Acquisition and Development of the Gut Microbiome in Lizards

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The gut microbiome plays a pivotal role in the health of an animal. While studies have addressed the acquisition of the gut microbiota in placental mammals, limited research has been conducted on the origin of reptilian gut microbiota, especially for oviparous species. Dietary changes in early life have also been shown to play a major role in determining the composition of mammalian gut microbiota; early development of the gut microbiota has positive lifelong health repercussions[1–4]. In this study, we address two pivotal questions in an emerging model of an oviparous lizard (*anolis sagrei*, brown anole): (1) is there microbial colonization in the embryonic gut, and (2) how does the diversity of the diet shape community composition in early life.

To answer these questions, we collected eggs from a captive laboratory colony of *A. sagrei*, extracted DNA from embryonic and hatchling gut tissue, and analyzed the resulting 16S rRNA gene sequences of each sample using the Oxford Nanopore minion 16S sequencing kit.

We first established a breeding colony of 76 wild-caught brown anoles from Palm Coast, Florida. The breeding colony's living conditions and the incubation conditions were similar to previous experiments except that our experimental eggs were being sanitized through bleach immersion. These animals were kept in breeding pairs and fed a diet of crickets and watered daily. The eggs were collected every 2 to 3 days, bleached in a 1:5 bleach to DI water solution to remove external bacteria, and placed in sterile petri dishes with autoclaved vermiculite within an incubator. Eggs were randomly assigned to one of three groups: (1) dissected at an embryonic stage, (2) hatchings fed a control diet, (3) hatchlings fed a diverse diet. Eggs assigned to the embryonic timepoint were dissected approximately five days before hatching. Eggs assigned a dietary treatment were fed their diet for approximately 5 weeks. Dietary treatments were either a control diet of only fruit flies or a diverse diet consisting of fruit flies, cockroaches, and crickets.

After dissecting all embryos and hatchlings to procure their intestinal tracts, the tissues were processed to isolate DNA using Zymo Quick-DNA Fecal/Soil Microbe Minikits. Eluates were amplified utilizing PCR with the Oxford Nanopore 16S whole gene amplicon primers, and the products were visualized on an agarose gel. The gel containing PCR product from embryonic guts contained no band at the ~1500bp region compared to a positive control of microbial DNA (Fig. 1).

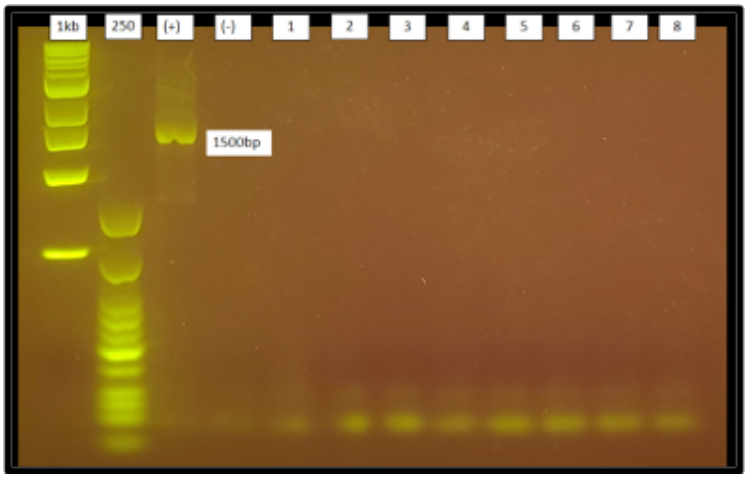

Fig. 1 Gel of the 16S PCR products of embryonic gut DNA. The first two lanes are ladders, the third lane is a positive control from a five-week-old hatchling, the fourth lane is the negative control showing only primer-dimer. Lanes 5-12 are embryonic samples showing no 1500 bp 16S PCR product.

Results from the PCR amplification of gut DNA suggested that bacteria were not present; thus, those samples were not sequenced. Additional validation was obtained through qPCR which showed that the amount of bacterial DNA in the embryonic gut samples were 1/159th the amount of DNA from a positive control sample and had an average Qc of 38 in a 40-cycle run that was not distinguishable from the negative controls.

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DNA extracted from the gut for both dietary treatments (n=10/treatment) along with four adults, was processed through the minION 16S gene sequencing protocol. After sequencing the 24 samples, over 1.4 million reads were base-called and classified using Oxford Nanopore's minION and Epi2me software. The data will be checked for quality and analyzed for future publication. Given the gene sequencing data we have collected, we are confident that our analysis will provide insight into the influence of dietary variation in gut bacterial community composition during early life.

While we cannot say for certain that there is no bacterial DNA in embryonic guts, the comparison between embryonic samples and controls suggests that if any are present the amount is biologically irrelevant. While poultry papers[5] showed colonization of the gut *in* ovo even in sterilized conditions, we did not see similar levels of colonization in our samples. Our results contrast research[6] utilizing field-acquired embryonic samples from both birds and lizards, which suggested maternal transmission but those studies utilized whole yolk samples rather than dissected intestinal tracts[6]. The addition of our research may alter the perception of microbial transmission in lizards, whereby the development of gut microbiota may occur following hatching rather than during embryonic stages.

Statement of Research Advisor

Microbiome research is a rapidly growing field and how the microbiome develops is a key question. Taylor has designed and implemented a study that will be foundational in our understanding of microbiome development in a reptile model system. Upon final analysis, Taylor will be submitting his findings for peer-review publication in a scientific journal.
- *Tonia Schwartz, Department of Biological Sciences. COSAM*

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Authors Biography

Taylor McKibben is a senior pursuing a B.S. degree in Microbiology. He has played key research roles in experimental design, literature review, and manuscript writing. He will be continuing his education at Auburn University as a masters student in the Department of Biology.

Kaitlyn Murphy is a 5th year Ph.D. Candidate under Drs. Daniel Warner and Mary Mendonça in the Department of Biological Sciences. She is interested in the influence of environmental variation on microbiota associated with reptiles, particularly in maternal transmission among oviparous species. She served as a graduate mentor to Mr. McKibben and will assist with the downstream analyses of bacterial sequences.

Dr. Tonia Schwartz is an Associate Professor in the Department of Biological Sciences. Her Functional Genomics Lab addresses questions about how animals respond to environmental stressors. She served as a mentor to Mr. McKibben as he conducted his research on the development of the microbiome in brown anoles.