity was calculated for number of observed ASVs (NumASVs), Shannon's index [58] (vegan [59] R package), Faith's phylogenetic diversity [38] (calculated with the picante [60] R package), and Pielou's evenness [61] (microbiome [62] R package). T-tests were conducted to test for difference in alpha diversity between fiber & no-fiber diet samples in the R statistical programming environment [63] (v4.0.0).

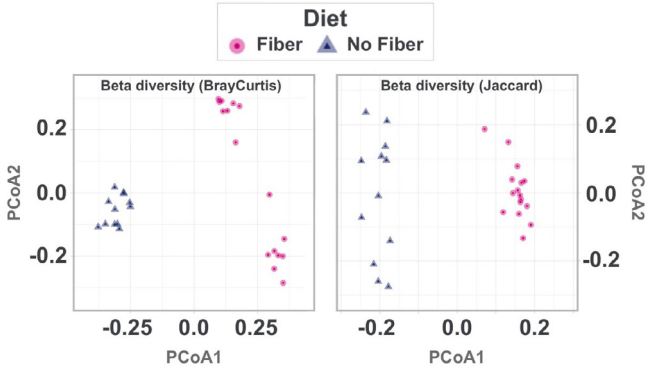

Fig. 5. Beta diversity as a function of high and zero fiber diets. The experiment was conducted as described in the caption for Fig. 3. Beta diversity was calculated for each pair of samples. The weighted (Bray-Curtis) Beta diversity was dramatically reduced in animals fed a zero-fiber diet, indicating that the number of predominant bacterial species was very limited in those animals compared to controls on a Western diet. In contrast, the unweighted (Jaccard) plot showed equal or greater Beta diversity in animals without fiber in their diet compared to controls, indicating that a wide range of species were present in low abundance in animals eating a fiber free diet. Beta diversity was calculated using the Phyloseq [64] package in R for Bray-Curtis [65] and Jaccard [66] on rarefied data. For the UniFrac and Weighted UniFrac metrics, a maximum likelihood tree was generated in the Qiime2 pipeline utilizing the RAxML [57] program. Principal coordinate analysis ordination (PCoA) was performed on all of the beta diversity matrices to provide a visualization of clustering of the data. A permutational multivariate analysis of variance (permanova) was performed to test for differences in beta diversity between fiber & no-fiber diets using the vegan package in R. In both cases, the p-value was less than 0.001.

who enjoy year-round availability of fresh produce from a global market [42].

The fact that the diet and the microbiota show seasonal variation in the ancestral (hunter-gatherer) state has implications for the evolution of the human microbiota. In particular, the ability of the microbiota to vary on a seasonal basis would suggest that components of the microbiota are adapted to "wait out" periods of months when their preferred food source is unavailable. Unfortunately, it is expected that this predicted feature of the human microbiota will be difficult to find in laboratory animal models. Given the rapid evolution of bacteria in the rodent gut as a response to changing environments [19,43], it is expected that any ability to maintain seasonal variation in community structure will have been lost after hundreds of generations on an unvarying laboratory diet.

6. Conclusions

Based on available evidence, we conclude that rapid evolution of the human gut microbiota happens in response to changes in the gut environment. Environmental changes expected to catalyze rapid evolutionary changes include the introduction of chronic inflammation and an altered Western diet. These evolutionary changes within the microbiota begin to occur within days, and, based on experimental evidence, continue to occur over a period of weeks to months. Such rapid changes occur in the presence of host evolution, which occurs over the span of hundreds of thousands of years and also affects evolution of the microbial community, as discussed by Knight and colleagues

Table 1
Diets used in this study.

Western Diet Dietary Composition	ST1 Diet Dietary Composition	ST1 Diet Quality Characteristics
Casein (19.5%)	Wheat (40%)	Humidity (12.5%)
Corn starch (40%)	Soybean meal (22%)	Nitrogenous substances (24%)
Maltodextrin (14.59%)	Fish meal (10%)	Fiber (4.4%)
Sucrose (10%)	Maize (6%)	Fat (3.4%)
Cellulose powder (5%)*	Wheat bran (5%)	Ash (6.8%)
DL-Methionine (0.1%)	Oat rice (3%)	Lysine (14 g)
L-Cystine (0.2%)	Lucerne meal (2.5%)	Methionine (4.8 g)
Vitamin premix (1%)	Feed yeast (2.5%)	Calcium (11 g)
Mineral & trace element mix (4.3%)	Dried milk (1.5%)	Phosphorus (7.2 g)
Ascorbic acid (0.1%)	Feed sugar	Sodium (1.8 g)
Choline chloride (0.2%)	Calcium carbonate	Vitamin A (28000 m.j.)
Butylated hydroxytoluene (0.01%)	Calcium dihydrogen phosphate	Vitamin D (2200 m.j.)
Soybean oil (5%)	Sodium chloride	Vitamin E (alpha-tocopherol) (100 mg)
		Copper (20 mg)
		Selenium (0.38 mg)

[44]. Given the vast disparities in the time frame of host evolution and microbial evolution, it is practical to consider these two processes independently from a human health perspective. Consistent with this view, the molecular processes associated with short-term evolution of the gut microbiota probably differ from molecular processes associated with long-term evolution of the microbiota. On the one hand, evolution of the gut microbiota over the short term probably happens through series of "soft sweeps", which involve the emergence of a variety of organisms rather than the dominance of a single organism [19,43]. In contract, evolution of the gut microbiota that accompanies host speciation probably involves repeated hard sweeps involving offspring outcompeting ancestral variants. In this manner, presumably neutral or non-adaptive changes in 16S rRNA are carried along during hard sweeps and thus can be used as markers for evolution driven by host adaptations to habitat over the course of tens of millions of years [45]. Thus, microbial evolution in the short-term probably needs to be considered for host health, whereas host evolution and its accompanying long-term microbial evolution are key factors that are likely responsible for differences in the microbiota across species [44].

As pointed out by Gordo, evolution of the microbiota by natural selection has been "vastly neglected" [15]. Although chronic inflammation profoundly affects the host/gut interface and the gut microbiota, the amount of microbial evolution that happens in response to that inflammation remains almost completely uncharacterized. Similarly, the Western diet constitutes a major change to the ecosystem of the gut compared to the ancestral state, but the effects of that change on microbial evolution have yet to be characterized in detail. Numerous studies have focused on changes in microbial community composition and metabolism associated with such conditions as obesity [46–48] and autoimmune disease [33], but the role of biological evolution in these processes, if any, is unknown. Further, studies in animal models testing the effects of changing diet on the microbiota are informative, but have limitations. For example, laboratory animal models are probably not appropriate for testing the effects of loss of seasonal variations in diet on the microbiota. Further, although a total loss of dietary fiber constitutes an extinction level event in the gut of laboratory animals, some fiber remains in the diet of almost all Western individuals, albeit at reduced levels. Thus, the complete loss of fiber is probably not a driving force for evolution of the microbiota in Western humans.

7. Future outlook

Given the reproducibility with which microbes adapt to changing environments, and given the changes to the human gut imposed by Western culture, microbial evolution imposes a potential source of diversity in the human gut microbiota that should not be ignored. This view has a very significant bearing on considerations for clinical practice. For example, the rapid responsiveness of the microbiota to their environment would suggest that modulation of the microbiota using microbiota-based therapeutics may be difficult to achieve without addressing the changes to the environment (evolutionary mismatches) that drove the evolution of unhealthy or dysbiotic microbial communities in the first place. Further, Carmody and colleagues argue effectively that, even if it were possible, it may not be a good idea to establish a "normal" or ancestral microbiota in an environment that has been modified by modern culture and may be incompatible with that ancestral microbiota [49]. With these ideas in mind, work is urgently needed with the aims of evaluating evolution of the microbiota *in vivo* and the effects of that evolution on health and disease. To the extent that such evolution is detrimental to human health, the only recourse for public health may be to identify and address the underlying factors driving that microbial evolution.

8. Methods

The Western diet was purchased from ssniff Spezialdiäten GmbH, Germany (product # E15720-04), and "feed mixture ST-1" (ST1) was purchased from Velaz, Lysolajské údolí 15/53, Praha 6 – Lysolaje. Although the Western diet is described as a "no fiber" diet, it does contain 5% cellulose as a binding agent in the food pellets. However, this fiber is not a "microbiota-accessible carbohydrate (MAC) [39], and is therefore not considered as fiber in the present study for practical intents and purposes.

The experiment was carried out with outbred female Wistar rats obtained when 13 weeks old and 180–220 g from Envigo

RMS B.V. (Horst, Netherlands; the supplier Anlab s.r.o., Prague, Czechia). All rats were housed under a controlled condition (22C, 12:12-hrs light–dark cycle), provided unlimited access to rat chow (Western and standard diet) and water. Diets were as described in Table 1. The health status of all animals was visually inspected at regular 24-h intervals, and animals were acclimated to the facility for seven days prior to initiation of the dietary changes.

Rats were held in individually ventilated isolator cages with HEPA filters for filtration of incoming air (Individually Ventilated Cages machine SealSafe 1291H, Techniplast s.p.a., Buguggiate, Italy; the supplier: Trigon Plus a.s., Čestlice, Czechia).

For purposes of microbial analyses, rats were randomly assigned to experimental treatment cages in pairs, taking care to change cage mates compared to the initial week-long acclimatization period to minimize microbial similarity within a cage.

The study was carried out in the strict accordance with the recommendations in the Czech legislation (Act No. 166/1999 Coll., on veterinary care and on change of some related laws, and Act No. 246/1992 Coll., on the protection of animals against cruelty) as well as the legislation of European Union. The experiments and protocols were approved by the Committee on the Ethics of Animals Experiments of the Biology Centre of the Czech Academy of Sciences (České Budějovice, Czechia, permit no. 33/2018) and by the Resort Committee of the Czech Academy of Sciences (Prague, Czechia).

Methods used in the analysis were as follows: Fastp [50] (v0.20.1) was used to verify that reads were adaptor-free. The fastx_quality_stats tool from Fastx-toolkit [51] (v0.0.14) was used to determine median base quality for each position of the reads for each region. Reads were imported into qiime2 [52] (v2020.2), and denoised and dereplicated with dada2 [53] (via q2-dada2). In dada2, reads were trimmed at the beginning or truncated at the end if the median base quality fell below a score of 30 as determined by Fastx-toolkit. Taxonomy was assigned to ASVs using the q2-feature-classifier classify-sklearn naïve Bayes taxonomy classifier [54] against the SILVA 132 database [55]. ASVs identified as uncharacterized, mitochondrial, chloroplast, or Eukaryota were filtered from the dataset, and samples with fewer than 1000 reads were also excluded. All remaining amplicon sequence variants (ASVs) are aligned with Mafft [56] (via q2-alignment, v.7.310) and used to construct a phylogeny with Raxml version 8 [57] (via q2-phylogeny). ASVs were filtered to exclude any ASVs that were not observed in at least two samples overall and did not have a relative abundance of at least 1% across either all fiber diet or all non-fiber diet samples; 31 ASVs remained after filtering.

Author statement

Authors WP and KJ-P designed the study, analyzed the data, and helped write the manuscript. Authors CY and DLC analyzed the data and helped write the manuscript. Authors MJ performed experiments and helped write the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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