Comparative metagenomics reveals host-specific functional adaptation of intestinal microbiota across hominids

Authors

Affiliations
1 Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany
2 Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School
3 Hannover, Germany
4 Applied Zoology and Nature Conservation, University of Greifswald, Greifswald, Germany
5 Helmholtz Institute for One Health, Greifswald, Germany
6 Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institute, Berlin, Germany
7 Viral Evolution, Robert Koch Institute, Berlin, Germany
8 Evolutionary Genomics, Max Planck Institute for Evolutionary Biology, Plön, Germany
9 Institute of Experimental Medicine, Kiel University, Kiel, Germany
10 Nutriinformatics Research Group, Institute for Human Nutrition and Food Science, Kiel University, Kiel, Germany
11 Training and Research Unit for in Medical Sciences, Alassane Ouattara University / University Teaching Hospital of Bouaké, Bouaké, Côte d’Ivoire
12 Comparative BioCognition, Institute of Cognitive Science, University of Osnabrück, Osnabrück, Germany
13 National Institute for Biomedical Research, National Laboratory of Public Health, Kinshasa, Democratic Republic of the Congo
14 Department of Primate Behavior and Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
15 Department of Human Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America
16 Institute of Cognitive Sciences, CNRS UMR5229 University Lyon 1, Bron Cedex, France
17 Tai Chimpanzee Project, CSRS, Abidjan, Côte d’Ivoire
18 Institute of Biology, University of Neuchatel, Neuchatel, Switzerland
Summary
Characterizing trajectories of the composition and function of hominid gut microbiota across diverse environments and host species can help reveal specific properties of the human microbiota, with possible implications for host evolution and health. Using shotgun metagenomic sequencing, we investigated taxonomic and functional diversity in the gut microbiota of wild-living great apes, including two gorilla subspecies (Gorilla gorilla gorilla, Gorilla beringei beringei), three chimpanzee subspecies (Pan troglodytes verus, P.t. troglodytes, P.t. schweinfurthii), and bonobos (Pan paniscus), together with human samples from Africa and Europe. We identified microbial taxonomic and functional adaptations convergent with host phylogeny at both the community and microbial genomic levels. We could show that repeated horizontal gene transfer and gene loss are processes involved in these adaptations. We hypothesize, that these adaptation processes and changes in the microbiome predispose the host to chronic inflammatory disorders, such as type 2 diabetes via altered histidine metabolism and inflammatory bowel disease indicated by adaptation of microbes to aerobic conditions. Additionally, we find multiple lines of evidence suggesting a widespread loss of microbial diversity and evolutionary conserved clades in the human microbiota, especially in the European population. Lastly, we observed patterns consistent with codivergence of hosts and microbes, particularly for the bacterial family Dialisteraceae, though we find that overall, co-phylogeny patterns are frequently disrupted in humans.

Introduction
Human gut microbiome research has demonstrated that numerous factors, including diet, environment, and lifestyle influence the structure of the human gut microbiota, which, in turn, has profound impacts on human health and disease (Johnson et al., 2019; Lloyd-Price et al., 2019; Vangay et al., 2018). To date, the majority of these studies have been conducted on sample collections from high-income countries and only recently, has interest shifted to include humans from diverse global populations, thereby providing an additional angle to investigate and evaluate shared and specific microbiome properties across human populations (McCall et al., 2020; Schaan et al., 2021; E. D. Sonnenburg and Sonnenburg, 2019; Vangay et al., 2018). These recent efforts provided an opportunity to discover microbiome signatures of host geography and lifestyle that go beyond differences in
microbiota diversity structures. For instance, they revealed elevated rates of horizontal gene
transfer (HGT) that correlate with the Human Development Index (HDI) of a population,
suggesting that gut microbiota constantly acquire new functions in conjunction with host
lifestyle changes (Groussin et al., 2021). However, the mechanisms that link changes in gut
microbial structure with host behavior and ecology remain largely unexplored.

Additional critical insight into understanding patterns of diversity and composition among
human gut communities can be obtained from comparative surveys of the hominid gut
microbiota. Humans, chimpanzees, bonobos, and gorillas show increasingly divergent gut
microbiota, with more distantly related species exhibiting more divergent community
composition (phylosymbiosis; (Brooks et al., 2016)). At the same time, the phylogeny of
some of their individual bacterial lineages parallels their own phylogeny (codivergence;
(Groussin et al., 2020; Suzuki et al., 2022)). Both patterns of phylosymbiosis and
codivergence are suggestive of long-term effects of hominid evolution on their communities
of symbionts (Groussin et al., 2017; Moeller et al., 2016; Ochman et al., 2010). Notably,
results from comparative marker-gene analyses suggest co-diversifying members of the
hominid gut microbial communities (both prokaryotic and phage) are lost and replaced with
human lineages, when animals leave their natural environments and are moved into
captivity (Gogarten et al., 2021; Nishida and Ochman, 2021). However, poor taxonomic
resolution and a lack of functional characterization precludes a deeper understanding of
processes driving these changes.

Functional analyses from shotgun metagenomic data revealed a conserved phylogenetic
signal across wild nonhuman primates (NHP), despite dietary changes over an individual’s
lifespan and between species, suggesting that the evolution of gut microbiota within wild
NHP is partially constrained by host genetics and physiology (Amato et al., 2019). A
comparative meta-analysis of microbial functions in NHP and diverse human populations
observed a comparable loss of biodiversity in captive NHP and human populations from
regions with higher HDI (Manara et al., 2019), supporting previous findings (Moeller et al.,
2014). However, overall, the functional characterization of NHP microbiota in previous
studies has been limited to only selected microbial taxa without addressing broader scale
functional changes and specific alterations especially in African great apes and humans. As
such, robust comparative functional analyses are still needed for a comprehensive understanding of how gut microbiota have evolved with hominids and shaped the current structure and functional capabilities (and deficits) of the human gut microbiome.

Here, we present a large-scale comparative study of wild NHP and humans from geographically distinct populations spanning two continents to better elucidate host-microbiome interactions in the hominid gut, within an evolutionary context. To functionally characterize hominid gut microbial communities, we performed shotgun metagenomic sequencing from the feces of wild-living great apes, including two gorilla subspecies (Gorilla gorilla gorilla, Gabon (GAB), n=8; Gorilla beringei beringei, Uganda (UGA), n=11), three chimpanzee subspecies (Pan troglodytes verus, Côte d’Ivoire (CIV), n=55; P. troglodytes, GAB, n=11; P. schweinfurthii, UGA, n=12), and bonobos (Pan paniscus, Dem. Rep. of the Congo (DRC), n=12). Further, we included published metagenomic data from samples of G. g. gorilla (n=28) and P. troglodytes (n=18) from the Republic of Congo (CG) (Campbell et al., 2020). We additionally sequenced human fecal samples from a rural, remote village from Côte d’Ivoire (n=12) and a rural community from the Democratic Republic of Congo (n=12), jointly representing a spectrum of African populations with lower HDI; samples from a German population were included in our analyses as a representative of populations with high HDI.

Using this extensive data resource, we created a comprehensive catalog of high-quality prokaryotic genomes assembled from metagenomic data, which we annotated on a taxonomic and functional level. Using this catalog we explored patterns of diversity, host-specificities in abundances of taxonomic groups and functions, and phylogenetic parallels between host and microbiota on a community level, but also for individual microbial clades. These analyses reveal intriguing patterns associated with human gut microbial communities, convergent functional adaptations across lineages and potential mechanisms driving these.

**An expanded catalog of microbial genomes from the hominid gut**

From 203 fecal samples of humans and non-human primates with shotgun metagenomic data, we reconstructed a total of 7,506 metagenome-assembled genomes (MAGs) ensuring maximum completeness and low contamination using multiple binning algorithms and
dedicated curation and scoring tools ([Rühlemann et al., 2022]; see Methods). The most
MAGs (quality score > 50%) were reconstructed for the most sampled subgroup of great
ape, P. t. verus (n=2,431), while the per sample on average the highest number of
reconstructed MAGs was the highest for P. t. schweinfurthii (mean=74.8). Library size /
number of sequencing reads was highly correlated with total assembly size ($\rho_{Spearman} =
0.644$), which in turn was directly correlated with the number of bins recovered for a sample
($\rho_{Spearman} = 0.966$).

Together with two large collection of reference species reconstructed from human fecal
metagenomes (UHG Gv2; [Almeida et al., 2021]), and non-human primate fecal
metagenomes (Manara et al., 2019), MAGs were clustered into 5,648 species-level genome
bins (SGBs; 95% ANI) using stringent criteria (Supplementary Table 1). These included 1,047
novel SGBs not covered by any of the two large references, which mostly originated from
NHP samples (n=954, 91.1%; Suppl. Figure 1b). The highest-quality MAG in each SGB was
chosen as representative. Overall quality of SGB representative genomes was high (median
quality score = 94.5%; Suppl. Figure 1c). SGB representatives were used as comprehensive
reference for the estimation of per-sample abundances (Methods, Suppl. Figure 1d, Suppl.
Table 2). In total 2,934 SGBs, encompassing 21 bacterial and two archaeal phyla (Suppl.
Figure 1a), were found present in the dataset. This newly curated catalog of SGBs from
gorillas, bonobos, and chimpanzees increases the number of microbial species genomes
previously reconstructed from feces by more than ten-fold, and increases mapping success
of fecal metagenomes from NHPs to reference genomes by two- to three-fold (Suppl. Figure
1d; [Manara et al., 2019]. Novelty in human fecal samples was comparably low, with 5.6%,
2.3%, and 1.1% of novel SGBs found in samples from CIV, DRC, and GER, respectively (Suppl.
Figure 1d). For both NHPs and humans, the highest percentages of novel diversity were
observed within the phyla Bacteroidota and Spirochaetota, and, to a lesser degree, within
Firmicutes and Firmicutes A for NHPs only (Suppl. Figure 1e). Generally, recovered clusters
were highly host specific. While the 7,506 MAGs spanned 1,759 of the final SGBs, only for 48
of these SGBs MAGs were reconstructed from samples of more than a single host genus.

Within sample diversity varied considerably between host (sub-)species and with
sequencing depth (Suppl. Figure 1f). The used SGB collection covers large proportions of the
diversity found in the human gut (Suppl. Fig. 1d) and Faith’s PD incorporates SGB relatedness in the diversity calculation, which enables a better estimate of total diversity of a community than simpler richness estimates from taxonomic group abundances. Phylogenetic diversity (PD) at an even mapping depth of 1 Mio. reads per sample showed significantly lower diversity in Humans compared to all African great ape hosts ($P_{\text{Wilcoxon}} = 6.9 \times 10^{-14}$; Figure 1a). Comparing individual groups, human from GER and DRC showed lower means than all NHP group (all $P_{\text{Wilcoxon}} < 0.05$), while Humans from CIV exhibited high variance and were found to have significant lower diversity than all NHP hosts ($P_{\text{Wilcoxon}} < 0.05$), except for $G. g. gorilla$ and $P. t. troglodytes$ ($P_{\text{Wilcoxon}} > 0.05$). These two great ape taxa were found to have the lowest library sizes, low numbers of recovered genomes and lowest mapping efficiency. Taken together, this suggests that phylogenetic diversity in these hosts may be biased by lower representation in the reference database and that the reduced diversity could be an artefact of this. Consequently, samples with less than 1 Million mapped reads were removed from further analyses. This resulted in the removal of samples from the analysis for the groups $G. b. beringei$ (n=1), $G. g. gorilla$ (n$_{\text{GB}}=5$, n$_{\text{CG}}=1$), $P. t. troglodytes$ (n$_{\text{GB}}=4$), $P. t. schweinfurthii$ (n=1) and humans from CIV (n=1). Of note, lowest mean values for within-sample phylogenetic diversity were found for the human subgroup from Germany, which however were not significantly different from that of humans from African communities (all $P_{\text{Wilcoxon}} > 0.05$), contradicting previous reports (McCall et al., 2020; Vangay et al., 2018). Additionally, the considerable differences in PD observed between CIV and DRC highlight the diversity found between human populations and the need to better characterize human diversity. Therefore, while total diversity of some host groups might not be exhaustively sampled, especially considering lower abundant microbial clades, the presented reference collection of high-quality metagenome reconstructed genomes likely represents the current best resource for an in-depth taxonomic and functional assessment of hominid fecal microbiomes and highlights that some human populations, including those sampled in this analysis, have lost considerable microbial diversity in their guts.
Microbial taxonomy, but not function, supports phylosymbiosis in hominids

Phylosymbiosis, a pattern in which microbial community divergence parallels that of the hosts, can be a sign of community-level co-evolution of host and microbiota indicating a close relationship over evolutionary timescales (Brooks et al., 2016). To investigate such patterns, we used six different measures of beta diversity. Four were based on phylogenetic or taxonomic distance metrics (weighted and unweighted UniFrac, as well as genus level Aitchison and Jaccard distance), two on functional capacities of the community (KEGG
ortholog (KO) abundance and presence/absence patterns; Supplementary Figure 2). Strong signals for phylosymbiosis were found for Aitchison, Jaccard and unweighted UniFrac distances (all $P < 0.001$, Figure 1b and c, Suppl. Figure 2, Suppl. Table 3), but not for the function-based distances and the weighted UniFrac distance (all $P > 0.25$). These results suggest phylosymbiotic divergence in the general structure of hominid microbiota (Jaccard and unweighted UniFrac are based on presence/absence of microbial clades), while abundance patterns of distinct microbial clades and functions do not seem to follow this trend, which is in line with previous observations in other host systems (Brooks et al., 2016). This pattern might be explained as the result of fluctuating clade abundances and functions in response to changing environmental factors, including diet, indicating a high functional plasticity of the hominid gut microbiome in response to immediate influences, while structural changes through acquisition and loss of microbial clades are rather the result of longer-term adaptations.

Assessing shared SGBs between host groups, we found that especially human-associated SGBs were strongly depleted across multiple NHP groups (Figure 1d), while no such signal was found in the opposite direction, suggesting the widespread acquisition of novel microbial clades in the human intestinal microbiome. A similar pattern was found for G. b. beringei and multiple Pan subspecies, however not for G.g. gorilla. G.g. gorilla and P.t. troglodytes are sympatric species and were sampled in the same environment in the Republic of Congo and Gabon. The absence of excess strong host-specific signals between these groups might suggest effects of shared environmental factors influencing microbiome structure. SGBs from human samples from lower HDI populations were enriched in all human groups, indicating a shared conserved core microbiota, however no overrepresentation of SGBs from higher HDI populations were found in the two other groups, suggesting that specific SGBs were introduced to the human gut in association with lifestyle changes.

**Fecal microbiome of high HDI populations marked by loss of evolutionarily conserved core microbiota**

Abundance difference in microbial clades between humans and NHPs and between human communities with differing environments, such as living in rural or urban regions, in regions
of the world with lower or higher HDI, can give insights into microbiome-mediated adaptations to environmental changes in the far and more recent past. We analyzed and compared the abundance profiles of gut microbes between NHPs and humans, including individuals living in rural, lower HDI areas of CIV and DRC as well as individuals residing in urban, higher HDI regions within GER. For all following taxonomic and functional comparisons, we restricted the analysis to human and Pan (chimpanzees and bonobos) samples to obtain focused insights into the microbiota divergence since their hosts diverged about 7-8 million years ago (Langergraber et al., 2012).

A total of 287 microbial genera were included in the analysis, of which 148 were found to be differentially abundant (Q_{Bonferroni}<0.05, Figure 1d, Suppl. Table 4) in at least one of these comparisons between human subgroups, or between humans and NHPs, and subsequently sorted into one of four groups. We identified 30 taxa with increased abundances among humans from Germany, such as Akkermansia, Bacteroides, and Alistipes (Figure 1d and e). We additionally found 30 taxa that are enriched in the two African populations such as Cryptobacteroides, Prevotella, and Succinivibrio (Figure 1e and f). Overall, the marker taxa of European microbiomes that we detected are in agreement with previous findings (Jha et al., 2018; Pasolli et al., 2019). Our approach allowed us to identify bacterial taxa that exhibit differential abundance profiles between humans and NHPs that are independent from the human population considered (Figure 1f). We found 63 taxa, such as SIG603 that have increased abundance in NHPs and 25 taxa, such as Coprococcus, with increased abundance in humans. Interestingly, taxa depleted in the microbiome of European individuals are more likely to be also abundant (>0.1%) in the microbiota of NHPs (P < 0.001; Figure 1g), suggesting a loss of evolutionary conserved clades in these populations.

**Widespread changes in fecal microbiome function between hosts and across human communities**

Taxon-specific changes reflect broad-scale differences between host-groups. A focused analysis of microbial functions can give insights into the specific driving forces of such community-level changes. We performed, analysis of abundance differences of 6,179 KEGG orthologs (KOs, (Kanehisa and Goto, 2000)) in NHP (genus Pan) vs. human fecal microbiota and humans in European and African societies and found significant abundance differences...
in 2,030 (32.9%) and 607 (9.82%) KOs, respectively (Q \text{Bonferroni}<0.05; \ Figure 1i, Suppl. Table 5). For the NHP vs. human comparisons, the KOs with the lowest Q-values included K20426 (acbU, 1-epi-valienol-7-phosphate kinase; \ Q = 6.03 \times 10^{-46}) and K17723 (preA; dihydropyrimidine dehydrogenase (NAD+) subunit PraA; \ Q = 2.25 \times 10^{-41}) with higher abundances in NHPs and K07267 (oprB; porin; \ Q = 2.11 \times 10^{-50}) and K08646 (MEP; peptidyl-Lys metalloendopeptidase; \ Q = 3.08 \times 10^{-45}) with higher abundances in humans. K22336 (bfrB, bacterioferritin B; \ Q = 3.43 \times 10^{-32}) and K17285 (SELENBP1, methanethiol oxidase; \ Q = 8.21 \times 10^{-21}) were strongly increased in Germans. The strongest signals for humans with African lifestyles were found for K12252 (aruH; arginine:pyruvate transaminase; \ Q = 1.01 \times 10^{-12}) and K12078 (ptlA; type IV secretion system protein PtlA; \ Q = 1.24 \times 10^{-12}).

Analysis of higher-level KEGG annotations including e.g. complete pathways overrepresented among differentially abundant KOs revealed three annotations with enrichment in NHPs compared to humans, and seven annotations differentiating humans in Europe and Africa (Q_{FDR}<0.05; \ Figure 1j, Suppl. Table 6). The enriched KEGG categories in NHPs were “V/A-type ATPase, prokaryotes” and “ABC transporters”. ABC transporters are a large family of ATP-dependent translocases (KO=377) with broad substrate spectra, including e.g. carbohydrates, metal ions, amino acids, that are involved in nutrient uptake (Davies et al., 2021) and export of toxic compounds (Davidson and Chen, 2004; Lee et al., 2014). The signal is driven by a broad range of ABC transporters found with increase abundance in NHPs (Suppl. Table 6), which might indicate adaptation to a diverse diet (Ejby et al., 2016; Tsujikawa et al., 2021) or metabolism of plant-derived xenobiotics (Rodríguez-Daza et al., 2021). Additionally, we found an enrichment of terms in “Microbial metabolism in diverse environments” (map01120), an extensive pathway that serves as an umbrella category for KEGG metabolic modules; thus, this signal can be interpreted as pointing to substantial changes in the abundance of non-specific metabolic functions between NHPs and humans.

Signals driven by differences between human subgroups included the KEGG module “Helicobacter pylori pathogenicity signature, cagA pathogenicity island” (md:M00564), driven by increased functional abundances in the fecal communities of humans living in Africa. Individuals infected with CagA-positive \text{\textit{H. pylori}} are at increased risk to develop
gastric cancer (Hatakeyama, 2014). Curiously, \textit{H. pylori} was not found in the dataset, however the KOs annotated for this pathway are found in the genus \textit{Succinivibrio}, belonging to the same phylum as \textit{Helicobacter} (Proteobacteria), which is depleted in Europeans. Whether \textit{Succinivibrio} in the gut can under certain circumstances exhibit a higher risk for other cancer types needs to be investigated.

In the European population, Vitamin B\textsubscript{12} / Cobalamin biosynthesis (md:M00122, M00924, M00925, and path:map00860) and “antimicrobial resistance genes” (br:ko01504) were found to be enriched. Increased antimicrobial functions may be explained by a higher use of antibiotics in human healthcare and animal husbandry, as well as by environmental pollution (Groussin et al., 2021). The increased abundance of Vitamin B\textsubscript{12}-producing microorganisms in the feces of Europeans may be driven by higher dietary intake of meat and dairy products from ruminants (Watanabe and Bito, 2018). These associations warrant further experimental studies to identify their driving factors and to understand their implications on the gut ecosystem.

**Success of taxa associated to European populations relates to oxidative carbohydrate metabolism**

To elucidate the functional basis of the success of taxa in the human gut of individuals residing within in Europe, we analyzed differences in the functional capacities of these in comparison to other human associated taxa either enriched in African societies or without association to this dichotomy. Compared to community-level functional analyses used to identify higher-level functional patterns, pangenome analysis can specifically identify individual genes and functions enriched or depleted in association with a trait of interest within a specific microbial clade. Analyses were restricted to five bacterial families, \textit{Acidaminococcaceae}, \textit{Bacteroidaceae}, \textit{Lachnospiraceae}, \textit{Oscillospiraceae}, and \textit{Ruminococcaceae}, for which sufficient numbers of SGBs (\textit{n}>=20) for pangenome analysis were recovered. SGBs in these family represent large proportions of the overall human microbiome (mean = 61.7%), with no significant differences between the subgroups (\textit{P\textsubscript{Kruskal-Wallis}} = 0.8). The analysis was conducted at the family-level, as higher taxonomic ranks would increase clade-specific functional biases. To account for between-family functional differences, functional differences of SGBs associated with Europeans were first analyzed
within families using Fisher’s exact test and subsequently subjected to unweighted meta-
analysis using Z-scores to leverage shared signals.

We identified 276 enriched and 50 depleted KOs in pangenomes associated with the
European population ($Q_{\text{Bonferroni}} < 0.05$; Suppl. Tables 7 and 8). Then, by using higher-level
KEGG annotations (modules, pathways, BRITE hierarchies), we found that 18 of these
annotations were overrepresented in the dataset ($Q_{\text{Bonferroni}} < 0.05$, Figure 1h, Table 1, Suppl.
Table 9).

Among these were multiple groups involved in carbohydrate metabolism enriched in taxa
enriched in Europeans, specifically pointing at aerobic breakdown of sugar molecules for
ATP generation (Citrate cycle: md:M00009, path:map00020; Pentose phosphate pathway:
md:M00004; V/A-type ATPase, prokaryote: md:M00159). These signals strongly suggest that
the selection for taxa in the gut of humans living in Europe is connected to the adaptation to
transient microaerobic conditions in the gut environment, and to the use of oxidative
phosphorylation as a mean to release energy from nutrients, which is more efficient than
strictly anaerobic fermentation (Jurtshuk, 1996). However, reduced fermentation can
impact short-chain fatty acid production, which can, in turn, potentially negatively affect the
host’s intestinal epithelial cells and metabolism (Deleu et al., 2021). Additionally, we found
multiple pathways connected to the metabolism of amino acids (Histidine metabolism:
path:map00340, md:M00026, md:M00045; Glycine, serine and threonine metabolism:
path:map00260). Especially gut microbial histidine metabolism has been discussed with
relevance to human health, as it was shown that an intermediary product of histidine
degradation, imidazole propionate, was increased in type 2 diabetic individuals in a large
study of almost 2,000 individuals, and that this increase was directly connected to the
microbiota and overall unhealthy dietary habits, however independent of dietary histidine
intake (Molinaro et al., 2020). This suggests a microbiome-driven pre-disposition to
metabolic disorders in European human communities.

We did not find any higher-level KEGG annotations significantly enriched among the taxa
not enriched in Europeans. the relative lower fitness of these taxa may not result from a
single mechanism associated to European lifestyles, but rather from multiple selective
forces specific to the individual clades.
Convergent host-specific adaptations are found across microbial families

Shared gene gains or losses across multiple microbial clades can indicate convergent adaptations to specific host intestinal environments. We performed a pangenome analysis of genera shared between humans and NHPs (n=37) to identify such patterns. To control for higher-level clade effects, functional repertoires (KEGG Ontology terms) were compared at the genus-level between SGBs from NHPs (Suppl. Table 10) and humans and then combined in a meta-analysis across genera. In total, 123 KO terms were identified as carrying signatures of convergent adaptation to the respective host group, with 44 of these signatures associating with humans and 79 associating with NHPs (Q_{Bonferroni}<0.05; Figure 2a, Suppl. Table S10). Among the human-associated KOs, we found multiple functional groups hinting again at an adaptation to increased oxygen by utilization of oxygen as an electron acceptor within the respiratory chain, such as cytochrome bd ubiquinol oxidase subunits (cydA, cydB), as well as adaptation to increased oxidative stress through ferritin (ftnA) and thioredoxin-dependent peroxiredoxin (BCP). Among the KOs enriched in NHP pangenomes we found 1-epi-valienol-7-phosphate kinase (acbU), phosphoserine phosphatase (rsbU/P), two KOs annotated as polyketide synthases (rhiA, pksN) and a phage terminase (xtmB).

Products produced by polyketide synthases have diverse functions, including antibiotic activity, virulence and support of symbiotic relationships (Ridley et al., 2008).
Prevotella represented the largest genus-level clade in the dataset (n=113, 5.2% of all SGBs). While we found this genus across all host species, it is largely decreased in Europeans. Thus, we selected this genus for further analysis to elucidate potential functional mechanisms driving the observed patterns. Enrichment analysis revealed 55 KOs with distinct prevalence patterns (Q<0.05; Figure 2b). The most striking difference was found for the cytochrome bd ubiquinol oxidases subunits 1 and 2 (cydA and cydB), which were ubiquitous in all 31 human-associated Prevotella SGBs, but present in only two out of 62 SGBs from NHPs. It is important to note that these prevalence differences are not driven by single lineages within the Prevotella genus that would have distribution ranges restricted to single hosts. Instead, they are observed across multiple sibling clades spanning the entire phylogenetic tree of the taxon. Cytochrome bd oxidases are involved in stress responses, most prominently in transiently microaerobic environments (Giuffrè et al., 2014). Comparison of the Prevotella species tree (reconstructed using all SGB representatives recovered from the dataset (n = 191; including UHGGv2)) and the cydA gene phylogeny exhibit widespread incongruencies between the tree topologies (Figure 2c). We performed a tree reconciliation using a duplication-transfer-loss (DTL; Kundu and Bansal, 2018) model between the Prevotella and cydA phylogenies, which revealed a high rate of gene transfer (\( \bar{T} = 32.227 \)) and loss (\( \bar{L} = 35.769 \)) driving the evolution of the cydA gene family. The gene transfer events and mappings were robust across 1,000 reconciliations with different starting seeds, with 94% of all events and 88% of all mappings found with 100% consistency. These results suggest that the enrichment of cytochrome bd ubiquinol oxidases observed in humans compared to NHPs are the result of multiple individual events of gene loss and horizontal gene transfer within the hominid gut.

Co-phylogeny is rare in spore-forming microbes and disrupted in humans
Patterns of co-phylogeny between host and microbes can result from close interaction, or even interdependence in extreme cases, and congruent metabolic pathways from co-evolutionary trajectories. Using stringent selection criteria, we subjected 222 subtrees of the SGB phylogeny for co-phylogeny analysis (see Methods). The subtrees spanned 45 families and 961 (32.8%) SGBs present in the dataset in addition to 90 SGBs from the UHGGv2 catalog. We used a Mantel-test based framework and permutation to detect co-phylogeny signals ([Hommola et al., 2009], see Methods for details). When defining co-phylogeny candidates based on a mean P-value < 0.05 across all permutations, 53 of 222 subtrees (23.9%) qualified as exhibiting co-phylogenetic patterns (Suppl. Table S11). These
subtrees cover 303 of the 1051 SGBs (28.8%) included in the analysis and 5.7% of the total 5,316 hominid SGBs (excluding the SGBs from Manara et al.). All results and subtrees can be inspected online (https://mruehlemann.shinyapps.io/great_apes_shiny_app). By visually inspecting subtrees with co-phylogeny signals, we find many candidates microbial phylogenies that do not follow host phylogeny, e.g., in a subgroup of the genus Cryptobacteroides (Figure 3a). Such signals suggest that co-phylogeny within the Gorilla and Pan clades can result in statistically significant Mantel tests, despite topological incongruences of human-derived sequences, for which no host sister (sub-)species from the same genus is available.

Human-derived genomes were found in 160 (72.1%) of the tested subtrees, of which 35 (21.9%) were co-phylogeny candidates. In comparison, 18 of 62 (29%) tested subtrees without human-derived representatives exhibited co-phylogeny signals, e.g., W0P29-013 spp. (Figure 3a). Overall, 16.3% (n = 66 out of 404) of human-derived SGBs in the analysis were found in co-phylogenetic subtrees, which is significantly less compared to 34.6% (n = 227 out of 656) of NHP-derived SGBs (P_{Fisher} = 6 \times 10^{-11}). Co-phylogeny signals, defined based on the ratio of SGBs in trees exhibiting co-phylogeny patterns and the total SGBs in the family, differed strongly. Six families showed excessive signals of co-phylogeny and four families a significant depletion (Q_{Fisher,FDR} < 0.05; Figure 3b, Suppl. Table S12). The strongest co-phylogeny ratio was found for the family Dialisteraceae (Cophyl-Ratio = 61.22%, Q = 4.44 \times 10^{-7}), which in the analysis were entirely represented by members of the genus Dialister. Dialister are a common, but rather neglected members of the gut microbiota which have been found increased (Vals-Delgado et al., 2021; Zheng et al., 2018) and decreased (Joossens et al., 2011) with various human diseases, hence their relation to human health remains unclear. However, on species, Dialister invisus, was found to be moderately transmissible between human mother-infant pairs and within households in a large meta-analysis (Valles-Colomer et al., 2023). The strongest depletions were found for e.g. the families Lachnospiraceae and Treponemataceae, the former confirming previous results for this clade (Moeller et al., 2016). The latter, Treponemataceae, especially the genus Treponema D, were found depleted in humans living in Germany and occur in anaerobic sediments (Thingholm et al., 2021), serving as (intermediary) reservoirs for transmission to
humans and NHPs, which can disrupt co-phylogenetic signals by constant re-introduction to
the community.

When comparing host groups, the proportion of SGBs with co-phylogeny signal is
significantly reduced in all human subgroups compared to the NHP hosts (all Q_{Wilcoxon} < 0.05; Figure 3c). Further, humans from Germany exhibited even lower proportions of co-
phylogeny SGBs compared to the two African human populations (Q_{Wilcoxon} < 0.05). The
human subgroups from Africa did not differ in their co-phylogeny proportions (P_{Wilcoxon} = 0.583). These results clearly suggest a loss of wild great ape associated clades with
“humanization” of the intestinal microbiota and the introduction of novel microbial partners
with changing environment and lifestyle, confirming again findings from previous analyses.

Previous analyses suggested that spore-forming clades are less likely to be exhibiting co-
phylogenic patterns, due to their ability to survive outside of the gut, facilitating dispersal
between hosts (Groussin et al., 2020; Moeller et al., 2016). We find a negative correlation
between the number of sporulation-associated genes and family-level cophylogeny-ratio (P < 1.4 x 10^{-4}, Figure 3d, Suppl. Table S13), confirming this hypothesis, also supporting
previous findings of increased direct host-to-host transmission of non-spore forming
microbial clades in baboons (Tung et al., 2015).

Although not enriched, we found multiple (n=10 out of 28) subgroups in Bacteroidaceae
with co-phylogeny signals, consistent with previous findings based on gyrB amplicon data
(Moeller et al., 2016). However, no evidence for strict co-phylogeny was found in
Bifidobacteriaceae, which is inconsistent with findings from the same previous report
(Moeller et al., 2016). Comparing the phylogeny of metagenome-derived gyrB sequences
and the GTDB marker-gene phylogeny for Bifidobacterium spp. revealed clear incongruences
between both approaches (Figure 3e), which can likely explain the differences in the
presented analysis and previous findings.

Discussion

Here, we present the largest curated dataset of fecal metagenomes derived from wild
African great apes and human populations. For this, we surveyed and reconstructed high-
quality microbial genomes from the feces wild nonhuman primates, including gorillas (Gorilla gorilla gorilla; Gorilla beringei beringei), chimpanzees (Pan troglodytes verus; P.t. troglodytes; P.t. schweinfurthii), and bonobos (Pan paniscus) as well as human populations from Africa and Germany. We identified signals of phyllosymbiosis across the included hominids, indicating a conserved evolutionary relationship of microbial communities with their host species. Moreover, by employing a comparative approach, we found extensive changes of microbial taxonomic and functional abundances across the intestinal microbiota of NHPs and humans. Previous studies have pointed to Western lifestyles as an important factor influencing the intestinal microbiota in humans. Within our human sample population, we were able to confirm differential signals of prokaryotes associated with the German human population. Importantly, using a comparative dataset of great ape taxa showed that microbial clades lost in the German population in comparison to African human populations are also found in wild great ape populations. Thus, we suggest that the loss of these taxa might be regarded as a mass extinction event of evolutionary conserved members of hominid-associated gut microbiota. While it is tempting to link these changes to industrialization (as previous studies have done), there are many differences between these human populations (e.g., genomic diversity, diet, exercise, sunlight exposure, exposure to antibiotics, population bottlenecks) and it was certainly not possible with the sampling regime here, to determine the particular factors responsible for the variation observed between the human populations sampled here. Despite this caveat, that fact that considerable variation exists between human populations is notable and highlights the need for much higher resolution sampling of human associated microbial diversity.

In a pan-genome analysis to identify individual genes or functions enriched or depleted in genomes of taxa associated with different human populations, we identified numerous functional traits involved in aerobic respiration associated with the German population in the analysis. We hypothesize that taxa found to be enriched in the fecal samples of humans from Germany might have a selective advantage via their clade-independent ability to survive or even utilize aerobic conditions in the intestinal tract. More specifically, we propose that these taxa have undergone convergent adaptation to tolerate high oxygen concentrations. Such aerotolerance could increase microbial fitness, whereby bacteria can withstand high oxygen concentrations to metabolize mucus layers for energy (Zheng et al.,
However, the depletion of this mucus by bacteria diminishes an important physical and immunological barrier that protects the human host against microbial assaults and allows for direct interaction between host epithelial cells and microbiota, potentially triggering (auto-)immune processes (Costa et al., 2016; Matute et al., 2023). Notably, we showed that the introduction of novel microbes associated with industrialization related to vast differences in the community assembly of fecal microbiota in the German population. Thus, it is possible that susceptibility to intestinal inflammation might be potentiated by specific taxa found in this population. Accordingly, we found increased abundances of well-characterized mucin-degrading taxa, including Akkermansia and Bacteroides, in the German cohort. These findings are congruent with previous reports suggesting that there is increased mucus degradation by intestinal microbiota in human populations with direct access to industrial food systems, which may relate to higher incidences of inflammatory bowels diseases observed in developed economies (E. D. Sonnenburg and Sonnenburg, 2019).

Comparatively, only a few pathways showed conserved enrichment in the opposite direction, suggesting clade specific mechanisms for their loss in some human societies. In particular, we found the taxon Prevotella is depleted in Germans but conserved across hominids, despite representing a diversity of host clades and diets. Prevotella is a major determining taxon of one of the human enterotypes, a concept used to define fecal microbial communities (Arumugam et al., 2011). It remains controversial as to whether the human gut microbiome is best classified using such discrete categories, or rather along a dynamic, continuous gradient (Cheng 2019, Knight 2014). Nevertheless, previous reports have shown that individuals who access industrialized food systems (i.e., consume so-called “Westernized” diets) generally display a Bacteroides-dominant enterotype. Bacteroides-enterotypes have been previously associated with a multitude of intestinal (Vieira-Silva et al., 2019) and extra-intestinal inflammatory diseases (Valles- Colomer et al., 2019). Conversely, individuals who rely on rural and traditional subsistence strategies (i.e., consume plant-rich diets) tend to exhibit a Prevotella-dominant enterotype (Vangay et al., 2018). This enterotype is also displayed in about 20% of individuals living within Western societies (Costea et al., 2018). Interestingly, there are conflicting reports concerning Prevotella and host health. While it has been shown that Prevotella may improve glucose
metabolism (Kovatcheva-Datchary et al., 2015), other reports have linked high abundances
of *Prevotella* spp. with autoimmune diseases and intestinal inflammation (Iljazovic et al.,
2021). While results from model systems have suggested *Prevotella* likely plays a role in
autoimmunity, these studies largely relied on mono-colonization of germ-free animals and
thus may be biased due to a lack of microbial interaction partners and an aberrant host
physiology (Iljazovic et al., 2021). Within human studies, no convincing link between
increased *Prevotella* spp. and inflammatory bowel disease has yet been shown (Iljazovic et
al., 2021).

Here, we used an evolutionarily-informed framework to extend the enterotype concept to
elucidate the functional dynamics involved in the assembly of the human gut microbiome
over evolutionary timescales. Such insights may better inform how changes in the gut
microbiome might affect human health. We find the taxon *Prevotella* to be conserved across
the sampled hominids. Moreover, the sheer diversity of *Prevotella* displayed across all
hominid clades clearly suggests an evolutionary conservation and long-standing interaction
of this microbial clade with the host, as further revealed by host-specific microbial functions
identified in the metagenomic pangenome analysis. In other words, we find the *Prevotella*
clade to be an integral member of the intestinal microbial community of all hominids.
Therefore, we propose that the *Prevotella*-enterotype represents an evolutionary ancestral
community state for the human gut microbiome. Rather than a discrete enterotype, a
reduced abundance and diversity of *Prevotella* may better regarded a key biomarker for
disease risk (Gorvitovskaia et al., 2016) or for microbiota insufficiency syndrome (J. L.
Sonnenburg and Sonnenburg, 2019), which seems to be partly driven by changes associated
with Western lifestyles. Additional research and large-scale strain collections for *Prevotella*
are needed for an in-depth analysis and evaluation of this diverse taxonomic group with
regard to host health and its role in inflammation. Such research must consider *Prevotella*
spp. as members of a complex consortium of interacting microorganisms and as, we argue, a
potential target for pre- and probiotic intervention in chronic inflammatory disorders.

Lastly, we leveraged our catalog of high-quality metagenome-assembled genomes from
hominid fecal samples together with existing data to investigate co-phylogenetic patterns
across the sampled hosts. Overall, co-phylogeny showed highly clade-specific enrichments
and depletions. In addition, human-derived MAGs were found significantly less often among co-phylogenetic groups than MAGs from NHPs. Since we included human-derived data from global reference datasets (Almeida et al., 2021), this effect is unlikely to be an artifact of non-exhaustive coverage of human microbiome members. Additionally, we provide evidence for the previously proposed effect of sporulation capabilities as a mechanism for prokaryotes to become independent of direct host-to-host transmission, disrupting co-phylogeny patterns, potentially through repeated environmental uptake of spore-forming bacteria (Groussin et al., 2020).

Our study has limitations. The co-phylogeny analysis relies heavily on genome-sequences recovered from shotgun metagenomic sequencing (MAGs), which are potentially contaminated and incomplete, which could bias tree structure and thus, co-phylogeny estimates, and also the can potentially under- (or over-)estimate the functional capacities of recovered microbial genomes. To address the potential shortcomings of MAGs, we implemented stringent data processing pipelines and quality control to achieve the high-quality MAGs and a host-specific pan-genome based functional annotation framework incorporating information from multiple MAGs per species representative to reduce potential genome gaps (see Methods). Additionally, the commonly used estimates of divergence times of the hominid hosts included in the analysis set the timeframe of the split from a shared ancestor to 8-19 million years ago (Langergraber et al., 2012; Scally et al., 2012). Bacterial speciation events happen in the timeframe of 10-100 million years, or longer (Marin et al., 2017; McDonald and Currie, 2017; Ochman et al., 1999), and thus, co-phylogeny in hominids is expected to be observed within microbial species or possibly genera. In the presented dataset, species-level sharing of MAGs between host genera was low (2.73%; n = 48 out of 1,759 newly reconstructed SGBs, not including Manara et al. and UHGGv2). SGBs were defined on 95% average nucleotide identity, a measure generally regarded as appropriate (Jain et al., 2018), but it is nevertheless prone to clade specific biases, potentially further influenced by altered speciation dynamics in association with (evolutionary) changes in host lifestyle (Lawrence and Retchless, 2009), i.e. previously demonstrated increases in horizontal gene transfer (HGT) within individual microbiomes (Groussin et al., 2021). Accounting for these potential biases, we relaxed the threshold to a shared genus-level annotations for subgroups to be included in the co-phylogeny testing,
while keeping the number of tested sub-phylogenies to a minimum through the definition of stringent inclusion criteria (see Methods). Despite these considerations, the observation of signals of co-phylogeny across hominids is supported by a robust statistical framework.

Our work here lays the foundation for the analysis of disease-associated changes in the human intestinal microbiome in an evolutionarily informed framework, thereby allowing researchers to evaluate microbiome-associated inflammatory disorders from a point of view that considers both proximal and evolutionary influences. Future investigations should consider in-depth analysis of horizontal gene transfer events within or even between primate hosts to shed further light on also cross-species dynamics and transition of microbes. Such analyses however require either microbial isolate genomes or at least long-read sequencing data to increase confidence in detection events. Additionally, time series data for host groups sharing the same habitat, e.g. G. g. gorilla and P. t. troglodytes, could give additional insights into cross-species sharing dynamics which cannot be appropriately elucidated based on single-timepoint data.

In summary, we present an in-depth taxonomic and functional description and analysis of hominid-associated fecal communities spanning about ten millions of years of evolution and host-microbiome interactions in the gut of humans and African great apes. Western lifestyle and maybe more precisely industrialization associated changes in human gut microbiota have been previously suggested as a driver of microbiome insufficiency syndrome, whereby an incompatibility between quickly adapting microbiota and slowly evolving host genes leads to chronic inflammatory diseases such as metabolic syndrome, type 2 diabetes, and inflammatory bowel disease (E. D. Sonnenburg and Sonnenburg, 2019; Wallenborn and Vonaesch, 2022). Thus, a comparative analysis of human and NHP intestinal microbiota that considers evolutionary forces as presented herein provides a powerful platform to advance our understanding of human-associated microbiota and guide the development of personalized, targeted interventions to prevent and treat chronic inflammatory disorders.
Tables

Table 1: KEGG functional groups with significant enrichment (Q < 0.05) in the gut microbiome of humans living in Germany.

<table>
<thead>
<tr>
<th>KEGG ID</th>
<th>Name</th>
<th># of KOs</th>
<th>Mean Z-Score</th>
<th>P-value</th>
<th>Q-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>path:map01230</td>
<td>Biosynthesis of amino acids</td>
<td>238</td>
<td>2.43</td>
<td>2.80E-17</td>
<td>2.29E-14</td>
</tr>
<tr>
<td>path:map00340</td>
<td>Histidine metabolism</td>
<td>47</td>
<td>4.69</td>
<td>3.92E-16</td>
<td>3.20E-13</td>
</tr>
<tr>
<td>md:M00159</td>
<td>V/A-type ATPase, prokaryotes</td>
<td>9</td>
<td>13.61</td>
<td>5.08E-15</td>
<td>4.15E-12</td>
</tr>
<tr>
<td>md:M00026</td>
<td>Histidine biosynthesis, PRPP =&gt; histidine</td>
<td>20</td>
<td>4.56</td>
<td>3.05E-13</td>
<td>2.49E-10</td>
</tr>
<tr>
<td>path:map00630</td>
<td>Glyoxylate and dicarboxylate metabolism</td>
<td>101</td>
<td>3.27</td>
<td>3.26E-11</td>
<td>2.66E-08</td>
</tr>
<tr>
<td>br:br01601</td>
<td>Enzymes of 2-oxocarboxylic acid metabolism</td>
<td>60</td>
<td>5.39</td>
<td>1.33E-08</td>
<td>1.09E-05</td>
</tr>
<tr>
<td>md:M00010</td>
<td>Citrate cycle, first carbon oxidation, oxaloacetate =&gt; 2-oxoglutarate</td>
<td>6</td>
<td>5.98</td>
<td>3.68E-07</td>
<td>3.01E-04</td>
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<tr>
<td>md:M00004</td>
<td>Pentose phosphate pathway (Pentose phosphate cycle)</td>
<td>15</td>
<td>7.29</td>
<td>8.40E-07</td>
<td>6.87E-04</td>
</tr>
<tr>
<td>md:M00045</td>
<td>Histidine degradation, histidine =&gt; N-formiminoglutamate =&gt; glutamate</td>
<td>8</td>
<td>4.7</td>
<td>1.68E-06</td>
<td>1.38E-03</td>
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<tr>
<td>md:M00631</td>
<td>D-Galactarotate degradation (bacteria), D-galactarotate =&gt; pyruvate + D-glyceraldehyde 3P</td>
<td>8</td>
<td>4.92</td>
<td>1.68E-06</td>
<td>1.38E-03</td>
</tr>
<tr>
<td>path:map00520</td>
<td>Amino sugar and nucleotide sugar metabolism</td>
<td>156</td>
<td>2.38</td>
<td>4.74E-06</td>
<td>3.87E-03</td>
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<tr>
<td>path:map00620</td>
<td>Pyruvate metabolism</td>
<td>132</td>
<td>3.41</td>
<td>6.86E-06</td>
<td>5.60E-03</td>
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<tr>
<td>path:map00260</td>
<td>Glycine, serine and threonine metabolism</td>
<td>109</td>
<td>2.54</td>
<td>9.95E-06</td>
<td>8.13E-03</td>
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<tr>
<td>md:M00009</td>
<td>Citrate cycle (TCA cycle, Krebs cycle)</td>
<td>42</td>
<td>3.81</td>
<td>1.37E-05</td>
<td>1.12E-02</td>
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<tr>
<td>path:map00020</td>
<td>Citrate cycle (TCA cycle)</td>
<td>66</td>
<td>4.35</td>
<td>1.94E-05</td>
<td>1.59E-02</td>
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<tr>
<td>md:M00008</td>
<td>Entner-Doudoroff pathway, glucose-6P =&gt; glyceraldehyde-3P + pyruvate</td>
<td>5</td>
<td>7.59</td>
<td>1.97E-05</td>
<td>1.61E-02</td>
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<tr>
<td>path:map00190</td>
<td>Oxidative phosphorylation</td>
<td>223</td>
<td>8.71</td>
<td>2.74E-05</td>
<td>2.24E-02</td>
</tr>
<tr>
<td>md:M00006</td>
<td>Pentose phosphate pathway, oxidative phase, glucose 6P =&gt; ribose 5P</td>
<td>6</td>
<td>8.44</td>
<td>3.90E-05</td>
<td>3.19E-02</td>
</tr>
</tbody>
</table>

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Data and code availability
All metagenomic sequencing data is available via the NCBI BioProject accession IDs PRJNA692042 and PRJNA539933. All code to process sequencing files to generate the presented results is available via https://github.com/mruehlemann/greatapes_mgx_scripts.

Conflicts of interest
All authors declare no conflicts of interest.

Material and Methods

Fecal Sampling, DNA extraction and data generation

Sampling procedures for collecting feces from humans (n=48) and wild non-human primates (n=109) have been previously described (Gogarten et al., 2021). Briefly, fecal samples were collected immediately after defecation, and, depending on the local infrastructure, either stored in RNAlater and frozen at -20°C or stored in a cryotube, cooled in a thermos until return to the field laboratory, and subsequently snap frozen in liquid nitrogen. Appropriate government permits and permission to conduct research on wild primates were granted by the relevant authorities (see Acknowledgments for site-specific details). Human fecal samples from the Democratic Republic of Congo (n = 12) were stored in RNAlater and frozen at -20°C. Human fecal samples from Côte d’Ivoire (n=12) were stored in a cryotube, cooled in a thermos until return to the field laboratory and subsequently snap frozen in liquid nitrogen. Human fecal samples from Germany were collected at home by the participant in standard fecal collection tubes, mailed to the study center, and stored at -80°C. Ethical approval was obtained from the Local Ethics Committee (Kiel, Germany; reference number A156/03). DNA extraction from fecal samples was performed from 200mg of stool transferred to 0.70mm Garnet Bead tubes (Qiagen) with 1.1 mL ASL buffer, followed by bead beating in a SpeedMill PLUS (Analytik Jena AG) for 45 s at 50 Hz. Samples were heated to 95°C for 5 min and centrifuged, retaining 200 µl of the supernatant for DNA extraction with the QIAamp DNA Stool Mini Kit (Qiagen) automated on a QIAcube system (Qiagen) according to the manufacturer’s protocol. DNA quality was assessed by Qubit and Genomic DNA ScreenTape (Agilent). Illumina Nextera DNA Library Preparation Kit was used to construct shotgun metagenomic libraries, and subsequently sequenced with either 2 x 125 bp reads on a HiSeq 2500 platform or with 2 x 150 bp reads on a HiSeq 4000 machine (Illumina).
Data processing, assembly and metagenomic binning

Raw sequencing FastQ files were quality controlled and preprocessed using the BBMap software suite ("BBMap," n.d.). Host reads were removed using a human reference database and a lenient threshold of 95% identity to account for a broader host range using bbmap.sh. Metagenomic contigs were assembled with metaSPAdes and contigs >= 2000 bp were retained (Bankevich et al., 2012). Reads were mapped to the contigs of the respective samples using Minimap2 (Li, 2018), converted to BAM files with Samtools (Li et al., 2009) and used to estimate per-contig mapping depth with the jgi_summarize_bam_contig_depths binary from the MetaBAT2 binning tool (Kang et al., 2019). Contig binning for individual samples was performed with MetaBAT2 (Kang et al., 2019), MaxBin2 (Wu et al., 2016) and CONCOCT (Alneberg et al., 2014). In addition, the VAMB binning tool (Nissen et al., 2021) was used on a cross-mapping catalog of the merged contigs from all samples within each host group. Individual binning results were refined using MAGScoT (Rühlemann et al., 2022) to acquire high quality metagenome-assembled genomes (MAGs) for each sample. Clustering of MAGs to species-level genome bins (SGBs) was performed with dRep (Olm et al., 2017) in a multi-step approach to control for inflated SGBS due to low MAG quality. First, MAGs were dereplicated to 97% similarity within each host group, choosing the MAG with the highest score (calculated by MAGScoT based on completeness and contamination) as cluster representative. High and good quality representatives (score >=0.7) from all host groups together with representative sequences from the UHGG v2 were then clustered into 95% SGBS using dRep, again selecting the highest quality MAG as representatives. Medium quality (scores between 0.5 and 0.7) 97% representatives from previous clustering step were then compared to SGB representatives using fastANI (Jain et al., 2018), assigning MAGs with high similarity (>=95%) to the respective SGB. Medium quality 97% representatives without hits to the high quality SGB library were then clustered into 95% SGBS and added to the catalog in the case of at least two genomes in the cluster, discarding singleton clusters. The final catalog of SGB representatives was used to quantify contig abundances in all samples using Salmon in metagenome mode (Patro et al., 2017). Taxonomic annotations were performed using the GTDBtk (v2.1) and GTDB release 207v2 (Chaumeil et al., 2022; Parks et al., 2022). For SGBS
without genus- and/or species-level assignments, the SGB ID was used as taxonomic label. GTDBtk marker gene alignments were used to generate a phylogenetic tree of all SGB representatives using the respective “infer” function of the GTDBtk. All data processing scripts are available online: https://github.com/mruehlemann/greatapes_sgb_scripts

**Pangeneome catalog creation, annotation and analysis**

All MAGs underwent calling of coding sequences using prodigal (v2.6.3) (Hyatt et al., 2010). Protein sequences were clustered based on 95% similarity using MMseqs (Hauser et al., 2016; Steinegger and Söding, 2017) and annotated using the emapper.py script of the eggNOG-mapper v2 (Cantalapiedra et al., 2021) annotation tool with the eggNOG 5.0 reference database (Huerta-Cepas et al., 2019). MAG level functional profiles based on KEGG Ortholog annotations were collapsed into SGB-level pangenomes for each host genus (Homo, Gorilla, and Pan). In the case that no MAGs of an SGB were recovered from a given host genus, functional profiles were inferred from MAGs across the other host groups, accounting for host-specificities in the inferred accessory genomes/functions by considering a function to be present if it was present in all host-specific pangenomes of the respective SGB with MAGs recovered from the metagenomic data.

**Calculation of microbial clade and functional abundances**

All downstream data processing and statistical calculations were performed in R v4.2 (R Core Team, 2022) and using the tidyverse library (Wickham et al., 2019). Per-sample contig abundances for the SGB representatives from Salmon were used to estimate SGB abundances. Salmon output includes total mapped reads per contig and mapping reads adjusted for library size and total sequencing depth as transcripts per million (TPM), a measure from the transcriptomics field which can be directly transferred to metagenomic libraries. Individual contig coverages were calculated from the number of mapped reads and the effective lengths of the Salmon mapping output, considering contigs with > 10% coverage as present. An SGB was considered present when at least 20% of its total length was in contigs marked as “present” and if at least 1,000 total reads and 250 TPM mapped to it. Final SGB abundances were calculated as TPM, calculated from the reads mapping to the SGBs present in the respective sample, thus representing a normalized abundance across all samples. Combining SGB abundances with taxonomic assignments, domain- to species-level
abundances were calculated as cumulative TPM abundances within the respective
taxonomic bins. Rarefactions were calculated based on 5-fold repeated subsampling of
contig level mapped reads at 100k, 250k, 500k, 1M, 2.5M, 5M, and 10M reads, followed by
TPM calculations as described above. By rarefying reads and not TPM we realistically
simulate sampling effects introduced by low coverage and low abundances of SGBs affecting
especially samples with small library sizes. Community level functional profiles were
calculated be multiplying TPM abundances of SGBs with the respective host-genus specific
functional profiles of the SGBs and summarizing the per-SGB values into a sample-level
abundance of functional annotations.

Alpha and beta diversity
Faith's phylogenetic diversity (PD) (Faith, 1992) was used as measure of alpha diversity,
calculated from the phylogenetic tree based on GTDBtk marker genes using the pd() function of the picante package for R (Kembel et al., 2010). Genus-level increase of PD from
novel SGBs was calculated from the differences of PDs with and without novel SGB
annotated as the respective genus. Sample level PDs were calculated from the SGB
presence/absence patterns. Beta diversity was assessed as unweighted and weighted
UniFrac distances (Lozupone et al., 2011) using the UniFrac() function of the phyloseq
package for R (McMurdie and Holmes, 2013) and SGB abundances and the phylogenetic tree
based on GTDBtk marker genes as input. Aitchison distance (Aitchison, 1982) was calculated
from the CLR-transformed genus-level TPM abundances obtained from the clr() function
from the compositions package for R (van den Boogaart and Tolosana-Delgado, 2008) and
adding a pseudocount of 1 to all abundances, setting all CLR-transformed abundances below
zero to zero. Jaccard distances (Jaccard, 1912) were also calculated on genus-level
presence/absence patterns using the vegdist() function from the vegan package for R
(Oksanen et al., 2022). Genus-level abundances were chosen for Aitchison and Jaccard
distance, as SGBs are highly host-specific, thus would lead to high beta-diversities simply
due to host exclusive SGBs, grouping at genus-level prevents from this and UniFrac distances
use phylogenetic relations between SGBs.

Cross-host sharing analysis
Analysis of excessive and reduced sharing of SGBs between host groups were based on the mean SGB abundances of the five rarefaction of 1M mapped reads to account for differences in library depth impacting SGB richness. For each host group, 100-fold sampling of five samples from this group were drawn and the SGBs found in the host were analyzed for their presence in five random samples of each of the other host groups, calculating the relative amount of shared SGBs. The mean of all 100 samplings was used as relative sharing coefficient for all host pairs in both directions. Excess and reduced sharing was analyzed by 1000-fold drawing of five random samples accounting for differences of host groups and the repetition of above calculations for relative sharing with all host groups. P-values were calculated from the proportions of random samplings exceeding/falling below the true sharing coefficients.

Phylosymbiosis analysis

Phylosymbiosis was assessed using five measures for community level diversity, unweighted and weighted UniFrac, genus-level Aitchison, and Jaccard distances, as well as KEGG ortholog (KO) abundance based Aitchison distance, and following the approach described in Brooks et al. (2016). Briefly, host group differences were used to infer microbiome dendrograms by UPGMA clustering. Branch support was calculated from 1000-fold jackknife sampling. Robinson-Foulds distances between microbiome trees and host phylogeny were calculated using the RF.dist() function from the phangorn package for R (Schliep, 2011). Significance of phylosymbiosis was assessed using the host phylogeny and 100,000 random trees as comparison for the microbiome trees. Tanglegrams were created with the ggtree and cowplot packages for R (Yu et al., 2017).

Assessment of between-group abundance differences

Taxonomic and functional abundance differences between Humans and NHPs, as well as between humans living outside and within industrialized systems were based on CLR-transformed abundances to account for the compositionality of microbiome data (Gloor et al., 2017). Included in the calculations were all genera with a prevalence > 20% and relative abundance (before CLR-transformation) of > 0.1% in at least of the host groups and all KO abundances with a prevalence > 20% and CLR-transformed abundance of > 1 in at least of the host groups. Abundance differences were assessed using linear regression. Log-fold
differences were calculated using group mean abundances and a pseudocount of 0.01. P-values were adjusted for multiple testing using Bonferroni correction. Genera with significant differences with lifestyle were grouped into BloSUM (Bloom or Selected in Societies of Urbanization/Modernization) and VANISH (Volatile and/or Associated Negatively with Industrialized Societies of Humans) taxa. Remaining genera with significant differences between humans and NHPs were grouped according to these patterns. Genera without abundance differences in any of these comparisons were grouped as “other”. Accordingly, significantly different KO abundances were grouped into association with human subgroups or humans vs. NHPs depending on the statistical significance and direction of the association.

Functional pan-genome differences between groups

Pangenome catalogs of human-associated SGBs were compared within microbial families between SGBs in BloSUM and non-BloSUM taxa, the latter comprising all VANISH taxa and taxa with higher abundance in humans compared to NHPs, however not associated with the industrialization gradient. KEGG Ontology (KO) term annotations were used as functional groups and their prevalence differences between groups were assessed using Fisher’s exact test. Effect sizes were calculated using the log2 of the ratio of prevalences in the two groups and a pseudocount of 0.01. Per-family Z-Scores of KOs were calculated from P-values and the direction of the effects. The sum of the Z-Scores were added and divided by the square-root of the total number of families the respective KOs were found in to obtain a Z

Meta for each KO term, used to calculated P

Meta. P

Meta-values were adjusted for multiple testing using Holm-correction. KO terms with Q < 0.05 and present in at least two of the microbial families in the analysis were considered as functions with differential prevalence. A similar approach was employed to assess functional differences between NHP- and Human-associated SGBs, however in this case, SGB pangenome differences were compared on genus level and the meta-analysis was performed combining signals from all genera, and specifically across the genera within particular phyla.

Tree reconciliation analysis

Proteins from the representative SGBs of the genus Prevotella annotated with the annotation “cydA” (cytochrome bd oxidase subunit 1) as “Preferred name” in the
emapper/eggNOG annotation were extracted from the unclustered protein sequence catalog. The same procedure was followed for *Paraprevotella*, which was included as an outgroup. Incomplete cydA sequences were removed using a length threshold of 200. Protein sequences were aligned using Clustal Omega (Sievers et al., 2011). The alignment was used to reconstruct the phylogenetic tree using IQTREE2 (Minh et al., 2020) and a WAG model (Whelan and Goldman, 2001), calculating branch support using UFBoot (Minh et al., 2013) and performing SH-aLRT test (Guindon et al., 2010). Alignments of GTDBtk marker protein sequences for *Prevotella* and *Paraprevotella* SGBs were used to reconstruct a genome-level species phylogeny in the same respective way as described above for the cydA sequences. Very low confident branches (< 60% bootstrap support) in the cydA phylogeny were resolved together with the species tree using the OptResolutions supplementary program of the RANGER-DTL 2.0 software (Bansal et al., 2018) resulting in 495 equally probable trees with optimized duplication-transfer-loss costs using default values (duplication: 2, loss: 1, transfer: 3). A randomly chosen output tree was using in the reconciliation analysis with the species tree in RANGER-DTL 2.0 using default values and 1,000 random starting seeds in parallel (Tange, 2011) to assess robustness. Resulting sampling outcomes were summarized using the AggregateRanger tool of the RANGER-DTL 2.0 software package.

**Co-phylogeny analysis**

Host phylogenetic trees were obtained from the 10kTrees website ([Arnold et al., 2010]; [https://10ktrees.nunn-lab.org/](https://10ktrees.nunn-lab.org/)). To assure high quality microbial phylogenies for the co-phylogeny analysis, family-level maximum-likelihood trees were reconstructed from the GTDBtk marker gene alignments with the IQTREE2 software (Minh et al., 2020) and a WAG model including a random SGB outside the respective families as outgroups. Family level trees were rooted and for each SGB traced from tip to root to identify for each SGB the smallest subtree which covered 4, 5, 6, and 7 host groups. Combining information from all SGBs, the overall set of smallest trees to be included in the co-phylogeny analysis were identified, discarding subtrees for which the inclusion criterion was fulfilled already for a smaller tree starting from a different tip. In addition, subtrees spanning more than a single genus were excluded from the analysis, as divergence times of microbial genera predate divergence of the included hosts (Ochman et al., 1999). For all subgroups included in the
analysis, maximum-likelihood distances and trees using a WAG model in IQTREE2 were inferred from the marker gene alignment of all MAGs assigned to the SGBs in the respective subgroups. Co-phylogeny of the subgroup was assessed by randomly selecting one MAG per host, calculating congruence with the host tree by Robinson-Foulds metric and by Mantel-test (Hommola et al., 2009). Tiplabels were permuted 999-fold and P-values calculated. This process starting from the random selection of one MAG per host was repeated 999 times to obtain final P-values. Family-level co-phylogeny ratios were calculated based on the ratio of SGBs within subtrees with co-phylogeny signal and total SGBs in the respective family that were included in the analysis. Enrichment of co-phylogeny for each microbial family was calculated by using Fisher’s exact test on the SGBs in the analysis dividing them into four groups based on family membership and being in a subtree with co-phylogeny signal. All P-values were adjusted using FDR correction.

Correlation between co-phylogeny ratio and sporulation genes

Genes were selected using the KEGG Ontology annotations, selecting KOs with the words “spore” or “sporulation” in their description. This resulted in a total of 149 unique KOs. The presence of these KOs in an SGB was assessed based on the pangenome annotations (see above). The number of KOs was summed up for each genome and genus-level averages were regressed against the family-level co-phylogeny ratios. The P-value of the correlation was calculated from the two-sided t-statistic calculated from regression coefficient and standard error.

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