The hidden costs of rapid adaptation: experimentally assessing the effects of standing variation on the pace and trajectory of evolution

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ABSTRACT

As the planet changes at an alarming rate, there is a great need to understand why some populations are better equipped to rapidly adapt to their new environment than others. Many factors contribute, but populations are ultimately limited in their pace by their genetic makeup—they either have variants that allow them to survive or they do not. But, in the race to adapt, all sources of variation are not equal, and standing genetic variation is theorized to be of the most benefit in contributing to rapid adaptation. Here I explore the role of standing variation, both in a population's ability to adapt at a rapid pace and in the potential long term evolutionary consequences that occur as a result. My work confirms expectations that increased standing variation in a population allows for a faster rate of adaptation, but although these populations are able to succeed in the short term, but this achievement comes at a significant cost to long term viability. All populations, across all experiments, that utilize standing variation as the genetic basis for rapid adaptation lose the ability to undergo sexual recombination, and therefore lose an important mechanism for maintaining variation in the long term. I begin by determining how the amount of standing variation present in a population correlates to the timing and rate of a successful adaptive response to a stressful environment. I assess how this result is intertwined with loss of sex and explore the mechanism for that loss. Then, I explore how the dynamics of the system change if the environmental shift occurs gradually rather than as a dramatic climactic event. Finally, I compare the variety of evolutionary strategies that develop in populations that began with standing variation versus mutation as their genetic substrate and evaluate their potential for success in the long term. Taken together, these results present a different picture of the role of standing variation than might be assumed. It does indeed allow for rapid adaptation, but the increased degree of genetic variation is not an evolutionary panacea and may send populations down evolutionary trajectories that are short-sighted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Research motivation

The ability of a population to rapidly adapt to its environment is often the difference between continued existence and extinction. Lineages that are able to evolve and change as the environment around them changes are the ones that are able to withstand the test of time. In a world where human activity is increasing the global rate of change at an alarming rate, understanding the dynamics of rapid adaptation is of the utmost importance.

Rapid adaptation can be dictated by many different factors, but a population's innate capacity for adaptation will be determined by its genetic variation. This was clearly and effectively stated by R.A. Fisher in 1930 in his fundamental theorem of natural selection: "The rate of fitness increase in any organism at any time is equal to its genetic variance in fitness at that time" (Fisher 1930). Genetic variation is the key to adaptation by natural selection, and therefore it stands that the more genetic variation present in a population the more quickly the pace by which evolution can proceed. This thought, while perhaps seeming intuitively obvious, has been often observed in natural populations but seldom tested in experimental ones. Is increased genetic variation always the key to a higher rate of adaptation or can populations that start with minimal variation also find routes to rapid success?

Additionally, is there a price to rapid success? One population may find a way to adapt to a changing environment more quickly than another, in which case it may seem as though the faster population has found a more adequate solution to an evolutionary puzzle. However, these differences in rate of adaptation bring with them separate consequences, and while rapid adaptation certainly brings benefits in regard to population size, there may be problems that come as well. Rapid adaptation is certainly better than no adaptation, which will ultimately result in extinction, but there can be benefits to taking a more leisurely route toward increasing population fitness. Do tradeoffs exist that might place rapid adaptation at odds with long term strategies for evolutionary success?

These questions are what this dissertation seeks to evaluate. By constructing populations with different initial genetic makeups and subjecting them to different environmental stressors, both the rate of adaptation and the potential consequences of that rate can be determined. The aim here is not to arrive at universal answers to these questions, but to show that situation is more complex than what the reader might presume it to be, and to show that assumptions—like that of more genetic variation always being better—should always be tested.

Definitions and experimental system

This work is primarily concerned with how the amount of genetic variation present in a population affects the pace at which that population is able to adapt. Before going further, it is necessary to define how this dissertation will be describing genetic variation. The ultimate source of all variation is mutation, but a distinction will be made hereafter between mutations that are accumulated prior to an environmental shift and those acquired after the environment has already changed. The former category will be referred to as standing genetic variation, or simply “standing variation”. The latter category will be referred to as *de novo* mutations, or simply “mutations”. One source of genetic variation is pre-existing in an organism’s genome and another is gained after an event of interest that changes the living conditions for the organism. The reason for this distinction and the differences between the two types of variation will be made apparent throughout the following chapters. The environmental shift used throughout these experiments is a move from ideal growth conditions to warmer growth conditions, but the phrase itself can be meant to be anything that causes a population to experience a difference in selection pressure.

The phenomenon that this dissertation seeks to understand—that of how some populations are able to adapt more rapidly than others—is universal to all populations, both natural and laboratory, and will be explored here in brewer’s yeast, *Saccharomyces cerevisiae*. The use of a microbial system allows evolution to be observed in a much shorter time frame than more complex organisms, which means that it is easier to see the effects of adaptation in both the short and long term. This particular yeast system offers additional benefits beyond just ease of care and quick generation times, and those will be detailed in the following chapters.

Chapter outline

This dissertation will assess the relative importance of standing variation and *de novo* mutations in contributing to rapid adaptation. To do so, populations with differing initial genetic makeups will be constructed, they will experience different rates of environmental shifts, and their rate of adaptation, as well as the manner of adaptive response, will be measured.

- Chapter 1 presents a comparison of adaptative responses when the substrate for evolution is either standing variation or new, *de novo* mutations. The experiments from this chapter illustrate that standing variation does allow for more rapid adaptation to an environmental stressor. It also describes an unexpected and potentially negative consequence associated with this rapid adaptation, specifically the loss of sexuality. Genetic analyses and viability assays are presented to explain possible mechanisms for this loss. Finally, the conditions that lead to this derived asexuality in this experiment are compared with similar conditions and consequences seen in a wide variety of organismal taxa.
- Chapter 2 assesses different conditions wherein standing variation may be of relatively greater or less significance to variation from *de novo* mutations. Here, the pace of the environmental change is altered to either be a dramatic, sudden shift or a gradual, incremental move towards the same level of selection intensity. This chapter reviews predictions from solely theoretical models related to the relative importance of standing variation in different scenarios of environmental change. The experiments here describe both the likelihood of success of populations with different initial genetic makeup and the probable routes by which they may achieve success. By using a real world system established with the same parameters as the theoretical models, this chapter is able to comment on the robustness of these models. The results presented here both support and

contradict model predictions, which stresses the importance of testing theories empirically.

- Chapter 3 focuses on the long term success of different evolutionary strategies that rely on either standing variation or *de novo* mutation. This chapter presents results from experiments where populations grew for more than 500 generations, and it describes the variety of evolutionary strategies that arose in populations as a result of being dependent on each type of genetic variation for adaptation. Adaptation from both standing variation and *de novo* mutations presents tradeoffs in terms of evolutionary success. Populations that began with mutations as their genetic substrate showed more variation in strategy and in success, with strategies that are both most and least likely to lead to long term evolutionary viability coming from this starting genetic makeup. Conversely, strategies from high standing variation populations initially perform well, however, their evolutionary outlook is not as optimistic if the environmental conditions were to shift in the future.

Taken together, these chapters show that the role of standing variation in adaptation is not as straightforward as might be assumed. More variation is often thought to consistently be a net good, but these results show that the dynamic is more complex than that, with standing variation sometimes leading to the adoption of strategies that prioritize short term success over long term survivability.

CHAPTER 1

Standing variation allows for rapid adaptation at the cost of sexual reproduction

ABSTRACT

Rapid adaptation to strong selection pressure can be facilitated by high levels of standing variation and sexual recombination. Recently, aneuploidy has also been revealed to be a mechanism for achieving a fast rate of adaptation. However, aneuploidy is known to have large costs. Here, we show a system where the costs and benefits of aneuploidy are explicit. Yeast populations that contain different levels of standing variation are introduced to a novel stressor and those populations with high variation use aneuploidy as a strategy to rapidly adapt. However, these populations were originally capable of sexual reproduction and their rapid adaptation comes at a cost to their ability to produce viable spores. We show that standing variation is of benefit in a strongly selective environment, but also that it can lead to the loss of sexual recombination. The loss of sex seen here connects this work to the larger pattern of derived asexuality seen across many eukaryote lineages, including natural populations. Our work shows the utility of experimental systems in adding to our understanding of complex evolutionary patterns.

1.1 INTRODUCTION

The need to understand the evolutionary processes that shape a population's ability to adapt has never been more important than at the current moment. Disentangling the factors that allow for rapid rates of evolutionary change is of particular urgency, given the rapid rate of environmental change. Lande & Shannon argued that genetic variation "can play the decisive role in allowing a population to persist and adapt in a changing environment" (1958). Genetic variation, though, is not a monolith and when used as the source for adaptation, different types of variation can have different consequences. The degree to which the genetic basis of an adaptation affects evolutionary trajectory in both the short and long term warrants further study.

When adapting, populations can wait for a new beneficial variant to arise via mutation or they can rely on a pool of pre-existing genetic variation. This variation, standing variation, is thought to be especially valuable when selection is strong and a rapid rate of adaptation is required to meet environmental challenges (Barrett and Schluter 2008). There are two reasons standing variation is thought to be more important when change is rapid—immediacy of use and initial frequency. Standing variants are already present in a population and therefore can be immediately accessed, whereas *de novo* mutations are random and a population could be left waiting a significant amount of time before one occurs. Additionally, new mutations necessarily enter a diploid population at a frequency of 1/2N (Kimura 1983). However, adaptive standing variants can be present in populations at much higher frequencies, which may give these populations a critical head start in the race to adapt. This higher frequency of standing variants is possible due to the dynamics of how they originate in a population. They enter a population as a new mutation but are initially neutral or nearly neutral with respect to fitness and only become beneficial when there is an environmental shift. This means that a standing variant's frequency is determined by drift and time; the longer the allele has to segregate in the population, the more potential it has to reach a higher frequency.

Standing variation may be of relatively less importance in asexual populations, as these organisms tend to have higher population sizes and thus larger reservoirs for potential new mutations. Recently, work has also uncovered the aneuploidy as a potentiator for rapid rates of change in microbial populations. Aneuploidy is typically thought of in a negative context since it throws off genomic equilibrium and often has a strongly detrimental impact on fitness in complex organisms (Siegel and Amon 2012). However, evidence has shown that aneuploidy can be an effective adaptive mechanism in certain situations (Wertheimer *et al.* 2016; Gilchrist and Stelkens 2019). In experimental systems, aneuploidy has been shown to be one possible solution in the race to adapt to a changing environment due to its large and immediate effects (Gerstein and Berman 2015; Berman 2016). Different stressors can cause chromosomal transmission fidelity to fail in different ways, resulting in a variety of karyotypes on which selection can act. In this way, different aneuploidies in an asexual population can themselves act as standing variants and facilitate very rapid adaptation.

Both asexual and sexual organisms can also adapt rapidly from standing variation when regulatory networks break down and allow cryptic variation to be revealed. Chaperone proteins can mask the effects of genetic variants, leaving them as neutral standing variants in a population (Rutherford and Lindquist 1998). When this buffering ability of chaperone proteins is compromised, the effects of previously hidden variation become apparent. Environmental stress has then been shown to allow chaperone proteins to act as capacitors for evolutionary change (Jarosz and Lindquist 2010). The inhibition of chaperones has also been shown to mediate a transition to aneuploidy in response to a selective environment (Chen *et al.* 2012a, b; Siegal and Masel 2012). Regulatory changes due to chaperone inhibition leads to rapid evolution in both animals and microbes alike and does not depend on the mode of reproduction of the population.

But mode of reproduction does play a role in adaptation to a changing environment, and the benefits of sexuality versus asexuality have long been contested (Williams 1975). Populations that reproduce sexually have more avenues for generating standing variation, which should give them a greater chance to weather changes, or to paraphrase J.T. Bonner, sex is an adaptation to future changes (1958). And sexual reproduction brings benefits when those changes are occurring rapidly, in particular (Crow 1992). Sex has been theorized to speed adaptation, and experiments have repeatedly shown this to be true (Rice and Chippindale 2001; Colegrave 2002; Goddard *et al.* 2005; McDonald *et al.* 2016). The advantages of sexual recombination are well-documented (reviews: Rice 2002; Barton 2009), and its widespread presence and persistence over time speak to its profound evolutionary advantages (Crow 1994). What benefit, then, would there be in losing the ability to sexually recombine once one has already established it? However, many times and over a broad range of diverse taxa, sexuality has been lost (Neiman *et al.* 2014; Jaron *et al.* 2021). The advantages of derived asexuality and its persistence are still under scrutiny, but one possibility is that the transition to asexuality happens as a consequence of exposure to a strongly selective environment (Glesener and Tilman 1978).

But this same scenario, one of a large change in selection pressure, is also when standing variation is thought to be of the most importance. This, then, raises the question of what possible relationship exists between standing variation and derived asexuality. Are they merely both related to strong selection in independent ways, or is there an interplay between the two? Standing variation's relationship to sex is more accessible. Sexual recombination acts to increase standing variation by recombining alleles onto different genetic backgrounds. Thus, it may seem as though sex and standing variation go hand in hand, but do they complement each other, or is their dynamic more complex?

Here we present a system where the two are opposed—where high standing variation aids rapid adaptation but is directly correlated to the loss of sex. We use brewers' yeast, *Saccharomyces cerevisiae*, which is capable of reproducing both asexually or sexually. Additionally, our yeast have been evolved to be multicellular rather than unicellular. The benefit here is that with increased complexity comes an increase in tradeoffs, and therefore these yeast are more likely to be sensitive to changes and will allow the effects of selection to be more readily seen. As with other yeast experiments, we see the sudden adoption of aneuploidy as a mechanism for rapid adaptation. But the same karyotype variations that give an immediate fitness benefit have the negative consequence of producing a chromosome imbalance that disrupts normal meiosis. We see this effect only in populations that initially contain high levels of standing variation. Low standing variation populations maintain the ability to undergo sex, but they do not show a quick adaptive response to the selective environment and their populations significantly decline. This confirms the idea that standing variation is important in scenarios of sudden environmental change, but offers a caveat to its benefits. The loss of sex is significant, but not entirely uncommon. In other systems, parthenogenesis is often seen in conjunction with karyotype variations, similar to what is shown here. This work may aid our understanding of why sex is lost in wild populations and shows the benefits of using experimental evolution to explain natural phenomena.

1.2 METHODS

1.2.1 ***Strains and media***

The yeast used in this study is an evolved multicellular lineage of *Saccharomyces cerevisiae*. This lineage was derived from a unicellular ancestor (Y55 strain) via selection for settling ability (Ratcliff *et al.* 2012). Under this regime, yeast daughter cells do not break away from the mother cell after reaching maturity, and as a result multicellular clusters are formed. Settling selection was continued for 14 weeks, allowing variation to accumulate as populations continually evolved to a constant pressure. This generated a population containing high variation from which experimental populations were derived. Two different types of starting populations were generated: one with high standing variation and one with low standing variation. There were 16 populations created for the low standing variation condition. These populations were created by dilution plating and selected isolated colonies from the original high variation population. Complementing these were 16 populations that contained high standing variation. Eight were 1% of original yeast population, and thus were a good proxy for sampling all of the variation present in the original population. Eight were 0.1% of the population. All populations were maintained in 10mL of YPD (per liter distilled water: 10g yeast extract, 20g bacterial peptone, and 20g dextrose) in 25mm × 150mm glass culture tubes.

1.2.2 *Control and experimental conditions*

All 32 populations were subjected to settling selection throughout the entire experiment. A brief description of this protocol: 1mL of cell culture was pipetted into a 1.5mL microcentrifuge tube and allowed to sit for 10 minutes. The top 900μL of culture was then carefully pipetted off and discarded. The bottom 100μL of culture was thoroughly mixed and transferred to new media. Populations were grown in a 30°C incubator shaking at 250 rpms. Transfers were made every 24 hours.

To ensure equal starting population growth, 32 populations were grown for 48 hours at 30°C, then 100μL was transferred to new media and grown for 24 hours. Experimental populations were then transferred and placed in a 37°C incubator shaking at 250 rpms. These populations were transferred every 24 hours with settling selection and returned to the 37°C incubator. Control replicates of all 32 populations were maintained throughout the experiment at 30°C.

1.2.3 *Scoring sporulation efficiency*

Yeast reproduces sexually by means of sporulation. This typically occurs when conditions are too stressful for normal growth, for example, in low nutrient environments. Diploid yeast undergo two rounds of meiosis to produce four haploid spores. The mother cell then collapses around these spores and forms an ascus. The spores then either conjugate within the ascus or the ascus can be broken down to release spores for outcrossing. Sporulation assays were done at the beginning of the experiment before exposure to heat stress, in the middle at five transfers, and at the end after ten transfers. For each population, 1mL was transferred to 10mL sporulation media (per liter distilled water: 20g potassium acetate, 2.2g yeast extract, 0.5g glucose, 870mg amino acid complex) and grown for 48 hours at 37°C, shaking at 250 rpms, in 25mm × 150mm glass culture tubes. One-tenth dilutions were made and observed under a light microscope. Percent sporulation was calculated as the fraction of cells present that showed any spore development, including dyads, triads, and tetrads, all counted equally as forms of sporulation. Approximately 200-400 cells were assessed per slide; three technical replicates were made per population.

Spores were then tested for viability. 50μL of the sporulated culture was heated at 55°C for two minutes using a thermocycler. This temperature should kill adult yeast cells but leave spores intact, so any amount of growth post-heat kill will be from spores. Additionally, this will test if improperly formed spores are able to develop into normal adult cells. After the heat kill, the samples were transferred to the normal growth conditions described above and population size was measured.

1.2.4 *Ploidy detection via whole genome sequencing*

Samples from high and low standing variation populations were sequenced to determine ploidy. Dilution plates were created from each population and grown for 24 hours. High standing variation populations produced both multicellular and unicellular colonies. These were isolated and a colony of each type were selected for sequencing. Low standing variation populations only produced multicellular colonies, so only one colony was sequenced. Samples were sent to the Microbial Genome Sequencing Center (MiGS) in Pittsburgh, PA. There, DNA was extracted, Illumina libraries were created for 151bp paired-end reads, and samples were sequenced via NextSeq 2000 platform. Adapters were trimmed by MiGS. Reads were assessed for quality via FastQC. Reads were mapped to the Y55 reference genome using the BWA-MEM alignment tool from BWA v0.7.17. Several functions were used from Samtools v1.12. *Fixmate* was used to add mate coordinates, *markdup* to remove duplicate alignments, and coverage depth was calculated using *depth* for all positions of the reference genome.

1.2.5 *Statistical analysis*

All analyses were performed and plotted in R v4.0.3. Individual treatment groups were fit with separate models: linear for the control group and log linear for experimental groups. The model for high standing variation populations was fit starting at the point of population increase, transfer 2. All data were fit for low standing variation. All models were fixed-effect and fitted using the glm function. Models were compared using AIC to distinguish among a set of possible models describing the relationship between population size (OD600) and transfer number, standing variation level, and individual sample. Only transfer number was found to be a useful factor when separating by standing variation level. Significance was tested via ANOVA. Change in sporulation ability over time within the same treatment was determined by Welch’s t-test. Coverage for ploidy plots was calculated as the ratio of coverage of the evolved samples to their individual ancestral samples. Ratio was then $\log_2$ converted and plotted as position in chromosomes.

1.3 RESULTS

1.3.1 *Amount of initial standing variation correlates directly to rate of adaptation*

All populations that initially contained high levels of standing variation showed signs of rapid adaptation to the novel stress, while all the low standing variation populations suffered in the heat (Figure 1.1: blue points and yellow points, respectively). Upon introduction to heat stress, all populations, regardless of standing variation level, experienced depressed population sizes as measured by optical density (OD600). Populations that contained high levels of standing variation experienced this initial dip but then an almost immediate increase in growth. By day three, population sizes began to rise. After four days, these populations all had a growth level greater than or equal to the growth level seen before introduction to the heat stress. Over the next few days, these populations continued to grow in size, eventually achieving a maximum size that was significantly higher than their initial state ($F = 302.3$, $p < 2.2e-16$). This pattern was remarkably consistent for every population that contained high amounts of initial variation. Low standing variation populations showed the opposite pattern, showing steady decline until day five, and then maintaining this population size. ($F = 260.7$, $p < 2.2e-16$). Control populations, those kept at 30°C growth conditions, did not show any decline or improvement over the ten transfers ($F = 0.127$, $p = 0.783$).

Table 1.1. AIC values for different models. High and low standing variation populations were modeled separately. TRAN refers to transfer number and IND refers to sample number.

Model	**df**	**AIC**	**Δ AIC**
LOW: TRAN	3	-53.26	0
LOW: TRAN + IND	18	-25.35	27.92
LOW: TRAN * IND	33	2.91	56.18
HIGH: TRAN	3	-148.39	0
HIGH: TRAN + IND	18	-120.10	28.28
HIGH: TRAN * IND	33	-92.91	55.48

The population growth in the high standing variation populations is driven largely by the presence of a unicellular phenotype. All the yeast in this experiment began as multicellular, but populations that did well under stress all developed a unicellular phenotype that coexisted in the population with the multicellular phenotype (Figure 1.2). The multicellular phenotype occurs when daughter cells do not break off from the mother cell, resulting in a snowflake shape (Figure 1.2A). When plated, these colonies tend to be large and have a wrinkled appearance (Figure 1.2B). After exposure to heat multicellular snowflakes and unicellular cells can be seen (Figure 1.2C). When plated, the unicellular colonies are smaller and smoother than the multicellular colonies (Figure 1.2D). The unicellular individuals are a wide range of sizes, which is typical of aneuploid cells.

In all populations with initially high standing variation, multicellular and unicellular individuals were present after three transfers. This presence showed a similar increase from transfer three to five in all populations. The appearance of the unicellular individuals in the populations appears to be the primary driver of the increase in population size. Multicellularity is the ancestral trait for these populations, and the transition to unicellular individuals is consistent across all populations that adapt to have high population sizes under heat stress. The multicellular phenotype was still present in all populations and was not completely replaced by the unicellular phenotype.

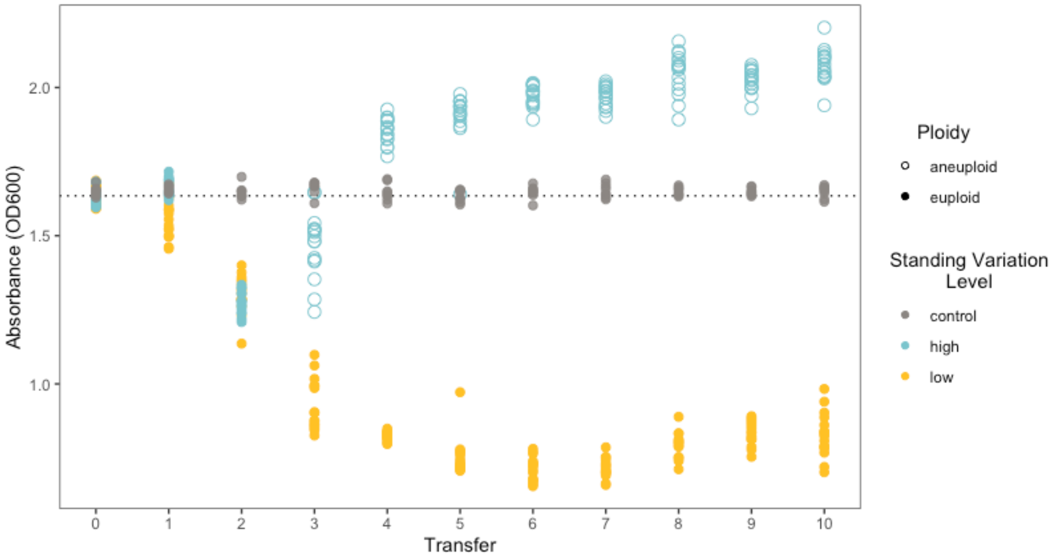


Figure 1.1. Population size over time. Points represent replicate populations (32 total). Increased population size correlates directly to level of standing variation and presence of aneuploidy. Aneuploids are denoted by an unfilled circle and start at transfer 3. Dotted line represents the average OD600 of all populations at the start of the experiment.

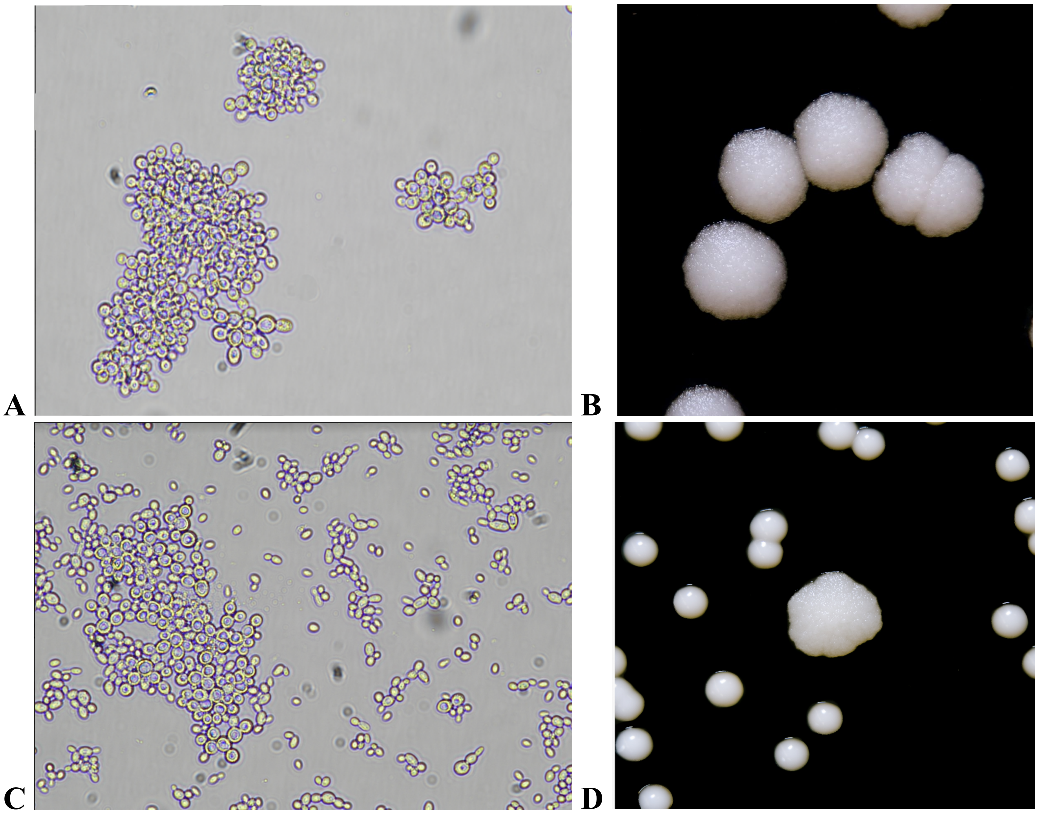


Figure 1.2. Photos of a high standing variation population. Only multicellular phenotypes are present in A & B. Multicellular and unicellular phenotypes are present in C & D. A) Microscope photo of starting population; B) Plate photo of starting population, wrinkly (multicellular) colonies are present; C) Microscope photo after introduction to heat stress; D) Plate photo after introduction of heat stress, smooth (unicellular) colonies and wrinkly colonies are present.

1.3.2 *Rapid adaptation leads to an inability to sporulate in high standing variation populations*

Coinciding with their increase in population size, the high standing variation populations lose the ability to sporulate. Prior to selection by heat stress, all populations were extremely efficient sporulators (Table 1.2). Normal sporulation in yeast involves the production of an ascus that contains four spores, called a tetrad. Multicellular clusters are capable of maintaining multicellularity and producing normal tetrad spores (Figure 1.3A). The low standing variation populations retained their ability to sporulate at nearly 100%

efficiency with no change throughout the experiment (t = -0.77, p = 0.446). High standing variation populations showed almost complete failure in their ability to properly sporulate after the introduction to heat stress (t = -165.97, p = 2.2e-16). These populations had low sporulation rates (mean = 2.71%, sd = 3.95%), produced incomplete tetrads (dyads or triads), or showed signs of loss of cellular structure and failure to replicate when grown in sporulation media (Figure 1.3B & C). The viability of the spores was assessed after heating the sample to a degree where only spores would remain. For the control and low standing populations, the spores went on to grow normally, but any spores produced from high standing variation populations failed to grow (Table 1.2). This indicates that even though these populations have some ability to grow incomplete spores, the spores themselves cannot continue a normal life cycle and will not go on to produce viable adult cells.

Table 1.2. Sporulation efficiency and viability of experimental populations. As described in Methods, sporulation efficiency is determined from visual observation of percent of surviving spores; spore viability is determined by the population size (OD600) after heat killing at 55°C because only properly formed spores are able to survive. Values are mean ± standard deviation.

Population	**Sporulation efficiency pre-heat exposure**	**Sporulation efficiency post-heat exposure**	**Population size prior to sporulation**	**Population size after sporulation & heat kill**	**Spore viability (% survival)**
Control	99.333 ± 1.434 (n=24)	99.375 ± 1.409 (n=24)	1.640 ± 0.011 (n=8)	1.369 ± 0.137 (n=8)	83.474 ± 8.486 (n=8)
Low standing variation	99.313 ± 1.323 (n=64)	99.447 ± 1.666 (n=64)	1.636 ± 0.031 (n=16)	1.463 ± 0.122 (n=16)	89.465 ± 7.416 (n=16)
High standing variation	99.688 ± 0.903 (n=64)	3.542 ± 5.105 (n=64)	1.633 ± 0.025 (n=16)	0.005 ± 0.021 (n=16)	0.315 ± 1.260 (n=16)

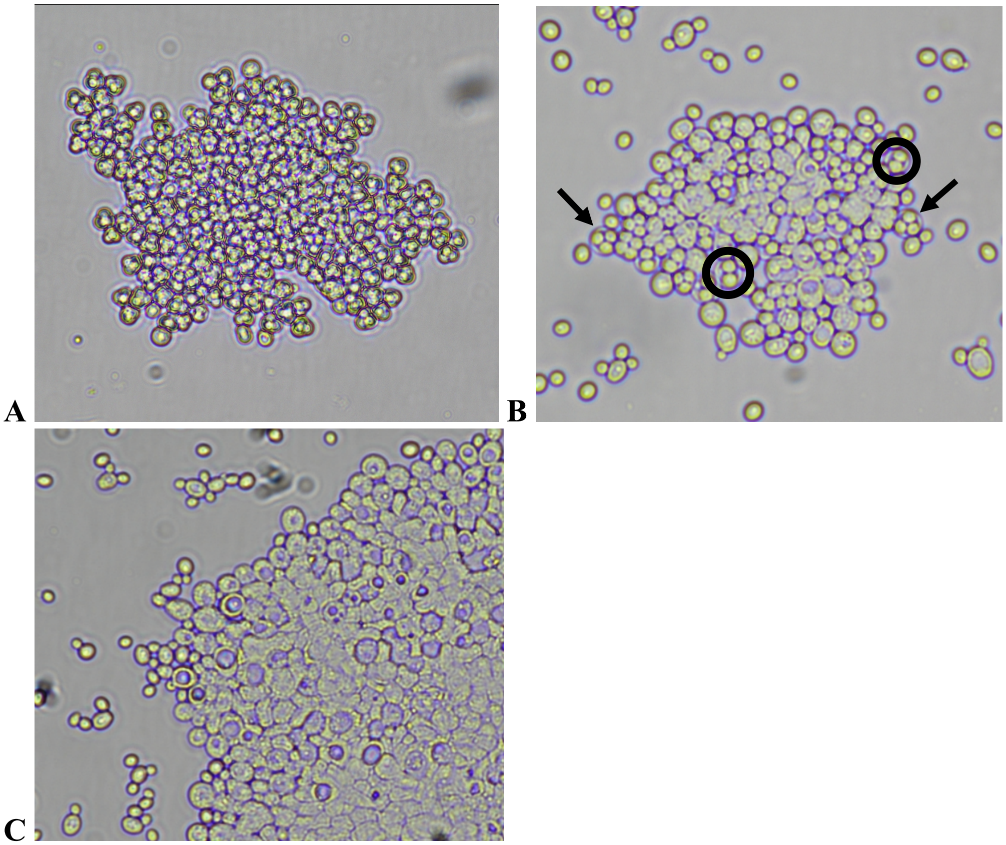


Figure 1.3. Pictures of normal and abnormal sporulation. A) normal sporulation in multicellular yeast, tetrads are in a tight pyramidal shape; B) incomplete sporulation with dyads (circle) and triads (arrows); C) failure to sporulate and failed cell division as seen by lack of discrete cell shapes.

1.3.3 *Loss of sex due to aneuploidy*

The loss of sex in the unicellular individuals suggests aneuploidy, and this was verified by whole genome sequencing to determine genome coverage. Populations at transfer 6 were compared to their initial populations before exposure to heat stress. All initial populations were euploid, and aneuploidy was not detected in the low standing variation populations (Figure 1.4A). High standing variation populations did show aneuploidy with multiple different karyotypes being revealed (Figure 1.4B). Aneuploids were detected in both the unicellular individual and in some of the individuals that remained multicellular. Two of four sequenced isolates showed aneuploidy in the multicellular individuals and all unicellular individuals isolates showed aneuploidy. Four chromosomes (I, VII, XII, and XIV) had additional copies in the aneuploid isolates. Of those, chromosomes I and XIV had additional copies in multiple populations in the selective environment. Two of the unicellular isolates each showed the gain of two separate chromosomes, including both sharing additional copies of chromosome I.

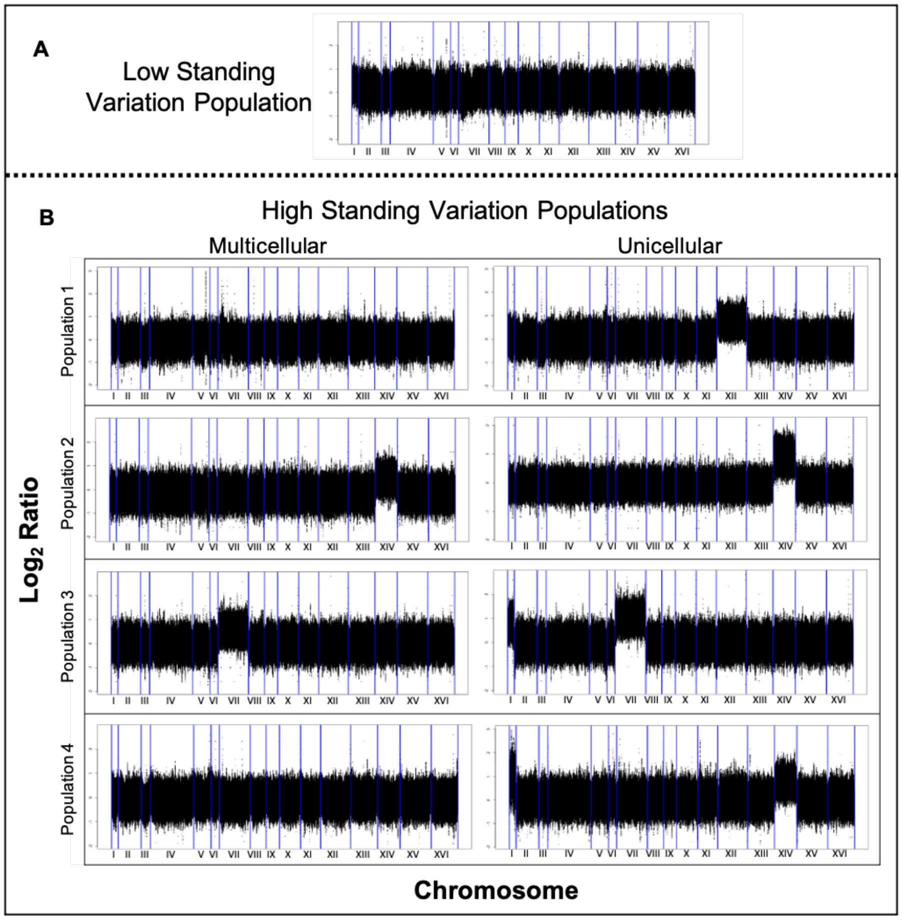


Figure 1.4. Log_2 ratio of coverage (Evolved/Ancestor). Evolved populations are from transfer 6 and are compared to their ancestral population at transfer 0 before introduction to heat stress. A value of zero represents no change from the starting population, 0.5 shows the gain of one copy of a chromosome, 1 shows the gain of two copies of the chromosome. A) representative low standing variation population (all populations are multicellular); B) high standing variation populations, both multicellular and unicellular individuals from the population are shown.

1.4 DISCUSSION

These results support the idea that standing variation allows for rapid adaptation, but may have long term costs in some systems. High standing variation allowed for rapid adaptation but resulted in the loss of sexual recombination. This work adds to the growing body of work—work from theoretical, laboratory, and natural populations—that contends that standing variation is the dominant genetic source for adaptation when an environmental shift is rapid (Barrett and Schluter 2008; Agashe *et al.* 2011; Gonzalez and Bell 2013; Hedrick 2013; Brawand *et al.* 2015; Ralph and Coop 2015; Bell 2017; Bitter *et al.* 2019; Thompson *et al.* 2019; Zhou *et al.* 2021). These results also reinforce experiments that have shown aneuploidy to be a useful mechanism in rapid adaptation (Gilchrist and Stelkens 2019). Previous insights into aneuploidy as an adaptive mechanism in yeast were investigating clonally reproducing yeast. This work used conditions that force sexual reproduction to show that aneuploidy has a clear cost. The loss of sex seen here connects this work to the larger pattern of derived asexuality seen across many eukaryote lineages (Neiman *et al.* 2014). Strong selection has been theorized to be a causative factor in the evolution of parthenogenesis (Glesener and Tilman 1978; Jaron *et al.* 2021), and our work explicitly shows derived asexuality as a result of a strongly selective environment. Additionally, this work shows the usefulness of experimental systems in adding to our understanding of complex evolutionary patterns that occur in natural populations.

1.4.1 *Loss of Sporulation*

High standing variation populations showed an almost immediate loss of sporulation when grown in a high temperature environment. These yeast began as diploids capable of sexual recombination. Diploid yeast can reproduce via asexual division, budding, or via sexual reproduction, creating haploid spores that combine with other spores to create a diploid cell. Poor environmental conditions lead to sporulation, specifically the lack of nitrogen and the lack of a fermentable carbon source. Like other fungal spores, *S. cerevisiae* spores are robust and can survive for long periods of time in very unfavorable conditions. Normal sporulation requires two rounds of meiosis and results in four haploid spores inside an ascus. The cells from evolved high standing variation populations largely failed to sporulate properly. The unicellular cells do not produce even partial spores and reproduce solely by budding, which indicates a failure to begin meiosis. Of the multicellular cells, some produce limited spores, but often two or three rather than the normal four spores (Figure 1.3B: circles and arrows). This indicates a failure in meiosis II. Others show a failure to reproduce even by normal budding and show a seeming loss of cytoskeletal structure (Figure 1.3C). There are many pathways for chromosome transmission fidelity to fail. Whole chromosome aneuploidy can result in an unequal number of chromatids for division. Aberrations in genes coding for kinetochores or centrosomes will result in errors in chromosome segregation and consequently aneuploid cells. Further work would need to be conducted to know the precise mechanism driving the meiotic failure in this system.

1.4.2 *Aneuploidy as an adaptive mechanism*

While aneuploidy is typically thought to have a negative effect on fitness, there is increasing evidence that it can be an effective adaptive mechanism (Selmecki *et al.* 2009, 2015; Pavelka *et al.* 2010; Chen *et al.* 2012a, 2015; Yona *et al.* 2012; Gerstein and Berman 2015; Morard *et al.* 2019; Kaya *et al.* 2020). Here, aneuploidy led to a successful strategy—the development of a unicellular phenotype—that allowed for rapid population growth in the face of strong selective stress. Unicellular individuals and multicellular individuals in initially high standing variation populations both showed aneuploidies (Figure 1.4). Aneuploidy was seen in all unicellular individuals but not all that were multicellular. This may explain the complete failure of unicellular sporulation and the partial sporulation seen in multicellular clusters. The mechanism that drives that transition from multicellular to unicellular phenotype cannot be known just from this work, nor can it be determined if it is due to a single mechanism or different processes in different populations. The original transition from unicellularity to the multicellular individuals used in this work was due to the homozygous loss of function of a single gene (Ratcliff *et al.* 2012), though many parallel mechanisms have since been uncovered (Gettle, in review).

There was not a single, consistent type of karyotype variation that was seen across populations, instead many different karyotypes were observed. This lack of a uniform change is congruous with other experiments. Evidence from *S. cerevisiae* experiments has shown multiple different karyotypes develop in response to the same selective environment (Chen *et al.* 2012a; Morard *et al.* 2019). Assays of different experimentally constructed aneuploids have shown fitness benefits associated with copy number variations of virtually every chromosome (see citations above and reviewed in Gilchrist and Stelkens 2019). Here all karyotype variations showed the addition of chromosomes, but chromosome loss has also shown to be beneficial (Gilchrist and Stelkens 2019). While we saw no loss, this could be due to sequencing only a subset of populations. The aneuploids seen here have been noted as having fitness benefits in other experiments: chromosome I from antifungal stress (Pavelka *et al.* 2010), chromosome VII from antifungal stress (Chen *et al.* 2012a), chromosome XII from ethanol (Morard *et al.* 2019) and antifungal stress (Pavelka *et al.* 2010; Chen *et al.* 2012a; Kaya *et al.* 2020), chromosome XIV from nutrient limitation (Hong and Gresham 2014) and antifungal stress (Kaya *et al.* 2020).

The selective environment used here was growth in an elevated temperature and heat shock has been shown to induce aneuploidy in other experiments with *S. cerevisiae*. Shen *et al.* used higher temperatures for briefer periods of time and found double stranded breaks to be the cause of incorrect segregation of sister chromatids (2020). Jarosz & Lindquist found that heat stress (growth in 39°C versus 37°C used here) was responsible for revealing the hidden effects of standing variants due to the depletion of the molecular chaperone Hsp90 (2010). These cryptic variants, including an open reading frame on chromosome XII, conferred resistance to antifungals. Hsp90 inhibition was also shown to cause trisomies in chromosomes VII and XII, which were also both affected in this experiment (Chen *et al.* 2012a). It is unknown whether the inhibition of Hsp90 or another chaperone protein is causal here, but it seems a likely mechanism that could be explored.

These works were all analyzing the adaptive potential for aneuploids. But, just because there are conditional benefits to aneuploidy, it does not negate the potential cost of chromosomal imbalance. Notably, the majority of work that shows the benefits of aneuploidy has been done in organisms that can reproduce asexually (Table 1.3). Aneuploidy is likely more easily tolerated in organisms that do not rely on the ability to undergo meiosis. In sexually reproducing organisms, karyotype variation can cause large incompatibilities and costs that are not so easily borne.

1.4.3 *Aneuploidy as a short-sighted evolutionary strategy*

When environmental change is rapid and selection pressure is strong, standing variation is often thought of as a panacea. This experiment, though, shows a case where high standing variation leads to a strategy that could prove detrimental in the long term. Aneuploid yeast adapt very rapidly to a change in environment, however they do so at the cost of sexual reproduction. This tradeoff benefits them well in their current, static environment but potentially sets these populations on a short-sighted evolutionary trajectory. Were the environment to change, these populations would no longer have access to sexual recombination, which is beneficial for adaptation in a shifting climate (Lande and Shannon 1958; Felsenstein and Yokoyama 1976; Crow 1992). Even small environmental changes can drastically effect asexual populations, while sexual populations are able to maintain high levels of fitness even in the face of quick shifts (Waxman and Peck 1999). In *S. cerevisiae*, which has the option to reproduce sexually or asexually, sexual recombination is still advantageous due to its ability to remove deleterious mutations and to allow populations to continuously adapt to a changing environment (Goddard *et al.* 2005; Gray and Goddard 2012; McDonald *et al.* 2016; Kosheleva and Desai 2018). The creation of environmentally-hardy spores also allows for the possibility of dispersal to other niches.

The loss of sex here represents a case where increased standing variation may actually be a detriment to the evolution of the population. This strategy could be a risky strategy if the environment experiences further changes and the current phenotype is no longer beneficial. This idea of short-sighted evolution has been evaluated in bacterial and viral systems, but the adaptation there is facilitated by high mutation rates rather than standing variation (Levin 1996; Martínez 2014; Lythgoe *et al.* 2017). To the authors' knowledge, the degree to which standing variation may serve as a limiting force by placing a population on a short-sighted evolutionary trajectory has not been evaluated. Without exception, populations in this experiment that contained significant amounts of standing variation adopted the aneuploid strategy that prohibits sexual recombination. High standing variation did indeed provide the basis for rapid adaptation, but, in the long run, the fastest strategy may not always be the best strategy.

1.4.4 *A move towards asexuality*

For yeast, the transition to aneuploidy has been shown to be reversible (Berman 2016), but that does not seem to be the case for other organisms that have lost sex through karyotype variations. Why this asexuality develops in sexual lineages has been a persistent question. Yet, across taxa we see many asexual offshoots appear, sometimes repeatedly within clades (Butlin 2002; Neiman *et al.* 2014). However, these phylogenetic branches are, for the most part, quite short-lived (Simon *et al.* 2003; for the exception, bdelloid rotifers, see Welch and Meselson 2000). The transition to asexuality can occur quite rapidly (Table 1.3), which is precisely what we see in this experiment. Asexuality in historically sexual lineages has no single mechanism, and it is possible that it could be the result of particular adaptive circumstances within individual life histories (Lodé 2013; Jaron *et al.* 2021). Instead of a generalized mechanism, individual periods of strong selection could lead to asexuality, and this has been seen in both laboratory and natural populations that exist in harsh climates (King and Hurst 2010; Table 1.3).

Rapid adaptation corresponding to variation in karyotype is seen most prominently in pathogens under strong selection for drug resistance. Fungal pathogens, yeast in particular, have been model systems for exploring the role of aneuploidy in rapid adaptation to stress. Experiments in several pathogenic trypanosomes have also shown that different aneuploid karyotypes act as sources of variation in the absence of sexual recombination, and that selection on haplotypes allows for rapid adaptation. These experiments shed light on the usefulness of aneuploidy when faced with strong environmental stress, but do not explore the potential costs of aneuploidy. Additionally, loss of sex would not be a cost in these systems because these organisms are already asexual, or they have the capacity for sex but reproduce asexually. The exception is an oomycete plant pathogen that rapidly develops drug resistance at the cost of a sexual lifestyle, similar to the work presented here (Hu *et al.* 2020).

The area where the cost of sex would be significant is the more puzzling case of parthenogenesis in animals. Far afield from the medical perspectives presented above, the transition from sexuality to asexuality is also well-characterized in the evolutionary histories of several animal taxa (Neiman *et al.* 2014). Many parthenogenetic lineages have been shown to have aneuploid karyotypes (Table 1.3). Parthenogenesis in animals is also correlated with strong selection in extreme environments, and developmental constraints suggest that it is likely the result of a rapid rather than gradual process (Galis and van Alphen 2020). Both of those factors, strong selection and rapidity, are seen in the work presented here. In nature, extremes like high altitudes, high latitudes, high temperatures, or high aridity have been shown to be the more common niches of asexual animals when compared to closely related sexual lineages (Glesener and Tilman 1978). The geographical distribution of parthenogenetic species suggests that they may be more prominent in locations where abiotic factors are harsh and dominate biotic interactions (Tilquin and Kokko 2016). In fact, a hotspot for parthenogenetic animals is the severely arid Australian desert (Kearney 2003). There, very distantly related lineages were shown to lose sex at the same point in time, following a dramatic environmental shift (Kearney *et al.* 2006). This parallel loss could suggest that populations drew upon standing variation to allow for rapid adaptation. Similar to the selective environment used in this experiment, heat stress, in particular, could be a driver of asexuality and has been linked to parthenogenesis in several species (Jose and Dufresne 2010; Liu *et al.* 2015; Alejandra Perotti *et al.* 2016).

Derived asexuality is often thought of as an evolutionary dead end due to low diversification rates, high extinction rates, and the existence of these lineages as “twigs at the tips of bountiful branches” (Vrijenhoek 1998). This leaves the repeated loss of sex as something of an evolutionary mystery. Our results show the loss of sex as a tradeoff for rapid adaptation to an extreme environment, and moreover show high genetic variation as a facilitator of that trade. One problem with trying to unravel the genetic mechanisms that lead to derived asexuality in natural populations is the difficulty in determining whether observed genetic patterns are a cause or consequence of the evolution of this trait. The ability to evaluate specific genetic changes over time is one of the great strengths of experimental evolution. This experiment provides an example of the benefits of losing sex and the genetic conditions under which this transition may occur.

Table 1.3. A review of experiments that have looked at a combination of the factors seen in this experiment. This experiment uses heat stress as the selection pressure and rapidly sees aneuploidy develop as an adaptive strategy, which in turn causes a loss of sex. Many experiments see portions of this pattern.

Organism	**Selection Pressure**	**Chromosomal changes**	**Rapid Adaptation**	**Sexuality**	**Reference**
Candida albicans (yeast)	Antifungal drug resistance	Multiple types of aneuploidy	✓	Asexual	(Selmecki *et al.* 2009)
Candida glabrata (yeast)	Antifungal drug resistance	Multiple types of aneuploidy, including entirely new chromosome	✓	Asexual	(Poláková *et al.* 2009)
Saccharomyces cerevisiae (yeast)	Ethanol tolerance	Multiple types of aneuploidy, large variation in strategy	✓	Capacity for sex, but reproduce asexually	(Voordeckers *et al.* 2015)
Cryptococcus neoformans (yeast)	Host environment & antifungal	Polyploid Titan cells produce daughter cells with multiple aneuploidies	✓	Asexual	(Gerstein and Berman 2015)
Leishmania genus (trypanosome)	Host environment	Aneuploidy is ubiquitous and essential	U	Capacity for sex, but reproduce asexually	(Mannaert *et al.* 2012)
Leishmania donovani (trypanosome)	Host environment	Multiple aneuploidies developed *in situ*	✓	Capacity for sex, but reproduce asexually	(Barja *et al.* 2017)
Trypanosoma cruzi (trypanosome)	Host environment	Multiple aneuploidies	U	Capacity for sex, but reproduce asexually	(Llewellyn *et al.* 2011; Minning *et al.* 2011)
Phytophthora capsici (oomycete)	Fungicide	"Dynamic extreme aneuploidy"	✓	Loss of sex due to aneuploidy	(Hu *et al.* 2020)
Meloidogyne incognita (nematode)	--	High karyotype variation across 220 populations	U	Parthenogenetic	(Triantaphyllou 1981)
Polycelis nigra (flatworm)	--	Aneuploid, polyploid, and euploid populations surveyed	U	Parthenogenetic	(Beukeboom *et al.* 1998)

Amblyomma cajennense (tick)	--	Aneuploid, polyploid, and euploid offspring from experiment	U	Loss of sex due to aneuploidy	(Gunn and Hilburn 1991)
Daphnia pulex (daphnia)	Temperature and pH	Limited differential survival of polyploids vs euploids	U	Parthenogenetic	(Jose and Dufresne 2010)
Drosophila genus (fruit fly)	--	Defects of chromosome alignment in meiosis I	U	Parthenogenetic	(Meyer *et al.* 2010; Markow 2013)
Brachionus calyciflorus (rotifer)	Seasonal change, aridity	Polyploidy	✓	Parthenogenetic	(Welch and Meselson 2000; Stelzer *et al.* 2010)
Carausius morosus (stick insect)	--	Aneuploid males	U	Parthenogenetic	(Pijnacker and Harbott 1980)
Tinema genus (stick insect)	--	Karyotype variation within and across species	✓*	Parthenogenetic	(Schwander and Crespi 2009)
Micromalthus debilis (beetle)	Temperature and drought	--	✓	Parthenogenetic †	(Alejandra Perotti *et al.* 2016)
Warramaba virgo (grasshopper)	Arid climate	--	U	Parthenogenetic	(Kearney *et al.* 2006)
Bombyx mori (silkworm)	Temperature	--	✓	Parthenogenetic	(Liu *et al.* 2015)
Myzus persicae (aphid)	Seasonal change	Chromosomal instability associated with Hsp90	✓	Parthenogenetic	(Mandrioli *et al.* 2019)
Darevskia rostombekowi (lizard)	--	Chromosomal rearrangement	✓	Parthenogenetic	(Spangenberg *et al.* 2020)
Heteronotia binoei (gecko)	Arid climate	Mitochondrial DNA duplication	✓	Parthenogenetic	(Kearney *et al.* 2006; Fujita *et al.* 2007)

✓ = Yes, U = unclear/not directly measured, * = sudden transition to apomixis, † = from parthenogenetic to sexual during experiment

CHAPTER 2

The relative importance of standing variation in adaptation to both sudden and gradual rates of environmental change

ABSTRACT

There are different expectations for how adaptation proceeds when there is a sudden shift in environmental conditions versus a gradual change over a longer period of time. The adaptive routes possible to populations in each scenario may depend heavily on their genetic makeup, specifically on whether they can immediately utilize standing variation or if they must rely on *de novo* mutations that take time to arise. Models have predicted when the benefits of one genetic source should outweigh the other based on the availability of genetic variants and assumptions about their relative effect size. Here, we evaluate how adaptation proceeds in conditions of both sudden and gradual change when populations contain different amounts of initial standing variation. We see similarities with model predictions, but our results suggest that beneficial alleles of large effect can come from standing variation. Additionally, we see a larger variety of adaptive strategies when *de novo* mutations are the genetic basis for adaptation. We discuss the implications these results may have for evolutionary rescue and how experimental tests of models can help better inform predictions.

2.1 INTRODUCTION

When taking a walk in an adaptive landscape, a population's ability to reach the summit is dictated by a number of factors. The summit symbolizes a fitness peak, a location where the combination of genotypes maximizes a population's chance for success. The quest for the summit occurs through steps across the landscape, with each step representing a different combination of genotypes and many practical considerations limiting step size and direction. The landscape can be rugged, containing many peaks and valleys, perhaps making it very difficult to reach a peak without falling into a pit of low fitness. And, as if this journey was not difficult enough, the landscape itself may be in constant flux, which means the path to success is ever changing. Navigating such landscapes is key to a population's survival and understanding the paths available to populations is key to those interested in unraveling the process of evolution.

The idea of adaptive landscapes comes from Sewall Wright's visualization of the process of moving from a low fitness valley to a high fitness peak, via steps that are the result of selection on genotypes. Ultimately, different steps are possible due to mutations, but selection can act on brand new mutations, *de novo* mutations, or on pre-existing variation, standing variation. Standing variants enter a population as neutral mutations but can transition to become beneficial when selection pressures change (Barrett and Schluter 2008). These different sources of genetic variation carry with them different assumptions about how they affect the rate and tempo of evolution. Standing variants are available for immediate use and therefore likely of importance when environmental change is rapid, that is to say, when selection pressures change rapidly resulting in a sudden shift from one fitness landscape to another. Of equal importance are the differences in theory of how adaptive landscapes are traversed when either of these genetic sources is used as the source for adaptation.

Adaptive walk models, those that use the mountain climbing metaphor described above, utilize *de novo* mutations as the mechanism by which steps are taken throughout the landscape. A population is placed into a new fitness landscape it has not adapted to, a neutral or detrimental allele is swapped out for a beneficial allele, and the effects of the adaptive substitution are observed (Gillespie 1984). The range of these effects was first formalized in R.A. Fisher’s geometric model, where he substituted mutations with different phenotypic effect sizes and evaluated the degree to which they were beneficial (Fisher 1930). Maynard Smith then updated this theory to fit within a genetic framework and use sequence differences as the determinants of step destinations (Maynard Smith 1962). Models have since shown the beneficial effects of mutations follow an exponential model where few mutations have large effects and these are the ones likely to fix in a population (Orr 1998, 2002). Evolution under this model, then, proceeds by large steps from few alleles—rare beneficial mutations have large fitness effects and these are what move a population around a fitness landscape.

The alternative to this is that alleles could have small fitness effects and many individual loci could cumulatively affect adaptation. This is the model put forward by Russell Lande and that has long been used by quantitative geneticists (Lande 1976). Here, standing variation is the dominant source for adaptation and mutation plays a drastically reduced role. Adaptation under this model is not the consequence of great fitness bounds resulting from single alleles, but rather the work of small shifts in the frequency of a large number of alleles. This view has typically been a “top down” approach where phenotype takes precedence and is not broken down into the effects of individual alleles, in part because of the difficulty teasing out the individual effects of many highly interconnected loci (Barton *et al.* 2002). However, there are a few models that aim to take a “bottom up” approach that is similar to the molecular perspective of the adaptive walk models but apply it to standing variation (Hermisson and Pennings 2005; Chevin 2011; Höllinger *et al.* 2019). These models did indeed show that under certain conditions standing variants have a stronger rate of fixation when compared to new mutations.

This leaves the conflict of how these two models intersect and when one model should be favored over the other. Under different regimes of selection and mutation, what is the distribution of substitutions that originate as standing variants or *de novo* variants? One thought is that standing variation is of more importance when there is a sudden shift in selection. There are two primary reasons: standing variation already exists and it exists at a relatively high frequency (Barrett and Schluter 2008). At the onset of an environmental shift, if previously neutral standing variants become beneficial they are immediately available for selection to act upon, whereas beneficial *de novo* mutations will arise randomly. Additionally, a *de novo* mutation will enter the population at a frequency of 1/2N in a diploid population, but a standing variant’s frequency at the time of benefit can be much higher. Standing variants are initially neutral or nearly neutral, so when they arise in a population they are expected to segregate via neutral processes and therefore can drift to frequencies that greatly exceed 1/2N. Hermisson & Pennings found that this increased frequency allowed for standing variants to fix even when their fitness benefits were not sizable (2005). Moreover, they found that adaptation from standing variation was likely to involve the fixation of many alleles of small effect, rather than single, large effect alleles. These model predictions have been substantiated in experimental systems that show standing variation is the more likely genetic basis for adaptation when the environment undergoes a sudden change (Teotónio *et al.* 2009; Burke 2012; Sheng *et al.* 2015).

The alternative to this, which has received relatively less attention, is the genetic basis for adaptation when environmental change is gradual. Here, instead of a single large change in the selection coefficient, there is a smaller coefficient that is continually applied to a population. This scenario brings with it different assumptions about the relative importance of standing variation and *de novo* mutations. Recent models have addressed the expectations for how adaptation proceeds in this scenario—with some focusing on *de novo* mutations as a starting point (Collins *et al.* 2007; Kopp and Hermisson 2009a; b) and others using standing variation (Matuszewski *et al.* 2014, 2015). When the rate of change is gradual, standing variation is thought to be of lesser importance than the supply of *de novo* mutations. The smaller change in selection pressure is thought to allow more time for a beneficial mutation of large effect to arise. The fitness decrease here is not so severe as to require the immediate benefits offered from standing variation, and the population can afford the waiting time for a large beneficial effect. However, little has been done outside of modeling to evaluate the relative importance of both genetic sources in a gradual change scenario.

The aim of the work presented here will be to evaluate how the constancy of an adaptive landscape (a sudden shift or a gradual change) affects which source of genetic variation (standing variation or *de novo* mutations) is used as the basis for adaptation. One source theoretically offers small but immediate benefits, while the other offers more benefits but with an undetermined wait time for their arrival. In terms of adaptive landscapes, this can be represented as a tradeoff between the ability to move immediately versus the ability to move far. We seek to assess this tradeoff using populations of experimentally evolved yeast that contain varying amounts of standing variation. These populations are subjected to two scenarios of selection—one that is a sudden change to a drastically different growth temperature and another that is a gradual change that eventually reaches the same drastic temperature change. The yeast used here are *Saccharomyces cerevisiae* that have been experimentally evolved to exist as multicellular clusters rather than individual single cells. The increase in complexity that accompanies multicellularity serves to highlight fitness tradeoffs and allows the effects of selection to be more readily seen. We find the degree of initial standing variation in a population to be of great relevance in both scenarios and furthermore see that it provides a large benefit rather than offering smaller advantages than *de novo* mutations.

2.2 METHODS

2.2.1 ***Strains and media***

All populations began from a clonal population of strain Y55 of *Saccharomyces cerevisiae*. To evolve multicellularity, these yeast were grown under conditions of settling selection as described in Ratcliff *et al.* 2012. Here, selection for greater ability to sink to the bottom of a tube results in yeast that do not break away from their mother cell when dividing, but rather stay attached to form snowflake-like clusters. After the onset of multicellularity in all individuals, growth continued in this selection regime for 14 weeks, allowing the populations to accumulate random mutations and build standing variation. From this ancestral population, populations were made that contained four different amounts of standing variation. All populations were maintained in 10mL of YPD (per liter distilled water: 10g yeast extract, 20g bacterial peptone, and 20g dextrose) in 25mm × 150mm glass culture tubes. Plated yeast were grown in the same YPD with 15g agar added. The initial growth condition for all yeast was incubation in a 30°C incubator shaking at 250 rpms.

2.2.2 *Creation of populations with different levels of standing variation*

Level 1 populations consisted of a single colony from the ancestral population. This population should have no standing variation as it is derived from a strain that is only facultatively sexual and therefore all cells will be clonal. The level 2 population was created by selecting and combining 5 colonies from the ancestral population. Level 3 was created from a 10μL sample of the ancestor and level 4 from a 100μL sample. Levels 1 and 2 are considered to be low standing variation populations while 3 and 4 are high standing variation (Figure 1).

Eight individual populations were made for each level of standing variation. For the high standing variation populations, each population is an independent genotype. For the low standing populations, each population has a replicate so that there are paired genotypes. The purpose of this is to see if the colonies of the same genotype behave similarly across the experiment. If there is an adaptation that occurs in one replicate but not the other, then it is more likely to have been the result of a random new mutation and not due to the genetic background of that individual. Conversely, if each pair of replicates reacts in the same way, then the reaction is more likely to be the result of standing variation. For example, the level 1 standing variation populations comprise four pairs, each of which represents a different single colony isolate. This means there are four genotypes represented in level one and all populations are starting with essentially zero standing variation because they are from single colonies. The same scheme is true for level 2, there are four pairs of distinct genotypes, but here there is more variation because each genotype is the combination of five colonies.

Four tubes from each standing variation level, totaling 16 tubes, were used as controls and were maintained in normal growth conditions and not subjected to the heat stresses below. These tubes yielded no differences in population size or phenotype when compared to the ancestral population and for simplicity will not be discussed below (see Supplemental).

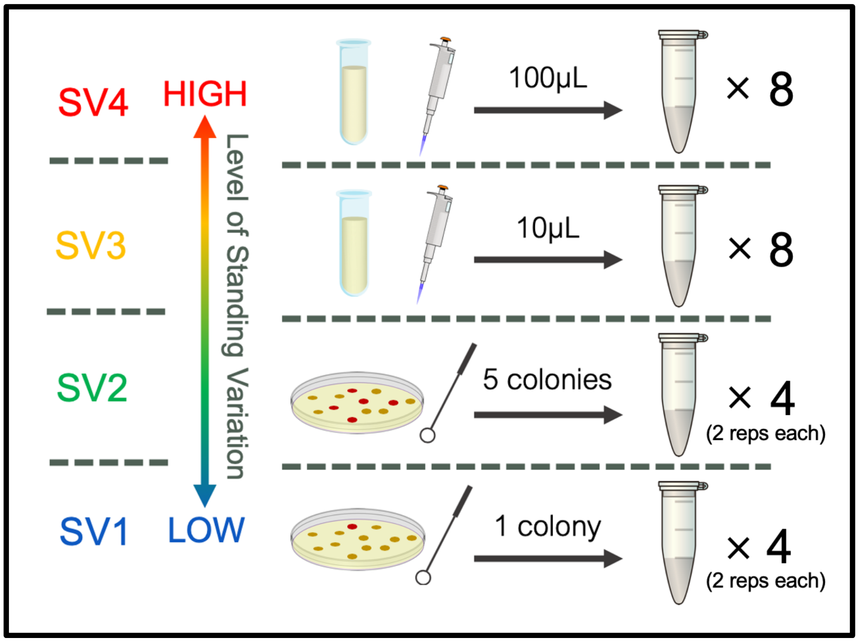


Figure 2.1. Schematic of how experimental populations were created from the ancestral population. SV1 represents the lowest level of standing variation, with all individuals being clonal; SV4 is the highest, containing approximately the same level of variation as in the ancestral population. Each level comprises eight total populations with the levels 1 and 2 having four genetically distinct populations, each with a replicate, and levels 3 and 4 being entirely distinct.

2.2.3 *Selection regime: sudden heat stress*

These 32 populations were grown for one day at 30°C, shaking at 250 rpms, in 25mm × 150mm glass culture tubes. From here, they were subjected to the same settling conditions as before so that settling is not a new environmental stressor. Upon removal from the incubator, 1mL of culture is pipetted from the culture tube into a 1.5mL microcentrifuge tube. This culture is allowed to settle undisturbed on the bench top for 10 minutes. The top 900μL of culture is then carefully pipetted off and discarded. The bottom 100μL of culture is thoroughly mixed and pipetted into fresh YPD media in glass culture tubes. Tubes were then placed in an incubator set at 37°C and shaking at the same 250 rpms. The abrupt change from 30°C to 37°C represents the novel heat stress. From this point tubes were always placed in the 37C incubator after being transferred. This process was repeated every 24 hours (+/- 30min) for 90 days.

2.2.4 *Selection regime: gradual heat stress*

This regime is largely similar to the above regime, but with a daily difference in incubator temperature. The populations start at 30°C and are transferred with settling in the same manner. However, after the first transfer the incubator is gradually heated every day. The first temperature change is from 30°C to 31°C, then 32°C, 32.5°C, and 33°C. From there the incubator temperature is increased by 0.2°C every transfer. It had been previously determined that there is no significant population change in size or phenotype before incubator temperature reaches 34°C (data not shown). The temperature was increased in this manner for 32 transfers, which means the highest temperature reached was 38.6°C.

2.2.5 *Population growth assay*

To assess population growth, absorbance measurements (OD600) were taken at every transfer for the first 14 transfers, then every other transfer. For the populations in the sudden temperature change regime the cultures were thoroughly vortexed and 300µL of sample was combined with 300µL of water. Samples were again mixed by pipetting immediately prior to taking absorbance readings because settling rate was very rapid in most samples. For populations in the gradual temperature change regime, samples were taken in the same manner as above and supernatant samples were also taken. For the supernatant samples, tubes were allowed to sit for ten minutes on the benchtop, then

300µL was taken from the top of each tube and again combined with 300µL water. These were again mixed before readings were taken. The reason for the supernatant samples will become apparent after reading results.

2.2.6 *Sizing particles within populations*

To show shifts from multicellularity to unicellularity, particle size was measured. A Beckman-Coulter Counter Multisizer 4 was used to determine the diameter of individuals, both multicellular and unicellular, within populations. Samples were measured at the start of the experiment before exposure to heat selection. The multisizer was fitted with a 70µm diameter aperture probe, which can measure particle sizes between 3 and 40 µm in diameter. A phosphate buffered saline solution (PBS; per liter distilled water: 8g NaCl, 0.2g KCl, 1.8g Na_2HGPO_4 at pH 7) was used to suspend cells. Yeast cultures at 10^3 dilutions were suspended in 5µl PBS for passage through the probe. The R package MultisizerToolkit was used for analysis. Particle counts and particle diameters were observed. Note that particle diameter cannot account for the number of individual cells per particle, only the overall diameter. Therefore, unicellular individuals composed of one cell and multicellular individuals composed of dozens of cells will both register as a single particle count but with different diameters.

2.2.7 *Statistical analysis*

Models were used to determine the statistical significance of population size changes as a factor of transfer number and standing variation level. Different scenarios required different model parameterization and models were compared using AIC. For the sudden change scenario, the initial population decrease was modeled using a linear model with only transfer number as a factor. The rebound in the high standing variation populations used a log linear model where again only transfer number was determined to be a factor. In the gradual change scenario, a linear model was used to fit populations until their point of divide, which was found to be transfer 15. From here, high and low standing variation populations then best fit a log linear model with transfer number, standing variation level, and individual population number as additive factors with no significant interaction effects. Significance was tested by ANOVA. Analyses are for full dataset, except where otherwise stated. Some analyses were performed with and without outlier strategies removed from standing variation levels 1 & 2. The reasoning for which will become apparent in the results. There was stronger significance with outliers removed but trends were the same regardless of their inclusion. All analyses were performed and graphed in R v4.0.3.

Table 2.1. AIC values for different models. High and low standing variation populations were modeled separately. TRAN refers to transfer number, SV to level of standing variation, and IND refers to sample number.

High SV Models	**df**	**AIC**	**Δ AIC**	**Low SV Models**	**df**	**AIC**	**Δ AIC**
HIGH: TRAN	3	-1227.58	44.17	LOW: TRAN	3	-1476.15	56.75
HIGH: TRAN + SV	4	-1229.11	42.64	LOW: TRAN + SV	4	-1480.53	52.36
HIGH: TRAN * SV	5	-1227.25	44.49	LOW: TRAN * SV	5	-1478.63	54.26
HIGH: TRAN + SV + IND	18	-1271.75	0	LOW: TRAN + SV + IND	18	-1532.90	0
HIGH: TRAN + SV * IND	33	-1248.60	23.14	LOW: TRAN + SV * IND	33	-1515.49	17.403

2.3 RESULTS

2.3.1 *Sudden change scenario*

This scenario is characterized by the sudden transition from normal growth in 30°C to growth in 37°C starting at transfer 1. This 37°C temperature was constant across all 34 transfers of the experiment.

2.3.1.i Rapid divergence of high and low standing variation populations

Upon introduction to the 37°C temperature incubator, all populations decreased in population size as expected due to heat stress (Figure 2). This initial decrease in population occurred similarly in all levels of standing variation for the first two transfers ($p = 0.591$, $F = 0.290$). After this initial drop, there was an immediate distinction between the high standing variation populations (levels 3 & 4) and low standing variation populations (levels 1 & 2). Beginning at transfer 4 and peaking by transfer 10, the high standing variation populations showed a dramatic increase in population size. The populations had fully recovered by transfer 4, and continued to increase until approximately transfer 10, where they exceeded their average initial population size (1.635 ± 0.025) by 29% (2.071 ± 0.057). The high standing variations, both levels 3 and 4, maintained this population size for the duration of the experiment. After transfer 10, there was no effect from transfer number on population size ($p = 0.477$, $F = 0.505$), nor was there a significant difference between levels 3 and 4 ($p = 0.468$, $F = 0.527$).

The low standing variation populations did not see the sudden increase in population size and suffered the effects of heat stress for the duration of the experiment. The population continued to decline until transfer 6 and there was no difference in severity of decline between levels 1 and 2 (p = 0.582, F = 0.306). After transfer 7, these populations began to tolerate the heat stress. When all low standing variation replicates are included, these populations show a slow process of adaptation, however, grouping all of the replicates together provides an erroneous view of this system (hence the large error bars in Figure 2). The next section will detail the multiple strategies occurring in the low standing variation populations, and further analysis will remove the outliers that evolved unique strategies. The low standing variation populations gradually increased in population size over the remaining transfers from their lowest point at transfer 6 (0.704±0.044) to a 52% increase at transfer 34 (1.067±0.038), but still showed a 43% decrease from their initial population size (1.642±0.025). There was no significant difference in recovery between levels 1 & 2 once the outliers were removed (p = 0.784, F = 0.075).

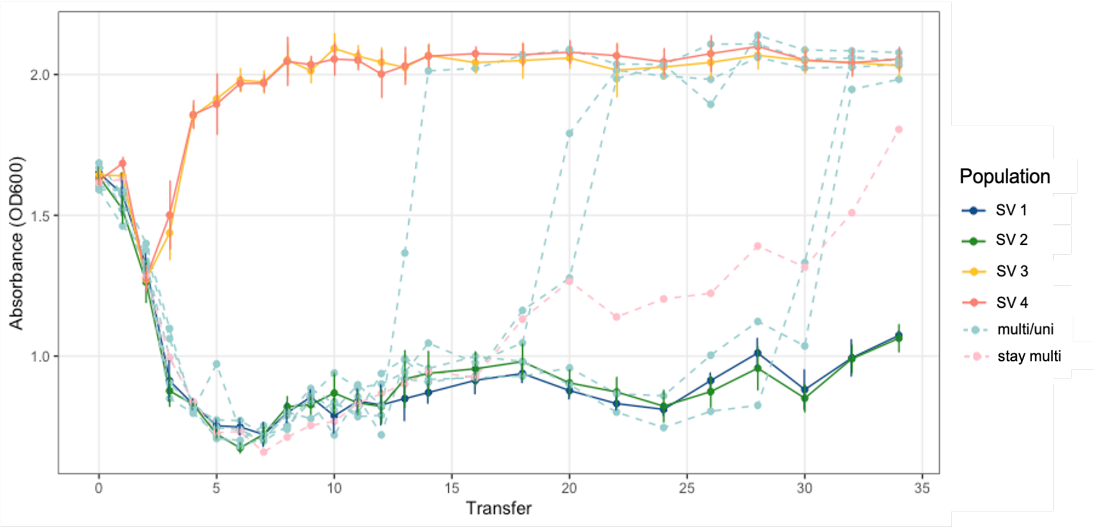


Figure 2.2. Relationship between population size (absorbance at OD600) and transfer number (time) in the sudden change scenario. High standing variation populations are SV3 and SV4. Low standing variation populations with outliers removed are SV1 and SV2. Outlier populations (represented by dotted lines) are defined by their final phenotype: either a mix of multicellular and unicellular individuals or a population that stayed entirely multicellular.

2.3.1.ii *Derived unicellularity in high standing variation populations*

Every replicate of the high standing variation populations, from both levels 3 & 4, showed the same unexpected response to heat stress: the addition of unicellular individuals to the population, here deemed the “multi/uni” strategy. All populations across every replicate, for high and low standing variation, began as multicellular clusters (Figure 3A). The driver of the differences that begin to occur at transfer 3 is a rapid progression towards a population that contains both multicellular and unicellular individuals. At transfer 3, all yeast are still largely multicellular but there is a transition toward a broader range of cluster sizes, due to an increase in smaller sized snowflakes, particularly snowflakes that are composed of 3 to 6 cells. By transfer 4, there are many unicellular cells present (Figure 3B). This pattern is seen in all high standing variation replicates.

The level of unicellular cells in the high standing variation populations was assessed by measuring the particle size before exposure to heat and after 34 transfers in heat stress (Figure 4). Ancestral multicellular clusters range in size but are typically between 20μm to 30μm wide. These clusters reproduce by breaking apart into smaller clusters and those are typically around 10μm wide. The populations at transfer 0 showed particle sizes consistent with these measurements (Figure 4A: purple). When measured at transfer 34, there was a noticeable shift in sizes (Figure 4A: gray). Unicellular yeast are typically around 6μm wide and we see a large peak in particle size at this point. Along with this, multicellular individuals show a divergence from their original size. They show both an increase in the diameter of large clusters and an increase in count of small multicellular clusters. Note that this method of size detection cannot distinguish between the number of cells in the particle being measured. So, while there is a higher count of unicellular individuals, there are more cells per particle in the multicellular particles. Regardless, this analysis shows the shift from entirely multicellular to multicellular and unicellular.

The low standing variation populations displayed a wider array of responses to the heat stress. Six of 16 populations evolved a strategy that dramatically increased their population size, while ten showed modest gains. The modest group will be discussed here and the outlier populations will be discussed below. This group (represented as SV1 and SV2 in Figure 2) did not evolve any unicellular individuals and retained multicellularity across the experiment. There were, however, some changes in phenotype. These clusters grew to be far denser than their ancestral counterparts (Figure 3D and 3A, respectively). Individual cells within clusters also appear smaller and less oblong than prior to heat stress. Overall cluster size changed as well, with a shift towards a larger diameter (Figure 4B). These populations saw a flatter curve centered around 30µm rather than a narrow distribution around 25µm as was seen in their ancestor populations. The maximum diameter of these clusters was similar to that in the large multicellular individuals in the high standing variation populations. Interestingly, the count for larger particle sizes was similar in both high and low standing variation evolved populations (note y-axis differences for 4A and 4B), indicating that overall multicellular counts are similar and it is the presence of unicellular individuals driving population size difference.

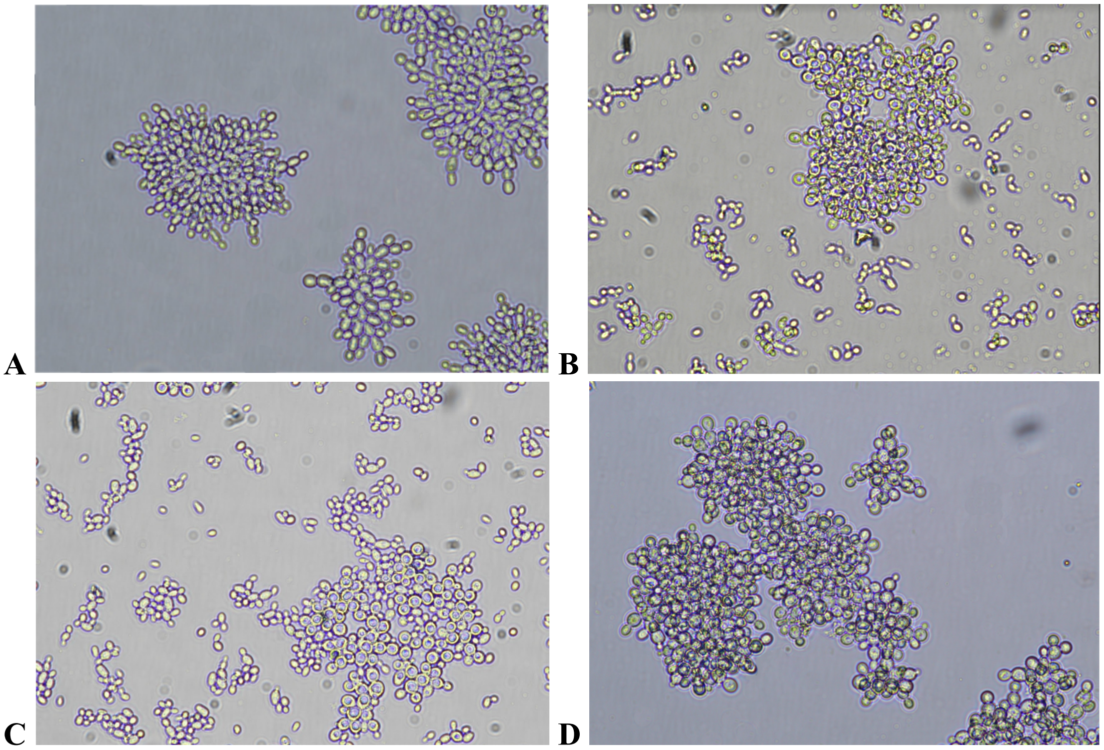


Figure 2.3. Photos of phenotypes of multicellular yeast. A) Ancestral phenotype, all others derived from this; B) Evolved "multi/uni" strategy in high standing variation; C) Evolved "multi/uni" strategy in low standing variation, D) Evolved "stay multi" strategy.

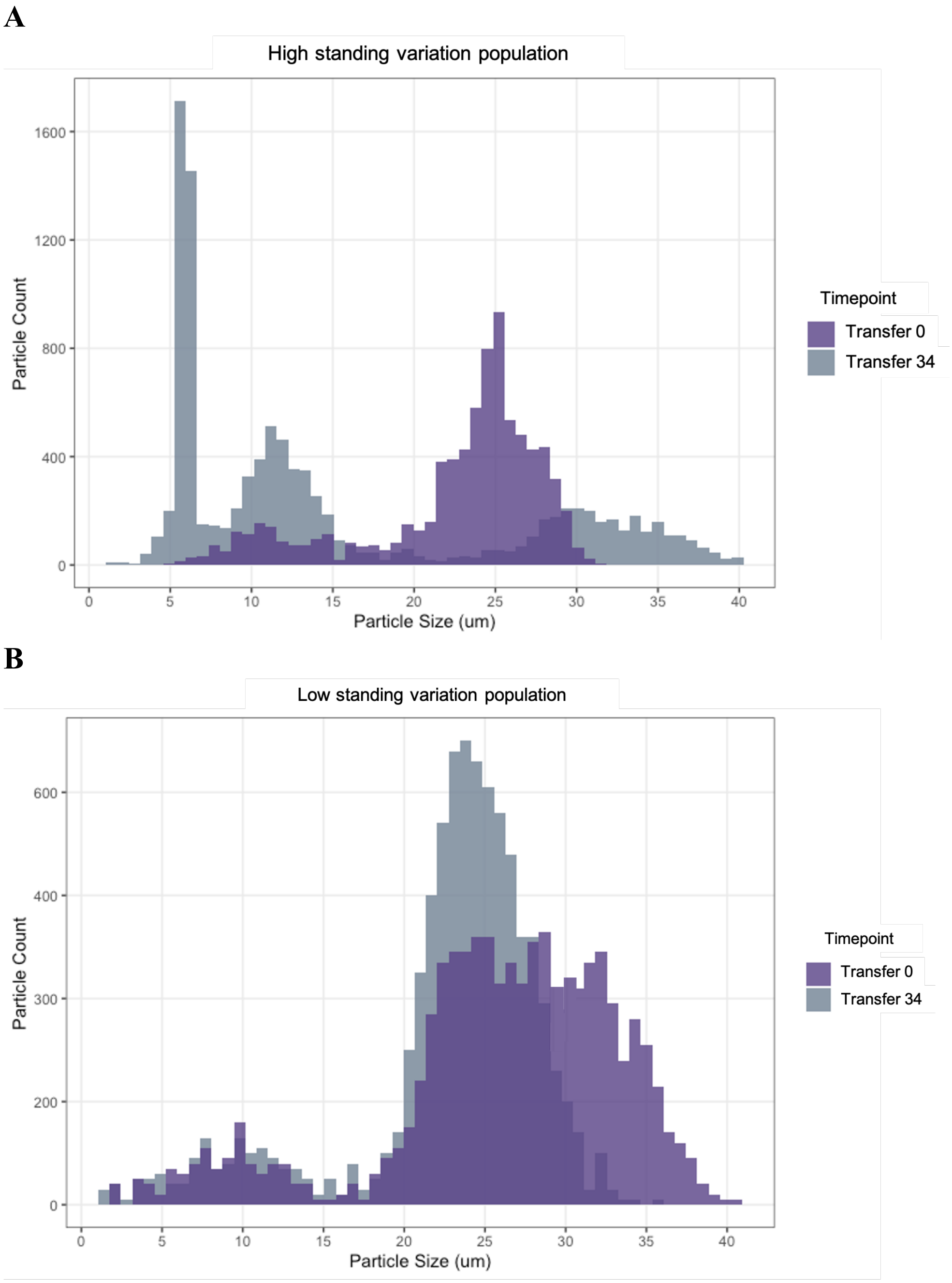


Figure 2.4. Particle size of individuals in representative high and low standing variation populations. Transfer 0 (purple) represents the individual population phenotype before introduction to heat stress; transfer 34 (gray) represents the evolved population phenotype at the end of the experiment. Particle size of ~6µm indicates a unicellular individual, ~10µm represents a small multicellular individual that has broken off of a mature multicellular cluster.

2.3.2 *Gradual change scenario*

The populations that underwent a gradual increase in temperature also underwent 34 transfers but instead of an immediate stress temperature of 37°C, they were gradually raised by 0.2°C every transfer (note: this began at 33°C, 30-33°C had larger increments). This resulted in their final temperature being 38.6°C, higher than the sudden change. These populations arrived at the same ending point as the sudden change populations but their path there was quite different (Figure 5). These populations did not see the immediate population increases or decreases that were seen in the sudden change populations.

2.3.2.i <u>Slow divergence of high and low standing variation populations</u>

The high standing variation populations began to moderately increase around halfway through the experiment (approximately transfer 15 or 35°C) rather than seeing the jump in population size that occurs over the course of two to three transfers in the sudden change populations (Figure 5A). There was no difference between standing variation levels 3 and 4 starting from the beginning of the experiment to transfer 15 ($F = 0.0045$, $p = 0.9465$), but standing variation level was a factor from transfer 15 to the end ($F = 4.5434$, $= 0.03414$). At 37°C (transfer 24), the size of these populations (1.833±0.027) was 10% below the size of the sudden change scenario populations. This increase over the remaining transfers leaves their final mean population size at 1.966±0.051, which was slightly, but significantly, smaller (4.4% decrease) than the ending population size of the sudden change scenario ($p < 1.31e-05$). However, the final temperature difference between the two populations is considerable at 1.6°C. After transfer 15, there was also an effect from individual samples in this scenario, independent of standing variation level (F = 5.447, p < 5.917e-09). This shows that in this scenario there is more variation due to individual populations without respect to their degree of standing variation.

Low standing variation populations also saw drastic differences when in the gradual change scenario (Figure 5A). They also had two outlier populations that are removed from the following analysis but discussed further below. The rest of populations, 14 out of 16, did not start showing large decreases in size until around transfer 23 at which point they were experiencing a growth temperature of 36.6°C, yet only showing a 2.4% decrease from their starting size (p = 0.006). There was a significant difference in performance between levels 1 and 2 of standing variation, with the slightly higher amount of standing variation showing a greater decline (F = 10.881, p = 0.001). After this point all low standing variation populations declined rapidly with there being a significant effect from level of variation (F = 10.882, p = 0.001) and from individual populations (F = 7.5248, p < 3.44e-12), even with outliers removed. This was the only occurrence of multiple independent populations having an effect outside of their level of standing variation.

2.3.2.ii *Derived unicellularity in high standing variation populations*

The divergence of the high and low standing variation population sizes was again driven by the presence of unicellular individuals in the high populations. Their presence was not immediate and they slowly accumulated in populations. This was most readily seen by observing the supernatant of sample tubes after they had been settled. Unicellular individuals do not settle quickly as multicellular individuals and as such give the media a cloudy rather than clear appearance. Absorbance readings were taken of only the supernatant from all gradual change populations. This revealed that the appearance of unicellular individuals began at approximately 34.8°C (transfer 13, Figure 5B). The appearance of the unicellular cells was slight at first but started to impact the population size by transfer 16, coinciding with the overall population increase of the high standing variation populations.

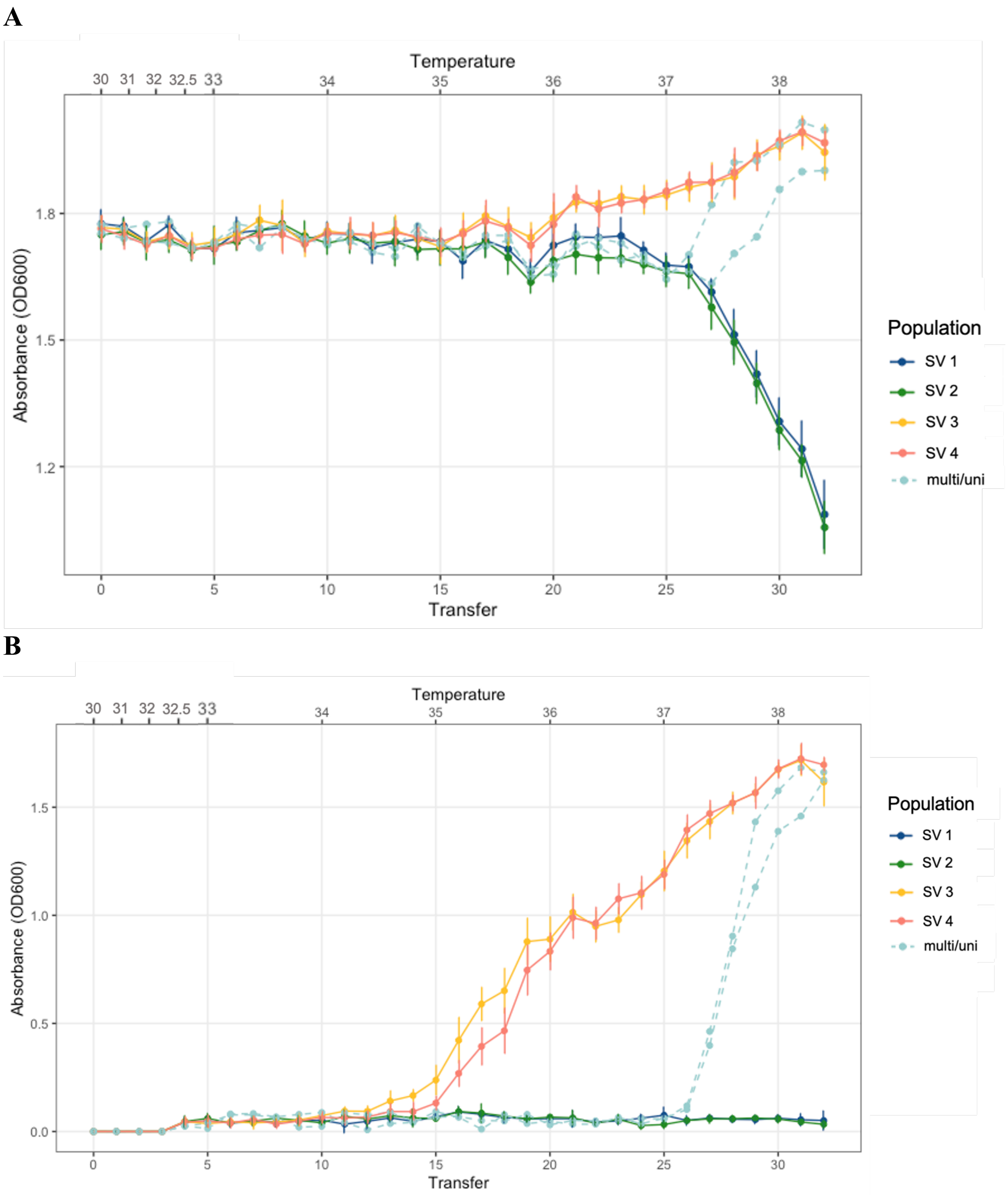


Figure 2.5. Relationship between population size (absorbance at OD600), transfer number (time), and temperature in the gradual change scenario. A) High standing variation populations are SV3 and SV4. Low standing variation populations with outliers removed are SV1 and SV2. Outlier populations (represented by dotted lines) are defined by their final phenotype: either a mix of multicellular and unicellular individuals or a population that stayed entirely multicellular. B) Only the supernatant of the populations shown in part A. This shows the presence of unicellular individuals more effectively.

2.3.3 *Evolved strategies*

The above analyses have had outliers removed from the low standing variation populations because these populations developed novel strategies that differentiated them from the rest of their experimental group. The high standing variation populations all utilized the same strategy, dubbed the "multi/uni" strategy. As described above, these populations uniformly derived a unicellular phenotype that persisted along with the multicellular phenotype.

This was the main strategy that was used in the outliers from the low standing variation populations. In the sudden change scenario, five out of 16 populations adopted this strategy (Figure 2: light blue, dotted lines). They did so at different time points throughout the experiment, but showed the same precipitous increase in population size when the unicellular individuals appear in the population. Of these five populations, three are from standing variation level 1 and two are from standing variation level 2, and there was no pattern to which derived this strategy first. The "multi/uni" strategy also appeared in the gradual change scenario (Figure 5: light blue, dotted lines). These both came from standing variation level 2 and population increases began at transfer 26 and 27, though the initial presence of unicellular individuals began at 26 for each (Figure 5B). Both of these populations adopted this strategy after they were past the 37°C mark. Their populations grew steadily over the next few transfers and reached a population size similar to that of the high standing variation populations. All of the low standing variations were comprised of pairs (eight pairs for each scenario) that were clonal replicates. In the sudden change scenario, two of the five "multi/uni" strategy populations were clonal replicates; the partners of the other three did not adopt this strategy. In the gradual change scenario, the two “multi/uni” populations were not clonal replicates.

The other strategy that arose was the “stay multi” strategy in the sudden change scenario. Only one population derived this strategy (Figure 2: pink dotted line). This strategy was characterized by the population remaining multicellular across the experiment but also seeing an increase in population size. This population began to increase at transfer 7 and continued to steadily rise, reaching a population size greater than its pre-stress size by transfer 34. This population came from standing variation level 1 and was not a replicate of any of the “multi/uni” populations.

2.4 DISCUSSION

This work supports the claim that high standing variation is of great value when adapting to a sudden shift in selection pressure, but finds that this occurs via the rapid fixation of a variant with a large effect size rather than the fixation of many alleles of small effect. To a degree we saw a similar result in the gradual change scenario, although here low standing variation populations were able to keep pace with high standing variation populations for a significant portion of time. In low standing variation populations we did see mutations of large effect have significant impacts on population size in both sudden and gradual change scenarios. Low standing variation populations also showed a greater number of adaptive strategies than were seen in high standing variation populations. Below we discuss how these results fit with model predictions and the possible implications of this work on the conditions necessary for evolutionary rescue to be a possibility for populations faced with drastic environmental changes. Our results agree with the predominant idea that standing variation is sufficient for populations to adapt very rapidly to evolutionary change, but they also show it is not strictly necessary.

2.4.1 *Single adaptive variant of large effect from standing variation*

Adaptation from standing variation has often been thought to occur through the fixation of many alleles of small effect size (Hermisson and Pennings 2005; Barrett and Schluter 2008). However, the results here suggest a large effect size. The transition from multicellular to mixed multicellular/unicellular populations resulted in a drastic increase in population size, though fitness was not directly measured here. The extremely rapid rate at which the beneficial variant swept through the population suggests only a single allele is responsible. For this result to have been caused by many alleles of small effect, the same set of alleles would have to be present in the genetic background of every initial high standing variation population. Additionally, these populations are clonally reproducing, making it impossible for independent small beneficial alleles from different backgrounds to come together through sexual recombination. There is the possibility of adaptation from many small beneficial alleles that are very tightly linked, though given the alternative, this seems unlikely.

Adaptation from standing variants of large effect has been predicted in at least one model (Matuszewski *et al.* 2015). The authors found that adaptive substitution from standing variation can allow populations to explore more phenotypic space given that the rate of change is fast rather than slow. The reasoning is that any large effect alleles in a population are likely to be immediately acted upon by selection if there is a sudden shift. However, when there is weak selection for a new optimum, these alleles will likely be removed by purifying selection, leaving smaller effect alleles to dominate adaptive substitutions when change is gradual. The population increases seen here were modest at first and these populations showed a significant effect from individual populations independent of standing variation level, which means there is a possibility that smaller effect alleles were reaching fixation within different populations. Interestingly, in the gradual change scenario, the population increase does not begin until 15 transfers in, yet the beneficial variant appears to be present in all populations and not lost to drift. Standing variants are assumed neutral or nearly neutral, so it might be expected that in some of these populations drift would have removed the beneficial allele from the population. Since all populations behaved similarly it is possible that the beneficial allele is linked to an allele that is under stabilizing selection or is just at a sufficiently high frequency due to neutral processes.

In both the sudden and gradual change scenarios presented here, the high standing variation populations likely increased in size due to a standing variant. In each scenario, there was a uniform response across populations, although the tempo of that response did differ between scenarios with the sudden change scenario eliciting a more rapid response. For all high standing variation populations it is unlikely that a *de novo* variant is driving change due to the improbability of independent *de novo* mutations resulting in the consistency seen across all 16 populations.

2.4.2 *Adaptation from de novo mutations*

Consistent with expectations on the relative importance of both sources of genetic variation, *de novo* mutations dominated when standing variation was low. In both sudden and gradual change scenarios, populations with low standing variation saw similar leaps in population size from what appear to be *de novo* mutations (light blue dotted lines in Figures 2 & 5). In the sudden change scenario, five populations developed the "multi/uni" strategy, which allowed them to reach the same population maximums as the high standing variation populations, all of which also used this strategy. The seemingly random time points at which low standing variation populations adopted this strategy suggest that the benefit is coming from mutations rather than standing variation. Additionally, the adoption of this strategy was not seen across replicate pairs, further indicating that the beneficial variants were not already present in the population's genetic background. The "stay multi" strategy also occurred in the sudden change scenario and also appears to use *de novo* mutations as the genetic basis for adaptation. This strategy was only seen in one population and was not characterized by the rapid increase in population size seen in the "multi/uni" strategy, but rather by incremental increases starting at transfer 7 and continuing for the course of the experiment. The slow, successional nature of this increase could indicate the fixation of many *de novo* mutations of small effect.

The gain of the successful "multi/uni" strategy was also seen in low standing variation populations in the gradual change scenario. Here, two populations that were not replicates adopted this strategy and saw rapid increases in population size. When change is gradual, models have shown that alleles of small or intermediate effect are more likely to fix before alleles of large effect (Bello and Waxman; Collins *et al.* 2007; Kopp and Hermisson 2007, 2009a). While we likely see the fixation of an allele of large effect after temperatures reach 37°C, the results do potentially show smaller fixation events along the way. The low standing variation populations in the gradual change scenario are able to maintain population sizes similar to high standing variation populations for much of the experiment, including up to the 37°C mark. Their ability to weather these temperatures with little negative effect could be due to the fixation of smaller alleles that allow them to stay near the fitness peak. Only when they are pushed further into population decline, further into a fitness valley, do we see the fixation of mutations of large effect.

2.4.3 *Fewer strategies in gradual change scenario*

In the sudden change scenario, three strategies were seen: the “multi/uni” strategy, the “stay multi” strategy, and the less successful strategy seen in the low standing variation populations that did not experience dramatic population size increases. In the gradual change scenario, only the “multi/uni” strategy and a less successful strategy emerged. A similar result was seen in Collins 2007, where a gradual change scenario led to less phenotypic diversity than seen in sudden change scenarios. In a sudden change scenario, the large shift in selection pressure leaves the ancestral phenotype at a large disadvantage and therefore allows any variant with higher fitness the chance to succeed. In a gradual change scenario the difference in selection pressure at any given point in time is modest compared to the sudden change and so the ancestral phenotype does not see the same decrease in fitness. This limits the ability of different phenotypes to offer significant advantages, making them more susceptible to drift, and consequently fewer novel phenotypes emerge.

Indeed, this assumption has been shown to be accurate in a study that saw standing variation hinders adaptation when the rate of environmental change is gradual

(Guzella *et al.* 2017). The authors showed that in *C. elegans* standing variants that are of the most benefit in an extreme environment (here salinity tolerance) are lost to either drift or founder effects when populations are gradually increased to that extreme. Additionally, they found that clonal interference from self-fertilizing *C. elegans* acted to further limit the frequency of the most beneficial variants and therefore increased their susceptibility to drift. This is also a possibility for the clonally reproducing yeast in this experiment, though the presence of competing beneficial variants cannot be assessed from these results alone. It is also unknown here whether the same standing variant was the underlying factor for population increase in both the sudden and gradual change scenarios. If it was, then this means that it was not lost to drift for a significant period of time since the adaptive strategy evolved around transfer 20 in the gradual scenario. This could mean that it was already at a relatively high frequency when the experiment began or it could be linked to a locus under stabilizing selection and not as affected by drift. Either way, the appearance of the "multi/uni" strategy in both the gradual and sudden change environments and in both high and low standing variation populations, agrees with previous findings that adaptive fixations of very large effect are likely to be beneficial no matter the genetic background (Lenski *et al.* 1998).

2.4.4 *Implications for evolutionary rescue*

When populations experience significant decline and are on route to extinction they can sometimes be saved by significant adaptations. This process of recovery is known as evolutionary rescue (Gomulkiewicz and Holt 1995). Decisive examples of evolutionary rescue in natural populations are rare, if they exist at all (Gomulkiewicz and

Shaw 2013), but models and laboratory experiments have shown that the potential for recovery from near collapse is there (Gonzalez and Bell 2013). It is generally believed that evolutionary rescue is possible when the rate of environmental change is gradual (Bell 2013; Lindsey *et al.* 2013; Carlson *et al.* 2014). When change is slow there is more time for a beneficial mutation of large effect to occur and drive adaptation. However, even as this occurs the resulting change in phenotype may lag behind the rate of environmental change, and if this "phenotypic lag" is too large the population will be at risk for extinction (Kopp *et al.* 2018). Similarly, the fixation of alleles of small effect from standing variation may also not be enough of a fitness boost to overcome lag. Thus, the slower the environmental change, the more potential for evolutionary rescue to occur. However, predictions from models have shown that evolutionary rescue is possible when selection pressures change rapidly, but the likelihood of rescue is severely decreased (Martin *et al.* 2013; Anciaux *et al.* 2018). In keeping with previous theory, these models show that when change is sudden the primary genetic source for adaptations is standing variation.

Here, we see that evolutionary rescue is not dependent on gradual change but is strongly correlated to standing variation. In the sudden change scenario, populations that had high amounts of initial standing variation were able to keep pace with the rapid change in selection pressure. However, a significant portion of low standing variation populations were also able to rebound even when the environmental change was sudden and there was a dearth of genetic variation. While the selection pressure here was not enough to drive any population to extinction, we believe the steep rate of decline followed by swift recovery in successful low standing variation populations is in keeping with the traditional U-shaped trajectory of evolutionary rescue (Carlson *et al.* 2014). This result of seeing rescue even with little standing variation is likely very dependent on the relatively large population sizes that accompany experimental evolution systems and has been noted previously in yeast (Samani and Bell 2010). The larger the population, the more opportunity for beneficial *de novo* mutations to arise, and consequently this outcome of evolutionary rescue without sufficient standing variation in conditions of sudden change may not be accessible to natural populations.

The most likely occasion for evolutionary rescue to occur is when there is sufficient standing variation and change in the environment is gradual. The results here, however, show that when change is gradual, standing variation was of little importance and even clonal populations kept pace with high standing variation populations. If we consider only the portion of the gradual scenario that reaches the same temperature as the sudden scenario, that is just until 37°C, there is only a small difference in outcome based on standing variation level. Low standing variation populations saw only modest negative effects, even at the extreme temperature that had caused their swift decline in the sudden change scenario. This shows that even populations with little to no standing variation are capable of adapting to large climatic shifts given that the change is sufficiently gradual.

2.4.5 ***Utility of experimental evolution in testing assumptions***

This experiment both supports and contradicts various predictions from different models. Experimental systems, such as the one used here, can help bridge the gap between natural populations and simulations and theory. Of particular use may be the ways that the added complexity of an organism sheds light on model assumptions. In gradual change models, the rate of change in the selection coefficient is often static. Here the rate of change in the temperature increase was also static, at an increase of 0.2°C per day. However, from these results it appears that this may not have resulted in a static selection coefficient. Populations in the gradual change scenario, whether they had high or low initial standing variation, saw minimal population decreases for a substantial amount of time, including up to the point of reaching the temperature peak for the sudden change scenario. However, there was a point at which strong divergence between populations began and the population trajectories resembled those of their counterparts in the sudden change scenario. The possibility, then, is that rather than experiencing a constant gradual change in selection pressure, there was a sudden point at which the heat took a sudden toll on the yeast physiology—a sudden point at which the selection pressure increased disproportionately to the environmental rate of change.

This shows that models for natural populations need to take into account the additional biological complexity of the system they are attempting to describe. A constant rate of environmental change may not have a constant effect on a population. The fixation of adaptive variants along the way can alter the selection pressure or there may be some point at which physiological limits have been reached and the selection pressure swiftly changes from moderate to intense. Models evaluating the role of standing variation in events of gradual change have only recently been developed, but a further consideration for these models should be determining the practicality of using a static rate of change for selection. More broadly, when considering slow environmental changes in natural populations, one cannot assume that the pressures on a population are parallel to the rate of environmental change. There is great value and importance in using a genetic perspective to inform our understanding of how populations adapt to climate change. The more we can interweave our theoretical knowledge of the mechanisms of adaptation with the biological realities of populations, the more power we will have for predicting their evolutionary fate.

CHAPTER 3

Standing variation and the long term consequences of short term solutions

3.1 INTRODUCTION

Adaptation may maintain populations despite sudden environmental change, depending critically upon available genetic variation. Of the two sources of genetic variation, standing variation and *de novo* mutation, the former is frequently thought to be of greater importance in adapting to rapid environmental change. However, the longer term impacts of each source of genetic variation on evolutionary trajectories remain uncertain (Pigliucci 2008). Standing variation may aid finding an immediate evolutionary solution to the problem, but how does it affect longer term evolutionary solutions?

Significant standing variation is thought to confer a large advantage in short-term evolution (Barrett and Schluter 2008). Standing variation is genetic variation that began as neutral mutations, exhibiting little to no impact on fitness. These variants spread through the population primarily via drift and their frequency is only dependent on how long they have been segregating in the population. The importance of standing variation becomes important at the time of an environmental shift. These previously neutral alleles may suddenly become beneficial in the new environment, and, therefore, the higher the amount of standing variation in a population, the higher the population’s chance of possessing an allele that allows for adaptation.

In contrast, populations with little standing variation are reliant on a new, *de novo* mutation for adaptation to sudden environmental change. Since a beneficial new mutation would occur randomly, this population would not have immediate access to alleles that would benefit fitness, and instead would have to wait an undetermined amount of time for them to arise. Additionally, when a beneficial variant does appear, it will necessarily enter a diploid population at a frequency of 1/2N (Kimura 1983). This means this allele will take time to reach the same frequency at which a standing variant may start. Due to both the immediacy of use and the higher relative frequency, standing variation is thought to be more critical to rapid adaptation than *de novo* mutation.

From this reasoning alone, one could begin to think of standing variation as a sort of panacea to the problem of a rapidly changing environment. However, there is a key component missing here, and that is an evaluation of how each genetic basis for adaptation may affect a population over time. Standing variation is useful for an immediate response, but it is thought to affect adaptation by the fixation of many alleles of small effect (Hermisson and Pennings 2005). *De novo* mutations are thought to have a larger effect size, but the waiting period must be endured. The assumptions for these different sources of genetic variation predict that they will be of effect on different time scales. Does utilizing standing variation for an immediate fitness benefit limit a population’s potential for evolution in the future? Are there long-term tradeoffs when adapting quickly versus slowly or is getting a head start always good?

Here I evaluate the long term consequences of short term solutions. By creating yeast populations with different levels of standing variation, I test the degree to which initial genetic variation affects the population's long term evolutionary response. I am interested in evaluating the extreme ends of the genetic variation spectrum and therefore create populations that can be distinctly classified as high or low standing variation, including populations that are solely dependent on new mutations. The yeast populations for this experiment are multicellular, rather than typical single cells. During cellular reproduction in multicellular yeast, daughter cells do not completely separate from their mother cells when they bud and form snowflake clusters as a result. Snowflake multicellular clusters reproduce primarily breaking into two clusters of differing sizes.

Multicellularity adds the benefit of complexity. Complex organisms are more likely to be limited by life history tradeoffs and therefore if there are constraints from different genetic starting points, a more complex organism is more likely to show these (Orr 2000). All experimental populations are from a founding population that gained the trait of multicellularity fairly recently in its life history as a result of selection pressure (Ratcliff *et al.* 2012). Here, this pressure will be maintained and the populations will be assessed by their ability to adapt to a novel stressor in the form of a sudden increase in the heat of their growth environment. The effects of this heat stress become immediately apparent and the reaction from populations with different levels of standing variation is assessed over a period of 90 transfers, or approximately 550 generations.

3.2 METHODS

3.2.1 *Strains and media*

The yeast used in this study is an evolved multicellular lineage of *Saccharomyces cerevisiae*. This lineage was derived from a unicellular ancestor (Y55 strain) via selection for settling ability (Ratcliff *et al.* 2012). Under this regime, yeast daughter cells do not break away from the mother cell after reaching maturity, and as a result multicellular clusters are formed. Settling selection was continued for 14 weeks, allowing variation to accumulate as populations continually evolved to a constant pressure. This generated a population containing high variation from which experimental populations were derived. Two different types of starting populations were generated: one with high standing variation and one with low standing variation. There were 16 populations created for the low standing variation condition. These populations were created by dilution plating and selected isolated colonies from the original high variation population. Complementing these were 16 populations that contained high standing variation. Eight were 1% of original yeast population, and thus were a good proxy for sampling all of the variation present in the original population. Eight were 0.1% of the population. All populations were maintained in 10mL of YPD (per liter distilled water: 10g yeast extract, 20g bacterial peptone, and 20g dextrose) in 25mm × 150mm glass culture tubes.

3.2.2 *Creation of populations with different levels of standing variation*

Level 1 populations consisted of a single colony from the ancestral population. This population should have no standing variation as it is derived from a strain that is only facultatively sexual and therefore all cells will be clonal. The level 2 population was created by selecting and combining 5 colonies from the ancestral population. Level 3 was created from a 10μL sample of the ancestor and level 4 from a 100μL sample. Levels 1 and 2 are considered to be low standing variation populations while 3 and 4 are high standing variation (Figure 1).

Eight individual populations were made for each level of standing variation. For the high standing variation populations, each population is an independent genotype. For the low standing populations, each population has a replicate so that there are paired genotypes. The purpose of this is to see if the colonies of the same genotype behave similarly across the experiment. If there is an adaptation that occurs in one replicate but not the other, then it is more likely to have been the result of a random new mutation and not due to the genetic background of that individual. Conversely, if each pair of replicates reacts in the same way then the reaction is more likely to be the result of standing variation. For example, the level 1 standing variation populations comprise four pairs, each of which represents a different single colony isolate. This means there are four genotypes represented in level one and all populations are starting with essentially zero standing variation because they are initiated from single colonies. The same scheme is true for level 2, there are four pairs of distinct genotypes, but here there is more variation because each population is the combination of five colonies. Additionally, there was a set of control tubes that were not subjected to novel selection. These consisted of four tubes from each standing variation level, so a set of 16 total tubes. These levels represent relative, not quantitative, measures of standing variation. The purpose of this experimental design is to assess the extremes of the genetic variation spectrum where there are strongly divergent expectations for where adaptive variants originate.

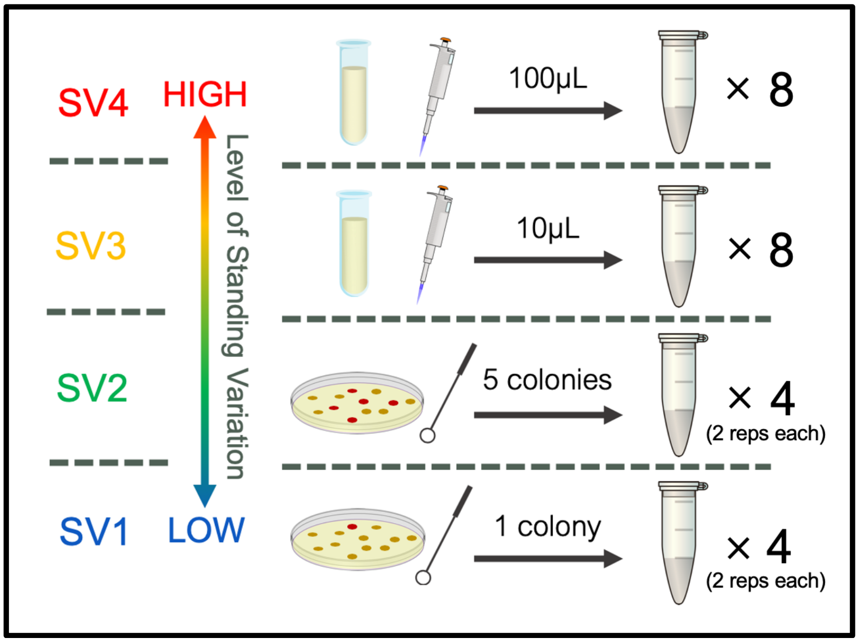


Figure 3.1. Schematic of how experimental populations were created from an ancestral population. SV 1 represents the lowest level of standing variation, with all individuals being clonal; SV 4 is the highest, representing all variation contained in the initial population. SV 3 & 4 represent eight populations each with a distinct genotype while SV 1 & 2 represent eight populations with paired replicates of four genotypes.

3.2.3 *Exposure to heat stress*

These 32 populations were grown for 24 hours at 30°C, shaking at 250 rpms, in 25mm × 150mm glass culture tubes. Then they were subjected to the same settling conditions as the ancestral population so that settling is not a new environmental stressor. Upon removal from the incubator, 1mL of culture is pipetted from the culture tube into a 1.5mL microcentrifuge tube. This culture is allowed to settle undisturbed on the bench top for 10 minutes. The top 900μL of culture is then carefully pipetted off and discarded. The bottom 100μL of culture is thoroughly mixed and pipetted into fresh YPD media in glass culture tubes. Tubes were then placed in an incubator set at 37°C and shaking at the same 250 rpms. The abrupt change from 30°C to 37°C represents the novel heat stress.

From this point tubes were always placed in the 37C incubator after being transferred. This process was repeated every 24 hours (+/- 30min) for 90 days. Control tubes were kept at 30°C and transferred daily in the same manner described above.

3.2.4 *Population growth assay*

To assess population growth, absorbance measurements (OD600) were taken at every transfer for the first 14 transfers, then every other transfer. For the populations in the sudden temperature change regime the cultures were thoroughly vortexed and 300μL of sample was combined with 300μL of water. Samples were again mixed by pipetting immediately prior to taking absorbance readings because settling rate was very rapid in most samples. For populations in the gradual temperature change regime, samples were taken in the same manner as above and supernatant samples were also taken. For the supernatant samples, tubes were allowed to sit for ten minutes on the benchtop, then 300μL was taken from the top of each tube and again combined with 300μL water. These were again mixed before readings were taken.

3.2.5 *Isolating phenotypes*

In order to assess differences that arise, isolates were obtained by repeated plating. The original samples had 1mL removed and then serially diluted to arrive at a concentration that would give 100-200 colonies per plate. The dilution factor was different based on each individual sample. The diluted sample was spread on YPD plates using glass beads. Plates were then grown for 24-48 hours at the same temperature that they had been growing at before isolation (this was different per experiment). Colonies that had distinct phenotypes were selected via inoculating loop and streak plated. These plates again grew for 24-48 hours. These plates were then visually assessed to see if all colonies were of the same phenotype; if not, the streak plating was repeated until only a single phenotype remained. These colonies were then transferred via inoculating loop to a tube of fresh YPD media and grown for 24 hours. Phenotype was then reassessed and characterized via microscopy of the liquid sample.

3.2.6 *Scoring sporulation efficiency*

Yeast reproduces sexually by means of sporulation. This typically occurs when conditions are too stressful for normal growth, for example, in low nutrient environments. Diploid yeast undergo two rounds of meiosis to produce four haploid spores. The mother cell then collapses around these spores and forms an ascus. Pairs of spores then either fuse within the ascus or the ascus can be broken down to release spores for outcrossing. Sporulation assays were done at the beginning of the experiment before exposure to heat stress, in the middle at five transfers, and at the end after ten transfers. For each population, 1mL was transferred to 10mL sporulation media (per liter distilled water: 20g potassium acetate, 2.2g yeast extract, 0.5g glucose, 870mg amino acid complex) and grown for 48 hours at 37°C, shaking at 250 rpms, in 25mm × 150mm glass culture tubes. One-tenth dilutions were made and observed under a light microscope. Percent sporulation was calculated as the fraction of cells present that showed any spore development, including dyads, triads, and tetrads, all counted equally as forms of sporulation. Approximately 200-400 cells were assessed per slide; three technical replicates were made per population.

3.2.7 *Statistical analysis*

Models were used to determine the statistical significance of population size changes as a factor of transfer number and standing variation level. Different timepoints and evolutionary responses required different model parameterization and models were compared using AIC. The initial population decrease was modeled using a linear model with only transfer number as a factor. The rebound in the high standing variation populations used a log linear model where only transfer number was determined to be a factor. A majority of the models also only showed transfer number as a factor for population size, with the exceptions noted below where individual population was also a factor. Significance was tested using an ANOVA. Analyses for the non-"minimal" low standing variation populations required outlier responses to be removed prior to analysis, the reasoning for which will become apparent in the results. There was stronger significance with outliers removed but response trends were the same regardless of their inclusion. Individual population growth rates were calculated for outlier responses using the R package growthcurver. Significant differences between population growth rates were compared using a Chow test. All analyses were performed and graphed in R v4.0.3.

Table 3.1. AIC values for different models. High and low standing variation populations were modeled separately. TRAN refers to transfer number, SV to level of standing variation, and IND refers to sample number.

High SV Models	**df**	**AIC**	**Δ AIC**	**Low SV Models**	**df**	**AIC**	**Δ AIC**
HIGH: TRAN	3	-1703.71	0	LOW: TRAN	3	-147.15	72.23
HIGH: TRAN + SV	4	-1702.20	1.51	LOW: TRAN + SV	4	-148.22	71.16
HIGH: TRAN * SV	5	-1700.22	3.49	LOW: TRAN * SV	5	-146.22	73.16
HIGH: TRAN + SV + IND	18	-1693.82	9.89	LOW: TRAN + SV + IND	9	-189.13	30.25
HIGH: TRAN + SV * IND	33	-1669.65	34.06	LOW: TRAN + SV * IND	15	-219.38	0

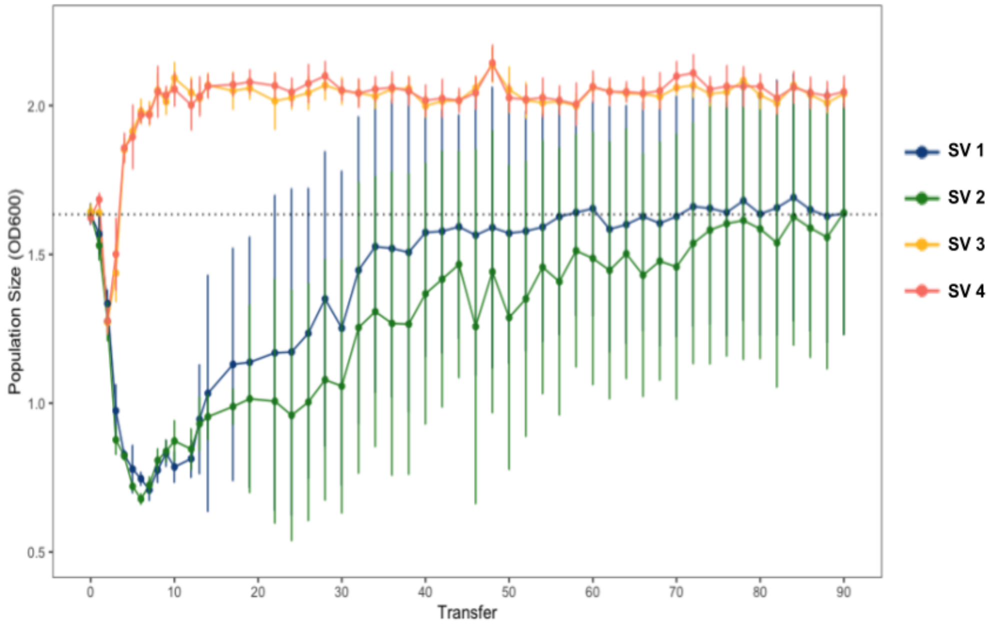
3.3 RESULTS

3.3.1 *Rapid divergence of high and low standing variation populations*

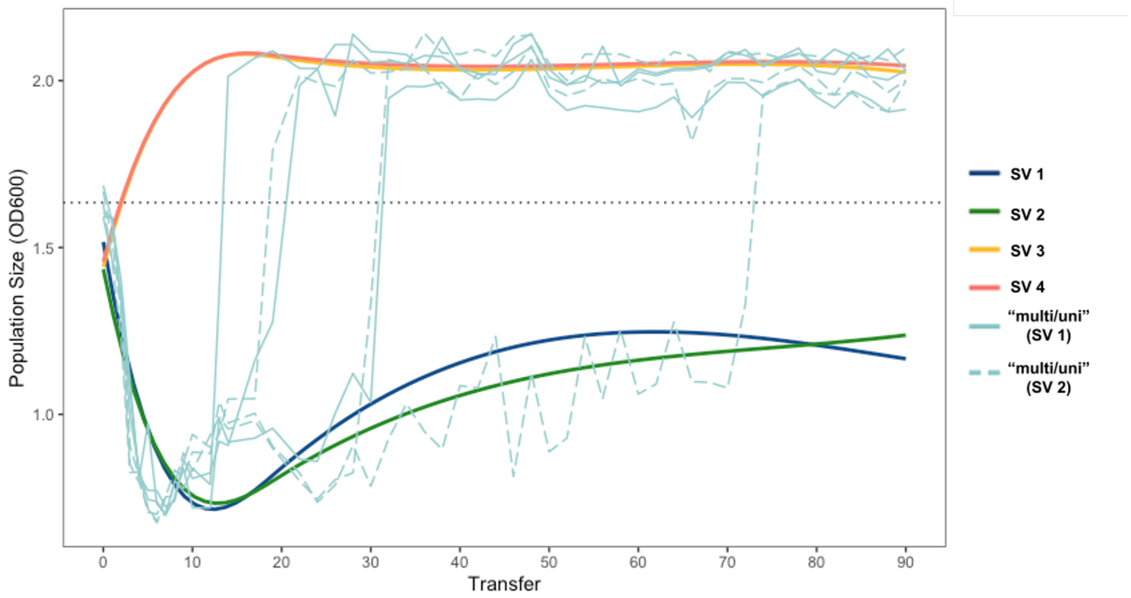
There was an immediately obvious difference between the high and low standing variation populations in response to heat stress. High standing variation populations showed an initial decrease followed by a rapid increase in population size. Low standing variation populations showed a similarly paced initial decline (p = 0.309, F = 1.046), but did not rebound after the first few transfers and continued their trajectory towards a depressed population size. There was no difference in rate of levels 3 and 4 (p = 0.468, F = 0.527).

The low standing variation populations did not see the sudden increase in population size and suffered the effects of heat stress for the duration of the experiment. These populations saw the same initial drop off that steadily continued until transfer 6. Within the low standing variation populations there was no difference in population decrease across levels 1 and 2 or among individual tubes (p = 0.582, F = 0.306). After transfer 7, these populations began to tolerate the heat stress. When all low standing variation replicates are included, these populations show a slow process of adaptation, however, grouping all of the replicates together obscures the responses of this system (hence the large error bars in (Figure 2A). The next section will detail the multiple evolutionary responses that arose in the low standing variation populations, and further analysis will remove the outliers that evolved unique strategies (Figure 2B). The low standing variation populations gradually increased in population size over the remaining transfers but overall showed decreased population size due to heat stress.

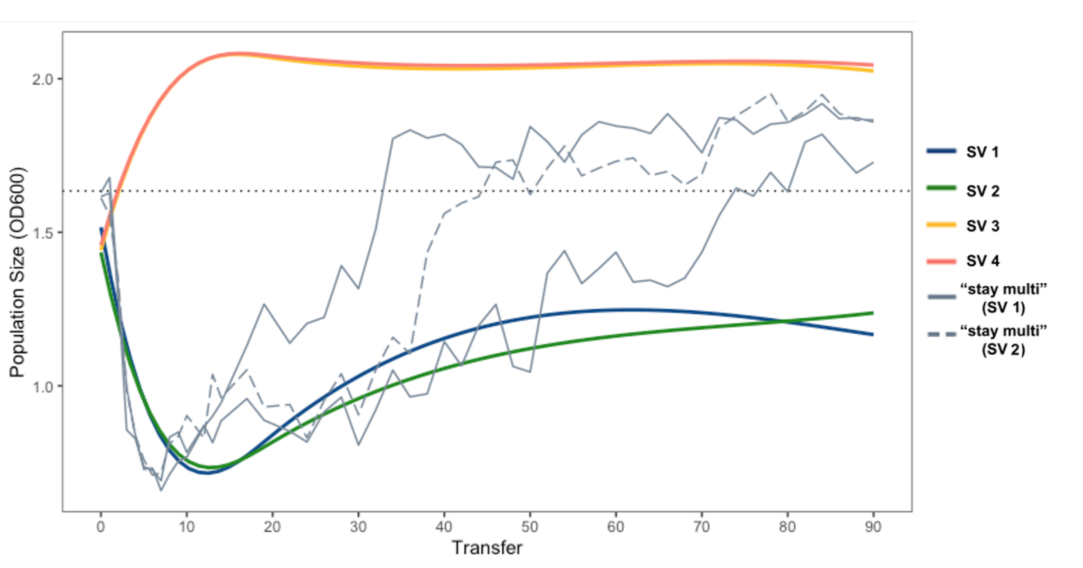
A


Population Size (OD600)
0.5
1.0
1.5
2.0
0
10
20
30
40
50
60
70
80
90
Transfer
SV 1
SV 2
SV 3
SV 4

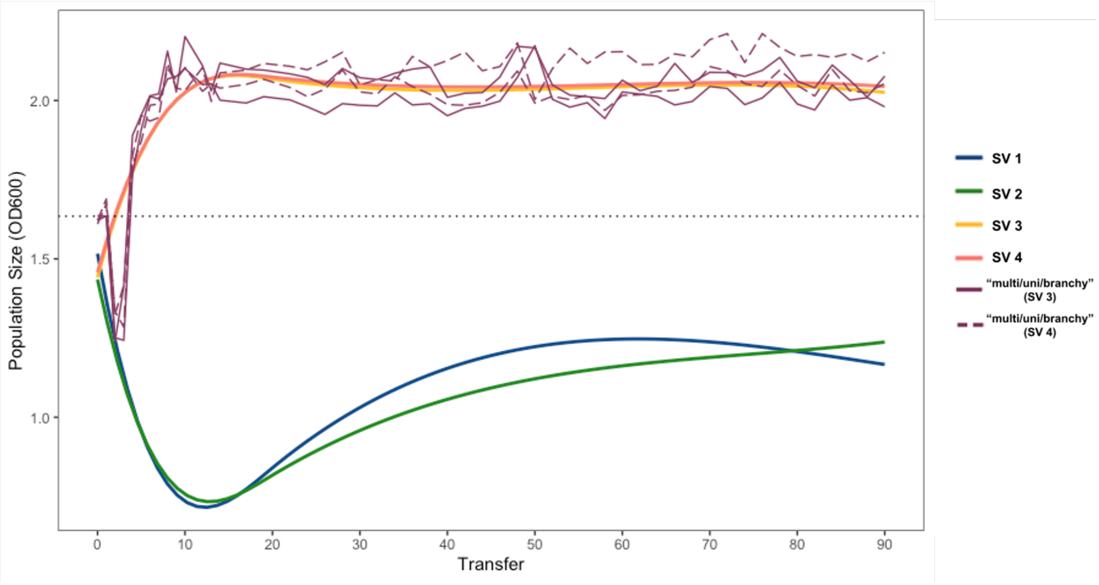
B


Population Size (OD600)
1.0
1.5
2.0
0
10
20
30
40
50
60
70
80
90
Transfer
SV 1
SV 2
SV 3
SV 4
“multi/uni” (SV 1)
“multi/uni” (SV 2)

C


Population Size (OD600)
2.0
1.5
1.0
0
10
20
30
40
50
60
70
80
90
Transfer
SV 1
SV 2
SV 3
SV 4
"stay multi" (SV 1)
"stay multi" (SV 2)

D


Population Size (OD600)
2.0
1.5
1.0
0
10
20
30
40
50
60
70
80
90
Transfer
SV 1
SV 2
SV 3
SV 4
"multi/uni/branchy" (SV 3)
"multi/uni/branchy" (SV 4)

E

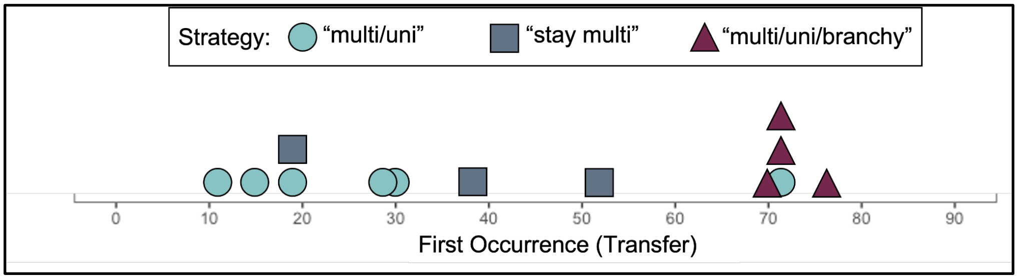


Figure 3.2. Relationship between population size (absorbance at OD600) and transfer number (time). Dotted black line is the average starting value across all populations. **A)** Color of line indicates level of standing variation. Each point is an average of 8 replicates. Larger error bars on SV1 & SV2 show different evolutionary strategies at play rather than experimental error (see text). **B)** Evolved strategies from low standing variation populations have been removed. Remaining populations are summarized by fitted splines (bold curves). Light blue lines are individual low standing variation populations that derived the “multi/uni” response. Dashed vs solid lines show SV level. **C)** Similar to B, but gray lines represent low standing variation populations that derived the “stay multi” response. Dashed vs solid lines show SV level. **D)** Similar to B & C, but maroon lines represent high standing variation populations that derived the “brancy/multi/uni” response. Dashed vs solid lines show SV level. **E)** Timeline showing when the phenotypes of each evolutionary response first appeared.

3.3.2 *Different evolutionary strategies for adaptation to heat stress*

Four distinct evolutionary responses were present at the end of this experiment, three of which were successful in fully recovering to initial population size. These will be labeled the “stay multi”, “multi/uni”, and “multi/uni/branchy” responses (Table 2). The relatively unsuccessful low standing variation populations that did not show great increases in population size will be referred to as having the “minimal” response. The “stay multi” was exclusive to the low standing variation populations. The “multi/uni” was shared by high and low standing variation populations. The “multi/uni/branchy” was exclusive to the high standing variation populations. Each response is described below.

Table 3.2. Populations with different successful evolutionary responses in adaptation to heat stress. Label refers to individual population number, Transfer is when the response phenotype first began.

Label	SV level	Evolutionary response	Transfer (Day)
1.1-1	1	multi/uni	12
1.1-2	1	multi/uni	30
1.2-1	1	multi/uni	18
1.4-1	1	stay multi	18
1.4-2	1	stay multi	52
2.1-1	2	stay multi	38
2.2-1	2	multi/uni	16
2.3-1	2	multi/uni	72
2.3-2	2	multi/uni	28
3.7	3	multi/uni/branchy	70
3.8	3	multi/uni/branchy	76
4.3	4	multi/uni/branchy	72
4.4	4	multi/uni/branchy	72

3.3.2.i *“Minimal” response*

This response was exhibited by 7 of the 16 low standing variation populations (blue and green splines in Figure 2B), and is characterized by a lack of recovery from heat stress. These populations showed the same initial decrease over transfers 1 through 7 as all low standing variation populations, but were never able to rebound to their initial population size. They were able to show some improvement over the course of the experiment but showed an average of 25% decline from start to finish when introduced to heat stress. These populations all stayed multicellular throughout the experiment and strongly resembled the ancestor phenotype (Figure 3B).

3.3.2.ii *“Stay multi” response*

This response was exhibited by 3 of the 16 low standing variation populations. It is characterized by gradual adaptation to the heat stress (maroon colored lines in Figure 2B). These populations were able to completely recover to the point of the initial population size while remaining multicellular the entire time (see dotted black line). The rate of increase in these populations is slower than other successful strategies and does not end with as high a final population size. The final population sizes averaged 12% higher than their initial population averages. This response developed in both levels 1 and 2 of the low standing variation populations. As the name suggests, these populations all stayed multicellular throughout the experiment. The clusters stayed approximately the same size but developed a more rounded phenotype than the ancestor (Figure 3C).

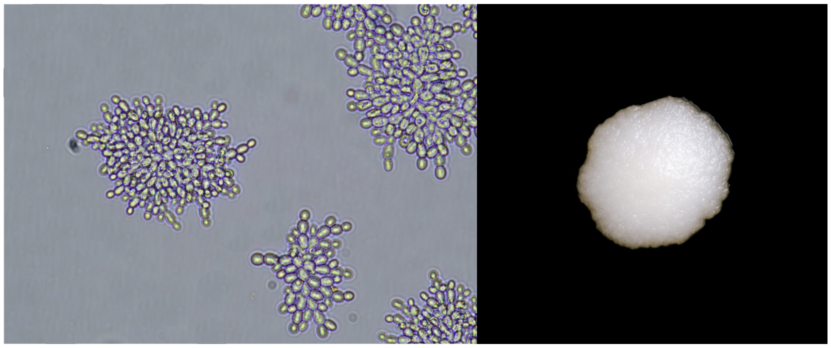
3.3.2.iii *“Multi/uni” response*

This response was seen in both high and low standing variation populations. Every replicate of the high standing variation populations, from both levels 3 & 4, quickly arrived at this response. These populations showed the same unexpected response to heat stress: a reversion to unicellularity. The advantage of this response is readily seen. The driver of the strong divergence that begins at transfer 4 coincides with the appearance of unicellular individuals. At transfer 4, all yeast populations are still largely multicellular but there is a transition toward a broader range of cluster sizes. Many clusters are still as large as the ancestor but smaller snowflakes begin to appear, particularly ones that are composed of fewer than 10 cells. By transfer 5, there are still large snowflakes, but there are many small snowflakes, now often of 3 to 6 cells, and there are also many unicellular cells present (Figure 3D). This pattern of declining cluster size is consistently seen across all high standing variation replicates. The presence of the unicellular cells can be easily seen while still in tubes after settling for ten minutes. The unicellular cells do not settle within the time frame and are observed as a cloudy supernatant in (Figure 5). By approximately transfer 10, the frequency of unicellular cells seems to peak but the majority of the transition occurs over three transfers. After the population sizes reach a maximum many multicellular clusters of all sizes are still present. The unicellular cells do not sweep to fixation in the population and do not dominate the population. Of the high standing variation populations, 12 out of 16 see this response at the end of 90 transfers while 4 populations derive the "multi/uni/branchy" response described below.

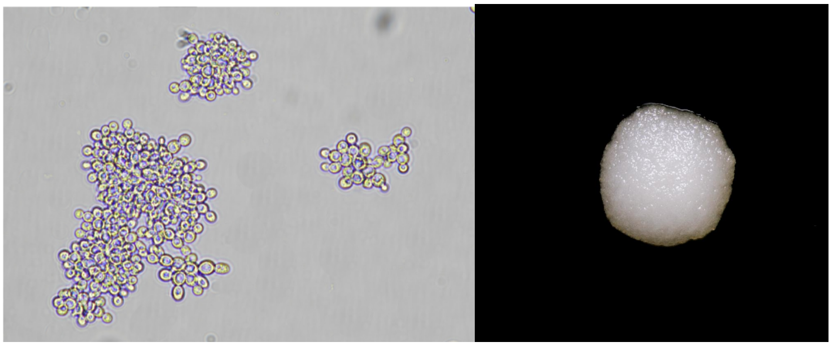
The appearance of unicellular individuals also occurs in 6 of the 16 low standing variation populations. When this occurs it follows the same process as the high standing variation populations: smaller multicellular clusters come first, then unicellular cells, and within approximately three transfers populations reach a maximum size. These populations also do not see the unicellular phenotype sweep and are indistinguishable from the high standing variation populations both on plates and under the microscope (Figure 3D vs 3E). The primary difference from the high standing variation populations is that these sudden bursts in population size do not occur simultaneously in the 6 low standing variation populations. Instead the changes seem to occur randomly across the experiment (light blue lines in Figure 2B, Table 2). The earliest population to develop this response gained it at transfer 13, while the latest gained it at transfer 72.

3.3.2.iv *“Multi/uni/branchy” response*

This evolutionary response was seen only in the high standing variation populations and occurred only towards the end of the experiment. The populations that developed this response, 4 out of 16, all originally had the “multi/uni” response. The innovation here is a new multicellular phenotype, called the “branchies” because of peripheral cells in the clusters developing an elongated phenotype (Figure 3F). When plated these cells also display a novel phenotype. Rather than appearing as colonies with a wrinkly surface and ragged edges, these colonies appeared smoother with a domed shape. This phenotype also does not sweep to fixation, and these populations show a relatively even coexistence of normal multicellular clusters, unicellular cells, and branchy phenotypes. These populations do not settle as compactly as the populations with no branchies (Figure 4). This response was similarly timed for all four populations, but did not affect their population size (maroon lines Figure 2D, vertical lines show onset of response).



A



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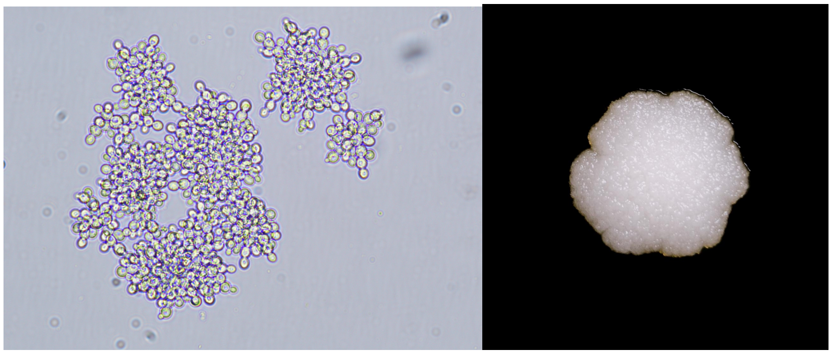

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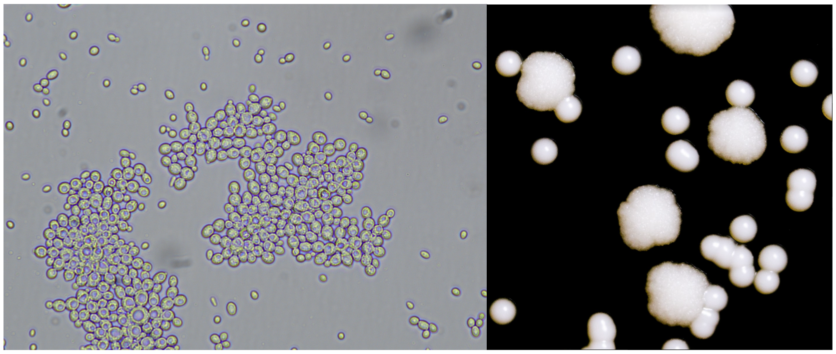

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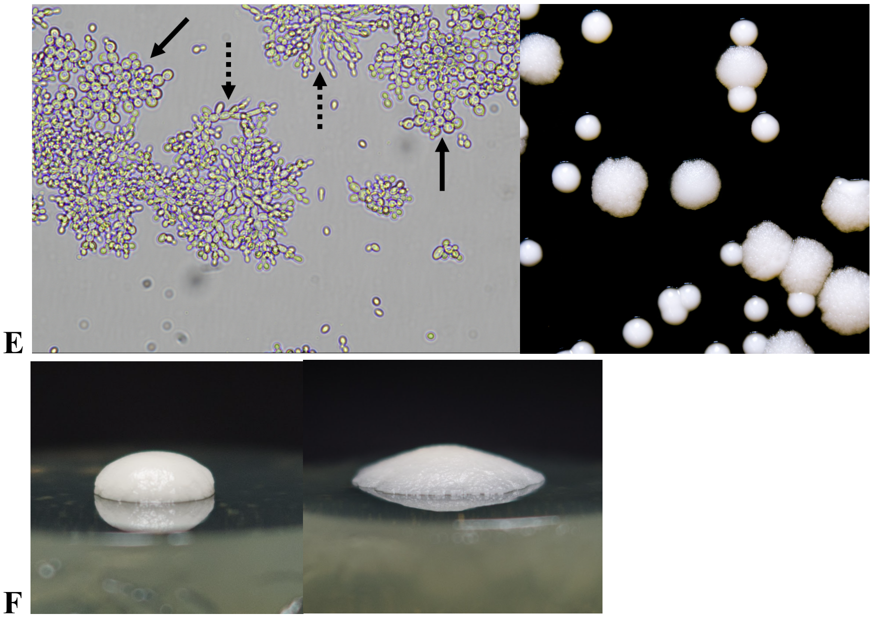


Figure 3.3. Photos of phenotypes under microscope and plated. **A)** Ancestral phenotype, only wrinkly multi colonies, all others derived from this; **B)** “minimal” response; **C)** Evolved “stay multi” response in low standing variation population, only wrinkly multi colonies; **D)** Evolved “multi/uni” response: wrinkly multis and small, smooth uni colonies; **E)** Evolved “multi/uni & branchy” response in high standing variation population. Solid arrows show normal multi, dotted arrows show branchy, budding unicellular cells are also present. **F)** Branchy multi colonies are more domed and not as wrinkly as normal multi colonies.

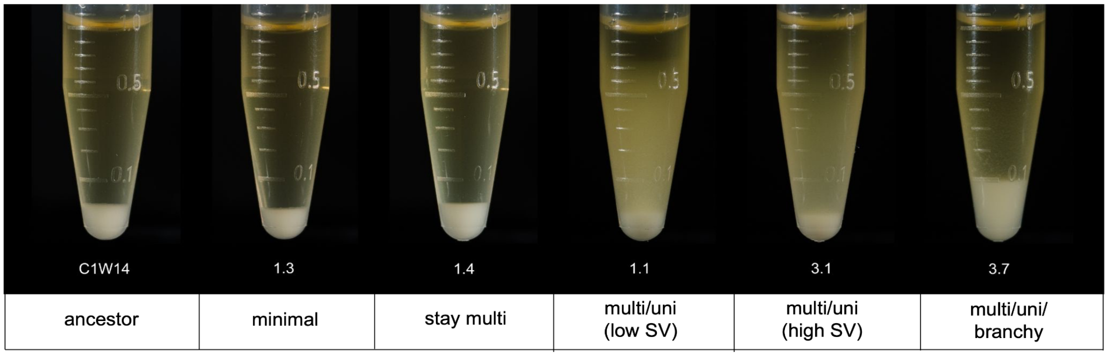


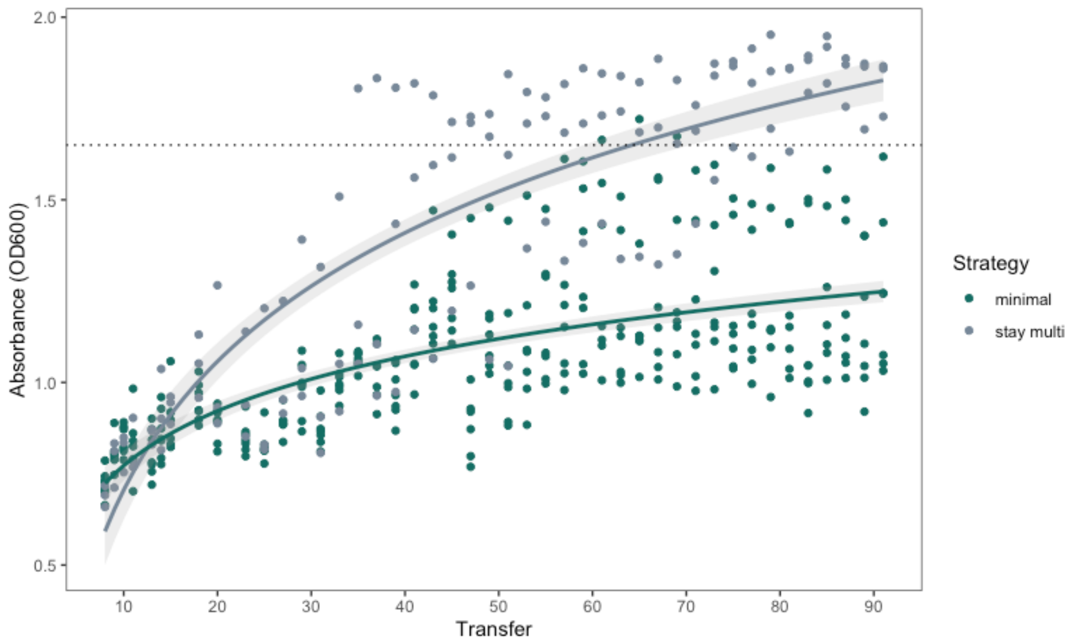
Figure 3.4. Difference in settling ability for each evolutionary response and the ancestral population. Clear liquid in the first three strategies indicates that the entire population was able to settle into a pellet at the bottom. The cloudiness seen in the latter three strategies indicates the presence of unicellular cells that do not settle.

3.3.3 *Rate of recovery by evolutionary response*

The different responses show strong differences in the rate of population increase across the experiment. The “stay multi” and “minimal” strategies showed a slow increase over the course of the experiment while the “multi/uni” response was characterized by swift increases in population size. The “minimal” response showed the least improvement. For these populations the best fit model was a log linear model using an interaction effect between transfer number and individual sample because two samples performed better than the other five after transfer 50; level of standing variation was not a significant factor (F=73.23, $p<2.2e-16$, Adj $R^2=0.7536$; Figure 5A, green line). The “stay multi” was also best fit by a log linear model with interaction between transfer and individual sample, with the most variation coming from one sample that did not increase in size quite as much as the other three (F=176.5, p-value < 2.2e-16, Adj $R^2=0.8701$; Figure 5A, gray line).

The “multi/uni” response was divided into high and low standing variation and reduced to reflect just the period of increase. For the high standing variation population, this means that the period from transfer 3 to transfer 9 was included. For the low standing variation population, the seven transfers past their initial date of increase were included. This date was different for each population (Table 2), and all populations were overlaid (Figure 5B). Each population was modeled separately by logistic curves determined using the growthcurver R package. There was no difference between the rate of increase for levels 3 and 4 of the high standing variation populations (F=2.16, p-value=0.213). They were analyzed together and their rate of growth is indicated by the dotted line in Figure 5B. A Chow test was then performed to compare the rates of increase between the high standing variation populations and “multi/uni” response populations from the low standing variation populations and they were significantly different (F=1.41e+01, p=2.93e-06). The growth rate parameters were consistently equal or higher in the low standing variation populations. Modeling the “multi/uni/branchy” response separate from the other high standing variation populations did not produce a significant result (Chow test: F=1.91, p=0.149).

A



B

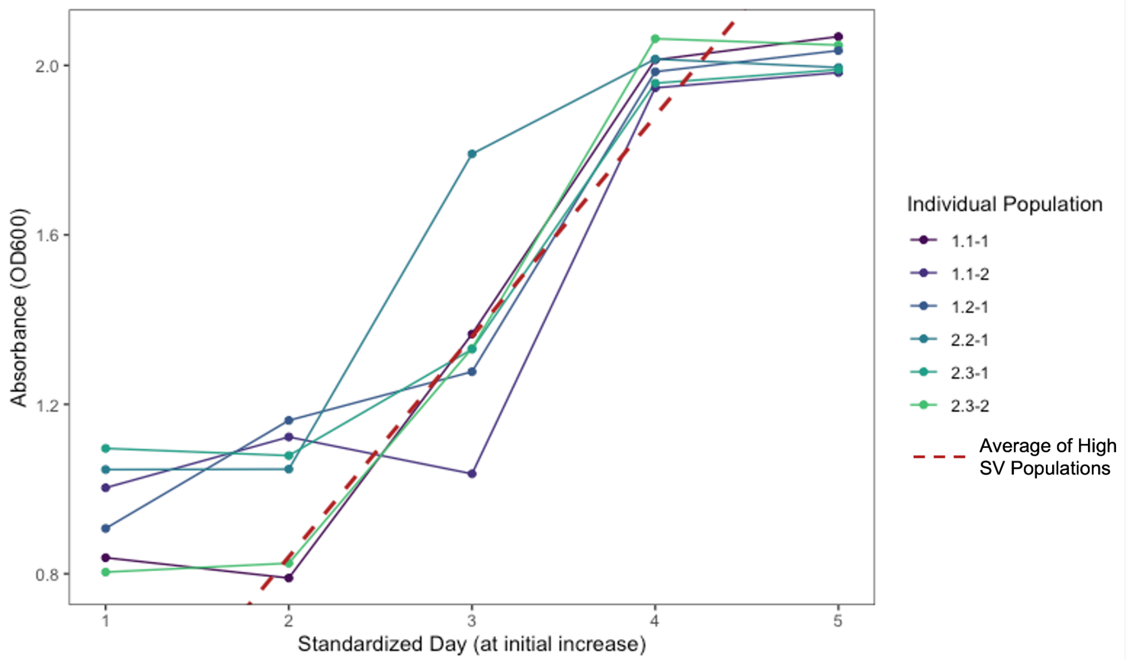


Figure 3.5. Rate of population size based on response type. **A)** Log linear regression models for the “minimal” and “stay multi” responses. Gray shading represents 95% confidence. Dotted black line is the average starting value across all populations. **B)** Individual populations from the low standing variation “multi/uni” responses. Date of initial increase is standardized for easier comparison. Dotted line is the initial growth rate for the high standing variation populations as determined by logistic regression.

3.3.4 *Loss of sporulation*

There is a stark difference in sporulation ability between strategies (Figure 6). The ancestral population sporulated with complete efficiency. The “multi/uni” and “multi/uni/branchy” populations completely lose the ability to sporulate. This directly coincides with the appearance of the unicellular cells. For high standing variation populations, this means they all lose the ability to sporulate at approximately transfer 5. For low standing variation, all populations were able to spore until they adopted the “multi/uni” response. The “minimal” response populations all retained the ability to completely sporulate, as did the “stay multi” response. Successful sporulation results in four haploid spores tightly packed in an ascus, which results in the cell having a pyramidal shape. It does not disrupt multicellular clusters. Unsuccessful sporulation does not result in any shape changes for cells and they will appear similarly to their appearance before introduction to sporulation media.

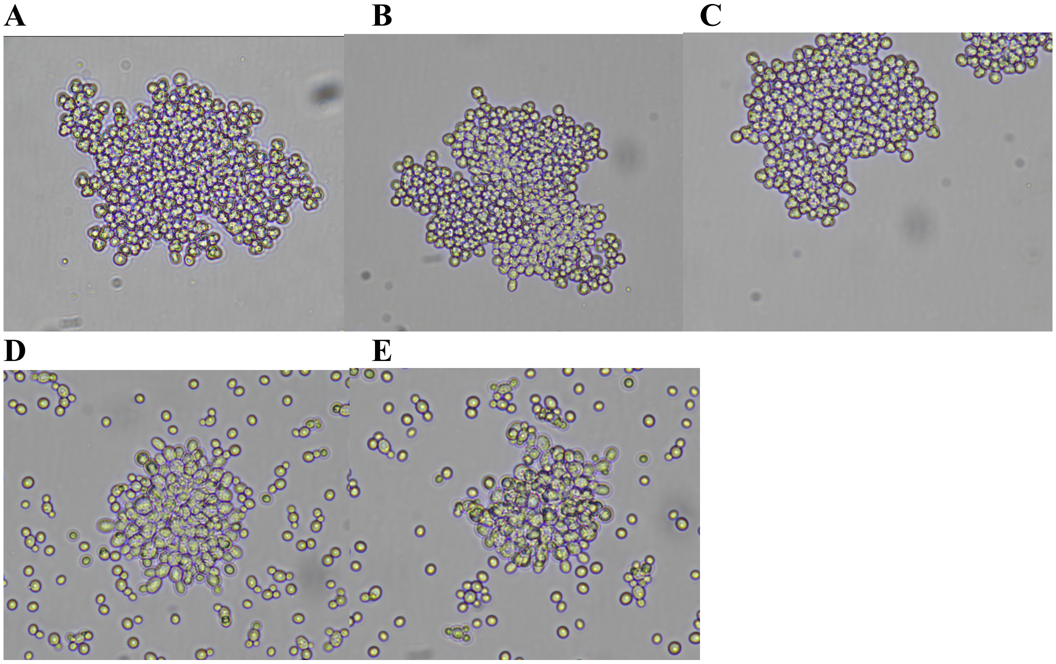


Figure 3.6. Sporulation ability of each response. **A)** ancestor: complete sporulation; **B)** “minimal” response: complete sporulation; **C)** “stay multi” response: complete sporulation; **D)** “multi/uni” response: no sporulation; **E)** “multi/uni/branchy” response: no sporulation.

3.3.5 *Individual phenotype is heritable, not plastic*

The unicellular, multicellular, and branchy phenotypes were isolated from populations from each experiment and were grown separately. It was found that the unicellular cells only produced unicellular offspring and the multicellular clusters only produced multicellular offspring. Multicellular clusters that were branchy also retained their branchiness when cultured separately. This remained true no matter the temperature—when they were grown at the stressful 37°C and when they were returned to a 30°C environment. The different phenotypes therefore do not seem to be the result of phenotypic plasticity.

3.4 DISCUSSION

Adaptation to an extreme environmental condition was seen to some degree across all populations. Those that had ample initial standing variation evolved a novel evolutionary response almost immediately, and that response was remarkably consistent across all populations. Low standing variation populations showed more diversity in response. Some languished in the heat but did show marginal population increases, while others were also able to arrive at the same phenotype that allowed for rapid increased population growth in the high standing variation populations. A third subset of the low standing variation populations evolved a response that resulted in full population recovery, albeit at a much slower rate. This slower rate response, though, may offer more long term benefit than the swift recovery: the retained ability to sporulate. All populations, high or low standing variation, that adopted the rapid increase response were subsequently not able to sexually recombine. The seeming tradeoff, here, between increased growth rate and sporulation ability signals different evolutionary consequences for these populations.

3.4.1 *Short term success*

High levels of standing variation in these yeast populations lead to a rapid adaptive response to environmental stress—within three days of exposure to heat these populations had regained their initial size and from there quickly surpassed it. Standing variation is readily available to these populations and its benefits are quickly favored by selection. This result is consistent with theoretical assumptions and with previous experimental work on the role of standing variation when environmental conditions undergo rapid changes (Barrett and Schluter 2008; Agashe *et al.* 2011; Gonzalez and Bell 2013; Brawand *et al.* 2015; Ralph and Coop 2015; Bell 2017; Bitter *et al.* 2019; Thompson *et al.* 2019). However, a rapid response was not limited solely to populations with high standing variation. Several low standing variation populations were also able to achieve this same response, the "multi/uni" response. Whether they used the same genetic mechanism as the high standing variation populations is not known, but it is very likely that they did not use the same source of genetic variation. These populations experienced rapid growth at seemingly random time points (Figure 2E), which is consistent with the gain of beneficial variants from *de novo* mutations. The majority of these gains do, however, occur earlier in the experiment, but rather than that timing resulting from standing variation, the authors believe this indicates that there may be multiple routes to achieve the same response. The transition from unicellularity to multicellularity in these yeast occurs via mutations in many different genes, so it is reasonable to assume the same in the opposite directions (Gettle, in review).

Understanding what allows for these rapid adaptive responses is of increasing importance as populations face daunting changes from climate change and habitat destruction. While no populations here underwent extinction, the distinct U-shape in population size—meaning the sharp decline and then equally fast paced recovery—is typical of populations experiencing evolutionary rescue (Gomulkiewicz and Holt 1995). Decisive examples of evolutionary rescue in natural populations are rare, if they exist at all (Gomulkiewicz and Shaw 2013), but models and laboratory experiments have shown that the potential for recovery from near collapse is there (Gonzalez and Bell 2013). Sufficient standing variation and gradual change has been thought to be the determining factor in whether or not populations will have even the potential for evolutionary rescue (Lindsey *et al.* 2013; Carlson *et al.* 2014). But here I see that rapid recovery is not limited to populations with high initial standing variation even when the pace of environmental change is instantaneous. Indeed, the rate of recovery was slightly, but significantly, faster in populations that used *de novo* mutations as the starting point (Figure 5B). If a highly beneficial phenotype can be reached by many distinct genetic mechanisms, then the relative importance of standing variation in allowing evolutionary rescue to occur may be diminished.

3.4.2 *Long term viability*

The downside of the rapid recovery strategies is that they come at a cost to sporulation. Both high and low standing variation populations that experienced rapid growth rates were no longer able to sporulate after the adoption of the successful "multi/uni" response (Figure 6).

However, low standing variation populations that take a longer road to recover their population size retain their ability to sporulate. The "stay multi" response did not impact sporulation and allowed populations to fully recover to their pre-stress size, but they took a significantly longer amount of time to do so. While this response did not offer immediate benefits, it may prove to be better in the long term. Sporulation is the only opportunity for these yeast to sexually recombine, so without it they lose the myriad of benefits that come with sex (Crow 1994; Rice 2002; Barton 2009). The future for these particular yeast is the autoclave, but were their lineage to continue, it would likely not fare as well as those that can sexually recombine if the environment continued to fluctuate (Crow 1992). Derived asexuality is often looked at as an evolutionary dead end and phylogenetic analyses do seem to support the idea that such lineages have reduced viability in the long term (Vrijenhoek 1998; Neiman *et al.* 2014).

Additionally, the populations that develop the “multi/uni” response and undergo rapid population growth are likely to have reduced genetic variation as a result of the rapid selective sweep, which could also affect them long term. The low standing variation populations already began with little to no genetic diversity, but strong selective sweeps can eliminate diversity even in populations with initially high standing variation (Barrick and Lenski 2013). Though unquantified here, the high standing variation populations would see a reduction in allelic variation in any sites that are linked to the beneficial variant (Stephan 2019). Sweeps from standing variation are deemed “soft” and these results indicate a partial sweep (see below), but this decrease in diversity combined with the reduced ability to generate diversity via sexual recombination likely leave the initially high standing variation populations in a very different position than where they started. These populations, however, did see the appearance of an additional response, the “multi/uni/branchy” response, that was not developed in low standing variation populations. While populations with this response still lack the ability to sporulate, they did show differences in settling ability (Figure 4). The branchier phenotype may allow clusters to link with one another more easily, and this increased size would speed their rate of descent. This phenotype developed at a similar timepoint in the three populations where it occurred (Figure 2E). It could be the result of independent *de novo* mutations or could indicate that multiple genetic backgrounds made it through the selective sweep and these three retained a variant allowing for branchiness. Either way, this shows the “multi/uni” response’s continued capacity for innovation in a consistent environment. The detrimental effects of loss of sexual recombination would likely only be seen were the environment to undergo further shifts.

3.4.3 *Lack of fixation of adaptive variant in rapid adaptation strategies*

The benefits of the “multi/uni” response coincide with the appearance of these unicellular individuals. However, over the course of the experiment, the populations that evolved the unicellular individuals did not see that phenotype sweep to fixation. Instead, this phenotype coexisted with multicellular clusters in all populations. In the high standing variation populations and the low standing variation populations with the “multi/uni” response, the unicellular phenotype increased rapidly over the first 6-7 transfers but then plateaued and never completely overtook the multicellular clusters. When the branchy phenotype was added in 4 high standing variation populations, all three phenotypes coexisted across the remaining transfers.

The unicellular individuals are able to reproduce at a more rapid rate than the multicellular individuals, which gives them a competitive advantage for the majority of each day. However, at the time of transfer, only the cells that can sink sufficiently quickly make it into new growth media, and therefore the multicellular clusters have an advantage at this stage. Thus, there are two selection pressures at play here: increased environmental temperature and settling selection. It is possible that neither selection pressure is sufficiently strong enough to outweigh the other: thus the multicellular clusters still have an advantage in settling and the unicellular individuals succeed at growth in heat. An incomplete sweep of a standing variant could be due to the balance between these two competing selection pressures. Incomplete sweeps, often referred to as "soft" sweeps, are thought to be a consequence of adaptation from standing variation, among other things, but they are often difficult to detect (Voight *et al.* 2006; Ferrer-Admetlla *et al.* 2014; Schrider *et al.* 2015). A soft sweep from standing variation seems likely here and has been observed in some populations (Garud *et al.* 2015; Xie *et al.* 2019; Ferreira *et al.* 2021).

There are other possibilities that might explain why no single phenotype fixes in these populations. Heterozygote advantage from a beneficial variant of sufficiently large effect size could explain this outcome. Heterozygote advantage through overdominance has been shown to be a common adaptive mechanism in *S. cerevisiae*, though it has been more associated with *de novo* mutations than standing variation (Sellis *et al.* 2016). The mechanism behind the "multi/uni" response is not necessarily the same in the high and low standing variation populations, and presence of unicellular individuals in the low standing variation populations is likely the result of new mutations. It is also possible that different phenotypes are being maintained as a consequence of a bet hedging strategy. This has been found to occur in experimentally evolved microbial populations as a consequence of alternating environmental conditions (Beaumont *et al.* 2009). Populations here do experience pressure both to reproduce in the heat and to quickly settle; however, bet hedging is typically shown in environments that are completely distinct and alternate across longer periods of time than occur here.

In all of these "multi/uni" populations, the inability to sporulate is consistent, and this does imply that this trait has fixed in these populations. Neither the multicellular or unicellular individuals are able to sporulate once this response has been derived. This seeming difference in fixation rates may imply that the two are not directly related—that the variant that induces unicellularity is not the same variant that causes the loss of sporulation. While the two are very strongly correlated in these results, more work would need to be done to determine the exact mechanisms driving each change. It is possible that a pleiotropic effect results in the complete fixation of one variant and then incomplete fixation of another in different genetic backgrounds.

3.4.4 *Adaptive loss of complexity and evolutionary trajectory*

The lack of fixation here means that the simpler unicellular phenotype and the more complex multicellular phenotype are both preserved. The ancestral yeast population of these populations was multicellular, but that ancestral population was the result of selection on a unicellular yeast strain, thus making multicellularity a recently acquired trait in the context of this experiment. Multicellularity has, perhaps surprisingly, proved relatively easy to evolve in a considerable number of laboratory organisms, many of which require few genetic changes for its onset (Libby *et al.* 2016). However, while beneficial in certain selective conditions, new multicellular forms are also seen to be at a fitness disadvantage when that selection pressure is removed (Libby and B Rainey 2013; Ratcliff *et al.* 2015). This suggests that even amongst longer established multicellular lineages a reversion to unicellularity could provide fitness benefits. Indeed, the loss of multicellularity can be seen across the fungal phylogeny. Distantly related fungal lineages show convergence on a unicellular form, including multiple independent reversions to unicellularity within yeast (O'Malley *et al.* 2016). So, while the unicellular yeast here are unable to sporulate, this phenotype has proved to be valuable over time. This simpler form may be better suited for future evolvability due to the constraints of multicellularity (Teotónio and Rose 2001). Complex multicellular organisms tend to evolve by the fixation of alleles of small effect and consequently adapt more slowly; and this becomes more true with each additional characteristic (Orr 2000). Unicellular organisms, on the other hand, are more able to tolerate mutations of large phenotypic effect because they do not have the level of genetic dependency of more complex organisms.

The reversion to unicellularity seen here could be of future benefit even despite its costs. If there were another dramatic environmental shift, it remains to be seen whether the simpler unicellular form may offer more advantages than would come with the ability to sexually recombine. This contrast again pits the benefits of *de novo* mutations against those of standing variation. In the event of an environmental shift, a unicellular organism would be in a better position to benefit from a new large effect mutation, but a multicellular organism capable of sexual reproduction would have more standing variation to pull from. In effect, the end points of this experiment reflect the reverse of the starting conditions: the initially high standing variation populations arrived at a unicellular phenotype that is more likely to benefit from *de novo* mutations and the initially low standing variation populations arrived at a multicellular phenotype that through sexual recombination is more able to utilize standing variation.

My results suggest that the dynamics of adaptation from standing variation are complicated and should continue to be evaluated by experimental work. While the assumption might be that more variation is always better, that may not be true in every scenario. Standing variation undoubtedly offers short term benefits, but the long term consequences of adaptation from standing variation merit further consideration.

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