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Cospeciation of gut microbiota with hominids

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Abstract

The evolutionary origins of the bacterial lineages that populate the human gut are unknown. Here we show that multiple lineages of the predominant bacterial taxa in the gut arose via cospeciation with humans, chimpanzees, bonobos, and gorillas over the past 15 million years. Analyses of

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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strain-level bacterial diversity within hominid gut microbiomes revealed that clades of Bacteroidaceae and Bifidobacteriaceae have been maintained exclusively within host lineages across hundreds of thousands of host generations. Divergence times of these cospeciating gut bacteria are congruent with those of hominids, indicating that nuclear, mitochondrial, and gut bacterial genomes diversified in concert during hominid evolution. This study identifies human gut bacteria descended from ancient symbionts that speciated simultaneously with humans and the African apes.

Cospeciation is a hallmark of intimate and ancient symbiotic relationships (1, 2). Humans and other mammals host communities of bacterial symbionts, which are essential for normal postnatal development and adult health. Gut bacterial community membership and abundance profiles are shaped by host genetics (3) and evolutionary history (4, 5), but also by diet (6), geography (7), and medical intervention (8). External factors exert a strong influence on the composition of gut microbial communities, which are assembled anew in each host generation. It is unknown whether lineages of gut bacteria persist within individual host lineages over timescales long enough to lead to cospeciation. Here, we tested whether gut bacteria residing within present-day humans are descended from ancestral bacterial symbionts that cospeciated with humans and the African apes.

To test for cospeciation between hominids and their gut bacteria, we assessed the congruence between hominid and bacterial phylogenetic trees. Although DNA sequence data sets are available for gut microbiomes of hominids (4, 5, 9, 10), these are based on short ribosomal RNA (rRNA) amplicons or shotgun-metagenomic data, which lack the resolution required to detect codiversification between bacterial and hominid lineages. Bacterial rRNA sequences diverge too slowly to track diversification over the time scale of hominid evolution (11, 12), and shotgun metagenomic sequencing does not reliably capture orthologous genetic regions from related bacteria in different host species. Therefore, we used an amplicon sequencing approach that assays quickly evolving protein-coding regions in bacterial genomes (phyloTags) (13) to profile strain diversity within the gut microbiomes of humans, chimpanzees, bonobos, and gorillas. This fine-scale resolution allows inference of the phylogenies of closely related bacterial lineages, thereby enabling tests for cospeciation between gut bacteria and the Hominidae.

We amplified a variable region of the DNA gyrase, subunit B (*gyrB*) gene from bacteria present in fecal samples collected from humans living in Connecticut, USA (*Homo sapiens*; $n = 16$); wild chimpanzees from Gombe National Park, Tanzania (*Pan troglodytes*; $n = 47$); wild bonobos from three field sites in the Democratic Republic of the Congo (*Pan paniscus*; $n = 24$); and wild gorillas from two field sites in Cameroon (*Gorilla gorilla*; $n = 24$) (table S1 and fig. S1). We used multiple sets of primers, each designed to target one of three bacterial families prominent in the gut microbiome (13): the Bifidobacteriaceae, the Bacteroidaceae, or the Lachnospiraceae. Amplicons were sequenced on the Illumina MiSeq platform, generating 4,578,632 reads averaging 41,249 reads per sample. Sequences were screened for quality in QIIME (14) and filtered to eliminate sequencing errors (15) (data files S1 to S3), and the relative frequencies of Bacteroidaceae, Bifidobacteriaceae, and Lachnospiraceae strains recovered from each sample were recorded (tables S2 to S4). Phylogenetic analyses

were performed separately for each bacterial family. Sequences were aligned with ClustalW as implemented in MEGA 6.0 (16), and maximum-likelihood trees were constructed with a general time-reversible plus invariant sites (GTR+I) model of nucleotide substitution. Relative node ages of maximum-likelihood trees were estimated in BEAST (17).

The phylogenetic relationships among the Bacteroidaceae strains from the Hominidae mirror the relationships of their host species (Fig. 1). Previous studies of the microbiota of the Hominidae compared the overall composition of their microbial communities (i.e., beta diversity) (4, 5, 9, 10), but our phylogenetic analysis allowed us to trace the evolution of individual bacterial lineages. We recovered three well-supported clades (i.e., clades in which the relationships among the bacterial lineages derived from different host species were supported by more than 50% of bootstrap replicates) that contain strains from more than two host species (Fig. 1, A to C). The topology of each of these clades indicates ancient cospeciation between the Bacteroidaceae and the Hominidae: Within each of these clades, strains recovered from bonobos and chimpanzees form separate sister groups, which together form a clade that is sister to either gorilla-derived (Fig. 1, A and B) or human-derived (Fig. 1C) strains.

The dominant pattern of diversification has been the parallel cospeciation of multiple Bacteroidaceae lineages with their hominid host lineages (Fig. 1A). However, in one case along the branch leading to *Pan*, two Bacteroidaceae lineages arose from a single ancestral lineage without a host split, and each subsequently codiversified with chimpanzees and bonobos (Fig. 1B). This instance of strain divergence within a single host lineage is analogous to a gene duplication event, in which a single ancestral gene gives rise to two distinct loci within a genome. In some cases (e.g., Fig. 1, A and B), bacterial lineages have been lost from the human population, consistent with previous observations that humans have a depleted microbiome relative to those in wild-living African apes (5). No close gorilla-derived relatives of the strains represented in Fig. 1C were detected. This bacterial group was either acquired on the lineage leading to humans, chimpanzees, and bonobos, or lost from the lineage leading to gorillas. The lack of detection of human-derived representatives from the clades depicted in Fig. 1, A and B, and of gorilla-derived representatives from the clade depicted in Fig. 1C is unlikely to be caused by primer bias, as the Bacteroidaceae primers used captured a broad diversity of human-derived and gorilla-derived Bacteroidaceae lineages from closely related clades (data file S4). Reanalysis of a previously reported data set (18) that includes the V4 region of 16*S* ribosomal DNA sequences recovered from sympatric chimpanzees ($n = 9$) and gorillas ($n = 15$) (10,028 to 110,324 reads per sample) living in Cameroon revealed no shared 99% Bacteroidaceae operational taxonomic units (OTUs) between these sympatric populations. Because *gyrB* phyloTAGs are hierarchically embedded within 16*S* OTUs (13), the observation that sympatric chimpanzees and gorillas harbor entirely nonover-lapping sets of 16*S* Bacteroidaceae OTUs indicates that sympatric chimpanzees and gorillas maintain distinct Bacteroidaceae *gyrB* lineages, although we cannot rule out the possibility that some lineages are shared at low, undetected abundances.

Other well-supported clades of Bacteroidaceae were recovered from more restricted (i.e., two or fewer) sets of host species; however, the relationships among these clades could not

be resolved, and they could not be used to test for cospeciation. These included two clades from bonobos, three clades from chimpanzees, three clades from humans, four clades from gorillas, and three clades from humans and chimpanzees (data file S4). In all but two of these clades, strains derived from the same host species constitute monophyletic groups, confirming that bacterial strains have diversified within their respective host species. In the other two cases, unique strains recovered from chimpanzees fall within a clade recovered from humans (fig. S2), indicative of past rare strain transfers between humans and chimpanzees. The short branch lengths of the chimpanzee-derived Bacteroidaceae lineages of human origin imply that the transfers were relatively recent.

Results for the Bifidobacteriaceae resemble those for the Bacteroidaceae. The phylogeny of the 307 Bifidobacteriaceae strains recovered from the Hominidae also mirrors the phylogeny of the host species (Fig. 1D and data file S5). However, one clade of gorilla-derived Bifidobacteriaceae strains is sister to the clade of bonobo-derived strains. Hence, there appears to have been a transfer of a Bifidobacteriaceae lineage from *Pan* into gorillas (Fig. 1D). Branch lengths indicate that this transfer occurred soon after the divergence of chimpanzees and bonobos. Identical to what was observed for Bacteroidaceae, analysis of the previously reported data set (18) for sympatric chimpanzees and gorillas living in Cameroon revealed no shared 99% Bifidobacteriaceae OTUs between these sympatric populations.

For the Lachnospiraceae, the phylogenetic history contrasts with that observed in the Bacteroidaceae and the Bifidobacteriaceae. The present-day host associations of the 746 Lachnospiraceae strains indicate at least four between-host-species transfer events since the common ancestor of the Hominidae (fig. S3 and data file S6). These results corroborate previous observations that Lachnospiraceae 16*S* OTUs are shared across African ape species (18). The Lachnospiraceae, unlike Bacteroidaceae and Bifidobacteriaceae, are spore-forming and can survive outside the gut, which may enhance their ability to disperse and transfer among host species. The distinct evolutionary pattern of the Lachnospiraceae indicates that human and ape gut microbiomes are composites of cospeciating and independently diversifying bacterial lineages (fig. S4).

We expect, under a neutral model, the degree of sequence divergence between bacterial strains derived from different host species to be proportional to the divergence dates of their corresponding host species. For the two bacterial families that displayed evidence of cospeciation with hominids, we estimated the divergence times within bacterial clades whose relationships recapitulated the host phylogeny (data files S7 and S8). For each bacterial clade, we estimated the divergence dates of *Homo*- and *Pan*-derived strains or of Hominini-(*Pan* + *Homo*) and *Gorilla*-derived strains based on the assumption that the nested chimpanzee-and bonobo-derived strains diverged 2.2 million years ago (Ma). Using the chimp-bonobo split as calibration, the mean estimated divergence dates of bacterial clades derived from the two deeper splits correspond closely to hominid divergence dates estimated from fossil and genomic evidence (Fig. 2). Based on the sequence divergence of gut bacteria, we date the split of humans and chimpanzees at 5.3 Ma (Fig. 2A), coincident with estimates based on host mitochondrial genomes (5.2 to 6.4 Ma) (19) but later than some recent estimates based on nuclear genomes (7 to 13 Ma) (20). In contrast, the human-gorilla

split based on the sequence divergence of their gut bacteria is dated to 15.6 Ma (Fig. 2A), older than estimates based on mitochondrial genomes (7.1 to 9.2 Ma) (19) but within the range of estimates based on nuclear genomes (8 to 19 Ma) (20).

The history of cospeciation between the Hominidae and their gut bacteria provides robust host-derived calibration dates for estimating the rate of DNA sequence evolution in the Bacteroidaceae and Bifidobacteriaceae (Fig. 2B). Rates of molecular evolution are lowest in the mutualistic Bifidobacteriaceae, in which sequence divergence has accumulated at a rate of ~0.7 and 0.07% per million years at synonymous and nonsynonymous sites, respectively (Fig. 2B), estimates that agree well with those derived from comparisons of *Salmonella* and *Escherichia* gene sequences (21). In contrast, rates of molecular evolution are highest in the Bacteroidaceae clade containing the human-derived commensal and opportunistic pathogen *Bacteroides vulgatus* (displayed in Fig. 1C) at ~7.0 and 2.2% per million years at synonymous and nonsynonymous sites, respectively (Fig. 2B). This Bacteroidaceae clade also displayed the lowest GC content at 45%, compared with 50 to 56% GC in all other Bacteroidaceae clades. Together, fast rates of molecular evolution at both synonymous and nonsynonymous sites alongside relatively high AT content are indicative of an elevated mutation rate in the clade containing *B. vulgatus*.

Applying the same time calibration points for bacterial and host sequences, rates of synonymous site divergence of bacterial gyrase B genes (0.7 to 7%/Ma) are faster than those of host nuclear DNA (topoisomerase I, 0.2%/Ma) but similar to those of host mitochondrial DNA (e.g., NADH1, 1.4%/Ma). The observation that genes within cospeciating bacterial symbionts evolve faster than host genes may be useful for inferring the evolutionary and biogeographical relationships among recently diverged host species.

We next tested whether cospeciating gut bacterial lineages are also present in human populations living in Africa. We queried 23 previously reported gut metagenomes of humans from Malawi (7) with a representative *gyrB* sequence from each chimpanzee-derived clade. The top five *e*-value hits of each search were extracted and added to either the Bacteroidaceae, Bifidobacteriaceae, or Lachnospiraceae alignment, and phylogenetic analyses were reperformed (data files S9 to S11). The phylogenetic placements of all the Malawi-derived Bacteroidaceae and Bifidobacteriaceae *gyrB* sequences indicate a history of cospeciation. Among the bacterial strains detected, humans from Malawi harbor a cospeciating lineage of Bacteroidaceae not detected in humans from the USA (fig. S5), as well as distinct lineages within the cospeciating Bifidobacteriaceae clade depicted in Fig. 1 (fig. S6). The loss of bacterial lineages is in line with the general reduction in gut microbiome diversity that has been observed in USA humans (5, 7). In contrast, the phylogenetic placements of the Malawi-derived Lachnospiraceae lineages are consistent with a history of transfer of Lachnospiraceae among host species (fig. S7).

Codiversification between the Hominidae and their gut bacteria shows that symbiotic associations arose in a common ancestor to all African great apes and have persisted over evolutionary time scales. Our comparisons only reveal the minimum age of these symbioses, and it is possible that diversification alongside ancestral bacterial lineages is common to all vertebrates. Evidence from experimental systems has revealed the roles that gut bacteria play

in host development and immune-system function (22, 23), indicating that hosts have evolved in response to their bacterial counterparts. Conversely, many bacterial taxa have adapted to their respective hosts (24, 25). Our results represent a step toward understanding the coevolutionary history of vertebrates and their gut bacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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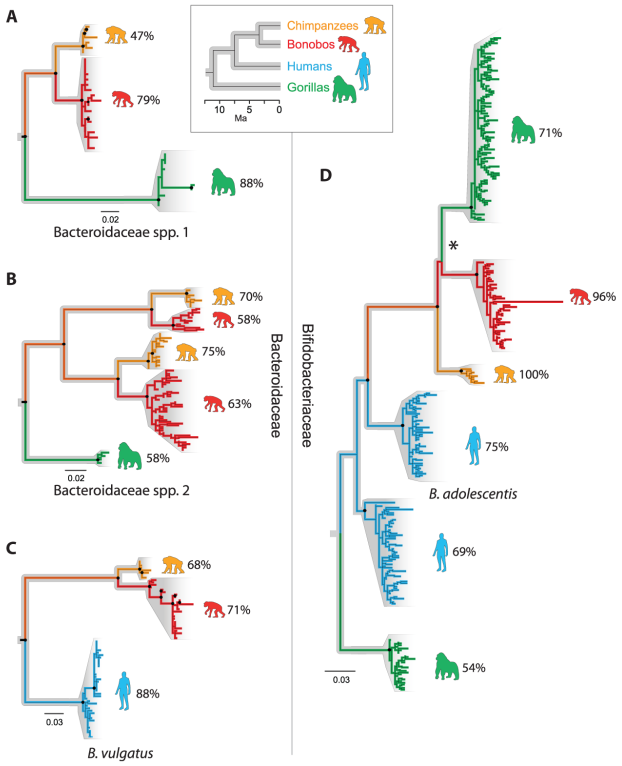

Fig. 1. Cospeciation between gut bacteria and hominids

Inset contains a phylogeny showing the relationships among humans and the African apes. (**A**) Maximum-likelihood phylogeny of a clade of Bacteroidaceae lineages that codiversified with the African apes but that was lost from the lineage leading to humans. In (A) and subsequent panels, black dots denote nodes supported in >50% of bootstrap replicates, colors correspond to the inset and denote the host species from which each bacterial lineage was recovered, and percentages indicate the percent of host individuals from which each clade was recovered. (**B**) Maximum-likelihood phylogeny of a clade of Bacteroidaceae lineages that codiversified with the African apes but that was lost from the lineage leading to humans. Note that this Bacteroidaceae lineage bifurcated in an ancestor of chimpanzees and bonobos, giving rise to two, paralogous cospeciating bacterial lineages. (**C**) Maximum-likelihood phylogeny of a Bacteroidaceae clade that cospeciated with humans, chimpanzees, and bonobos. No gorilla-derived representatives of this clade were recovered. (**D**) Inferred relationships among Bifidobacteriaceae *gyrB* sequences recovered from humans and African apes. Black asterisk indicates the transfer of a *Bifidobacterium adolescentis* relative from bonobos into gorillas.

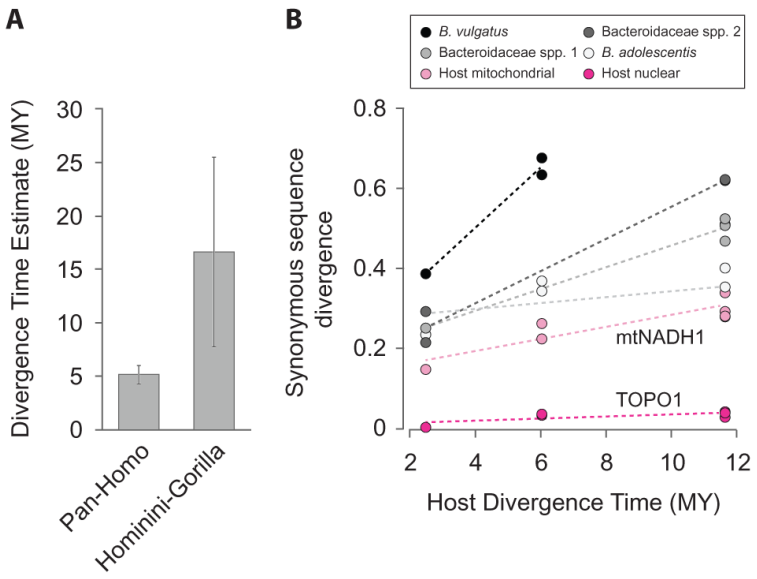

Fig. 2. Bacterial time scale for hominid evolution

(**A**) Divergence times of Hominidae species estimated from Bacteroidaceae and Bifidobacteriaceae *gyrB* sequences in BEAST. Error bars represent SDs of the mean divergence times estimated from each clade that cospeciated with Hominids. (**B**) Color-coded trend lines indicate rates of synonymous site divergence of *gyrB* in each bacterial clade displaying evidence of cospeciation, the host mitochondrial NADH1 (mtNADH1) gene, and the host nuclear topoisomerase I gene (TOPO1). Trend lines correspond to bacterial clades depicted in Figs. 1 and 2, and named bacterial species for each clade are shown when available.