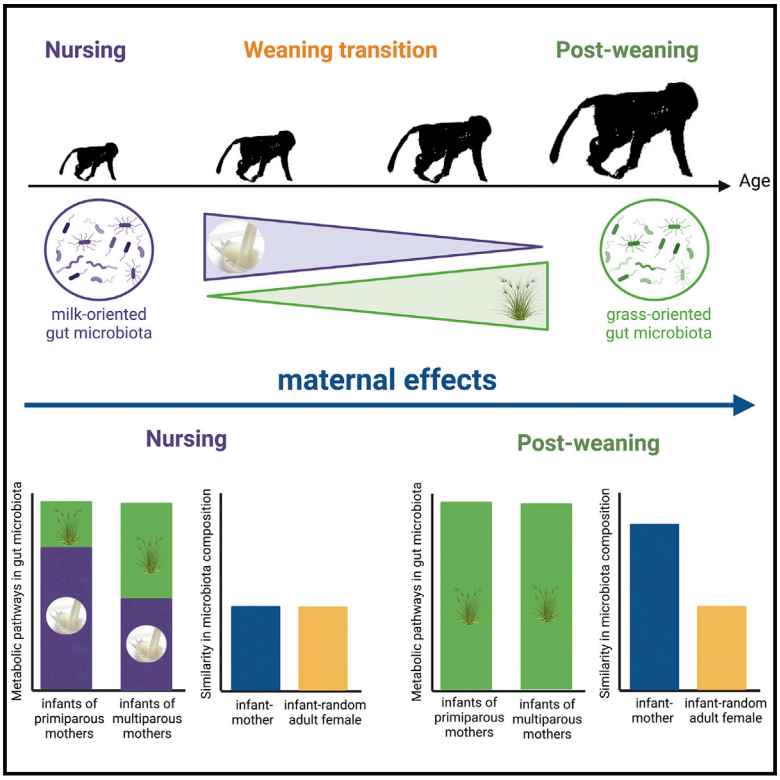
Report

Current Biology

Maternal effects on early-life gut microbiota maturation in a wild nonhuman primate

Graphical abstract



Authors

Alice Baniel, Lauren Petrullo, Arianne Mercer, ..., Thore J. Bergman, Noah Snyder-Mackler, Amy Lu

Correspondence

alice.baniel@gmail.com (A.B.), nsnyderm@asu.edu (N.S.-M.), amy.lu@stonybrook.edu (A.L.)

In brief

Baniel et al. report on the gut microbiota maturation in a wild nonhuman primate, the gelada. Maternal effects shape the postnatal assembly of the gut microbiome during nursing and past the weaning transition. Such microbially mediated maternal effects could contribute to phenotypic plasticity in mammalian development.

Highlights

- Gelada and human infants have similar microbial taxa and colonization trajectories
- Transition from milk to solid food drives early-life microbial composition changes
- Microbiota maturation is influenced by maternal effects throughout development
- Nursing infants of first-time mothers have slower functional microbiota maturation

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Report

Maternal effects on early-life gut microbiota maturation in a wild nonhuman primate

Alice Baniel,[1,2,10,14,*] Lauren Petrullo,[3] Arianne Mercer,[4] Laurie Reitsema,[5] Sierra Sams,[4] Jacinta C. Beehner,[3,6] Thore J. Bergman,[3,7] Noah Snyder-Mackler,[1,2,4,8,11,13,*] and Amy Lu[9,12,13,*]

[1]Center for Evolution and Medicine, Arizona State University, E Tyler Mall, Tempe, AZ 85281, USA
[2]School of Life Sciences, Arizona State University, E Tyler Mall, Tempe, AZ 85287, USA
[3]Department of Psychology, University of Michigan, Church St., Ann Arbor, MI 48109, USA
[4]Department of Psychology, University of Washington, Okanogan Ln., Seattle, WA 98195, USA
[5]Department of Anthropology, University of Georgia, Jackson St., Athens, GA 30602, USA
[6]Department of Anthropology, University of Michigan, S University Ave., Ann Arbor, MI 48109, USA
[7]Department of Ecology and Evolutionary Biology, University of Michigan, N University Ave., Ann Arbor, MI 48109, USA
[8]School for Human Evolution and Social Change, Arizona State University, Cady Mall, Tempe, AZ 85287, USA
[9]Department of Anthropology, Stony Brook University, Circle Rd., Stony Brook, NY 11794, USA
[10]Twitter: @AliceBaniel
[11]Twitter: @SMack_Lab
[12]Twitter: @sbululab
[13]These authors contributed equally
[14]Lead contact
*Correspondence: alice.baniel@gmail.com (A.B.), nsnyderm@asu.edu (N.S.-M.), amy.lu@stonybrook.edu (A.L.)
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SUMMARY

Early-life microbial colonization is an important process shaping host physiology,[1–3] immunity,[4–6] and long-term health outcomes[7–10] in humans. However, our understanding of this dynamic process remains poorly investigated in wild animals,[11–13] where developmental mechanisms can be better understood within ecological and evolutionarily relevant contexts.[11,12] Using one of the largest developmental datasets on a wild primate—the gelada (*Theropithecus gelada*)—we used 16S rRNA amplicon sequencing to characterize gut microbiota maturation during the first 3 years of life and assessed the role of maternal effects in shaping offspring microbiota assembly. In contrast to recent data on chimpanzees, postnatal microbial colonization in geladas was highly similar to humans:[14] microbial alpha diversity increased rapidly following birth, followed by gradual changes in composition until weaning. Dietary changes associated with weaning (from milk- to plant-based diet) were the main drivers of shifts in taxonomic composition and microbial predicted functional pathways. Maternal effects were also an important factor influencing the offspring gut microbiota. During nursing (<12 months), offspring of experienced (multi-time) mothers exhibited faster functional microbial maturation, likely reflecting the general faster developmental pace of infants born to these mothers. Following weaning (>18 months), the composition of the juvenile microbiota tended to be more similar to the maternal microbiota than to the microbiota of other adult females, highlighting that maternal effects may persist even after nursing cessation.[15,16] Together, our findings highlight the dynamic nature of early-life gut colonization and the role of maternal effects in shaping this trajectory in a wild primate.

RESULTS

Early-life gut microbiota colonization varies across individuals[17–24] and is shaped by maternal microbial ecology.[24–29] In mammals, direct vertical transmission of maternal microbial lineages occurs at birth[24–27,30,31] and continues as infants ingest milk microbes during lactation[27,32,33] and acquire maternal gut and skin microbes through body contact.[34,35] Maternal milk glycans further influence the colonization of offspring microbial communities by promoting the growth of beneficial microbes (e.g., *Bifidobacterium* and *Bacteroides*) that influence infant nutrition and immunity.[36–38] Given such vertical transmission of microbes and microbe-promoting factors (i.e., milk glycans), mothers with varying physical and social backgrounds may differentially influence offspring gut microbiota, resulting in maternal effects.[28,39–41] Indeed, maternal effects on offspring development, survival, and physiology are well documented, with poor maternal condition,[42–44] maternal inexperience (i.e., parity),[45–50] and low maternal dominance rank[47,50–54] often negatively impacting offspring development and fitness. In captive vervet monkeys, maternal effects on the offspring microbiome have also been identified: during lactation, first-time mothers vertically transmitted more bacteria that process milk, promoting faster postnatal growth in offspring and compensating for poor milk production.[55] These results suggest that variation in vertical transmission may be a key feature impacting offspring development and

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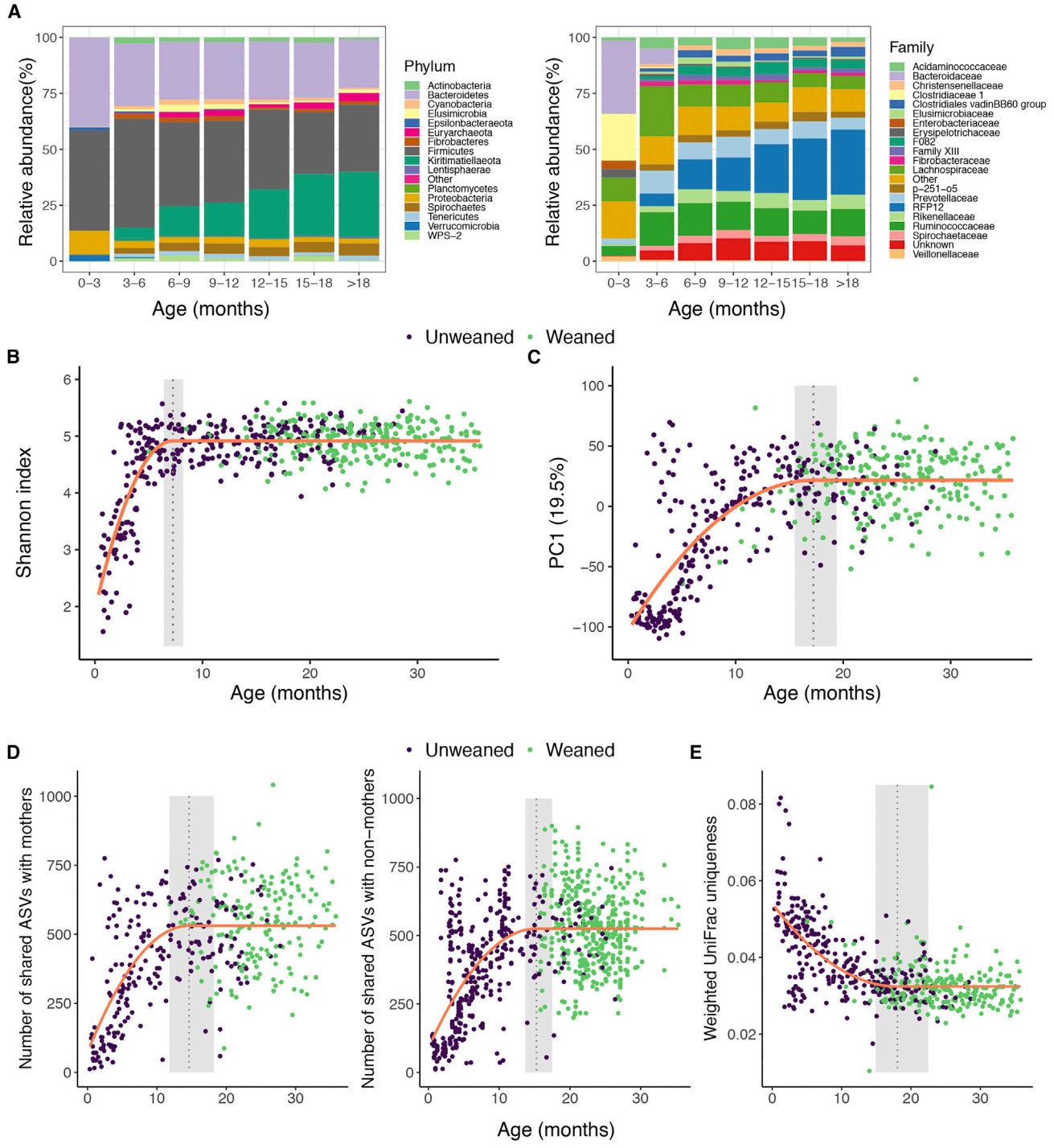

Figure 1. Gut microbiome taxonomic assembly in the first 3 years of life in immature geladas

(A) Relative abundance of the 15 and 19 most abundant phyla and families per age category.

(B) Age-associated pattern of alpha diversity (Shannon index of ASVs) within samples. The dotted vertical line represents the critical point of inflexion (7.3 months, 95% CI = [6.4–8.2] in shaded gray, nonlinear quadratic plateau model [QPM]: $R^2 = 0.62$) representing the age at which alpha diversity converges to adult-like patterns. Immatures are considered "weaned" when their mother has resumed reproductive cycles. The dataset was rarefied at 20,000 reads for the figure.

(C) Age-associated pattern of beta diversity. A principal component analysis (PCA) was used to ordinate samples based on the Aitchison dissimilarity index. (C) represents the projection of the first principal component (PC1) that is best explained by the age of immatures. The dotted vertical line represents the critical point of inflexion (17.2 months, 95% CI = [15.5–19.4] in shaded gray, nonlinear QPM between PC1 and age: $R^2 = 0.55$) representing the age at which immatures beta diversity (i.e., overall composition) converges to adult-like patterns.

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survival.[39,40,55,56] Additionally, mothers may influence offspring gut microbiota indirectly through their shared social and physical environment.[34,57,58] However, while direct and indirect maternal effects on the developing gut microbiome are an understudied and overlooked mechanism impacting developmental plasticity in offspring,[39,40,56] our understanding of these maternal effects has been largely limited to findings from humans[59–63] and captive animals.[55,64–68] Only a handful of studies from wild populations exist—all of which have limited sampling of infants.[14,16,69]

Here, we characterized early-life gut microbiota development in a wild primate—the gelada (*Theropithecus gelada*)—and investigated if maternal effects shape offspring gut microbiota assembly. Using 16S rRNA amplicon sequencing, we generated the largest gut microbiome dataset of immature (i.e., infant or juvenile) individuals in a wild, nonhuman mammal, including cross-sectional and longitudinally collected fecal samples from 89 immature geladas during the first 3 years of life (n = 525 samples; 5.9 ± 5.5 per individual, range: 1–18; Figure S1A). We subsequently examined if immatures shared more gut bacteria with their mothers than with other adult females of their social group and whether such similarities varied across development (i.e., pre- versus post-weaning). Fecal-fecal comparisons are expected to partly capture milk vertical transmission in early life, because microbes that colonize the mammary gland likely originate from the female's gastrointestinal tract ("enteromammary pathway"[32,70]). While 16S data lack the taxonomic resolution to infer direct vertical transmission,[25,26,29–31] more similar infant-mother gut microbiota while infants are still nursing would likely reflect greater mother-offspring microbial transfer through milk and/or body contact. This is more likely for geladas due to core social groups having highly overlapping home ranges,[71] a reliance on a homogenous grass-based diet,[72] and the existence of strong mother-offspring social bonds,[73] which may dampen the role of shared mother-offspring environments in generating strong maternal-offspring similarities prior to weaning. Finally, we investigated whether maternal attributes (such as dominance rank or parity) altered the development of the infant gut microbiota. We predicted that immature geladas born to resource-limited mothers (low-ranking or primiparous[74,75]) would harbor an early-life microbiome better equipped to digest milk to compensate for poorer maternal energetic allocation during lactation—similar to recent findings in vervet monkeys.[55]

Gut microbiome diversity during development

We identified 3,784 amplicon sequence variants (ASVs) from 19 phyla and 76 families across 525 samples (mean ± SD ASVs per sample: 728 ± 261, range: 65–1,498). Gut microbiome composition changed quickly after birth (Figure 1A). Alpha diversity (i.e., within-sample diversity) increased rapidly with age (Shannon index: generalized additive mixed model [GAMM]: effective degrees of freedom [edf] = 8.1, $p < 2.0\times10^{-16}$; Data S1A), converging to adult-like levels at 7.3 months of age (95% confidence interval [CI] = [6.4–8.2]; Figures 1B, S1B, and S1C). Age was the strongest predictor of the differences in microbial composition among immature samples (Aitchison β-diversity PERMANOVA: $R^2 = 0.74$, $p = 1.0\times10^{-04}$; Data S1B), with samples clustering tightly by age on the first principal component of Aitchison β-diversity (first principal component [PC1]; Pearson's $R_{age,PC1} = 0.62$, $p < 2.2\times10^{-16}$; Figure 1C). Compared to alpha diversity, beta diversity reached adult levels later in development at 17.2 months (95% CI = [15.5–19.4]; Figure 1C), which matches the mean age at weaning defined by the mother's resumption of reproductive cycles.[76]

As offspring got older, they shared an increasing number of ASVs with their mothers (GAMM, effect of immature age: edf = 4.5, $p < 2.0\times10^{-16}$; n = 398 mother-offspring pairs sampled within one day of each other; Data S2A; Figure 1D), plateauing at 14.6 months (95% CI = [11.8–18.2]; Figure 1D) when similarities cease to increase further. Immatures also become more similar in composition to their mother as they aged (pairwise β-diversity dissimilarity between mother-offspring pairs; Data S2A; Figure S1D), converging toward an adult-like microbiome at 15.2–17.2 months (Figure S1D). This pattern of maturation was similar when comparing immatures to other adult females (Figures 1D and S1D). Despite these strong developmental trajectories, there was substantial inter-individual variability in microbial composition in early life: gut microbiota were more individualized prior to ~18 months of age (95% CI = [14.9–22.5]; Figure 1E), with a few ~3–6-month-old infants harboring gut microbiota that were typically found in much older immatures (Figure 1C).

Taxonomic and functional changes reflect nutritional shifts during development

We characterized age-associated changes in the abundance of microbial taxa (families or genera) and in functional profiles based on predicted metabolic (Kyoto Encyclopedia of Genes and Genomes [KEGG] orthologs [KOs]) and enzymatic (Enzyme Commission [EC] numbers) microbial pathways inferred using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 [PICRUSt2].[77] Most microbial taxa (96% of families and 91% of genera) and predicted functional pathways (89%–91% of KOs and 93% of ECs) exhibited significant changes in abundance with age (autoregressive integrated moving average [ARIMA] models; Data S3). Taxa (Figures 2A and S2A) and pathways (Figures 2B, S2B, and S3A) with similar age trajectories were subsequently grouped into four different clusters, each characterized by a distinct temporal colonization

(D) Number of shared ASVs between mothers and offspring (n = 398 matched pairs of fecal samples collected on the same or next day) and between non-mothers and immatures from the same social group (n = 761 pairs of fecal samples collected within 20 days). The dotted vertical line represents the critical point of inflexion (14.6 months, 95% CI = [11.8–18.2] in shaded gray for mother-offspring pairs and 15.3 months, 95% CI = [13.4–17.9] in shaded gray for non-mother-immature pairs, nonlinear QPM: $R^2 = 0.36$ in both cases) representing the age at which the number of shared ASVs stabilizes to its maximal value. The dataset was normalized using cumulative sum scaling (CSS) transformation.

(E) Age distribution of inter-individual variability in gut microbiomes of immatures using weighted UniFrac distance. Higher "uniqueness" values indicate a more distinct gut microbiome from the immature cohort. The dotted vertical line represents the critical point of inflexion (18.0 months, 95% CI = [14.9–22.5] in shaded gray, nonlinear QPM: $R^2 = 0.43$) representing the age at which inter-individual variation reaches adult-like values. The dataset was normalized using CSS transformation prior to calculation.

See also Figure S1 and Data S1.

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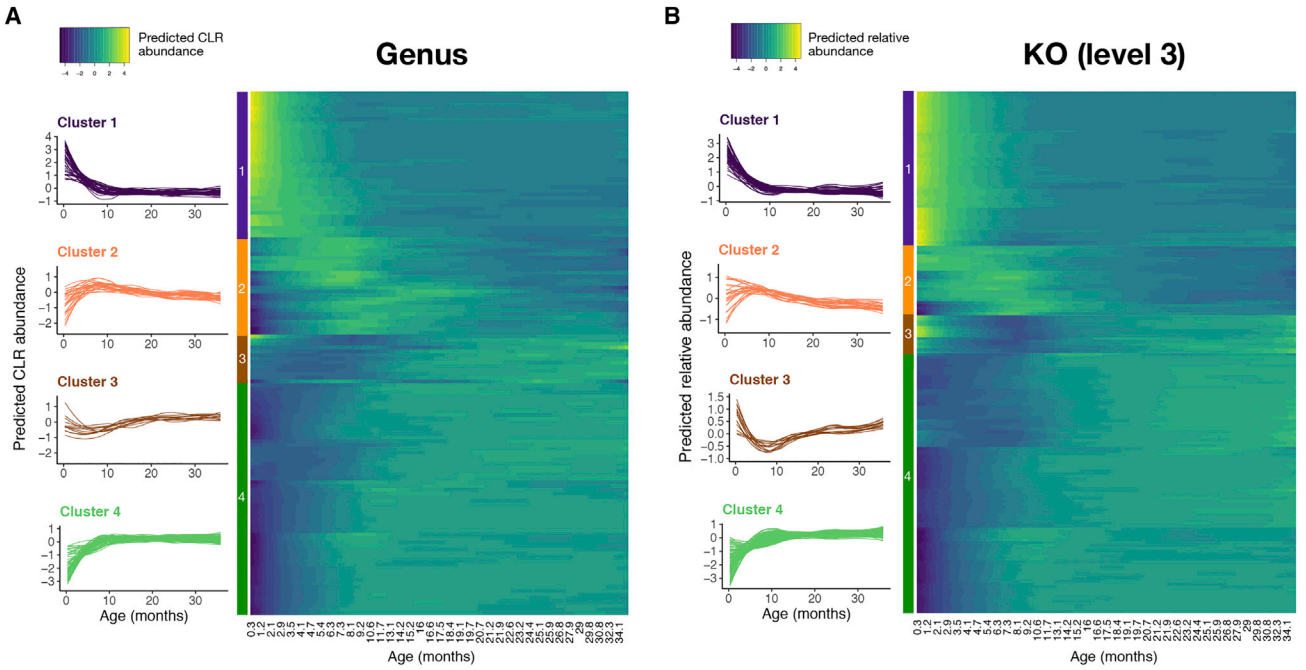

Figure 2. Age-associated changes in microbial composition and predicted functional profile in geladas

(A and B) Heatmaps of the (A) microbial genera and (B) predicted KEGG orthologs metagenomes (KO level 3) exhibiting a significant chronological trend as a function of age (fitted values from ARIMA models and predicted using locally estimated scatterplot smoothing (LOESS) regression per taxa or pathway). Values represent *Z* score normalized counts after centered-log-ratio (clr) transformation for genera and relative abundance transformation for pathways. Hierarchical clustering was used to group these age-dependent trajectories into four clusters exhibiting similar chronological trends. Color bar on the left side represents the delimitation of the clusters. Taxa or pathways (i.e., rows) are ordinated on the heatmap using correlation as distance function. The full list of taxa and pathways in each cluster can be found in Data S3.

See also Figures S2 and S3 and Data S3.

pattern (Data S3). These four microbial clusters succeeded and replaced each other during development, reflecting progressive nutritional shifts from a milk-dominant to plant-dominant diet (Figure 3).

Many of the early colonizers of the gelada gut (cluster 1; Figures 2A, 3A, and S2A; Data S3A and S3B) were similar to the core microbial taxa found in human newborns (reviewed in Milani et al. [2017][2]), including milk glycan degraders (*Bifidobacterium* and *Bacteroides*),[2,78–80] lactose degraders and fermenters (*Streptococcus* and *Veillonella*),[81] keystone saccharolytic and short-chain fatty acids producers (*Lachnoclostridium*, *Blautia*, *Faecalibacterium, Butyricicoccus,* and *Butyricimonas*),[2,81–83] and mucin degraders involved in activating the host immune system and intestinal barrier function (*Akkermansia, Ruminococcus gnavus*, and *R. torques*) (Figures 3A and S2C).[84,85] At the ASV level, we found that *Bacteroides fragilis* (a milk glycan degrader[78,86]) and *Ruminococcus lactaris* (a lactose fermenter[87]) were highly abundant in the youngest infant samples (Figure S2D). Functional analyses (Figures 2B and S2B; Data S3C and S3D) similarly revealed that the early-life gut microbiome contained high levels of bacterial genes involved in carbohydrate metabolism (e.g., degradation of milk sugars:[88] fructose, mannose, and galactose) and the conversion of sugars to energy (Figure 3B). The early-life microbes also encode key enzymes necessary to cleave complex milk glycans, including glucosidase, galactosidase, fucosidase, sialidase, and beta-hexosaminidase (Figure S3A; Data S3E).[2,37,79,86,89] *Bacteroides*—the most abundant genus in early life (Figures 3A and S2C)—was the main microbial group encoding these enzymes (Figure S3B), highlighting its central role in milk glycan degradation in geladas. By contrast, *Bifidobacterium*—the main glycan degrader in humans[90,91]—was rare in the developing gelada gut (Figure S2C; <0.01% at 1 month in geladas versus ~40% in humans[29]).

As typically found in human newborns,[20,21,25] the early-life gelada microbiome contained a higher proportion of oxygen-tolerant genera compared to later-life microbiomes (cluster 1 had 18%, while later-life clusters 2, 3, and 4, described below, contained 3.8%, 0%, and 1.5% facultative anaerobes or aerobes; Data S3B). This early-life cluster was further characterized by a high number of potentially pathogenic bacterial groups, including oxygen-tolerant (*Actinobacillus*, *Escherichia-Shigella*, *Helicobacter* and *Mannheimia*) and anaerobic (*Hungatella*, *Eggerthella*, *Clostridioides* and *Clostridium sensu stricto 1*) genera (Figure S2C; Data S3B), some of which cause disease and diarrhea in human newborns and captive animals (*C. difficile*, *C. butyricum and perfringens* and *H. macacae*).[92–96] In line with this, the early-life microbial metabolic pathways were also more involved in processes related to the host immune system (Data S3C and S3D).

Around 10 months of age (i.e., 5–7 months before nursing cessation), there was an important compositional and functional rearrangement of the gelada gut microbiota, characterized by a small number of taxa and metabolic pathways peaking (cluster 2; Figures S2A and S2B) or decreasing (cluster 3; Figures S2A and

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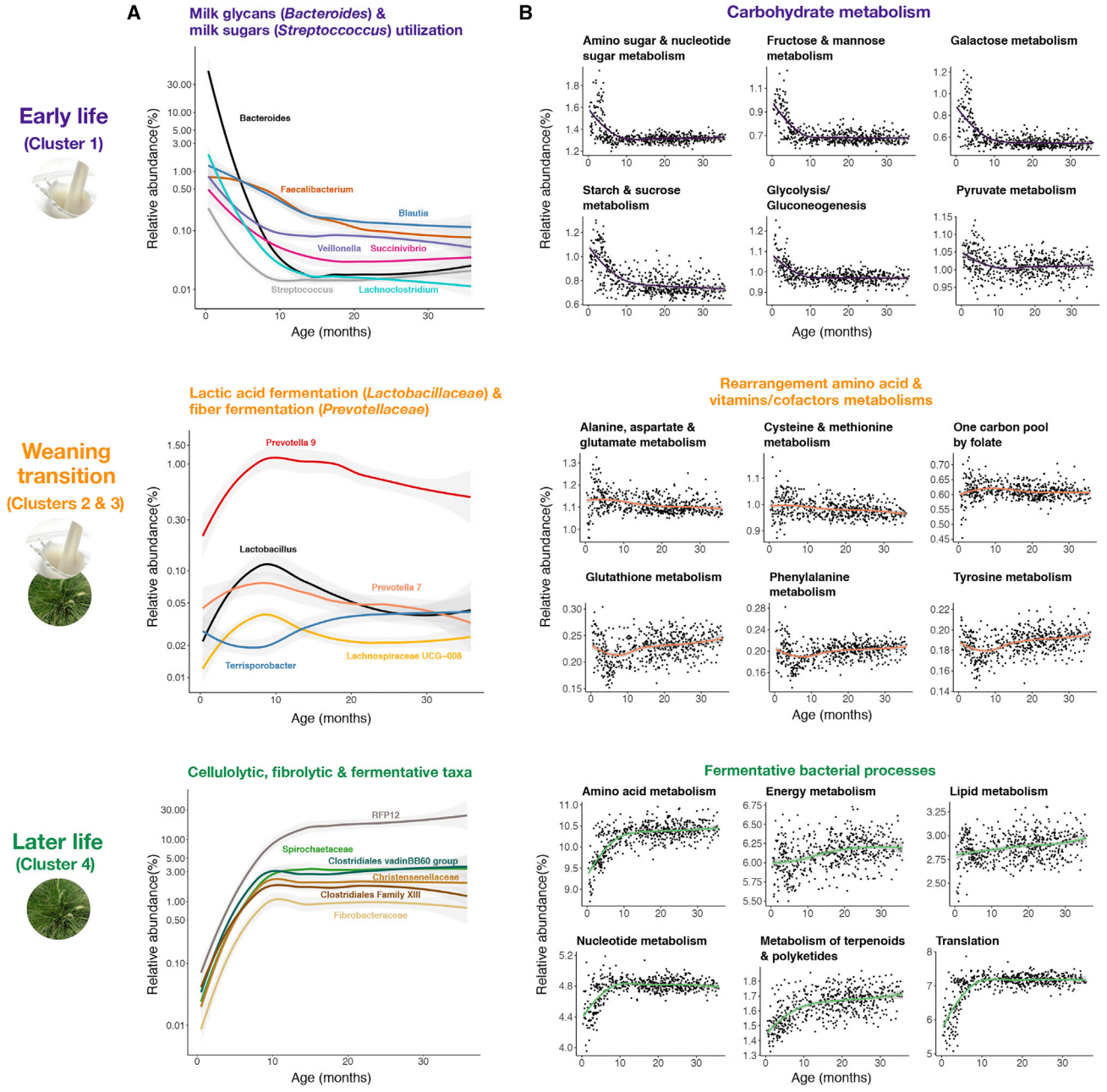

Figure 3. Composition and predicted functions of the gut microbiome in early life, at the weaning transition, and in later life in geladas

(A) Relative abundance of representative microbial genera in early life (cluster 1) and at the weaning transition (clusters 2 and 3) and of microbial families in later life (cluster 4), as a function of age. The age-dependent trajectories were calculated on clr-transformed counts, but here for visualization purposes, we represent the LOESS regression on the raw relative abundance across samples. Relative abundance is represented on a log scale to accommodate high and low abundance genera together.

(B) Relative abundance of representative predicted KEGG metabolic pathways enriched in early life (cluster 1, KO3), during the weaning transition (clusters 2 and 3, KO3), and in later life (cluster 4, KO2). In all plots, the average curve is the LOESS regression on the raw relative abundance across samples.

See also Figures S2 and S3 and Data S3.

S2B) in abundance (Data S3). *Bacteroides* became much less abundant, while other taxa—such as *Lactobacillus*, *Prevotella*, and several genera from the Lachnospiraceae family—became more abundant (cluster 2; Figure 3A; Data S3A and S3B). *Lactobacillus* is a keystone lactic acid bacterial family producing large amounts of lactate from milk sugars,[80,97] but *Prevotella* and Lachnospiraceae are fiber-degrading genera.[98,99] These transient shifts highlight the role of the gut microbiota in digesting both milk and plant items at this age. Taxonomic changes were also accompanied by important predicted functional changes, including remodeling of amino acid metabolism, vitamins, and cofactors (Figure 3B; Data S3C and S3D).

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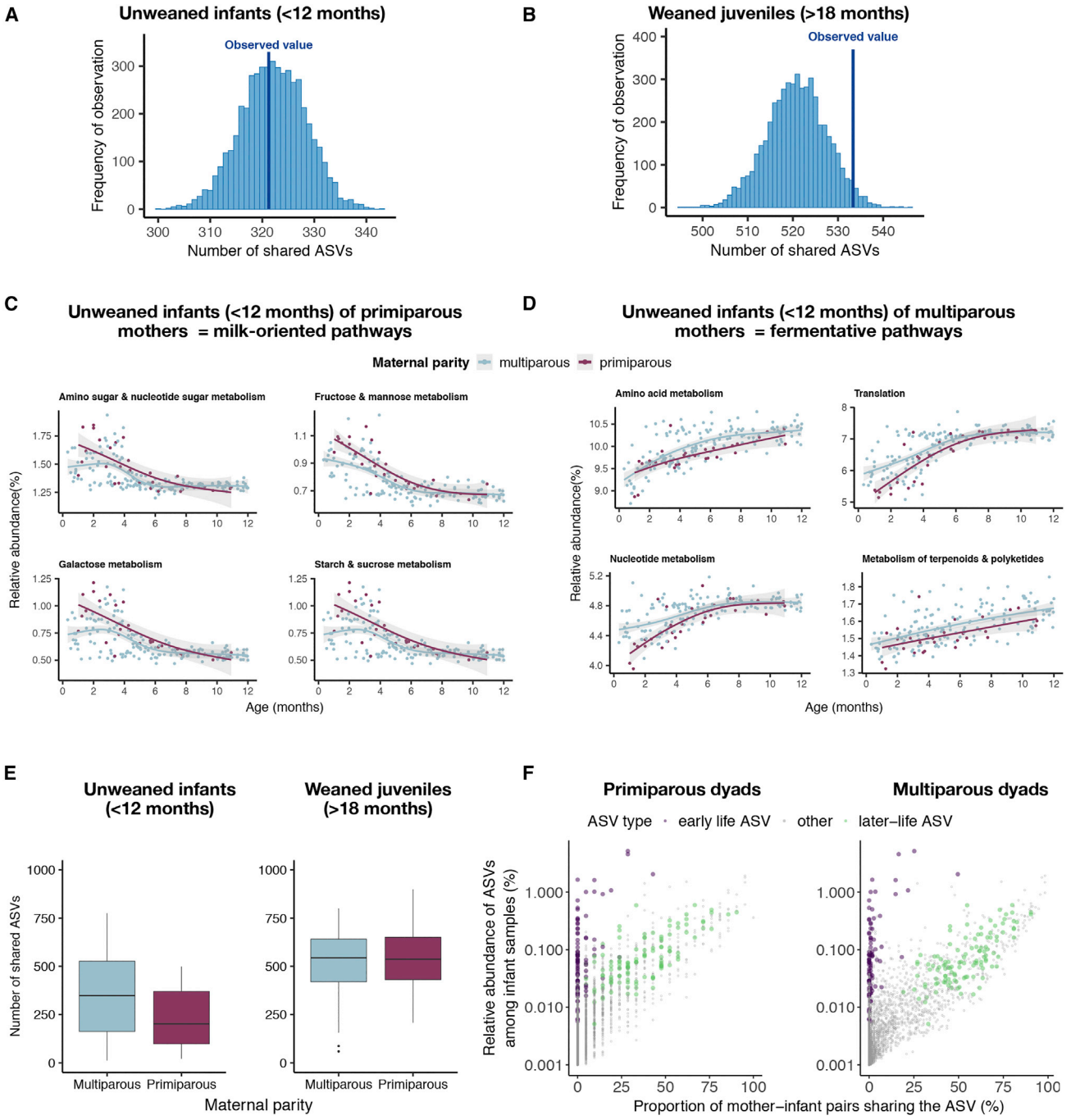

Figure 4. Maternal effects on offspring's gut microbiota in early and later life

(A and B) Results of the nonparametric resampling approach testing if offspring share more amplicon sequencing variants (ASVs) with their mother than with other adult females of their social group (considering all maternal and non-maternal samples collected 0–20 days apart from immature samples). Analysis was performed separately on (A) young nursing infants (aged 0–12 months, n = 127 samples) and (B) old immatures (>18 months, n = 193) that were likely weaned. The histograms show the distribution of the metric for non-mother-immature pairs (with 5,000 repetitions). The vertical line shows the value of the metric for mother-offspring pairs (averaged across 5,000 repetitions).

(C) The gut microbiota of infants (<12 months) born to primiparous females is characterized by a higher abundance of several KEGG pathways (level 3) related to milk digestion, which are typically observed in early life (see cluster 1 of Figure 3B and Data S3D).

(D) By contrast, the gut microbiota of infants (<12 months) born to multiparous females has higher abundance of KEGG pathways (level 3) related to plant-fermentation, which are typically observed in later life (see cluster 4 of Figure 3B and Data S3D).

(E) In early life (0–12 months), infants born to primiparous females share fewer ASVs with their mother compared to offspring born to multiparous females, but this pattern disappears later in life (>18 months).

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Later in development, when immatures begin to eat graminoids (e.g., grasses),[72] there was a rapid increase in microbes that metabolize plant complex polysaccharides, which plateaued by 10 months of age (cluster 4; Figures 2A, S2A, and 3A; Data S3A and S3B). This included cellulolytic (Spirochaetaceae, Fibrobacteraceae, and *Cellulosilyticum*)[98,100,101] and fermentative taxa (Lachnospiraceae, Clostridiales Family XIII, Prevotellaceae, and Ruminococcaceae),[98,99] as well as RFP12 (Figure 3A), all of which are commonly found in the adult gelada gut.[102] Functional profiles also typically converged toward an adult gelada pattern,[102] with more bacterial genes involved in energy, amino acid, and lipid metabolism, and the regulation of genetic expression and bacteria growth (cluster 4; Figure 3B; Data S3C and S3D).

Mother-offspring similarity in gut microbiome composition during development

We next examined mother-offspring gut microbiome similarity and determined if such similarities varied in strength from the pre- to post-weaning period. We used nonparametric resampling tests (n = 5,000 resampling iterations) to compare the number of shared ASVs and beta diversity dissimilarity values between mother-offspring pairs (n = 510 possible pairs) and random non-mother adult female-immature pairs drawn from the same social group (n = 1,587 possible pairs), with all pairs collected within 20 days of each other (see STAR Methods). In the overall dataset, immatures shared significantly more ASVs with their mothers (n = 510 samples; ~1.5% more ASVs, mean ASVs shared between mother-offspring = 459, non-mother-immature = 452, p = 0.02) and were 1.2% (weighted UniFrac dissimilarity between mother-offspring = 5.2×10^{-2}, non-mother-immature = 5.3×10^{-2}, p = 0.05) more similar compositionally to their mothers than to another adult female in the same group sampled around the same time (Data S2B). However, this effect was not statistically significant in the youngest infants with a primarily milk-based diet (n = 126 samples <12 months: mean ASVs shared between mother-offspring = 321, non-mother-immature = 322, mean difference = 2.3, p = 0.41; weighted UniFrac dissimilarity between mother-offspring = 6.8×10^{-2}, non-mother-immature = 6.8×10^{-2}, p = 0.65; Figures 4A and S4A; Data S2B) but was significant in older immatures that likely rely more on solid foods and less on milk (n = 193 samples >18 months: number of shared ASVs between mother-offspring = 533, non-mother-immature = 521, p = 0.01 and weighted UniFrac dissimilarity between mother-offspring = 4.4×10^{-2}, non-mother-immature = 4.5×10^{-2}, p = 0.03; Figures 4B and S4B; Data S2B). Note that this pattern of mother-offspring similarity in old juveniles did not remain statistically significant when the dataset was subset to the same size as the young infant dataset (see STAR Methods) or when a more stringent matching criteria of maternal and non-maternal pairs collected within 10 days (rather than 20 days; Data S2B) was applied. Nevertheless, the effect size of pair identity (mother-offspring versus not) remained the same, suggesting greater mother-offspring similarity after, rather than before, nursing cessation. Importantly, sharing maternal-offspring sharing of gut microbes at this later life stage is likely mediated by non-nursing interactions following weaning.

While there was no evidence that mother-offspring pairs shared significantly more microbes than expected by chance in the pre-weaning period, we hypothesized that vertical transmission might specifically involve taxa specialized in processing milk (especially milk glycan degraders such as *Bifidobacterium* and *Bacteroides*), which are not common in adult geladas[102] and therefore less likely to be environmentally or socially acquired. Contrary to our expectations, the shared bacterial community between mother-offspring dyads during the nursing period (<12 months) was not specific to early-life microbes that are likely functionally important to processing milk but instead belonged to the typical adult microbiome of geladas that are likely involved in grass digestion (Figure S4C). Collectively, (1) the lack of similarity with the maternal microbiota during the nursing period and (2) the predominance of adult-like microbes in the ASVs that were shared suggest that 16S fecal-fecal comparisons do not adequately capture vertical transmission through nursing.

Maternal parity influences predicted functional microbial profiles of their offspring

Despite our inability to detect early-life vertical transmission using 16S data, we might still be able to measure the phenotypic consequences of maternal environmental variation on offspring gut microbiota during development. We tested whether two maternal attributes—maternal rank and parity—predicted offspring gut microbiome composition and function (using all immature samples; n = 525). Maternal dominance rank did not predict alpha or beta diversity (Data S1A and S1B) nor the abundance of any microbial taxa or predicted functional pathway of offspring (Data S4 for <12 months; results not shown for >18 months). Maternal parity was also not associated with offspring alpha diversity but explained ~0.3% of the variance in beta diversity between immatures (Data S1A; Figure S4D). At the taxonomic level, parity was not associated with the abundance of any microbial families or genera (Data S4A and S4B for <12 months; results not shown for >18 months). Nonetheless, several milk-related microbes exhibited a trend to be more abundant in infants (<12 months) born to primiparous mothers (Figure S4E; Data S4A and S4B; see p values before false discovery rate [FDR] correction). At the functional level, parity was associated with the abundance of several predicted metabolic and enzymatic pathways but only in the first 12 months of life (Data S4C and S4D). Specifically, the gut microbiota of nursing infants (<12 months) born to primiparous females was enriched in carbohydrate pathways (galactose, fructose, and mannose

(F) Relative abundance (log transformed) of the shared ASVs between mother-offspring pairs during the first 12 months of life according to parity status of the dyad (primiparous vs multiparous). A subset of ASVs were classified as typically observed in early life (i.e., those with a loading score < −0.025 on PC1; see Figure S2D) versus typically observed in later life (i.e., those with a loading score > 0.04 on PC1). The ASVs that are commonly shared between mother-offspring pairs (e.g., among >70% of the pairs) are typically those found in later life ("early-life" ASVs are not universally shared between mother-offspring dyads), and this is true for both primiparous and multiparous dyads.
See also Figure S4 and Data S2 and S4.

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metabolism; Figure 4C; Data S4C and S4D) and enzymes (Figure S4F; Data S4E) directly involved in milk digestion and belonging to the early developmental period (i.e., cluster 1 in Figures 3B and S3B). By contrast, nursing infants (<12 months) born to multiparous females had a more functionally "mature" gut microbiota for their age, with a higher abundance of pathways involved in amino acid and nucleotide metabolisms (Figure 4D; Data S4C and S4D) typically belonging to the later life cluster (cluster 4 in Figure 3B).

Despite the effect of maternal parity on predicted microbial functions during the weaning period, we found that infants of primiparous females shared fewer ASVs ($\beta = -99.2$, $p = 9.0\times10^{-3}$; Figure 4E; Data S2A) and were more dissimilar to their mother (weighted UniFrac: $\beta = 7.9\times10^{-2}$, $p = 0.05$; Data S2A) in the first 12 months of life compared to infants of multiparous females. However, this effect of greater dissimilarity in primiparous-infant dyads disappeared later in life (>18 months of age) when the effect of maternal parity was no longer detected (number of shared ASVs: $\beta = 2.13$, $p = 0.92$, weighted UniFrac: $\beta = 8.5\times10^{-2}$, $p = 0.73$; Figure 4E; Data S2B). Furthermore, similar to the pattern for all females, the shared ASVs between infants and primiparous mothers during the nursing period (n = 21 pairs) did not belong to the typical early-life ASVs focused on digesting milk but rather to adult-like ASVs associated with a grass-based diet (Figure 4F). Taken together, these results suggest that despite maternal parity effects on the function of the early-life gut microbiome, they are not strongly mediated by differences of shared microbes between primiparous versus multiparous dyads as detected using 16S fecal-fecal comparisons of mother-infant dyads.

DISCUSSION

The dynamics of microbial colonization in geladas shares many similarities with patterns previously reported for humans[18,21,24] and other mammals[55,103,104] (but see Reese et al. [2021][14]). First, gut microbiota composition changed quickly following birth, with rapid taxonomic diversification and succession during the first 7 months of life, followed by more gradual changes until ~17 months, which corresponds to the age at weaning in geladas.[76] Thus, similar to humans,[21,27,105] the cessation of nursing—rather than solely the introduction of solid foods—appears to drive the developing gut microbiome to an adult-like composition. Second, shifts in gut microbiome composition and function closely followed progressive dietary transitions: gut bacteria that facilitate milk glycan and lactose utilization were dominant during early infancy while cellulolytic and fibrolytic bacteria that metabolize plant complex polysaccharides were dominant later in development as graminoids were progressively introduced in the diet.[72] Many of the early colonizers of the gelada gut were similar to those found in humans, with the exception that Bacteroides (specifically *B. fragilis*) instead of *Bifidobacterium* (common in human newborns[24,106–108]) appears to be the main group of milk glycan degraders in geladas. The absence of *Bifidobacterium* but abundance of *Bacteroides* in some nursing infants has in fact been documented in several human populations[19,29,109–111] and some nonhuman mammals.[55,103,112,113] Such individual and species-specific differences in the predominance of *Bifidobacterium* or *Bacteroides* may be linked to their different glycan-use profiles mapping on to species and individual specific differences in milk composition (specifically, the structure and relative abundance of different milk glycans).[114–116]

Importantly, our results suggest that early-life gut microbiome composition and function may be influenced by maternal effects, both during nursing and after weaning. However, these early versus later life maternal effects are likely driven by different mechanisms. During the first 12 months of life when infants remain heavily reliant on nursing, functional PICRUSt2 analyses revealed that infants of primiparous mothers harbored more bacteria that were functionally relevant for processing milk sugars. Previous work on captive vervet monkeys showed that the effect of maternal parity on microbial function could be explained by primiparous females transferring more milk-oriented microbes to their offspring (via milk) to potentially compensate for poor maternal milk production.[55] However, in our study, we did not find evidence for strong vertical transmission in the youngest period of life—either for all females or for primiparous females specifically. In other words, while offspring of first-time mothers harbored a microbiome abundant in milk-processing taxa, this abundance was not shared with their mothers. One potential explanation for these results is that fecal-fecal comparisons are less reliable for approximating vertical transmission via milk, and 16S sequencing itself may not have adequate resolution to identify rare taxa that may be shared.[25,26,29–31] In the absence of milk samples, however, evidence for such mechanisms remains unclear in geladas.

Despite our inability to pinpoint the mechanism for this pattern, we suggest that the more milk-oriented microbiome associated with first-time mothers could also simply reflect a slower pace of offspring gut microbiome maturation. By contrast, the greater similarity between multiparous mothers and their infants may be generated by accelerated gut microbiome development, suggesting that these infants are undergoing the weaning transition at a faster pace than their peers. Infants from multiparous females could be growing faster, gaining nutritional independence earlier (e.g., start eating grass earlier) and becoming socially integrated earlier than their similar-aged peers—all of which could explain why offspring of multiparous females also show greater microbial resemblance to mothers (and other adults) before weaning. This interpretation is supported by evidence that primiparous mothers wean their offspring about 5 months later than multiparous females in our study cohort (multiparous = 17.1 months, primiparous = 21.9 months).

Although there was little evidence for shared microbes prior to weaning, we found some evidence for greater mother-offspring similarities in gut microbiome composition after weaning. As immature gut microbiomes became more similar to adults in general, offspring were even more similar to their mothers than expected by chance, a pattern previously documented in other wild mammals[14,16] (but see Moeller et al. [2016][69]). Thus, while parity-associated maternal effects prior to weaning were not linked to shared microbes, evidence for shared microbes were identified following weaning, albeit unassociated with any clear maternal attributes. Maternal-offspring gut microbiome similarities beyond the early postnatal period may be explained by host genetics, post-weaning vertical transmission via non-nursing mechanisms, and shared maternal and offspring

environments.[35] Indeed, not only is gut microbiome composition highly heritable[15] but transmitted through more frequent social contacts, shared diets, and shared physical substrates, all of which are likely to characterize mother-offspring dyads that reside in the same social group.[34,57] Further work is needed to understand the relative importance of these mechanisms in explaining mother-infant similarity in gut microbial composition during juvenility.

Our study comes with a few limitations. First, age differences in functional profiles in relation to parity may have been better assessed using an interaction term rather than testing separately within each age group. However, to account for the non-linear relationship between age and microbial metrics, we used GAMMs, which cannot account for interaction terms. Thus, our models, while optimized for their distribution, were not able to capture an interaction in the full dataset. Second, our functional results may be limited because functional pathways generated from predicted bacterial metagenome profiles are contingent on how closely ASVs match reference databases. In our developmental data, the average weighted Nearest Sequence Taxon Index (NSTI: a measure of how similar bacteria from a sample are to reference genome sequences) was relatively high (mean ± SD = 0.49 ± 0.19, range: 0.01–0.89) compared to other mammals,[77] suggesting that our functional results should therefore be interpreted with caution (see further discussion in STAR Methods). Confidence in our functional analyses is nonetheless bolstered by the fact that our observed functional changes were consistent with those that are expected to occur during development and across dietary transitions. Future studies incorporating shotgun metagenomic data are needed to confirm the functional signal of "immaturity" among primiparous mother-offspring dyads.

Our results demonstrate maternal effects on the early-life gut microbiome from infancy past the weaning transition in a wild primate. Maternal parity in particular was associated with the functional maturation of the microbiome in offspring, likely reflecting faster developmental pace of infants born to reproductively experienced mothers. As infants age, they converge toward an adult-like gut microbiome that tends to be more similar to the maternal gut microbiome than expected by chance. The long-term consequences of such microbially mediated maternal effects remain unknown but could potentially influence phenotypic outcomes such as growth and immune function.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.08.037.

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AUTHOR CONTRIBUTIONS

Conceptualization, data curation, formal analysis, investigation, visualization, A.B., A.L., and N.S.M.; methodology, laboratory work, A.B., S.S., and A.M.; writing – original draft, A.B, A.L., and N.S.M; writing – review & editing, all authors; funding acquisition, A.L., N.S.M., J.C.B., T.J.B., and L.R.; supervision, A.L. and N.S.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Gelada fecal samples	Simien mountains gelada research project	https://geladaresearch.org/
Critical commercial assays		
DNeasy PowerLyzer PowerSoil Kit	QIAGEN	Cat #: 12855-100
NEBNext® Ultra™ II Q5® Master Mix	New England Biolab	Cat #: M0544
Deposited data		
Immature fecal sample 16S rRNA gene sequence data	This study	NCBI Sequence Read Archive. BioProject ID: PRJNA772269 (http://www.ncbi.nlm.nih.gov/bioproject/772269)
Adult female fecal sample 16S rRNA gene sequence data	Baniel et al.[102]	NCBI Sequence Read Archive. BioProject ID: PRJNA639843 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA639843)
Oligonucleotides		
16S V4 amplicon primers	Gohl et al.[117]	515F: GTGYCAGCMGCCGCGGTAA 806R: GGACTACNVGGGTWTCTAAT
Software and algorithms		
QIIME2 version 2019.1	Bolyen et al.[118]	https://qiime2.org/
DADA2	Callahan et al.[119]	https://benjjneb.github.io/dada2/index.html
SILVA database (version 132 - updated December 2017)	Quast et al.[120]	https://www.arb-silva.de/
R version 3.5.2	Team[121]	https://www.r-project.org/
PICRUSt2 v.2.1.3-b	Douglas et al.[77]	https://github.com/picrust/picrust2
R package EloRating	Neumann et al.[122]	https://cran.r-project.org/web/packages/EloRating/index.html
R package phyloseq	McMurdie et al.[123]	https://www.bioconductor.org/packages/release/bioc/html/phyloseq.html
R package picante	Kembel et al.[124]	https://cran.r-project.org/web/packages/picante/index.html
R package vegan	Oksanen et al.[125]	https://cran.r-project.org/web/packages/vegan/index.html
R package mgcv	Wood[126]	https://cran.r-project.org/web/packages/mgcv/index.html
R package zComposition	Palarea-Albaladejo and Martín-Fernández[127]	https://cran.r-project.org/web/packages/zCompositions/index.html
R package zoo	Zeileis et al.[128]	https://cran.r-project.org/web/packages/zoo/index.html
R package forecast	Hyndman et al.[129]	https://cran.r-project.org/web/packages/forecast/index.html
Other		
16S V4 rRNA library prep via dual-indexing protocol		https://smack-lab.com/protocols/
data and R code to reproduce the analyses	This study	https://doi.org/10.5281/zenodo.6984416

RESOURCE AVAILABILITY

Lead contact

Requests for additional information or resources should be directed to and will be fulfilled by the lead contact, Alice Baniel (alice.baniel@gmail.com).

Material availability

This study did not generate new reagents.

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Data and code availability

- All 16S sequence data used in this study are available at the NCBI Sequence Read Archive under BioProject ID PRJNA772269: http://www.ncbi.nlm.nih.gov/bioproject/772269 for the immature samples and PRJNA639843: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA639843 for the adult female samples.
- Data (including the ASV table and metadata) and R code to reproduce all the analyses are available at: https://doi.org/10.5281/zenodo.6984416.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The data for this study were collected between Jan 2015 and Jan 2019 from a population of wild geladas living in the Simien Mountains National Park in northern Ethiopia (13°15′N, 38°00′E). All research was conducted with permission of the Ethiopian Wildlife and Conservation Authority (EWCA) following all laws and guidelines in Ethiopia. Samples were conducted with approval by the Institutional Animal Care and Use Committees (IACUCs) of the University of Washington (protocol 4416-01), Arizona State University (20-1754 R), Stony Brook University (773805) and University of Michigan (PRO-00008871). This research conformed to the American Society of Primatologists/International Primatological Society Code of Best Practices for Field Primatology. Geladas live in multi-level societies, where several reproductive units (comprising a leader male, several adult females, their offspring, and occasionally 1-2 follower males) aggregate together during the day to forage and sleep together forming a "band" with a shared homerange.[71] Since Jan 2006, the Simien Mountains Gelada Research Project (SMGRP) has collected behavioral, demographic, and genetic data on a near-daily basis from over 600 individuals living in 2 separate bands of the area. All gelada subjects were habituated to human observers on foot and were individually recognizable (using natural body and facial features). Data were derived from 89 infants and juveniles (0-3 years of age) with known birth dates and 83 adult females living in 23 different reproductive units. The reproductive state of each adult female was monitored during group visits and recorded as cycling (as indicated by the presence of sex skin swellings on the neck, chest, and perineum), lactating (if she had a nursing infant), or pregnant (the date of conception was inferred by removing 183 days from the date of birth of subsequent offspring).[76] Records of female reproductive history were used to assign maternal parity status for each infant (first-time mother: primiparous or multi-time mother: multiparous) and to establish the date at which the mother resumed cycling following the infant's birth. We also used mother's resumption of cycling to estimate the approximate age at weaning for each infant. For 8 infants, age at weaning was assigned on the date of maternal death.

METHOD DETAILS

Fecal sample collection

Fecal samples (N=534; 307 females, 227 male samples) from 89 immature geladas (i.e., infants and juveniles sampled pre-reproductive maturity; female: N=51; male: N=38, mean±SD=6.00±5.69 samples per individual, range=1-18) were collected opportunistically from 2015-2016, and then regularly from 2017 to 2018 using targeted protocols (Figure S1A). Samples were derived from individuals residing in 17 different reproductive units (mean±SD=5.65±4.44 number of individuals sampled per unit, range=1-17). For a subset of immature samples (N=398 samples from 61 infants), we also collected a matched fecal sample from the mother (N=398 samples from 44 mothers) on the same day or on the following day of the immature sample collection. Fecal samples of additional known adult females in all reproductive states were also routinely collected (N=222 samples from 79 females) and were used to generate a random distribution of gut microbiome composition similarity between immatures and females of the same unit. Immediately upon defecation, approximately 1.5 g of feces was collected in 3 ml of RNA later[130] (observers wore sterile gloves during sample collection and avoided contact with soil to minimize contamination with soil microbes). Samples were stored at room temperature for up to 2 months, and subsequently shipped to the University of Washington (UW). At UW, samples were stored at -80°C until the sequencing libraries were prepared.

Maternal dominance ranks

Female dominance ranks were established using *ad libitum* and focal observations of agonistic interactions between all adult females belonging to the same unit with an Elo-rating procedure[131] implemented in the R package EloRating.[122] Agonistic interactions included physical aggression (hit, bite), chase, threats (vocal threats, non-vocal gestures), approach-avoid interactions (displacements) and submissive behaviors (fear bark, crouch, grimace). In geladas, agonistic interactions usually consist of a sequence of several behaviors emitted and received by both parties. Since it can be difficult to establish the winner of each agonistic sequence, we consider each behavior of a sequence as a separate event and assign the winner and loser based on the directionality of the behavior. We obtained a daily Elo-score that we then averaged over the course of each month. Since Elo-scores can be sensitive to differences in sampling effort, we then converted this monthly Elo-rank into a monthly proportional rank that controlled for female group size (0=lowest-ranking females and 1= highest ranking female). In the analyses, we used maternal dominance rank during the month of the infant's birth since we expect microbially-mediated maternal effects to be the strongest in the postnatal period (during nursing). However, we also investigated maternal rank during pregnancy and at the date of immature sample collection, which led to similar results (not reported here).

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Environmental data

The study area is located at 3200 m above sea level and is characterized as an Afroalpine grassland ecosystem, consisting of grassland plateaus, scrublands, and Ericaceous forests.[132] The climate in the Simien Mountains National Park exhibits marked inter- and intra-annual fluctuation in rainfall and temperature and can be broadly divided into 3 distinct seasons: a cold-dry season (Oct to Jan), a hot-dry season (Feb to May) and a cold-wet season (Jun to Sep).[133] Fecal samples of immatures and adult females were collected across the year, with roughly equal coverage across seasons (406 in cold-dry, 426 in cold-wet and 313 in hot-dry season). Daily cumulative rainfall and minimum and maximum temperature are recorded on a near-daily basis by the SMGRP. Geladas are graminivorous, with up to 90% of their diet composed of graminoids.[72] They eat primarily graminoid leaves (i.e., grasses and sedges) all year long, but increase their consumption of underground storage organs (rhizome, corms, roots) in the dry season, as above-ground graminoid leaves become less abundant.[72] A previous study established that the gut microbiome composition of adults shifts in response to environmental variation, in particular with cumulative rainfall over the previous month, which is a good proxy of diet.[102] Thus, in all models we controlled for the total cumulative rainfall over the 30 days prior to the date of fecal sample collection (as a proxy for grass availability) and the average minimum daily temperatures in the 30 days preceding the date of sample collection (as a proxy of thermoregulatory constraints).

16S rRNA gene sequencing

We performed 16S rRNA gene amplicon sequencing on the immature and adult female fecal samples to establish gut microbial composition. We first extracted microbial DNA using Qiagen's PowerLyzer PowerSoil DNA Isolation kit (Qiagen #12855) following standard protocols. We then amplified the hypervariable V4 region of the 16S rRNA gene using PCR primer set 515F and 806R from The Human Microbiome Project and a dual-indexing approach.[117] Details of the amplification protocol can be found in[102] (see also: https://smack-lab.com/wp-content/uploads/2020/07/16S_library_prep_v1.pdf). The libraries were then pooled in roughly equimolar amounts (each with their own unique indexing primer combination), spiked with 10% PhiX to increase library complexity, and sequenced together on a single Illumina NovaSeq 6000 SP 250 bp paired-end sequence flowcell at the Northwest Genomics Sequencing Core at the University of Washington, Seattle.

Data were processed using the Quantitative Insights Into Microbial Ecology 2 (QIIME2) platform[118] using the demux command to demultiplex raw reads and the DADA2 pipeline[119] to generate amplicon sequence variants (ASVs) feature tables. Forward and reverse reads were trimmed to 220 and 180 bases, respectively, to remove the low-quality portion of the sequences. After filtering, trimming, merging, and chimera removal, we retained a total of 224,855,675 reads across the 534 immature fecal samples (421,078±642,783 reads per sample, range=21,325-7,990,434) and 293,437,402 reads across 620 adult female fecal samples (473,286 ±870,388 reads per sample, range= 20,109-10,735,575). ASVs were taxonomically assigned using the q2-feature classifier in QIIME2 against version 132 of the SILVA database (updated December 2017)[120] based on 100% similarity.

QUANTIFICATION AND STATISTICAL ANALYSIS

The count and taxonomy files generated by QIIME2 were imported into R version 3.5.2[127] using the qiime2R package.[134] We initially obtained 29,686 ASVs (75% were singleton, i.e. observed in only one sample, and had very few reads) and subsequently filtered the count table to retain only ASVs that had at least 500 reads total in the dataset to eliminate potentially artifactual sequences. Nine immature samples were further removed at this step (one because it contained less than 20,000 reads following observation of rarefaction curves and 8 because they were clear sequencing outliers). With these filtering criteria, 3,784 different ASVs were found across 525 immature fecal samples (mean±SD number of ASVs per sample: 728±261, range: 65-1498), while the 620 adult female samples contained 3,679 ASVs (mean±SD number of ASVs per sample: 829±248, range: 98-1761). Most ASVs could be taxonomically assigned to the phylum (100%), class (99%), and order levels (99%), with assignments decreasing substantially at the family (88%) and genus (63%) levels.

Alpha-diversity analyses

We calculated three complementary metrics of alpha diversity for each sample: the observed richness (the total number of unique ASVs per sample), Shannon Index (taking into account both richness and evenness in abundance of ASVs), and Faith's phylogenetic diversity (a measure of the diversity of phylogenetic lineages within a sample) using the "phyloseq"[123] and "picante" package.[124] To assess which predictors affected immatures' gut microbial alpha diversity, we used generalized additive mixed models (GAMMs) with the 'mgcv' package in R.[126] Such models allow fitting of a nonlinear relationship between the response variable and the fixed effect (by adding a smooth term), such as between alpha diversity and immature age (Figure 1B). Fitted predictors included: immature age (in months) at the date of fecal sample collection (continuous and modeled as a smooth term), immature sex, the parity status of mother (primiparous versus multiparous), maternal dominance rank in the month of infant's birth (continuous, between 0 and 1), cumulative monthly rainfall over the previous 30 days (in mm), average monthly minimum temperature over the previous 30 days (in ^{O}C) and the log-transformed sequencing depth (i.e., the number of reads per sample). The use of rarefaction (i.e., subsampling of the read counts in each sample to a common sequencing depth) has been strongly discouraged on microbiome datasets because it discards too much sequencing information and leads to a high rate of false positives,[135] so we calculated alpha diversity on raw counts but controlled for sequencing depth in our model. Graphical representations of alpha diversity metrics are nonetheless displayed using a rarefied dataset at 20,000 reads. Individual identity and unit membership were included as random effects. Model residual checks

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were performed using the qq.gamViz and check.gamViz functions. Given that GAMMs cannot accommodate the test of the interaction between a smooth (age) and fixed (e.g rank, parity) term, we ran these models including (i) all immature samples (0-3 years, N=525), (ii) only on young infants (<12 months, relying largely on milk, N=184), and (iii) only on older juveniles (>18 months, relying largely on plants, N=259) to test for early versus later life maternal attributes separately. Since the later life dataset is larger compared to the early life dataset, we randomly subsetted 5000 times the later life dataset to 184 samples and report the 2.5% and 97.5% quantiles of each covariate's estimate to ensure that we have the same results in the full and subset datasets.

To quantitatively assess the age at which alpha diversity reaches a plateau (i.e., converges to adult-like pattern), we used quadratic plateau models (formula: y ~ (a + b * x + c * I(x^2)) * (x <= -0.5 * b/c) + (a + I(-b^2/(4 * c))) * (x > -0.5 * b/c)) fitted using the nlsfit() function of the easynls package[136] and extracted the critical point of inflexion and r-squared of the optimized model. 95% confidence intervals (CI) for the age at inflexion were calculated by subsampling the alpha diversity metric with replacement in the dataset 1000 times. Since it is not possible to control for covariates in those analyses (e.g., sequencing depth), we ran these models on a rarefied dataset at 20,000 reads. We also run those quadratic plateau models when including both immature and adult samples to confirm the age at convergence to adult-like values.

Beta-diversity analyses

Beta-diversity (between-sample dissimilarity in composition) among immature samples was computed as the Aitchison distance,[137] which is simply the Euclidean distance between samples after centered log-ratio (clr) transformation of the raw counts (a pseudo-count was added to the zeros using the imputation based on a Bayesian-multiplicative replacement from the cmultRepl() function in the package zCompositions[127]). The clr transformation allows us to account for differences in sequencing depth between samples and is a better practice than rarefaction of the counts.[138] Principal components analysis (PCA) on the Aitchison dissimilarity matrix (function "prcomp") was used to examine how immatures samples clustered by age. The first Principal component (PC1) of the PCA was strongly predicted by age, thus we extracted the loading scores of each ASV on PC1 to determine which specific ASVs have the highest influence on the clustering by age of samples (ASVs with the most negative scores are characteristic of early life and ASVs with the most positive scores are characteristic of later life, see Figure S2D). A quadratic plateau model was implemented to find the age at which Aitchison beta diversity reaches a plateau (using only immatures samples or both immature and adult samples).

Marginal Permutational Multivariate Analysis of Variance (PERMANOVA) was then carried out on the Aitchison dissimilarity matrix using the adonis2 function in the vegan package[125] (with 10,000 permutations) to test for associations among gut microbial beta-diversity and the variables of interest (immature age, sex, maternal parity, maternal rank, environmental variables, the log-transformed sequencing depth, and unit membership). Individual identity was included as a blocking factor in the permutation design to control for repeated sampling among individuals. To test for differences on the effect of maternal attributes in early vs later life, we ran another PERMANOVA while including interactions between age and maternal parity and age and maternal rank. We also replicated these PERMANOVA analyses using more classical measures of beta diversity (Bray-Curtis, unweighted and weighted UniFrac dissimilarity) on a dataset normalized using Cumulative Sum Scaling (CSS) (a median-like quantile normalization method which corrects for differences in sequencing depth) from the metagenomeSeq package.[139,140] We found essentially similar results (Data S1B).

Mother-infant comparison of gut microbiome composition

To assess the compositional maturation of the gut microbiome of immature geladas relative to the maternal gut microbiome across age, we calculated (1) the number of shared ASVs across maternal and immature fecal-fecal communities, and (2) the beta diversity dissimilarity (Bray-Curtis, unweighted and weighted UniFrac distances) between the matched infant-mother fecal samples collected the same or next day. The dataset of immature and mother fecal samples was normalized using the CSS method to calculate these metrics since sequencing depth affects the similarity between paired samples. Quadratic plateau models were implemented on the 4 metrics to identify the age at which infants converged toward the maternal (i.e., adult-like) gut microbial composition.

To assess which predictors and maternal attributes (rank, parity) affected the compositional similarity between mother-offspring pairs, we used GAMMs to model these 4 metrics as a function of immature age (as a smooth term), immature sex, maternal parity and maternal dominance rank, climatic variables (cumulative monthly rainfall and average monthly minimum temperature) and the log-transformed sequencing depth (total number of reads in the maternal and immature samples), while individual identity and unit membership were included as random effects. Since we collected matched pairs over the first 3 years of an immature life, mothers could either be lactating (usually when infants <12-15 months) or non-lactating (i.e., cycling or pregnant with the next infant, usually when immatures>15-18 months). We did not add maternal reproductive state as a predictor in the model because it was too collinear with immature age and because female reproductive state exerts a small and mostly non-significant effect on adult female gut microbiome composition in geladas.[102] These GAMMs were run (i) on all mother-offspring pairs (0-3 years, N=398) and then separately (ii) only on young infants (<12 months, N=136) or (iii) only on older juveniles (>18 months, N=201) to assess how maternal attributes potentially affect differently the microbial similarity between mother-offspring dyads in early vs later life. We also ran the GAMM after randomly subsetting 5000 times (iii) to 136 pairs to ensure that later life results were not due to a higher sample size in the later- vs early-life dataset (we report the 2.5% and 97.5% quantiles of each covariate's estimate). Note that we ran separate analyses for each age group because it is not possible to fit an interaction between a smooth term (i.e., age) and covariates (i.e., maternal attributes) in GAMMs.

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Individuality of the microbiomes in immatures

To capture the compositional divergence between immature samples, we calculated a measure of "individuality" of the microbiomes among the 525 immature samples, as defined in,[141] which corresponds to the beta diversity dissimilarity value between a sample and the most similar sample (i.e., the minimum pairwise values from a beta diversity dissimilarity matrix, based on Bray-Curtis, unweighted and weighted UniFrac metrics). The higher the value, the more distinct the gut microbiome composition is from all other immature samples in the cohort. This was calculated using the CSS normalized dataset.

Age-associated changes in microbial taxonomic composition

To identify the microbial taxa that vary significantly in abundance as immatures age, we used a statistical framework that is commonly used to analyze time series (and, in our case, longitudinal dataset). Autoregressive Integrated Moving Average (ARIMA) models allowed us to model and test for chronological trends in temporal data.[142] First, raw microbial counts were aggregated at the family or genus level, normalized using a clr-transformation, and z-transformed per taxon (i.e., across samples) to correct for variation in library size and unaccounted variance due to other covariates. Only microbial families or genera > 0.01% relative abundance across the samples were selected for further analyses. Second, the counts were averaged across samples belonging to the same chronological age and converted into z-ordered objects (using R package zoo[128]) and into time series objects. Formatted time series were then analyzed using auto.arima (from the forecast R package[129]), using stepwise search and Akaike Information Criterion (AIC) to select the best model. This algorithm scans a wide range of possible ARIMA models and selects the one with the smallest AIC. ARIMA models that exhibited significant non-stationary trends (as opposed to unstructured "noise" fluctuations indistinguishable from stationary data) were selected following the criteria in[142]: (1) the difference order from stationary was higher than zero, and (2) at least one autoregressive (AR) and moving average (MA) coefficient was included in the model. LOESS regressions were then fitted to re-predict the count of each taxon as a function of age.

We then grouped microbial taxa into clusters based on similarities in age-associated abundance trajectories. Pairwise distances between microbial taxa trajectories (i.e., the predicted values of the LOESS regression) were computed using correlation coefficients as a distance measure,[143] and hierarchical clustering was performed using the complete method (using the function hclust from the stats R package). The optimal number of clusters was determined using the Elbow method (i.e., choosing a number of clusters so that adding another cluster does not highly improve the total within-cluster sum of squares).[144] Results of hierarchical clustering were visualized using the R package heatmap3[145] to provide an overview of gut microbiome composition changes with age.

We used the Genomes OnLine Database (GOLD) (https://gold.jgi.doe.gov/)[146] to broadly characterize the oxygen-tolerance of the 140 genera belonging to the 4 different clusters. Using a script (kindly provided by Dr. Laura Grieneisen), we extracted all organisms (bacteria/archaea) for which oxygen requirement information was available in GOLD (N=12,211 Operational Taxonomic Units, OTUs). For each gelada genus, we investigated the oxygen requirement of the representative organisms of the same genus in GOLD (classified as obligate anaerobe, anaerobe, facultative anaerobe, microaerophilic and aerobe) and reached (usually easily) a consensus as to the most frequent type of aerotolerance for the genus. For the missing genera, we looked at the original publications describing the organism in NCBI taxonomy Browser (see Data S3B).

AGE-ASSOCIATED CHANGES IN MICROBIAL FUNCTIONAL COMPOSITION

To predict the microbial functional metagenomes of each sample from 16S rRNA data, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) v.2.1.3-b software[77] with default options (picrust2_pipeline.py). We then computed the relative abundance of Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs (KOs) (agglomerated at level 2, KO2, or 3, KO3, of the BRITE map) and of Enzyme Commission (EC) numbers for each sample. The age-related temporal trajectory of each predicted KO pathway and EC was assessed using ARIMA models in a similar fashion than described above. The only difference is that the raw metagenome counts were transformed into relative abundance (instead of clr transformed). Only predicted microbial pathways > 0.01% relative abundance across the samples were included. Hierarchical clustering was used to group the predicted pathways with similar aging trajectories.

16S metagenome prediction tools like PICRUSt2 are known to predict *average* gene profiles relatively well (they miss a large percentage of bacterial genes compared to shotgun sequencing, but the actual predicted genes are not particularly "wrong" either), but perform poorly at predicting between-individual or sample differences in functional profiles.[77,147] Here, we use those metagenome inference solely to interpret the broad functions of the early life vs later microbial community (clusters) that have vastly different taxonomic composition (and the early life microbiome functional profiles picked up a relevant biological signal). The relatively high NSTI score of the immature samples indicates that gelada ASVs are not very close to reference bacterial genomes, which is likely because we assigned functional categories at the level of ASVs, while other studies do this at the level of 97% OTUs, which removes 3% of the variation across sequences and leads to closer matches to known databases. Note, however, that predicted microbial functional profiles had higher accuracy in early life (when they resemble more to human-like newborn microbiota, NSTI=0.34±0.22) than later in life (as microbiota become more specialized into grass digestion, NSTI=0.58±0.10).

Mother-infant gut microbiota similarity

To assess if maternal and offspring gut microbiome communities were more similar than expected by chance, we took a resampling approach (with 5000 repetitions) to compare the number of shared ASVs and beta diversity dissimilarity metrics (Bray-Curtis,

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unweighted and weighted UniFrac calculated on CSS transformed counts) between (1) mother-offspring pairs and (2) pairs of fecal samples from the same immature to another adult female. In this analysis, we only considered maternal and non-maternal samples collected within 0-20 days of a given immature sample to be candidate samples for resampling (in order to avoid introducing environmental variability in gut microbiota composition). Since mother-offspring pairs always shared the same social unit, we further restricted the non-maternal samples to those involving females living in the same social group as the immature. Only 377 immature samples were available for this analysis (i.e., with at least one random possible maternal and non-maternal sample). In total, we had mean±SD=1.3±0.7 [range=1-5] possible maternal samples and 4.2±2.3 [range=1-15] per infant sample. The number of days between all possible infant-mother pairs was 3.5±6.1 days and 5.8±6.4 between and infant-non-maternal pairs.

The resampling procedure randomly selects one maternal and one non-maternal sample per immature sample at each iteration. After we created this set, we use GAMMs to compare the distribution of metrics (number of shared ASVs or beta diversity dissimilarity, response variable) by fitting a variable (''pair type'') coding whether the value comes from a mother-offspring pair (1) or a non-mother-immature pair (0), and controlling for immature age (as a smooth term), immature sex, the reproductive state (lactating versus non-lactating) of the female sample of the pair (to avoid introducing systematic bias, e.g., if mothers are more often lactating than the random females and if immatures are closer to lactating females), the difference in the number of days of collection between the samples involved in the pair, and the log-transformed total number of reads in both immature and female samples involved in the pair (since some pairwise metrics still exhibited some effect of sequencing depth). Immature and female identity were included as random effects to account for repeated observations of the same individuals. We extracted the estimate of the ''pair type'' variable for the model and re-ran the model on a different set of random maternal and non-maternal pairs (5000 times in total). We thus obtained a distribution of 5000 estimates for the ''pair type'' variable. We report the exact p-value (calculated as the proportion of models with positive estimates for the number of shared ASVs and the proportion of models with negative estimates for beta dissimilarity) and the 95% confidence interval of the estimates of the ''pair type'' variable. Like above, these analyses were run including (i) all immatures (0-3 years, N=377 pairs), (ii) only young infants (<12 months, N=127), or (iii) only older juveniles (>18 months, N=193) to compare the strength of the effect among the different age categories. Since we found stronger maternal effects in later life, we needed to ensure that this was not a bias due to relying on a higher sample size of paired samples in later vs early life. Thus, during the resampling approach we also ran a fourth test where we resampled the dataset (iii) to 127 observed and random pairs at each iteration. To ensure the robustness of our biological signal (Data S2B), we re-reran the same exact resampling approach but only including random maternal and non-maternal pairs collected within 10 days of each other. We also re-ran the same exact resampling approach at 20 days, but including all ASVs > 50 reads and all ASVs > 100 reads (our filtering criteria to keep only ASVs with > 500 reads might have removed important rare non-artifactual ASVs that would weaken the signal of similarity between mother-offspring dyads) and found similar results.

We then examined the nature of the shared microbes between mother and offspring in early life (using mother-offspring pairs matched within 0-1 day). For each ASV found in infant samples (<12 months, N=3,877 ASVs total), we computed its relative abundance and prevalence (% samples containing it) in the infant dataset (<12 months, N=184) and adult female dataset (N=620 samples). We then simply plotted the relative abundance and prevalence of those ASVs according to the percentage of mother-offspring pairs sharing this ASV (N=136 matched pairs within 0-1 day where infants <12 months, N=1,870 shared ASVs). We also computed those metrics separately for primiparous (N=21) and multiparous (N=115) mother-offspring pairs.

Maternal attributes on offspring's gut microbiota taxonomic and functional profiles

We examined how maternal attributes (dominance rank, parity) were associated with differences in offspring gut microbiome (1) composition (at the family and genus levels) and (2) predicted function (KO2 and KO3 pathways and EC numbers) using GAMMs. We modelled the relative abundance of each taxon and each functional pathway as a function of maternal parity and maternal dominance rank in the month of infant's birth, while controlling for immature age (as a smooth term), immature sex, climatic variables (cumulative monthly rainfall and average monthly minimum temperature. For (1), the logarithm of the relative abundance of each taxon was fit (adding a pseudo-count of 0.001% to include zero counts). In all models, individual identity and unit membership were included as random effects. Only taxa that had an average relative abundance across samples > 0.01% were tested. Given the number of predicted metabolic pathways and the correction of p-values for multiple testings, only pathways that had an average relative abundance across samples > 0.10% were tested. P-values were adjusted for multiple hypothesis testing by calculating the Benjamini-Hochberg FDR multiple-test correction and taxa or predicted functional pathways with a corrected p-value < 0.05 were considered statistically significant. Since we predicted that maternal effects would be strongest in early life (when infants are still nursing), we ran these analyses using (i) all samples (0-3 years, N=525, results not shown), or only focusing on (ii) young infants (<12 months of age, N=184) and (iii) old immatures (>18 months, N=259).