Do environmental factors influence the development of the gut microbiome in young birds?
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Environmental impact on gut microbiome development in birds

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**food scarcity**

Food scarcity during development occurs regularly in the wild, resulting in chicks receiving less food. This may enhance the competition between microbes in their developing gut microbiome, which may change gut microbiome composition. Often food scarcity also leads to diet changes, which affects gut microbiome composition.

To disentangle this, we investigated the effect of food restriction in captive Rock pigeons (*Columba livia*). Rock pigeons fed chicks with crop milk during the first week, thereafter chicks were fed with regurgitated grains and pellets. By food restricting the parents during the first week, chicks received less food, i.e. crop milk, without an accompanying diet change.

Cloacal swabs were taken at 10 ages (0 to 38 days after hatching; fecal sample at day 0) from both chicks of 3 nests without and 3 nests with food restriction during the first week. Food restricted chicks were 23% lighter at day 8. This difference was maintained during development.

Cloacal microbiome was determined by sequencing the 16S rRNA V4/V5 region (Illumina MiSeq), and the data was analyzed with QIIME and R (packages Phylsoeq<sup>2</sup>, Vegan and ANCOM<sup>3</sup>).

**diet variation**

Chick diet may vary with age. E.g., many insectivorous birds feed their younger chicks a higher proportion of spiders than older chicks<sup>4</sup>. In addition, chick diet may also vary with time over the breeding season, e.g. due to the peak availability of insects<sup>5</sup>. We investigated the effect of diet variation over the breeding season on the development of gut microbiomes of pied flycatcher chicks (*Ficedula hypoleuca*) of early and late nests.

Chick feces were collected 5, 7, 10 and 12 days after hatching of all chicks of 10 early and 10 late nests. We analyzed the data of 2 chicks of nests of which at least 2 chicks had samples over the full age range (7 early and 7 late nests).

Being born in a early of late nest did not affect body mass development (R, lme, repeated measures; with age, timing and their interaction term in the model).

Fecal microbiome was determined by sequencing the 16S rRNA V4/V5 region (Illumina MiSeq), and the data was analyzed with QIIME and R (packages Phylsoeq<sup>2</sup>, Vegan and ANCOM<sup>3</sup>).

**conclusions**

Both food restriction and timing of breeding (diet) had no effect on the alpha- and beta-diversity of the cloacal and gut microbiomes of developing birds. This suggests a limited to no impact on the development of the gut microbiome composition. Food restriction and timing of breeding did however affect relative abundances, resulting in a difference in about half of the classes. The limited effect of food restriction and timing of breeding on gut microbiome development may be due to the fact that these altricial chicks were fed by their parents, which results in a continuous transfer of gut microbes (saliva). This is especially true in rock pigeons, that feed their chicks with crop milk and regurgitated food.

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