MICROBIOTA

Transmission modes of the mammalian gut microbiota

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Mammals house a diversity of bacteria that affect health in various ways, but the routes by which bacterial lineages are transmitted between hosts remain poorly understood. We experimentally determined microbiota transmission modes by deriving 17 inbred mouse lines from two wild populations and monitoring their gut microbiotas for up to 11 host generations. Individual- and population-level microbiota compositions were maintained within mouse lines throughout the experiment, indicating predominantly vertical inheritance of the microbiota. However, certain bacterial taxa tended to be exchanged horizontally between mouse lines. Consistent with evolutionary theory, the degree of horizontal transmission predicted bacterial genera with pathogenic representatives responsible for human infections and hospitalizations.

All mammals associate with bacteria (*1*) in relationships that affect the hosts' digestive (*2*), immune (*3*), and neuroendocrine (*4*) systems. However, the routes by which specific bacterial lineages within the microbiota are transmitted between hosts remain poorly understood. Mammalian microbiotas are acquired both vertically from mother to offspring (*5*–*7*) and horizontally among non-kin through social interactions and shared environments (*8*–*10*). Quantifying the relative contributions of these transmission modes has been hindered by a lack of experiments designed to disentangle the transmission of individual bacterial lineages. Differentiating between vertical and horizontal transmission in humans, for example, is complicated by the difficulty of monitoring microbiotas for multiple host generations (*11*, *12*).

To differentiate between vertically and horizontally transmitted bacterial lineages in the mouse gut microbiota, we derived 17 mouse lines from wild populations in Tucson, AZ, and Edmonton, Alberta, Canada, and monitored their gut microbiotas during 3 years of inbreeding in a common laboratory environment (5 to 11 generations per line) (Fig. 1A and fig. S1). Mice from Tucson and from Edmonton were housed on different racks in the same room, and mice from different lines never came into direct contact. Every 1 to 2 weeks, mice were provided with new cages, food, and bedding. All cages were autoclaved, and food and bedding were sterilized. Cages were changed in random order with respect to population of origin. This experimental setup allowed us to quantify the fidelity of gut bacterial lineages to mouse lines and to identify gut bacterial lineages that were horizontally transmitted (through the mouse facility and animal handlers).

Cecal contents were extracted from wild-caught mice and subsequently from breeding individuals of each inbred line every generation, yielding a total of 212 samples (table S1). Illumina sequencing of the V4-V5 region of the 16S ribosomal DNA gene produced 15,353,040 sequences, averaging 72,420 reads per sample. To evaluate the relative contributions of vertical and horizontal transmission to the composition of the mouse microbiota, the binary Sorensen-Dice coefficient was calculated for every pairwise comparison between cecal samples across all mice. The binary Sorensen-Dice coefficient indicates the proportion of lineages that are shared by two communities and does not consider the relative abundances of lineages. Here, this coefficient is the proportion of amplicon sequence variants (ASVs) shared by two microbiotas. We defined binary Sorensen-Dice dissimilarity (BSDD) as one minus the binary Sorensen-Dice coefficient, such that a high BSDD indicates little overlap between the microbiotas' community memberships and a low BSDD indicates substantial overlap between the microbiotas' community memberships. Comparing BSDD within and between mouse lines allowed us to infer the contribution of vertical and horizontal transmission to the community membership of the mouse gut microbiota.

Comparing BSDDs within and between mouse lines indicated that vertical inheritance was the primary mode of gut bacterial transmission during the transition from the wild to the laboratory and subsequent inbreeding. Population- and individual-level compositional differences among wild-derived gut microbiotas were maintained within inbred mouse lines for 10 generations (Fig. 1). We found that Edmonton and Tucson mice that were sampled in the wild harbored compositionally distinct gut microbiotas [analysis of similarity (ANOSIM) P value = 0.001] (Fig. 1B). Edmonton and Tucson mice that were sampled in the laboratory maintained compositionally distinct gut microbiotas that reflected their wild population of origin (ANOSIM P value = 0.001) (Fig. 1C). The microbiotas of laboratory-born mice tended to be more similar to those of individuals sampled in their wild population of origin than they were to those of individuals sampled in the other wild population (t test P value = 3.15×10^{-23} for Edmonton, and t test P value = 7.58×10^{-7} for Tucson) (Fig. 1D). The distinctiveness of Edmonton and Tucson microbiotas was still evident in the 10th generation of the experiment (Fig. 1E). We observed that each mouse line, including sublines derived from the same wild-caught founders, maintained a compositionally distinct gut microbiota community membership throughout the experiment (Fig. 1F and table S2). The wild microbiota is not present in laboratory lines of mice that have been rederived under sterile conditions, such as the mouse model C57BL/6, but previous work has shown that germ-free mice inoculated with a wild gut microbiota can maintain the wild microbiota for four generations in gnotobiotic isolators (*12*). Our results indicate that individual-specific gut microbiota compositions of wild mice can be maintained in descendants for more than 10 generations in a mouse facility without gnotobiotic isolators.

Patterns of BSDD between samples indicated that vertical inheritance was the dominant mode of gut bacterial transmission, but they also indicated the horizontal transmission of some gut bacteria. Despite remaining distinct for 10 generations, the community memberships of the microbiotas of Tucson and Edmonton mice converged over the course of the experiment (Fig. 2A). We divided the experiment into seven time periods of equal length and found that BSDD between Tucson and Edmonton mice from the same period decreased with increasing time in the laboratory (nonzero linear coefficient, $P = 1.24 \times 10^{-8}$). This trend may indicate the exchange of bacteria between Tucson and Edmonton mice or parallel selection in the laboratory environment. To differentiate between these alternatives, we compared the microbiotas of later generations of laboratory-bred mice with the microbiotas of the founders of the lines derived from the other location (Edmonton for Tucson lines and Tucson for Edmonton lines). We found that their dissimilarity decreased with time (Fig. 2B). These results indicate that the gut microbiotas of laboratory-bred mice diverged from those of wild-caught mice initially, most likely due to a combination of drift and selection in the laboratory environment. Subsequently, the convergence of the gut microbiotas of laboratory-bred mice with those of wild-caught line founders from the other geographical origin indicated that gut bacteria were transmitted horizontally between lines derived from the different wild populations.

Given that the gut bacteria of wild-derived mouse lines displayed evidence of both vertical and horizontal transmission, we next investigated how specific bacterial genera were transmitted

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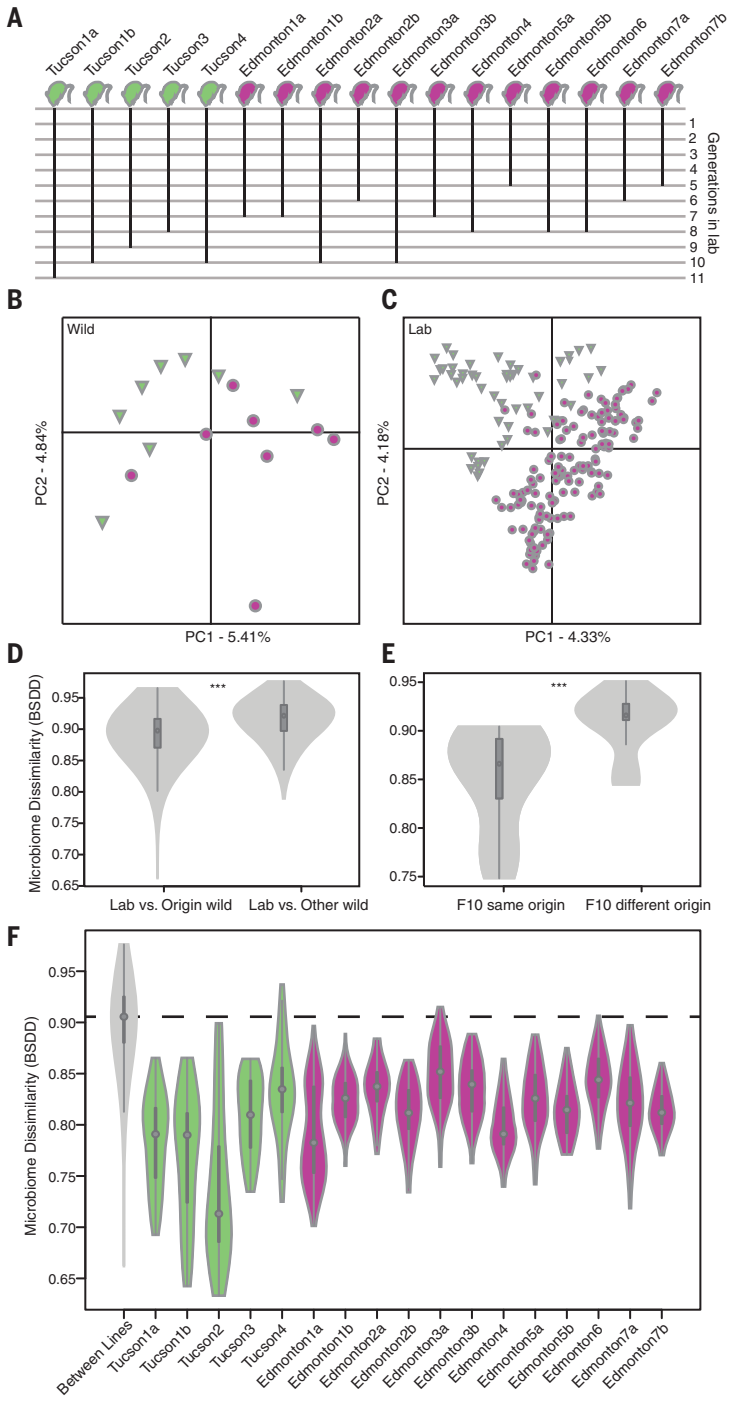

Fig. 1. Population- and individual-specific microbiotas of wild mice are vertically inherited for 10 generations in the laboratory. (**A**) Mouse cartoons and vertical lines represent inbred mouse lines. Sublines are labeled "a" and "b." Green and purple indicate Tucson and Edmonton, respectively. (**B**) Principal coordinates (PC) plot of BSDDs among mice sampled in the wild. (**C**) Principal coordinates plot of BSDDs among mice sampled in the laboratory. In (B) and (C), triangles and circles represent Tucson and Edmonton mice, respectively. (**D**) The violin plot on the left displays BSDDs between laboratory mice and wild mice from the same geographical origin. The violin plot on the right displays BSDDs between laboratory mice and wild mice from different geographical origins. (**E**) BSDDs among F_{10} mice descended from the same geographical origin (left) and from different geographical origins (right). Asterisks in (D) and (E) indicate significance; ***$P < 0.001$. (**F**) BSDDs among mice from different lines derived from the same geographical origin (gray), from the same Tucson line (green), and from the same Edmonton line (purple). The dashed line indicates the mean between-line BSDD. Rectangles in (D) to (F) delineate inner-quartile ranges. Circles in (F) indicate means.

between hosts. For each bacterial genus, we calculated the mean BSDD between mice from different lines and between mice from the same line. The ratio of between-line to within-line BSDD for each genus, which we call the transmission mode (TM) score, provided information about the degree of vertical versus horizontal transmission of ASVs belonging to the genus (fig. S2). A TM score of >1 indicated that ASVs of the genus tended to be restricted to specific mouse lines (i.e., were vertically inherited), a score equal to 1 indicated that ASVs were distributed equally among mice irrespective of line, and a score of <1 indicated that ASVs were more often shared by mice from different lines than by mice from the same line (i.e., were horizontally transmitted). TM scores of <1 were consistent with the rapid spread and decline of ASVs in the mouse colony (leading to increased similarity of the gut microbiotas of mice from different lines), as well as the epigenetic inheritance of mouse immune response [e.g., (*13*)] to specific ASVs (leading to decreased similarity of the gut microbiotas of mice from the same line). For example, bacterial genera within the Mollicutes (e.g., *Mycoplasma*) displayed TM scores of <1, and mice from different lines sampled in the same time period tended to share ASVs of these genera (fig. S3).

Comparing TM scores across bacterial genera produced a ranking of the fidelity of gut bacterial genera to mouse lineages (table S3). The majority of the bacterial genera displayed a tendency for vertical transmission. However, the distribution of TM scores across bacterial genera was bimodal (Hartigan's dip test P value = 0.001252), with a dip around 1 (Fig. 3A). Thus, bacterial genera in the mouse gut microbiota tend to be either exchanged horizontally or inherited vertically, with relatively few genera displaying mixed transmission strategies.

Transmission mode was associated with bacterial phylogenetic history. Three bacterial classes displayed a mean genus-level TM score significantly different from 1 (based on 95% confidence intervals): the Bacilli (0.954), the Clostridia (1.106), and the Bacteroidia (1.113) (Fig. 3B). In the Bacilli and the Clostridia, genera from the same class displayed TM scores that were more similar than were the TM scores of bacterial genera from different classes (F tests; $P = 0.0504$ for Bacilli, and $P = 0.00231$ for Clostridia). These results indicate that the two dominant classes of Firmicutes within the mammalian gut have evolved divergent transmission strategies. Horizontal transmission of the Bacilli and vertical inheritance of the Bacteroidia are evident in principal coordinates plots of the distributions of Bacilli and Bacteroidia ASVs within and between mouse lines (fig. S4).

Transmission routes were also associated with bacterial lifestyle (Fig. 3C and supplementary materials). Obligate anaerobes tended to be transmitted vertically (TM score > 1; t test P value = 0.01503), whereas obligate aerobes were transmitted horizontally (TM score < 1; t test P value = 0.01537) (Fig. 3C). These results suggest that oxygen tolerance enables the horizontal transmission

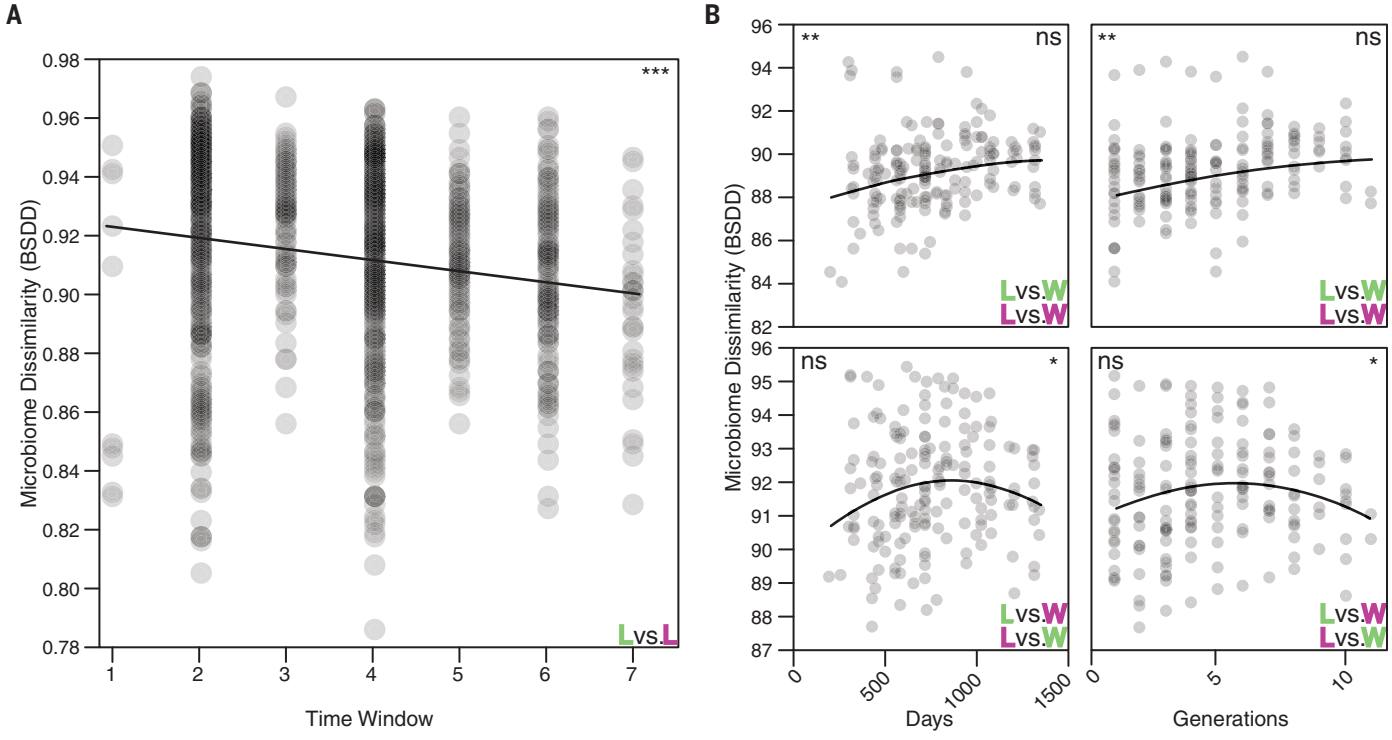

Fig. 2. Horizontal transmission of gut microbiota among mouse lines. (**A**) Dot plot displaying BSDDs between Tucson and Edmonton mice sampled in the same time period of the experiment. Each point represents a comparison between two laboratory mice descended from different geographical origins, denoted in the bottom left corner by the label "L vs. L," colored to correspond to Fig. 1. (**B**) Dot plots display the relationship between the number of days (left column of plots) or generations (right column of plots) in the laboratory and microbiota BSDDs between laboratory-bred mice and wild-caught line founders. The top row of plots displays comparisons between laboratory-bred mice and ancestral wild-caught line founders from the same geographical origin. The bottom row of plots displays comparisons between laboratory-bred mice and wild-caught line founders from the other geographical origin. "L" indicates laboratory-bred mice, "W" indicates wild-caught line founders, and colors correspond to Fig. 1. In (B), the curves indicate best-fit polynomial regressions, and the significance of nonzero linear and quadratic parameters is shown in the upper left and upper right corners of each plot, respectively. ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

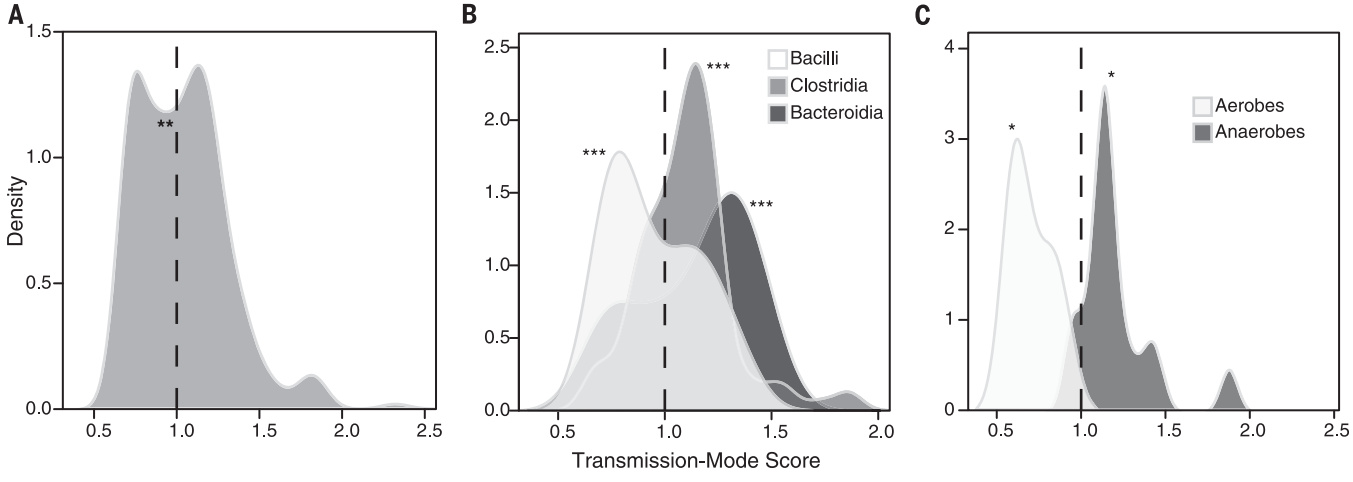

Fig. 3. Gut bacterial taxa follow divergent transmission strategies associated with evolutionary history and lifestyle. Kernal density plots show distributions of TM scores for (**A**) all bacteria; (**B**) the Bacilli, the Clostridia, and the Bacteroidia; and (**C**) obligate aerobes and obligate anaerobes. In (A) to (C), dashed lines denote a TM score of 1, and lighter to darker shades of gray indicate tendencies for horizontal and vertical transmission, respectively. The asterisks in (A) indicate the significance of Hartigan's dip test for unimodality. The distributions in (B) were all consistent with unimodality (Hartigan's dip test for unimodality, $P > 0.05$). Asterisks in (B) and (C) indicate the significance of t tests for mean TM scores different from 1. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

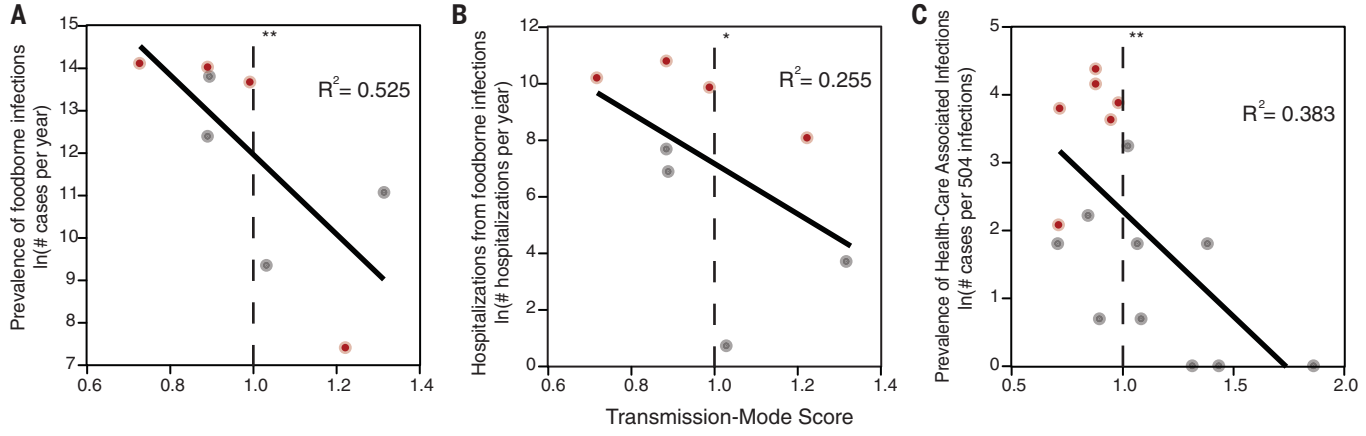

Fig. 4. TM scores predict infection and hospitalization rates for human pathogens. Dot plots display the relationships between the TM score and the number of foodborne infections (**A**), the number of hospitalizations from foodborne infections (**B**), and the number of HAIs (**C**) in the United States caused by bacterial genera as reported by (*18*) and (*19*). Red points indicate bacteria monitored by the Centers for Disease Control and Prevention's Emerging Infections Program. In (A) to (C), each point represents a bacterial genus, dashed lines denote a TM score of 1, and trend lines indicate linear regressions, with the significance of the nonzero slope denoted by * ($P < 0.05$) and ** ($P < 0.01$). R^2, coefficient of determination.

of gut bacterial lineages. However, spore-forming bacterial genera did not display any tendency for vertical or horizontal transmission ($P > 0.05$) (fig. S5).

Evolutionary theory suggests that transmission mode influences the evolution of virulence, with horizontal transmission favoring increased virulence relative to strict vertical transmission (*14*–*18*). To examine the relationship between TM score and virulence, we obtained epidemiological data on the number and severity of hospital-associated and foodborne infections in humans caused by gut bacterial genera in the United States. The genera currently monitored by the Centers for Disease Control and Prevention's Emerging Infections Programs displayed a mean TM score significantly less than 1 (Fig. 4) (*t* test *P* value = 0.0345). Moreover, the TM score was significantly associated with the number of infections and hospitalizations in humans caused by different genera. Linear regression analyses indicated that the TM score explained 52, 25, and 38% of the variance among genera in the rate at which they cause foodborne infections, hospitalizations from foodborne infections, and health care–associated infections (HAIs), respectively, in humans in the United States, on the basis of epidemiological data [(table 2 of (*19*) and table 3 of (*20*)]. Therefore, bacterial pathogens in humans belong to genera that appear to be adapted for transmission through the indoor environment. Previous work has shown that pathogenic bacteria are often generalists capable of persisting in both host-associated and external environments (*21*). Our results suggest that horizontally transmitted bacterial genera in mice (table S3) and humans are more likely to exhibit virulence than are vertically transmitted bacterial genera.

We conducted a long-term, multigenerational assessment of the transmission modes of bacterial genera in the mammalian gut microbiota. Our data showed that the majority of the murine gut microbiota was vertically inherited, such that the compositional identities of the gut microbiotas of individual mice were maintained for 10 generations of inbreeding in laboratory conditions. We also discovered that a minority of the gut microbiota was horizontally transmitted, leading to convergence in gut microbiota composition among mouse lines over time. Consistent with evolutionary theory, we found that the propensity for horizontal transmission of bacterial genera in mice is associated with the pathogenicity of the genera in humans, on the basis of epidemiological data (*19*, *20*).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/362/6413/453/suppl/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S3
References (*22*–*25*)

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Transmission of the gut community

Natural transmission of the mammalian microbiota is poorly understood. Some genera of bacteria are transmitted from mothers to offspring, whereas others are acquired from the wider environment. Moeller *et al.* derived inbred mouse lines from two wild populations of mice with distinct microbiota and monitored the populations' microbiomes for 3 years while they were kept in the same animal facility. The microbiota of the two mouse lineages remained distinct even after 10 generations. Most microbiota genera transmitted vertically. Those taxa that transmitted horizontally through the shared environment of the animal facility tended to be those that include pathogens.

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