Developing an Experimental Model to Study Natural Variation and Genetic Robustness

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Abstract: Cellular processes are the result of a complex network of molecular interactions, scripted for within the organism's DNA. The study of these interactions and their properties characterize an essential aspect of network biology. Networks within natural life can be defined by mathematical characteristics and computational modeling common to all networks, but certain properties of biological networks, such as robustness, are subject to greater variability. Robustness, ability of an organism to withstand change, can be environmental, as well as genetic. I sought to develop an experimental model to study the relationship of environmental and genetic robustness, as it pertains in natural, not theoretical, systems displaying variability. To accomplish this, a sample of 18 diverse, wild strains of Saccharomyces cerevisiae were selected for study. S. cerevisiae is a strong model organism, as its metabolic network is well-documented in literature, subject to influence by genome and environment, diversified across strain in carbon breadth, and fundamentally similar to other metabolic networks. Single-cell sorting and measurement with optical density were done on mutagenized populations. Differences in survivorship (p-value = 4.7772×10^{-6}) and growth rates between mutant and control populations were analyzed with scientific computing in R. It was demonstrated that the breadth of a sample's variation needs to be wide, for significance within studies on robustness in natural systems to be established. Works to improve the experimental model for studying environmental and genetic robustness, as well as in exploration into the genomics behind natural variation, are ongoing.

Personal Statement

I have always tried my best to be a young woman fueled by her imagination and driven up by her desire to innovate. Through my young life, each book page nimble fingers turned opened a new chamber in the labyrinth of my mind. Each impromptu science experiment or mad technological tinkering was a feather at my back. I found happiness in exploration and in loving those around me, but things changed. I was a 4th grader. My mom was diagnosed with severe lymphatic cancer, and, before my eyes, the wax of my base was melting away. I was a child, and I had to stand by, as my mother battled her way through revolving treatments of chemotherapy and radiation. I felt helpless. The science behind her medicine was a mystery to me. All I knew was her treatments made her ill, and my hope for her began to fall away with the strands of her hair. I thought I was losing my mother and losing my chance to soar.

But, yet again, things changed. Though she was weak, my mother held fast to her life. While I was feeling helpless, my mother was being lifted by a team of Herculean doctors. Behind those doctors, there were hardworking scientists. Their work was swift, silent. It is the work of ingenious scientists and doctors, which produced, for me, the miracle of my mother's continued life. My hope was reborn. The path I was made to travel, this chapter of my personal bildungsroman, connected my passion for innovation with my love for those around me. I want to make the world a better place, as cliché as it sounds. My passion in bettering my part of the universe is a symptom of my hope's renewal. I still hold my imaginative spirit, and I strive to innovate in the names of those I love. Now, I strive specifically to innovate at the intersection between biology and computer science.

However, as childhood faded to young adulthood, the STEM fields were only passing interests and areas of exploration of mine. Like most underclassmen in high school, I had nothing but vague imaginings of what my future would entail. I knew I wanted to make it into a good college. I knew I wanted a very self-driven career. I knew I loved writing, but I did not think I wanted to be a writer. I took time my freshman year to explore what my high school offered, and I fell in love with our *FIRST* Robotics

team. I caught a real bug for technology and engineering. My robotics team helped me recognize my own potential to create. Beyond robotics, I looked up to the seniors on my Academic Quiz Bowl team. I found many of them were members of my school's Science and Technology Research Program. My 9th grade Earth Science teacher would encourage me to attend an informational session on the program. That session had the auditorium abuzz with excited freshman. We were crowded in, as a petite, blonde woman took hold of the microphone in the front. She introduced herself as the program's coordinator. I was stunned by the eloquence and confidence of this educator, Maria Zeitlin. I was stunned by her stories of scientific discovery. More so, I was stunned by the concept of students, only a few years older than myself, achieving national recognition for their original work. Ms. Zeitlin promised her captivated audience that she helped students who were motivated to think big, and thinking big is what I wanted. When I was selected by Ms. Zeitlin to join that Science and Technology Research program, my life was changed.

I always try to search for educational opportunities that meet my ambitions and provide me with likeminded peers and guides. I found shining opportunities consistently in Ms. Zeitlin's research room. I was allowed to thrive in a symposium of my peers under the guidance of a dedicated teacher and mentor. I was allowed to explore my interests using in-house research projects and local science competitions like the Long Island Science Congress. All the while, Ms. Zeitlin instilled in me perseverance and a respect for the scientific method. Biology became an intensifying interest of mine there and through my 10th grade AP Biology class. My sense of awe for the medicine that saved my mother deepened, as I learned the intricacies of life. The power of man to explore the means of his physical existence drew me further and further in. My passion for robotics still lingered, however, seemingly confusing my path. All questions of my earnest academic devotion withstanding, Ms. Zeitlin made science real for me and equipped me with tools to take flight in university level research.

I searched far and wide for mentorship in a university lab before finding success. I reached out to microbiologists, roboticists, and researchers of every discipline in-

between. I wanted research that sparked my imagination. Ms. Zeitlin had taught me that passion is a requirement for true success in research. This fundamental lesson held me back from pursuing a lab for the sake of merely having a lab. My search took months of reaching out to professors without success. I reached a point of nearly giving up, before I stumbled upon the page for the Laufer Center for Physical and Quantitative Biology at SUNY Stony Brook. There, I found the page for the Rest Lab in the Department of Ecology and Evolution. When I read about the work being done in this lab, I knew it would be the right place for me to unleash an unbounded spirit of inquiry. The innovative methods of research in the lab joined cutting-edge technologies, like next-gen sequencing and robots that regulate growth environment, and the classical essence of biology, with investigation of variation and evolution. As if by fate, this would be the lab, where the researchers actually got back to me. After a single meeting with Dr. Joshua Rest, I found my research home for the summer of 2015.

In the lab, I would gain role models, such as my mentor Dr. Rest, postdocs Dr. Christopher Morales and Dr. Dana Opulente, and research support specialist Kash Bandaralage, for the kind of scientist I aspire to be. They guided me through moments where mistakes became revelations. Early in one experimental protocol, a population of the yeast I used as an experimental model died. In the same way I debug code, I stepped through the procedure to isolate where my mistake occurred. I regrettably learned the importance of having enough amino acid in a growth media. Episodes of learning through mistakes, experiential moments, that are reflected upon are irreplaceable. In particular, it is the thinking that matters. Nobel Prize winner Albert Szent-Gyorgyi said, “Discovery consists of looking at the same thing as everyone else and thinking something different.” It was a tenant of my high school research program, one which resonates with me deeply. This was one of many mantras instilled in me by that all-star teacher, Ms. Zeitlin. I learned the importance of looking beyond the journal, the lab bench, and the next deadline in scientific research. One needs a breadth of knowledge of local and world events, for one never knows from where or whom ideas and inspiration will be derived. In the lab, I gained an inclusive, safe space, where

acceptance was modeled and encouraged. Members of the lab would summon forth an atmosphere of sundry ideas whenever we met. My lab exemplified the value of diversity: collaboration. In lab, collaboration is a key component to success. I was able to consult my lab associates to ensure ownership of my experiments, especially of my mistakes. I began to further believe in mindfully powering through obstacles, because the unexpected will always arise to slow one down. Early on in the plate reads for my very first replicate, I noticed clumping occurring within 3 of my strains of yeast. I thought I had erred and contaminated my plates. Through trial and confusion, it became clear that this was an undocumented part of the strain morphologies, which appeared unnoticed in previous experiments. I had done nothing wrong; I discovered something new. I value, more now than ever, having a strong sense of curiosity. All this was sparked by my high school lab experience and the amazing attributes of the budding yeast I studied!

There is a movement of machines and computers to study the universe. It is, at its base, human, and I demand to be a part of it. One cannot dance in shallow sea waves and gain, instantaneously, clarity of the ocean's magnitude, but nor can one gaze into an expansive night sky and understand immediately the composition of the stars. Scientists of old would struggle to find meaning of the universe without technology as a reliable tool, yet goliath data loads plague my contemporaries. We are lost in cyber-enabled space. I aim to be part of the rescue team. Moore's Law states that computational processing power has and will double every two years. As this is the case, it is the unique duty of each class of new scientists to be doubly innovative in how we implement the processing power allowed to us. I believe scientific computing is key.

Computational biology, hereby, fascinates me, and I am glad I took the leap and pursued it as high school research. Now, I search for it in every available avenue of my life. My lab experience soothed the tear I felt between my passions in technology and engineering and biology. My research project showed me that I will not need to sacrifice either of my passions in pursuing a career in biological research. Rather, the combined force of technology and science will have a multiplicative effect on the scope of research

which I can perform. I have had other opportunities to explore these fascinations. Beyond my lab, I was lucky enough to be in a groundbreaking program, through Brookhaven National Labs, to teach scientific computing to promising students. I have learned to never take educational opportunities for granted. With these moments treasured in mind, I hope to be a professor and a scientific researcher in Computational Biology one day. Already, I have experienced the radiance of cross-generational mentorship and collaboration. I imagine passing the torch of investigation's excitement to younger minds will be a part of life I, one day, love. It will be my way to change the world, and I know my journey starts with the following research venture.

Research Introduction

Literature Review

Network biology is an ongoing investigation into the complexes of interactions behind each and every cellular function (Barabási and Oltvai 2004). Computer science and the laws of mathematics have become critical computational tools in understanding network structures in living samples, but maintaining a characterization of living things by their subjectivity to natural change is vital. Nodes are units within a network which connected, linked by pathways. Network biology can be broad in what it describes; biological networks can be as networks of species within ecosystems to the networks of proteins in cells. All biological networks can gain or lose their nodes, experience changes in the property of their nodes, and be affected by external elements. Changes to cellular networks entail changes on biological systems, whether cells, tissues, systems, organisms, species, or ecosystems, via changes to the processes defined by the networks' interactions (Proulx et al. 2005).

Metabolism is the collection of chemical processes required to maintain life in any organism, and network organizations for all organisms' metabolisms have been found to be fundamentally similar Metabolic networks are scale-free networks, which function by design principals of robustness, such as abilities to withstand internal defects, environmental fluctuations, and ecological niches (Jeong et al. 2000). Robustness is generally defined as the ability of a biological system to withstand disturbances. The high tolerance of metabolic networks to environmental perturbations is due to the high connectivity of the nodes within their complexes. If 80% of a scale-free network's nodes are removed at random, the remaining 20% should withhold within a compact, fully-connected cluster, as the removal of numerous small degree nodes is of relative unimportance in a robust system (Barabási and Oltvai 2004). Multiple interconnected pathways exist, within an organism, for metabolism to occur, so, typically in spite of the fluctuation of available metabolites in environments, change is withstood (Soyer and Pfeiffer 2010). This elucidates genetic robustness, the maintenance of phenotypic stability in the presence of mutation and a widespread

property of biological systems (Lehner and Kaneko 2010). A leading hypothesis for the evolution of genetic robustness is that it is a by-product of environmental robustness (de Visser et al. 2003). Environmental heterogeneity, the variety of conditions typical to an environment, explains diversity seen in wild isolates of species, as specialists evolve in environments of static condition, environmental homogeneity, and generalists evolve in more temperamental environments (Kassen 2002).Although the correlation between environmental and genetic robustness should exist across special lines, this hypothesis has not been tested within naturally occurring genotypes (Szöllősi and Derényi 2009). Environmental robustness has been demonstrated as possessing a non-monotonic relationship with evolvability (Draghi et al. 2010) (Fig. 1). This non-monotonic relationship and its implications confound the theoretical correlation of environmental robustness to genetic robustness, as the noisy gene expression typical of genetically robust models is often more responsive and evolvable than that of non-genetically robust models (Lehner and Kaneko 2011). In genetically robust systems, mutations fail to affect vitality, and, as a result, they accumulate. A stock of cryptic genetic variation, available to be revealed in the face of major perturbation, is built up, conferring evolvability (Masel and Siegal 2009). Analysis across of these relationships, across a spectrum of environmental robustness, is called for within a single species.

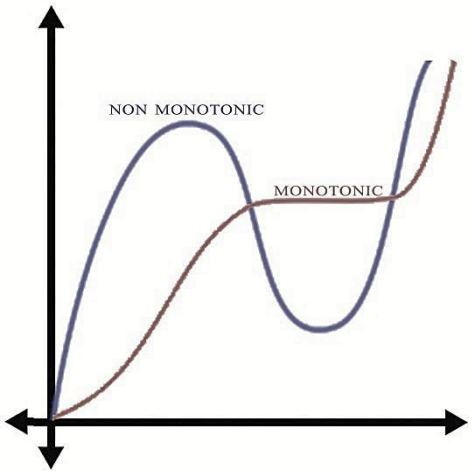

Fig.1. A function is monotonic, if it is either entirely non-increasing or non-decreasing. A monotonic function may plateau, but the direction of its slope may never change.

(Image created by student researcher)

Strains of *Saccharomyces cerevisiae*, a fungi commonly known as budding yeast, present expansive ecological, geographical, clinical and industrial distributions. They are a model for the study of natural diversity as it relates to adaptation in changing environments, environmental robustness (Carreto et al. 2008). Their metabolic networks are directly tied, through the availability of necessary metabolites, to immediate environment. A wide variety of carbon sources beyond glucose can be used to satisfy their metabolic needs (Bergman 2001). Strains that are able to implement a diverse collection of metabolites are considered generalists. Generalist strains are environmentally robust, as they can tolerate growth in highly variable environmental conditions. Within its plethora of metabolically diverse strains, *S. cerevisiae* has high conservation of metabolic and regulatory mechanisms (Sherman 2002). The study of budding yeasts' metabolic networks alongside their genomes hereby provides model framework for investigation into the diverse metabolic networks and their relationships to environmental and genetic robustness. *Saccharomyces cerevisiae* was the first eukaryote to have its genome fully sequenced and has since become a model organism in the field of evolutionary genomics. The genome of *S. cerevisiae* can be manipulated with ease (Sherman 2002). Its underlying gene–function relationships of metabolism have been established to a larger extent than most organisms (Gerlee et al. 2009). Only approximately 19% of budding yeast's genes are essential to its survival in lab conditions (Deustcher et al. 2008). This is because the genotypes of yeast hold hidden genetic variation, in the way of alternative network pathways (de Visser et al. 2003). This availability of alternative metabolic pathways is very influential on genetic robustness. When *Saccharomyces cerevisiae* were tested in complex, rich mediums, alternative pathways were more influential on genetic robustness than CNV or GCV (Deustcher et al. 2008).

Rational

I sought to investigate how generalist strains of *Saccharomyces cerevisiae* withstand mutation compared to specialist strains. I wanted to explore how the genetic robustness of environmentally robust strains compared with that of non-environmentally robust strains. Is there a relationship between environmental and genetic robustness, and, if so, how is that relationship defined? Multiple replicates of a chemical mutagenesis experiment would be carried out for this investigation, though it would be unclear what mutations occurred, until a model with confidence was achieved and genome-wide sequencing carried out. Initially, I hypothesized that the genetic robustness of environmentally robust strains would be greater than that of non-environmentally robustness strains, if alternative pathways, derivative of genetic robustness, underlie environmental robustness. When subjects are mutagenized, decreases in survivorship and growth rates could be expected. As environmental robustness of a strain increases, the likelihood of the decreases in its mutant survivorship and growth rate being significant would decrease.

Methodology

EMS Mutagenesis Preparation

Lab personnel and I altered the protocol initially implemented for mutagenesis from Mabel and Otto 2001. 18 strains of *Saccharomyces cerevisiae* were chosen, on the basis of being wild isolates with unique environmental robustness, previously quantified by an associate within my lab through strain ability to metabolize different carbon sources (Table 1). Each strain was streaked out onto Synthetic Defined Media without Tryptophan (SD-Trp, Sunrise Scientific) agar plates from frozen glycerol stocks and allowed to grow for 48 hours in a Binder BF400 Incubator at 30°C. The strains, frozen in stock, had originally been obtained from the CBS Fungal Biodiversity Centre. Streaked samples were inoculated into 5mL of SD-Trp and allowed to grow in a New Brunswick Scientific Lab Bacterial Tissue Culture Roller Drum, within the 30°C incubator for 12 hours. Samples underwent serial dilutions for 48 hours within SD-Trp, in order to ensure normalization after the freezing process.

Strain	Carbon Breadth	Environmental Breadth
BC 187	8	48
CBS 4054	4	21
CBS 6131	9	48
CBS 6872	4	21
CBS 7764	8	48
DBVPG 1106	10	49
DBVPG 1788	11	–
DBVPG 6044	10	–
DBVPG 6765	10	–
L_1374	9	–
NCYC 110	4	23
SK1	9	31
UWOPS03-461.4	11	–
Y12	10	51
Y55	8	–
YJM 975	11	55
YJM 978	11	51
YJM 981	8	–

Table 1. 18 diploid wild isolates of *Saccharomyces cerevisiae* were selected, on the basis of their wide variation in carbon and environmental breadths.

(Data from Opulente et al. 2013)

EMS Mutagenesis

Cultures were vortexed (Scientific Industries Vortex-Genie 2), and optical density, as a measure of cell concentration, was recorded using a Tecan Infinite® F500 plate reader. Each culture was diluted to a concentration of 7.39×10^7 cells/mL, in approximately 5mL of SD-Trp. Diluted cultures were centrifuged at maximum RPM for 2 minutes at room temperature in an Eppendorf™ Model 5810 Centrifuge. Supernatants were poured off, and each cell pellet was resuspended, while on ice, in 3mL of sterile 1X Phosphate Buffer Saline (1X PBS). 1.5mL of resuspended cells were used as ancestral populations, and 1 .5mL of resuspended cells were to be mutated. Samples designated for mutation were treated with 50uL of the ethyl methanesulfonate (EMS). Trained lab personnel carried out this step, within a BSL II hood, using an Eppendorf™ Combitip. These precautions were taken, as EMS is an alkylating mutagen and a known carcinogen in mammals. At no point during the experiment did I personally handle the EMS. Both cell populations were placed within the 30°C incubator for 1 hour. 40uL of each mutant population was pipetted into 800uL of 5% (w/v) sodium thiosulfate solution, to inactivate the mutagen, while 40uL of each ancestral population was pipetted into 800uL of 1X PBS, to maintain dilution factors. Tubes with 5% (w/v) sodium thiosulfate were set up during incubation, to ensure the quick inactivation of EMS. 500uL of diluted cells were transferred into 500uL of 1X PBS. Samples were brought over, on ice, to a BD FACSAria™ III cell sorter, for a single cell sorting into a 96-well cell culture plate for each strain. Three full replicates of this mutagenesis procedure were carried out, resulting in a total of 240 mutant samples and 48 ancestral samples for each strain.

Plate Set-Ups and Data Collection

There were 18 plates sorted per experimental replicate, with one strain sorted per plate. Strains were randomly assigned to plates in each replicate. Each plate was prepared with 150uL of autoclaved minimal growth media per well. The growth media used was composed of 2X yeast nitrogen base— with ammonium sulfate and without amino acids— at a final concentration of 6.7g/L, D-Glucose at a final concentration of 20g/L, as well as uracil at a final concentration of 20mg/L. In each replicate, ancestral cells were sorted into two randomly designated columns, while mutated cells were sorted into the remainder of columns. Also, two randomly selected wells within every plate were left without cells, in order to serve as controls with blank growth media.

Following cell sorting, cells were allowed to grow overnight in the 30°C incubator. The optical densities of the sorted plates were read in the Tecan Infinite F500 plate reader, at set time increments, through 72 hours— every 2 hours for hours 1-12, every 4 hours for hours 13-24, and every 8 hours for hours 25-72. This timeframe would allow each living sample to reach a saturation point. After the final plate reading of each replicate, glycerol, at a final concentration of 20% (w/v), was added before freezing plates at -80°C.

Initial Results

Data was gathered, via optical density readings, within each of the experimental replicates. It would be analyzed predominately with code, which I was responsible for developing in R (Rv3.2.2). These optical densities were used to determine survivorship and growth curve for each sample. The survivorship of each sample was determined by their final saturation level. Absolute survivorship for each strain was determined by the number of its individuals that survived relative to the total number of its individual cells deposited. Absolute survivorship would be separated out into measures for ancestral samples and mutant samples. For each strain, total ancestral sample size was 48 and mutant sample size was 240. In this initial experiment, there was no clear trend in the differences exhibited between ancestral and mutant survivorships across the 18 strains (Fig 2). Within some strains, absolute mutant survivorship was actually greater than absolute ancestral survivorship, which would be illogical with samples produced by the random point mutations of alkylation. The lack of trend and number of unfounded differences occurring in absolute survivorship hinted at the possibility of a significant failure in mutagenesis procedures. To investigate if mutagenesis occurred,

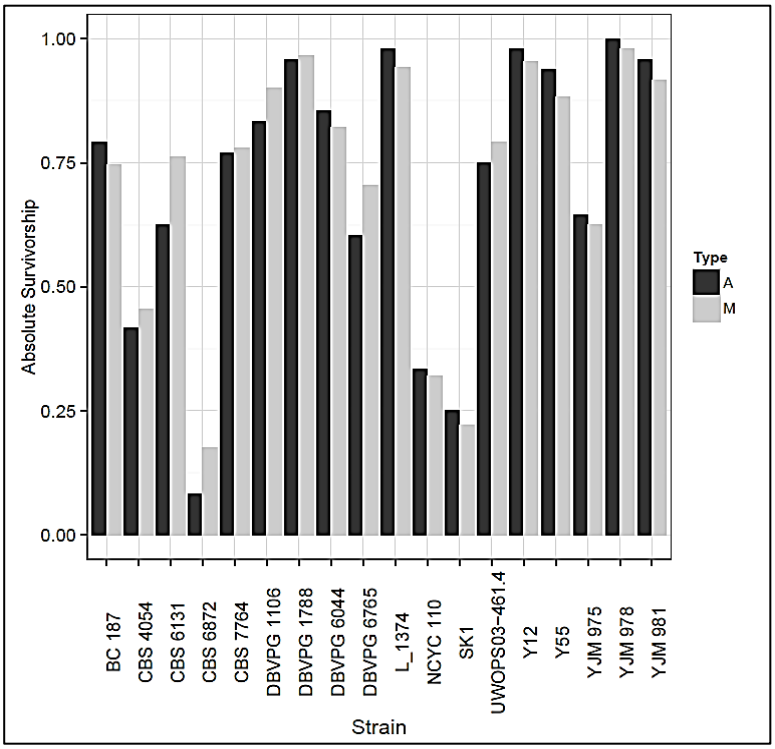

Fig.2. Graph demonstrating absolute survivorship of ancestors and "mutants" across the 18 strains within the initial experiment. Ancestral survivorship is displayed in dark gray; "mutant" survivorship is displayed in light gray.

(Figure produced by student researcher)

treatment with EMS having no effect on survivorship across strains was established as a null hypothesis. Treatment with EMS lowering survivorship across strains was established as an alternative hypothesis, and this alternative hypothesis was rejected (p-value = 0.5007). Mutagenesis did not occur on a significant level, due to some inherent flaw in protocol.

To further investigate the initial experiment, I also analyzed absolute survivorship across each of the experimental replicates carried out (Fig 3). It became clear that there were problems with strains surviving the protocol. 5 strains— CBS 6872, a non-environmentally robust strain, NCYC 110, a non-environmentally robust strain, CBS 6131, a relatively low-environmentally robust strain, SK1, a relatively low-environmentally robust strain, and YJM 975, an environmentally robust strain— experienced total failure of ancestral populations within certain replicates of the experiment The cause of their total failure under the stresses of this protocol remains unclear and is under continued investigation.

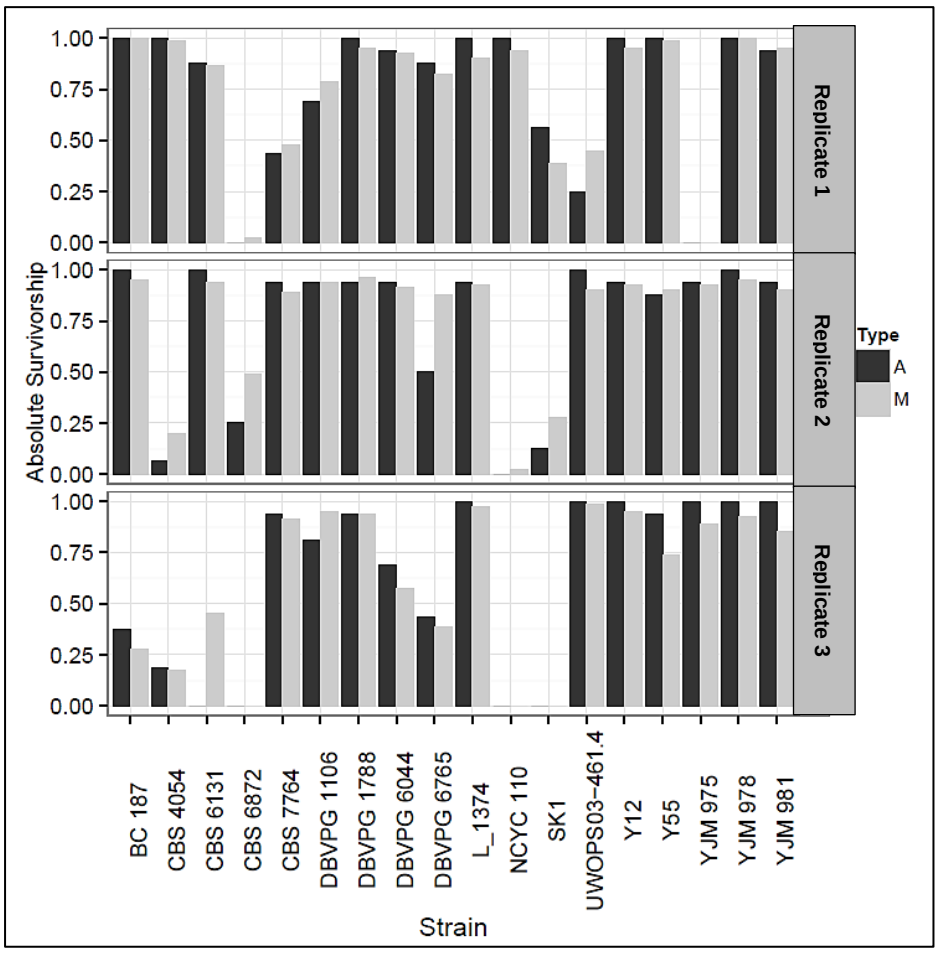

Fig. 3.
Graphs of survivorship of ancestors and mutants across the 18 strains in each replicate of the initial experiment. Ancestral survivorship is displayed in dark gray; mutant survivorship is displayed in light gray.

(Figure produced by student researcher)

Altered Mutagenesis Pilot

Protocol would need to be adapted to ensure successful mutagenesis. Achieving successful mutagenesis with EMS would be the sole focus of a novel pilot. It was realized that how significant the density gradient was between the yeast suspended in PBS and the EMS. In this pilot, to ensure a thorough mix of the samples and mutagen, samples would be inverted following the addition of EMS. All samples would be kept in a spinning drum throughout their hour-long incubation period with EMS. Dilution of samples before the addition of EMS and concentration of EMS implemented would be kept constant from the previous protocol.

Data from the pilot mutagenesis was gathered in the same way as the initial experiment, with regular optical density readings. Differences in absolute survivorship between ancestors to mutants, across 11 surviving strains in this pilot, indicate that mutagenesis successfully occurred (Fig. 4). Treatment with EMS having no effect on survivorship across strains would remain my null hypothesis in investigating absolute survivorship. Treatment with EMS lowering survivorship across strains would remain my alternative hypothesis, and, within the pilot, the alternative hypothesis would be accepted (p-value = 4.7772×10^{-6}). The significance of the difference observed across strains, between ancestral absolute survivorship and mutant absolute survivorship, clearly denotes that treatment with EMS worked within the pilot. Mutagenesis occurred, lowering absolute survivorship for mutants, as was expected. In the pilot mutagenesis, 2 of 3 non-environmentally robust, 4 of 7 low-environmentally robust, and 1 of 8 environmentally robust strains experienced total failure. Protocol now simply requires alteration to address the stress tolerance levels of all strains being used.

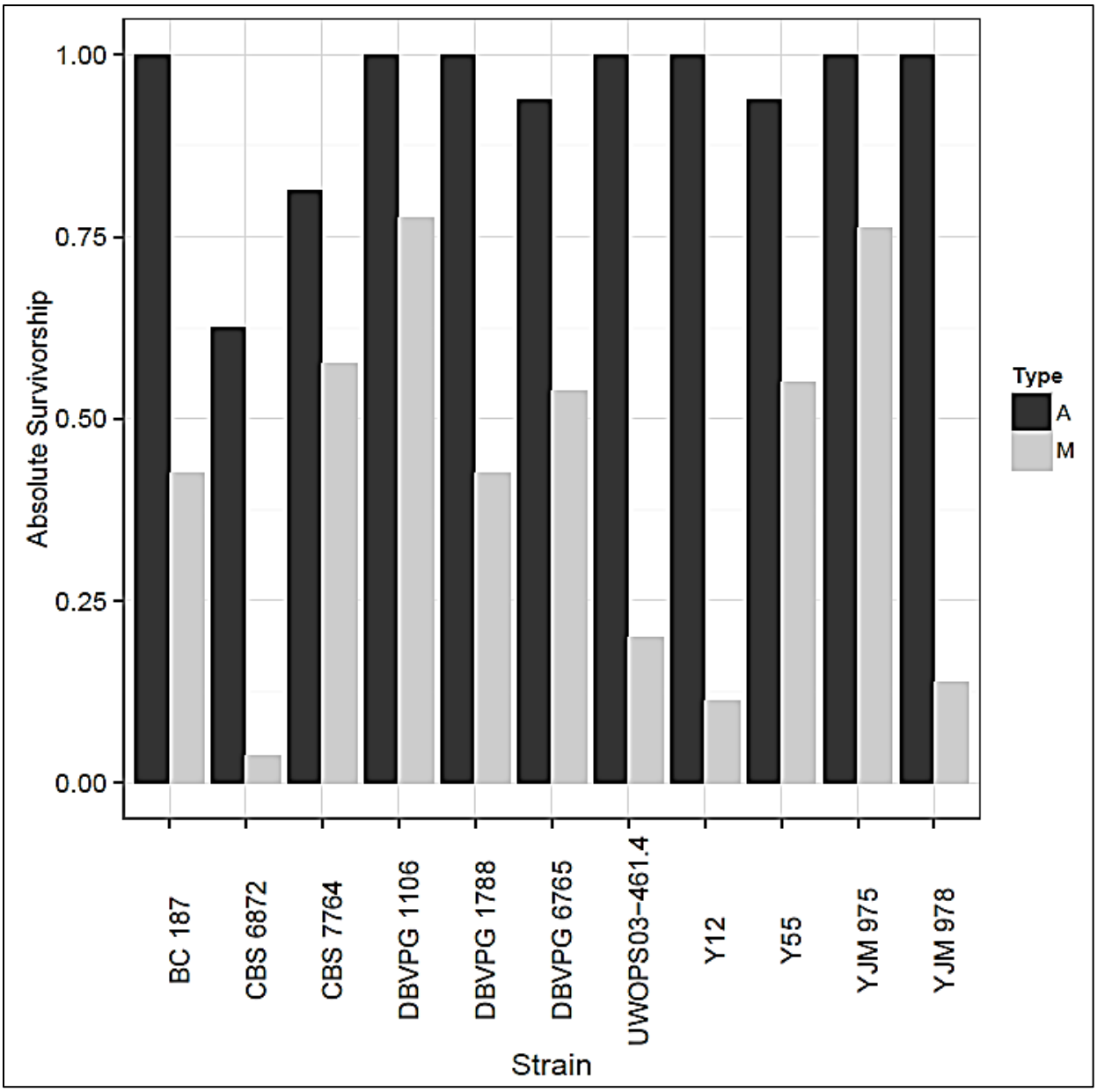

Fig.4. Graph demonstrating absolute survivorship of ancestors and mutants across the 11 surviving strains in the pilot mutagenesis. Ancestral survivorship displayed in dark gray; mutant survivorship displayed in light gray.

(Figure produced by student researcher)

The slope of each sample's growth curve was calculated using the 'grofit'(version 1.1.1) package in R. Slope was defined as: ΔOptical Density/ Time (hours). Mean ancestral and mutant growth rates for each strain were determined for surviving strains within the pilot (Fig. 5). In all strains, with the exception of DBVPG 1106, mean mutant growth rates was less than those of strain ancestors, as was predicted. Within 4 of the strains, the difference in growth rate was significant (Table 2). It may be noted that these 4 strains were all environmentally robust. A possible explanation for this trend may relate to the higher error seen across mean growth rates for non-environmentally and low-environmentally robust strains, as measures of significance are affected by error.

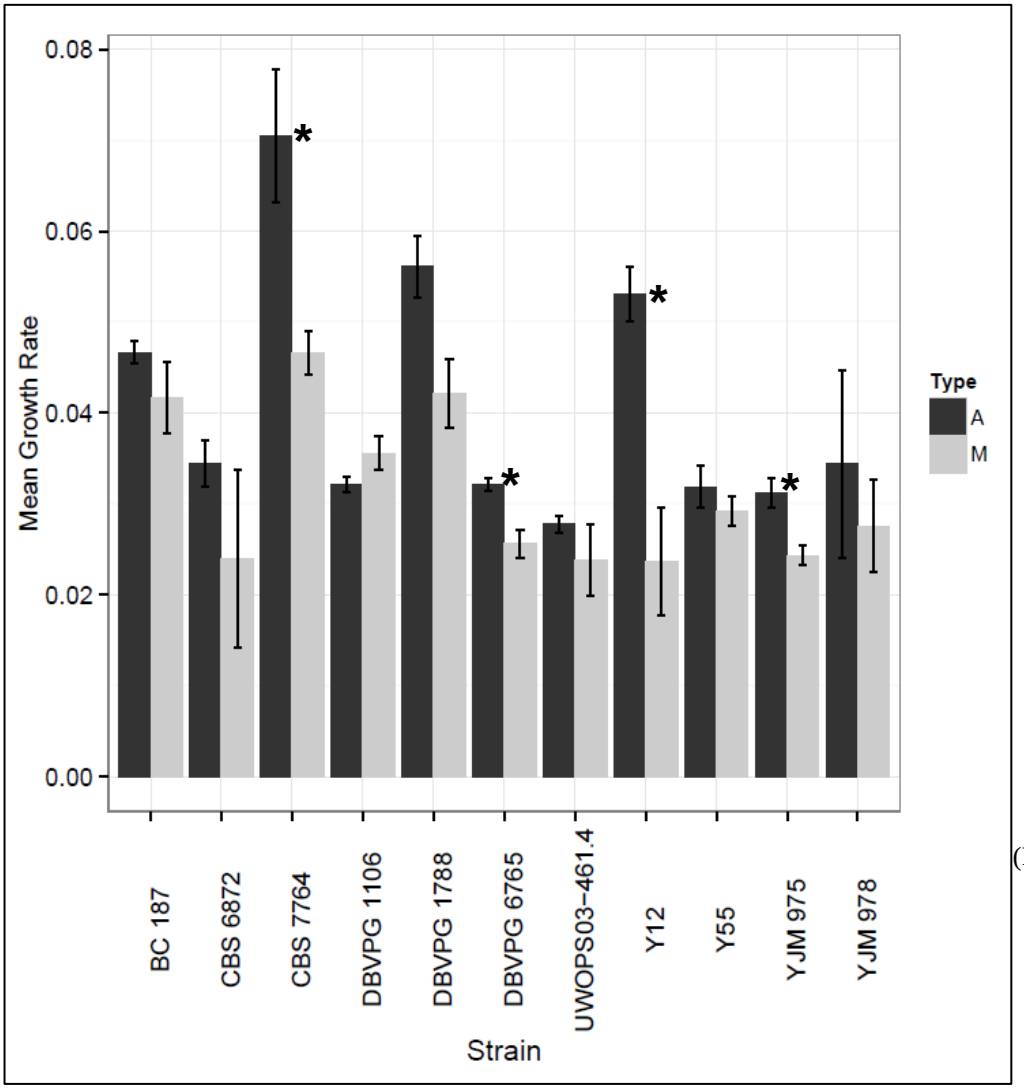

Fig.5. Graph demonstrating mean growth rates across 11 strains within pilot mutagenesis. Ancestral growth is displayed in dark gray; mutant growth is displayed in light gray. Strains, for which the difference in growth rate between ancestors and mutants was significant, are denoted with an asterisk (*).

(Figure produced by student researcher)

Strain	p-value
CBS 7764	**0.000166***
DBVPG 6044	**0.000658***
Y12	**0.000392***
YJM 975	**0.001293***

Table 2. Table containing significant p-values between strains' ancestral and mutant mean growth rates.

(Table produced by student researcher)

This possibility would fit predictions of the model's trends, as non-environmentally robust strains, if non-genetically robust as predicted, would experience a greater decrease in vitality following mutagenesis. This decrease in vitality could manifest, for some samples, in a major decrease in growth rate.

Relative survivorship, defined as absolute survivorship of mutants over ancestors, was used as a measure of genetic robustness, since it was confirmed that mutagenesis with EMS was successful in this pilot. It's correlation to environmental breadth, as a measure of environmental robustness, was observed (Fig. 6). In this pilot mutagenesis, there was a positive correlation between number of carbon and growth environments and relative survivorship, but, with only one replicate of data and a limited breadth of strains, it demonstrated poor confidence and no significance. The model for the number of viable carbon environments and relative survivorship demonstrated greater confidence than that for the number of all viable environments and relative survivorship.

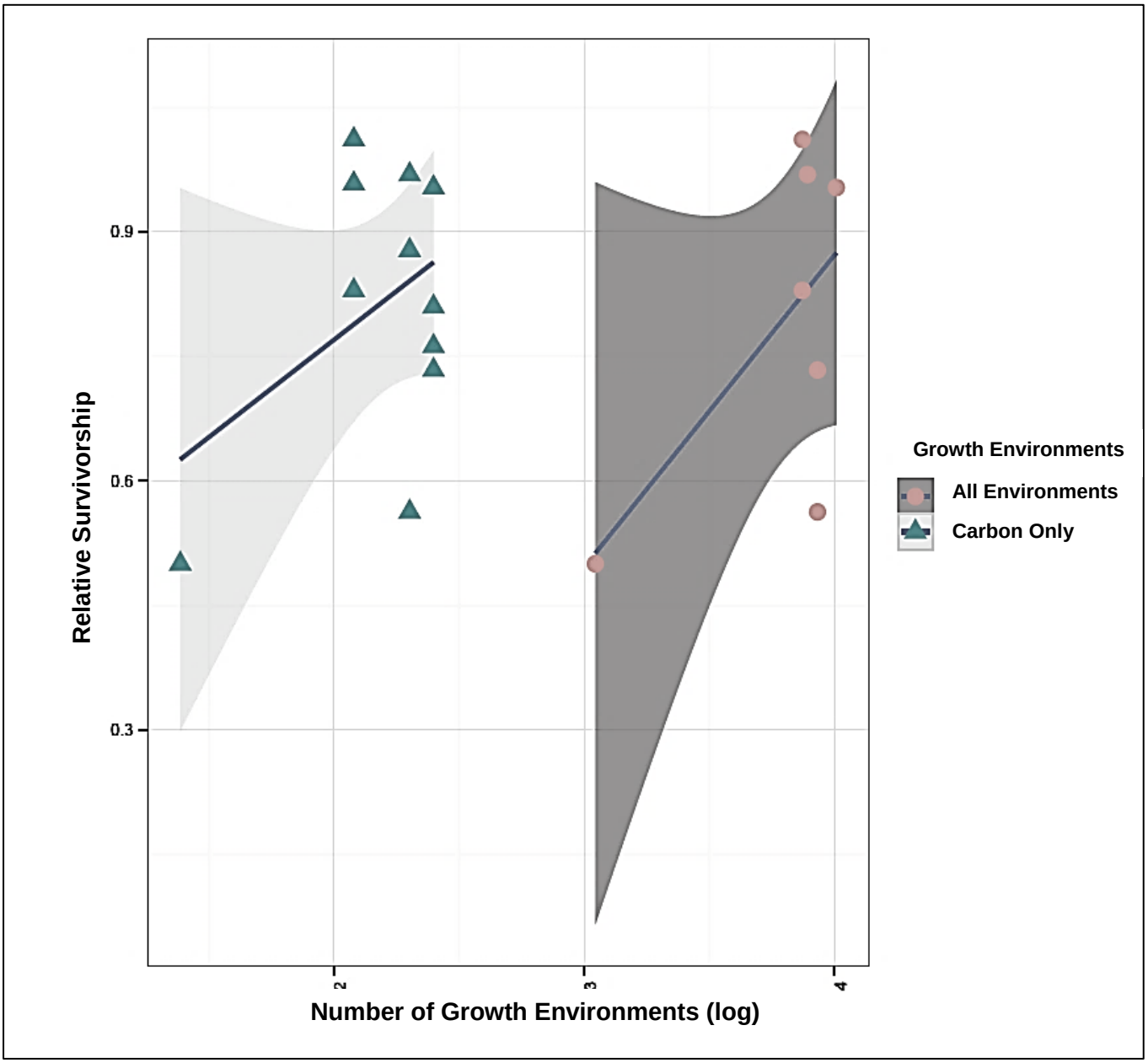

Fig. 6. Graph demonstrating relative survivorship, survivorship of mutants over that of ancestors, as it relates to the number of viable growth environments, expressed in a log scale, across the 11 strains which survived the pilot mutagenesis. . Total number of viable growth environments is displayed in circles with dark gray confidence intervals; total number of viable carbon environments is displayed in triangles with light gray confidence intervals.

(Figure produced by student researcher)

Conclusion

The metabolic network, in its already thorough documentation by scientists and its fundamentality in organisms, is well fit for investigating robustness of networks. Using the metabolic networks of diverse, wild samplings of *Saccharomyces cerevisiae*, there is much to be gained in understanding networks and how they function in biology. Network structures are complex, especially in their degrees of connectivity. A thorough analysis of these complexities is vital to building understanding of molecular, cellular, and special underpinnings. Adaptations and evolution can be better explained with network biology, but applications of network biology span beyond abstract notions of evolution. Within humans, key environmental disturbances uncover complex genetic diseases. The way in which these changes unbridle cryptic genetic variation becomes clearer with comprehensive knowledge of what robustness confers (Gibson 2009). Mutations are responsible for complicating interaction between hosts and pathogens in cases of with infectious disease (Abdulovic et al. 2006). The incredible demonstration of robustness by cancer cells, despite their appearance by stochastic perturbation, is clarified with understanding of genetic robustness in natural systems (Stelling et al. 2004).

Future works are in progress to improve an experimental model for the study of natural variation and genetic robustness. A model is required which accounts for both the intricacies of mutagenesis and the varying tolerance thresholds of strains to artificial environmental stresses. Troubleshooting and resolving potential sources of error will allow for the preservation of my model's environmental breadth. Furthermore, I plan to develop a method of normalizing mutagenized cells based on their position relative replication in the cell cycle, since GC-AT base pair replacement from EMS alkylation occurs with replication (Brown 2002). This will improve the integrity of my model and the integrity of other experiments which implement EMS as a mutagen for *S. cerevisiae*. As the cell cycles of diploids cannot be synchronized, they can, alternatively, be categorized and gated during sorting. I will relate cell size, measured by flow cytometry, to the cell cycle in each strain, after staining samples with DAPI, a

fluorescent stain which binds to chromatin. If I find a correlation between position in cell cycle and cell size, I will be able to gate the mutagenized cells before single cell sorting, to normalize for cell cycle position. After all mutagenesis experiments with EMS are complete, I plan to develop and carry out protocols for other types of mutagenesis, in order to ensure found correlations are consistent across mutagens. Methyl methanesulfonate, another alkylating agent, is less effective, because of DNA repair, at producing large mutant populations than EMS, despite a higher mutation rate, so it would not be a strong choice (Rhaese and Boetker 1972). The potential of protocols using Ultra Violet Light are being investigated, as UV Light has been shown to produce many types of mutation, from frameshifts to base-pair deletions (Miller 1985).

To understand the mechanisms behind genetic robustness, I plan to investigation the results of mutagenesis with genomics. A cascade of novel questions is introduced. For example, how many genes and nucleotides were hit by mutations, on average, in each strain? Within robust strains, do mutations occur in pathways that have known high connectivity or other measures of significant interest? Candidate samples, which will best serve to explain how robust strains handle mutation compared to non-robust strains, will continue to be selected for each from each mutagenesis. Having developed code in R (Rv3.2.2), I have and will select and pool mutant samples whose growth rate is within a set variation from their respective strain's ancestral median. Much work can still be done in improving experimental models for the study of natural variation and robustness, as experimental models must be thorough and exact, as was documented herein, to manage the variation which gives them their strength.

Works Cited

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