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Early-life gut microbiota assembly patterns are conserved between laboratory and wild mice

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Assembly of the mammalian gut microbiota during early life is known to shape key aspects of organismal development, including immunity, metabolism and behaviour. While house mice (*Mus musculus*) are the major laboratory model organism for gut microbiota research, their artificial lab-based lifestyle could fundamentally alter ecological processes of microbiota assembly and dynamics, in ways that affect their usefulness as a model system. To examine this, here we directly compared patterns of gut microbiota assembly in house mice from the lab and from the wild, making use of a tractable, individually-marked wild population where we could examine patterns of gut microbiota assembly during early life. Despite lab and wild mice harbouring taxonomically distinct communities, we identify striking similarities in multiple patterns of their gut microbiota assembly. Specifically, age-related changes in both alpha and beta diversity, as well as the abundance of predominant phyla and aerotolerance of the microbiota followed parallel trajectories in both settings. These results suggest some degree of intrinsic programme in gut microbiota assembly that transcends variation in taxonomic profiles, and the genetic and environmental background of the host. They further support the notion that despite their artificial environment, lab mice can provide meaningful insights into natural microbiota ecological dynamics in early life and their interplay with host development.

The mammalian gut microbiota has diverse impacts on host biology with key roles in modulating processes ranging from metabolism to behaviour¹⁻³. The composition and diversity of this internal ecosystem varies between individuals, populations, and species and also within individuals over time⁴⁻⁶. The gut microbiota matures in early life, during which marked changes in composition and diversity occur⁷⁻⁹. This maturation is guided by both ecological interactions within the microbial community¹⁰ and selective forces imposed by the hosts' physiology as it undergoes key developmental changes including development of the immune and central nervous systems^{11,12}. Perturbation of the gut microbiota during this critical period has been linked to altered functioning of the immune system in early life^{13,14}, indicating an active role for the gut microbiota in host development. Understanding the patterns and drivers of this microbiota maturation process is therefore essential for a comprehensive understanding of host development.

At birth, the gut is first colonised by microbes from the mother (through birth and breastfeeding) and the environment, after which the microbiota matures following a stereotypical pattern in which the community becomes more diverse within individuals but more homogenous

across individuals^{15,16}. In humans, it is well-established that environmental context can alter the microbiota's maturation trajectory at various stages, with potentially long-lasting consequences^{13,14,17}. Delivery mode affects the transmission of maternal microbes at birth, with C-section delivery predisposing infants to greater colonisation by microbes with pathogenic potential from the hospital environment over specialist beneficial symbionts from the mother^{7,15,18}. Subsequently, gut microbiota maturation can be altered by care practices such as feeding mode (breastfeeding/formula) and the timing of breastfeeding cessation^{7,15,18}, which affect both the transmission landscape and selective forces shaping the developing microbiota. Microbiota maturation is also known to be sensitive to environmental influences later in childhood¹⁹⁻²¹.

The gut microbiota's stereotypical trajectory of maturation has been well-characterised in humans, but in other mammals and especially in wild contexts, it is far less well-known. Studies of gut microbiota assembly in wild primates have reported somewhat conflicting patterns, with research on gelada baboons finding patterns that largely reflect those in humans^{22,23}, while a study in wild chimpanzees reported declining microbiota richness during early life²⁴. This suggests the broad patterns of microbiota assembly

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observed in humans may not be universal among mammals, and may vary with host genetic background or environmental context. However, to our knowledge there are currently no studies of mammals other than humans which directly compare patterns of gut microbiota assembly across populations or environmental contexts, such that we currently do not know the extent to which context shapes gut microbiota assembly patterns for a given host species.

Whether or not gut microbiota maturation patterns are consistent or variable across contexts is a particularly pertinent question for laboratory-reared model organisms. The laboratory mouse is the major model species for fundamental and preclinical research on mammals, and aspects of its gut microbiota maturation have been studied in several laboratory strains^{25–32}. In line with patterns detected in other mammals, existing studies suggest that as lab mice mature, the gut microbiota increases in diversity within individuals while becoming more similar across individuals^{25,26,31,32}.

Importantly, the dynamics of gut microbiota assembly in wild house mice have not yet been characterised, such that it remains unknown whether gut microbiota assembly in laboratory mice is representative of that occurring in natural populations. In the wild, many factors that could affect both microbial colonisation and subsequent within-host selective processes will differ from and be far more variable than in a laboratory. These include which specific microbes are present, how and when mice are exposed to them, as well as diet and host genetics. As such, wild mice and their microbiota is likely to be more representative of humans than lab mice, whose microbiota has been altered through domestication in ways that have changed its structure and effects on host functions, such as immunity³³. Given the preclinical model status of the lab mouse and the known influence of the developing gut microbiota on so many aspects of physiology, understanding how accurately lab mice recapitulate basic patterns of microbiota assembly in their wild counterparts, is an important goal.

Here, using standardised microbiota profiling methods we directly compare the dynamics of gut microbiota assembly in young laboratory mice of the most commonly used strain (C57BL/6) and completely wild house mice sampled from the island of Skokholm, UK. In contrast to inbred lab mice living under consistently stable environmental conditions, this wild population are outbred and exposed to highly variable environmental conditions. Given the gut microbiota is thought to play a key role in developmental processes that should be conserved across these contexts, we hypothesised that despite some differentiation in community composition between lab and wild mice, some broad trends of microbiota assembly would be conserved.

Materials and methods

Sample collection

Faecal samples were collected from 39 individual C57BL/6J^{OlaHsd}Oxuni laboratory mice (*Mus musculus*) aged between 7 and 124 days (mean 39), in October 2021 at the Biomedical Services Building, Oxford, UK. The C57BL/6J^{OlaHsd} strain was imported to the breeding facility 17-years ago and has been maintained as a closed colony since that date. Mice were housed under specific-pathogen-free conditions, where various pathogenic taxa were excluded (Supplementary Table 1). Mice were not subject to any interventions before or during sample collection. Mice were housed in Tecniplast GM500 IVC cages at 19–24 °C as per the Home Office Code of Practice under ASPA 1986, provided Eco-Pure Aspe Chip 4 premium (Datesand, UK) and Sizzle nest (Datesand, UK) as bedding as well as a Mouse Play Tunnel S (Datesand, UK) as enrichment. Stocking density was a maximum of 5 individuals per cage. Mice were fed Teklad Irradiated Global 16% Protein Rodent Diet *ad libitum*. Mice were separated from dams at 19–22 days of age. Faecal samples were collected from a sterile surface on which mice were temporarily placed. Pellets were collected with sterile forceps, preserved in DNA/RNA Shield (Zymo Research, USA) and stored at –80 °C until DNA extraction (≤12 months).

Wild house mice (*Mus musculus domesticus*) were live-trapped on Skokholm Island (UK; Fig. S1) in September–October 2019 and August–September 2020. House mice have been living relatively

independent from humans on Skokholm since their introduction around the turn of the 20th century, and there are no other small mammals on the island^{34,35}. Trapping was carried out in two sampling sites on the island (Fig. S1) using small Sherman traps provisioned with peanuts, non-absorbent cotton wool for bedding, and with a spray of sesame oil outside the trap used as a lure. On each trapping night, 150 traps were set at dusk and checked at dawn at one of the sampling sites. Visited traps (where signs of a mouse were detected, whether captured or not) were washed and sterilised with 20% bleach solution before re-use. Captured mice were tagged through subcutaneous injection of passive integrated transponder (PIT) tag (diameter: 2.12 mm, length 12 mm) for permanent identification, or identified through PIT tag detection upon recapture. All captures were therefore individually identified, sexed, scored for reproductive status, and weighed to the nearest 0.1 g before release at their trapping point. Sex was determined based on measurement of anogenital distance. Faecal pellets were collected from traps, and preserved in DNA/RNA Shield. Samples were stored at –20 °C during fieldwork (≤6 weeks), after which they were transported frozen to the laboratory and stored at –80 °C until DNA extraction (≤17 months).

Since birthdate is unknown in our wild mouse study system, and a lab-based epigenetic clock fails in accurate prediction of chronological age³⁶, these wild mice cannot be precisely aged with currently available methods. Because of this, we used body mass as a proxy for age, as among young individuals it has been shown to predict age relatively accurately in both wild and laboratory mice (Fig. S2)^{37–40}. For this purpose, samples from wild mice for which body mass had not been recorded were removed. Female mice showing signs of pregnancy (bulging central body; $n = 15$) were excluded, as these were associated with heavier body mass. We also excluded female mice ≥ 25 g ($n = 65$), with the aim of excluding pregnant females that may not have been recognised as such. Applying these criteria resulted in a set of 433 faecal samples from 230 individual wild mice (1–10 samples each; mean 2 samples per animal; Fig. S3, Supplementary Data 1).

Ethical statement

We have complied with all relevant ethical regulations for animal use. Laboratory mice sampled in this study were maintained at the Medical and Scientific Departments of the University of Oxford (Establishment licence number XEC303F12). Wild mouse data collection was done under Home Office licence PPL PB0178858 held at the University of Oxford, and with a research permit from the Islands Conservation Advisory Committee (ICAC), and Natural Resources Wales. Apart from PIT-tagging, mice in this study were not subject to intervention.

DNA extraction, library preparation, and sequencing

DNA extraction was performed using the ZymoBIOMICS DNA MiniPrep Kit (spin-column format) according to manufacturer's instructions (Zymo Research, USA). Samples were randomised into 53 extraction batches of up to 23 samples. A negative extraction control (DNase-free H₂O) was included in each extraction batch at a varying position with the exception of one extraction batch, in which a negative control was not included. The microbiota of each faecal sample was characterised using amplicon sequencing, targeting the V4–V5 region of the 16S rRNA gene using primers 515F and 926R^{41,42}. Library preparation was conducted in 16 plates of up to 95 samples. Amplicons were sequenced in four sequencing runs using the Illumina MiSeq platform (Reagent kit v3, 2 × 300 bp chemistry). Library preparation and sequencing was conducted at the Integrated Microbiome Resource, using the protocol described in Comeau et al. (2017)⁴³. Each sequencing run included a PCR reaction negative control and a separate negative control for sequencing. All extraction controls were sequenced intermixed with true samples.

Data processing

Data processing and subsequent analyses were carried out using R v4.1.2. Demultiplexed sequencing reads were processed through the DADA2 pipeline⁴⁴ to infer amplicon sequence variants (ASVs) and assign taxonomy

using the SILVA rRNA database v138.1. A total of 41 unique ASVs were detected across all negative DNA extraction and library preparation controls ($n = 66$), with a maximum read count of 363 for any given control, with the exception of one library preparation control. This control contained 255 unique ASVs and 29,300 reads (mean read count of biological samples was 29,235). All extraction controls ($n = 5$) from this control's 96-well plate had <20 reads each, indicating the entire plate was not contaminated during library preparation (negative controls were included on the plate in a randomised order, thus several rows and columns had negative controls). Instead, the most likely explanation is that a biological sample was mistakenly pipetted into the control well in addition to its designated well. As all extraction controls on this plate showed very low read counts indicating no systemic contamination, we retained this plate of samples in our analyses.

We tested for the presence of any potential contaminants using the R package *decontam*⁴⁵ using the 'prevalence' method, in which the prevalence of each sequence in biological samples is compared to that in negative controls (excluding the library preparation control that was contaminated with a biological sample, see above). A sequence was considered a contaminant if it reached a probability of 0.1 in the Fisher's exact test used in *decontam*. 31 contaminants were identified and filtered from the data before subsequent analysis. These contaminants were from families Aeromonadaceae, Azospirillaceae, Beijerinckiaceae, Burkholderiaceae, Comamonadaceae, Cryomorphaceae, Erysipelotrichaceae, Lachnospiraceae, Moraxellaceae, Muribaculaceae, Oscillospiraceae, Rhizobiaceae, Sutterellaceae, Weeksellaceae and Xanthomonadaceae, with each of the families being associated with 1–3 of the identified contaminant ASVs with the exception of Muribaculaceae, which associated with 10 contaminant ASVs. While the source of these contaminants cannot be confirmed, they could have originated from laboratory kits used when processing samples.

ASVs assigned as chloroplast were removed. The package *iNEXT*⁴⁶ was used to generate sample completeness and rarefaction curves separately for lab and wild mouse datasets. Based on these curves, lab and wild mouse samples with a read depth below 7500 and 4000, respectively, (where the curves plateaued) were excluded from further analysis. *iNEXT* was further used to measure asymptotic observed ASV richness and Shannon diversity. In each of the lab and wild mouse datasets separately, singleton and doubleton ASVs were subsequently removed to reduce the possible effect of contamination as well as PCR and sequencing errors. ASV counts were normalised to proportional abundance using package *phyloseq*⁴⁷. A phylogenetic tree was built using packages *DECIPHER* and *phangorn*. Information on bacterial aerotolerance was extracted from Bergey's Manual of Systematics of Archaea and Bacteria or additional references when this information was not available in the manual (Data S2). Aerotolerance was determined based on genus-level taxonomy, unless aerotolerance information was not available or genus-level taxonomy not assigned, in which case family-level information was inspected and used if all genera within a family were stated to have the same aerotolerance. Bacteria were categorised as *aerotolerant* or *obligate anaerobe*. If a given genus included both obligate anaerobes and aerotolerants, a Nucleotide BLAST search was conducted for the associated ASVs and if a 100% species identity match was found, aerotolerance category was determined based on this species.

Functional profiles

Functional pathways were predicted from the 16S rRNA data using PICRUSt2, pipeline v 2.5.0⁴⁸. Read counts of pathways were normalised to proportional abundance. Functional profiles were characterised at pathway-level only, as functional profiles characterised at category level varied depending which database was used to assign functional categories (MetaCyc vs KEGG ortholog). Data presented is based on MetaCyc pathways.

Statistics and reproducibility

Principal coordinate analysis was used to investigate the effect of source on microbiota composition. To explore different aspects of compositional microbiota variation in these analyses we used four different measures of

beta diversity: Jaccard, Aitchison, unweighted UniFrac and weighted UniFrac distances. Beta diversity metrics were calculated with packages *vegan*⁴⁹ and *microbiome*⁵⁰. Statistical differences in alpha and beta diversity were tested with Wilcoxon rank sum tests (with 1000 permutations for beta diversity analyses).

Predictors of beta diversity variation were investigated using marginal permutational multivariate analysis of variance (PERMANOVA) run using the *adonis2* function in *vegan*⁴⁹. Quadratic plateau models run in package *easynls*⁵¹ were used to assess how microbiota alpha diversity, and gut microbiota maturity (distance to a juvenile reference gut microbiota) changed over time. To enable direct comparison of age-related patterns across systems in these models, changes in microbiota traits over time in these models and their associated figures are presented as a function of body mass. Although individual body mass measurements were not available for the laboratory mouse dataset, age is tightly correlated with mass among juvenile lab and wild house mice^{32,37}, such that we used estimated body mass values for lab mice from published age-mass relationships (Fig. S2). For mice ≤ 21 days of age, body mass was estimated using mean body mass for each unit of age reported in Fig. 3 in Spangenberg et al. (2014)³⁷ (no separation based on sex). For mice > 21 days of age, body mass was estimated separately for male and female mice by using the mean body mass for each unit of age reported in 'Body weight information for C57BL/6J' table on The Jackson Laboratory website³⁸. Here, mean standard deviation for mice aged 3–18 weeks (oldest sampled lab mouse in our study was 124 days old) is reported to be ± 0.9 – 2.5 grams for females and ± 1.4 – 2.6 for males³⁸.

The reference community of early life microbiota was estimated separately for laboratory and wild mice. This estimation was based on the average abundance observed in mice that were under 15 days of age (estimated age for wild mice). This corresponds to an estimated body mass ≤ 6.5 g for lab mice, and a measured body mass ≤ 7.9 g for wild mice^{37,40}. The choice of age cut-off (14 days) was made by identifying the youngest age at which there were at least six samples available in both laboratory and wild mouse data. With this cut-off, 6 lab mice (body mass range 3.5–6.5 g, mean 5.4 g) and 16 wild mice (body mass range 5.4–7.8 g, mean 6.9 g) were included in estimating the reference communities. Relative abundances of taxonomic groups or phenotypic subsets of bacteria were modelled and plotted as a function of body mass using locally weighted scatterplot smoothing (LOESS) regression in package *ggplot2*⁵².

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results

After data processing, a total of 7821 ASVs were recovered across lab and wild mice. Taxonomic assignment rates were comparable across the two systems, indicating lab and wild mice have similar numbers of identifiable bacterial genera (89.9% and 84.3% ASVs assigned to family, 50.0% and 52.3% assigned to genus, for lab and wild mice respectively). The lab mouse colony (strain C57BL/6, a single animal facility) is representative of several other lab mouse strains sampled in various animal facilities in terms of gut microbial diversity and composition⁵³. Similarly, the gut microbiota of the wild mouse population (Skokholm Island, Wales) is representative of that of other wild house mouse populations from various mainland and island locations⁵³.

Laboratory and wild mice have distinct gut microbiotas

To facilitate statistical comparisons between the two settings, we compared gut microbiota of lab and wild mice using a randomly selected cross-sectional subset of the data (39 samples from each system). In this subset, only 18.5% ASVs (391 of 3183) were detected in both lab and wild mice. However, these shared taxa were common and abundant in both lab and wild mice, comprising on average 41.9% of the wild mouse microbiota and 44.7% of the lab mouse microbiota (standard deviation 11.1% and 20.1%, respectively). Wild mice had higher alpha diversity, both in terms of

estimated ASV richness and Shannon diversity (Wilcoxon rank sum tests, ASV richness: $p < 0.001$, Shannon diversity: $p = 0.006$, Fig. 1A, B). Lab and wild mouse gut microbiotas were broadly similar at the phylum level, but showed clear differences in composition at lower taxonomic levels (Fig. 1C, D). Compositionally, samples clustered by source using both non-phylogenetic and phylogenetic distance metrics and using various levels of taxonomic resolution (Figs. 1E, S4). In line, source explained a considerable amount of compositional variation in the dataset overall (10.7% in Jaccard dissimilarity, PERMANOVA, $F = 9.157$, $p = 0.001$; beta dispersion, $F = 2.519$, $p = 0.096$; 17.2% in Aitchison dissimilarity, $F = 15.770$, $p = 0.001$; beta dispersion, $F = 68.872$, $p = 0.001$). Wild mouse individuals were much more variable in microbiota composition than lab mice, although lab–wild sample pairs had the highest dissimilarity (Fig. 1F, all permutational Wilcoxon rank sum tests $p < 0.001$).

Conserved taxon-independent gut microbiota assembly patterns

As birthdates of wild mice are unknown and even modern epigenetic age estimates do not yet capture chronological age very well³⁶, we used body mass as a proxy of age. Previous studies have shown that in the first 30 days of life the relationship between age and body mass is very similar in lab, wild and wild-derived but lab-housed *Mus musculus*^{37–40} (Fig. S2). Body mass was strongly associated with alpha diversity in both lab and wild mice, with ASV richness increasing to a plateau in both systems. Parameters from quadratic plateau models indicated richness plateaued at 281 ASVs in adulthood among wild mice, and at 213 ASVs in lab mice (maximum values of γ). In lab mice, the plateau in ASV richness was estimated to occur at a body mass of 13 grams (Fig. 2A), or 27 days of age (Fig. S2). The plateau in ASV richness was more subtle in wild mice, and occurred later around 21 grams (Fig. 2B). Quadratic plateau models fit the relationship between body mass and alpha diversity better than linear models in both lab and wild mice, albeit with a smaller improvement to fit for wild mice (*lab*: $\Delta AIC = 25.12$; *wild*: $\Delta AIC = 2.13$).

Body mass was also associated with gut microbial composition in both systems. We investigated gut microbiota maturation by measuring each sample's compositional distance to a "reference microbiota of a juvenile mouse" (see Methods). Distance to the early life microbiota in each system increased with age, with a plateau reached at around 13 g body mass in both systems (corresponding to ~27 days of age in both lab and wild mice; ΔAIC vs linear model: *lab* 35.14, *wild* 79.53; Fig. S2; Fig. 1C, D). Highly comparable patterns were detected when including only one sample per wild mouse (Fig. S5). Microbiota variability among individuals decreased with age in both systems, although the pattern showed a more linear decline with increasing body mass in wild mice than lab mice (Fig. 2E).

Conserved taxon-dependent gut microbiota assembly patterns

Since the major bacterial phyla were conserved across lab and wild mice (Fig. 1C), we compared early life dynamics of the three predominant phyla (Firmicutes, Bacteroidota, and Proteobacteria). These phyla displayed very similar early life trends in both systems, with the relative abundances of Firmicutes (now known as Bacillota) and Proteobacteria (now known as Pseudomonadota) decreasing and that of Bacteroidota increasing during early life (Fig. 3A, B). The ratio between Firmicutes and Bacteroidota relative abundance became more equal at around 10–15 grams for both lab mice and wild mice. The microbial family Muribaculaceae (previously known as S24–7; unknown genus-level taxonomy) was a key driver of Bacteroidota relative abundance increase in both lab and wild mice (Fig. S6C, D). Declines in the relative abundance of Firmicutes appeared to be driven by a decline in the genus *Ligilactobacillus*, particularly in lab mice (Fig. S6A, B). In addition to *Ligilactobacillus*, taxa from *Limosilactobacillus* and Lachnospiraceae NK4A136 group were key drivers of the reduction in Firmicutes in wild mice (Fig. S6B).

The relative abundance of Proteobacteria decreased in both systems and stabilised at around 10 g estimated body mass for lab mice and 15 g body mass for wild mice (Fig. 3A, B). In both systems, this reduction in Proteobacteria was driven by taxa belonging to the Enterobacteriaceae

family (namely *Escherichia/Shigella*, *Muribacter* and an unknown genus in Enterobacteriaceae; Fig. S7). Finally, shared patterns were also observed in within-phylum ASV richness. In both lab and wild mice, ASVs richness within the phyla Firmicutes and Bacteroidota increased in early life while Proteobacteria ASV richness remained consistently low (Fig. 3C, D). Similar phylum-specific age patterns were also observed when only ASVs that were unique to each system were included (Fig. S8).

Age-related changes in gut microbial phenotypes and functional pathways

As the mammalian gut becomes increasingly anoxic in early life⁵⁴, we hypothesised that the ratio between aerotolerant and anaerobic bacteria would shift in early life, favouring anaerobes. In lab mice, we detected a clear age-related pattern in bacterial aerotolerance consistent with this expectation. Aerotolerant bacteria initially dominated the gut microbiota but declined in relative abundance while anaerobes increased, eventually dominating over aerotolerant taxa in relative abundance by a ratio of approximately 3:1 by ~15 g body mass (Fig. 3E). In wild mice, we similarly observed an increase in anaerobes and a decrease in aerotolerant taxa, although the pattern was less pronounced. Further, aerotolerant taxa retained a higher relative abundance in wild compare to lab mice in adulthood (mean relative abundance of aerotolerant taxa in mice >20 g: 34.1% in wild vs 23.1% in lab; Fig. 3F). Given the finding of a clear compositional shift in lab mice during the weaning period, we re-assessed trends in phylum composition and aerotolerance in lab mice according to whether they had been separated from dams and thus were eating an entirely solid food-based diet. This indicated that much of the observed age-related gut microbial changes we observed occurred before pups were separated from dams, and therefore fully weaned (Fig. S9).

Finally, we investigated predicted functional profiles of the gut microbiota. The profile of functional pathways showed a clear shift at around 8 g body mass (17d of age) in lab mice (Fig. 4C). This shift occurred 2–5 days before pups were separated from their mother and coincided with a clear compositional shift in the bacterial genera present (Fig. 4A). No clear shift in overall functional profile or bacterial genus composition was detected in wild mice (Fig. 4B, D). We further considered which functional pathways showed the greatest fold-change in relative abundance between juvenile (≤ 7 g) and adult mice (>20 g) in each setting, and how their relative abundance changed with body mass (Fig. S9). Interestingly, none of the pathways identified in this way overlapped between lab and wild mice. Moreover, pathways with the largest age-related fold-changes in lab mice often showed distinct patterns of change with body mass in lab and wild mice, for example often showing an initial decrease rather than increase with body mass (Fig. S10A, B). Similarly, pathways that showed the greatest age-related fold-change in wild mice typically did not show similar mass-related patterns in lab mice, with the exception of PWY-5971 (Palmitate biosynthesis II) which showed an initial decrease followed by an increase with body mass in both settings (Fig. S10C, D).

Discussion

Considering laboratory mice are widely used in research on the gut microbiota and its links to host physiology, we set out to investigate how well the dynamics of gut microbiota maturation in lab mice represent those occurring in wild members of the same species whose microbial exposure patterns more closely resemble those of humans. Several key patterns in microbial community assembly were conserved across settings, despite wild mice having higher microbiota richness and distinct taxonomic composition, as reported in previous studies comparing adult lab and wild mice^{33,55–57}.

Our results illustrate how in both the lab and the wild, the gut microbiota of house mice matured during the first month of life. During this time, the microbiota increased in alpha diversity, changed in taxonomic composition, and became more homogenous in composition among individuals. These patterns resemble those of gut microbiota maturation reported for humans and wild gelada baboons^{15,23,25,58,59} but contrast those

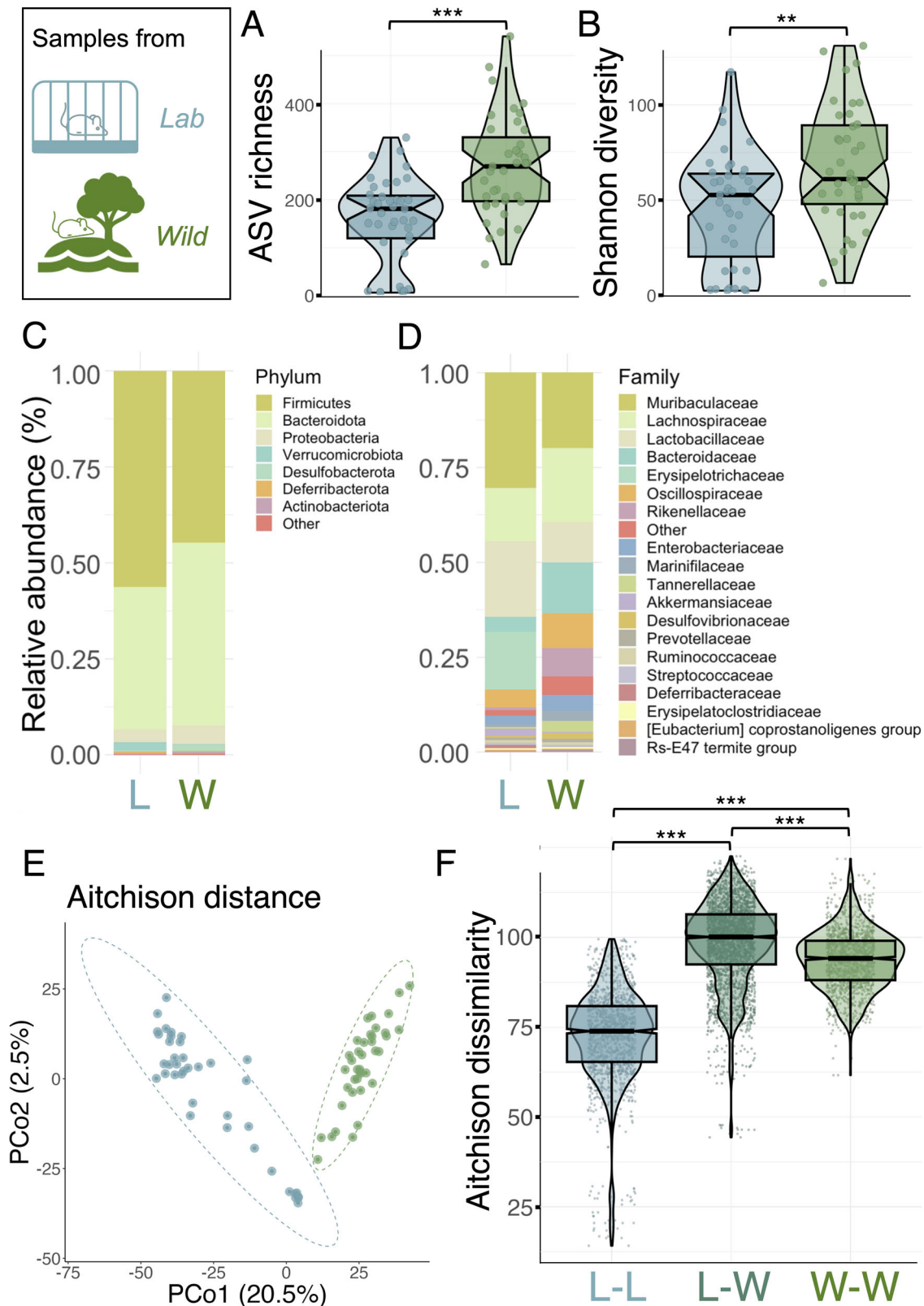


Fig. 1 | Comparison of laboratory and wild mouse gut microbiotas using faecal samples from 39 laboratory and 39 wild mice (1 randomly selected sample per mouse). Alpha diversity; (A) asymptotic ASV richness and (B) asymptotic Shannon diversity. Statistical differences between lab and wild mice were tested with Wilcoxon rank sum tests (***, $p < 0.001$; **, $p < 0.01$). Mean relative abundance of bacterial (C) phyla and (D) families in laboratory (L) and wild (W) mice. E Principal

coordinates analysis (PCoA) on Aitchison distance. F Pairwise comparison on gut microbial dissimilarity on Aitchison distance in lab–lab (L–L), lab–wild (L–W) and wild–wild (W–W) sample pairs. Statistical differences between lab and wild mice were tested with permutational Wilcoxon rank sum tests (***, $p < 0.001$). Colour in (A, B and E, F) indicates sample source: blue = laboratory, green = wild, teal = lab/wild.

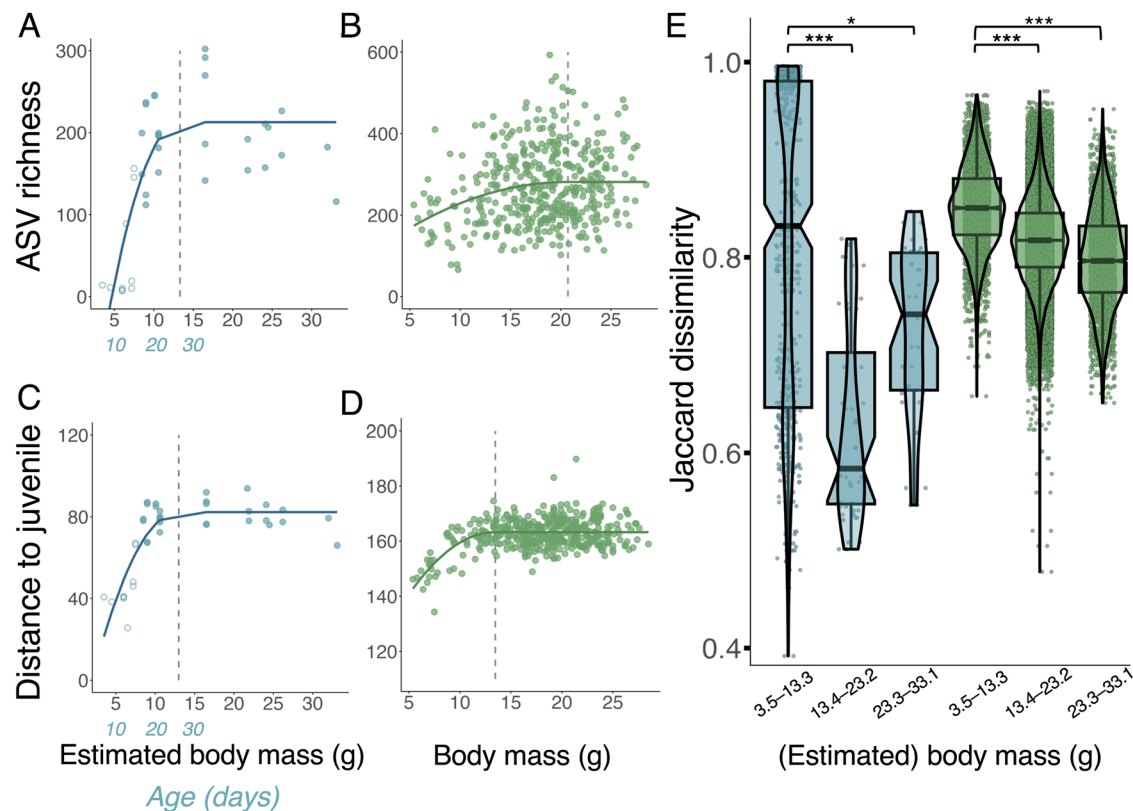


Fig. 2 | Taxon-independent trends in laboratory (blue) and wild house mouse (green) gut microbiota alpha and beta diversity using body mass as a proxy of age. Body mass ranges 3.5–33.1 g for lab mice and 5.4–28.5 g for wild mice. Chronological age is shown in blue for the first 30 days for lab mice, in which age–body mass relationship is very clear and not yet strongly influenced by sex differences. Circles in A and C indicate maternal separation status (*hollow* = not yet separated, *filled* = separated). **A, B** Quadratic plateau models on asymptotic ASV richness. Vertical lines indicate critical points of plateau. **A** Lab mice: $R^2 = 0.596$, critical point of inflexion = 13.3 g. **B** Wild mice: $R^2 = 0.049$, critical point of inflexion = 20.7 g. **C, D** Quadratic plateau models on the relationship between Aitchison distance to reference juvenile microbiota and body mass. Reference juvenile microbiota was

measured separately for laboratory and wild mice by taking the mean of all taxon abundances in mice ≤ 14 days of age (laboratory mice, $n = 6$; body mass ≤ 6.5 g), or ≤ 7.9 g of body mass (wild mice, $n = 16$), which is the estimated equivalent of 14 days of age in wild mice. Vertical lines indicate critical points of inflexion. **C** Lab mice: $R^2 = 0.709$, critical point of inflexion = 13.0 g. **D** Wild mice: $R^2 = 0.249$, critical point of inflexion = 13.5 g. **E** Beta diversity (based on Jaccard distance) in lab and wild mice. Samples were assigned into classes by dividing the range of body masses across all (lab and wild) samples into three at equal intervals: 3.5–13.3 g, 13.4–23.2 g, and 23.3–33.1 g. Significance was tested with permutational Wilcoxon rank sum test (***, $p < 0.001$; *, $p = 0.002$).

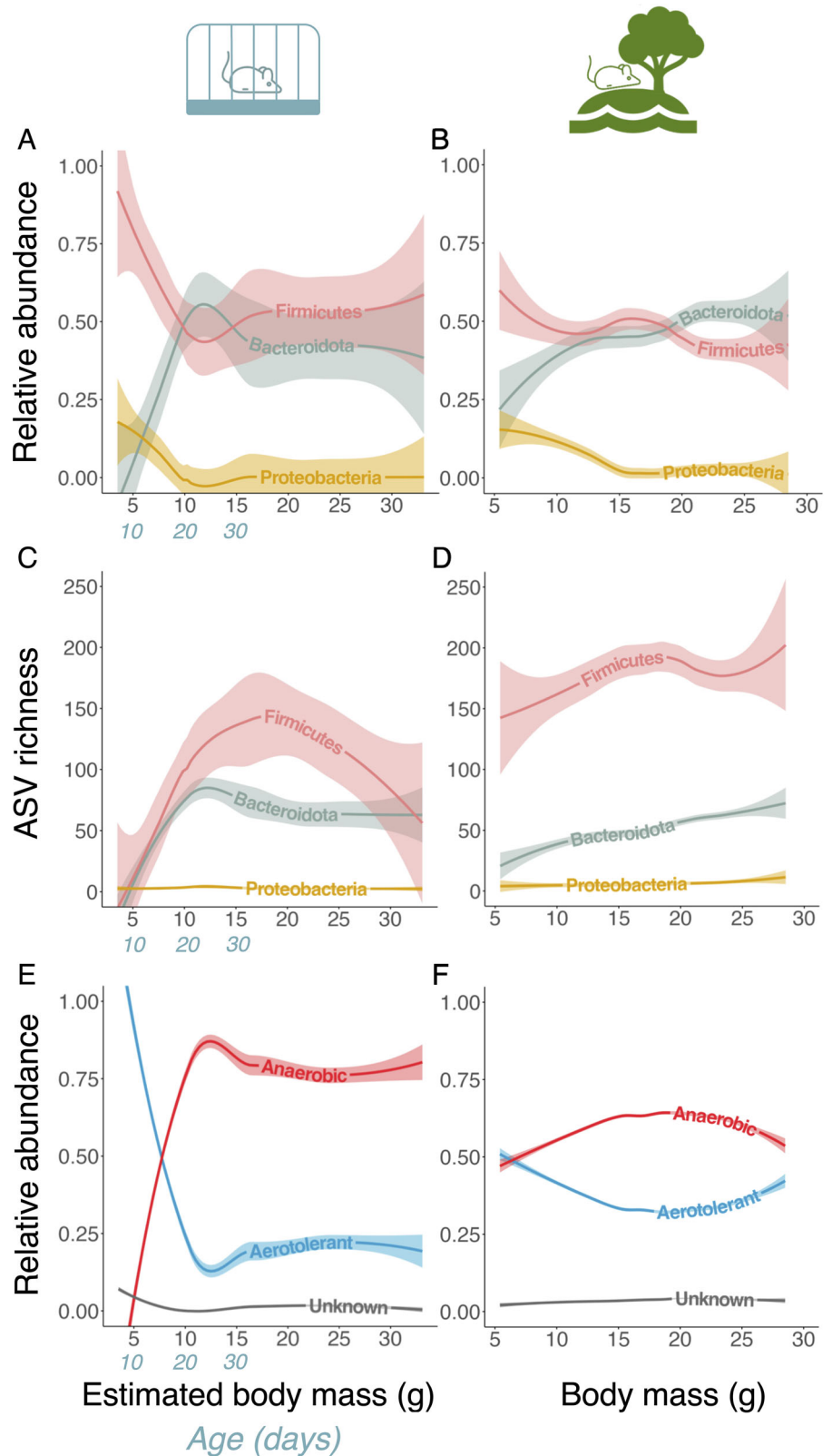
reported in a study of wild chimpanzees, where richness declined during early life²⁴. As previously documented in humans^{15,60}, anaerobic bacteria also increased in relative abundance during early life, likely because they are best adapted to the increasingly anoxic conditions of the developing gut.

Despite having taxonomically distinct gut microbiotas at nearly all levels examined, phylum-level composition remained similar among lab and wild mice, as reported by previous studies^{33,56}. We show that these phyla also show remarkably consistent age-related changes in relative abundance and richness, irrespective of whether mice were lab-housed or wild. Moreover, while shared taxa (ASVs detected in both lab and wild mice) made up nearly half the gut microbiota in both settings, age-related patterns in phylum relative abundances and richness were unchanged when only taxa unique to each study system were analysed. These consistent taxonomic shifts are thus not solely driven by a set of shared ASVs, but appear to reflect general trends occurring in members of these phyla. In both systems, the ratio between Firmicutes and Bacteroidota decreased as young mice matured, coinciding with an increase in the ratio of anaerobic to aerotolerant bacteria. The decrease in Firmicutes relative abundance with age was primarily driven by declining *Ligilactobacillus* in lab mice and *Ligilactobacillus* and *Limosilactobacillus* in wild mice. These aerotolerant taxa have been detected in the milk of various mammal species including mice^{61–64} and may be transmitted by lactation, such that their reduction may be driven by declining milk consumption in weaning mice⁶⁵. *Ligilactobacillus* had remarkably low relative abundance in wild mice of all ages, which

would be expected if this taxon was primarily transmitted through lactation, as wild mice must have been either weaning or fully weaned to be trapped in this study. The age-related increase in Bacteroidota relative abundance was largely driven by Muribaculaceae in both systems. These are anaerobic fermentative bacteria with a broad repertoire of carbohydrate metabolising capabilities⁶⁶ that appear to be socially and vertically transmitted in wild wood mice^{67,68}. While we can only speculate on the mechanisms underlying this increase, two possibilities are increased consumption of a plant-based diet and increasing social interaction as young wild mice mature.

Changes in the relative abundance and richness of proteobacterial taxa were also very similar in lab and wild mice. Consistent with documented patterns in humans, primates, and mice^{15,22,26,69}, the relative abundance of Proteobacteria decreased with age, with Enterobacteriaceae playing a prominent role in this trend. The precise drivers of this pattern remain to be explored, but could include declining oxygen levels in the gut (as Enterobacteriaceae are facultative aerobes⁷⁰), or immune maturation, as this family harbours several genera with pathogenic potential, and early life immune maturation should increase the host's ability to suppress such pathogenic taxa. Indeed, *Escherichia*, a genus containing well-known pathogenic species, was a key driver of the early-life decline in Enterobacteriaceae among lab mice. Interestingly, although broad patterns of diversity and taxon relative abundances often showed parallel age-related changes in lab and wild mice, age-related differences in predicted functional pathways appeared to vary across settings (Figs. 4, S10). This suggests that

Fig. 3 | Age-related gut microbial dynamics in lab (left) and wild (right) mice using body mass as a proxy of age. Body mass ranges 3.5–33.1 g for lab mice and 5.4–28.5 g for wild mice. Chronological age is shown in *blue* for the first 30 days for lab mice, in which age–body mass relationship is very clear and not yet strongly influenced by sex differences. **A, B** Relative abundance of predominant phyla (only top three phyla with highest total abundance in both systems are presented; abundances were measured from all taxa). **C, D** ASV richness (total count of unique ASVs) in predominant phyla. Lines are locally estimated scatterplot smoothing (LOESS) lines with 95% confidence interval bands. Relative abundance of aerotolerant and obligate anaerobic bacteria across body mass in **(E)** lab and **(F)** wild mice respectively. Lines are locally estimated scatterplot smoothing (LOESS) lines with 95% confidence interval bands. *Anaerobic* = obligate anaerobes, *aerotolerant* = everything else with known aerotolerance, *unknown* = bacteria with unknown aerotolerance.



differences in gut microbiome assembly between lab and wild mice may still have functional consequences, a possibility that warrants future investigation with dedicated metagenomic and metatranscriptomic approaches.

While several broad patterns of alpha and beta diversity change during early life were conserved among lab and wild mice, microbiota maturation appeared to be more gradual among wild mice. In lab mice, alpha diversity

reached a plateau rather abruptly at the same time compositional maturation was completed at around 27 days of age. In wild mice, however, alpha diversity appeared to increase up to a body mass of 20 g, at which point wild mice are expected to be ~40–80 days old. Several factors may underlie the more gradual patterns of gut microbiota maturation observed in wild mice. One possibility is that weaning (gradual transition from a purely milk-based

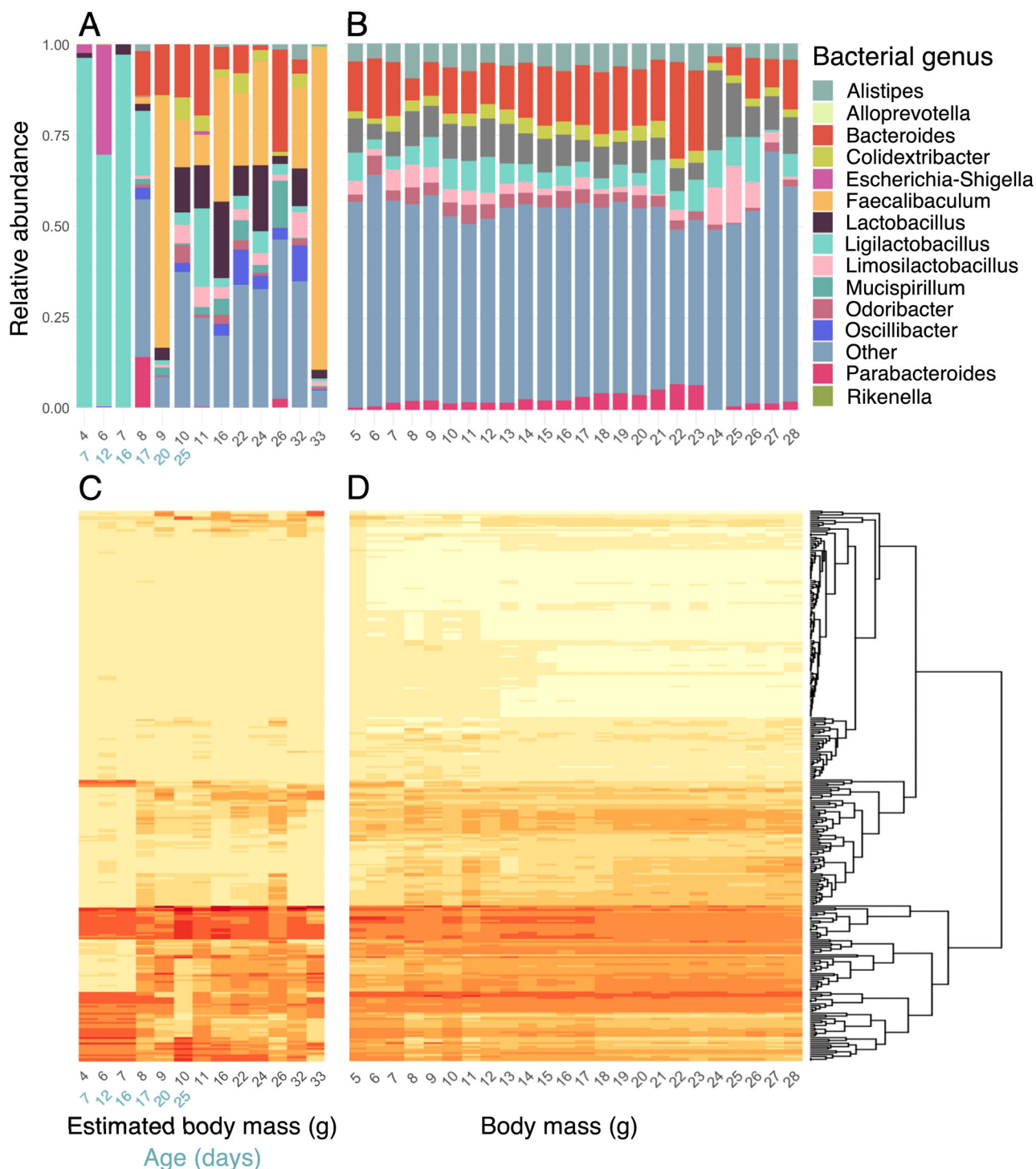


Fig. 4 | Composition of bacterial genera and functional pathways in laboratory and wild mice. Body mass (used as a proxy of age) ranges 3.5–33.1 g for lab mice and 5.4–28.5 g for wild mice. Chronological age is shown in blue for the first 30 days for lab mice, in which age–body mass relationship is very clear and not yet strongly influenced by sex differences. Mean relative abundance of bacterial genera across each available unit of (estimated) body mass in (A) lab and (B) wild mice. Mean

relative abundance of predicted functional pathways across each available unit of age/body mass in (C) lab and (D) wild mice. Rows are unique MetaCyc pathways ordered by hierarchical clustering as indicated with dendrogram. Colour darkness increases with relative abundance (range 0.0–2.7%).

to a purely solid food diet), a crucial process driving gut microbiota maturation in mammals^{15,22,71}, was captured more completely in our lab compared to wild mouse dataset. Laboratory mice have been shown to start nibbling solid food from 12 days of age⁷² and have been shown to wean between days 17 and 22 of age⁷³. While alpha and beta diversity in the lab plateaued a few days after pups were separated from their mothers, shifts in

microbiota composition and diversity had already begun before the expected start of solid food consumption and several aspects of maturation were completed before maternal separation. In particular, the lab mouse gut microbiota displayed a clear shift in predicted microbiota functions at 17d of age when weaning is expected to have ramped up, which coincided with a marked shift in the microbial genera present, including a stark decrease in

milk-associated *Ligilactobacillus*. Together these patterns support previous findings that the introduction of solid food or cessation of milk consumption alone can induce certain changes^{15,22,31,71}, but also suggest that the overall maturation of the gut microbiota is driven by the gradual transition from a milk-based diet to a solid food diet.

In our study, the lab dataset included mice still on a purely milk-based diet, while wild mice included were independent enough to be trapped and thus expected to be either fully weaned or in the late stages of weaning. As such, we may have captured only the tail-end of gut microbial maturation in wild mice, which could contribute to the maturation appearing more gradual in the wild, and explain why the age-related functional shift was more pronounced and rapid in lab mice. Further studies where wild/semi-wild pups can be sampled in nests, allowing capture of the full successional process, would be useful to investigate whether and when a similar functional shift occurs in more natural settings, and whether gut microbiota assembly is indeed more gradual in the wild. Further, our analysis of functional profiles was limited by the approach used (predicting functional pathways from 16S rRNA data). Profiling microbial function from metagenomic sequencing data would provide a more accurate and comprehensive identification of functional genes and metabolic pathways, and any functional parallels or differences during microbiome assembly in lab and wild mice.

Another factor that could contribute to more gradual trends of microbiota maturation among wild mice is differing patterns of microbial exposure. Highly controlled lab conditions (including confined space, pathogen exclusion, a small number of cage mates, and a homogeneous chow diet) should lead to a restricted pool of microbes available for colonisation, which could generate a faster saturation of alpha diversity as the available microbial pool is explored. In contrast, young wild mice should interact with a much more dynamic, variable microbial landscape as they disperse, consume a variable diet, and establish their adult home ranges and social relationships. This could extend their exposure to a variety of environmental and conspecific microbial reservoirs, potentially contributing to a more gradual rise in alpha diversity during early life.

Finally, due to unknown birthdates of wild mice sampled here, we used body mass as a proxy of age. Body mass is an accurate predictor of age across wild and lab mice in juvenile mice, but decreases in accuracy after first month of life (at which point mice weigh >15 g). Variation in the relationship between body mass and age is observed within both lab and wild mice. Notably, the relationship is expected to vary more among wild mice, as nutritional and infection status as well as exposure to environmental stressors (such as weather) vary more in the wild. These factors will also vary across wild populations, such that prediction of age based on age–body mass relationship from another study system (as done here) may increase error in mass-derived age estimates. As such, our findings (particularly those from mice >15 g body mass, such as the exact age at which maximal alpha diversity is reached) may be influenced by the inaccuracy of body mass as a proxy of age.

Another notable difference in the early life gut microbiota of lab and wild mice involved aerotolerance phenotypes of the constituent bacteria. In both settings the relative abundance of anaerobes increased while that of aerotolerant bacteria declined with age. This pattern was again much more striking in lab mice than wild mice, while in wild mice anaerobes had higher relative abundance than aerotolerant taxa after brief dominance of aerotolerant taxa in very early life. This may again be largely due to a lack of unweaned mice in our wild dataset, such that the earliest part of microbiota maturation (when the gut is dominated by aerotolerant taxa) was not unobserved. Once maturation was complete, however, lab and wild mice showed a clear difference in the ratio of aerotolerant to anaerobic microbes, with the relative abundance of aerotolerant gut microbes remaining much higher in wild than lab mice. Several factors may underlie this difference. The abundant aerotolerant taxa in wild mice were not obviously pathogenic species, primarily comprising of members of the families Bacteroidaceae and Lactobacillaceae. As such, increased pathogen exposure in the wild does not appear to explain this difference. However, differences in microbial

exposures (if wild mice are exposed to a greater number of aerotolerant microbial taxa that persist in their complex natural environment) or diet differences (if lab mouse chow favours a more anaerobic fermentative environment than would occur in wild mice) could both play a role. Further work to characterise the diet of wild mice, and microbes in their abiotic environment could shed further light on this interesting difference.

Together, our finding that several major trends in gut microbiota assembly are conserved across lab and wild mice indicates that to a large extent, the broad patterns of gut microbiota maturation follow an intrinsic host programme that transcends contrasting genetic and environmental backgrounds, and the existence of different component species. Our results therefore indicate that meaningful insights can be drawn from lab models to understand the developing gut microbiota's significance in early life. Some apparent differences between lab and wild gut microbiota maturation warrant further attention, however, such as the potentially more gradual changes in wild animals and greater abundance of aerotolerant taxa into adulthood. Further understanding these differences and the drivers of gut microbiota assembly patterns in wild mice could allow adjustment of lab mouse husbandry to achieve assembly of gut microbiota that more closely resembles that occurring in natural populations, allowing more accurate inference about the impact of this key microbial developmental stage on mammalian biology.

Data availability

The 16S rRNA amplicon sequencing data used in this study have been deposited in GenBank under SRA accession: PRJNA1028479. Source data for Figs. 1–4 are included as Supplementary Data files 3–6 and are also available at https://github.com/eveliinahanski/mus_assembly. All other data are available from the corresponding author on reasonable request.

Code availability

R code is available at https://github.com/eveliinahanski/mus_assembly.

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Author contributions

E.H. and S.C.L.K. set up the wild mouse study system. E.H., A.R., and S.C.L.K. collected the data. E.H. conducted the laboratory work and analysed the data. E.H. wrote the manuscript. All authors contributed to the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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