(ur) DIMENSIONS

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Marks of a CSUF graduate from the College of Natural Sciences and Mathematics

GRADUATES FROM THE COLLEGE OF NATURAL SCIENCES AND MATHEMATICS:

Understand the basic concepts and principles of science and mathematics.

Are experienced in working collectively and collaborating to solve problems.

Communicate both orally and in writing with clarity, precision, and confidence.

Are adept at using computers to do word processing, prepare spreadsheets and graphs, and use presentation software.

Possess skills in information retrieval using library resources and the internet.

Have extensive laboratory, workshop, and field experience where they utilize the scientific method to ask questions, formulate hypotheses, design and conduct experiments, and analyze data.

Appreciate diverse cultures as a result of working side by side with many people in collaborative efforts in the classroom, laboratory, and on research projects.

Have had the opportunity to work individually with faculty members in conducting research and independent projects, often leading to the generation of original data and contributing to the research knowledge base.

Are capable of working with modern equipment, instrumentation, and techniques.

DIMENSIONS

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ABOUT THE COVER

We would like to give a special thanks to Fernando Del Rosario and his graphic design class. The cover for Volume 26 was designed by Manami Kobayashi. The artist's statement is as follows: "There are departments' icons in a light bulb. Color uses university color for title and the ones (circle and stick) around the light bulb. The light bulb represents inspiration and analysis. The ones around bulb and the right corner also represents light of the bulb".

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Letter from the Editor-in-Chief:

Dimensions is a testament to the incredible and insightful research that our undergraduate students complete during their time here at CSU Fullerton. The papers collected in this journal reflect the motivation, inspiration, and knowledge of Titans on their journey to study this world and improve their communities. For most of these students, this is a steppingstone on their educational path as they eventually move on to graduate school or careers outside academia.

Dimensions would like to thank the College of Natural Sciences and Mathematics faculty, for their guidance of these students' research and for encouraging them to submit their papers. Your research inspires your students to get involved in research labs and become better scholars. We also would like to thank the NSM Deans' Office for supporting this publication and their assistance in getting the word out to students and faculty about this opportunity. And as Editor-in-Chief, I would like to thank our student editors and submitters for continuing to support the mission of *Dimensions* through their involvement.

This journal would not exist without curiosity and the willingness to share research with the campus community. If you are reading this, no matter where you are in your educational journey, we hope that it inspires you to complete your own research project. Share this publication with your peers, your family, and your community. Get involved with *Dimensions*, apply to be a student editor or encourage others to submit their research papers. Most of all, be curious, and chase answers to questions you have. When you find those answers, *Dimensions* will be here to help you bring visibility to your work and push the field you study further forward.

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Biological Sciences

The Impact of Microbes on the Evolution of *Drosophila melanogaster* Development Time

Burgundy Davison, Sonia Garcia, Shelly Li, Urja Nandu

Advisor: Dr. Parvin Shahrestani

Abstract

Microbiome communities that inhabit animals or exist on their surfaces have historically been overlooked in laboratory evolution studies, yet they are essential for understanding the evolution of life history traits. Microbiota influence host traits, for example, affecting the developmental time of *Drosophila melanogaster*. Direct selection on development can result in divergence of this trait among laboratory populations and divergence of the host's microbiota. However, it remains unclear whether changes to development time evolve before changes in the host's microbiota or if alterations in the host's microbiota occur before the evolution of the host's life history traits. This project focuses on altering the host's developmental time through selection pressure while manipulating their microbiota through supplementation with acetic acid and lactic acid bacteria**.** This will be done by measuring bacterial abundance and development time before and after imposing selection pressure for fast development in different microbial conditions. Generation 0 data included in this study show that the development time of A-type flies is quicker than that of C-type flies. It also demonstrates that Acetic Acid Bacteria are more abundant in A-type flies whereas Lactic Acid Bacteria are more abundant in C-type flies. Anticipated results suggest that acetic acid-producing bacteria will alleviate selection pressure on the host genotype, leading to a slowdown in the evolution of fast development. Conversely, we expect that lactic acid-producing bacteria will buffer selection pressure on the host genotype,

accelerating the evolution of fast development. These findings will help elucidate how the host's evolution impacts and relies on the microbiota.

Introduction

Adaptive evolution occurs through natural selection, with the key requirements of heritable phenotypic differences within the population resulting in fitness differences. Within adaptive evolution, variation may result in advantageous and adverse alleles that lead to selection of phenotypes and accordingly, their subsequent genotypes. Adaptive evolution can occur in the wild or in a laboratory, where it is known as experimental evolution (Garland, T. & Rose, M. R., 2009). Within a laboratory setting, experimental evolution is a common practice used for phenotype-to-genotype mapping. Organisms used in experimental evolution are held under controlled conditions and environments, where variables such as temperature, humidity, and food can impact results (Garland, T., & Rose, M. R. 2009). The study of the microbiota within animal experimental evolution is often ignored and under-explored, leading to our inquiry on adaptive evolution within *Drosophila melanogaster* in relation to its microbiota.

D. melanogaster, alongside humans, is one of the most extensively studied organisms in biology due to sharing around 75% of the disease-causing genes within humans (Mirzoyan et al. 2019). *D. melanogaster* is considered a model organism for experimental, genomic, and evolutionary studies as they have a rapid life cycle, high reproductive rates, and are extremely versatile (Mirzoyan et al. 2019). The specific *D. melanogaster* populations used in this study come from the Drosophila Experimental Population Resource, also known as the Rose populations (e.g. Rose et al. 2004). Specifically, we used the C-type and the A-type *D. melanogaster.* C-type *D. melanogaster* have a 28-day generational cycle (one generation cycle is from one egg to the next egg), are slow developing, and long-lived. A-type *D. melanogaster* has

a 10-day generation cycle, are fast developing, and short-lived (Graves et al. 2017). Both C-type and A-type *D. melanogaster* contain five replicate populations, with the C-type being referred to as CO_{1-5} and the A-type is referred to as ACO_{1-5} (Graves et al. 2017)

The microbiota of an organism (microorganisms that live within and on the fly) are known to influence the host's traits (Matthews et al. 2021, & Gould et al. 2018). The components of the microbiota are also heavily influenced on *D. melanogaster* development time. Due to the strong influence the microbiota holds on the *D. melanogaster* development time, the experimental manipulation of this factor is the focus of this study. The two predominant bacterial types found within the *D. melanogaster* microbiota are lactic acid bacteria (LAB), and acetic acid bacteria (AAB) (Chaston et al. 2016). The A-type populations are abundant in both LAB and AAB, whereas C-type populations are less abundant in both bacteria (Walters et al. 2019). Our study seeks to determine if microbiota or genotype has stronger effects on the evolution of the organism's life history traits.

With continuous research, the question of the microbiota function and its overall ability to relieve or buffer selection pressure on the host genotype arises. We work to address this question through the laboratory selection of both the faster and slower developing flies in conventional and supplemented conditions with AAB and LAB. We hypothesize that the development time of the A-type and C-type populations of *Drosophila melanogaster* will have varying response times to the different selections based on their bacterial abundance. This study can help lead to a better understanding of the interactions between bacteria and development time of *D. melanogaster.*

Methods

Experimental Design

To investigate our hypothesis, we use experimental evolution to study the evolutionary relationship between development time and the microbiota in *D. melanogaster* populations. This experiment is done under three conditions: conventional, supplemented with AAB, and supplemented with LAB. For the first and last generations, we measure the host's ability to control its microbiota and its development time. The last generation is determined once the flies have reached A-type conditions. These are measured compared to the control and experimental populations. By measuring their bacterial abundance and development time, we gain further insight into the role that microbes play in the response to selection.

Selection

Drosophila melanogaster populations are kept on 24-hour light cycles on a banana molasses diet. The A-type flies (ACO) are on 10-day generation cycles, and the C-type flies (CO) are on 28-day generation cycles. nACO populations (new ACO populations) develop from CO ancestors by transferring the first 20% of emerged flies in each generation from rearing vials to cages. Then, they are allowed to lay eggs for 18 hours for the next generation. This continues until A-type flies are achieved. This is done under three different conditions. The first experimental population is kept under conventional conditions (nACO) with no bacterial manipulation. The second experimental population (nACO-AAB) is supplemented with acetic acid bacteria (*Acetobacter* species) at every generation. The third experimental population (nACO-LAB) is supplemented with lactic acid bacteria (*Lactobacillus* species) at every generation. Each of the ancestral and experimental populations has five replicates (ACO_{1-5}, CO_{1-5}, $nACO_{1-5}$, $nACO\text{-}AAB_{1-5}$, $nACO\text{-}LAB_{1-5}$).

Phenotyping

Bacterial Preparation

Bacterial stocks of *Acetobacter pasteurianus (Ap), Acetobacter tropicalis (At), Lactobacillus brevis (Lb),* and *Lactobacillus plantarum (Lp)* are stored separately in glycerol solution diluted at -80℃. Approximately two days before inoculation, bacterial stocks are streaked onto Criterion Lactobacilli MRS agar plates. Per Liter of Pure Life Distilled Water, 20g dextrose, 10g meat peptone, 10g beef peptone, 5g yeast extract, 5g sodium acetate, 2g disodium phosphate, 2 grams ammonium citrate, 1g tween 80, 0.1g magnesium sulfate, and 0.05g manganese sulfate is added to make agar plates. *Acetobacter* species grow under aerobic conditions, while *Lactobacillus* species are placed in tightly sealed containers with added CO_2 to create anaerobic conditions. A day before inoculation, a single colony from each species is isolated from the agar plates and mono-inoculated into sterile Criterion Lactobacilli MRS broth tubes before being vortexed. The *Acetobacter* species are incubated while shaking at 30°C, and the *Lactobacillus* species are incubated stagnant at 30°C. A day later (24 hours), 1mL of each bacterial suspension is vortexed and pipetted into cuvettes to receive absorbance readings on the spectrophotometer. Using 1.7 mL microcentrifuge tubes, the bacterial suspensions are diluted to receive a normalization absorbance reading of OD600=1, vortexed, and later centrifuged. The supernatant is removed, and the pellet is resuspended in PBS. After vortexing the pellet in PBS, the bacteria is mixed to create different treatments. *500 µL of Acetobacter pasteurianus* and *500 µL* of *Acetobacter tropicalis* is added to a microcentrifuge tube to create the AAB treatment. *500 µL of Lactobacillus brevis* and *500 µL* of *Lactobacillus plantarum* are added to a separate microcentrifuge tube to create the LAB treatment. The LAB+AAB treatment consists of 250 µL of each of the 4 bacteria species mixed in one microcentrifuge tube. All treatments are then placed in the fridge until inoculation.

Dechorionation

Half-filled banana food plates are placed inside the cages with a yeast paste in a micro-petri dish 6 hours before dechorionation. After 6 hours, the eggs laid by adult flies are washed off the food plate with autoclaved DI water into a sterile bushing. The bushing is washed in 0.6% bleach solution for 2.5 minutes outside of the sterile hood. Then, the bushing is placed in a new cup filled with 0.6% bleach, and the washing process is repeated for another 2.5 minutes. The bushing is then placed inside the sterile hood, where a third bleach is done with a new solution for another 2.5 minutes. Then, autoclaved DI water is used to wash the bushing three times by dunking it three times at each wash. Finally, once the last wash is done, the eggs that are on the walls of the bushing are transferred to sterile food with a paintbrush. Solutions are discarded after every wash.

Bacterial Inoculation

After dechorionation in the first and last generation, eggs from the CO, ACO, nACO, nACO-AAB, and nACO-LAB are inoculated with 50μL of the 4 species bacterial mixture described in the "selection" section above. We will inoculate eggs from the CO, ACO, nACO, nACO-AAB and nACO-LAB population with 100μL of *Acetobacter pasteurianus* and *Lactobacillus brevis* directly into each tube. The caps of the tubes are left a quarter turned, kept at room temperature with a 24-hour light in an incubator.

Homogenization & Plating

We prepare sterilized microcentrifuge tubes by filling them with 125 µL of sterile mMRS broth and 0.1 mL of ceramic mixing beads. Adult flies are removed from their respective food vials in the incubator and anesthetize them 5 days post-eclosion with CO_2. Five females and five males are chosen randomly from vials of each population and placed separately into the microcentrifuge tubes. The flies go through two rounds of homogenization at 6.5 m/s, with each

round being 60 seconds. We then plate the homogenate on mMRS agar plates and incubate it at 30°C for 48 hours. After 48 hours, we remove the plates from the incubator and count the grown colonies under a microscope. We estimate the number of Colony Forming Units (CFU) using the equation:

$$CFU = colonies\ counted \left(\frac{dilutions}{\mu L\ plated}\right) x \left(\frac{voume\ of\ fly\ homogenete\ total}{\text{number of flies homogenized}}\right).$$

Development Time

In the first and last generation, from each population, we will collect a sample of 10 vials with 60 eggs in each to measure development time. The last generation is determined once the flies have reached A-type conditions. Once the flies emerge from the pupal casing, the flies are checked every 6 hours. Freshly enclosed flies are removed from the vials, which are sexed and counted.

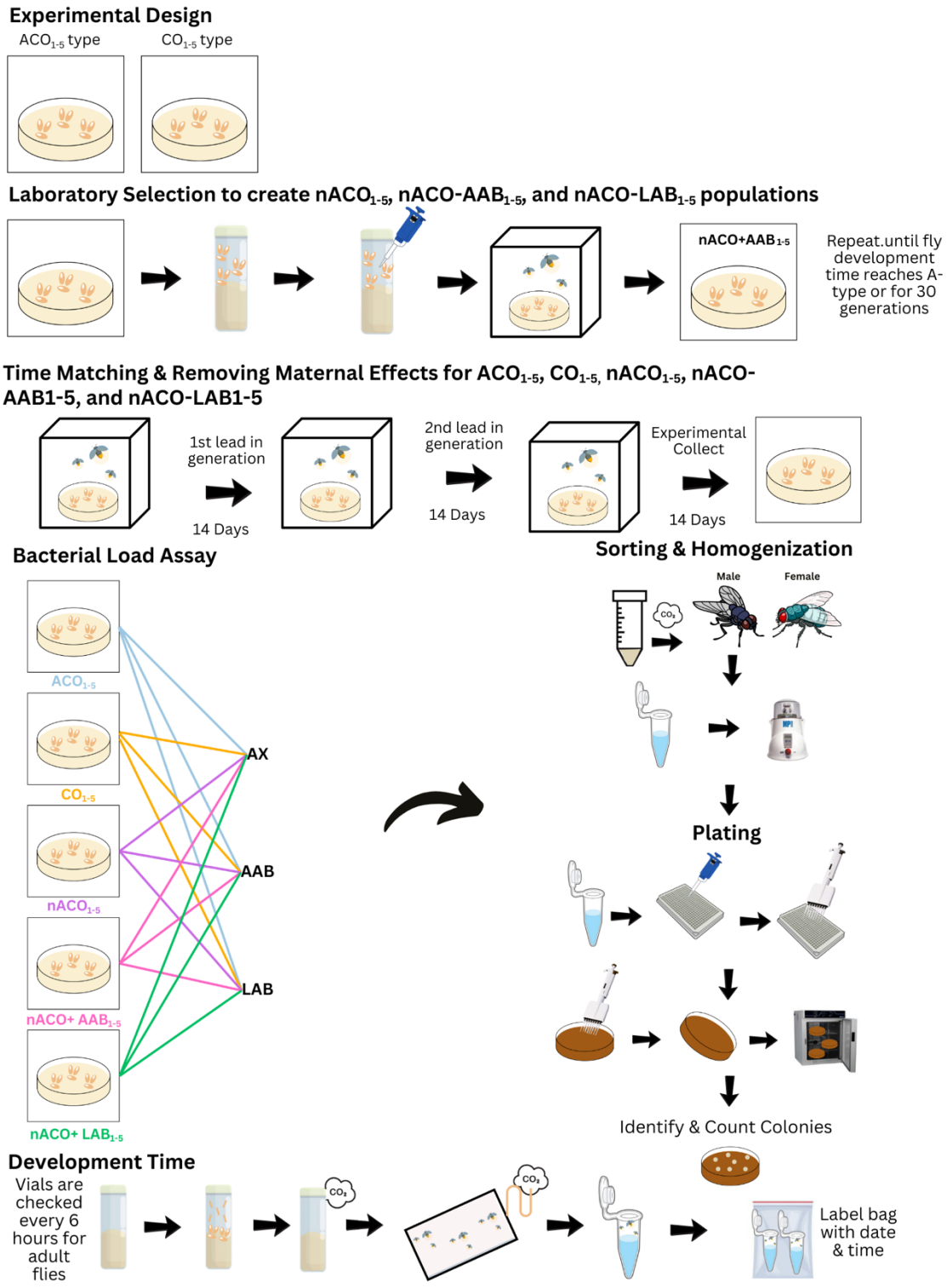

Figure *1*. The overall experimental design shows the control populations (ACO_{1-5} and CO_{1-5}) are laboratory selected to create experimental populations: $nACO_{1-5}$, nACO-AAB_{1-5}, and nACO-LAB_{1-5}. To time match different populations, flies are treated with two 14-day lead-in generations before experimental collection. Bacterial load is quantified by sorting, homogenizing, plating,

and colony counting of male and female flies. Development time is determined by counting vials every 6 hours for emergence of adults to evaluate the microbiota effects on host traits. Selection is continued until the development time equivalent to A-type flies or for 30 generations.

Results

Preliminary results

Generation 0 data from conventional ACO and CO populations were used to determine their bacterial abundance and the development time to predict experimental population results. At Generation 0 CO populations had more bacterial abundance overall compared to ACO populations. However, Figure 2 demonstrates that AAB is more abundant in ACO populations, whereas LAB is more abundant in CO populations.

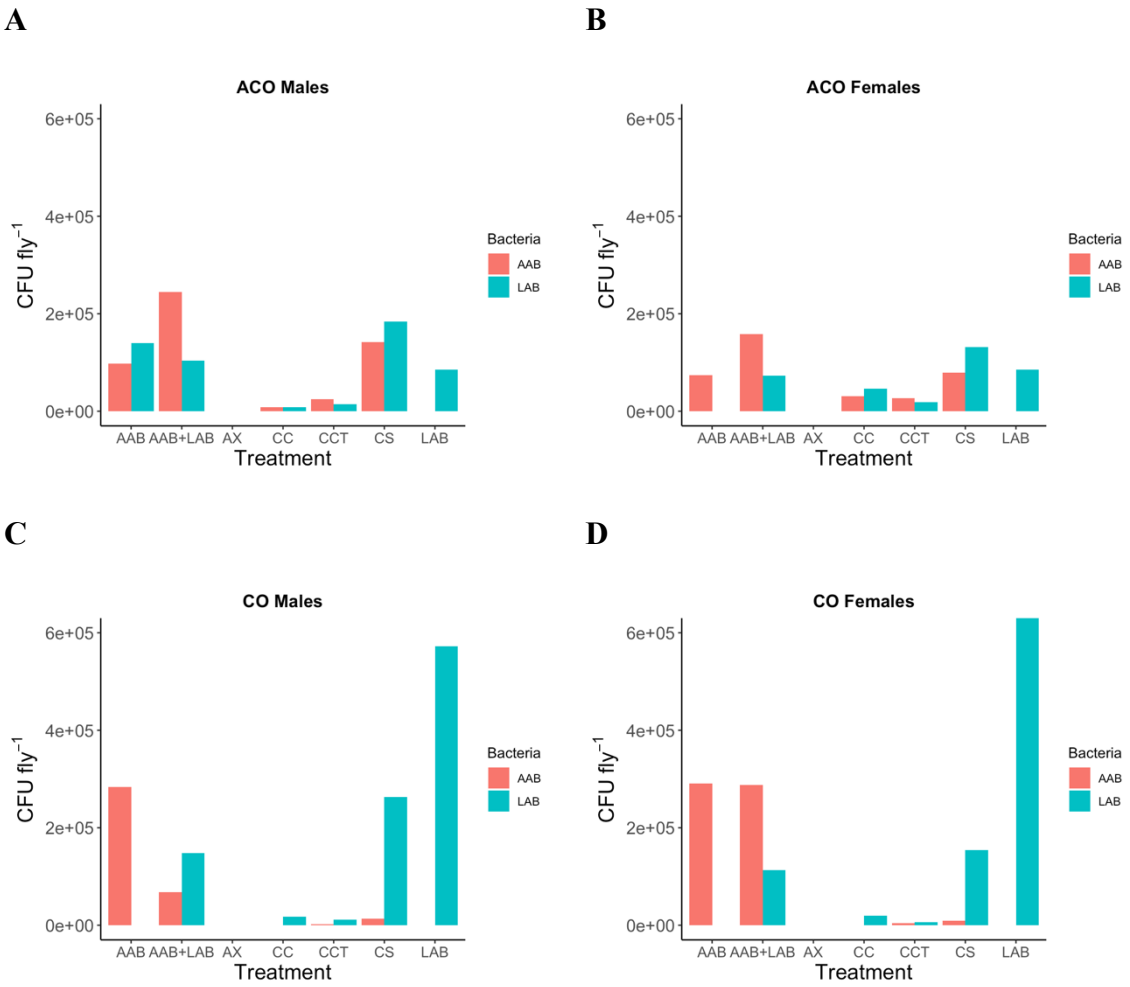

***Fig 2. D. melanogaster* Acetic Acid Bacteria and Lactic Acid Bacteria Abundance for Generation 0 using the CFU equation per fly for conventional conditions.** A) ACO males demonstrating more abundance for AAB. Total sample size per treatment is AAB=53, AAB+LAB=75, AX=75, CC=75, CCT=52, CS=21, LAB=75. B) ACO females demonstrating more abundance for AAB. Total sample size per treatment is AAB=53, AAB+LAB=75, AX=75, CC=72, CCT=53, CS=26, LAB=75. C) CO males demonstrating more abundance for LAB. Total sample size per treatment is AAB=75, AAB+LAB=63, AX=75, CC=71, CCT=75, CS=75, LAB=75. D) CO females demonstrating more abundance for LAB. Total sample size per treatment is AAB=75, AAB+LAB=60, AX=75, CC=66, CCT=75, CS=75, LAB=75.

Generation 0 data also demonstrates that flies under conventional conditions develop more quickly for A-type flies compared to C-type flies. Figure 3A demonstrates that ACO flies take fewer hours for flies to emerge as adults compared to CO flies which take longer to develop as seen in Figure 3B. This aligns with previous research by Chippindale et al. 1997 which indicates that ACO populations develop a lot quicker compared to CO. The research also shows that ACO populations are quickly developing, but short-lived lived and CO populations are long developing and long-lived (Chippindale et al. 1997). The difference in bacterial abundance for the two populations and their development time help explain the evolutionary relationship between microbiota and life history traits.

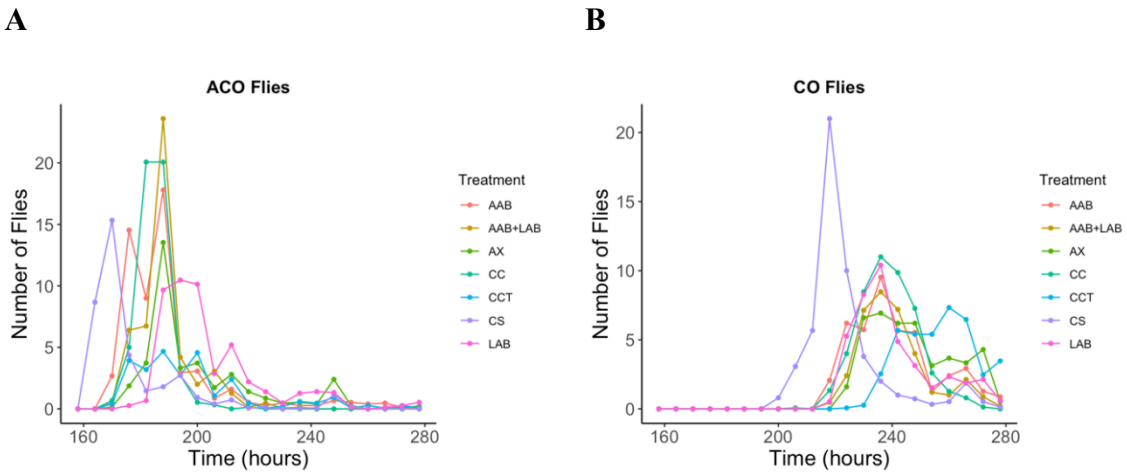

***Figure 3. D. melanogaster* development time for Generation 0 under conventional conditions.** A) Development time for ACO populations show flies under CS treatment are developing quicker compared to flies under LAB treatment which develop slower. AAB+LAB treatment shows the largest number of flies emerged as adults compared to CCT treatment showing the least number of flies emerged. B) Development time for CO populations show flies under CS treatment are emerging into adult flies the quickest compared to CCT flies developing the slowest. CS treatment also shows the largest number of flies emerged as adults compared to AX treatment showing the least number of flies emerged.

Discussion

The preliminary results for Generation 0 fruit flies show that under conventional conditions, A-type flies develop more quickly than C-type flies. Given the differences in development time and the bacterial abundance between A-type and C-type populations, we can infer that as flies evolve, their bacterial abundance will also change. Using Generation 0 as the baseline of the project, we can compare changes in the phenotype and genotype of different populations throughout the experiment. It is expected that by Generation 15, the conventionally evolved nACO populations would have the same bacterial abundance as A-type flies. Compared to conventionally evolved flies, the additional supplementation of AAB and LAB is expected to show a difference in response times for nACO populations to achieve A-type bacterial abundance.

The acetic acid-supplemented populations (nACO+AAB) are expected to have a slower response to selection for fast development, as the selection pressure is relieved. This means that the population supplemented with AAB will take longer time to show changes in life history phenotype. Under constant supplementation with acetic acid, nACO populations will evolve the ability to regulate their AAB abundance more rapidly. The lactic acid supplemented populations (nACO + LAB) are expected to respond more quickly to selection for fast development, since LAB buffers selection pressure. Under constant supplementation of lactic acid, the populations will evolve the ability to control their LAB abundance more gradually. These predicted results

indicate that the supplementation of AAB and LAB influences the response to rapid selection, showing that the microbiome affects life history phenotypes in fruit flies.

These findings should introduce new insights into the interactions between microbiomes and life history traits, which have previously been shown to affect nutritional phenotypes (Chaston et al., 2016). Since earlier studies indicated a trade-off between lifespan and reproduction, our projected outcomes suggest that bacteria may reduce the necessity for a fly's genotype to evolve, as some selection pressure is mitigated by the bacterial supplementation (Mathews et al., 2021; Gould et al., 2018). If the results reveal a significant difference as predicted, it will provide compelling evidence supporting the strong impact of bacteria and corroborate previous investigations. Comparisons with similar studies may address the long-standing question of which factor is more crucial, genotypes or microbiomes. Understanding the mechanisms driving the evolution of bacteria and fruit flies offers valuable insights into the broader dynamics of evolution. This knowledge can help expand practical applications of fast adaptive evolution in healthcare, agriculture, and conservation. Given that the results could have potential applications in other fields, it is essential to complete the project.

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A Biological Lens on Breast Tumor Classification Using Machine Learning Techniques

Vivian Duong, Sabrina Ly

Advisor: Dr. Sunny Le

California State University, Fullerton

Abstract

Breast cancer remains one of the most prevalent and life-threatening cancers among women worldwide. Early and accurate classification of tumors as benign or malignant is essential for effective treatment. In this study, we applied logistic regression modeling and the cross-validation method to the Breast Cancer Wisconsin (Diagnostic) dataset to investigate the classification of tumors based on nuclear features. After addressing multicollinearity and standardizing variables, our final model achieved high predictive accuracy (97.28% on a test set and 92.79% through leave-one-out cross-validation). Significant predictors included smoothness, texture, radius, compactness, and symmetry. As undergraduate students majoring in biology, this research deepened our understanding of statistical modeling in the biomedical context and demonstrated the powerful interdisciplinarity between data science and medicine.

Key words: breast tumor classification, cross validation, machine learning, cancer research

Introduction

As undergraduate students preparing to enter the medical field, we were drawn to studying breast cancer due to its prevalence and impact on public health. Recognizing the importance of early detection and diagnosis, we sought to explore how data science and statistical modeling can contribute to medical advancements. Through our coursework in statistics, we developed an appreciation for how medicine can be greatly supported by mathematical and statistical modeling and decided to explore these interdisciplinary areas. The course also inspired us to undertake a project that integrates our growing skills in data analysis with a critical biomedical issue - breast cancer classification and data science. By conducting this research, we intend to increase our knowledge of breast cancer diagnosis and classification and improve our understanding of data analysis and coding.

According to the American Cancer Society, the most common cancer diagnosed among women in the US is breast cancer. Breast cancer arises from uncontrolled cell growth in breast tissue, which frequently starts in milk ducts or glands. About 1 in 8 women (13.1%) get diagnosed with invasive breast cancer in their lifetime, and 2.3% pass away from the disease, making breast cancer a serious public health concern in the United States (American Cancer Society, 2024).

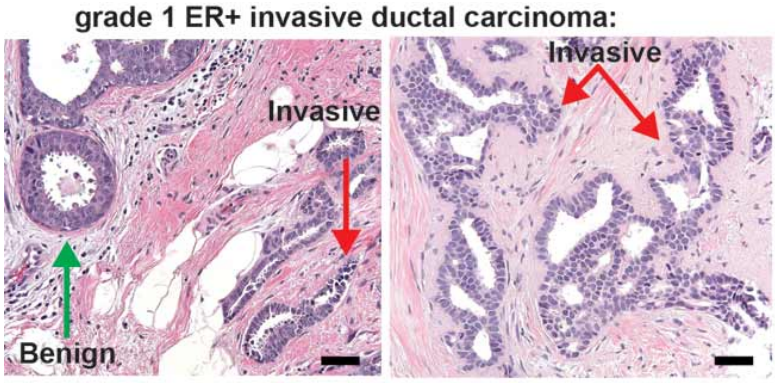

Figure 1. Types of breast cancer. The most common include non-invasive forms like ductal carcinoma in situ, where cancerous cells are contained, and invasive forms that spread outside of ducts or glands (Perry, 2014).

A clinical breast exam is usually the first step in the diagnosis process. Doctors use imaging techniques such as mammograms, ultrasounds, or MRIs to identify irregularities in size, shape, or density. If a suspicious mass is found, a biopsy is performed to determine whether the tumor is benign or malignant by examining tissue samples under a microscope (American Cancer Society, 2024).

Benign tumors are non-cancerous, slow-growing, and do not spread to other parts of the body. At the cellular level, the nuclei of benign tumors are usually smoother, smaller, and more uniform. Malignant tumors, on the other hand, are cancerous, fast-growing, and metastasize or spread to other body parts. The nuclei of malignant tumors tend to have larger radii, irregular textures, higher concavity, and more concave points due to abnormal growth (Raha et al., 2024).

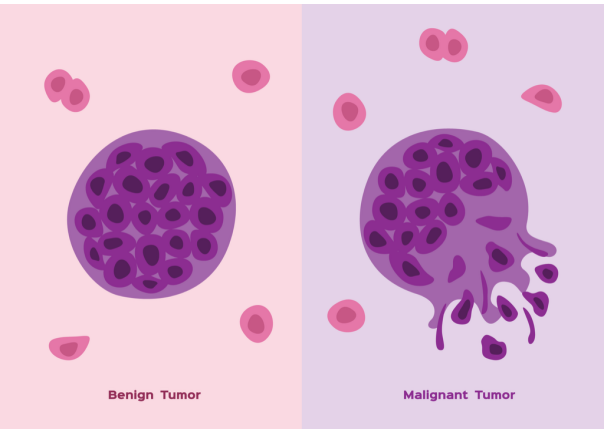

Figure 2. Visual comparison of benign (left) and malignant (right) tumor cell structures (Admac Oncology, 2021).

Additionally, digital mammograms play a crucial role in early detection. However, identifying and correctly classifying benign and malignant patterns in digital mammograms is challenging for radiologists as the appearance of such tumors is subtle and varied, especially in the early stages. Studies such as Sakai et al. (2020) have shown that even experienced radiologists can misclassify tumors based on visual features alone.

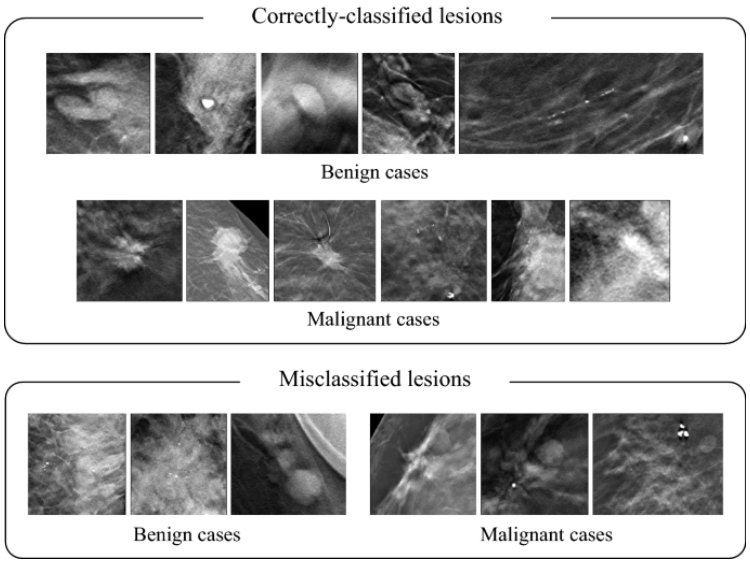

Figure 3. Ultrasound images of correctly classified and misclassified breast lesions for both benign and malignant cases. Visual overlap in features causes misclassification to occur (Sakai et al., 2020).

To support radiologists, machine learning techniques have been developed to improve classification accuracy. Typically, radiologists characterize masses by their location, size, shape, and margins when judging the likelihood of being cancerous. Benign masses are compact and limited to a circular or oval shape, while irregularly shaped masses with ill-defined margins are generally suggestive of malignancy. Verma et al. (2010) proposed that a soft, cluster-based direct learning classifier can significantly enhance and aid radiologists in accurately classifying suspicious areas and diagnosing breast cancer. Their model achieved over 97% classification accuracy, outperforming traditional methods and highlighting the potential of machine learning in diagnostic imaging.

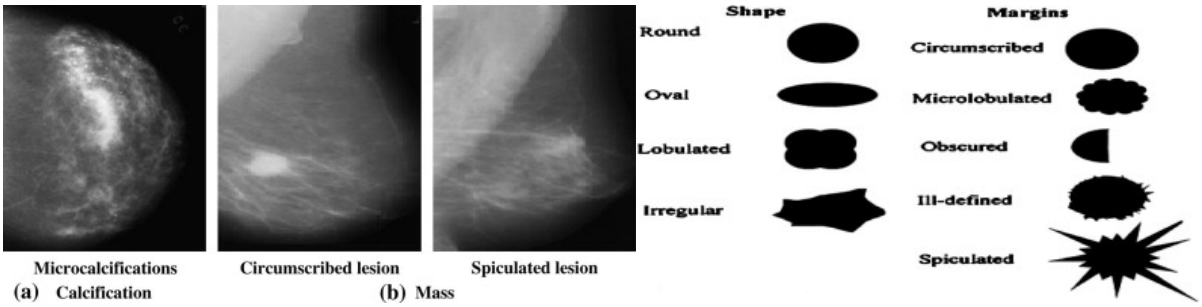

Figure 4. Modified image demonstrating breast abnormalities in mammograms, and different mass shapes and margins (Verma et al., 2010).

Other research suggests that tumor characteristics, being highly correlated with nuclear features, may be predicted at the cellular level using machine learning, a form of artificial intelligence that enables computers to learn through algorithms without explicit programming (IBM, 2025). The study by Kalaiyarasi et al. (2020) explores the classification of tumors using machine learning algorithms to improve medical diagnosis and aid physicians in making decisions about cancer. The authors emphasize the significance of machine learning-based early detection, describing how machine learning algorithms can be used to automate tumor identification. Similarly to our study, the authors stressed the importance of data preprocessing and feature extraction based on correlation.

This study aims to address the question: How can tumors be classified as benign or malignant using data analysis techniques? We apply logistic regression and assess model performance using cross-validation to ensure the robustness and reliability of our models.

Methodology

For this study, we selected the Breast Cancer Wisconsin (Diagnostic) dataset from the UCI Machine Learning Repository, a trusted source for datasets from various fields. This dataset was chosen for its suitability for tumor classification, compatibility with logistic regression, and the absence of missing values, which allowed for efficient preprocessing and analysis. Thus, this

dataset is ideally suited for our research as it offers a strong foundation for analyzing whether tumors are benign or malignant based on various characteristics.

We began by examining the dataset's overall tumor type distribution. Figure 4 below displays the distribution of tumor diagnoses, in which 33% of the tumors are malignant, while roughly 67% are benign. This distribution falls within the range (20-40%) that is considered mildly imbalanced, which is acceptable for training machine learning models (Google).

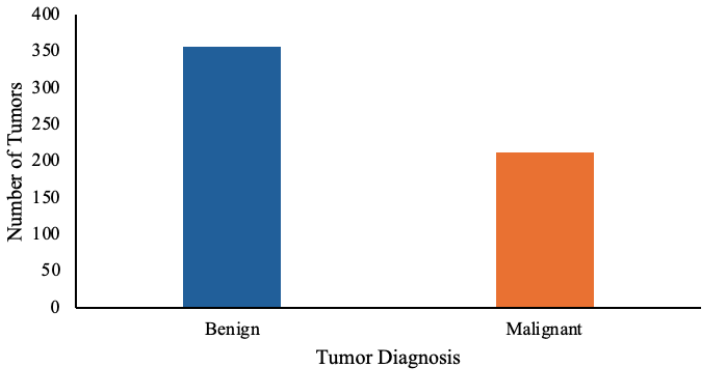

Figure 4: Tumor counts in the dataset.

The dataset includes 10 numerical variables that measure the nuclear features of tumor cells, which will be used as predictor variables for classifying tumor types (Table 1).

Table 1: *Independent Variable List*

Variable	Type and Level
Area	Numerical
Compactness	Numerical
Concave Points	Numerical
Concavity	Numerical
Fractal Dimension	Numerical
Perimeter	Numerical
Radius	Numerical
Smoothness	Numerical
Symmetry	Numerical
Texture	Numerical

All variables are numerical, and larger values are generally associated with malignancy. We conducted an exploratory data analysis (EDA) to explore this relationship by creating numerical summaries for each variable. Table 2 presents each nuclear feature's means and standard deviations (SD) for benign and malignant tumors.

Table 2: *Mean and SD of Tumor Nuclear Features*

Benign	Area	Compactness	Concave Points	Concavity	Fractal Dimension	Perimeter	Radius	Smoothness	Symmetry	Texture
Mean	462.7902	0.0801	0.0257	0.0461	0.0629	78.0754	12.1465	0.0925	0.1742	17.9148
SD	134.2871	0.0337	0.0159	0.0434	0.0067	11.8074	1.7805	0.0134	0.0248	3.9951

Malignant	Area	Compactness	Concave Points	Concavity	Fractal Dimension	Perimeter	Radius	Smoothness	Symmetry	Texture
Mean	978.3764	0.1452	0.0880	0.1608	0.0627	28.0826	17.4628	0.1029	0.1929	21.6049
SD	367.9380	0.0540	0.0344	0.0750	0.0076	21.8547	3.2040	0.0126	0.0276	3.7795

As expected, most nuclear features have higher average values in malignant tumors. However, some variables, such as perimeter, showed exceptions where benign tumors (mean = 28.083) had unexpectedly high values (malignant mean = 21.855), warranting further investigation.

To investigate the presence of outliers in the dataset, we calculated five-number summaries (minimum, Q1, median, Q3, and maximum) for each variable by type of tumor (Table 3 and Table 4). For benign tumors, values beyond the lower limit should be of no concern; however, values greater than the upper limit may be an issue when determining the "cut-off" value in classifying whether a tumor is malignant or benign based on that specific nuclear feature. If higher values are indicators of malignancy, observed benign tumor cells with unusually large areas may be predicted as malignant in the classification model, if considered a

stand-alone factor in determining tumor type. Thus, outliers may influence the accuracy of the classification model and should be carefully examined before creating the model.

Table 3: *Five Number Summary of Benign Tumors*

	Area	Compactness	Concave Points	Concavity	Fractal Dimension	Perimeter	Radius	Smoothness	Symmetry	Texture
LB	9.89	12.39	62.78	278.2	0.0737	0.0309	-0.0046	0.0052	0.1425	0.0542
min	6.981	9.71	43.79	143.5	0.0526	0.0194	0	0	0.1060	0.0519
Q1	11.08	15.15	70.87	378.2	0.0831	0.0556	0.0203	0.0150	0.1580	0.0585
median	12.18	17	78.01	451.1	0.0914	0.0728	0.0351	0.0227	0.1735	0.0614
Q3	13.37	19.76	86.1	551.1	0.1007	0.0976	0.0600	0.0325	0.1890	0.0658
max	17.85	33.81	114.6	992.1	0.1634	0.2239	0.4108	0.0853	0.2743	0.0958
UB	14.47	21.61	93.24	624	0.1090	0.1147	0.0748	0.0402	0.2045	0.0686

Table 4: *Five Number Summary of Malignant Tumors*

	Area	Compactness	Concave Points	Concavity	Fractal Dimension	Perimeter	Radius	Smoothness	Symmetry	Texture
LB	12.81	17.0225	83.02	433.55	0.0853	0.0696	0.0578	0.0477	0.1541	0.0511
min	10.95	10.38	71.9	361.6	0.0737	0.0461	0.0240	0.0203	0.1308	0.0500
Q1	15.075	19.3275	98.745	705.3	0.0940	0.1096	0.1095	0.0646	0.1741	0.0566
median	17.325	21.46	114.2	932	0.1022	0.1324	0.1514	0.0863	0.1899	0.0616
Q3	19.59	23.765	129.925	1203.75	0.1109	0.1724	0.2031	0.1032	0.2099	0.0671
max	28.11	39.28	188.5	2501	0.1447	0.3454	0.4268	0.2012	0.3040	0.0974
UB	21.84	25.8975	145.38	1430.45	0.1191	0.1952	0.2449	0.1248	0.2257	0.0721

Next, we examined multicollinearity among the predictors by computing a correlation matrix (Figure 5). Here, multicollinearity involves the presence of high correlations among predictor variables, which can reduce regression model reliability by raising the coefficient

estimate variance. For example, tumors with a larger radius also have a higher perimeter mean, resulting in a stronger linear relationship between these variables. This creates redundancy and makes it more challenging to determine the specific effect of each variable. To address the issue of multicollinearity in our dataset, we computed the correlation matrix between the 10 predictor variables and identified pairs of variables with a correlation coefficient greater than 0.8. The size and color intensity of the circles reflect the strength and direction of the correlations, where stronger positive correlations are indicated by deeper blue circles. We removed one variable from each highly correlated pair, keeping only the first variable in each pair, to reduce redundancy. By doing so, multicollinearity is reduced, making the model more stable and effective in classifying tumors.

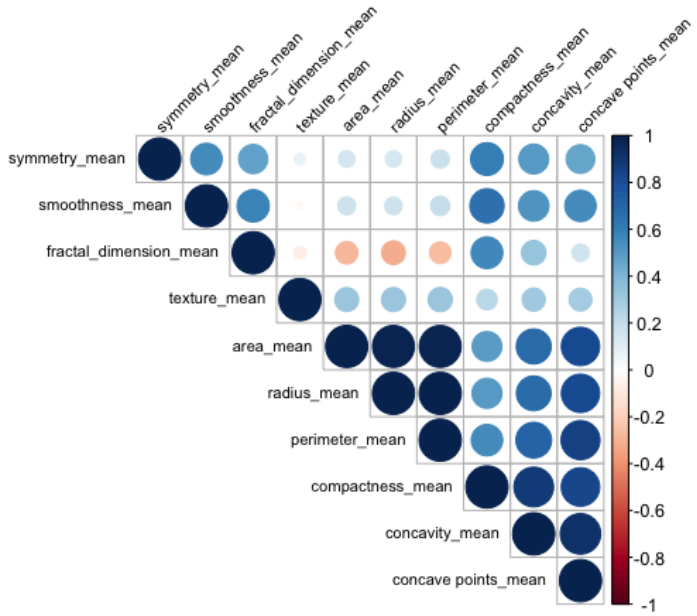

Figure 5. Correlation matrix of predictors from the dataset.

Finally, we applied logistic regression to classify breast cancer tumors as either benign or malignant based on the nuclear features of tumors. Logistic regression is well-suited for binary classification problems in which the outcome variable has two possible categories— in this case,

benign (0) or malignant (1). The goal of logistic regression is to find the best-fitting curve that estimates the probability of an outcome based on predictor values such as tumor area, radius, symmetry, or other nuclear features. The model uses the logistic or sigmoid function to produce an S-shaped curve, to ensure predicted probabilities are bound between 0 and 1. The Maximum Likelihood Estimation (MLE) determines the logistic regression model's coefficients (intercepts and slopes). MLE identifies the set of coefficients that maximizes the likelihood of observing the given data, ensuring that the model's predicted probability for tumor classifications closely aligns with actual outcomes in the dataset.

To evaluate the logistic regression model's performance, we assessed the significance of each predictor through its p-values and created a confusion matrix to calculate classification accuracy. We also applied leave-one-out cross-validation (LOOCV) to further validate the model on unseen data and assess generalizability. Overall, this methodology allowed us to develop a model capable of predicting tumor types based on nuclear features with high accuracy and reliability.

Results

Following the correction of multicollinearity, a logistic regression model was implemented to determine whether a tumor was benign or malignant using standardized predictors. The initial model included all ten nuclear features, and statistical significance was assessed by calculating the p-values for each predictor. Table 5 displays the coefficient estimates and the corresponding p-values for each variable in the initial model to identify the predictors strongly related to tumor classification.

Table 5: *Initial Model Significant Values*

Coefficients:	Estimate	Pr(>|z|)
(Intercept)	-7.260	0.567
radius_mean	-2.049	0.5813
texture_mean	0.385	2.5e-09
perimeter_mean	-0.072	0.885
area_mean	0.0398	0.017
smoothness_mean	76.432	0.017
compactness_mean	-1.462	0.9427
concavity_mean	8.469	0.297
concave points_mean	66.822	0.0192
symmetry_mean	16.278	0.126
fractal_dimension_mean	-68.337	0.424

Predictors such as texture_mean, area_mean, and smoothness_mean were among the variables that showed statistically significant p-values ($p < 0.05$), indicating a possible association with malignancy classification. However, variables such as radius_mean, perimeter_mean, and symmetry_mean had higher p-values, suggesting that their associations with tumor diagnosis were not strong or significant.

To strengthen the model's reliability and clarity, we removed either highly correlated or statistically insignificant variables. The remaining predictors in the model were centered and scaled. The final model included the six variables: radius_mean, texture_mean, smoothness_mean, compactness_mean, symmetry_mean, and fractal_dimension_mean. Table 6 shows the p-values and coefficient estimates for the final model. Among these predictors, malignancy was strongly associated with radius_mean, texture_mean, smoothness_mean, and symmetry_mean (all $p < 0.05$), but not compactness_mean or fractal_dimension_mean.

Table 6: *Final Model Significant Values*

Coefficients:	Estimate	Pr(>|z|)
(Intercept)	-37.564	7.11e-14
radius_mean	1.235	7.31e-14
texture_mean	0.326	1.48e-07
smoothness_mean	77.556	0.0014
compactness_mean	13.312	0.053
symmetry_mean	22.616	0.0340

Residual deviance: 146.12 on 416 degrees of freedom AIC: 158.12

The final model demonstrated high predictive performance, with a low residual deviance (146.12, df = 416) and AIC (158.12), indicating the model reliably differentiated benign and malignant diagnoses (Table 6). A confusion matrix generated from a 74/26 training/test split showed an overall accuracy of 97.28%, correctly classifying 89 benign and 54 malignant tumors, with only 4 misclassifications (Table 7). The 74% training and 26% testing division was determined arbitrarily, within the commonly accepted range of 70-80% for training data, balancing the need for model learning with reliability of performance evaluation (Gholamy et al., 2018).

Table 7: *Final Model Confusion Matrix*

	Actual	
Predicted	B	M
B	89	1
M	3	54

Accuracy: 0.9728

LOOCV further confirmed the model's reliability, achieving an accuracy of 92.79% (Table 8). The LOOCV confusion matrix shows that the model correctly predicted 341 benign and 187 malignant tumors, with 41 misclassifications overall. Classification was based on a

probability threshold (alpha) of 0.5, where tumors with predicted probabilities above 0.5 were labeled malignant.

Table 8: *LOOCV Confusion Matrix*

	Actual	
Predicted	B	M
B	341	25
M	16	187

Accuracy: 0.9279

To assess variable contributions beyond p-values, we fitted the final model on the full dataset and examined feature importance. Smoothness_mean had the highest importance score (86.909), followed by fractal_dimension_mean (33.546), and the least being texture_mean (0.337). Notably, fractal_dimension_mean – despite being statistically insignificant – showed a substantial influence on model predictions, suggesting that feature importance may capture predictive value not always reflected by significance testing alone.

Discussion

The final logistic regression model achieved high classification accuracy, both on the test data (97.28%) and in cross-validation (92.79%), indicating its strong ability to differentiate between benign and malignant tumors. Among the predictors, smoothness_mean emerged as the most influential features, as shown by both statistical significance and feature importance. Interestingly, some discrepancies were noted between p-value significance and feature importance rankings. For instance, fractal_dimension_mean was determined to be the second greatest important feature in prediction outcomes, despite the fact that it was considered statistically insignificant based on its p-value ($p > 0.05$). This suggests that relying solely on p-values for variable selection may overlook valuable predictive information. On the other hand, texture_mean, which had a highly significant p-value, contributed relatively little to model

predictions when considered alongside other variables. This emphasizes the importance of evaluating multiple metrics – such as coefficient size, p-value, and feature importance – when determining a variable's overall impact on classification accuracy.

Evaluating the performance of the final model after cross-validation, an accuracy of 0.9279 indicates that it is successful in correctly classifying tumors roughly 93% of the time based on the features chosen. These odds are fairly good, especially considering that the applications of this model would be to the diagnosis of breast tumors, which can lead to better patient prognosis if it can be identified early and accurately for treatment. However, it should be noted that using an alpha threshold of 0.5 assumes equal costs for false positives and false negatives. In clinical settings, misclassifying a malignant tumor as benign poses a greater risk than the reverse. Adjusting the classification threshold to prioritize sensitivity could reduce the risk of overlooking potentially harmful tumors, though at the expense of more false positives.

Overall, the findings highlight the effectiveness of logistic regression in tumor classification while revealing opportunities for refining variable selection methods and optimizing classification thresholds for practical applications.

Limitations and Future Research

While our logistic regression model demonstrated high accuracy, several limitations must be acknowledged. First, the Breast Cancer Wisconsin (Diagnostic) dataset was initially collected in 1993. Developments in pathology, medical imaging, and our knowledge of tumors over the last three decades may have led to new discoveries on how tumors develop and are classified. As a result, models developed using this dataset may not fully reflect recent diagnostic standards or newer tumor characteristics. Second, the dataset is cross-sectional and static, meaning it only contains one-time measurements of the nuclear characteristics of each tumor. Tumor features

such as size, texture, and shape may evolve over time in hospitals, especially when treatment varies. Due to the lack of longitudinal data, this study cannot account for the potential effects of these features' development on classification accuracy.

Third, the dataset includes each nuclear feature's standard deviation (SD) and worst (highest) values, which could offer additional predictive power. Including these variables in future models may improve classification accuracy and robustness.

Last, our model was developed and validated on a single dataset. Although LOOCV provides a reliable internal estimate, external validation on independent datasets is essential to ensure generalizability across diverse patient populations and clinical settings. Future research should explore incorporating additional feature types, validating the model across datasets, and optimizing classification thresholds to better match real-world clinical needs.

Conclusion

This study demonstrated the effectiveness of logistic regression in classifying breast tumors as benign or malignant based on nuclear features, achieving high accuracy both on test data and through cross-validation. Our findings highlight the valuable role that data-driven approaches can play in supporting early cancer diagnosis and improving patient outcomes.

As undergraduate students, this project allowed us to bridge the gap between statistical theory and real-world biomedical applications. Through conducting this research, we strengthened our skills in data analysis, coding, and critical evaluation of model performance — experiences that will be essential as we pursue future careers in medicine and research. Moving forward, we hope to build on this foundation by incorporating more advanced methods and working with up-to-date clinical datasets to further enhance diagnostic accuracy.

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We especially would like to express our gratitude and appreciation to our mentor, Dr. Sunny Le. We thank you for performing the coding for the various tests in this paper. We also thank you for your guidance and knowledge that strengthened our data analysis skills. As students, we are extremely grateful for your keen eye in spotting our potential to grow and encouraging us further as we would not have gained these valuable experiences without it.

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Chemistry and Biochemistry

Development of fluorescent methods of determining Dissociation Constant (Kd) for ssDNA-aptamer-based biosensors

Amanda Reyes and Dr. Stevan Pecic

Department of Chemistry and Biochemistry, California State University, Fullerton

Abstract

Biosensors are designed devices that come in contact with an analyte to provide a measurable signal. Recently, there has been research interests in developing single stranded DNA (ssDNA) aptamers as biosensors. In our lab we use a biochemistry technique called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to identify ssDNA aptamer candidates. Once SELEX yields possible aptamers their dissociation constants, Kd, is evaluated through fluorescent assay analysis. Most accurate testing of aptamers is using the 6-carboxyfluorescein (6-FAM)/DABCYL system. However, this process is expensive and labor intensive. Thus, there is a need for a fast and robust screening fluorescent assay to determine best aptamer candidates for 6-FAM/DABCYL testing. In this project we aim to develop a fluorescent assay method by using fluorescent dyes, Thiazole Orange (TO) and Thioflavin T (ThT), to determine the Kd for single stranded DNA aptamers. Using a commercially available ssDNA library of 36 random nucleotides flanked by two primers and through multiple rounds of SELEX, we isolated several aptamers for Kavain and Aldosterone. We have finished ThT and TO fluorescent testing on each aptamer, and we compared each dye's ability to bind to the ssDNA. From these experiments, we concluded that ThT is a better dye to use when testing preliminary ssDNA aptamer's Kd. We expect that our study will enable development of the more advanced biosensors for detection of challenging small molecules of interest.

Introduction

Aptamers are short oligonucleotide sequences that recognize or bind to a target with high affinity and specificity they can be either single stranded DNA (ssDNA) or RNA or peptides [1]. They are isolated from the process of Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers are similar to antibodies however they have unique characteristics that allow them to surpass antibodies capabilities such as straight forward chemical synthesis, cost effectiveness, and adaptability [2]. RNA aptamers targeted for amino acids can distinguish between different polarities, side chains, and different shapes. Specifically, the aptamer, Tyr 1, binds to amino acid L-Tyrosine has been shown to be stereo-specific [3]. Additionally, an aptamer called enantioselective can distinguish L-arginine from D-arginine with a 12,000-fold stronger affinity for the L conformation of the amino acid [4]. However, RNA libraries are generated from double stranded DNA; therefore, after each round of PCR, the library is reverse transcriptase is used to regenerate the library, and this step can introduce synthesis mistakes in the RNA sequence [1].

DNA aptamers have high stability, which allows the molecules to have a longer shelf life and can be stored at room temperature [5]. Aptamer identification occurs without the use of live animals though in vitro processes. Since the identification is going through *in vitro* selection, the aptamers can be used to identify molecules toxic to animals, or molecules produce an immunogenic response [6]. To quantify an aptamer's quality, they are often measured by their cross reactivity and their binding affinity. Binding affinity is reported as the dissociation constant (K_d) and ideally has a range of picomolar to micromolar.

The process to identify an aptamer is called SELEX (Fig. 1). This process requires the immobilization of the DNA library on to the column through non-covalent methods but through the use of a capture. A capture is a complementary base sequence that binds to a primer on the

library. Biotin is attached to the capture sequence because biotin strongly interacts with the medium streptavidin agarose. This method does not require a structural change to either the ssDNA or the target but instead relies on the structure switching capabilities of DNA and the weak hydrogen interactions with the complementary capture sequence. Target is introduced, and the library and target are eluted as the aptamer-target complex [7, 8]. Target concentration is decreased every few rounds of SELEX to increase aptamer affinity. [9]. The library is then introduced to a target to create the aptamer-target complex. Then the aptamer-target complex is eluted and collected for PCR amplification. PCR amplification will result in double stranded DNA, which will need to be reduced to single strands. After the strand separation step, the library is used in a new round to further enrich the library. This process may continue for 5-20 rounds of SELEX. After the last round of SELEX, the aptamer candidates are cloned and sequences for characterization [1, 10].

Results and Discussion

In this work, we used 14 rounds of SELEX and 4 rounds of counter SELEX to identify an aptamer for the target kavalactone, kavain. Our library consisted of 10^{14} unique ssDNA sequences with a random region of 36 nucleotides bookended between two primers (Table 1). We maintained the concentration of kavain until round 14 and introduced counter target, Pipermethystine (Fig. 2). Through immobilizing the library on a column, the target was added and based on structure switching capabilities aptamer- target complex was eluted and collected. Each round the quality of DNA was monitored using gel electrophoresis to determine how many rounds of PCR should be used to amplify our library. Through evolutionary pressure we isolated several possible aptamer sequences (Table 2). These sequences tested with Thioflavin T (ThT) did not produce an aptamer that resulted in a low K_d. We hypothesized that this may be due the target kavain's structure not

containing epitopes or recognizable functional groups. A similar problem was seen in the research to find an aptamer for glucose. The research team chose to create an aptamer that will bind to glucose and a receptor that changes conformational shape when interacting with glucose. Researchers used Shinkai's receptor bound to glucose as a target for SELEX. Shinkai's receptor was chosen due to the modification to override the preference the receptor would have for fructose and instead bind to glucose. The receptor when in contact with glucose then undergoes a conformational change, which allows a slight new arrangement of epitopes. The protocol included using receptor and target complex for SELEX until the library pool showed a high enough affinity for the complex. Then counter SELEX was run with the Shinkai receptor used as a counter-target, which reduces the aptamer's cross reactivity. This resulted in an aptamer capable of identifying glucose [11]. Therefore, future directions will change the target from kavain alone to a protein that is highly selective for kavain and then using counter-SELEX against the receptor to isolate an aptamer that binds specifically to kavain. As well as testing those sequences with the 6-FAM Dabcyl system and ThT to determine their dissociation constants.

We pivoted to creating a new protocol for fluorescent assays for determining a dissociation constant. A new preliminary step will rapidly narrow the large pool of potential ideal aptamer sequences. Reducing the costs to run the more expensive and more accurate 6-carboxyfluorscein (6-FAM) and 4-((4-(dimethylamino) phenyl) diazenyl)benzoic acid] (DABCYL) quenching method. Using previously published and unpublished aldosterone aptamer sequences we decided to create a preliminary step using fluorescent dyes Thiazole Orange (TO) or Thioflavin T (ThT). Using preciously published and unpublished aptamer sequences allowed us to validate our preliminary step by comparing dissociation constants from ThT or TO with the 6-FAM Dabcyl

system. The two sequences tested resulted in a K_d of 33 nM and 338 nM were respectively aptamers 14 and 23 seen in Table 3. Each sequence was truncated for optimal binding.

These assays show that the good fluorescent dye for preliminary testing dissociation constant of aptamers is Thioflavin T. This dye gave the best results in that the K_d values are smaller values and best correlate to the 6-FAM Dabcyl system that indicated aptamer 14 was an ideal aptamer for aldosterone. ThT also gave the best results for dissociation curve (Fig. 3). These tests indicate that ThT can be accurate enough to narrow the list of potential aptamers. Thiazole Orange showed less consistency with dissociation values in relation to the previous 6-FAM tests, the graphs produced do not follow the curve to indicate decreased binding, and the trends from secondary analysis are less correlated than with ThT data. This may be an indication that TO binds more tightly to DNA so that it cannot structure switch to bind with its target.

Conclusion

In the future, we will optimize the ThT assay by measuring different concentrations of dye and aptamer. We will also need to further validate the test by using more previously published aptamers that were tested using the 6-FAM Dabcyl system. With the rising interest in biosensors as aptamers a new preliminary step can be useful in cutting down time on testing and narrow the field of aptamer sequences so that only the most ideal candidates are tested reducing costs. Our results show, ThT is the best cost-effective fluorescent dye comparatively to the most sensitive and accurate standard, 6-carboxyfluorscein (6-FAM) and 4-((4-(dimethylamino) phenyl) diazenyl)benzoic acid] (DABCYL) quenching method. This preliminary step would precede the most precise assay 6-FAM Dabcyl to narrow aptamer candidates. With this work more aptamers can be tested and therefore aid in publishing new sequences for a widening array of targets.

Tables and Figures

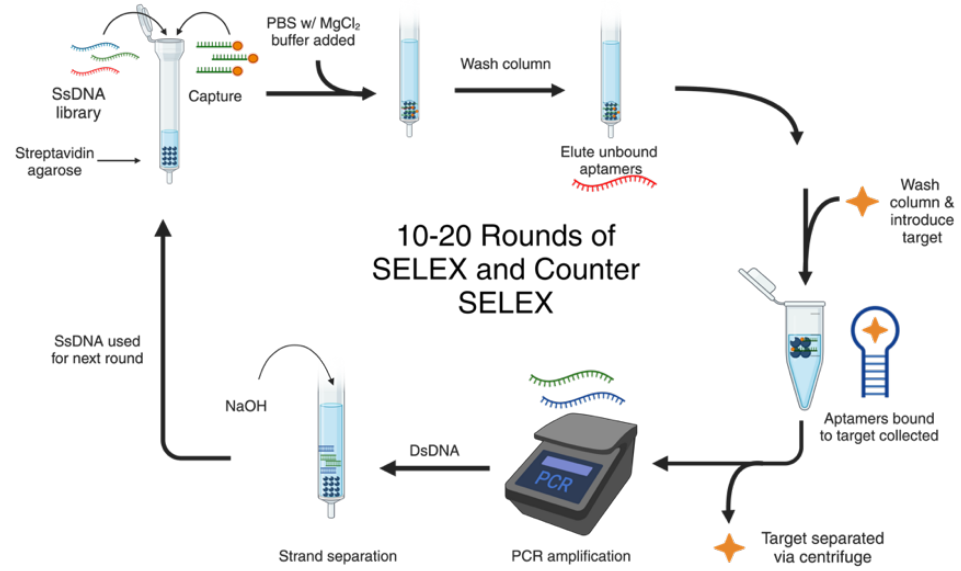

Figure 1. Shows SELEX schematic process for each round.

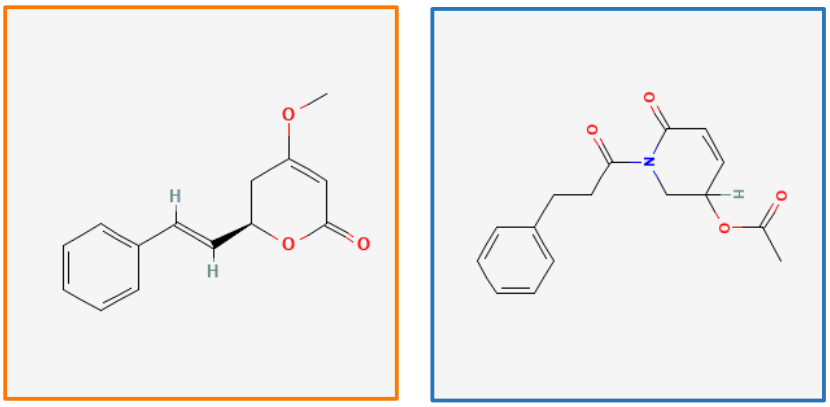

Figure 2. Chemical strucutres of Kavain and Pipermethystine.

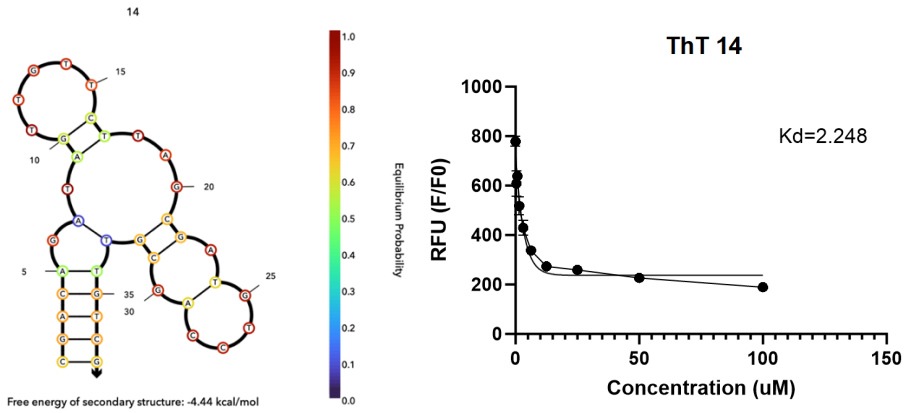

Figure 3. 2D Structure of Aldosterone 14 aptamer (NuPack) and ThT fluorescent assay results.

Table 1. Shows sequences used for expirement.

Primers	***Sequence***
Primer 1(forward)	GGA GGC TCT CGG GAC GAC
Primer 2(reverse)	CTG TAA ATC CTA AAG GCG GGA CGA C
P2_biot: /5Biosg/	CTG TAA ATC CTA AAG GCG GGA CGA C

Table 2. Shows sequences resulting from SELEX.

Number	*Sequence (without primers)*
1	GGAGGCTCTCGGGACGACCACAGGATCGTTGGTACACTGGTTCGGGGAGTCGTCCCGCCTTTAGGATTTACAG
4	GGAGGCTCTCGGGACGACGATGCGTCCTTGCTCTCTCTTTCGTGTCGTGTCGTCCCGCCTTTAGGATTTACAG
5	GGAGGCTCTCGGGACGACGGCATGCAAGGTTCTTTGTTTCGTGCTTCAGTCGTCCCGCCTTTAGGATTTACAG
6	GGAGGCTCTCGGGACGACGGGGCACTTTGTTGTTTTGTTTTTTGTCCTGTCGTCCCGCCTTTAGGATTTACAG
7	GGAGGCTCTCGGGACGACCAGGAGCTTCATTGTGTACTTTTCTGCTGTGTCGTCCCGCCTTTAGGATTTACAG
8	GGAGGCTCTCGGGACGACCGGCGTAGTTATCTTTCGGTCTTTTGTCGTGTCGTCCCGCCTTTAGGATTTACAG
9	GGAGGCTCTCGGGACGACCGAAGAGGGGGTGTGTTTGTGTTGGTCTAGGTCGTCCCGCCTTTAGGATTTACAG
10	GGAGGCTCTCGGGACGACGGGCATGTGTTATCTACCCTCCTTTAGCCAGTCGTCCCGCCTTTAGGATTTACAG
11	GGAGGCTCTCGGGACGACCATAGTGCAGTTCTTCGTAGTTTGTTGCTGGTCGTCCCGCCTTTAGGATTTACAG
12	GGAGGCTCTCGGGACGACCACGTGCTCTCTTGTTTTCTTGTTTAGTGTGTCGTCCCGCCTTTAGGATTTACAG
14	GGAGGCTCTCGGGACGACCATGATGCCTCTTTCCCTTTTTGCTCGTGCGTCGTCCCGCCTTTAGGATTTACAG
15	GGAGGCTCTCGGGACGACGGGGGGTTCTGTCTTGTTCTATTTACCGCTGTCGTCCCGCCTTTAGGATTTACAG
16	GGAGGCTCTCGGGACGACGGGTGCAGTTGTTCGTTCTTCTTTCACCTTGTCGTCCCGCCTTTAGGATTTACAG
17	GGAGGCTCTCGGGACGACGGGCAGGTGGCTGGTAGGGTAGGGCTGTATGTCGTCCCGCCTTTAGGATTTACAG
19	GGAGGCTCTCGGGACGACGGCCGTGTTGTCTTTTCTCGCTTTTGGATCGTCGTCCCGCCTTTAGGATTTACAG

20	GGAGGCTCTCGGGACGACCAACGGCTTGTACTTCTTTCGTTTTGTTGTGTCGTCCCGCCTTTAGGATTTACAG
22	GGAGGCTCTCGGGACGACGGAACAGGCACGTGGAATGGTGTACTTGAGGTCGTCCCGCCTTTAGGATTTACAG
23	GGAGGCTCTCGGGACGACCATGCCGGATGTGGTGATCGTGTTGCTTGTGTCGTCCCGCCTTTAGGATTTACAG
25	GGAGGCTCTCGGGACGACCAGCTCAGAGGATGCTCCGGTGTGTGTTGCGTCGTCCCGCCTTTAGGATTTACAG
27	GGAGGCTCTCGGGACGACGGGTCACCGATGTATCATGTGTCGGGGATGGTCGTCCCGCCTTTAGGATTTACAG

Table 3. Shows aldosterone aptamers tested and their resulting dissociation constants.

Number ALDO	*SEQ*	*ThT K_d (nM)*	*TO K_d (nM)*
14	CGACAGATAGTTGTTCTTAGCGATGTCCAGCGTTGTCG	2.248	29.13
15	ACGACGACAGTGCCTTGATATACGTTGGGCTGGTAGTCGT	26.2	33698
05	GACGACGAGACCCTTGATACTTCAAGCGTCAGCGAAGTCGTC	0.7306	0.4091
	CTGGGGTGCAATTCTTATGTAACGGGTCCCGG	3994244	60.57
17	CGACCGGAGTGTCTTAGTGTATAACTGAGTTTAGGTCG	29.57	6345158
23	CGACGGTAGGTAGGCCAACTGGGTATTTACTGGTGTCG	281.8	179.4
16	CGACAAATCTTTGGTGTGATGGATGTTGTCTTGTGTCG	2632540	1739195
22	ACGACGACCGGTCCCTGTTGTTAGGAATAGGGGGAGTCGT	4945384	3002228
06	ACGACGATGGGCCCCGATATGTACATTTCGGGTGAGTCGT	0.3785	0.3008
13	GGACGACGGAGACCCCATGTTTTCTGGTGTCAGCGTAGTCGTCC	225.7	83.76
10	ACGACGGAGGGTCCCGTTGTTCTACGATGGGTTTAGTCGT	36.68	47414
02	CGACGGGACACTTTGTATGTAAAGTGAGGTTCACGTCG	107369	2948616
07	ACGACGGGTGAGGTTCTTTATAACTGATGGGCCCTGTCGT	3332006	5084175

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Geology

Cement paragenesis of septarian concretions of the Holz Shale

Ms. Jamie Hoffman
Faculty Mentor: Professor Sean Loyd
Geology (B.Sc.), 2025, Department of Geological Sciences
714-356-5236
jlhoffman@csu.fullerton.edu

Abstract

The late Cretaceous Holz Shale hosts calcite concretions of ellipsoidal morphology up to 1 m in diameter. Some of these concretions exhibit septarian veins that are filled with multiple generations of cement. Septarian varieties of Holz Shale concretions exhibit up to four distinct calcite phases including 1) an initial gray cemented shale body, 2) a light brown early fringe outer phase, 3) a dark brown late fringe inner phase, and 4) a latest course crystalline vein filling white spar phase. Cement phases were analyzed for their carbon isotope composition ($\delta^{13}C$). The phase-specific $\delta^{13}C$ ranges are as follows: 1) body, 0 to -16‰, 2) light brown early fringe, 0.5 to -11‰, 3) dark brown late fringe, -8‰, 4) crystalline vein phase, -10 and -18‰. These changing compositions suggest that each exhibited a unique formation pathway with shifting contributions from organic carbon. The $\delta^{13}C$ ranges for each phase imply fractional contributions of organic matter that range from 0.08 to 0.7 with body phases incorporating the least organic-derived carbon and spar phases incorporating the most organic-derived carbon. The paragenetic sequence and δ13C-derived organic carbon contributions reveal that successive cement phases precipitated at deeper depths, primarily within the sulfate reduction zone. These findings provide further support concretions (and authigenic carbonates in general) form across a range of mineralization depths through progressive cementation.

Introduction

Past studies on concretions have concluded ways to determine their timing and formation mechanisms (Mozley & Burns, 1993). There remain many gaps in knowledge for the study of concretions. One major issue is the lack of modern analogues, as no modern concretions are identical to ancient concretions (Coleman, 1993). Modern concretions have only been found in nonmarine environments like marshes, while many ancient concretions were formed in marine settings, further complicating our understanding of concretion formation mechanisms (Loyd and Berelson, 2016). Concretions vary greatly in shape, size, and chemical composition (e.g., Mozley and Burns, 1993). The causes of these variations are not fully understood (Coleman, 1993). It is believed that existing interpretations of concretions may underestimate the depth and duration of concretion growth (Raiswell & Fisher, 2000). Septarian concretions are concretions with branching veins that are often filled with diagenetic minerals such as calcite (Rainswell & Fisher, 2000). Septarian concretion formation is also not completely understood. The origin of septarian fissures is still debated but may be the result of tensile fractures (Astin, 1986) or dehydration (Duck, 1995; Raiswell & Fisher, 2000).

It is important to continue to study septarian concretions. Septarian concretions record the environmental conditions at the time of formation, providing insights into how waters evolve through progressive diagenesis. Concretions provide a proxy reservoir for shallow diagenetic environments and provide information on transient processes that would not be accessible otherwise. Indeed, carbonate concretions have been used to reconstruct changes in shallow diagenetic environments even through deep geological time (e.g., Loyd et al., 2023). Organic matter is a common constituent of sediments, and its degradation leads to the release of carbon as dissolved inorganic carbon. This dissolved inorganic carbon drives concretion formation. As

such, concretion formation represents the product of organic matter degradation. With increasing amounts of CO_2 in the atmosphere, it is essential to more completely understand the mechanisms whereby natural systems sequester carbon. With the increasing effects of climate change, it has never been more important to create a greater understanding of environments that exhibit extensive carbon cycling.

Geologic Background

The Holz Shale is the uppermost member of the Ladd Formation which crops out in the Santa Ana Mountains of California (Figure 1, Buck and Bottjer, 1985). Diagnostic fossils indicate that the Holz Shale formed during the Upper Cretaceous, approximately 70 million years ago (Buck and Bottjer, 1985). The Holz Shale concretions range from ~2 to 100 cm in diameter and make up ~4-5% of the outcrop area. The presence of chute deposits, gully deposits, specific fossils, and turbidites indicates that the Holz Shale formed in a near-shore marine environment (Buck and Bottjer, 1985). The Holz Shale was likely deposited within an anoxic to sulfidic environment as evidenced by the presence of laminated sediments, absence of bioturbation, and evidence of thin-shelled bivalve fossils (Buck and Bottjer, 1985).

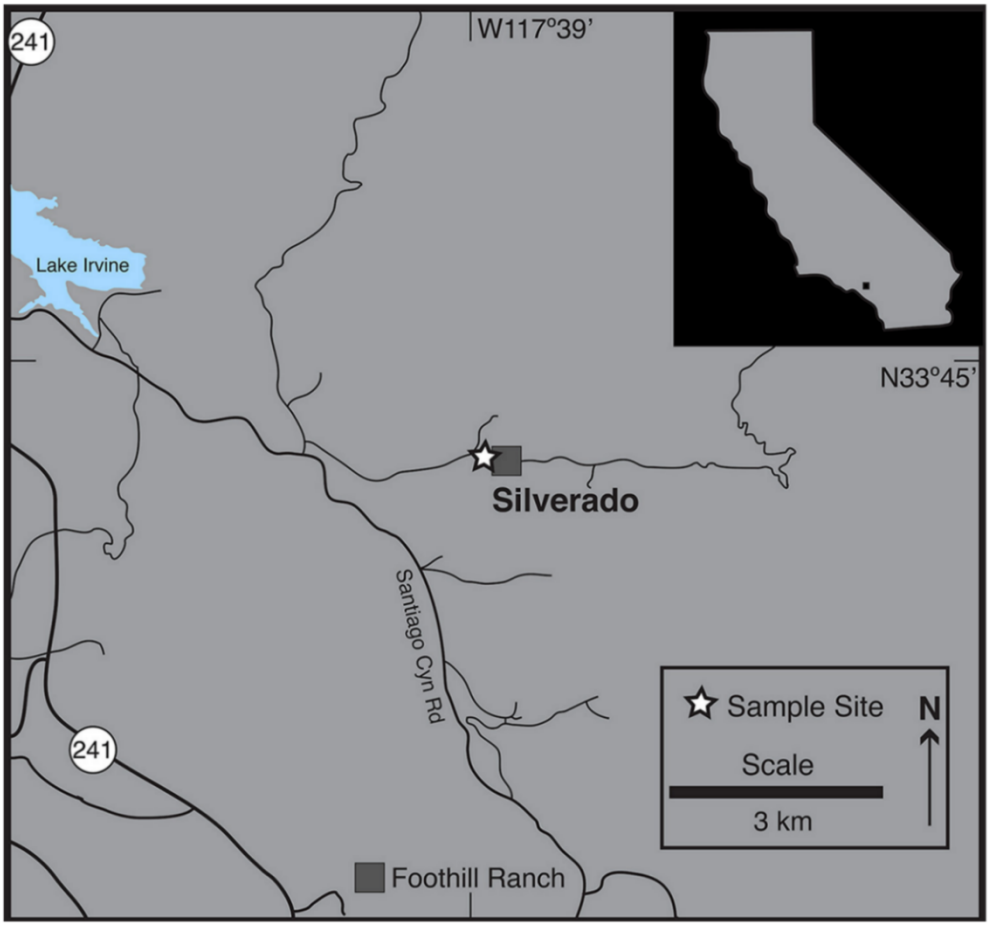


Figure 1 Study Site location in the Santa Ana Mountains, California. Figure after Loyd et al., 2017.

Research Question and Hypothesis

The goal of this study is to better understand the formation conditions of Holz Shale septarian concretions through petrographic and carbon geochemical analysis. Petrographic analysis allows identification of the different cement phases that occur in the septarian concretions. If phases express significantly different carbon isotope compositions, then different mechanisms of formation and/or variations in carbon sources can be inferred (e.g., Coleman, 1993). This study's hypothesis is that the different phases of Holz Shale septarian concretions formed via unique pathways and/or incorporate carbon from different sources, as indicated by unique $\delta^{13}C$ ranges.

Materials and Methods

Seven samples of Holz Shale septarian concretions were taken. Where present, each phase was drilled and powdered using a Dremel Rotary Tool. Multiple drill sites were collected for each phase. Produced powders were weighed and collected into Exetainer vials. The sampled powder from each drill site was run in triplicate. After powdered samples were placed in Exetainer vials, the ambient atmosphere was removed by vacuum. 10% phosphoric acid was added into each vial to convert the powdered carbonate to CO_2 gas. The CO_2 was passed into a Picarro G2121-*i* Cavity Ringdown Spectrometer via Automate Carbonate Preparation Device and measured for total inorganic carbon (TIC) content and carbon isotope composition ($\delta^{13}C$). TIC contents are reported as weight percent (wt%), and $\delta^{13}C$ values are reported in permil (‰) relative to the VPDB standard.

Results

Four calcite cement phases are identified in Holz Shale Septarian concretions. They include the body, outer fringe, inner fringe, and spar phases as shown by Figure 2. Most of the concretions are composed of gray cemented shale and have septarian veins lined with fringing cements and filled with white sparry calcite. Within most veins lies an outer fringe, inner fringe, and vein-occluding spar phase; not all veins contain all phases, and sometimes only show inner fringe cements. The outer fringe or early fringe, is light brown and formed first as indicated by its position closest to the initial body phase. The inner fringe or late fringe is dark brown and formed after the outer fringe and body phases. The last phase to form is the white crystalline calcite spar phase.

After the data was processed, $\delta^{13}C$ and total inorganic carbon weight percent were calculated for each phase. The body phase has the largest variability with $\delta^{13}C$ ranging from 0 to -16‰ and TIC from 7-12 wt.%. The early fringe has a $\delta^{13}C$ range from +0.5 to -11‰ with a TIC range from 7-12 wt.%. The late fringe has a $\delta^{13}C$ range from -8.5 to -15‰ and a TIC range from 8-10 wt.%. The spar phase has a $\delta^{13}C$ range between -10 and -18‰ and a TIC content of around 12 wt.%.

Petrographic photos of the Holz Shale give additional insight into phase spatial relationships and reveal a potential latest-stage dolomite phase shown in Figure 3. At the microscopic level, phases appear to have filled in directly after the sparry calcite phase has been found in septarian concretions from other locales (Thyne and Boles, 1979).

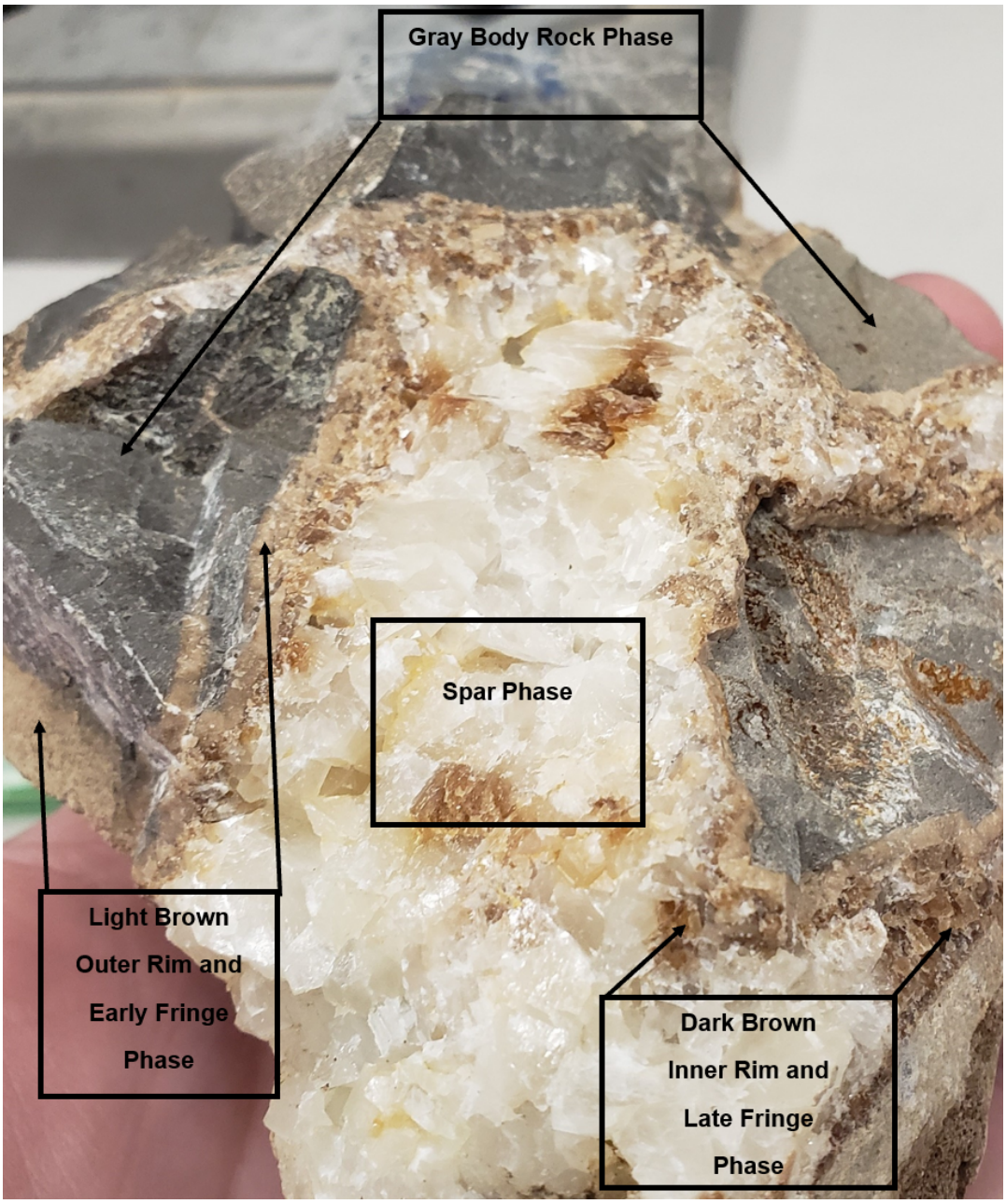
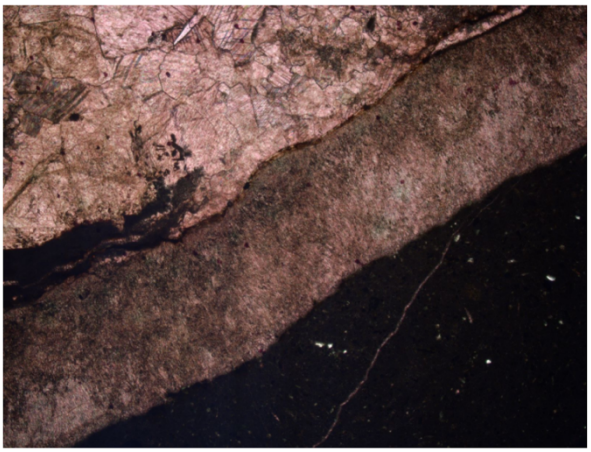
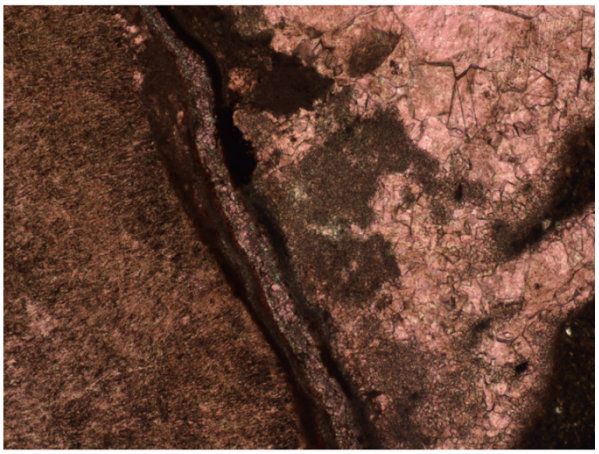


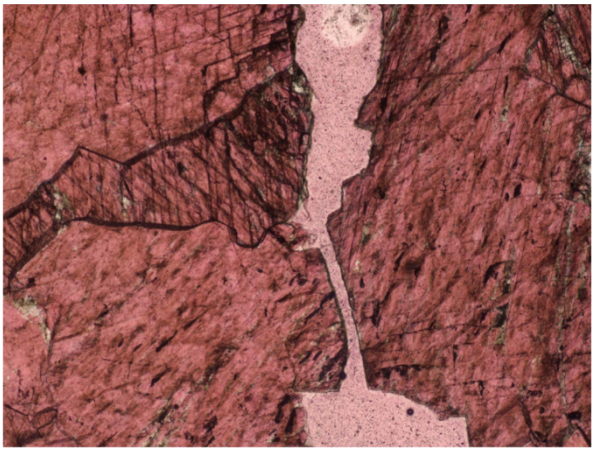
Figure 2: Photograph of Holz Shale septarian concretion with each of the four phases labeled. The four phases are a gray body rock, a light brown outer rim phase, a dark brown inner rim phase, and a crystalline spar phase.



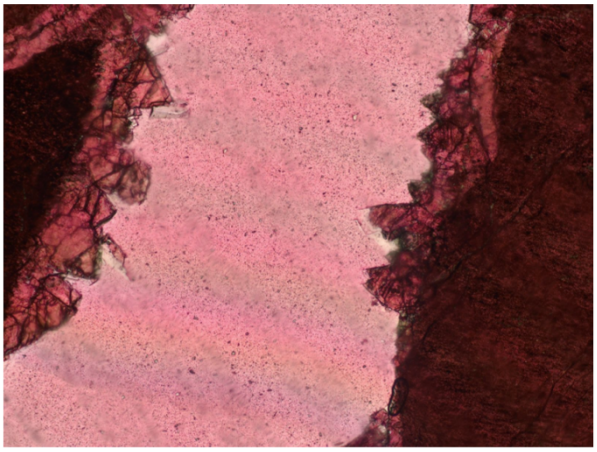
HSC1-4 PPL 2x: Multiple Phases



HSC1-4 PPL 5x: Border between phases



HSC 6-8 PPL 5x: Dolomite within Spar



HSC 6-9 PPL 2x: Dolomite within Spar

Figure 3: Petrographic photos of the Holz Shale under plain polarized light. Width of view for upper left and lower right is 4464 μm, width of view for upper right and lower left is 1936 μm.

Discussion

The paragenesis of the Holz Shale Septarian concretions began with the body cement followed by the outer fringe phase, the inner fringe phase, and lastly the vein-occluding crystalline phase, as indicated by Figure 2. The body must have formed first and then fractured, followed by the formation of the early outer light brown fringe phase. Fractures were further filled with a second layer of dark brown fringing cement. Lastly a crystalline calcite spar phase formed, including septarian veins.

Figure 4 displays each of the six samples with their corresponding TIC contents and $\delta^{13}C$ compositions. Figure 5 groups the six samples of the Holz Shale septarian concretion by phase, to allow more direct data comparison. Figure 5 shows that most $\delta^{13}C$ values for the spar phase are similar, and most are clustered together between -10 and -18‰ and TIC around 12 wt.%. As most spar phases have a similar range, it is likely that the spar phases from different concretions formed under similar conditions. The $\delta^{13}C$ and TIC values for other phases are less distinct and show some overlap.

Figure 5 displays that the late fringe cements express $\delta^{13}C$ values of ~ -8 to -15‰ and TIC between 8-10 wt.%. Although most of the data for late fringe are tightly grouped, a few data points of late fringe are outside of this range. It is possible that most late fringe formed together under the same conditions or formation mechanisms, and that some of the late fringe formed under different conditions.

Figure 5 does not indicate a clear correlation between total inorganic carbon contents and isotope compositions for the early fringe. The early fringe displays wide ranges in TIC and $\delta^{13}C$. The wide ranges for the early fringe cements imply a range of conditions for formation pathways. It is important to note that some of the early fringe data cluster together with 7-8 wt.

% TIC and -7 to -5‰ $\delta^{13}C$. This cluster of data points could indicate that some of the early fringe from different concretions formed at about the same time and under similar conditions.

Like the early fringe, Figure 5 does not show a clear correlation between TIC and $\delta^{13}C$ for the body phase. Like the early fringe, the body phases exhibit appreciable data ranges. The body phases range from 7- 12 wt.% TIC and from 0 to -16‰ $\delta^{13}C$. It is important to note that there is little overlap between the spar and body phases, further suggesting that there was a time gap or significant change in conditions between the times each was forming.

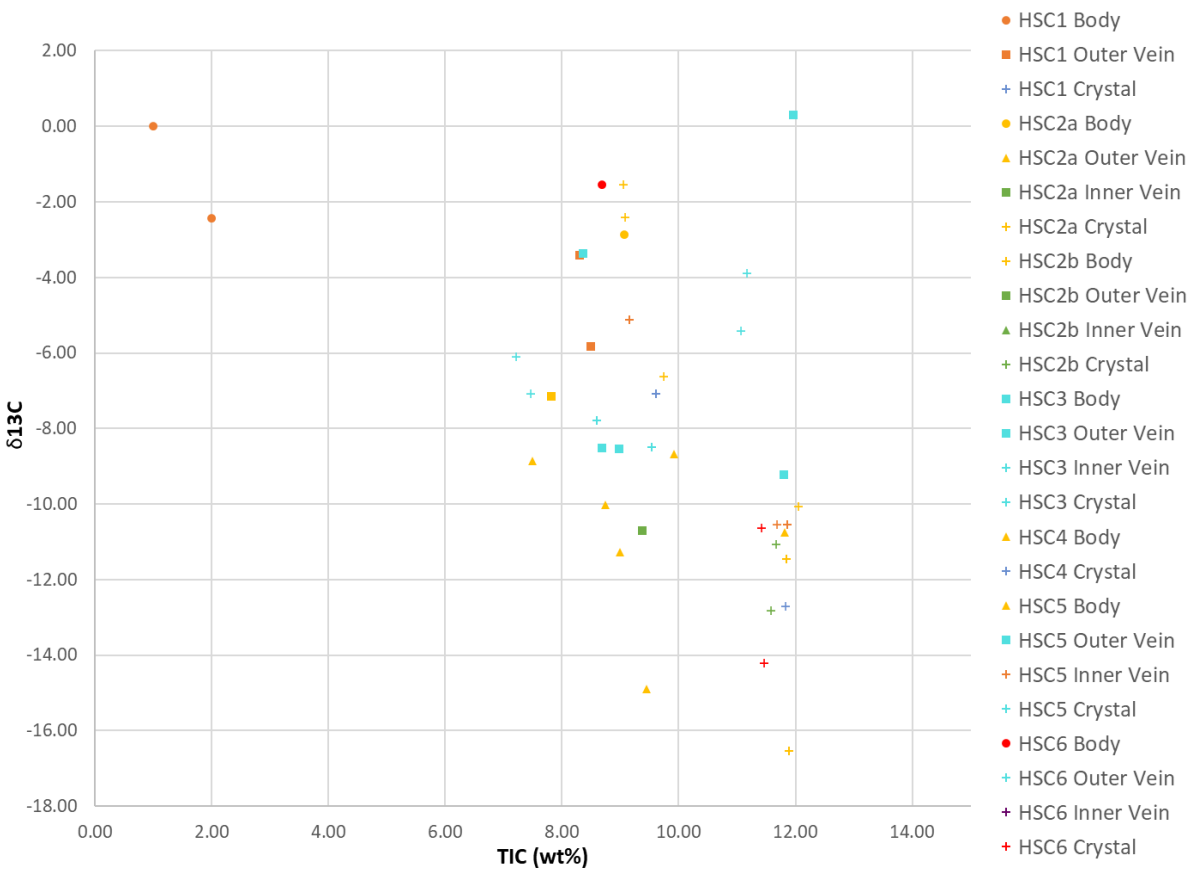


Figure 4: $\delta^{13}C$ compared to TIC of Holz Shale septarian concretions.

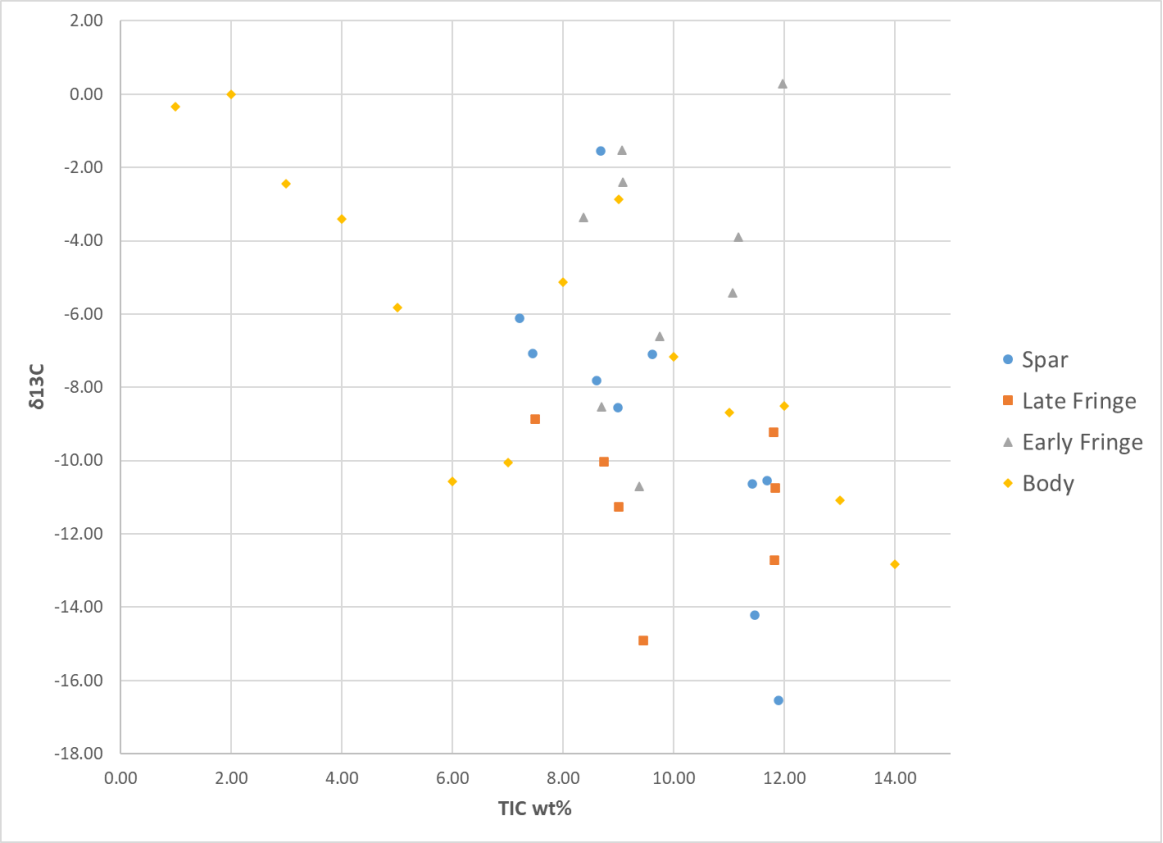


Figure 5: $\delta^{13}C$ compared to TIC of Holz Shale septarian concretions highlighting each phase.

Although the body and spar cements express disparate data ranges, most of the phases have some overlap (Figure 5). The overlap among the phases could indicate that formation conditions changed slowly over time, and that cement phases precipitated under somewhat similar but evolving diagenetic conditions. Indeed, the pore water profile shown in Figure 6 demonstrates that dissolved inorganic carbon expresses a smooth, consistent change with depth. In shallow sediments, this change is manifested as a decrease that represents an increase in the contribution of organic-degradation-derived inorganic carbon with depth and through time, primarily resulting from sulfate reduction diagenetic pathways.

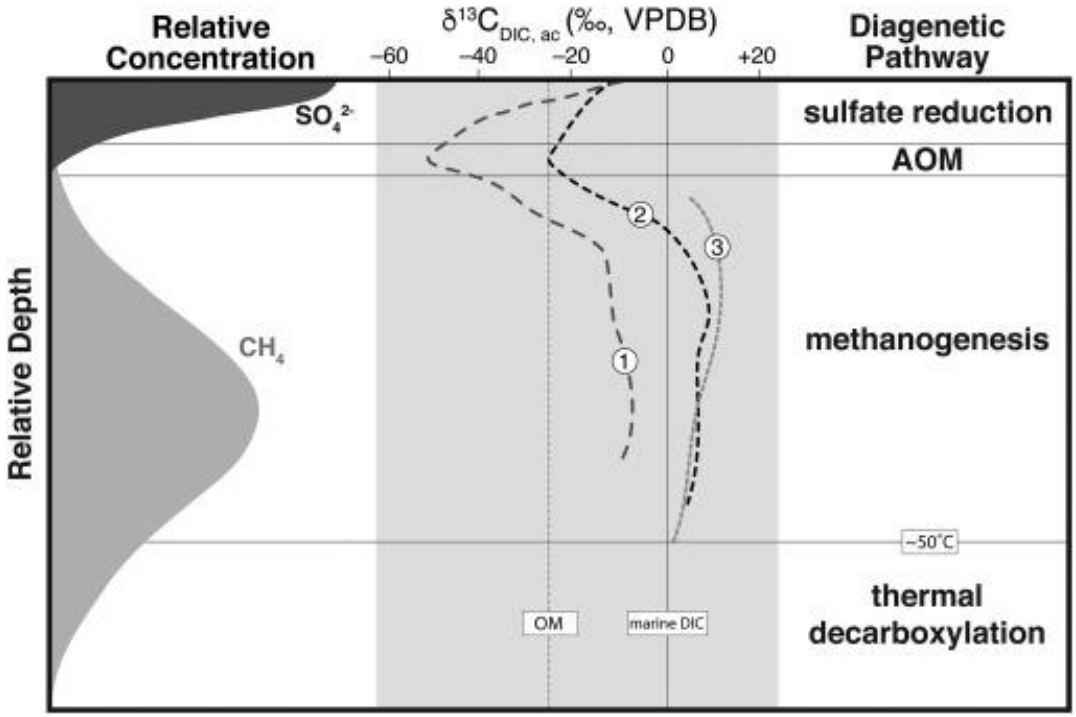


Figure 6: Summarized marine pore water profile. Middle column depicts changing $\delta^{13}C$ compositions that correlate to depth changes and associated diagenetic pathways. Figure from Loyd and Smirnoff (2021).

Figure 7 displays a range of the fractional contribution of organic matter to the four Holz Shale septarian concretion phases. The fractional contribution of organic matter or F_{org} is calculated using the following equation:

$$F_{org} = (\delta^{13}C_{cement} - \delta^{13}C_{sw}) / (\delta^{13}C_{org} - \delta^{13}C_{sw}).$$

Where $\delta^{13}C_{cement}$, $\delta^{13}C_{sw}$, and $\delta^{13}C_{org}$ are the isotope compositions of the concretion cements, late Cretaceous seawater, and organic matter, respectively. Late Cretaceous seawater $\delta^{13}C$ was ~ +1.8‰ (Veizer et al., 1999). The organic matter $\delta^{13}C$ value is -25‰, consistent with Holz Shale-contained organic matter data presented by Loyd (2017). The concretion's cement $\delta^{13}C$ values are those that were measured here. This equation assumes that the dissolved inorganic carbon in Holz Shale pore waters was derived from seawater and organic matter (converted upon diagenetic degradation) and does not take into consideration potential contributions from other carbon sources (e.g., methane).

After the body phase formed and then fractured, the early fringe could precipitate within the fracture. After the early fringe formed, the late fringe could precipitate within the remaining fracture void space. Lastly, after the late fringe formed, the crystalline spar phase precipitated and potentially completely occluded the fracture. As a period must have elapsed between the formation of the body phase and the spar phase, it makes sense that these phases are most geochemically divergent. Furthermore, it is likely that the organic material contributions to and formation mechanisms of these two phases were different.

The formula to determine F_{org} is dependent on the $\delta^{13}C$ value of the concretion cement. As such there is a clear separation between ranges of F_{org} of body and spar phases. The variation

between F_{org} of each of the phases of the Holz Shale indicates different carbon sources. There is a consistent trend of increasing dissolved organic carbon with increasing depth in marine sediments, driven by the addition of remineralized organic carbon. The $\delta^{13}C$ of each phase is different, suggesting that the $\delta^{13}C$ value was decreasing with time and thus depth. This trend is consistent with an increase in organic matter contribution to the dissolved inorganic carbon pool with depth. Therefore, each of the four phases of the Holz Shale formed with progressive burial of host sediments, an assertion supported by Figure 7.

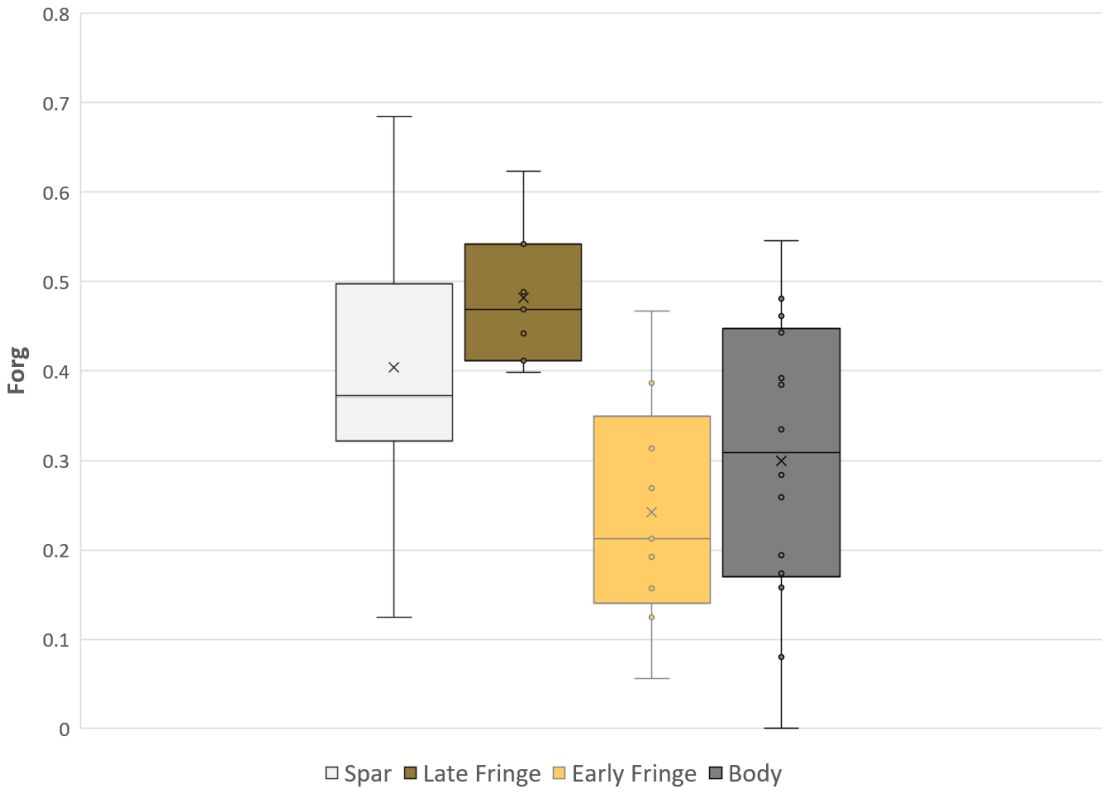


Figure 7- Box and whisker plot of the fractional contribution of organic matter (F_{org}) of each of the four phases of the Holz Shale septarian concretions.

Figures 6 and 8 can be used to determine the diagenetic pathway of each phase based on their $\delta^{13}C$ values. The body phase $\delta^{13}C$ ranges from ~0 to -16‰, which is consistent with the diagenetic pathway of sulfate reduction (Figure 6). The early fringe phase $\delta^{13}C$ ranges from 0.5 to -11‰, which likewise agrees with the zone of sulfate reduction. The late fringe phase $\delta^{13}C$ is about -8‰, which is also attributable to sulfate reduction. The spar phase $\delta^{13}C$ ranges from -10 to -18‰, which may also reflect sulfate reduction with a minor contribution from the AOM pathway. As some data ranges are large, the exact diagenetic pathway cannot be determined by $\delta^{13}C$ alone, but the diagenetic pathway was likely limited to these two possibilities. The occurrence of pyrite within Holz Shale concretions supports a sulfate reduction origin (Loyd et al., 2012, Loyd, 2017).

The continuous depletion in $\delta^{13}C$ compositions across the different phases within Holz Shale septarian concretions implies that the precipitation of cements was progressive. This supports the idea that concretions and authentic marine carbonates experience progressive formation with burial (e.g., Loyd and Smirnoff, 2022). Perhaps most intriguing, however, is the relationship between progressive cementation and septarian fracturing. The data presented here suggest that septarian fracturing occurred relatively early in the paragenetic sequence, and that all of the cementation occurred at relatively shallow sediment depths. This has broad implications for the mechanisms driving septarian fracturing in other units as well.

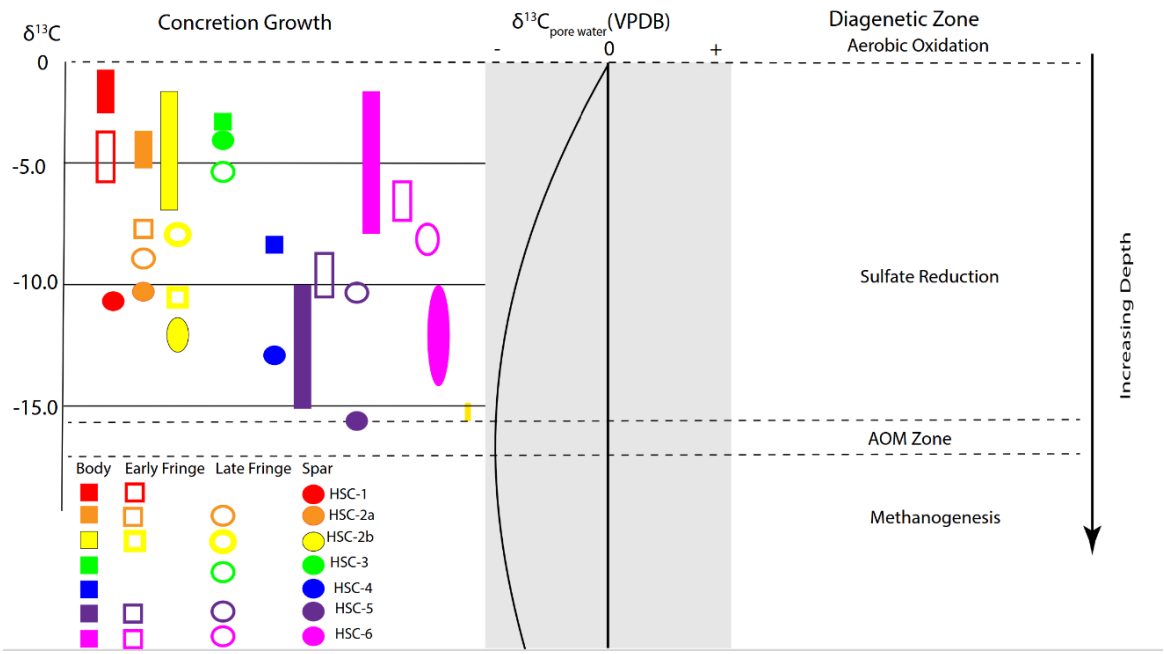


Figure 8: $\delta^{13}C$ ranges for each sample and interpreted diagenetic zone of formation.

Conclusions

The original hypothesis was not supported, as the $\delta^{13}C$ variations across the Holz Shale were not a result of unique formation pathways of each phase. $\delta^{13}C$ values showed overlap across phases. However, F_{org} analysis indicates an increasing contribution of organic matter-derived carbon in later paragenetic phases. This indicates that each successive phase formed at increasing depth, with increasing organic matter contributions. Despite increasing contributions from organic matter and increasing depth of formation, all of the phases within the septarian concretions of the Holz Shale precipitated at shallow depths within the sulfate reduction and perhaps anaerobic oxidation of methane zones.

Acknowledgements

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Mathematics

Bits and Primes: Exploring Digits of Prime Numbers in Binary

Brianna Castillo
Bobby Orozco
Advisor: Mr. Francisco Zepeda

Abstract

This paper explores the patterns that can be observed in the digits of prime numbers in binary. To analyze these patterns, we looked at subsets of prime numbers in binary. Our research found that patterns in the digits of prime numbers in binary exist for Mersenne Primes, Sexy Pair Primes, Fermat Primes, and Germain Primes. The patterns we observe in these subsets give us a broader understanding of primes. These patterns can give us insight into properties of prime numbers and lead to new conjectures about prime number theory. We conjectured the following: the lengths of Mersenne primes, a property of the relation between Sexy Prime Pairs, the number of zeros in Fermat primes, and the form of Germain Primes. Learning more about prime numbers can enhance theoretical and practical applications in mathematics.

1 Introduction

Patterns of prime numbers in base 10 have been extensively researched. This research has led to a better understanding of the properties and relationships of prime numbers. Previous works such as [3] and [4] have explored prime distribution in base 10 and explain the asymptotic density of primes. These works also cover the various patterns amongst primes such as Sophie Germain primes and Mersenne Primes, both of which are special classes of prime numbers that have specific relationships to other prime numbers. [2] conducted further exploration in the applications of prime numbers in fields such as cryptography. These works also explore sieves and probabilistic methods used to study and generate primes. Although this research has brought forth great discoveries and conjectures about prime numbers, it focuses only on observations made in base 10. Extending our research of primes to other bases, such as binary, can lead to new conjectures about patterns of primes numbers.

We asked the question: *What patterns can be observed in the digits of binary representations of prime numbers?*

Analyzing prime numbers in binary form can lead to new discoveries or conjectures about the properties or distribution of primes. This extends our

current knowledge on prime number properties because the binary representation of primes may reveal patterns not seen in other bases. By observing the patterns of the distribution of primes in binary, we can learn more about how prime gaps behave. The binary representation of prime numbers can simplify examination of certain properties such as their divisibility and parity. This can also help with primality testing, used to determine whether a number is prime, and prime generation which is the process of producing primes. Due to the nature of binary representation, analyzing primes in binary can be beneficial in computational applications. This can allow us to create more compact data and will enhance our understanding of the mathematical properties of prime numbers.

2 Background

Our conjectures focus on the patterns that may occur in the binary representation of prime numbers. To address these, we looked at the digits of different types of primes in binary and observed to see if any patterns occurred. We focused on looking for patterns in some prime numbers rather than patterns for all prime numbers. Analyzing these patterns may help us identify and make note of patterns that occur in all prime numbers.

Let's recall our definition of a prime number defined in [1].

Prime Numbers. *A prime number, p, is a positive integer greater than* 1*, such that the only factors of p are* 1 *and p.*

Note that 1 is not prime. Also, the representation for the number "1" in binary is the same as in base 10, written also as "1."

Now, let's consider other special primes as defined in [1], [5], and [6].

Palindrome Primes. *Palindrome Primes are prime numbers in any base such that the reverse order of that number's digits is also prime.*

Mersenne Primes. *Mersenne Primes are prime numbers written of the form*

$$M_n = 2^n - 1,$$

where n must be a prime number. Note that not all values of n will generate Mersenne Primes.

Sexy Primes. *Sexy Primes are pairs of prime numbers (p, q), such that $q - p =$* 6*.*

Fermat Primes. *Fermat Primes are prime numbers of the form*

$$F_n = 2^{2n} + 1,$$

where n is a non-negative integer.

Germain Primes. *Germain Primes are prime numbers contained in the set of primes,* $\mathbb{P}$*, such that*

$$\mathbb{P}_G = \{p \in \mathbb{P} : p \text{ and } 2p+1 \in \mathbb{P}\}.$$

Twin Prime Pairs. *Twin Prime Pairs are pairs of prime numbers,* $(p, p+2)$*, such that* p *and* $p+2$ *are both prime.*

3 Exploration Findings

In this section, we will present the patterns that we found in the digits for different types of prime numbers in binary. For this paper, a subscript of 2 denotes a number is expressed in binary, and the subscript of 10 denotes a number is expressed in base 10.

3.1 Mersenne Primes in Binary

The first type of primes that we will look at in binary are the Mersenne Primes defined in §2.

3.1.1 Case 1: n = 2

$M_2 = 2^2 - 1 = 3_{10} = 11_2$. Thus, 3_{10} is a Mersenne prime.

3.1.2 Case 2: n = 3

$M_3 = 2^3 - 1 = 7_{10} = 111_2$. Thus, 7_{10} is a Mersenne prime.

3.1.3 Case 3: n = 5

$M_5 = 2^5 - 1 = 31_{10} = 1111_2$. Thus, 31_{10} is a Mersenne prime.

3.1.4 Case 4: n = 7

$M_7 = 2^7 - 1 = 127_{10} = 11111_2$. Thus, 127_{10} is a Mersenne prime.

3.1.5 Case 5: n = 11

$M_{11} = 2^{11} - 1 = 2047_{10} = 111111_2$, which is not prime. Thus, 2047_{10} is <u>not</u> a Mersenne prime.

3.1.6 Case 6: n = 13

$M_{13} = 2^{13} - 1 = 8191_{10} = 1111111_2$. Thus, 8191_{10} is a Mersenne prime.

Theorem 3.1. *Let* p *be a prime number, where* $p \leq 13_{10}$*. Then, the Mersenne Primes will have the binary digits of* 1*'s a number of* p *times.*

3.2 Conjecture for Mersenne Primes

We believe that for all Mersenne Primes in binary will be written as

$$M_n = \underbrace{111\cdots1_2}_{n \text{ times}}.$$

3.3 Sexy Primes in Binary

We now consider a different set of prime numbers called Sexy Primes, defined in §2. Consider Table 1.

Table 1: Sexy Prime Pairs

Decimal	Binary
(5, 11)	(101, 1011)
(7, 13)	(111, 1101)
(11, 17)	(1011, 10001)
(13, 19)	(1101, 10011)
(17, 23)	(10001, 10111)
(31, 37)	(11111, 100101)
(37, 43)	(100101, 101011)
(41, 47)	(101001, 101111)
(47, 53)	(101111, 110101)

3.4 Reversing with Digits

Let's consider the reverse of these prime digits in binary to determine if they are prime themselves and whether they belong to the set of Sexy Prime Pairs. Note that being in the set does not necessarily mean that these numbers are pairs with each other.

- 101_2 reversed is still 101_2, so we're done.
- 1011_2 reversed is 1101_2, which are both prime and are both in the set of Sexy Prime pairs.
- 111_2 reversed is still 111_2, so we're done.
- 1011_2 reversed is 1101_2, which are both prime and are both in the set of Sexy Prime pairs.
- 10001_2 reversed is still 10001_2, so we're done.
- 10011_2 reversed is 11001_2, which are both prime and are both in the set of Sexy Prime pairs.
- 10111_2 reversed is 11101_2, which are both prime and are both in the set of Sexy Prime pairs.

- 101011_2 reversed is 110101_2, which are both prime and are both in the set of Sexy Prime pairs.

We see that these are all Palindrome Primes, which occur when the reverse order of the digits are prime as well as the forward direction, as explained in §2.

3.5 Conjecture for Sexy Prime Pairs

We deduce that every decimal representation of Sexy Prime pairs in binary have a pattern where the reverse order of digits are in the set of Sexy Prime pairs too.

For example, let's consider the decimal Sexy Prime Pair $(17, 23)$. In binary, this is represented as $(10001, 10111)$. If we reverse the order of digits of this binary Sexy Prime pair we have 10001_2 and 11101_2. Both these values are prime and in the set of Sexy Prime pairs.

3.6 Fermat Primes

Let's consider Fermat Primes, defined in §2. The first few Fermat primes are:

$$\begin{aligned}
F_0 &= 2^{2^0} + 1 = 3_{10} = 11_2, \\
F_1 &= 2^{2^1} + 1 = 5_{10} = 101_2, \\
F_2 &= 2^{2^2} + 1 = 17_{10} = 10001_2, \\
F_3 &= 2^{2^3} + 1 = 257_{10} = 100000001_2, \\
F_4 &= 2^{2^4} + 1 = 65537_{10} = 10000000000000001_2.
\end{aligned}$$

These are all the known Fermat primes. These numbers are also palindromic primes. Notably, each Fermat prime begins and ends with a "1," with varying numbers of zeros in between. Specifically, we observe the following number of zeros:

- For $n = 0$: 0 zeros
- For $n = 1$: 1 zero
- For $n = 2$: 3 zeros
- For $n = 3$: 7 zeros
- For $n = 4$: 15 zeros

3.7 Conjecture for Fermat Primes

We conjecture that the number of zeros between the ones in the binary representation is given by the formula

$$2^n - 1.$$

Beyond $n = 4$, it has been shown that F_n is composite for $n = 5, 6, 7, 8$, and higher values, so we do not consider these although it remains an open question whether any additional Fermat primes exist for larger values of n.

3.8 Germain Primes in Binary

We will look at a specific subset of prime numbers called Germain Primes, defined in §2.

3.8.1 First Sequence Case

Consider the sequence starting at 2_{10} with the terms growing by a recursive formula $a_{i+1} = 2a_i + 1$. We get the sequence (in base 10): $2, 5, 11, 23, 47, 95, \ldots$ However, $95 \notin \mathbb{P}$, which means that $47 \notin \mathbb{P}_G$. Thus, this first sequence is finite. In particular, the sequence (in decimal) is

$$2_{10}, 5_{10}, 11_{10}, 23_{10},$$

which converts to

$$10_2, 101_2, 1011_2, 10111_2.$$

Now, let's consider more cases.

3.8.2 Second Sequence Case

Similarly, consider the sequence starting at 3_{10} with terms growing by the same recursive formula, $a_{i+1} = 2a_i + 1$. So the sequence we have is

$$3_{10}, 7_{10}, 15_{10}, \ldots$$

Since $15_{10} \notin \mathbb{P}$, then $7_{10} \notin \mathbb{P}_G$. Therefore, this sequence is 3_{10}, which is finite. Note that this sequence case, in binary, is 11_2.

3.8.3 Third Sequence Case

Next, let's consider the prime 29_{10}, which is Germain. Now consider the sequence starting at 29_{10} with the same recursive formula, $a_{i+1} = 2a_i + 1$. So the sequence is

$$29_{10}, 59_{10}, 119_{10}, \ldots$$

Since $119_{10} \notin \mathbb{P}$, then $59_{10} \notin \mathbb{P}_{\mathbb{G}}$. Therefore, the sequence is 29_{10}, which is finite. Note that this sequence case, in binary, is 11101_2.

3.8.4 Fourth Sequence Case

Finally, consider the prime 41_{10}, which is also Germain. Using the same recursive formula, $a_{i+1} = 2a_i + 1$, the sequence is

$$41_{10}, 83_{10}, 167_{10}, 335_{10}, \ldots$$

Since $335_{10} \notin \mathbb{P}$, then $167_{10} \notin \mathbb{P}_{\mathbb{G}}$. Therefore, the finite sequence is $41_{10}, 83_{10}$, which in binary is $101001_2, 1010011_2$.

Theorem 3.2. *Let p be a prime number in base 10 such that $p \leq 83_{10}$. Let p_g be the primes numbers p that are Germain Primes. These Germain Primes have the binary representation where the consecutive terms in the sequence will add a digit of "1" at the end of the number.*

3.9 Conjecture for Germain Primes

In binary, we observe that primes add a "1" to the sequence each time. This occurs because multiplying a prime number p by 2 shifts it binary representation left, adding a "0" at the end. When we add "1", we convert that 0 to a 1. This works similarly to decimal: when we multiply by ten, we add a zero in the ones place.

3.10 Twin Prime Pairs

Now, we will consider Twin Prime Pairs, defined in §2. Consider Table 2 of such pairs in decimal and binary. We don't see any distinct patterns in these pairs, but we encourage researchers to further analyze patterns among binary representation of prime numbers.

Table 2: Twin Prime Pairs

Decimal	Binary
(3, 5)	(11, 101)
(5, 7)	(101, 111)
(11, 13)	(1011, 1101)
(17, 19)	(10001, 10011)
(29, 31)	(11101, 11111)
(41, 43)	(101001, 101011)
(59, 61)	(111011, 111101)
(71, 73)	(1000111, 1001001)

4 Conclusion

We considered what patterns can be observed in the digits of binary representations of prime numbers. We explored various prime numbers in binary to identify digit patterns. By examining smaller subsets, we aimed for a broader understanding of primes. We conjectured that Mersenne Primes are always a string of 1s that are of length n, where n is the term number. We also conjectured that Sexy Prime pairs and their reversed digits are also in the set of Sexy Prime pairs. We conjectured Fermat Primes have $2^n - 1$ zeros between the 1s at the ends of their binary representations. Finally, we conjectured that consecutive Germain Primes add a "1" at the end of the proceeding Germain Prime in the sequence. We did not state a conjecture for Twin Prime Pairs.

Observing the digits of prime numbers in binary and in any base can help us create theorems or conjectures about the digits of prime numbers and their properties. Mathematicians have been trying to understand prime numbers and patterns that are hidden within prime numbers for centuries.

Areas for future research include Fermat Primes and Twin Prime Pairs (§3.6 and §3.10). There are only five Fermat numbers known to be prime for $n = 0, 1, 2, 3, 4$. Other Fermat numbers have been found, but are composite (i.e., not prime). Twin Prime Pairs are directly connected to the Twin Primes Conjecture, which states that there are an infinite number of pairs of Twin Primes; this remains an open question. Not only can this conjecture be an area of continued research, but possible patterns could emerge in the binary representation of Twin Prime Pairs.

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Methods for Finding the Second Moment of Insurance Payment

Aaron Kim
Department of Mathematics
California State University
Fullerton CA 92831, USA
aaronskim@fullerton.edu

Justin Nguyen
Department of Mathematics
California State University
Fullerton CA 92831, USA
20jnguyen39@csu.fullerton.edu

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Abstract: While the formula for evaluating the first moment of insurance payment are widely available, the formula for evaluating its the second moment and hence variance are quite limited. Within this project, we are investigating several methods for this purpose, We then apply these methods when loss follows either a uniform, exponential, gamma, or Pareto distribution.

1 Introduction

Let X denote the loss amount, d represent deductible and Y^L be the insurance payment when a loss occurs. Thus

$$Y_L = \begin{cases} 0 & X \leq d \\ X - d & X > d. \end{cases}$$

Mathematically, we can write $Y_L = \max(0, X - d)$. This random variable is also known as (insurance) *payment per loss*. It is straight forward to see that $Y_L = X - X \wedge d$. In this formulation, the first moment of Y_L can be readily obtained. In fact, $E(Y_L) = E(X) - E(X \wedge d)$. The formula for $E(X \wedge d)$ are widely available when X follows either a uniform, exponential or pareto distribution. See, for example, "Loss Models" by Panjer and et el. This book however does not report such formula when X follows a gamma distribution. We will present the formula in this case along with our main results in calculating the second moment of Y_L.

2 Second Moment of Y_L - First Method

Let u be the limit for the insurance policy, then

$$Y_L = \begin{cases} 0 & X \leq d \\ X - d & d < X \leq u \\ u - d & X > u. \end{cases}.$$

Hence, $Y_L = (X \wedge u) - (X \wedge d)$ and

$$\begin{aligned} Y_L^2 &= [(X \wedge u) - (X \wedge d)]^2 \\ &= (X \wedge u)^2 - 2(X \wedge u)(X \wedge d) + (X \wedge d)^2 \\ &= (X \wedge u)^2 + (X \wedge d)^2 - 2(X \wedge d)[X \wedge u - X \wedge d] - 2(X \wedge d)^2 \\ &= (X \wedge u)^2 - (X \wedge d)^2 - 2(X \wedge d)[X \wedge u - X \wedge d]. \end{aligned}$$

Since we are only considering the case where the insurance policies do not have a limit, $u = \infty$ and

$$\begin{aligned} 2(X \wedge d)(X \wedge u - X \wedge d) &= 2(X \wedge d)(X - X \wedge d) \\ &= 2X(0) + 2d(X - d) \\ &= 2d(X - d) \\ &= 2dY_L. \end{aligned}$$

Therefore, we have

$$E(Y_L^2) = E(X^2) - E[(X \wedge d)^2] - 2dE(Y_L).$$

Mathematically, we can also write

$$\begin{aligned} Y_L^2 &= (X - X \wedge d)^2 \\ &= X^2 - 2X(X \wedge d) + (X \wedge d)^2. \end{aligned}$$

Therefore,

$$\begin{aligned} E(Y_L^2) &= E[(X - X \wedge d)^2] \\ &= E(X^2) - 2E[X(X \wedge d)] + E[(X \wedge d)^2]. \end{aligned}$$

2.1 Uniform Distribution

For a loss modeled by the uniform distribution $X \sim unif(0, b)$, its density function will be $f(x) = \frac{1}{b}$. Thus,

$$\begin{aligned} E(X^2) &= \int_0^b x^2 \frac{1}{b} dx \\ &= \frac{1}{b} \frac{x^3}{3} |_0^b \\ &= \frac{1}{3b} b^3 \\ &= \frac{b^2}{3}. \end{aligned}$$

$$\begin{aligned} E[X(X \wedge d)] &= \int_0^b x(x \wedge d) \frac{1}{b} dx \\ &= \int_0^d x^2 \frac{1}{b} dx + \int_d^b x d \frac{1}{b} dx \\ &= \frac{1}{b} [\frac{x^3}{3} |_0^d + d \frac{x^2}{2} |_d^b] \\ &= \frac{1}{b} [\frac{d^3}{3} + \frac{d(b^2 - d^2)}{2}]. \end{aligned}$$

$$
\begin{aligned}
E[(X \wedge d)^2] &= \int_0^b (x \wedge d)^2 \frac{1}{b} dx \\
&= \int_0^d \frac{x^2}{b} dx + \int_d^b \frac{d^2}{b} dx \\
&= \frac{1}{b}[\frac{d^3}{3} + d^2(b-d)] \\
&= \frac{1}{b}\frac{d^3 + 3d^2(b-d)}{3} \\
&= \frac{3bd^2 - 2d^3}{3b}.
\end{aligned}
$$

With these values calculated, we can now determine $E(Y_L^2)$.

$$
\begin{aligned}
E(Y_L^2) &= E(X^2) - 2E[X(X \wedge d)] + E[(X \wedge d)^2] \\
&= \frac{b^2}{3} - \frac{2}{b}[\frac{d^3}{3} + \frac{d(b^2 - d^2)}{2}] + \frac{3bd^2 - 2d^3}{3b} \\
&= \frac{b^2}{3} - \frac{1}{b}(\frac{2d^3}{3} + b^2 d - d^3) + \frac{3bd^2 - 2d^3}{3b} \\
&= \frac{b^2}{3} - \frac{2d^3 + 3b^2 - 3d^3}{3b} + \frac{3bd^2 - 2d^3}{3b} \\
&= \frac{1}{3b}(b^3 - 2d^3 - 3b^2 d + 3d^3 + 3bd^2 - 2d^3) \\
&= \frac{1}{3b}(b^3 - 3b^2 d + 3bd^2 - d^3) \\
&= \frac{1}{3b}(b-d)^3.
\end{aligned}
$$

2.2 Exponential Distribution

$X \sim exp(\theta)$, $f(x) = \frac{1}{\theta} e^{-\frac{x}{\theta}}$

$$
\begin{aligned}
E(X^2) &= \int_0^\infty x^2 \frac{1}{\theta} e^{-\frac{x}{\theta}} dx \\
&= -x^2 e^{-\frac{x}{\theta}} - 2x\theta e^{-\frac{x}{\theta}} - 2\theta^2 e^{-\frac{x}{\theta}} |_0^\infty \\
&= 0 - (-2\theta) = 2\theta.
\end{aligned}
$$

$$
\begin{aligned}
E[(X \wedge d)^2] &= \int_0^d x^2 \frac{1}{\theta} e^{-\frac{x}{\theta}} + \int_d^\infty d^2 \frac{1}{\theta} e^{-\frac{x}{\theta}} dx \\
&= I_1 + I_2 \\
&= 2\theta^2 - 2\theta d e^{-\frac{d}{\theta}} - 2\theta^2 e^{-\frac{d}{\theta}}
\end{aligned}
$$

$$
\begin{aligned}
I_1 &= -x^2 e^{-\frac{x}{\theta}} - 2x\theta e^{-\frac{x}{\theta}} - 2\theta^2 e^{-\frac{x}{\theta}} |_0^d \\
&= -d^2 e^{-\frac{d}{\theta}} - 2d\theta e^{-\frac{d}{\theta}} - 2\theta^2 e^{-\frac{d}{\theta}} + 2\theta^2
\end{aligned}
$$

$$
I_2 = -d^2 e^{-\frac{x}{\theta}} |_d^\infty = d^2 e^{-\frac{d}{\theta}}
$$

We know that the expected value of the first moment is $E(Y_L) = \theta e^{-\frac{d}{\theta}}$. Therefore,

$$
\begin{aligned}
E(Y_L^2) &= E(X^2) - E[(X \wedge d)^2] - 2dE(Y_L) \\
&= 2\theta^2 - (2\theta^2 - 2\theta d e^{-\frac{d}{\theta}} - 2\theta^2 e^{-\frac{d}{\theta}}) - 2\theta d e^{-\frac{d}{\theta}} \\
&= 2\theta^2 e^{-\frac{d}{\theta}}.
\end{aligned}
$$

2.3 Pareto Distribution

$X \sim Pareto(\alpha, \theta)$, $f(x) = \frac{\alpha\theta^\alpha}{(\theta+x)^{\alpha+1}}$

$$
\begin{aligned}
E(X^2) &= \int_0^\infty x^2 \frac{\alpha\theta^\alpha}{(\theta+x)^{\alpha+1}} dx \\
&= \alpha\theta^\alpha \int_\theta^\infty \frac{u^2 - 2\theta u + \theta^2}{u^{\alpha+1}} du \\
&= \alpha\theta^\alpha [\int_\theta^\infty u^{1-\alpha} du - 2\theta \int_\theta^\infty u^{-\alpha} du + \theta^2 \int_\theta^\infty u^{-\alpha-1} du] \\
&= \alpha\theta^\alpha [-\frac{u^{2-\alpha}}{\alpha-2}|_\theta^\infty + 2\theta \frac{u^{1-\alpha}}{\alpha-1}|_\theta^\infty - \theta^2 \frac{u^{-\alpha}}{\alpha}|_\theta^\infty] \\
&= \alpha\theta^\alpha [\frac{\theta^{2-\alpha}}{\alpha-2} - \frac{2\theta^{2-\alpha}}{\alpha-1} + \frac{\theta^{2-\alpha}}{\alpha}] \\
&= \theta^\alpha [\frac{2\theta^{2-\alpha}}{(\alpha-2)(\alpha-1)}] \\
&= \frac{2\theta^2}{(\alpha-2)(\alpha-1)}.
\end{aligned}
$$

$$
\begin{aligned}
E[(X \wedge d)^2] &= \int_0^d x^2 \frac{\alpha\theta^\alpha}{(x+\theta)^{\alpha+1}} dx + \int_d^\infty d^2 \frac{\alpha\theta^\alpha}{(x+\theta)^{\alpha+1}} dx \\
&= I_1 + I_2.
\end{aligned}
$$

$$
\begin{aligned}
I_1 &= \alpha\theta^\alpha \int_0^d \frac{x^2}{(x+\theta)^{\alpha+1}} dx \\
&= \alpha\theta^\alpha \int_\theta^{d+\theta} \frac{u^2 - 2\theta u + \theta^2}{u^{\alpha+1}} du \\
&= \alpha\theta^\alpha [\int_\theta^{d+\theta} u^{1-\alpha} du - 2\theta \int_\theta^{d+\theta} u^{-\alpha} du + \theta^2 \int_\theta^{d+\theta} u^{-\alpha-1} du] \\
&= \alpha\theta^\alpha [-\frac{1}{\alpha-2} u^{2-\alpha}|_\theta^{d+\theta} + \frac{2\theta}{\alpha-1} u^{1-\alpha}|_\theta^{d+\theta} - \frac{\theta^2}{\alpha} u^{-\alpha}|_\theta^{d+\theta}] \\
&= \alpha\theta^\alpha [\frac{\theta^{2-\alpha} - (d+\theta)^{2-\alpha}}{\alpha-2} + \frac{2\theta(d+\theta)^{1-\alpha} - 2\theta^{2-\alpha}}{\alpha-1} + \frac{\theta^{2-\alpha} - \theta^2(d+\theta)^{-\alpha}}{\alpha}] \\
&= \frac{2\theta^2}{(\alpha-2)(\alpha-1)} + \theta^\alpha [\frac{\alpha d^2 - 2\alpha\theta d - \alpha^2 d^2 - 2\theta^2}{(\alpha-2)(\alpha-1)(d+\theta)^\alpha}].
\end{aligned}
$$

$$
\begin{aligned}
I_2 &= d^2 \int_d^\infty \frac{\alpha\theta^\alpha}{(x+\theta)^{\alpha+1}} dx \\
&= d^2 S_X(d) \\
&= d^2 (\frac{\theta}{d+\theta})^\alpha \\
&= \theta^\alpha [\frac{\alpha^2 d^2 - 3\alpha d^2 + 2d^2}{(\alpha-2)(\alpha-1)(d+\theta)^\alpha}].
\end{aligned}
$$

$$
\begin{aligned}
E[(X \wedge d)^2] &= I_1 + I_2 \\
&= \frac{2\theta^2}{(\alpha-2)(\alpha-1)} + 2\theta^\alpha [\frac{d^2 - \alpha d^2 - \alpha\theta d - \theta^2}{(\alpha-2)(\alpha-1)(d+\theta)^\alpha}].
\end{aligned}
$$

$$
\begin{aligned}
E(Y_L) &= E(X) - E(X \wedge d) \\
&= \frac{\theta}{\alpha - 1} - \frac{\theta}{\alpha - 1}[1 - (\frac{\theta}{d+\theta})^{\alpha-1}] \\
&= \frac{\theta}{\alpha - 1}(\frac{\theta}{d+\theta})^{\alpha-1} \\
&= \theta^{\alpha}[\frac{d+\theta}{(\alpha-1)(d+\theta)^{\alpha}}] \\
&= \theta^{\alpha}[\frac{\alpha d - 2d + \alpha\theta - 2\theta}{(\alpha-2)(\alpha-1)(d+\theta)^{\alpha}}].
\end{aligned}
$$

$$
\begin{aligned}
E(Y_L^2) &= E(X^2) - E[(X \wedge d)^2] - 2dE(Y_L) \\
&= 2\theta^{\alpha}[\frac{(d+\theta)^2}{(\alpha-2)(\alpha-1)(d+\theta)^{\alpha}}] \\
&= \frac{2\theta^2}{(\alpha-2)(\alpha-1)}(\frac{\theta}{d+\theta})^{\alpha-2}.
\end{aligned}
$$

2.4 Gamma Distribution

If $X \sim gam(\alpha, \theta)$, then $f(x) = \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha}\Gamma(\alpha)}$.

$$
\begin{aligned}
E(X^2) &= \int_0^{\infty} x^2 \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha}\Gamma(\alpha)} dx \\
&= \theta^2\alpha(\alpha+1)\int_0^{\infty} \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+2}\Gamma(\alpha+2)} dx \\
&= \theta^2\alpha(\alpha+1).
\end{aligned}
$$

$E[(X \wedge d)^2] = \int_0^d x^2 f(x)dx + \int_d^{\infty} d^2 f(x)dx = I_1 + I_2.$

$$
\begin{aligned}
I_1 &= \int_0^d x^2 \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha \Gamma(\alpha)} dx \\
&= \int_0^d \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^\alpha \Gamma(\alpha)} dx \\
&= \frac{\theta^{\alpha+2}\Gamma(\alpha+2)}{\theta^\alpha \Gamma(\alpha)} \int_0^d \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+2}\Gamma(\alpha+2)} dx \\
&= \theta^2\alpha(\alpha+1) \int_0^d \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+2}\Gamma(\alpha+2)} dx \\
&= \theta^2\alpha(\alpha+1)P(X^* \leq d) \\
&= \theta^2\alpha(\alpha+1)F_{X^*}(d) \\
&= \theta^2\alpha(\alpha+1)[1 - P(Y \leq \alpha+1)] \\
&= \theta^2\alpha(\alpha+1) - \theta^2\alpha(\alpha+1)P(Y \leq \alpha+1).
\end{aligned}
$$

Note that here, $X^* \sim gam(\alpha+2, \theta)$, and $Y \sim Poi(\lambda = \frac{d}{\theta})$.

$$
\begin{aligned}
I_2 &= d^2 \int_d^\infty f(x) dx \\
&= d^2 S_X(d) \\
&= d^2 P(Y \leq \alpha - 1).
\end{aligned}
$$

Note that here, $S_X(d)$ is the survival function of the function X beyond the value of the deductible d. Thus,

$$
\begin{aligned}
E[(X \wedge d)^2] &= I_1 + I_2 \\
&= \theta^2\alpha(\alpha+1) - \theta^2\alpha(\alpha+1)P(Y \leq \alpha+1) + d^2 P(Y \leq \alpha - 1).
\end{aligned}
$$

$$
\begin{aligned}
E(X) &= \int_0^\infty x \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha \Gamma(\alpha)} \\
&= \frac{\theta^{\alpha+1}\Gamma(\alpha+1)}{\theta^\alpha \Gamma(\alpha)} \int_0^\infty \frac{x^{(\alpha+1)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+1}\Gamma(\alpha+1)} \\
&= \theta\alpha.
\end{aligned}
$$

$$\begin{aligned}
E(Y_L) &= E(X) - E(X \wedge d) \\
&= \theta\alpha - [\alpha\theta[1 - P(Y \leq \alpha)] + dP(Y \leq \alpha - 1)] \\
&= \theta\alpha - \alpha\theta[1 - P(Y \leq \alpha)] - dP(Y \leq \alpha - 1) \\
&= \theta\alpha P(Y \leq \alpha) - dP(Y \leq \alpha - 1).
\end{aligned}$$

$$\begin{aligned}
E(Y_L^2) &= E(X^2) - E[(X \wedge d)^2] - 2dE(Y_L) \\
&= \theta^2\alpha(\alpha+1)P(Y \leq \alpha + 1) - 2d\theta\alpha P(Y \leq \alpha) + d^2P(Y \leq \alpha - 1).
\end{aligned}$$

3 Second Moment of Y_L - Second Method

Let $I_{(X>d)}$ denote a dummy variable that is 1 when the condition $X > d$ is met and 0 otherwise. We can see that

$$(X-d)^2 I_{(X>d)} = \begin{cases} 0 & X \leq d \\ (X-d)^2 & X > d \end{cases} = Y_L^2.$$

Thus $E(Y_L^2) = \int_{\mathcal{D}} I_{(X>d)}(x-d)^2 f(x)dx$.

3.1 Uniform Distribution

$X \sim unif(0, b)$, $f(x) = \frac{1}{b}$

$$\begin{aligned}
E(Y_L^2) &= \int_0^b (x-d)^2 I_{(X>d)} \frac{1}{b} dx \\
&= \frac{1}{b}\int_d^b (x-d)^2 dx \\
&= \frac{1}{b}\int_0^{b-d} u^2 du \\
&= \frac{1}{b}\frac{1}{3}u^3|_0^{b-d} \\
&= \frac{1}{3b}(b-d)^3.
\end{aligned}$$

3.2 Exponential Distribution

$X \sim exp(\theta)$, $f(x) = \frac{1}{\theta}e^{-\frac{x}{\theta}}$

$$\begin{aligned}
E(Y_L^2) &= \int_0^\infty (x-d)^2 I_{(X>d)} f(x) dx \\
&= \int_d^\infty (x-d)^2 \frac{1}{\theta} e^{-\frac{x}{\theta}} dx \\
&= \int_0^d y^2 \frac{1}{\theta} e^{-\frac{(y+d)}{\theta}} dy \\
&= e^{-\frac{d}{\theta}} \int_0^\infty \frac{1}{\theta} y^2 e^{-\frac{y}{\theta}} dy \\
&= e^{-\frac{d}{\theta}} (-y^2 e^{-\frac{y}{\theta}} - 2y\theta e^{-\frac{y}{\theta}} - 2\theta^2 e^{-\frac{y}{\theta}} |_0^\infty) \\
&= e^{-\frac{d}{\theta}} (0 + 2\theta^2) \\
&= 2\theta^2 e^{-\frac{d}{\theta}}.
\end{aligned}$$

3.3 Pareto Distribution

$X \sim Pareto(\alpha, \theta)$, $f(x) = \frac{\alpha\theta^\alpha}{(\theta+x)^{\alpha+1}}$

$$
\begin{aligned}
E(Y_L^2) &= \int_0^\infty (x-d)^2 I_{(x>d)} \frac{\alpha\theta^\alpha}{(x+\theta)^{\alpha+1}} dx \\
&= \alpha\theta^\alpha \int_d^\infty \frac{(x-d)^2}{(x+\theta)^{\alpha+1}} dx \\
&= \alpha\theta^\alpha \int_{d+\theta}^\infty \frac{(u-\theta-d)^2}{u^{\alpha+1}} du \\
&= \alpha\theta^\alpha [\int_{d+\theta}^\infty u^{1-\alpha} du - 2(d+\theta)\int_{d+\theta}^\infty u^{-\alpha} du + (d+\theta)^2 \int_{d+\theta}^\infty u^{-1-\alpha} du] \\
&= \alpha\theta^\alpha [\frac{1}{2-\alpha} u^{2-\alpha}|_{d+\theta}^\infty - \frac{2(d+\theta)}{1-\alpha} u^{1-\alpha}|_{d+\theta}^\infty - \frac{(d+\theta)^2}{\alpha} u^{-\alpha}|_{d+\theta}^\infty] \\
&= \alpha\theta^\alpha [\frac{(d+\theta)^{2-\alpha}}{(\alpha-2)} - \frac{2(d+\theta)^{2-\alpha}}{(\alpha-1)} + \frac{(d+\theta)^{2-\alpha}}{\alpha}] \\
&= \alpha\theta^\alpha [\frac{2(d+\theta)^{2-\alpha}}{(\alpha-2)(\alpha-1)\alpha}] \\
&= \theta^2 [\frac{2\theta^{\alpha-2}}{(\alpha-2)(\alpha-1)(d+\theta)^{\alpha-2}}] \\
&= \frac{2\theta^2}{(\alpha-2)(\alpha-1)} (\frac{\theta}{d+\theta})^{\alpha-2}.
\end{aligned}
$$

3.4 Gamma Distribution

$X \sim Gamma(\alpha, \theta)$, $f(x) = \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha \Gamma(\alpha)}$

$$
\begin{aligned}
E(Y_L^2) &= \int_0^\infty (x-d)^2 I_{(x>d)} \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha\Gamma(\alpha)} dx \\
&= \int_d^\infty (x-d)^2 \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha\Gamma(\alpha)} dx \\
&= \int_d^\infty \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^\alpha\Gamma(\alpha)} dx - 2d\int_d^\infty \frac{x^{(\alpha+1)-1}e^{-\frac{x}{\theta}}}{\theta^\alpha\Gamma(\alpha)} dx + d^2 \int_d^\infty \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^\alpha\Gamma(\alpha)} dx \\
&= I_1 - 2dI_2 + d^2 I_3.
\end{aligned}
$$

$$
\begin{aligned}
I_1 &= \int_d^{\infty} \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha}\Gamma(\alpha)}dx \\
&= \frac{\theta^{\alpha+2}\Gamma(\alpha+2)}{\theta^{\alpha}\Gamma(\alpha)}\int_d^{\infty} \frac{x^{(\alpha+2)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+2}\Gamma(\alpha+2)}dx \\
&= \alpha(\alpha+1)\theta^2 S_{X^*}(d), X^* \sim Gamma(\alpha^* = \alpha+2, \theta^* = \theta) \\
&= \alpha(\alpha+1)\theta^2 F_Y(\alpha+1), Y \sim Poisson(\lambda = \frac{d}{\theta}) \\
&= \alpha(\alpha+1)\theta^2 \Sigma_{k=0}^{\alpha+1} P(Y=k) \\
&= \alpha(\alpha+1)\theta^2 P(Y \leq \alpha+1).
\end{aligned}
$$

$$
\begin{aligned}
I_2 &= \int_d^{\infty} \frac{x^{(\alpha+1)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha}\Gamma(\alpha)}dx \\
&= \frac{\theta^{\alpha+1}\Gamma(\alpha+1)}{\theta^{\alpha}\Gamma(\alpha)}\int_d^{\infty} \frac{x^{(\alpha+1)-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha+1}\Gamma(\alpha+1)}dx \\
&= \alpha\theta S_{X^{**}}(d), X^{**} \sim Gamma(\alpha^{**} = \alpha+1, \theta^{**} = \theta) \\
&= \alpha\theta F_Y(\alpha), Y \sim Poisson(\lambda = \frac{d}{\theta}) \\
&= \alpha\theta \Sigma_{k=0}^{\alpha} P(Y=k) \\
&= \alpha\theta P(Y \leq \alpha).
\end{aligned}
$$

$$
\begin{aligned}
I_3 &= \int_d^{\infty} \frac{x^{\alpha-1}e^{-\frac{x}{\theta}}}{\theta^{\alpha}\Gamma(\alpha)}dx \\
&= S_X(d), X \sim Gamma(\alpha, \theta) \\
&= F_Y(\alpha-1), Y \sim Poisson(\lambda = \frac{d}{\theta}) \\
&= \Sigma_{k=0}^{\alpha-1} P(Y=k) \\
&= P(Y \leq \alpha-1).
\end{aligned}
$$

$$
E(Y_L^2) = \alpha(\alpha+1)\theta^2 P(Y \leq \alpha+1) - 2d\alpha\theta P(Y \leq \alpha) + d^2 P(Y \leq \alpha-1).
$$

4 Second Moment of Y_L - Third Method

Let $E(Y_P^2)$ be the second moment of insurance payment, given that the loss exceeds the deductible. Thus, $E(Y_P^2) = E(Y_L^2|X > d)$. In addition to this, by the law of total expectation we can see that

$$\begin{aligned} E(Y_L^2) &= E(Y_L^2|X \leq d)P(X \leq d) + E(Y_L^2|X > d)P(X > d) \\ &= E[(X-d)^2|X > d]P(X > d) \\ &= E(Y_P^2)P(X > d). \end{aligned}$$

Additionally, we can find the CDF of Y_P

$$\begin{aligned} F_{Y_P}(y) &= P(Y_P \leq y) \\ &= P(X - d \leq y|X > d) \\ &= P(X \leq y + d|X > d) \\ &= \frac{P(X \leq y + d \cap X > d)}{P(X > d)} \\ &= \frac{P(d \leq X \leq y + d)}{P(X > d)} \\ &= \frac{F_X(y+d) - F_X(d)}{S_X(d)}. \end{aligned}$$

If X is a random variable where $X \sim unif(0, b)$, then we can see

$$\begin{aligned} F_{Y_P}(y) &= \frac{F_X(y+d) - F_X(d)}{S_X(d)} \\ &= \frac{\frac{y+d}{b} - \frac{d}{b}}{1 - \frac{d}{b}} \\ &= \frac{\frac{y}{b}}{\frac{b-d}{b}} \\ &= \frac{y}{b-d}. \\ &\therefore Y_P \sim unif(0, b-d) \end{aligned}$$

If X is a random variable where $X \sim exp(\theta)$, then we can see

$$\begin{aligned} F_{Y_P}(y) &= \frac{F_X(y+d) - F_X(d)}{S_X(d)} \\ &= \frac{1 - e^{-\frac{y+d}{\theta}} - 1 + e^{-\frac{d}{\theta}}}{e^{-\frac{d}{\theta}}} \\ &= \frac{e^{-\frac{d}{\theta}} - e^{-\frac{y+d}{\theta}}}{e^{-\frac{d}{\theta}}} \\ &= \frac{e^{-\frac{d}{\theta}}(1 - e^{-\frac{y}{\theta}})}{e^{-\frac{d}{\theta}}} \\ &= 1 - e^{-\frac{y}{\theta}}. \\ &\therefore Y_P \sim exp(\theta) \end{aligned}$$

If X is a random variable where $X \sim pareto(\alpha, \theta)$, then we can see

$$\begin{aligned} F_{Y_P}(y) &= \frac{F_X(y+d) - F_X(d)}{S_X(d)} \\ &= \frac{1 - (\frac{\theta}{\theta+y+d})^\alpha - 1 + (\frac{\theta}{\theta+d})^\alpha}{(\frac{\theta}{\theta+d})^\alpha} \\ &= \frac{(\frac{\theta}{\theta+d})^\alpha - (\frac{\theta}{\theta+y+d})^\alpha}{(\frac{\theta}{\theta+d})^\alpha} \\ &= 1 - (\frac{\theta+d}{\theta+d+y})^\alpha. \\ &\therefore Y_P \sim pareto(\alpha, \theta+d) \end{aligned}$$

4.1 Uniform Distribution

If $X \sim unif(0, b)$, then $Y_P \sim unif(0, b-d)$.

$$E(Y_L^2) = E(Y_P^2)P(X > d).$$

$$\begin{aligned} E(Y_p^2) &= \int_0^{b-d} y^2 \frac{1}{b-d} dy \\ &= \frac{1}{b-d}\frac{1}{3} y^3 |_0^{b-d} \\ &= \frac{(b-d)^2}{3}. \end{aligned}$$

$$\begin{aligned} P(X > d) &= \int_d^b \frac{1}{b} dx \\ &= \frac{b-d}{b}. \end{aligned}$$

$$\begin{aligned} E(Y_L^2) &= E(Y_p^2) P(X > d) \\ &= \frac{(b-d)^2}{3}\frac{b-d}{b} \\ &= \frac{1}{3b}(b-d)^3. \end{aligned}$$

4.2 Exponential Distribution

If $X \sim exp(\theta)$, then $Y_p \sim exp(\theta)$ as well, as seen above. Thus,

$$E(Y_L^2) = E(Y_P^2) P(X > d).$$

$$\begin{aligned} E(Y_p^2) &= \int_0^{\infty} y^2 \frac{1}{\theta} e^{-\frac{y}{\theta}} dy \\ &= -y^2 e^{-\frac{y}{\theta}} - 2y\theta e^{-\frac{y}{\theta}} 2\theta^2 e^{-\frac{y}{\theta}} |_0^{\infty} \\ &= 2\theta^2. \end{aligned}$$

$$\begin{aligned} P(X > d) &= \int_d^{\infty} \frac{1}{\theta} e^{-\frac{x}{\theta}} dx \\ &= -e^{\frac{x}{\theta}} |_d^{\infty} \\ &= e^{-\frac{d}{\theta}}. \end{aligned}$$

$$\begin{aligned} E(Y_L^2) &= E(Y_p^2)P(X > d) \\ &= 2\theta^2 e^{-\frac{d}{\theta}}. \end{aligned}$$

4.3 Pareto Distribution

If $X \sim Pareto(\alpha, \theta)$, then $Y_p \sim Pareto(\alpha, \theta + d)$.

$$E(Y_L^2) = E(Y_P^2)P(X > d)$$

$$\begin{aligned} E(Y_P^2) &= V(Y_P) + E^2(Y_P) \\ &= (\frac{\theta + d}{\alpha - 1})^2(\frac{\alpha}{\alpha - 2}) + (\frac{\theta + d}{\alpha - 1})^2 \\ &= (\frac{\alpha}{\alpha - 2} + 1)(\frac{\theta + d}{\alpha - 1})^2 \\ &= \frac{2(\alpha - 1)}{\alpha - 2}(\frac{\theta + d}{\alpha - 1})^2 \\ &= \frac{2(\theta + d)^2}{(\alpha - 2)(\alpha - 1)}. \end{aligned}$$

$$\begin{aligned} P(X > d) &= S_X(d) \\ &= (\frac{\theta}{\theta + d})^\alpha. \end{aligned}$$

$$\begin{aligned} E(Y_L^2) &= \frac{2(\theta + d)^2}{(\alpha - 2)(\alpha - 1)}(\frac{\theta}{\theta + d})^\alpha \\ &= \frac{2\theta^2}{(\alpha - 2)(\alpha - 1)}(\frac{\theta}{\theta + d})^{\alpha - 2}. \end{aligned}$$

4.4 Gamma Distribution

If $X \sim Gamma(\alpha, \theta)$ then

$$\begin{aligned} F_{Y_P}(y) &= \frac{F_X(y+d) - F_X(d)}{S_X(d)} \\ &= \frac{S_{W^*}(\alpha-1) - S_W(\alpha-1)}{F_W(\alpha-1)}, W^* \sim Poisson(\lambda^* = \frac{y+d}{\theta}) \;\; W \sim Poisson(\lambda = \frac{d}{\theta}) \\ &= \frac{P(W \leq \alpha-1) - P(W^* \leq \alpha-1)}{P(W \leq \alpha-1)} \\ &= 1 - \frac{P(W^* \leq \alpha-1)}{P(W \leq \alpha-1)}. \end{aligned}$$

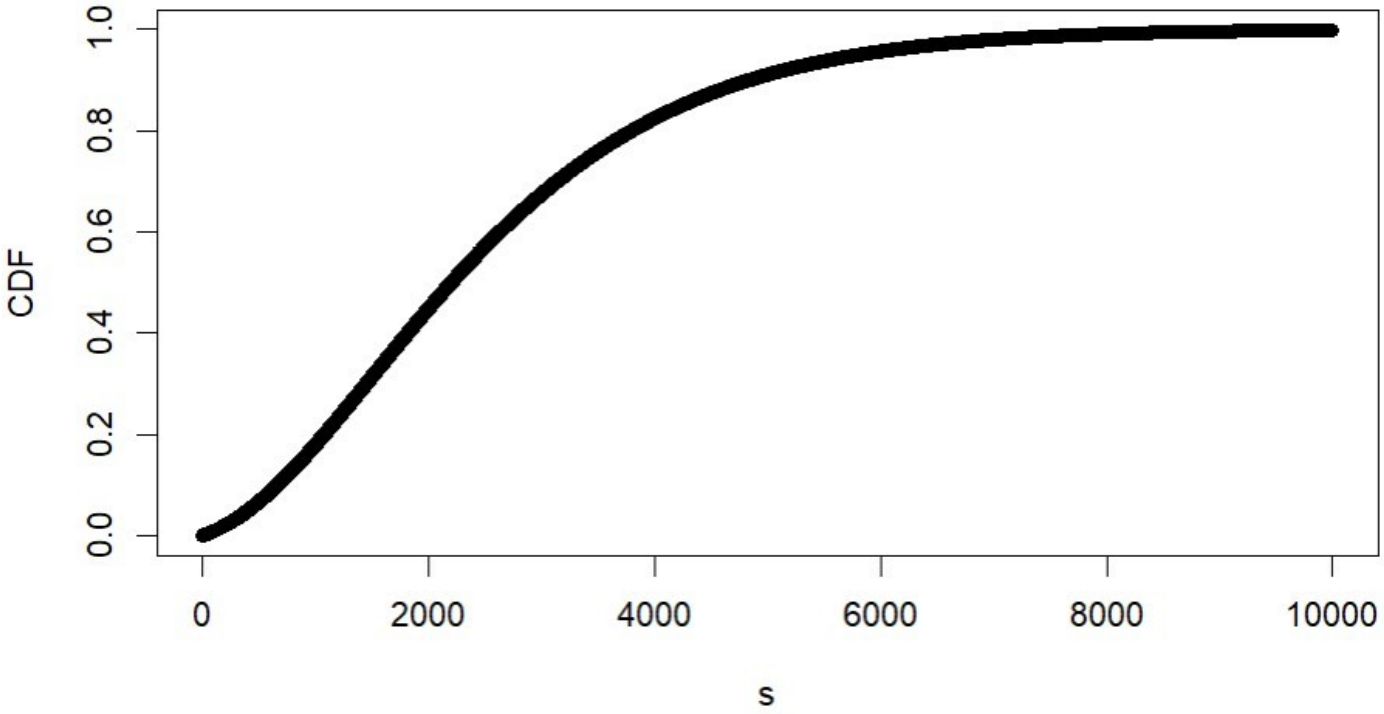
Unfortunately, for this method there is no concise formula for $E(Y_P^2)$, when the loss follows a gamma distribution. This is mainly due to the fact that the Y_P of a gamma distribution cannot be expressed in the form of a recognizable distribution. As seen above, the $F_{Y_p}(y)$ does not match any distribution's density function.

Although we were not able to derive a formula for $E(Y_P^2)$ using this method, we still found a result worth sharing. Since $F_{Y_P}(y) = 1 - \frac{P(W^* \leq \alpha-1)}{P(W \leq \alpha-1)}$ where W and W^* are Poisson distributions with the respective expected values of $\frac{d}{\theta}$ and $\frac{y+d}{\theta}$. We were able to create and define a function in R which gives us the cumulative probability of Y_P when X, the loss, follows a gamma distribution.

```
‘‘‘{r}
pgamma.yp <- function(a, t, d, y){

  b = (d + y)/t
  c = d/t

  1 - (ppois(a - 1, b)/ppois(a - 1, c)
       )
  }
pgamma.yp(3, 1000, 500, 2000)

s <- seq(from = 0, to = 10000, by = 1)
```

```
CDF <- pgamma.yp(3, 1000, 500, s)

plot(s, CDF)
```

In this R chunk, we are defining the function with the parameters "a" as α, "t" as θ, "d" as the deductible, and "y" as the point where we want to find the cumulative probability up to. Next, we test the function by plugging in 3 for α, 1000 for θ, 500 for d, and 2000 for y, which gives us back the probability of 0.4482485. After that, we create a sequence from 0 to 10000 in increments of 1, which we will use as input for y to make 10001 different cumulative probabilities. Lastly, we plot the sequence on the x-axis and the corresponding probabilities on the y-axis to visually display the cumulative distribution function.


CDF
0.0
0.2
0.4
0.6
0.8
1.0
0
2000
4000
6000
8000
10000
s

We can verify that the cumulative distribution function is valid by checking if it is monotonically increasing, if it converges to a probability of 1 as $s \to \infty$, and if the probability is 0 when $s \to -\infty$. Based on the graph, we can see that the cumulative probability is 0 when n = 0. Additionally, we can see that the function is monotonically increasing. Lastly, we can observe that as $S \to \infty$, the cumulative probability approaches 1.

5 Conclusion

During this research, three different methods were explored to calculate the second moment of insurance payout Y_L, in situations where the loss amount followed a uniform, exponential, gamma or Pareto distribution. The first method relied on using the existing formula $Y_L = (X \wedge u) - (X \wedge d)$, then squaring both sides and expanding. As an extension of the most common method used to find the first moment of the insurance payout, this is conceptually straightforward. However, it is computationally very heavy, with some distributions relying on integration by parts to be fully integrated. This can make the actual use of this first method impractical.

The second method used the indicator variable, a variable that was set equal to 1 when condition $X > d$ is met, and was set equal to 0 when it was not. Although indicator variables are more commonly seen in data analysis and regression modeling, their usage here makes the integration process much easier, streamlining the computational process of finding the second moment for the various distributions.

The third method, which involves creating a new variable Y_p and relating it to Y_L, requires the introduction of several new concepts. It involves the creation of an entirely new variable, then finding the CDF of said variable and attempting to match it to an existing distribution. This conceptual difficulty, however, is rewarded by being the easiest method to use computationally. For some distributions (uniform, Pareto), using Y_p allows $E(Y_L^2)$ to be calculated without needing to use an integral at all. It should be noted that this method does not result in finding a common distribution associated with the CDF of gamma's associated Y_p. This makes the third method less useful for finding the second moment of the gamma distribution specifically compared to the other methods found.

The development of these new methods should make it much easier to evaluate the variance of insurance payments when they take on the four dif-

ferent distributions evaluated here. This will make it easier to evaluate and analyze the worth of various insurance packages. Additionally, offering more and easier ways to calculate the second moment should make learning about them in classroom settings more approachable. Moment-generating functions are a key concept that appears repeatedly in upper-division statistics courses, and these new methods will ideally make learning and computing them easier for students unfamiliar with them. Further research could be done by investigating the validity of these new methods on other common continuous distributions, such as the beta distribution.