Establishment and Stability of the Murine Oral Microbiome

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Appendix

Appendix Materials and Methods

Experimental design of mouse experiments

For oral microbiome establishment experiments, parents (breeders, two different pairs) were housed alongside their pups until day 21, in which pups were weaned and moved to their own cage.

For the experiments assessing the stability of the oral microbiome, 8 week old mice were obtained from Taconic Biosciences and The Jackson Laboratory. We allowed them to acclimate in our animal facility and their oral cavity was sequentially sampled at 10, 32 and 52 weeks of age. At 52 weeks, oral swabs and gingival tissues were collected for microbiome analyses.

For co-housing experiments, 8 week old mice were obtained from Taconic Biosciences and The Jackson Laboratory. We allowed them to acclimate in our animal facility for a week, then performed the baseline oral sampling and proceeded to merge one Taconic and one Jackson cage, originating two cohoused cages that were kept for a total of 8 weeks. Another set of one Taconic and one Jackson cage were also sampled at baseline and were kept as sentinel cages (non-cohoused) and resampled after 8 weeks, to account for possible time-dependent microbial variation.

16S rRNA gene library preparation and sequencing

Briefly, primers utilized for library preparation were 515F and 806R, which targeted the V4 region of the 16SrRNA gene, included the adapter for MiSeq sequencing (Illumina) and barcodes. Afterwards, amplicon libraries were pooled and sequenced according the manufacturer instructions for the 2x250 bp paired end sequencing (Illumina), yielding paired-end reads, as described previously (Dutzan et al. 2018).

16S rRNA gene amplicon sequence analysis pipeline

Prior to Operational Taxonomic Unit (OTU) definition and classification, unique sequences were aligned using the SILVA database (Pruesse et al. 2007), release 132, as implemented in mothur. Reads were then classified using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007), release 11.5, training set version 16, as implemented in mothur, with a cutoff=80. For OTU analyses sequences were clustered using a 97% similarity cutoff. OTUs were classified up to genus level based on the consensus taxonomy using the default cutoff (51%).

Additionally, we further informed our taxonomical classification down to species-level by blasting the reference sequence of each OTU against the NCBI 16SrRNA database (accessed on September and October of 2019) using BLAST and the top match with at least 97% similarity and coverage is reported in parenthesis as part of the OTU name, as previously described (Abusleme et al. 2017). On the occasion that a representative sequence matched to multiple species, we selected the most likely oral species based on the literature (when possible). Our species level classification is reported in parenthesis because it is an approximation and it is not definitive. There is a possibility of incorrect assignments of species identity and finding multiple hits per representative OTU sequence, due to the reduced length of V4 region reads.

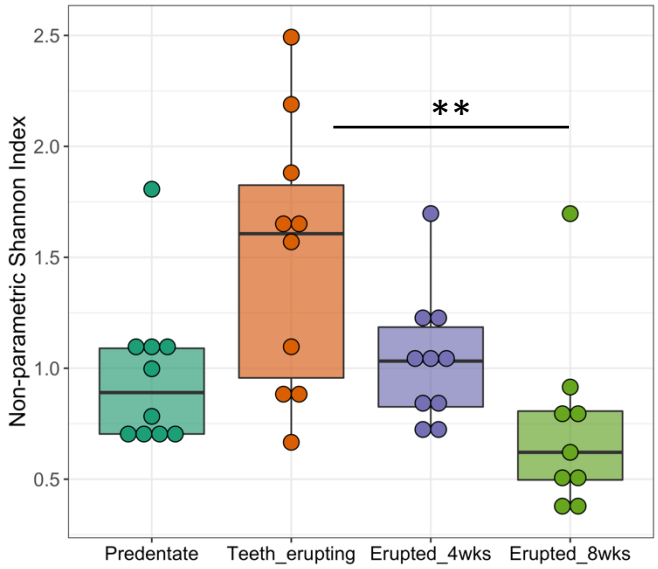
Software utilized for statistics and data visualization

LEfSe (Segata et al., 2011) was utilized to test for differences in relative abundance considering 0.01 as the α value for significance. All other statistical analyses and all graphs were generated using R (version 3.5.2, https://www.r-project.org) and RStudio (Version 1.1.463, http://www.rstudio.com/). To perform the Dunn's test, we used the R package 'dunn.test', version 1.3.5 (https://cran.r-project.org/web/packages/dunn.test/dunn.test.pdf). For data visualization, we used the R packages 'ggplot2', version 3.2.1 (http://ggplot2.org), 'RColorBrewer' version 1.1-2 (https://CRAN.R-project.org/package=RColorBrewer) and 'ggrepel' version 0.8.1 (https://CRAN.R-project.org/package=ggrepel).

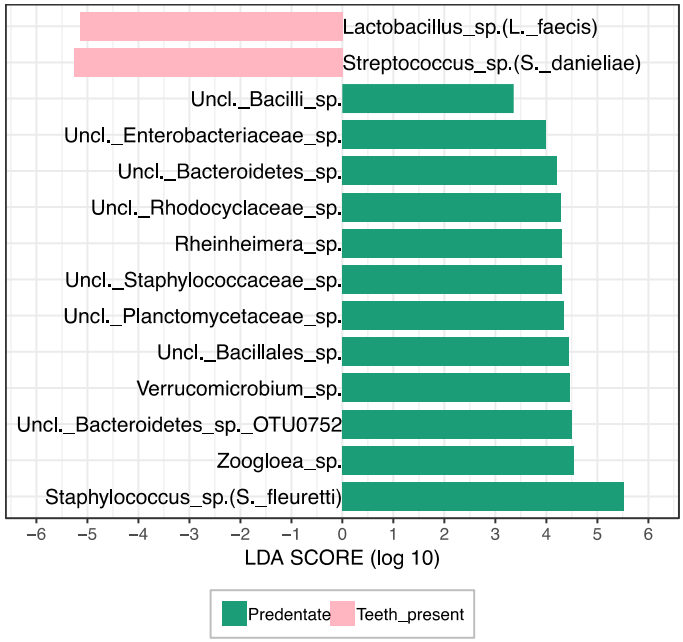
Appendix Table 1: Summary of read counts

Data-set	Total number of reads after preprocessing
Establishment of oral communities (Figure 1)	803,011
Vertical transmission of oral microbiome (Figure 2)	969,314
Stability of oral communities with age (Figure 3)	2,413,388
Jax and Tac gingival microbiome (Figure 4)	211,556
Co-housing of Jax and Tac mice (Figure 5)	695,438

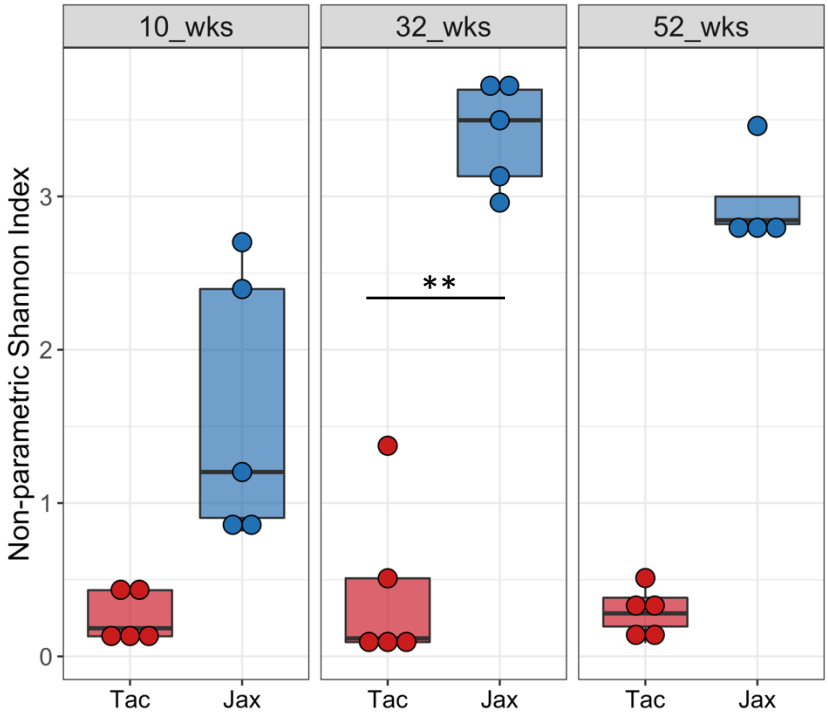
A



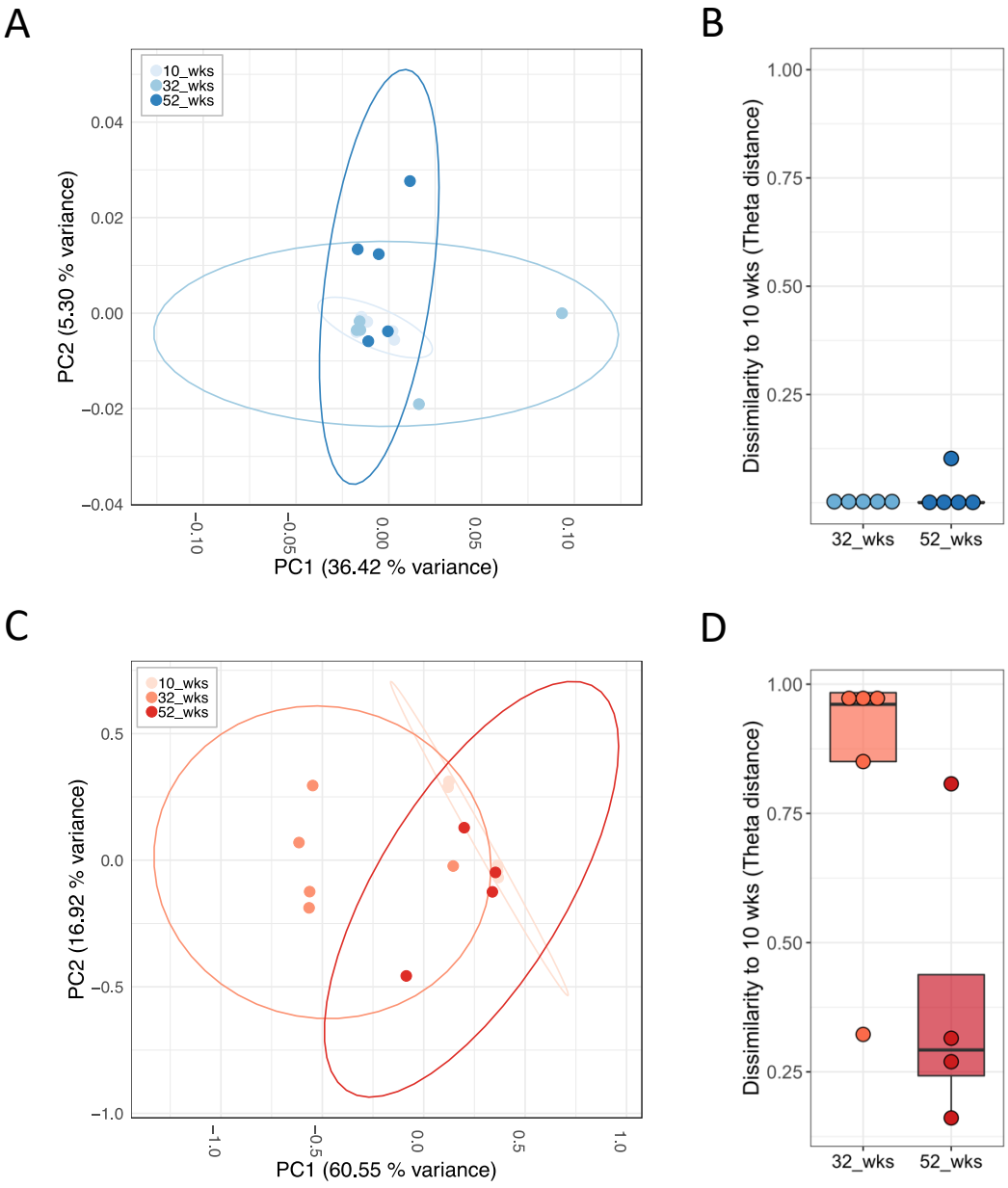
B



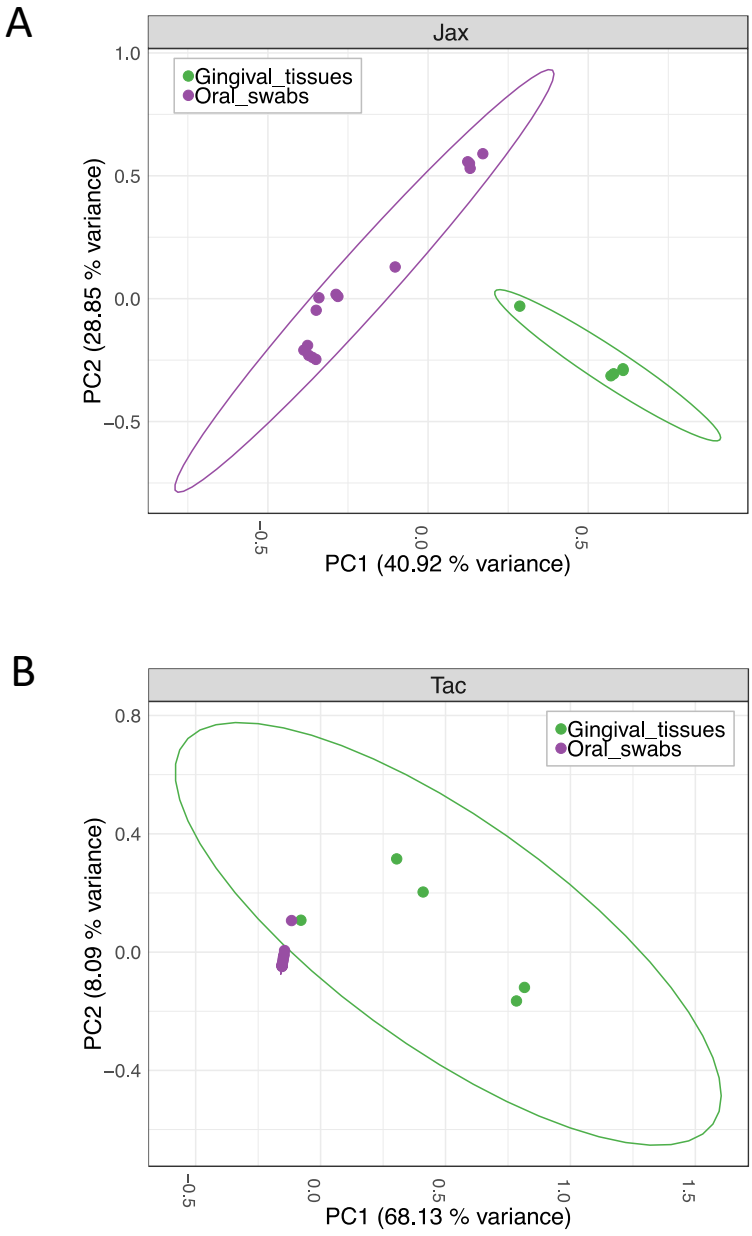
Appendix Figure 1. *Related to Figure 1. Tooth eruption is a critical event during establishment of oral microbiome communities.* (A) Microbial diversity based on the non-parametric Shannon Index. Differences among groups were tested using Friedman Test and Dunn's multiple comparisons test. ** P = 0.0076, between Teeth erupting and Erupted 8 wks timepoint. (B) Differentially represented OTUs in predentate samples versus all during and post-teeth erupted timepoints combined, determined via LEfSe analysis.



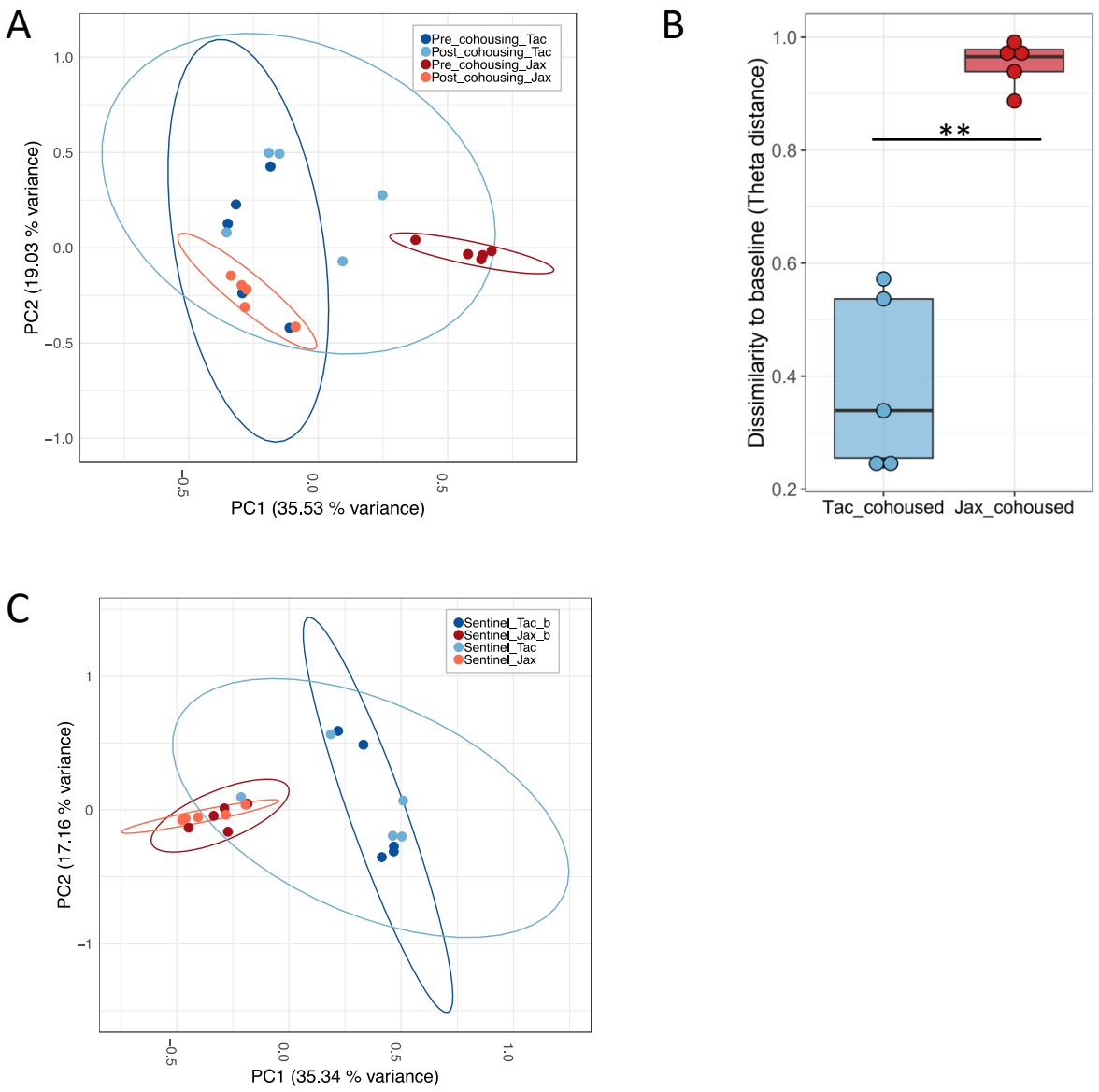
Appendix Figure 2. *Related to Figure 3. Stability of oral microbial communities with age.* Microbial diversity based on the non-parametric Shannon Index. ** *P < 0.001*, determined via Kruskall-Wallis and Dunn's multiple comparisons test.



Appendix Figure 3. *Related to Figure 3. Stability of oral microbial communities with age.* (A) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing oral microbial communities of longitudinally sampled mice obtained from Taconic Biosciences (Tac). ** *P = 0.004* as determined by AMOVA, comparing Tac samples at all time-points. Each sphere represents one sample from an individual mouse, some data points are not visible due to tight clustering. 95% confidence ellipses are also depicted. (B) Theta dissimilarities of Tac microbial communities at each time point compared to 10 weeks of age. P = 0.1250 (not significant) determined via Wilcoxon rank test. (C) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing oral microbial communities of longitudinally sampled mice obtained from The Jackson Laboratories (Jax). P = 0.588 (not significant) as determined by AMOVA, comparing Jax samples at all time-points. (D) Theta dissimilarities of Jax microbial communities at each time point compared to 10 weeks of age. P = 0.625 (not significant) determined via Wilcoxon rank test.



Appendix Figure 4. *Related to Figure 4. Jax and Tac mice have significantly different microbiomes but comparable susceptibility to periodontal bone loss.* (A) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing gingival and oral swabs communities of Jax mice at 52 weeks of age. *** *P < 0.001* as determined by Analysis of Molecular Variance (AMOVA), comparing gingival tissues versus oral swab samples. (B) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing gingival and oral swabs communities of Tac mice at 52 weeks of age. *** *P < 0.001* as determined by Analysis of Molecular Variance (AMOVA), comparing gingival tissues versus oral swab samples . Each sphere represents one sample from an individual mouse, some data points are not visible due to tight clustering. 95% confidence ellipses are also depicted.



Appendix Figure 5 . *Related to Figure 5. Oral microbiome changes during cohousing of Jax and Tac mice.* (A) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing oral microbial communities of pre and post-cohoused Jax and Tac mice ** *P = 0.001* as determined by AMOVA. Each sphere represents one sample from an individual mouse, some data points are not visible due to tight clustering. 95% confidence ellipses are also depicted. (B) Theta dissimilarities of Jax and Tac cohoused microbial communities after 8 weeks of cohousing compared to their baseline. ** *P < 0.05* determined via Mann Whitney test. (C) Principal coordinates analysis (PCoA) plot of community structure (based on Theta YC distances), showing oral microbial communities of sentinel (non-cohoused) Jax and Tac mice at baseline and after 8 weeks. P > 0.05 (non significant) as determined by AMOVA. Each sphere represents one sample from an individual mouse, some data points are not visible due to tight clustering. 95% confidence ellipses are also depicted.

Appendix References

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