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Measuring environmental & genetic effects on cell differentiation kinetics in yeast

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Measuring Environmental & Genetic Effects on Cell Differentiation Kinetics in Yeast

Tiara Johnson, and Dr. Daniel Pollard
Biology department at Western Washington University

Abstract
The MAPkinase pathway is commonly involved in cell differentiation and growth processes. To study the kinetics of this pathway in regards to the effects of several genetic and environmental factors, we have chosen the popular model of pheromone response in yeast, Saccharomyces cerevisiae. Our data showed significant differences based on strain that seem to match previous data. Surprisingly, there were no measurable trends based on concentration, but results indicate that temperature may be positively correlated with response rate and may vary between strains. Future experiments will be aimed towards statistically defining the effects of each variable and creating a mathematical model that can be used to predict the outcomes of unstated temperature and concentration conditions.

Background
Yeast
- Two mating types (a, α) only differentiate into mating-competent shmoo in response to the other’s mating pheromone.
- Process controlled my a MAPK pathway (Edelman & Snyder 2001)

MAPK Pathway
- Mitogen Activated Protein Kinase
- Pheromone response in yeast is a very popular model for study.
- Errors in this type of pathway are commonly linked to cancer. (Burotto et al. 2014)

Hypotheses
- We expected S288C to have a higher rate of response because of its known increase in mating efficiency.
- Temperature’s effect on the response rate was expected to be similar to its effect on growth. The lowest rate of response was at 30°C with a strain dependent interaction causing a higher relative rate in YML45 at 35°C.
- Based on given expression results, we expect higher concentration will increase response in both strains.

Methods
- Overnight Cultures: Cells from a single colony are suspended in YPD media and incubated overnight at 30°C.
- Morning Cultures: A sample from the overnight culture is added to YPD media to make 10x cultures of equivalent concentrations and incubated at 30°C until culture has doubled twice.
- Mating Pheromone Added: Mating pheromone added to each morning culture at the specified concentration and incubated at specified temperature.
- Sample Taken: A sample is taken from each morning culture every ~20 min. loaded onto a hemocytometer and the proportion of shmooing cells counted.

Results

<table>
<thead>
<tr>
<th>Sample Regression Model</th>
<th>Full Regression Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>S288C</td>
<td>YML45</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
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<td>100</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>-3.01*</td>
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<td>-3.61</td>
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<tr>
<td>-3.84</td>
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</tbody>
</table>

What's Next?
- Expand dataset with more replicates to increase the power.
- Explore the use of additional interaction terms to improve the model.
- Improve estimates of magnitude and timing of maximum response proportion across strains and conditions.
- Test novel conditions to see if the results fit with the model’s predictions.

Acknowledgements
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Works Cited

Sample Regression Model
- \( y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \epsilon \)

Strains
- Response at the molecular level has been shown to vary between strains (Pollard et al. 2016, Zheng et al. 2009). Is cellular differentiation from a morphological standpoint insensitive to large scale variation in gene expression responses to mating pheromone?
- The lack of a trend with concentration is unexpected based on published gene expression results. (Umulis et al 2015). Is cellular differentiation from a morphological standpoint insensitive to large scale variation in gene expression responses to mating pheromone?
- Our original log odds model was a useable, but poor fit to the data and did not capture the measurable trends based on concentration, but results

Figure 1: Image of yeast cells showing shmooing and non shmooing cells.
Figure 2: Diagram of the mating pheromone response pathway.
Figure 3: Example data points and corresponding best fit log odds model with Time² and Time³ terms.
Figure 4: Plots of full regression model showing differences between conditions listed and averaged over all other variables.

• Only strain specific differences were found to be significant with S288C showing more of a response delay (negative intercept) but then a higher rate (slope) than YML45 on average.
• Temperature shows trends towards higher temperatures eliciting a higher rate of response: both a less negative intercept and greater slope as temperature increases.
• Concentration shows no clear trends.

Discussion/Conclusions
- Strain specific differences in rate of differentiation fit with our prediction, though our data lacks the power to see how strain differences relate to changes in other conditions.
- Temperature shows trends towards a faster response rate as temperature increases. This partially supports our hypothesis, but is inconsistent with the prediction that response would peak at 30°C. The effect may become clearer with more data allowing us to separate temperature effects between strains.
- The lack of a trend with concentration is unexpected based on published gene expression results (Falconnet et al. 2011). Is cellular differentiation from a morphological standpoint insensitive to large scale variation in gene expression responses to mating pheromone?
- Our original log odds model was a useable, but poor fit to the data and did not capture the reversal or provide a way to estimate maximum proportion. Adding terms with powers of time seems to correct that problem and more accurately capture the trends shown in the data.
- Maximum proportion magnitude and timing vary across strains and conditions motivating further investigation into more accurate estimation and the biological implications.

What’s Next?
- Expand dataset with more replicates to increase the power.
- Explore the use of additional interaction terms to improve the model.
- Improve estimates of magnitude and timing of maximum response proportion across strains and conditions.
- Test novel conditions to see if the results fit with the model’s predictions.