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## Life History Trade-offs Between Growth and Reproduction in Wild Yeast

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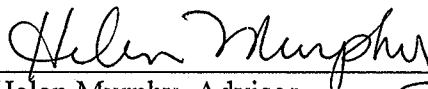
Life History Trade-offs Between Growth and Reproduction in Wild Yeast

A thesis submitted in partial fulfillment of the requirement  
for the degree of Bachelor of Science in Biology from  
The College of William and Mary

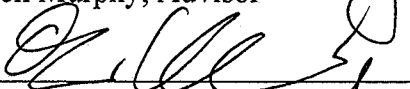
by

Simone M. Giovanetti

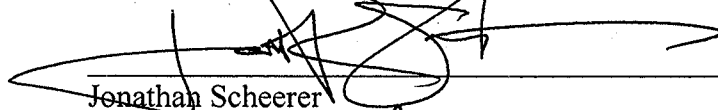
Accepted for Honors



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Williamsburg, VA  
May 1, 2012

## ABSTRACT

Life history trade-offs are an organism's balancing of energy allocations to necessary strategies of life: growth, reproduction, and survival. The success of a life history strategy depends on both the environment and the developmental constraints of an organism. The yeast *Saccharomyces cerevisiae* is an ideal organism to investigate microbial life history trade-offs. Since these yeast are found in a variety of environments with niche-specific strategies and the *S. cerevisiae* life cycle involves both sexual and asexual phases, I expected to see a life history trade-off between growth and reproduction within and between populations of yeast. A strain more efficient at mating should be a slower grower while a slow mater should proceed through the cell cycle quicker. To study this trade-off, I measured the relative rates of mating and asexual growth. I found a significant difference in both the speed of sexual and asexual reproduction within a single population as well as between populations from different environments. The results presented here are still preliminary, as the assays used to measure the relevant life history traits were prone to experimental error. Given that caveat, within the woodland population of yeast, which represents three distinct haplotypes, there appears to be an inverse relationship between the length of the cell cycle and the rate of mating, indicating a trade-off between the efficiency of each strategy. No pattern was found in strains of other ecotypes.

## I. INTRODUCTION

Life history trade-offs represent the balancing of energy allocation to growth, reproduction, and survivorship based on an organism's limited resources. The amount of energy put into each of these competing categories represents a life history strategy (Schluter, Price, and Rowe, 2004). The success of a life history strategy depends on both the environment and the developmental constraints of the organism and is subject to natural selection. In isolated populations of an organism, different environmental pressures can lead to the selection of different energy allocations; since environments vary, this can lead to divergence, reproductive isolation, and eventually speciation (Gudelj et al., 2010).

In many species, the cost of reproduction strongly influences the evolution of life history traits and trade-offs (Harshman and Zera, 2006). In sexually reproducing organisms, variation in life history strategies can result in differences in opportunities to mate and survive, thus providing the opportunity for selection to occur. In organisms that can switch between reproducing asexually and sexually, selection can shift the frequency of each mode of reproduction as well as determine the mode of reproduction that optimizes the environmental conditions. As selection favors one mode of reproduction over another, the amount of outcrossing in the population will change correspondingly (Magwene et al., 2011). For example, in the California oak *Quercus lobata*, the cost of sexual reproduction was shown to affect vegetative growth: the level of acorn production and the amount of local growth were inversely related, demonstrating a trade-off between reproduction and growth (Sanchez-Humanes, Sork and Espelta, 2011).

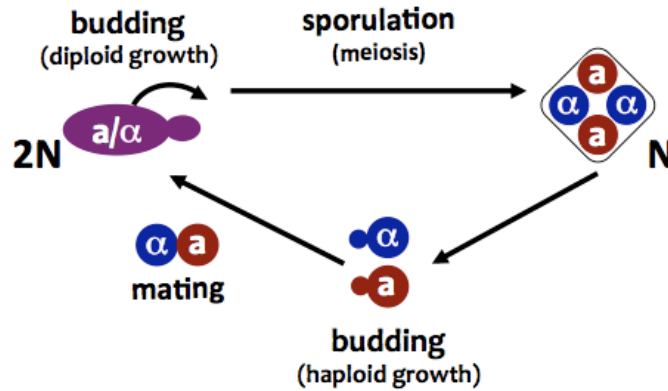
Not much is known about life history tradeoffs in microbial species. Of the work that has been done, most has focused on metabolic trade-offs (Gudelj et al., 2010). Unlike bacteria, many unicellular eukaryotes contain a sexual component to their life cycle (Landry et al., 2006) and could therefore be subject to some of the same trade-offs found in plant and animal species, especially those involving sexual reproduction and growth. The research presented here investigates the potential tradeoff between reproduction and growth in the budding yeast, *Saccharomyces cerevisiae*, specifically the relationship between the ability to efficiently undergo sexual reproduction and the ability to grow asexually.

## ***Saccharomyces cerevisiae* as a Model Organism**

The yeast *Saccharomyces cerevisiae* has been an important model organism for genetic and molecular research and, recently, it is increasingly being used for ecological and evolutionary studies as well (e.g., Botha, 2011; Cutter and Moses, 2011). *S. cerevisiae* is an ideal model organism. It can be cultured and manipulated in the lab using well-known genetic tools and its cellular machinery is similar to that of other higher eukaryotes (Replansky et al., 2008). Well-characterized lab strains of yeast have been used to address many questions about cellular processes including replication, recombination, cell division, and metabolism, as well as larger scale questions like gene interactions and longevity (e.g., Richard, Kerrest, and Dujon 2008; Prakash et al., 2009). In recent years, more work has been conducted using yeast from diverse ecological backgrounds because gaining a more complete understanding of the natural environment and the life history traits of this organism would put all that we have learned about its cell and molecular biology into an evolutionary context.

### ***S. cerevisiae* Life Cycle**

*S. cerevisiae* has a complex life cycle that responds directly to environmental conditions and includes reproduction in both the haploid and diploid state via budding (Figure 1). Under ideal conditions, diploid cells are predominant; however when nutrients are depleted, cells enter meiosis and sporulate, producing four haploid spores that are enveloped in an ascus. The spores are divided equally between the **Mata** and **Mata $\alpha$**  mating types and remain dormant until stimulated by environmental conditions. Haploid cells can reproduce vegetatively through budding or can mate with the opposite mating type when pheromone is detected (Bardwell, 2004; Greig and Leu, 2006; Landry et al., 2006). During the cell cycle, a yeast cell reaches the G<sub>1</sub> checkpoint, where it must either commit to growth and proceed through the cell cycle or arrest growth and begin mating (see Figure 1; Wittenberg and La Valle, 2003).



**Figure 1.** The life cycle of *Saccharomyces cerevisiae*.

While there appears to be a preference for diploidy, the ability to mate is maintained in natural populations (Greig and Leu, 2009). Mating can either be between two individuals from a similar genetic background or via outcrossing. In a woodland population of *S. paradoxus*, sexual reproduction was estimated to occur once every 1000 divisions (Tsai, Burt, and Koufopanou, 2010). This included all forms of sexual reproduction: outcrossing, mating with a sister spore, and mating-type switching (when a mother cell mates with a daughter cell after switching mating types). Outcrossing, the mating between two genetically divergent strains of yeast, appears to occur infrequently in natural populations of yeast. One extreme estimate, which was made using a common lab strain, a vineyard isolate, and a clinical strain, suggests that, based on sequence divergence, two strains will have undergone about 16 million cell divisions since their last common ancestor, with only about 300 outcrossing events having occurred (1 outcrossing event every 50,000 divisions; Ruderfer et al., 2006). As infrequent as outcrossing appears to be, the mixing of genetically distinct backgrounds would still have important consequences for genetic variation and the population structure (Ruderfer et al., 2006). Although sexual reproduction (recombination and outcrossing) can disrupt favorable gene combinations and requires a large expenditure of time and energy, it is maintained in all populations of yeast studied thus far. Sex increases genetic variation thus allowing for more variants on which selection may act (Burt, 2000). Additionally, evolution experiments have shown that sex increases the rate of adaptation to a new harsh environment, while it has little to no effect in stable environments (Goddard, Godfray,

and Burt, 2005), thus maintenance of sexual reproduction in yeast may allow for faster adaptation in changing environments.

### **Ecological Diversity of *Saccharomyces* Yeasts**

In general, *Saccharomyces* yeasts can be found in a wide variety of ecological niches- sometimes in association with human activities- including vineyards, forests, as a pathogen, and in commercial processes (Replansky et al., 2008). In vineyards, yeast are commonly found on damaged fruit (Mortimer and Posinelli, 1999). Yeast are also found on the bark and in the soil surrounding oak trees (Sniegowski et al., 2002) in North America and Europe and have been isolated from exudates of trees and fruits in eastern Asia (Landry et al., 2006). Additionally, yeast have been isolated from mushroom fruiting bodies, spoiling mangoes and peaches, as well as in the fungal diversity of beetle guts (Diezmann and Dietrich 2009). In immunocompromised individuals, *Saccharomyces cerevisiae* has been isolated and identified as an opportunistic pathogen (Landry et al., 2006; Diezmann and Dietrich, 2009). Additionally, *Saccharomyces* yeasts have been considered a domestic organism due to its use for centuries in brewing, baking, distilling, and wine-making (Landry et al., 2006).

In a genomic analysis of a set of over 70 wild and cultivated isolates of *Saccharomyces cerevisiae* and its sister species *Saccharomyces paradoxus*, *S. paradoxus* was found to be more genetically diverse but phenotypically similar while *S. cerevisiae* was genetically similar and much more phenotypically diverse. Most *S. paradoxus* isolates were collected from woodlands whereas the *S. cerevisiae* isolates were collected from a wider range of ecological niches. *S. cerevisiae* was found to consist of a limited number of well-defined, geographically isolated lineages: Malaysia, West Africa, sake and related strains, North America, and a mixed source group including European and many wine strains. Some of these lineages are correlated with geographic origin however some, specifically those used commercially for the same function, do not have a singular geographic niche. Additionally, there were a number of mosaic strains of the five lineages, representing cross-population outcrossing between lineages (See Figure 3; Liti et al., 2009). Because *S. cerevisiae* resides in a large number of habitats with diverse

environmental pressures, their life history strategies should reflect adaptation to the local growth conditions and competition.

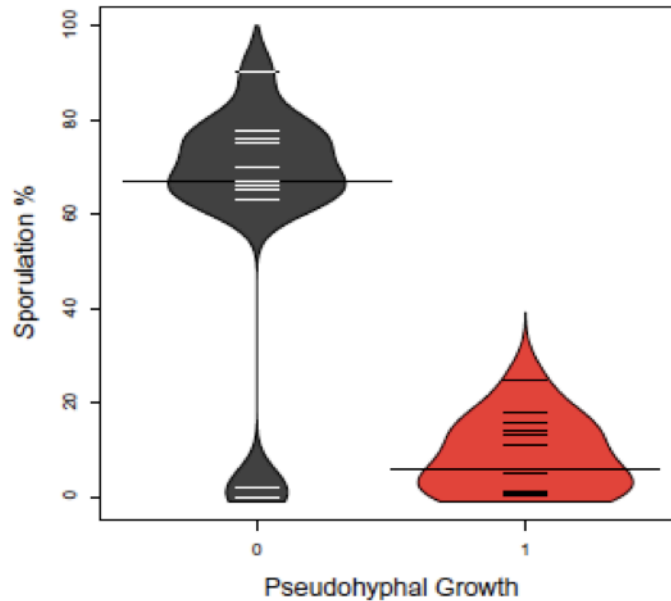
### **Life History Trade-offs in *S. cerevisiae***

Recent work has begun to investigate the life-history trade-offs of *Saccharomyces cerevisiae*. The first study to look at life history trade-offs in *S. cerevisiae* compared variation in growth profiles based on strain origin (Spor et al., 2008). An apparent trade-off between the rate of glucose consumption and carrying capacity resulted in the identification of the “ant” and “grasshopper” strategies. Strains originating from industrial settings were found to consume glucose at a high rate, have large cell size and thus a low carrying capacity and are an example of the “grasshopper” strategy. Conversely, forest and laboratory strains tended to consume glucose at a low rate, have small cell size, and a higher carrying capacity and are an example of the “ant” strategy. The divergence in life history traits in these yeast show a significant relationship with habitat of origin: strains from a similar habitat had similar life history strategies. This niche-driven evolution indicates phenotypic convergence in similar but geographically isolated habitats.

In a different study, life history trade-offs involving asexual and sexual reproduction under nutrient stress in *Saccharomyces cerevisiae* were also correlated with the type of environment with which the yeast were associated (Magwene et al., 2011). This trade-off is demonstrated by the inverse relationship between the ability to sporulate efficiently versus grow via pseudohyphae (Figure 2). Sporulation, a strategy for long-term nutrient stress, was more frequently associated with homozygosity and found in wild populations. Pseudohyphal growth, the formation of a series of connected budded cells, represents a foraging strategy. It was more commonly found in human associated environments where outcrossing is more likely to occur and was associated with a higher rate of heterozygosity. Strains of *S. cerevisiae* that are well suited to pseudohyphal growth to deal with nutrient starvation have likely been selected for in environments where nutrient starvation occurs predominantly for short time periods. This differential preference indicates a selective pressure dependent on the environment for the reproductive mode as well pressure altering the frequency of sexual and asexual cycles



(Magwene et al., 2011). Furthermore, the similarity between strains of yeast originating from the same type of habitat, potentially independent of actual geography and physical proximity, suggests that ecology is the main driving force of the evolution of these strains, resulting in convergence between populations (Diezmann and Dietrich, 2009).



**Figure 2.** The inverse relationship (trade-off) between efficient sporulation and pseudohyphal growth. (Magwene et al., 2011).

The niche-specific preference for one strategy of survival over another indicates selection on different geographically located (and potentially human versus non-human associated environments) populations for different reproductive strategies. In environments where sexual reproduction is more common, the benefits of sexual reproduction (adaptation to a changing environment) must then outweigh the costs (energy input into sporulating, hibernating, and mating). In yeast, sexual reproduction requires the production of a pheromone signal to attract a haploid of the opposite mating type; this pheromone is energetically expensive to make and may be made in different quantities as an indication of the genetic quality of the pheromone producer (Smith and Greig, 2010). This finding suggests that when energy is not being directed towards the production of a sexual signal, the energy can be allocated to survival, exhibiting the

principle of life history trade-off in the context of finding a mate and reproducing versus fitness.

## **My Research**

I am investigating at the differences of reproductive strategies, specifically the potential trade-off between growth and reproduction in yeast isolated from different geographic and ecological niches. Depending on the origin of the strain, the specific environmental pressures of each habitat may influence the yeast's ability and efficiency to undergo sexual reproduction. Therefore, as the environmental demands differ between populations of yeast, the selection pressures for the likelihood of sexual and asexual reproduction between populations of yeast will also differ. The result of this divergent selection is that some populations will be better (faster) at reproducing sexually while other populations will be better (faster) at reproducing asexually.

Mechanistically, this trade-off would likely be controlled at the G<sub>1</sub> checkpoint, at which point cells must either commit to growth or mating (Elion, 2000). While arrested at this checkpoint, cells are receptive to pheromone from the opposite mating type. Therefore, strains that are selected to be more efficient maters might spend more time at this checkpoint, increasing the opportunity to detect pheromone and mate while extending the length of the cell cycle. Alternatively, strains that move through the checkpoint quickly will be less likely to detect pheromone (and be less efficient maters) while proceeding through the cell cycle at an overall faster rate.

In accordance with the principle of trade-offs, a yeast's limited resources must be divided between all processes of survival and reproduction, thus as one mode of reproduction is being consistently selected to be optimized in a specific environment, the efficiency in completing the alternative mode of reproduction will decrease. I expect to see an inverse relationship between the speed of sexual and asexual reproduction in each strain.

Furthermore, the strategy should be dependent on the origin of the strain and thus the environmental pressures that necessitate and determine the frequency of each mating strategy. Since harsher and more variable environments necessitate more sex and outcrossing (leading to faster adaptation), I predict that strains that are from wild

environments should be optimized to reproduce sexually, as indicated by the higher frequency of outcrossing and recombination previously found. Alternatively, human-associated strains should be better at asexual reproduction, which would be consistent with their tendency for clonal growth. I will also investigate a number of strains from within one woodland population to determine the amount of variation in these phenotypes and determine whether multiple life history strategies exist in one environment. To test for a trade-off, I measured the length of the cell cycle (asexual growth) and mating speed in strains from various genetic and geographic backgrounds.

Additionally, I investigated the relationship between sporulation and mating. Previously published results (Magwene et al., 2011) suggested a relationship between heterozygosity (an indicator of a recent sexual cycle) and inefficient sporulation, potentially indicating differences in effective mating rates. Although sporulation is a prerequisite for mating in the wild, the relationship between the ability to sporulate efficiently and the speed of mating is unclear. I investigated this possible relationship by measuring sporulation efficiency and comparing it to mating speed.

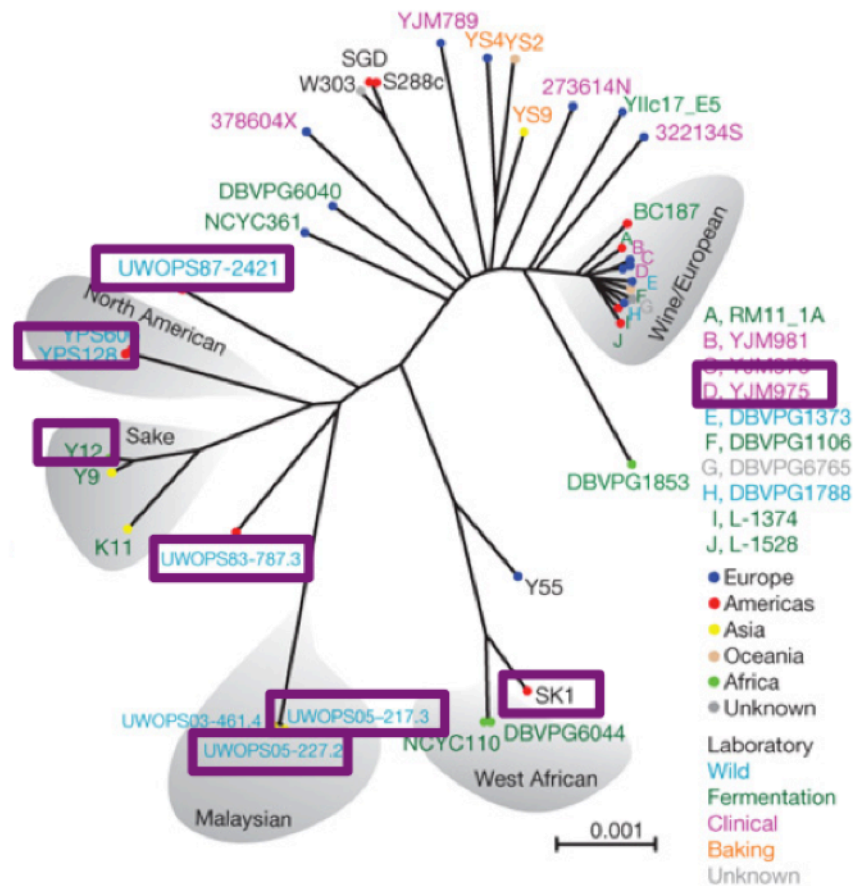
## II. METHODS

### Yeast Strains

Thirteen strains of *Saccharomyces cerevisiae* were analyzed in this study (Table 1 and Appendix A). All strains were previously transformed to be heterothallic and thus are unable to switch mating types. Six strains, representing three haplotypes (A, B, and C) were from one woodland population of yeast collected by Paul Sniegowski and Heidi Kuehne from the bark of oak trees in second growth forests in Pennsylvania and New Jersey (Murphy and Zeyl, in press). The remaining seven strains were from the Sanger Institute's *Saccharomyces* Genome Resequencing Project (SGRP: <http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html>) and are from a variety of locations and ecological settings from around the globe, representing a variety of different habitats (described in detail in Liti et al., 2009). The phylogenetic relationship between these strains can be seen in Figure 3.

ORIGINAL STRAIN	LOCATION	ECOLOGICAL NICHE	HAPLOTYPE (woodland strains)
YPS 133	PA, USA	Bark of oak tree	C
YPS 681	PA, USA	Bark of oak tree	C
YPS 615	PA, USA	Bark of oak tree	B
YPS 623	PA, USA	Bark of oak tree	B
YPS 630	PA, USA	Bark of oak tree	A
YPS 670	PA, USA	Bark of oak tree	A
SK1	USA	Initially from soil, lab strain	
Y12	Ivory Coast, Africa	Palm wine	
UWOPS83-787.3	Great Inagua Island, Bahamas	Fruit of prickly pear cactus	
UWOPS87-2421	Maui, Hawaii	Cladode of prickly pear cactus	
UWOPS05-217.3	Telok Senangin, Malaysia	Nectar of bertam palm	
UWOPS05-227.2	Telok Senangin, Malaysia	Bee near bertam palm	
YJM975	Bergamo, Italy	Clinical strain	

**Table 1.** The thirteen strains used in this study.



**Figure 3.** The *Saccharomyces cerevisiae* strain phylogeny; the color of the name indicates the source and the color of the dot indicates the geographic origin. (Liti et al. 2009). The strains highlighted with a purple box indicate strains used in this experiment. YPS128 is a representative of the North American woodland population.

## Media

All growth was conducted in either YPD (2% peptone, 2% dextrose, 1% yeast extract) or SOE (Synthetic Oak Exudate: 1% sucrose, 0.5% fructose, 0.5% glucose, 0.15% peptone, 0.1% yeast extract); solid medium was prepared by adding agar to 2%. When appropriate, media was supplemented with antibiotics (G418: 40µg/mL, hygromycin: 200µg/mL, nourseothricin: 100µg/mL) and/or amino acids (uracil: 20µg/mL).

## Mating Speed: Initial Measure

A rough measure of the rate of sexual reproduction was conducted over a five-hour period (Murphy et al., 2007) for the six woodland strains and two strains from

outside of the woodland population (SK1 and Y12). Each haploid strain had been previously transformed to contain different antibiotic resistances (G418, hygromycin B, or nourseothricin resistance; see Appendix A) such that the *Mata* and *Mat $\alpha$*  of each strain contained different antibiotic resistances.

*Mata* and *Mat $\alpha$*  haploids were grown overnight in YPD at 30°C. Each strain was washed and sonicated to disrupt any cell clumps, allowing *Mata* and *Mat $\alpha$*  cells access to one another when mixed. Approximately equal amounts of each corresponding haploid were mixed and plated onto SOE plates. After the mating plates air-dried, an initial sample (start time) and a sample every hour for the following five hours were taken. When samples were not being taken, the plates were incubated in a 30°C. Samples were taken by pipetting 10  $\mu$ L of water onto the mating plate; the water was immediately taken up with scrapings of yeast from the lawn. A 1:10,000 dilution was plated onto YPD plates. All plates were grown overnight at 30°C.

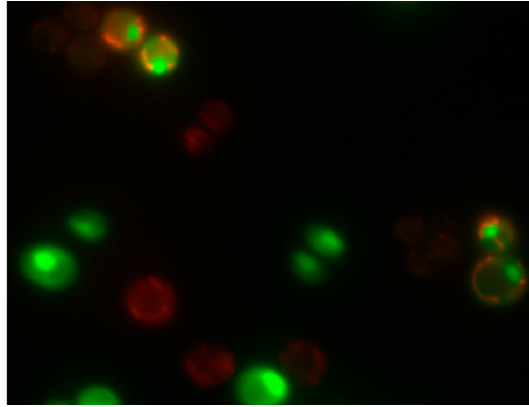
Once colonies had grown large enough to see, the total number of individual colonies per strain per time point was counted; plates from the initial time point were replica plated to YPD + G418 plates to determine the proportion of *Mata* cells, while all other time points were replica plated to double antibiotic plates to determine the proportion of mated colonies. To correct for unequal starting proportions of *Mata* and *Mat $\alpha$* , the proportion of colonies growing on the antibiotic plates for each time point was divided by the average proportion from the start plates.

This assay was repeated four times on independent days for different combinations of the nine strains. The results suggest that the assay is plagued by a large experimental error because it is not possible to determine how many cells are being plated at any given time and the number of colonies that can be counted per time point is limited. As a result, it can only detect large differences in mating propensity.

### **Mating Speed: A more precise measure**

In order to obtain a finer-scale measurement of the rate of sexual reproduction, I attempted to devise a new assay in which fluorescent proteins could be used to monitor the rate of mating. Similar to the previously described assay, each mating type would contain a different color fluorescent protein, and using FACS (Fluorescence-Activated

Cell Sorting) analysis, the proportion of cells mated over time could be monitored with a larger sample size and finer time scale measurements (Figure 4 demonstrates the concept of the assay).

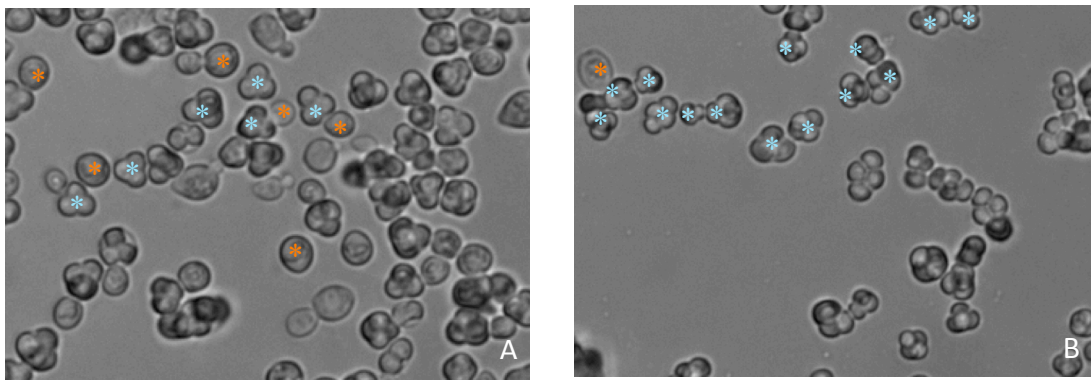


**Figure 4.** Example of mating assay: haploid yeast (cells expressing only one fluorescent protein: green or red) and diploid yeast (the product of sexual reproduction; cells expressing both fluorescent proteins).

To accomplish this goal, each haploid strain would need to express a different fluorescent protein. First, I attempted this assay using fluorescent proteins localized to the mitochondria. Once sample strains were engineered with red fluorescent protein (RFP), yellow fluorescent protein (YFP), or cyan fluorescent protein (CFP), I tested the procedure using a BDFACS Array Flow Cytometer; the various combinations of red, yellow, and cyan fluorophores could not be differentiated using the available equipment. I also tried using a yellow fluorescent protein that is localized to the nucleus in combination with the other fluorescent proteins but differentiation was still not possible. In order for the assay to work, the BDFACS Array Flow Cytometer required one of the fluorescent proteins to fluoresce in the infrared range. The second attempt at this assay utilized a recently published infrared fluorescent protein (IFP1.4) with excitation and emission maxima of 684 and 708 nm, respectively and requires biliverdin as the chromophore (Shu et al., 2009). Unfortunately, this attempt did not work, as incorporating biliverdin into the medium made differentiating between the IFP1.4 and YFP impossible. For details on all plasmids, bacterial and yeast transformation procedures and resulting strains, see Appendix B.

### Ability to Sexually Reproduce: Sporulation Efficiency

To investigate the relationship between sporulation and mating, the sporulation efficiency was measured for each strain. Three independent cultures from freezer stocks of diploid cells were grown overnight in YPD at 30°C. The following day, each culture was pelleted, resuspended in 500 µL of water, and 250 µL was spread onto a sporulation plate (1% potassium acetate). 48 hours later, 4 µL of water was dispensed onto the plate and taken up with scrapings from the lawn. At 40x, four pictures for each plate were taken with as many non-clumped cells in the frame as possible (Figure 5). The cells in each picture were then identified and demarcated as either sporulated or not sporulated. Totals for each culture (3-4 per strain) were counted and graphed as the proportion of sporulated cells after 48 hours.



**Figure 5.** Two representative pictures taken to determine the sporulation efficiency after 48 hours. Asci are demarcated with a teal tag, diploids with an orange tag. A. Y12, a relatively slow sporulator. B. SK1, a relatively efficient sporulator.

### Cell Cycle: Asynchronous Cells

To compare the growth rate between haploid strains, three separate scrapings from stocks of Mata cells of each strain were grown overnight in YPD. The cultures were growing asynchronously, following their natural growth pattern. The following day, the optical density of each strain was taken using a NanoDrop 1000 (Thermo Fischer Scientific) spectrophotometer to ensure the strains were in log phase (exponential growth;  $OD_{600} = 0.5 - 1.0$ ). 1 mL samples of each strain was transferred to an Ependorff tube, centrifuged and aspirated. 1mL of 70% ethanol was added to each strain and the pellet was resuspended. Samples were stored at 4°C until prepared for Flow Cytometry



Analysis, which measures the amount of DNA present in each cell and indicates the phase of the cell cycle ( $G_1$ , S, or  $G_2/M$ ). The proportion of cells in each phase of the cell cycle indicates the relative length of that phase for each strain's cell cycle (Delaney et al., 2011).

All samples were stored for up to a week and subsequently prepared for a budding index analysis and cell sorting. Each sample was pelleted and the ethanol was removed. Samples were resuspended in 1 mL of 50 mM sodium citrate, pelleted, and the supernatant was removed. Each sample was then resuspended in 500  $\mu$ L of 500 mM sodium citrate and sonicated for 30 seconds. A 50  $\mu$ L aliquot was set aside and stored at 4°C for microscopy purposes. Samples were then brought to a total volume of 1 mL by adding 50 mM sodium citrate and RNase A (final concentration of .25mg/mL) and were incubated at 50°C for 60 minutes. Next, 50  $\mu$ L of proteinase K (20 mg/mL) was added and samples were incubated at 50°C for an additional 60 minutes. Finally, the cells were pelleted, the supernatant was removed, and the cells were resuspended in 1 mL of 50 mM sodium citrate. At this point, samples were stored at 4°C. Approximately 16 hours before running the samples, each sample was again sonicated for 30 seconds. Propidium iodide (final concentration of 10mg/mL), which interacts with DNA by intercalating between the nucleic acid bases, was added to each tube (Hasse and Lew, 1997). A sample for each strain was left unstained to use as a blank. Prior to running the samples, 200  $\mu$ L of each sample was loaded into a 96 well plate and the plate was run using a BD FACSAArray Flow Cytometer.

### **Cell Cycle: Synchronous Cells**

To assess progression through the cell cycle, each *Mata* strain was analyzed over a time course after synchronization by  $\alpha$ -factor. Samples were taken at least every 15 minutes for 120 minutes and subsequently analyzed using Flow Cytometry.

Strains were grown overnight in YPD, pH=3.9 at 30°C. The following morning, the optical density was measured using a NanoDrop 1000 (Thermo Fischer Scientific) spectrophotometer and adjusted to 0.4 via the addition of YPD, pH=3.9. 2 mL were transferred to a new tube and  $\alpha$ -arrest factor ( $10^{-3}$  stock  $\alpha$ -factor in 0.1N HCl) was added to a final concentration of 2.0  $\mu$ g/mL. Cells were incubated at 30°C for 60 minutes at

which point another 1  $\mu\text{g}/\text{mL}$  of  $\alpha$ -arrest factor was added. Cells were incubated at 30°C for another 30-60 minutes or until, when checked under the microscope, about 90% of cells had shmoos or a small bud, indicating that the cells were arrested in the G<sub>1</sub> phase. Cells were pelleted, washed twice with regular YPD, and resuspended in 1.3mL of YPD and stored in a 30°C incubator. At time points 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 minutes, a 120  $\mu\text{L}$  sample from each strain was resuspended in 280  $\mu\text{L}$  of 70% ethanol and stored at 4°C (Iaouk et al., 2002). For comparison when analyzing the FACS data, each Mata strain was grown for 2 days in YPD at 30°C, allowing the cells to deplete the nutrients in the medium and stop actively dividing.

Samples were prepared according to same protocol described above for Flow Cytometry analysis.

### **Cell Cycle: Microscopy of Synchronous Cells**

As an additional analysis of cell cycle progression, the  $\alpha$ -factor synchronized samples were analyzed under the microscope. For each sample, 40  $\mu\text{L}$  of the 50  $\mu\text{L}$  sample was transferred to a new tube, pelleted, and about 38  $\mu\text{L}$  of the supernatant was removed; the pellet was resuspended in the remaining 2  $\mu\text{L}$  of sodium citrate and placed on a clean microscope slide. At 40x, four pictures for each time point were taken with as many non-clumped cells in the frame as possible. The non-clumped cells in each picture were then identified and demarcated as having 1) no bud/G<sub>1</sub> (perfectly round cells with no protrusions) 2) beginning to bud/S (cells have a small, nearly pointy, oval protrusion extending from one end of the parent cell) or 3) large bud/G<sub>2</sub> or M (bud is rounder and half the size of the parent cell). Totals for each time point were counted and graphed as the proportion of cells per time point in each of the three stages.

The set of samples analyzed via microscopy are from a different day than the samples analyzed via FACS analysis. Samples from both days were treated the same (except for the use of ethidium bromide instead of propidium iodide) and had similar trends when analyzed using the data from the Flow Cytometer.

## **FACS Analysis**

Data from each Flow Cytometry run was downloaded and analyzed with WEASEL (Walter and Eliza Hall Institute of Medical Research) using the Cell Cycle fit. In order to standardize from one well to another, the parameters were modified such that the approximate G<sub>1</sub> peak was consistent. The number of events fit to each phase of the cell cycle was exported and analyzed using Microsoft Excel and JMP.

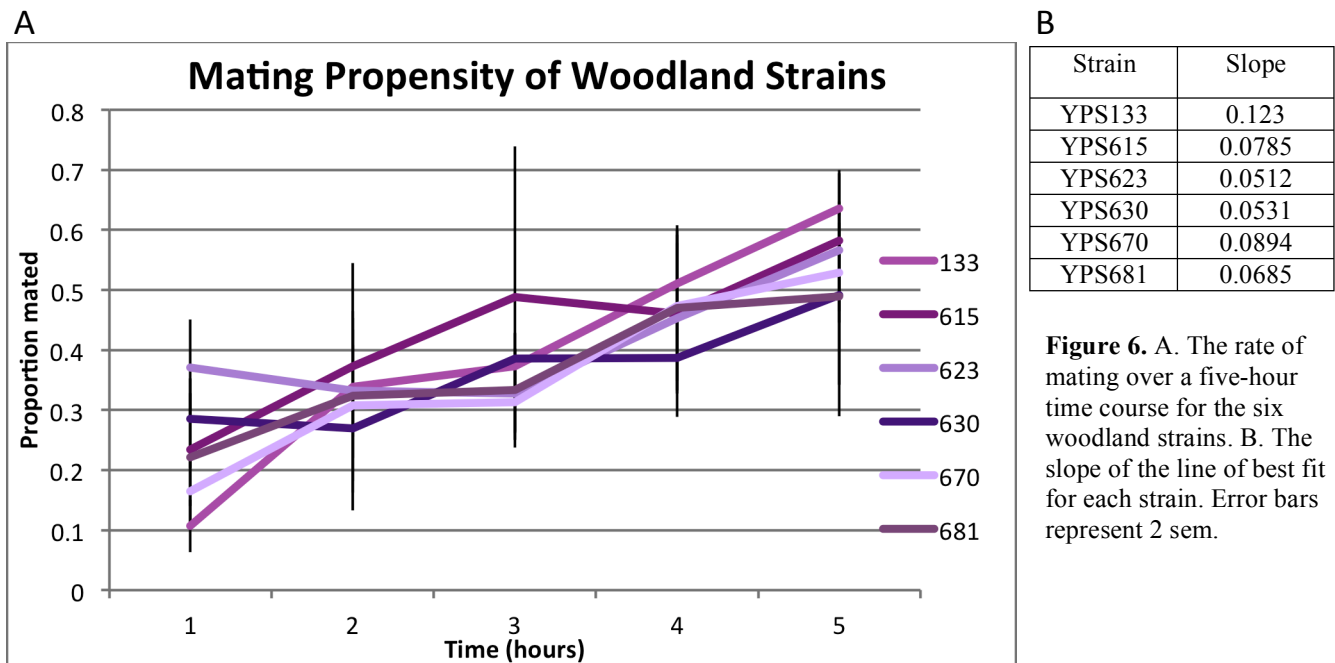
### III. RESULTS

#### Mating Speed: Initial Measure

The proportion of colonies that were the result of sexual reproduction was first assayed within the woodland population (Figure 6A) and analyzed using ANOVA. There was no significant difference in the interaction between time and strain ( $p=0.4884$ ), which would indicate a difference in mating speed between the strains. However, there were significant differences among the strains, indicating differences in overall mating probability. Using a Tukey's Honestly Significant Difference test, YPS 630 was found to be significantly different from the other five woodland strains.

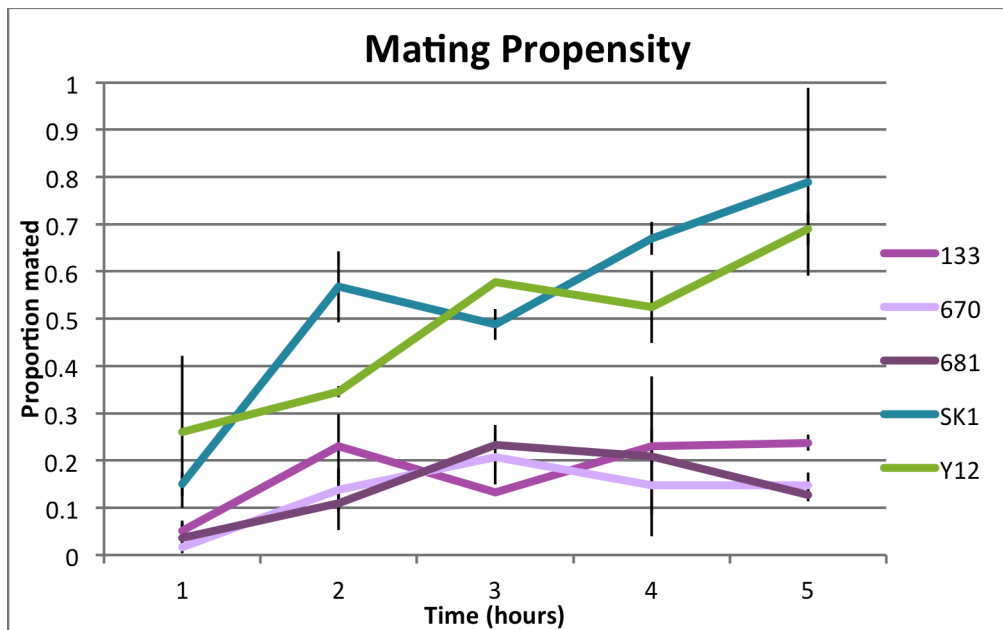
ANOVA (Woodland Populations Only)				
Source	DF	Sum of Squares	F ratio	Prob > F
Strain	5	0.5023133	10.3816	<.0001
Time	4	0.61218731	15.8155	<.0001
Time*Strain	20	0.19385256	1.0016	0.4884
Error	29	0.2806334		
C. Total	58	1.5954628		

As a relative measure of mating speed, the slope of the line of best fit for each line was calculated (Figure 6B).



To expand the analysis, two additional strains of yeast were assayed in comparison with three representative woodland strains (Figure 7) and analyzed using an ANOVA. There was a significant difference due the interaction of time and strain (indicating a difference in the speed of mating;  $p=0.0022$ ), as well as a significant difference between the strains. Using a Tukey's HSD test both Y12 and SK1 were found to have a significantly different rate of mating than the woodland strains.

ANOVA (Woodland and Two Outside Population Strains)				
Source	DF	Sum of Squares	F ratio	Prob > F
Strain	4	1.7705379	66.1613	<.0001
Time	4	0.69999047	26.154	<.0001
Time*Strain	16	0.3882062	5.3799	0.0022
Error	25	0.1672558		
C. Total	49	3.0209045		



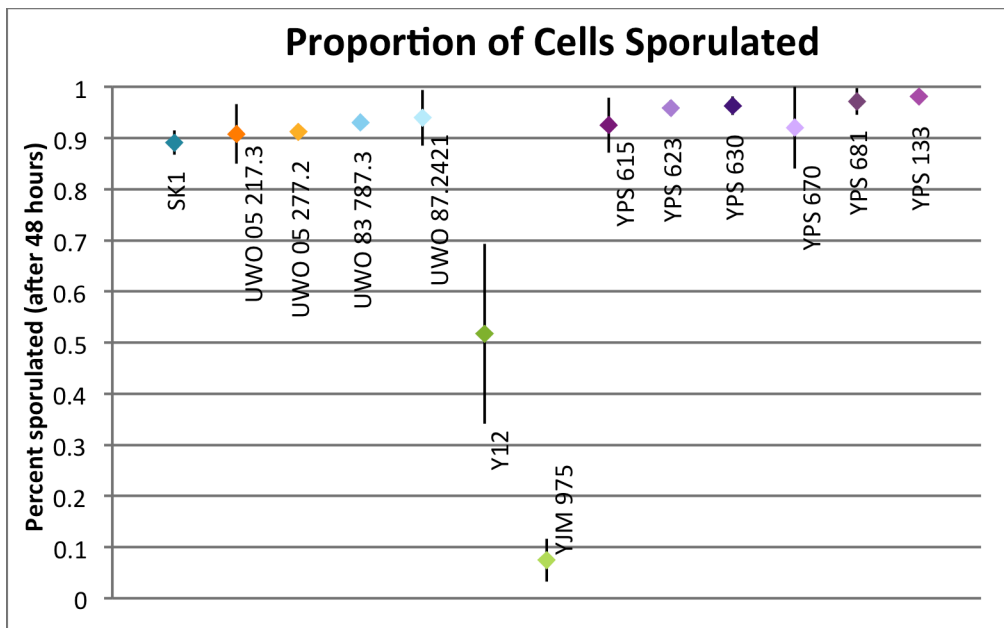
**Figure 7.** The rate of mating over a five-hour time course for three representative woodland strains, a lab strain (SK1), and a strain used in the fermenting of Palm wine from Ivory Coast, Africa (Y12). Error bars represent 2 standard error measures.

This assay was performed numerous other times, but due to the difficulties with the unpredictability of the number of colonies sampled at a given time point, the results were not reliable. The assays run on separate days were analyzed individually because the

assay is not precise enough to provide an absolute measure of mating speed, but rather is reasonable at providing an assessment of the relative differences between strains assayed in concert.

### Ability to Reproduce Sexually: Sporulation Rate

The sporulation efficiency after 48 hours was estimated; of the strains used for this study, only Y12 and YJM975 appeared to have low sporulation efficiency (Figure 8).

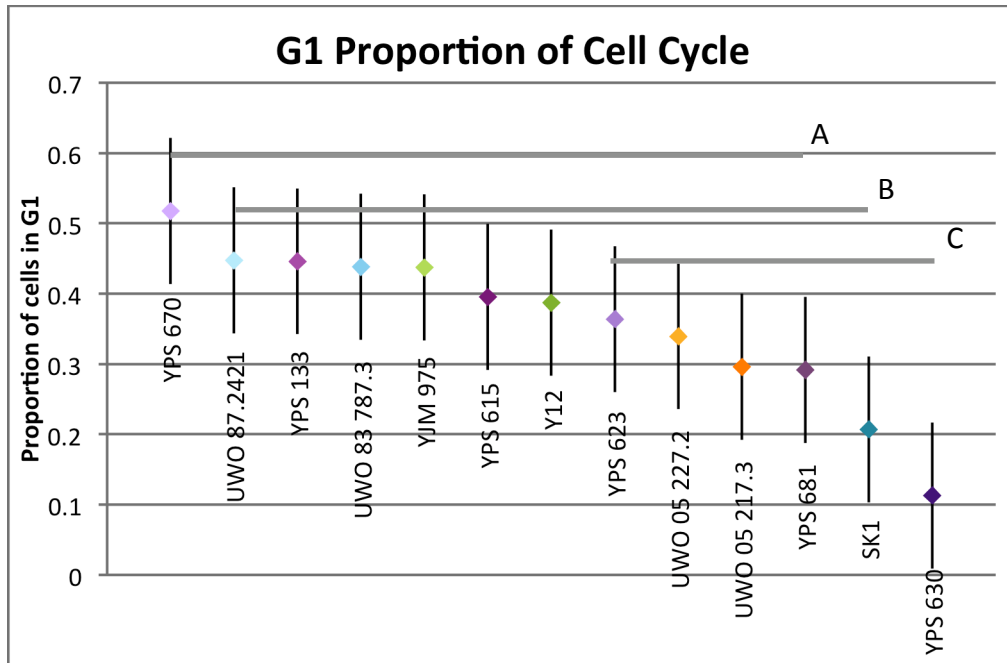


**Figure 8.** After 48 hours of exposure to sporulation medium, the number of cells that had sporulated was counted and graphed as a proportion of total cells for each strain. Error bars represent 2 standard error measures.

### Growth Rate: Asynchronous Cells

The proportion of cells in the G<sub>1</sub> phase of the cycle from each strain growing asynchronously (Figure 9) was analyzed using an ANOVA. There was a significant difference between strains ( $p=0.008$ ). Using a Tukey's HSD test, the strains of yeast were grouped into three groups (A, B, C); strains in the same group are not significantly different from one another.

ANOVA (G <sub>1</sub> proportion of the cell cycle)				
Source	DF	Sum of Squares	F ratio	Prob > F
Strain	12	0.44274919	4.3768	0.0008
Error	26	0.21917467		
C. Total	38	0.66192386		



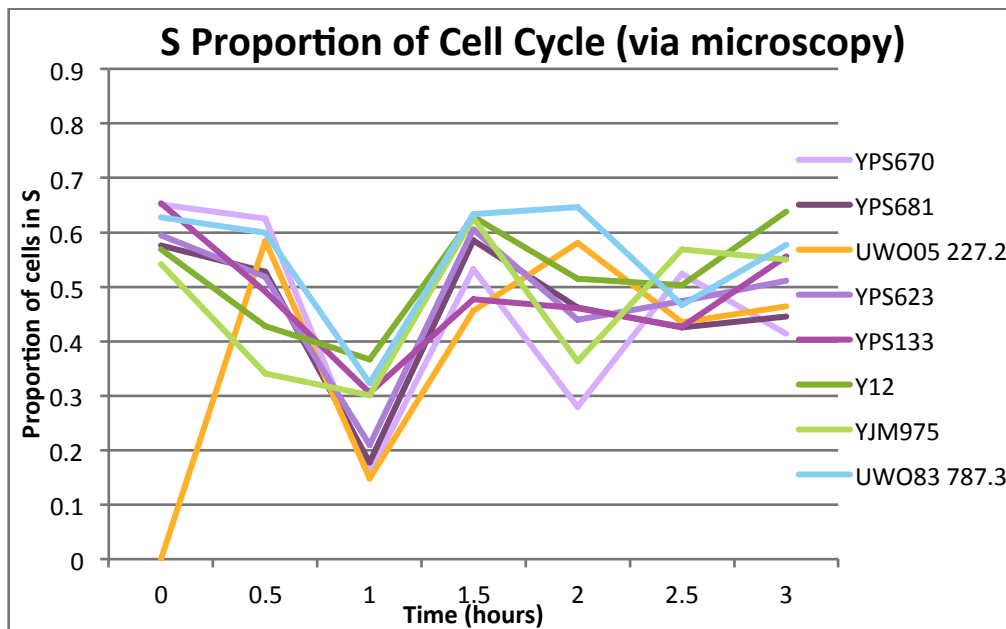
**Figure 9.** Cells grew asynchronously until they reached exponential growth, at which point they were harvested and analyzed. The proportion of cells in the G<sub>1</sub> phase of the cell cycle was plotted by strain. Strains under the same line indicate no significant difference between the strains within the group. Error bars represent 2 MSE (mean squared error).

### Growth Rate: Synchronous Cells

The proportion of cells in both S and G<sub>2</sub> during the first 90 minutes for each synchronously growing strain was analyzed using an ANOVA. In fitting each time point with the Cell Cycle fit, there was difficulty in detecting the difference between G<sub>1</sub>, S and G<sub>2</sub>. Multiple peaks, potentially indicating clumped cells detected as a single event, prevented clear delineation between the phases of the cycle. Based on estimates of each phase using the Cell Cycle fit, neither the S nor G<sub>2</sub> phase was significantly different between the strains (p=0.9936, p=0.9965, respectively).

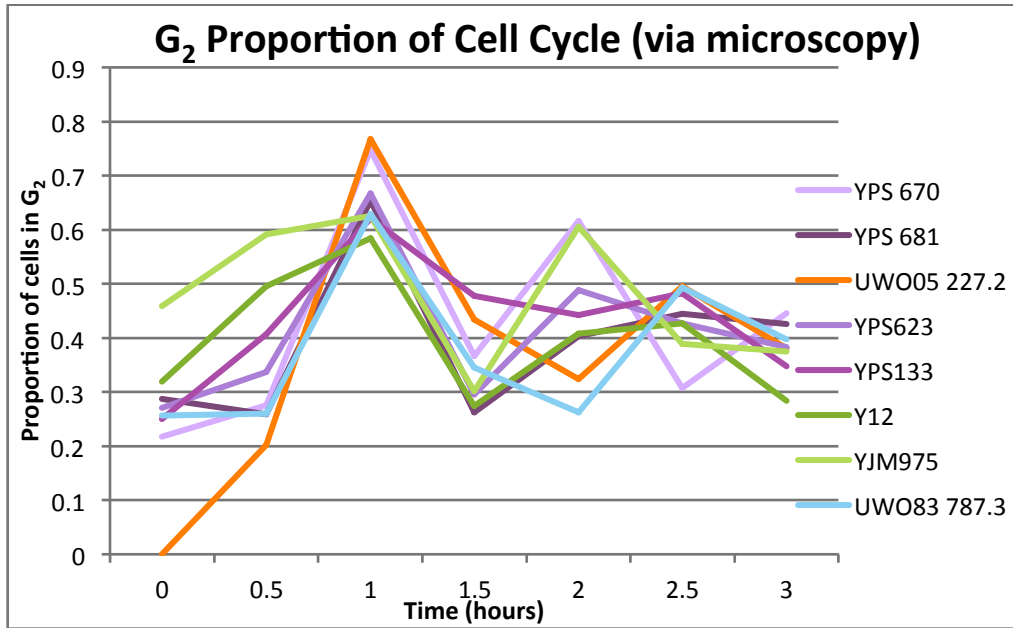
ANOVA (S phase for synchronized cells)				
Source	DF	Sum of Squares	F ratio	Prob > F
Strain	10	0.18752039	0.4639	0.9059
Time	1	0.25440932	6.2934	0.0152
Time*Strain	10	0.08794314	0.2175	0.9936
Error	54	2.18292989		
C. Total	75	2.7121883		
ANOVA (G <sub>2</sub> for synchronized cells)				
Source	DF	Sum of Squares	F ratio	Prob > F
Strain	10	0.27929468	0.5828	0.8208
Time	1	0.81259553	16.9566	0.0001
Time*Strain	10	0.08981303	0.1874	0.9965
Error	54	2.5877995		
C. Total	75	3.7678806		

The analysis of the synchronous cells via microscopy was conducted on a set of FACS samples from another day, although the results of both were similar. When the proportion of cells in both the S (Figure 10) and G<sub>2</sub> phase (Figure 11) from the budding index is graphed, the strains appear to move through these phases in at a similar rate.



**Figure 10.** The proportion of cells in S from the budding index (microscopy) samples. The same general trend is apparent for all strains, indicating that the S phase is maintained between strains. After 90 minutes (the estimated length of the cell cycle), the samples become less synchronized within and between strains.





**Figure 11.** The proportion of cells in G<sub>2</sub> from the budding index (microscopy) samples. The same general trend is apparent for all strains, indicating that the G<sub>2</sub> phase is maintained between strains. After 90 minutes (the estimated length of the cell cycle), the samples become less synchronized within and between strains.

## **IV. DISCUSSION**

Life-history traits are the balancing of energy between different processes in an organism. Due to developmental constraints and environmental conditions, selection often favors one life-history trait at the expense of another. Because the life cycle of *Saccharomyces cerevisiae* contains both sexual and asexual stages, I investigated the trade-off between modes of reproduction as dependent on the origin of the strain of yeast as well as within a singular woodland population. Specifically, I investigated the efficiency of mating and growth by measuring the relative rate of each. I expected to see an inverse relationship between growth and mating indicating a trade-off. Due to niche-driven evolution, I predicted a convergence between strategies in strains of yeast from geographically distinct but environmentally similar origins.

### **Sexual Reproduction**

Within the Pennsylvania woodland populations, although isolated from the same environment, I expected differences between the six strains, which represent three genetic haplotypes. The existence of separate, genetically divergent haplotypes in the same geographic location may indicate varied outcomes to similar environmental conditions (due to, for example, random mutation being acted upon by selection). These differences could lead to temporal reproductive isolation that leads to further divergence. Within the Pennsylvania woodland population, YPS 630 was the only strain to exhibit a significant difference in mating speed. However, there are likely to be differences in mating speed that are too fine to measure with the mating propensity assay used.

Between populations of yeast with diverse origins, I expected to see distinct mating rates. Geographically isolated strains have encountered varied environmental pressures since their divergence from a common ancestor. As a result, there are likely to be large differences in their life-history traits, including the rate of reproduction. As predicted, when SK1 (lab strain) and Y12 (vineyard isolate) were compared to the three representative woodland strains, the woodland populations had no internal significant difference but overall the woodland population was significantly different from SK1 and Y12 in terms of mating speed.

Although the variation in mating speed was observable, the technique could not capture fine-scale differences and the results contained error that prevented precise quantification of the rate of mating between similar strains. It is very likely there are differences that remain undetected. Using an assay that allows for a clearer identification of the proportion of cells that are diploid at each time point would allow a more exact measure of mating. For example, yeast containing fluorescent proteins with non-overlapping emission wavelengths and thus distinguishable by FACS analysis would allow for a more precise measure of the rate of mating. Proteins that are tagged to the nuclei of *Mat $\alpha$*  and *Mat $\alpha$*  cells would allow the identification and exact number of haploids and diploids in each sample. FACS machines are also able to look at tens of thousands of events (cells) per sample, allowing for a much larger and thus potentially more accurate measure of the rate of reproduction. Additionally, taking samples more frequently (smaller time intervals) could result in clearer results.

Sporulation efficiency was also assayed and used as an indicator of mating ability, as meiosis is a prerequisite for mating. Furthermore, previously published results suggested differences in sporulation ability were correlated to levels of heterozygosity, and therefore potentially indicated differences in effective mating rates (Magwene et al., 2011). Sporulation efficiency was only notably different for Y12 (vineyard isolate) and YJM975 (clinical isolate), which both had relatively low rates of sporulation compared to the other strains in this study. Although sporulation is necessary for mating to occur, the relationship between the ability to sporulate efficiently and the *speed* of mating is unclear (Appendix C). Strains that are fast maters could be less efficient sporulators. This inverse relationship could be explained by a trade-off: a high rate of mating could indicate less pressure to produce many haploids (less efficient sporulation) in order to produce successors. Similarly, strains that produce a relatively large number of haploid spores (strong sporulators) might be less efficient at mating but have been selected to increase the opportunity for mating by increasing the number of possible maters. Alternatively, the control of mating and sporulation could be linked such that selection could not favor a higher efficiency of sporulation without also favoring a higher efficiency of mating (or vice versa), thereby indicating a positive relationship between sporulation and mating. Without further study it is difficult to interpret the sporulation data and the relationship

between sporulation and mating efficiency. If a finer measure of the rate of mating was developed (as discussed previously), a more complete understanding of the relationship between sporulation and mating could be determined, helping to elucidate the ways in which selection can act on the pathways of sexual reproduction in *Saccharomyces cerevisiae*.

## **Growth Rate**

In *S. cerevisiae*, the pathway regulating the cell cycle is well characterized (Wittenberg and La Valle, 2003) with the length to complete one cycle estimated to be about 90 minutes (Chen et al., 2004). Due to differential environmental pressures and thus selection, slight changes in the regulation of the cell cycle could result in changes in phase length.

The measures of the growth rate in synchronized and asynchronized cells suggest, in accordance with the presence of a G<sub>1</sub> checkpoint, that if there is variation between the strains, G<sub>1</sub> would be the variable phase of the cell cycle. There were significant differences between the strains in the proportion of cells in the G<sub>1</sub> phase of the asynchronous cultures. Strains that spend longer in G<sub>1</sub> are likely to be slower growers (take longer to proceed through the cell cycle) while strains with shorter G<sub>1</sub> phases should be faster growers. In order to verify this relationship, I analyzed the progression through the cell cycle of synchronized cultures. These exhibited no significant differences in the S and G<sub>2</sub> phases of the cell cycle during the time course and thus the length of these phases across strains appear to be maintained; however, these data were not what we expected to see from a synchronized culture and should be interpreted with caution.

The data from the FACS analysis of the length of each phase for each strain (Appendix C) is unclear. The length of the cell cycle of budding yeast is estimated to be about 90 minutes (Chen et al., 2004). Although some variation is expected and would be consistent with my predictions, the range in length of the cell cycle that I have observed is large and therefore doesn't seem likely. Some strains show data consistent with the estimated cell cycle length with the time for completion of the cell cycle around 90 minutes. However, some strains appear to complete the cell cycle in 30 minutes.

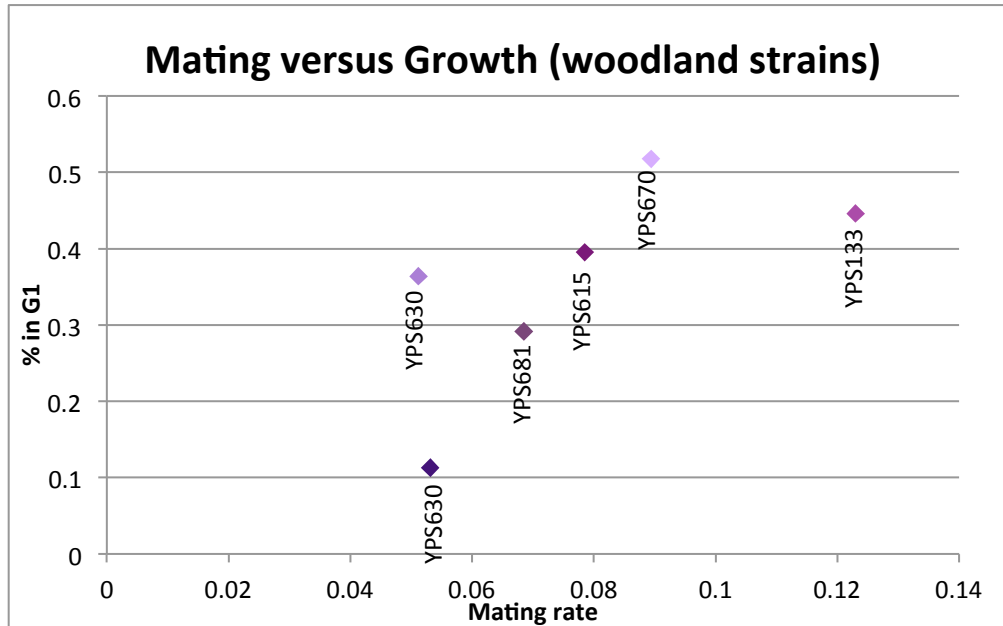
To improve the cell cycle data, using a treatment that allows for the incorporation of a DNA stain while the cells are dividing would result in a more exact measure of the S phase and thus the overall cell cycle. Haploids could be again be arrested in the G<sub>1</sub> phase and strained with propidium iodide. Upon release from arrest, bromodeoxyuridine (BrdU) would be incorporated into the DNA during synthesis. Time point samples could be taken, exposed to a fluorescent BrdU antibody, and analyzed using flow cytometry. Both total DNA content and newly synthesized DNA could be detected, allowing for the determination of the period of DNA synthesis (Rieseberg et al., 2001). As a result, the cell cycle length and doubling time for each strain could be better estimated and compared.

### **A Trade-off Between Growth and Reproduction?**

While the measures of reproduction and growth were not as precise as planned, to address the possible life-history trade-off, the relationship between the estimates of the relative measures of mating and growth that were obtained in the woodland populations of yeast were compared. Measures of the other non-woodland populations were incomplete and thus could not be included in this part of the analysis.

In this study, the slope of the line of best fit from the mating propensity assay was taken as the experimental measure of mating used for the trade-off analysis (Figure 12). A larger slope indicates a faster relative rate of mating. The percentage of cells in the G<sub>1</sub> phase, which was determined to be the variable phase of the cycle, was used as the relative measure of asexual growth. A higher percentage of cells in G<sub>1</sub> indicates that it is a prolonged phase and should therefore take longer for the yeast to complete the cell cycle. When plotted, an inverse relationship between mating and growth is suggested: strains that have a faster mating speed spent more time in G<sub>1</sub> and thus were slower growers (and vice versa). When the two non-woodland strains were included in the trade-off analysis (Appendix C), Y12 (vineyard isolate) also seemed to exhibit the same trade-off while SK1 (lab strain) seemed to be both a strong mater and grower. Since I'm specifically predicting convergence between strains from similar ecological niches, it is impossible to determine if this trend exists since I only had a complete set of data for a single lab and a single vineyard strain and thus could not identify a trend with a single

data point representing those populations. While these results are still very preliminary, they do suggest an overall pattern in the direction originally predicted.



**Figure 12.** The overall trend among the woodland strains when comparing the rate of mating (from the slope of the line of best fit for the mating propensity assay) and the proportion of cells in the G<sub>1</sub> phase of an asynchronous sample indicates a trade-off. Strains better at mating (higher mating rate) are slower growers (spend more time in G<sub>1</sub>) and faster growers are less efficient at mating.

To strengthen this result, using the finer measures of both mating and growth discussed would perhaps show suspected differences in strains and the expected inverse relationship. Additionally, expanding the analysis to strains outside of the woodland population might reveal whether the life-history strategy optimized by a strain of yeast is environmentally determined such that yeast occupying similar habitats experience convergent evolution. If genetically distinct strains that are found in similar habitats have similar relative measures and patterns of mating and growth, the evolution of this trade-off might be, as other trade-offs in *S. cerevisiae* have been demonstrated to be, niche driven.

The trade-off between growth and reproduction had not yet been explored in microbes, including *Saccharomyces cerevisiae*. This preliminary investigation suggests the presence of trade-off between two strategies for producing a successor generation. Further investigation and quantification of this trade-off between genetically distinct

strains of yeast from different environments as well as from the same environment would add to the growing body of research about the evolutionary history of *S. cerevisiae*.

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## APPENDIX A

Original Strain	Transformed Strain	Mating Type	Genotype	Assay
YPS133	YPS2067	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS133	YMZ10	Mata	$\Delta$ ho::natMX	Mating propensity
YPS615	YPS2057	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS615	YPS3326	Mata	$\Delta$ ho::natMX	Mating propensity
YPS623	YPS2066	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS623	YMZ45	Mata	$\Delta$ ho::hygMX	Mating propensity
YPS630	YPS2073	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS630	YMZ53	Mata	$\Delta$ ho::hygMX	Mating propensity
YPS670	YPS3060	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS670	YPS3340	Mata	$\Delta$ ho::natMX	Mating propensity
YPS681	YPS3137	Mata	$\Delta$ ho::kanMX	Mating propensity
YPS681	YPS3318	Mata	$\Delta$ ho::natMX	Mating propensity
Y12 a	YSG1	Mata	$\Delta$ ho::natMX, $\Delta$ ura3::kanMX	Mating propensity
Y12 a	YSG5	Mata	$\Delta$ ura3::kanMX, M309-pgal1-cox4-YFP	FACS mating propensity trial
Y12 a	YSG8	Mata	$\Delta$ ura3::kanMX, SLX8-YFP	FACS mating propensity trial
Y12 $\alpha$	YSG4	Mata	$\Delta$ ura3::kanMX, M304-pgal1-cox4-CFP	FACS mating propensity trial
Y12 $\alpha$	YSG16	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial
SK1 a	YSG2	Mata	$\Delta$ ho::natMX, $\Delta$ ura3::kanMX	Mating propensity
SK1 a	YSG9	Mata	$\Delta$ ura3::kanMX, M309-pgal1-cox4-YFP	FACS mating propensity trial
SK1 $\alpha$	YSG7	Mata	$\Delta$ ura3::kanMX, M363-pgal1-cox4-RFP	FACS mating propensity trial
SK1 $\alpha$	YSG15	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial
YJM975 a	YSG3	Mata	$\Delta$ ho::natMX, $\Delta$ ura3::kanMX	Mating propensity
YJM975 a	YSG6	Mata	$\Delta$ ura3::kanMX, M363-pgal1-cox4-RFP	FACS mating propensity trial
YJM975 a	YSG10	Mata	$\Delta$ ura3::kanMX, M309-pgal1-cox4-YFP	FACS mating propensity trial
YJM975 $\alpha$	YSG17	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial
UWOPS 05 217.3 a		Mata	$\Delta$ ura3::kanMX	Growth assay
UWOPS 05 217.3 a	YSG11	Mata	M309-pgal1-cox4-YFP	FACS mating propensity trial
UWOPS 05 217.3 $\alpha$	YSG18	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial
UWOPS 05 227.2 a		Mata	$\Delta$ ura3::kanMX	Growth assay
UWOPS 05 227.2 a	YSG12	Mata	M309-pgal1-cox4-YFP	FACS mating propensity trial
UWOPS 05 227.2 $\alpha$	YSG19	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial
UWOPS 83 787.3 a		Mata	$\Delta$ ura3::kanMX	Growth assay
UWOPS 83 787.3 a	YSG13	Mata	M309-pgal1-cox4-YFP	FACS mating propensity trial
UWOPS 87.2421 a		Mata	$\Delta$ ura3::kanMX	Growth assay
UWOPS 87.2421 a	YSG14	Mata	M309-pgal1-cox4-YFP	FACS mating propensity trial
UWOPS 87.241 $\alpha$	YSG20	Mata	$\Delta$ ura3::kanMX, IFP1.4 TOPO	FACS mating propensity trial

**Table 2.** All strains used in this experiment.

## APPENDIX B

### Details on the Rate of Reproduction Assay using Fluorescent Proteins

The plasmids containing the fluorescent proteins utilized URA3, which is necessary for the synthesis of uracil, as a selectable marker. All strains needed to be engineered to contain a *ura3* deletion. All strains from the Sanger collection (Cubillos et al., 2009) were  $\Delta$ ura3::KANMX. Genomic DNA from strain YJM975 was used as a template and the *ura3*::KANMX cassette was amplified for use in transformations of the woodland strains. PCR was performed using a standard protocol for Phusion (New England Biolabs, Inc) with an annealing temperature of 72°C and 55°C extension time and sequence specific primers (URA3-for: CAACTGCACAGAACAAAAACCTGC; URA3-rev: GTTTTTTAATCGATGAATTCGAGCTCG). All strains were then transformed using PCR product with a standard LiAc protocol (Gietz et al., 1992) and selected on YPD plates supplemented with kanamycin (final concentration of 40 µg/mL).

### *Fluorescence Proteins Targeted to the Mitochondria*

The M309-pgal1-cox4-YFP plasmid (generously provided by Hiromi Sesaki, Johns Hopkins University) was first transformed into TOP10 chemocompetent *E. coli* cells (Invitrogen). Competent cells were mixed with 1 µL M309 plasmid and allowed to stand on ice for 30 minutes. The cells were then heat shocked at 42°C for 45 seconds and 450 µL of room temperature SOC medium was added. Samples were incubated at 37°C for 60 minutes and then plated on LB plates containing carbenicillin (final concentration of 50 µg/mL), allowing only the growth of colonies that had been successfully transformed with the M309 plasmid. Successful transformants were used to inoculate liquid medium and subsequently harvest the plasmid using the QIAprepSpin Miniprep kit.

All Mata  $\Delta$ ura3::KANMX strains were transformed with the plasmid containing the M309 plasmid. Each strain was grown in YPD; once in log phase (exponential growth), cells were harvested and resuspended in 800 µL of LiAc/TE and incubated for 30 minutes at 30°C. Cells were then centrifuged, the supernatant was removed, and the cells were mixed with 5 µL of ssDNA, 2 µL of M309, and 200 µL of LiAc/TE/PEG with

DTT. Cells were incubated at 30°C for 30 minutes, heat shocked at 42°C for 14 minutes and finally plated on selective plates (ura- plates) and allowed to grow at 30°C overnight. Successful colonies were re-streaked on selective plates and, after growth in the presence of galactose, were viewed under a microscope with UV light to ensure the fluorescence was visible.

The same procedure was repeated for sample Mata and Mat $\alpha$  strains using the M363-pgal1-cox4-RFP and M304-pgal1-cox4-CFP plasmids (generously provided by Hiromi Sesaki, Johns Hopkins University). Additionally, Y12 was transformed with the plasmid SLX8-YFP (generously provided by Oliver Kerscher, College of William and Mary), which is localized to the nucleus.

#### *Infrared Fluorescence Protein*

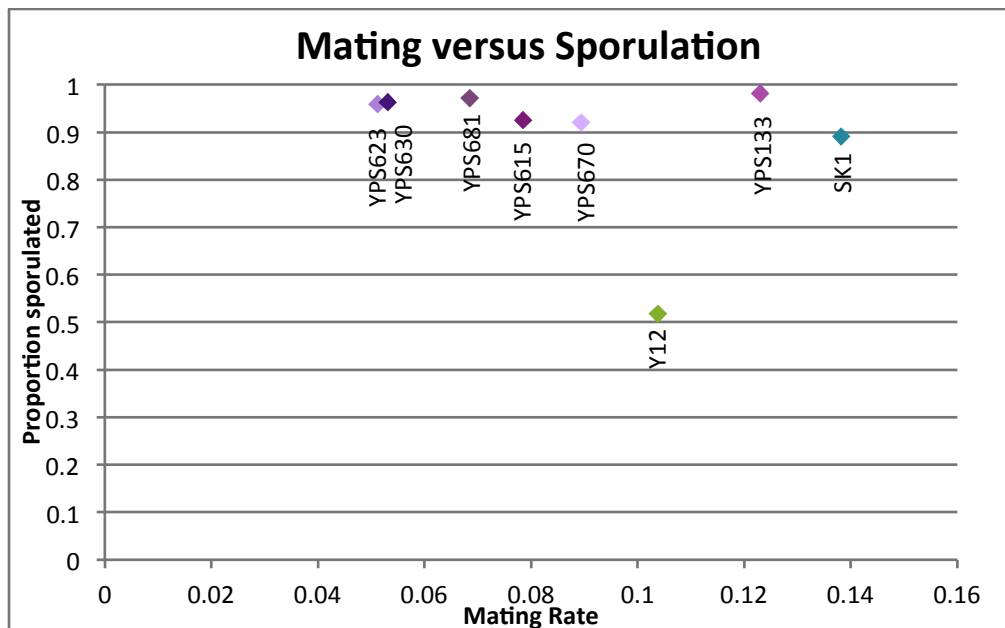
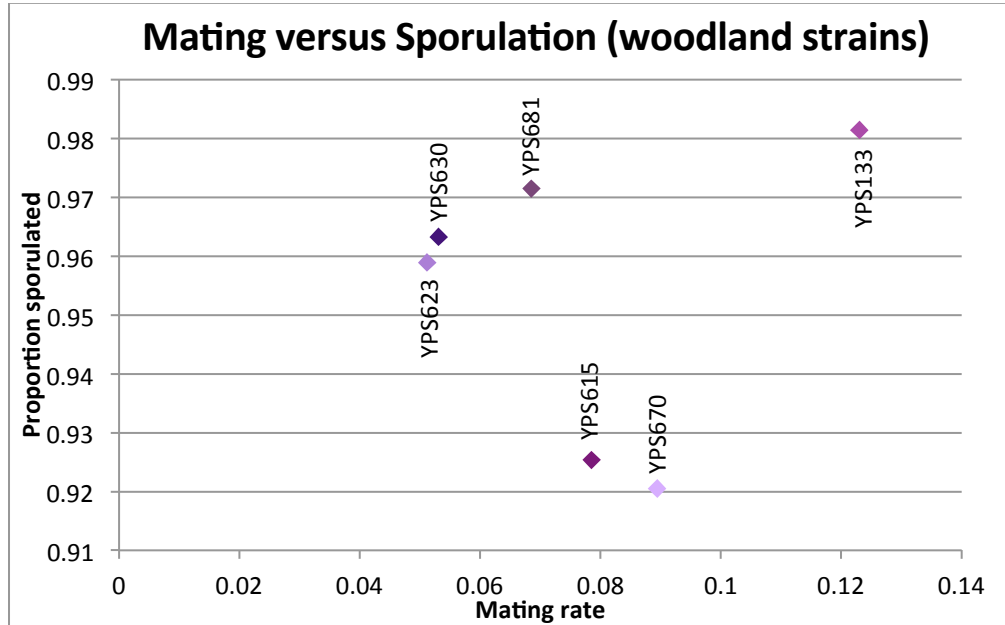
The pGZ108 plasmid (generously provided by Zhen Gu, Massachusetts Institute of Technology, Boston, MA) contains the IFP1.4 gene (Gu et al., 2011). After the *E.coli* containing the plasmid was grown in liquid LB media containing kanamycin, the DNA was extracted using a QIAprepSpin Miniprep kit. PCR with elongase and sequence specific primers (forward- 5' ATG GCT CGG GAC CCT CTG C; reverse- 5' CAT TTA TAC AGC TCG TCC ATT CCG GCT TC) were used to amplify the desired IFP1.4 gene while creating A overhangs. The PCR product was then transformed into a pYES2.1/V5-His-TOPO vector using the TOP10 One Shot Chemical Transformation protocol and plated onto LB plates containing carbenicillin. The plasmid was then extracted from the successful colonies using the Hi-Speed Plasmid Extraction Kit (IBI Scientific). The presence of the IFP1.4 gene was verified via PCR with the same sequence specific primers.

The  $\Delta$ ura3::KANMX Mat $\alpha$  strains were transformed with the IFP1.4 TOPO plasmid using the same protocol as described above. To screen for successful colonies, the transformation mix was again plated on ura- plates. The plasmid DNA from successful colonies from each strain was extracted using a E.Z.N.A Yeast Plasmid Kit (Omega Bio-Tek) to verify, via PCR with gene specific primers, that the strain contained the correct gene.

## APPENDIX C

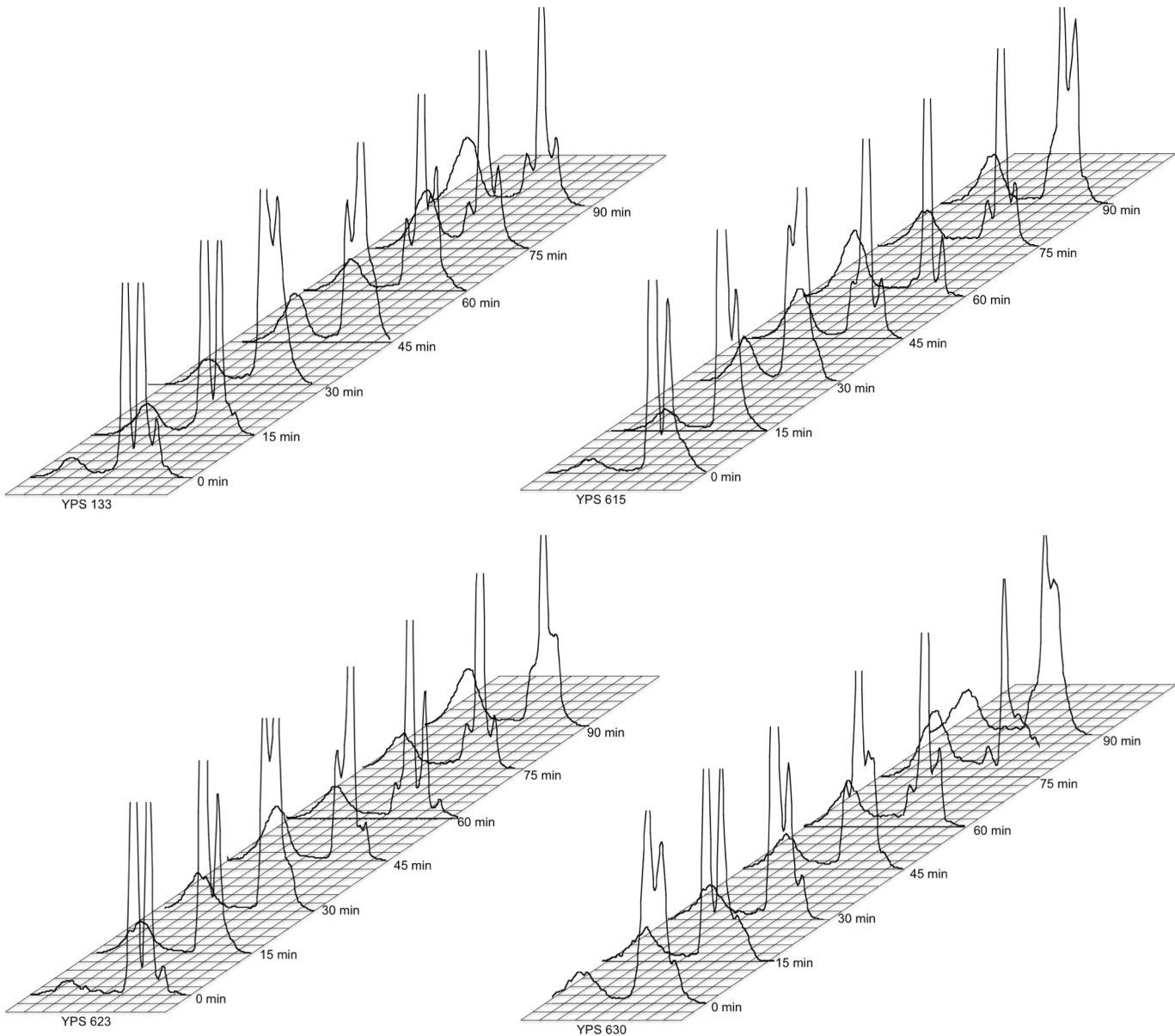
### Rate of Reproduction

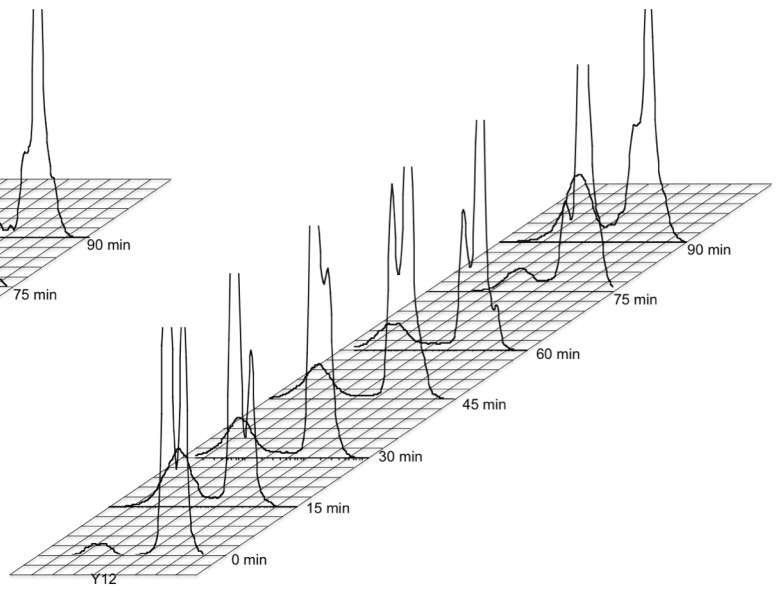
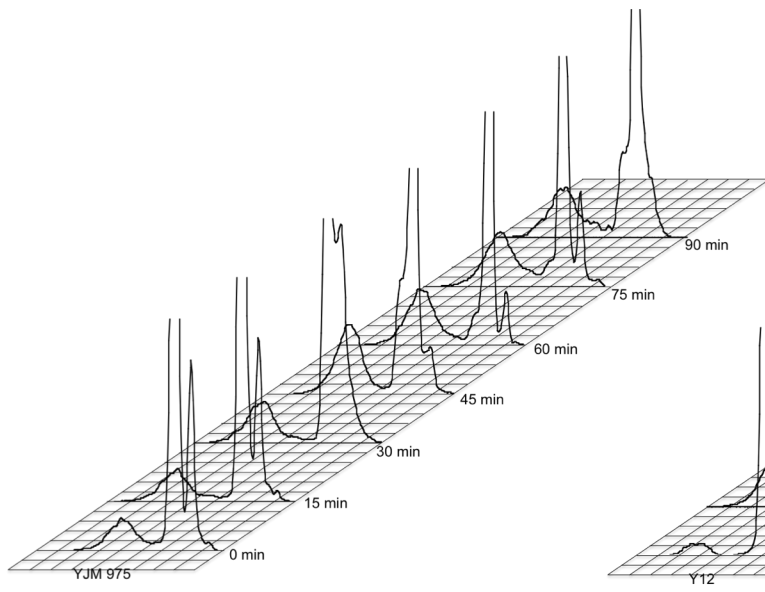
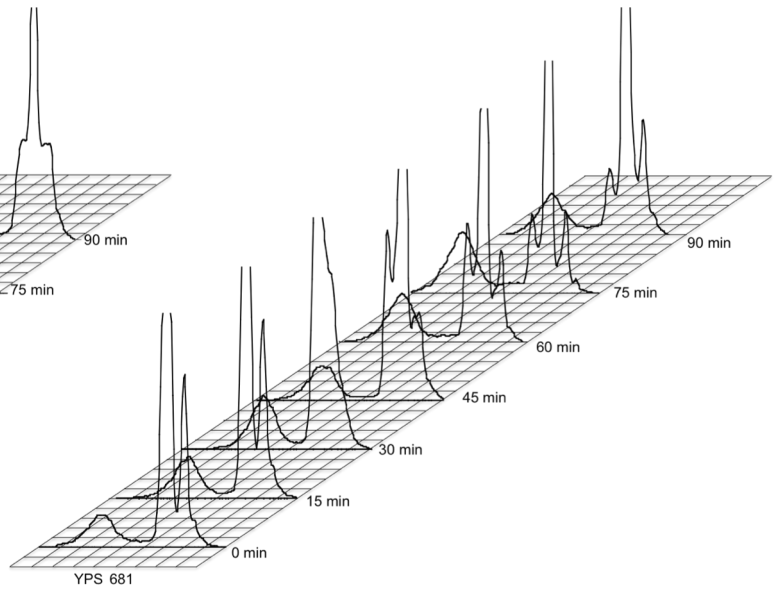
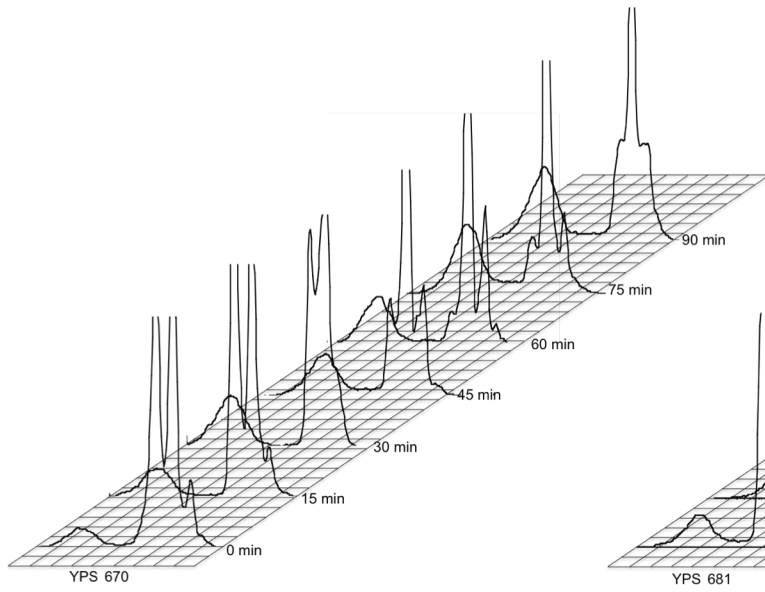
The proportion of sporulated cells was plotted against the rate of sexual reproduction for evidence of a relationship. No obvious pattern exists either in the woodland population or in the expanded environment strains.



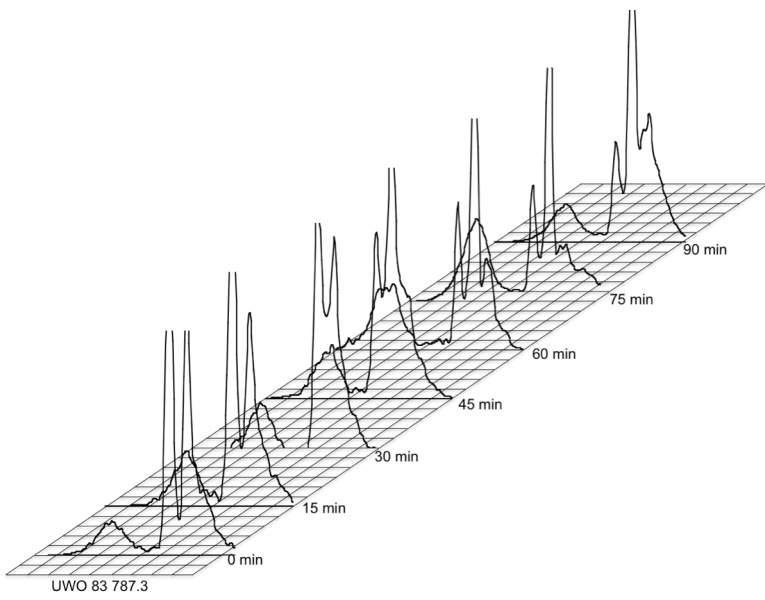
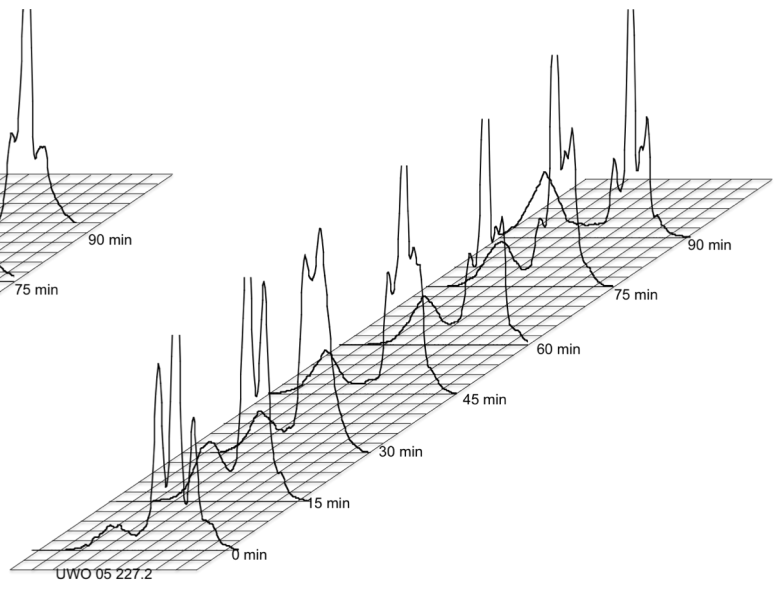
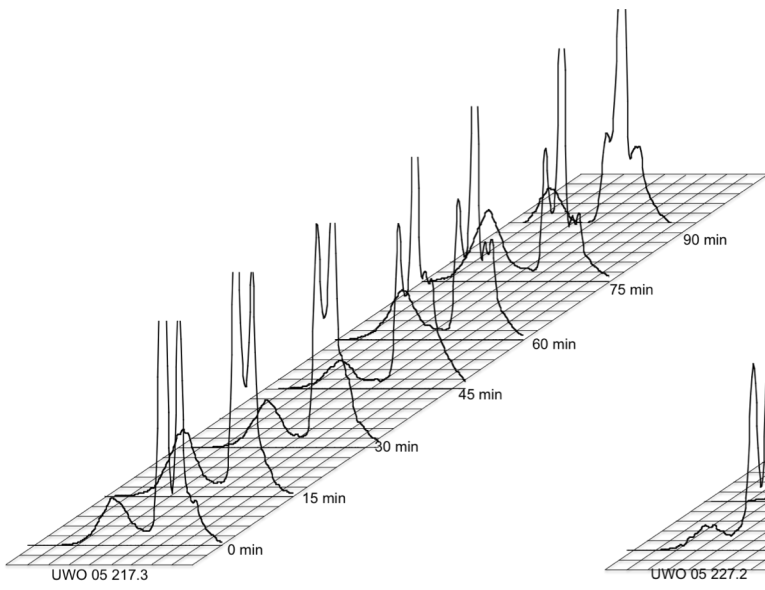
## Growth Rate: Synchronous Cells

Each plot shows the raw data from the Flow Cytometer for the cell cycle progression for each strain. Strains were initially arrested in G<sub>1</sub>. Once freed from G<sub>1</sub>, samples were taken every 15 minutes. The x-axis represents the amount of fluorescence while the height of the curve indicates the amount of cells.









### A Trade-off Between Growth and Reproduction?

The following graph shows the relationship between growth and mating for the six woodland strains, SK1, and Y12.

