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The Effect of High Terperature on Mating: Developmental Buffering of S. cerevisiae to the Environment

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The Effect of High Temperature on Mating: Developmental
Buffering of *S. cerevisiae* to the Environment

A Capstone Project Submitted in Partial Fulfillment of the
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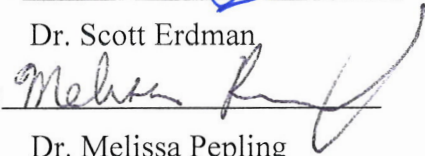and Renée Crown University Honors
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Honors Capstone Project in Biochemistry

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Abstract

In *Saccharomyces cerevisiae*, cellular polarization is an essential structural and functional aspect of growth and development. It is responsible for yielding and maintaining cellular asymmetry, and allows for cells to function. Mating in *S. cerevisiae* is a process that incorporates cell-to-cell signaling, signal transduction, cellular polarization, plasmogamy, karyogamy, and many other cellular processes. Each of these steps is mediated by a myriad of signaling proteins that are involved in a signaling cascade that is regulated by both extracellular and intracellular signals. Much is known about the mating process and pathway in *S. cerevisiae*. However, this project aimed to target that pathway and determine how much it is developmentally buffered to its environment.

The environment that *S. cerevisiae* most successfully grows and mates in is an optimal temperature of 30°C. In this project, it was hypothesized that elevating the temperature from the optimal 30°C up to between 39°C to 41°C would inhibit mating. This is an environmental stress that is easily applied in a controlled manner and that may allow for weaknesses in the mating process under such conditions to be exploited for their identification genetically. Both the ability of the cells to mate and their ability to form mating projections at elevated temperatures were studied. It was determined that the strains used are not capable of mating at 41°C and that polarization in the form of mating projections is greatly reduced at 41°C.

Executive Summary

My project involves several aspects of cellular polarization observed in the organism *Saccharomyces cerevisiae*. To put it very generally, cell polarity is the culmination of all of the properties of the cell that allows one end to be different from the other. In *S. cerevisiae*, polarization becomes prominent during mating and budding, both of which are aspects of yeast's cellular cycle. My Capstone project centers around the effect that high temperatures have on the cellular processes behind polarization in *S. cerevisiae.*

The core theory that is the driving force behind this project was that of the evolutionary theory of canalization. First presented by Waddington in 1942, this theory suggests that organisms are able to buffer themselves to perturbations in their environments without impairing the processes that allow them to live and grow. This project is interested in the upper limits of the ability of *S. cerevisiae* to buffer against the environmental stress of an elevated temperature. Two questions that it considers are (1) at what temperature is yeast no longer able to successfully mate and (2) which step(s) in the process are affected?

Many of the methods used are possible due to the nature of my model organism. *S. cerevisiae* is a single celled, eukaryotic fungi with a fully sequenced genome (its entire DNA sequence has been determined). Most know *S. cerevisiae* by the more common name of baker's yeast, which is used to make fermented beverages and bread. As a eukaryotic cell, it has many similarities to human cells (for example cell cycle and cell division, among others). Its fully sequenced genome allows for "knockout collections" to be formed. In a knockout collection, there are strains that have particular genes removed. This provides the ability to examine what phenotypic effect those particular genes have on the cell by showing the phenotype when the gene is absent. There are also many mutants that are auxotrophic. That means that the strain is

not able to make an essential nutrient necessary for growth on its own. This is useful for screening strains to determine if they are wild type (most commonly found in nature) or the mutant type.

The aspect of cellular polarization that I am currently studying is the process of mating. As with most fungi, baker's yeast can reproduce asexually (budding) or sexually, through mating. The two mating types (essentially "sexes") are MATa and MATα. The mating process is initiated when a cell senses the mating pheromones (a special type of secreted chemicals) of the opposite mating type. This is a feedback mechanism in which, once a cell senses the pheromone, it in turn releases more pheromone itself. However, baker's yeast cells are non-motile. Therefore, in order to contact the other cell, they must form what is referred to as a "mating projection" towards the other through chemotaxis. Chemotaxis is the process by which an organism "moves" towards a chemical stimulus by changing its morphology. These mating projections are regions of pronounced polarization. In mating, the cytoskeleton is rearranged to form the projection, the cells fuse (involving even more rearrangement), and a new cell with two sets of genes (a diploid) is formed. This process proceeds with the highest efficiency at 30°C. My research involves finding the temperature threshold at which the cells are no longer able to mate.

In baker's yeast, polarization is also seen in the process of budding. Budding occurs when a haploid (a cell with a single set of genes) reproduces asexually to form another, almost identical daughter cell. This happens through a process where a small bud forms on the parent cell. The bud will grow until it is large enough to separate from the parent cell. At this time, they will break apart and become two cells. In order for this progression to happen, the entire cytoskeleton (the network of protein filaments that supports the cell's shape and structure) must be rearranged. As in mating, this process usually proceeds efficiently at approximately 30°C,

which is the temperature at which the cellular mechanisms involved with budding prefer to function. High temperatures are expected to affect this process.

Over the course of my project, I have used several methods to accomplish my research. A few examples of these methods are tetrad dissecting and replica plating, among others. I have also recently developed a procedure to test the effect that high temperatures have on yeast cell's ability to mate. This procedure involves producing cultures of cells of each mating type at several concentrations and combining them in complex liquid media (containing all compounds necessary for growth) at both optimal and increased temperatures. The strains I am using are auxotrophs. For example, on their own, the MAT a strain cannot make the amino acid histidine and the MAT α cell cannot make the amino acid tryptophan. These individual strains cannot grow on media that lacks these amino acids. Therefore, if the mating has occurred properly, the diploid cells produced from these will be able to grow on dropout media lacking both histidine and tryptophan because, once their genes have combined, they will be able to produce both of the amino acids that are not in the plate. This allows me to determine whether or not cells have been able to mate at increased temperatures.

In addition to this method, I have also been observing the effects of temperature on polarization by growing cultures of MAT a cells and adding varying concentrations of α factor (the pheromone excreted by MAT α cells that invokes the formation of the mating projection). These cells can be fixed (killed and preserved to maintain their morphology) and observed under a microscope for their polarization state.

This project is important to the field of cellular biology in many ways. The overall, future goal of a project such as this one is to push the boundaries of the mating process in these cells in order to determine which aspects of said process are affected. This can be shown in several

cellular different processes of mating. The steps that a baker's yeast cell goes through to mate and fuse is replicated to various degrees across a wide range of organisms, humans included. Firstly, mating involves extensive cell-to-cell signaling, as cells need to be able to communicate in order to survive. Hand in hand with cell signaling is the ability of neighboring cells to recognize those signals and respond to them in an appropriate manner. Signal recognition plays a role in almost all cells, but it is perhaps most important when it comes to neurological signals between neurons in our bodies. It is possible that the high heat the cells in my experiments are exposed to are no longer able to send, receive, and respond to signals correctly. This project is also important in terms of reproduction. When two yeast cells mate, they undergo both cytoplasmic and nuclear fusion (karyogamy). This is seen in humans (and many other species) through the fertilization of a female egg cell by a male sperm cell. Overall, the work that I am doing will provide the framework for finding new components in these processes, reevaluating those already known, and expanding on the pool of knowledge about *S. cerevisiae*.

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Acknowledgements

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Introduction

Cellular polarization in *Saccaromyces cerevisiae*, as in many other cell types, is an essential structural and functional aspect of growth and development. Cellular polarization occurs as a response to extra and intracellular signals, and it is responsible for yielding and maintaining both a chemical gradient and structural cellular asymmetry. The morphological changes that occur allow for specific tasks to be performed. For example cell division, differentiation, growth, among others all rely upon polarity in various ways. These changes are a result of many different pathways within the cell, each of which work together to allow individual cells to perform specific functions.

In *Saccharomyces cerevisiae*, polarization is greatly involved in the processes of budding and mating. This project focuses on the mating system. Mating in *S. cerevisiae* occurs between MATa and MATα haploid cells, which are two different cell types. These cells produce mating pheromones (a factor and α factor, respectively), which are secreted from the cell and will diffuse to other cells (McKinney et. al., 1993). This begins the chain of events that is the mating pheromone response signaling cascade that serves to differentiate mating cells.

Upon receiving the pheromone signal, a cell will begin to grow in the direction of the extracellular pheromone. As yeast cells are nonmotile, this represents a form of polarized cell growth through chemotaxis (Arkowitz, 2009). This process is primarily regulated by Cdc42, which is a GTPase in the Rho family of proteins (Merlini et. al., 2013). Cdc42 has been shown to be a key player in polarization and mating. In 1995, Simon et. al. determined that when Cdc42 is mutated cells are not able to mate. One of the reasons for this can be explained when the relationship between Cdc42 and the actin cytoskeleton is examined. In yeast, actin is responsible for many functions, such as directing growth, transporting secretory vesicles, and assisting in the

separation of a budding daughter cell from the mother cell. Cdc42 plays an essential role because it is responsible for organizing the actin cytoskeleton and influences the cascade that controls the mating process.

Once contact has been made between two mating partners, they will adhere to each other due to components present in the lipid bilayer and cell walls (Erdman et. al., 1998). When mating is successful, one MATa and one MATα cell will undergo plasmogamy and karyogamy to produce one diploid MATa/MATα cell. In comparison to the mechanisms of polarization themselves, these two processes are not very well understood, but they have many broad implications in the fields of fertilization and development (Merlini et. al. 2013). It is understood that fusion must be very highly regulated because if it does not occur at the correct stage for both cells, one or both of them are likely to die due to lysis. There are many components involved in cell and nuclear fusion, some designated specifically for the task (cell-fusion machinery) and others that are also related to polarization. For example, Cdc42 (as previously discussed) and actin are thought to be involved with fusion. Karyogamy, also known as nuclear fusion, occurs a short time after cytoplasmic fusion is complete (Gibeaux et.al., 2013). It is also carried out in large part by cytoskeletal components (microtubules and their motor proteins dynenin and kinesin). After cytoplasmic and nuclear fusion steps are finished, the mating process is complete.
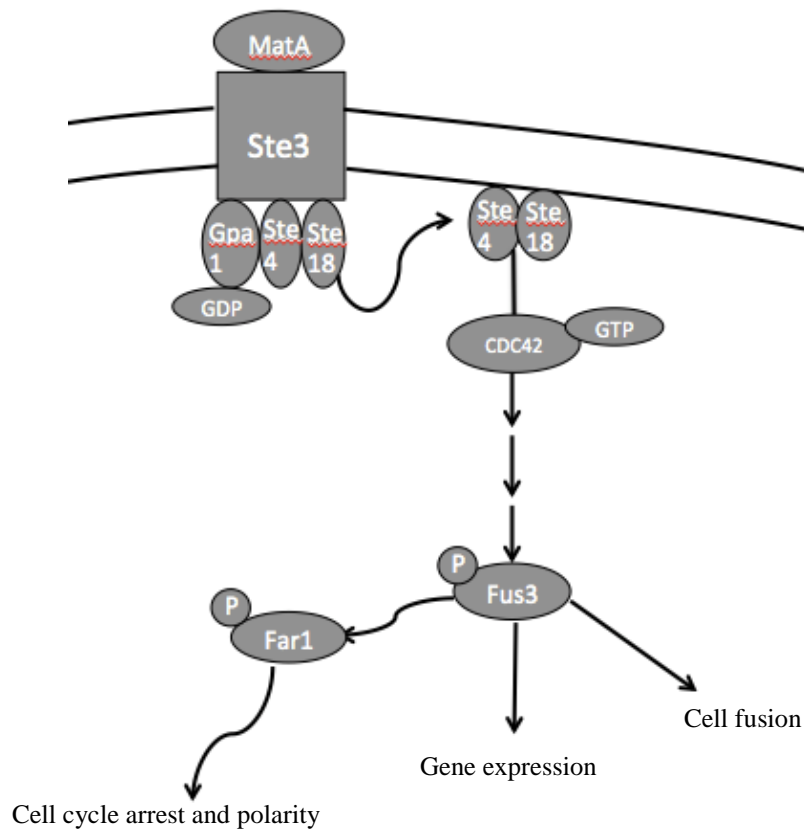
Figure 1. A simplified diagram of the yeast mating response signaling cascade. Adapted from Quiagen.

All of these processes from pheromone secretion to nuclear fusion are, in part, initiated and

regulated by the yeast mating pheromone response pathway. The yeast mating pheromone

response pathway is stimulated by mating pheromone peptides secreted by neighboring cells.

After a cell has received the extracellular signal in the form of the mating pheromone, the rest of

the pathway is an intracellular signaling process (Bardwell, 2005). Secreted pheromones are first

received by the G-protein-coupled pheromone receptors on the surfaces of cells of the opposite

mating type **(Figure 1)**. When these receptors are stimulated, they cause the dissociation of the

Gα protein complex from the G$\beta\gamma$ heterodimer. This invokes the signaling cascade involving

proteins such as Cdc42, Bem1, and the Ste proteins through the the MAP kinase, Fus3. Fus3 is

an important part of this process due to the fact that it plays a role in several processes in the mating pathway.

The mating signaling pathway results in polarized cell growth towards the mating partner and the mating pheromone the opposite partner is secreting, arrest of the cell cycle at the G_1 phase, and amplified expression of the genes that are required for the mating process (van Drogen, 2001). The arrest of the cell in the G_1 phase is very specific. G_1 is the phase where a cell decides if division is the best course of action, so if progression through the G_1 phase occurs, the cell must go into DNA replication (S) and mitosis (G_2 and M) (McKinney and Cross, 1995). Once the yeast pheromone mating response pathway has been triggered, Far1 is phosphorylated by Fus3. Far1, a key protein, plays a critical role in ensuring that a cell that has found a potential mating partner does not enter mitosis. This process occurs in a cycle. For example, if a cell is in the S phase when it senses mating pheromone, the cell will continue through the division process and divide until the two new cells reach G_1, at which point their cell cycles will be arrested (Pope and Pryciak, 2013).

The basis of this project surrounds many steps of the mating process that have just been described. As seen with the signaling cascade, it is a process that has been widely studied and is well understood. This project is the beginnings of research that can be done on the mating pathway. In cell biology, the first step in making discoveries is often taking a well understood pathway and using a method (in this case temperature) to find a weakness in that pathway. Once the pathway fails, that failure can be exploited through genetic screens to find which genes and proteins are responsible for failure. The goal of this project is overall to determine the cutoff temperature at which the mating process is inhibited in *S. cerevisiae*. From this discussion comes the question of which part, if not all of the process is being affected? In this project, several

experiments have been performed to examine specific steps in the mating process and their reactions to high temperatures.

A topic in biology that must be considered when looking into research involving developmental pathways and their buffering to the enviromnent is canalization. Canalization is an evolutionary theory first presented by Waddington that is concerned with the "constancy of the wild type" irrespective of the organism's genotype or environmental factors. As Waddington states in his 1942 paper in *Nature*, "The constancy of the wild type must be taken as evidence of the buffering of the genotype against minor variations not only in the environment in which the animals developed but also in the genetic make-up" (Waddington, 1942). This has some relevance to the concept of the inheritance of acquired characteristics in the fact that certain characteristics will be selected if they are favorable. An optimum developmental path is formed, and the result is a fixed response without an apparent genetic influence required. It allows for changes in phenotype that will not affect an organism's developmental progress towards a certain goal. This is related to the project at hand in the sense that the optimum growth temperature of *S. cerevisiae* at 30°C is a form of the canalization effect. This project is attempting to explore the upper boundaries of the temperature range at which *S. cerevisiae* can mate. This will allow the point where canalization is no longer possible due to too much environmental strain to be determined.

To perform this study, two different strain backgrounds of wildtype *S. cerevisiae* were used to carry out a series of experiments. The first objective was to determine the temperature at which the mating process did not occur. This discovery set the framework for the rest of the experiments. Following this, the second objective was to look more closely at the polarized cell growth towards mating pheromone to determine if high temperatures affect this part of the

process. The next step in this project would involve the cell cycle arrest portion of the mating

process being studied at a higher temperature to find if cells will properly cell cycle arrest when

exposed to high temperatures.

Materials and Methods

Strain Background. YSE430 MAT a met15 LYS2 and YSE431 MAT α MET15 lys2 are strains used in this experiment. YSE21 MAT a his3 TRP1 and YSE22 MAT α HIS3 trp1 were also used. All strains are S228c derivations. Therefore, the selection plate for YSE430 and YSE431 mating is met-lys- and the selection plate for YSE21 and YSE22 is his-trp-. Any growth on the dropout selection plates will indicate that mating has occurred.

Mating Procedure. In order to determine how high temperatures affect the ability of *S. cerevisiae* to mate at higher temperatures, a procedure was created that would allow for a qualitative determination of mating potential. Thirty degrees Celsius is the optimal temperature for mating, so the first high temperature tested was 39°C. Each strain was grown in a test tube in 4mL of YPD at 30°C overnight. Tubes were placed on a rotator to ensure continuous mixing so that cells did not become concentrated. At the same time, an incubator was set to the desired temperature of 39°C and a Styrofoam holding container was placed into the incubator to ensure that it would also be at the desired temperature.

The following day, a BioRad Smart Spec spectrophotometer was used to test the optical density (OD) of the cultures. Using the same bottle of YPD, the solutions were diluted until a 1mL culture at approximately 1.0 OD was obtained. The wavelength used for the reading was 600 nm.

This experiment was done using a serial dilution. The highest concentration was 1.0 OD, followed by 0.1 OD, 0.01 OD and 0.001 OD. Both MAT a and MAT α dilutions were made in test tubes using YPD to dilute the cultures. For both the experimental and control experiments 4 Corning 15 mL centrifuge tubes were pre-labeled. The tubes were labeled with the MAT a strain

number and the concentration. The MAT a strain dilutions were added to the appropriate conical tubes.

To begin the reactions, the 1mL dilutions of the 430 MAT α strains were added to the conical tubes containing 1 mL of MAT a and 1 mL of YPD. The tubes were vortexed and immediately moved to the 30°C (control) incubator and the 39°C (experimental) incubator for 4 hours. No centrifugation was done, and the cells were allowed to fall out of solution at a slower rate over time. After the cells began to incubate, two his-trp- plates and two YPD plates were labeled and one of each was put into the two different temperature incubators so that they would be at the appropriate temperatures.

After 4 hours, the tubes were removed from the incubators and put on ice so as to ensure no further mating occurred. The tubes were spun down in a Sorval clinical centrifuge at low speed for 1 minute, and the supernatant was removed. The cells were resuspended in 500 µL of sterile deionized water. 5 µL of each of the dilutions was dotted in a quadrant on each of the 4 plates (**Figure 2**). The plates were grown overnight at 30°C and 39°C, respectively, and mating success was observed. This procedure was performed for matings involving both strain backgrounds at 30°C, 39°C, and 41°C. The results were imaged with a Kodak camera.
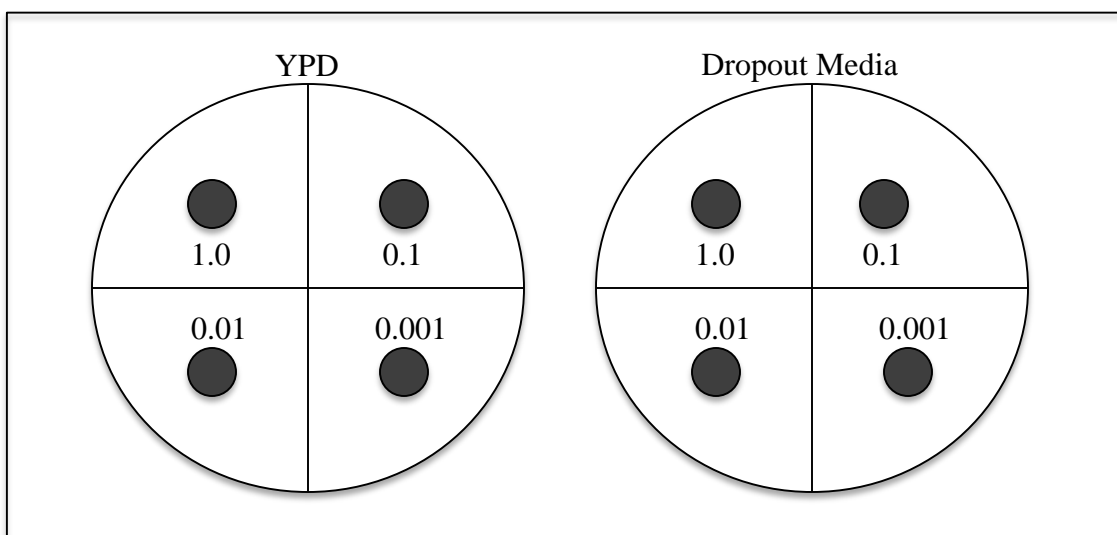
Figure 2. Diagram of mating proceedure plates. Each plate was divided into quadrants and 5μL of each concentration was dotted onto the plate with a micropipetter.

Alpha factor treatment. The next aspect of the mating cycle that was tested was the ability of

MAT a cells to polarize towards alpha factor. This experiment was set up by first growing 1 mL

cultures of the two MAT a strains (430 and 21) in test tubes overnight at the optimal growing

temperature of 30°C. The next day, the BioRad Smart Spec spectrophotometer was used at a

wavelength of 600 nm to determine the OD and dilute the solutions to an OD of approximately

0.3. A low concentration was required for this experiment because a low concentration of alpha

factor was added and its effects would be difficult to observe in a high concentration of cells.

 One mL of the diluted culture was placed in a test tube, and 1/10 volume (or 1 μL) of α factor

from a stock solution of 5μg/μL was added and the tubes were vortexed. Based on the previous

experiments, the temperature the experimental cultures were placed at was 41°C. One hour later,

the tubes were removed from the control 30°C incubator and the experimental 41°C incubator.

In order for the cells to be observed under the microscope for evidence of pheromone

pathway signaling develoment, they had to be fixed in the state they were in after 1 hour of

exposure to the temperatures. Therefore, 100 μL of 37% formaldehyde was added to each of the

1 mL cultures for 45 minutes. After that time, the cultures were moved into 1.5 mL Eppendorf

tubes and centrifuged using an Eppendorf 5424 centrifuge at 6000 RPM for 1 minute. The

supernatant was drawn off, and the cells were resuspended in 500 μL of a 1:1 mixture of 2M

sorbitol and 1x PBS. At this point, they were stored in a 4°C refrigerator.

To quantify how many cells were polarized, approximately 0.9 μL of the fixed cells were

dotted onto a slide and covered with a 15mm x 15mm glass coverslip. A Nikon TE 300

microscple was used to observe the cells at 1000x magnification and to determine the amount

that were polarized, partially polarized, or non polarized. The rubric for this determination can be

seen in **Figure 3**. Cells that did not appear to have a mating projection but were also not entirely

round were categorized as partially polarized.

Polarized   **Nonpolarized**
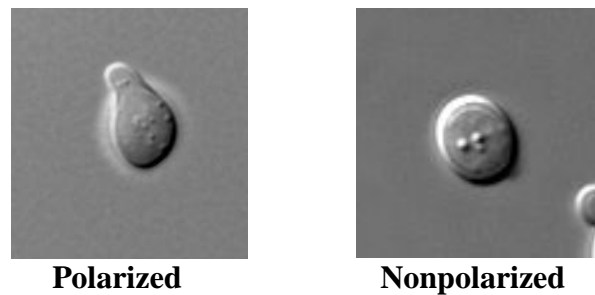
Figure 3. Examples of polarized and nonpolarized cells.
Adapted from Nash (2013) and Held (2010).

Results

Mating is dramatically reduced between YSE430 MATa met15 LYS2 and YSE431 MATα MET15 lys2 at 41°C. The mating experiment procedure was first performed between YSE430 MAT a met15 LYS2 and YSE431 MAT α MET15 lys2 at 39°C. This temperature was chosen due to the fact that it is high enough above the optimum temperature 30°C and would be expected to be outside of the "canal" and, therefore, unable to withstand the environmental pressure. It was performed at four different cell densities in order to maximize the chances of observing an effect of temperature. **Figure 4A** and **Figure 4B** show the results of both the control at 30°C and the experimental 39°C matings. As can be seen in the control **(Figure 4A)**, mating is clear at all four mating densities employed. This is expected, as 30°C is the optimal mating temperature. When considering the experimental, mating has occurred at all four mating densities, but considerably (although not quantifiably) less at a mating density of 0.001. As these results did not define a conclusive cut off point for mating capability, it was clear that it would be useful to examine the results at a higher density. The temperature was raised to 41°C, and the results of this experimental factor can be seen in **Figure 4C**. **Figure 4A** can still be used as a control. However, in this experiment, another control was introduced. As seen in **Figure 4D** the matings were also plated on YPD media to ensure that vegetative growth could normally occur at the higher temperatures. This was done so that, if the presence of cells was not detected on the dropout plate, it could undoubtedly be contributed to an inability to mate rather than an inability to grow vegetatively. The results of this experiment were more conclusive, and it was determined that the success of mating was dramatically reduced between YSE430 and YSE431 at 41°C.
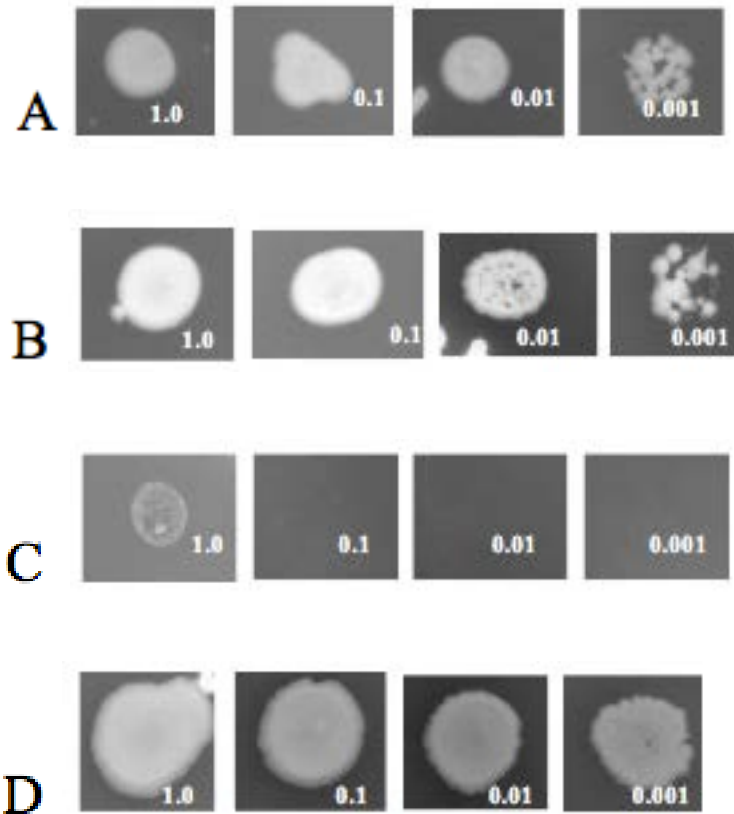
Figure 4. YSE430 and YSE431 results. (A) YSE430 and YSE431 on met-lys- media at 30°C. Control used to ensure that mating has occurred between the two strains under the optimum temperature. **(B)** YSE430 and YSE431 on met-lys- media at 39°C. Mating occurred at all densities. **(C)** YSE430 and YSE431 on met-lys- media at 41°C. As seen in 1.0, mating only occurred at the highest density. This indicates a dramatic reduction in the success of mating at 41°C. **(D)** YSE430 and YSE431 on YPD media at 41°C. Control used to ensure that growth can occur at elevated temperature. Therefore, when growth does not occur, this provides proof that it is mating that can not happen, not vegetative growth.

Mating is not successful between YSE21 MATa his3 TRP1 and YSE22 MATα HIS3 trp1 at 39°C. The second half of this experiment followed the same procedure using the YSE21 MATa his3 TRP1 and YSE22 MATα HIS3 trp1 strains. This strain background is different from that of YSE430 and YSE431 and it was hypothesized that these strains might be more temperature sensitive based on previous observations. Mating was first attempted at 41°C and then the lower temperature of 39°C was tested from there. **Figure 5A** displays the control that was performed on dropout media at 30°C to ensure that mating was possible. **Figure 5B** shows the experimental results of the YSE21 and YSE22 mating on his-trp- media at 41°C, while **Figure 5C** shows the results of the same mating on YPD media at 41°C. As expected, mating was not successful at 41°C. This was expected because it was previously thought that the YSE21 and YSE22 strains would be more sensitive to temperature changes than YSE430 and YSE431. Therefore, if the latter could not mate at this elevated temperature, the prediction proved true.   The next step was to lower the temperature to 39°C to determine if temperature still strongly affected the success of mating in YSE21 and YSE22 strains. As seen in **Figure 5D** the mating procedure was done at 39°C on the experimental dropout plate. As seen at 41°C, mating was greatly inhibited at 39°C in the YSE21 and YSE22 strain. It was also shown through the 41°C control that vegetative growth was possible at 39°C, as growth would not be able to occur at 41°C and 30°C, but not 39°C.
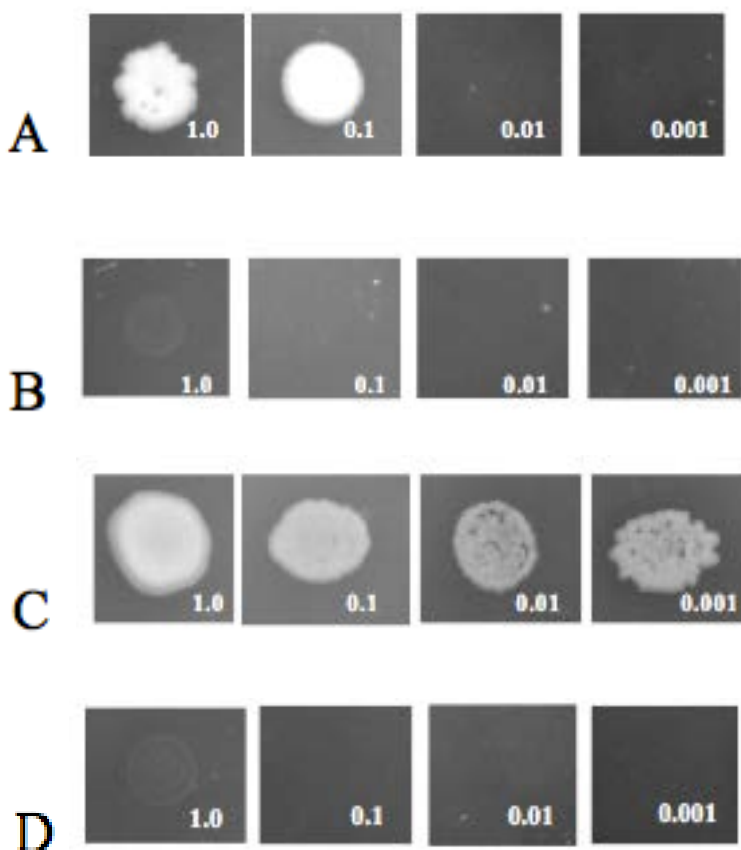
Figure 5. **YSE21 and YSE22 results.** **(A)** YSE21 and YSE22 on his-trp- at 30°C. In this control, it was expected that mating would occur at all densities. However, it is still seen that mating is possible between YSE21 and YSE22 at higher concentrations. **(B)** YSE21 and YSE22 on his-trp- at 41°C. The slight spot that can be seen at 1.0 is the cells that were put onto the plate. They have not mated and are not growing on the dropout media. **(C)** YSE21 and YSE22 on YPD at 41°C. These results show that vegetative growth is possible at 41°C, validating the results of **Figure 4B**. **(D)** YSE21 and YSE22 on his-trp- at 39°C. These results show that, like the 41°C experiment in **Figure 4B**, mating was not successful at 39°C.

Mating projections form less frequently at elevated temperatures. The

α □□□□□□□□□□□□□□□□□□ results showed quite clearly that mating projection formation

is greatly affected by a temperature of 41°C. For each of these experiments, the procedure was

repeated three times. A total of 330 cells was counted to obtain each set of data. **Table 1** and

Table 2 showsthe results of these data collections. **Figure 6** and **Figure 7** show two histograms

that allow for visualization of the results. The results indicate that, though polarization occurred

at an elevated temperature of 41°C, the process is significantly impaired among both strains that

were tested.

YSE430		**Polarized**	**Partially Polarized**	**Nonpolarized**
30°C	**Test 1**	125	54	145
	Test 2	150	53	127
	Test 3	131	81	118
41°C	**Test 1**	53	73	204
	Test 2	47	78	205
	Test 3	48	72	210

Table 1. α Factor Induced Polarization of YSE430. Shown here are the numbers of polarized,
partially polarized, and nonpolarized cells that were observed.

YSE21		**Polarized**	**Partially Polarized**	**Nonpolarized**
30°C	**Test 1**	128	84	118
	Test 2	133	68	129
	Test 3	120	77	133
41°C	**Test 1**	41	81	208
	Test 2	43	93	194
	Test 3	46	79	205

Table 2. α Factor Induced Polarization of YSE21. Shown here are the numbers of polarized,
partially polarized, and nonpolarized cells that were observed.

Figure 6. YSE430 results. The control is shown on the left in orange and the experimental is shown on the right in blue.
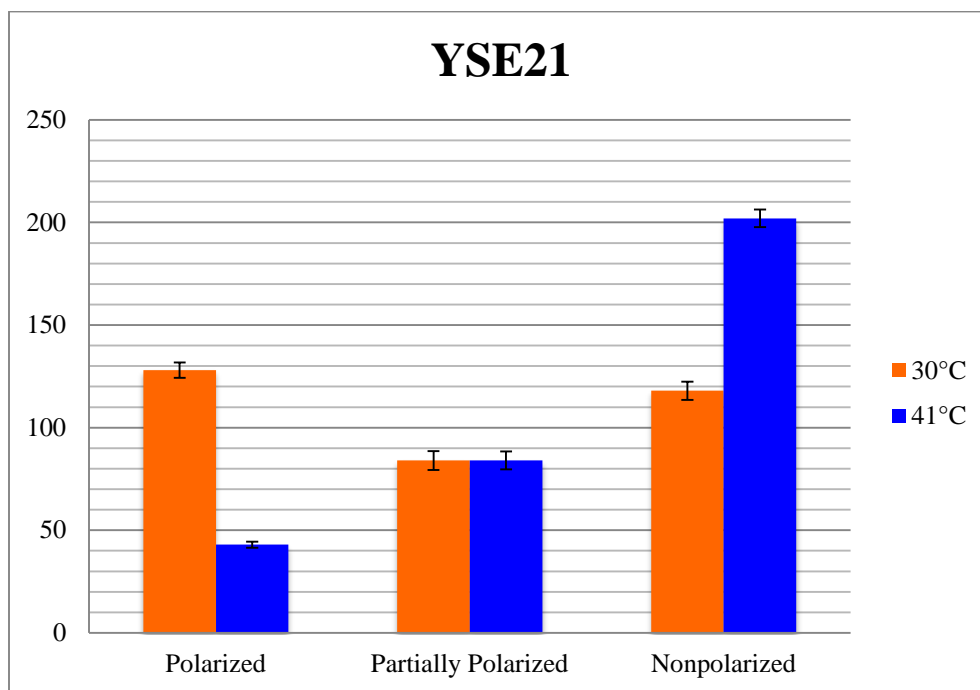
Figure 7. YSE21 results. The control is shown on the left in orange and the experimental is shown on the right in blue.

Discussion

Implications. In considering this project, it is important to note that the most significant part did not specifically involve temperature. Although it is important, and one of the central focuses of this project, it is merely the environmental factor chosen to push the limits of the chosen system. Temperature was chosen because it is very easy to manipulate and control in an experimental setting. The goal of a project such as this is not really determining the effect that high temperatures have on mating, it is to determine a point at which the process of mating is inhibited. At this point, there are parts of the cells that are not able to correctly execute the developmental program of mating. Genetic screens can be done to find genes and proteins that are involved in mating and to determine their function. That is the future of this project; finding the genes in the mating process that fail when environmental stress is introduced and therefore discovering their specific functions in the system.

Overall, these experiments and results define the boundaries of environmental temperature to which the mating process is buffered. These studies lay the groundwork for using genetic screening methods to define the genes that are responsible for buffering the process as well as specific gene products participating in the mating process that are sensitive to the stress of elevated temperature. Since these aspects of the mating process have not been systematically explored before, the approaches may reveal new gene products and components that function in these developmental processes at a cellular level.

Temperature limits of mating. The results from the mating temperature experiments were relatively conclusive qualitatively. In the YSE430 and YSE431 strains, the temperature threshold

at which mating was no longer possible occurred in between 39°C and 41°C. As the optimal

temperature for mating is 30°C, this is significantly above the optimum temperature. This

indicates that, as stated in the introduction, canalization is no longer possible due to

environmental strain. The phenotypes of the YSE430 and YSE431 strains are no longer able to

accommodate mating at an elevated temperature in between 39°C and 41°C. This is significant

for several reasons. As the goal of this project was to push the boundaries of mating, this

experiment will become a starting point into an exploration of the limits of the mating process. In

the future, knowledge such as this will indicate the temperature range at which to look for

defects in the pathway.

In considering the YSE21 and YSE22 strain, it can be said that the strain is more

temperature sensitive than YSE430 and YSE431. As expected, no mating occurred at 41°C. As

seen in **Figure 5D** very minimal signs of mating were present at 39°C. The presence of cells that

can grow on the dropout media indicates that mating was possible; however, if more studies were

done on the strains, it is hypothesized that a fewer amount of cells would have mated. That being

said, this provided insights into the fact that different strains of cells have different abilities to

canalize aspects of their phenotypes. Due to reasons that are currently unknown, these two wild

type strains have presented slightly different results. This would lead to future studies on the

matter.

Temperature limitations of mating cell polarization as determined by α Factor treatment.

The alpha factor experiment followed the observations from the experiments to determine the

temperature at which the cells can no longer mate. As part of the process of defining which

step(s) of the mating process are inhibited by elevated temperatures, the first step investigated

was the ability of the cells to form mating projections. This occurs as a cell detects mating pheromone of the opposite mating type; therefore, alpha factor and the MAT a cell lines (430 and 21) were used in this experiment.

When looking at **Figure 6** and **Figure 7** it is seen that, in the 30°C control for both strains, there is roughly an equal number of polarized versus nonpolarized cells. This allows for the experimental results to be put into context. It is seen in both strains that the number of nonpolarized cells decreased approximately by half when exposed to a temperature of 41°C. The number of partially polarized cells remains approximately the same, as those are the cells that don't appear to fall into either category. When observing the number of polarized versus nonpolarized cells at 41°C, it is clear that the cells had difficulty polarizing. These experiments were repeated on three different cultures of cells, and the results did not greatly vary as seen in **Table 1** and **Table 2**.

That being said, though polarization is greatly inhibited, it is clear in both strains that mating projections were still able to form at an elevated temperature. This is interesting when also considering the results of the mating temperature experiments. As seen in **Figure 4D** and **Figure 5B** the process of mating is extremely reduced at 41°C. Though mating projection formation was less likely at this temperature, it was not as dramatically reduced as the success of mating at the same temperature. Therefore, it can be concluded that the inability to form a mating projection formation is not the only cause of the lack of mating success at 41°C. It is likely that there are other steps in the mating process that are causing the results from **Figure 6** and **Figure 7** showing that approximately 50 polarized cells per experimental trial to occur.

Future Work. There are many directions that this project could be taken in the future. Much of this project was an assessment; that is, in order to make discoveries involving a system of which a lot is already known it must be pushed until it breaks. At that point, the system is assessed further. More information about factors such as genotype and phenotype can be determined through screening and further studies.

The next steps for this project would involve examining other steps in the mating process to continue determining which steps are affected by high temperature. In particular, after the alpha factor experiment the best thing to do would be to define whether or not cell cycle arrest in the G1 phase could still occur. Although an exact method would need to be worked out, it would generally involve exposing cells to a high temperature, most likely starting at 41°C, and observing the results. If arrest happened correctly, at any given point in time one would expect to see many vegetative cells and only several undergoing the process of budding (those being the cells that were already undergoing division before the heat was applied). Some quantative assessments could be performed, and insight could be gained into another aspect of the mating process. Additionally, aspects of cell fusion and nuclear fusion could be studied under high temperatures. Based on the determination that mating and polarization are affected by elevated temperatures, it would follow that cells have difficulty fusing under those conditions.

Importance. The importance of this project goes beyond that of studying mating in yeast. There are many reasons that *S. cerevisiae* is so often studied, one of which is that the processes that occur in this single-celled, eukaryotic fungus are representative of many more complex cell types. The steps and process of mating are mirrored in many different eukaryotic cells; some examples of these steps being cell-to-cell signaling, signal recognition, plasmogamy, and

karyogamy. It also provides insights on the cell cycle, as studies on yeast have been doing for many years.

Cell-to-cell signaling or communication, as demonstrated in this project in the mating pheromone response pathway, is an essential function of all cells. Communication is essential for growth, survival, and even cell death. This is very closely related to signal recognition, which is a very important part of mating projection formation. Both plasmogamy and karyogamy, though not studied in this project, have other implications in other cell types. Perhaps one of the most significant for mammalian systems is that of the fusing of a sperm cell with an egg cell. This is a relevant example because a lot of research is being done on the processes of plasmogamy and karyogamy in relation to fertilization and issues with infertility. Also, due to the fact that mating yeast cells arrest in the G_1 phase of the cell cycle, further studies can provide more information about the cell cycle in all organisms.

Overall, the goal of this project was to attempt to observe the effects of environmental stress on many different processes in *S. cerevisiae* that have many significant relationships to all organisms from yeast to humans. To bring this discussion full circle, the evolutionary theory of canalization must be considered. As Waddington postulated, organism's genomes can be buffered against environmental perturbations and persist under this pressure (Waddington, 1942). In these experiments, the ability of *S. cerevisiae* to buffer to the environmental pressure of high temperature was observed. This is important both because it demonstrates the theory that Waddington proposed many years ago, and because it shows that it is possible for the pathways that organisms need for survival to withstand unfavorable conditions. Canalization does occur in *S. cerevisiae*, and future work on this topic could provide many more insights in this field.

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