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June 28, 2025

FURSCA 2025

This summer, I had the opportunity to explore microbial ecology by using *Drosophila melanogaster* as a model system. My project originally focused on how oral infection with *Escherichia coli* K-12 alters the gut microbiome of *Drosophila*, but soon expanded to include other bacteralles like *Serratia marcescens* and *Lactobacillus*. Within 5 weeks, I moved through all stages of a molecular microbiology experiment (from designing infection protocols to dissecting flies, extracting DNA, optimizing PCR reactions, and beginning bioinformatics analysis). I'm a biochemistry student by training, so it was wonderful to gain hands-on experience in both a microbiology and genomics setting.

At the beginning of the summer, I conducted an extensive literature review to understand both the significance of *E. coli*–host interactions and the advantages of using *Drosophila* as a simplified model organism. I practiced techniques such as fly handling (I wasn't even aware of the necessity of yeast for breeding nor PBS for proper ingestion of food), anesthesia (CO2 and ice), and gut dissection under a microscope. I also learned how to prepare and sterilize sucrose and LB media, which quickly became critical to my oral infection setup. Within 2 weeks, I had conducted pilot infections using dyed sucrose to verify feeding behavior, then proceeded to optimize bacterial culture conditions. Using spectrophotometry (OD600), I adjusted my bacterial suspensions to consistent concentrations and confirmed growth with LB agar streaking.

The most transformative part of the summer came when I finalized and ran my full time-course infection experiment. Flies were starved and then orally infected via cotton balls soaked in a 5% sucrose solution containing *E. coli* K-12. I sampled gut tissue at 24, 48, 72, and 96 hours to capture early, mid, and late infection states. All dissections were performed with care to minimize stress, and guts were stored at −20°C for downstream processing. I extracted DNA using two protocols—first a modified squishing prep, and then the Qiagen DNeasy Blood & Tissue Kit, which I ultimately standardized across all samples for better purity and reproducibility.

To evaluate DNA quality and prepare for sequencing, I ran dozens of PCR reactions targeting the 16S rRNA gene using primers gifted by Dr. Ola Olapade. I tested four different master mixes, comparing amplification efficiency and clarity across reactions. Gels were prepared with 1.0% agarose and visualized under UV light. These technical steps demanded patience and iteration; early gels showed weak or inconsistent results, but through careful troubleshooting and reagent testing, I developed a protocol that consistently yielded strong, clean bands. I also repeated competition and behavioral assays that had originally produced confusing data and, through improved streaking technique and plating accuracy, generated results I felt much more confident in.

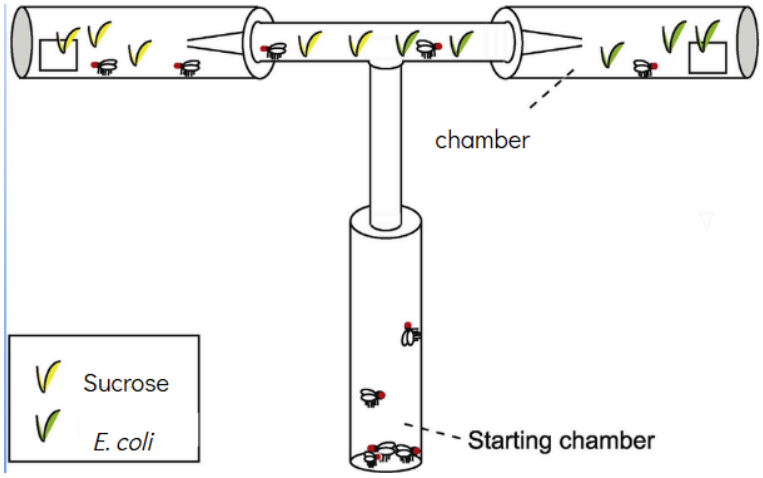
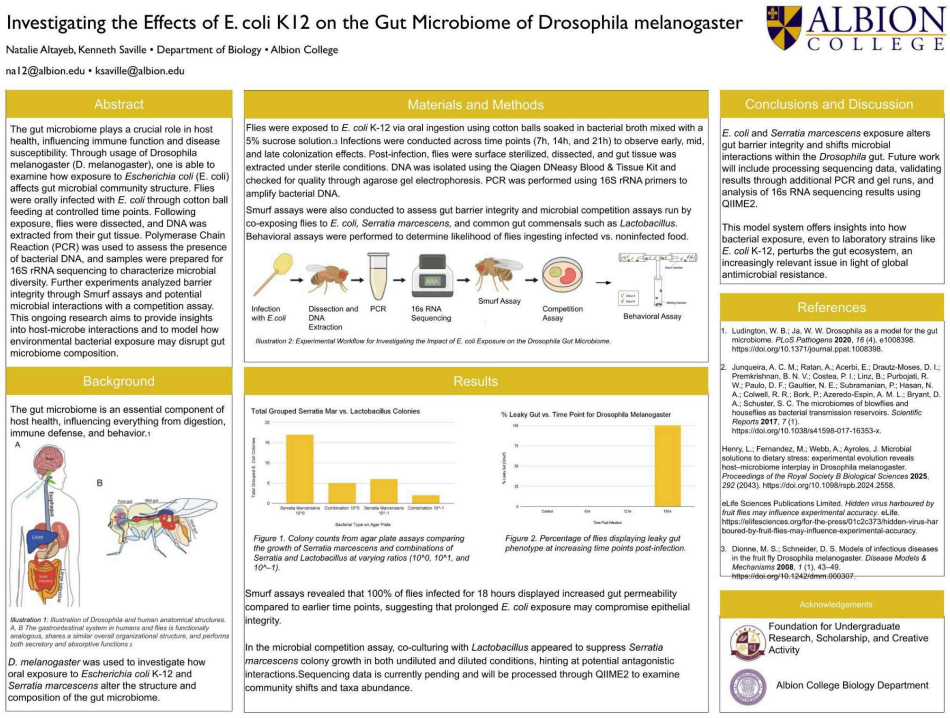


Illustration 1: Depiction of behavioral assay setup in which flies are given choice between non-infected and infected food.

Beyond benchwork, I dedicated time every day to bioinformatics training. I began with Mothur's MiSeq SOP, learning to import FASTQ files, quality filter sequences, align reads to the SILVA database, and cluster them into OTUs. I supplemented this training with tutorials in QIIME2 and plan to compare both pipelines once my own data is available. The transition from hands-on lab work to command-line programming and microbiome ecology was difficult but rewarding. I watched countless YouTube tutorials, practiced coding in Linux environments, and slowly gained confidence in my ability to analyze microbial community data.

Throughout the summer, I documented my work in a formal report and began outlining a scientific poster for the MI-SURE symposium. I used BioRender to create workflow figures

showing the infection timeline, gut dissection, and the DNA extraction–PCR–sequencing process. Although I have not yet received sequencing data, I created detailed placeholders for background, methods, hypotheses, and anticipated outcomes.



Investigating the Effects of E. coli K12 on the Gut Microbiome of Drosophila melanogaster

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Abstract

The gut microbiome plays a crucial role in host health, influencing immune function and disease susceptibility. Through usage of Drosophila melanogaster (D. melanogaster), one is able to examine how exposure to *Escherichia coli* (E. coli) affects gut microbial community structure. Flies were orally infected with *E. coli* through cotton ball feeding at controlled time points. Following exposure, flies were dissected, and DNA was extracted from their gut tissue. Polymerase Chain Reaction (PCR) was used to assess the presence of bacterial DNA, and samples were prepared for 16S rRNA sequencing to characterize microbial diversity. Further experiments analyzed barrier integrity through Smurf assays and potential microbial interactions with a competition assay. This ongoing research aims to provide insights into host-microbe interactions and to model how environmental bacterial exposure may disrupt gut microbiome composition.

Materials and Methods

Flies were exposed to *E. coli* K-12 via oral ingestion using cotton balls soaked in bacterial broth mixed with a 5% sucrose solution.[3] Infections were conducted across time points (7h, 14h, and 21h) to observe early, mid, and late colonization effects. Post-infection, flies were surface sterilized, dissected, and gut tissue was extracted under sterile conditions. DNA was isolated using the Qiagen DNeasy Blood & Tissue Kit and checked for quality through agarose gel electrophoresis. PCR was performed using 16S rRNA primers to amplify bacterial DNA.

Smurf assays were also conducted to assess gut barrier integrity and microbial competition assays run by co-exposing flies to *E. coli*, *Serratia marcescens*, and common gut commensals such as *Lactobacillus*. Behavioral assays were performed to determine likelihood of flies ingesting infected vs. noninfected food.

Infection with *E.coli* · Dissection and DNA Extraction · PCR · 16s RNA Sequencing · Smurf Assay · Competition Assay · Behavioral Assay

Illustration 2: Experimental Workflow for Investigating the Impact of E. coli Exposure on the Drosophila Gut Microbiome.

Conclusions and Discussion

E. coli and *Serratia marcescens* exposure alters gut barrier integrity and shifts microbial interactions within the *Drosophila* gut. Future work will include processing sequencing data, validating results through additional PCR and gel runs, and analysis of 16s RNA sequencing results using QIIME2.

This model system offers insights into how bacterial exposure, even to laboratory strains like *E. coli* K-12, perturbs the gut ecosystem, an increasingly relevant issue in light of global antimicrobial resistance.

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Background

The gut microbiome is an essential component of host health, influencing everything from digestion, immune defense, and behavior.[1]

A B

Illustration 1: Illustration of Drosophila and human anatomical structures. A, B The gastrointestinal system in humans and flies is functionally analogous, shares a similar overall organizational structure, and performs both secretory and absorptive functions.[2]

D. melanogaster was used to investigate how oral exposure to *Escherichia coli* K-12 and *Serratia marcescens* alter the structure and composition of the gut microbiome.

Results

Total Grouped Serratia Mar vs. Lactobacillus Colonies

% Leaky Gut vs. Time Point for Drosophila Melanogaster

Figure 1. Colony counts from agar plate assays comparing the growth of Serratia marcescens and combinations of Serratia and Lactobacillus at varying ratios (10^0, 10^1, and 10^–1).

Figure 2. Percentage of flies displaying leaky gut phenotype at increasing time points post-infection.

Smurf assays revealed that 100% of flies infected for 18 hours displayed increased gut permeability compared to earlier time points, suggesting that prolonged *E. coli* exposure may compromise epithelial integrity.

In the microbial competition assay, co-culturing with *Lactobacillus* appeared to suppress *Serratia marcescens* colony growth in both undiluted and diluted conditions, hinting at potential antagonistic interactions.Sequencing data is currently pending and will be processed through QIIME2 to examine community shifts and taxa abundance.

Acknowledgements

Foundation for Undergraduate Research, Scholarship, and Creative Activity

Albion College Biology Department

Illustration : A copy of the poster drafted for the MID-SURE research conference.

In terms of results, both my experiment with the dyed sucrose mentioned above (formally called a Smurf Assay) and different types of bacteria combined in food (Competition Assay) aligned with one of my hypotheses. The overall gut health of flies exposed to E. coli deteriorates after a period of over 12 hours.

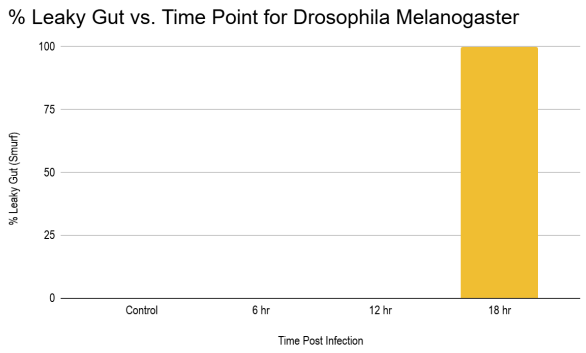

Figure 1:(A) Percent Leaky Gut vs. Time Point for D. melanogaster. The results of the graph depict that after a period of 18 hours, all flies exposed to infected food displayed a leaky gut/full body dyeing. (B) Total Grouped Serratia marcenscens vs. Lactobacillus Colonies. The results of the graph depict that Lactobacillus outcompetes Serratia marcescens on two diluted combination plates.

Image 1: (A) Infected flies post 18-hour incubation and (B) Infected flies post 12-hour incubation.

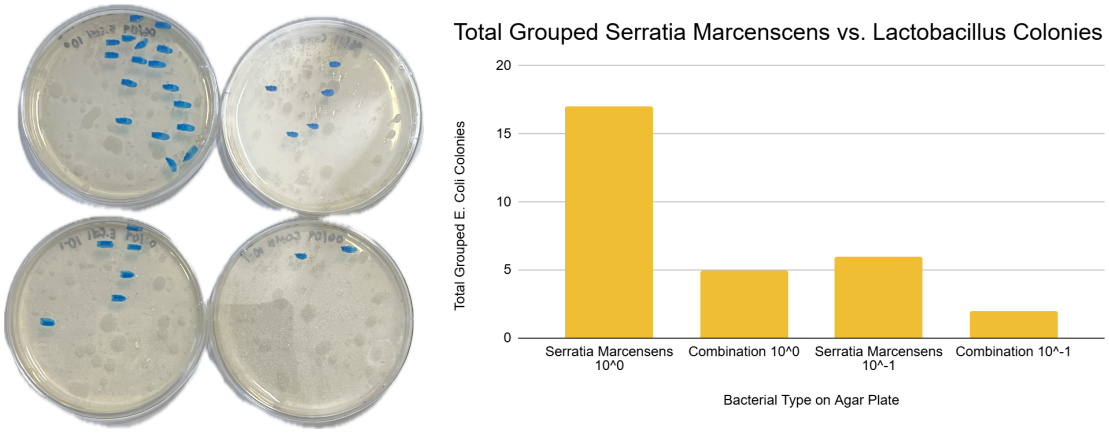

Image 2: LB plates of 10^0 and 10^-1 dilutions displaying the presence of Serratia marcescens displayed alongside Lactobacillus bacteria. The results indicate a decreased amount of E. coli colonies present in both the undiluted and diluted combination mixtures

Figure 2: Total Grouped Serratia marcenscens vs. Lactobacillus Colonies. The results of the graph depict that Lactobacillus outcompetes Serratia marcescens on two diluted combination plates.

One of the most impactful moments came from meetings with representatives from Thermo Fisher and PCR Biosystems. Speaking directly with gene sequencing professionals helped me understand the field's complexity—and made me realize how much I still have to learn. I often felt like a clueless undergrad in those conversations, but I am also left feeling inspired and curious. These interactions, along with the challenges I faced troubleshooting PCR reactions and adjusting protocols, taught me that science is built on iteration, humility, and asking good questions.

Looking ahead, I plan to finalize my PCR protocol and send extracted DNA to Zymo Research for 16S rRNA sequencing. Once results are returned, I will analyze them using both Mothur and QIIME2, comparing taxonomic profiles across infection time points to identify microbiome shifts induced by *E. coli*.

This project, with all its setbacks, still created work that has meaningful implications for how we understand gut-microbe interactions and bacterial colonization in *Drosophila melanogaster*. Though flies may seem far removed from human biology, they are a powerful model for studying microbiomes, disease, and immune responses.

Gut health begins with complexity, and our ability to parse through microbial communities using DNA extraction, PCR, and sequencing techniques allows us to ask deeper questions about how organisms interact with pathogens and with each other.

With this in mind, I plan to present my findings at Albion College's 2026 Elkin Isaac Research Symposium and hopefully other conferences (MIDSURE, ASBMB). I also hope to build on this foundation through a career in medicine and endocrinology research. The skills, knowledge, and mentorship I've gained this summer have truly been transformative.

None of this would have been possible without the generous support of the Kenneth Ballou, '47 Research Endowment for Biology. Research like this is made feasible through your investment in student discovery and education. I am deeply grateful for the opportunity to learn, grow, and contribute to science through your support!

With immense gratitude,

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