SPELMAN COLLEGE

Investigating the Interconnection Between Cellular Aging and Network Robustness

A thesis submitted in partial satisfaction of the requirements for the

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ABSTRACT

Cellular aging is a pleiotropic trait that is influenced by the intricate coordination of various components of gene networks. Despite vast research in this area to determine the specific mechanisms responsible for the complex nature of aging, its connection with network robustness has yet to be explored. Therefore, in this study we aim to dissect the relationship between network robustness and life history traits using budding *Saccharomyces Cerevisiae,* an effective model for the study of aging. We hypothesize that cellular aging is influenced by the configuration of gene and protein interaction networks for which robustness is a key factor in shaping the characteristics of the aging process. Using the R-statistical analysis tool, we evaluated the causal interactions of several robustness proxies including network connectivity, coefficients of variation of gene expression, evolutionary distance, fitness, morphological plasticity, and replicative lifespan using partial regressions. The results of our study showed significant correlations between replicative lifespan and several robustness proxies. Specifically, replicative lifespan is strongly negatively correlated to morphological plasticity and positively correlated to fitness robustness. Interestingly, we found that morphological plasticity is the causal factor for both replicative lifespan and growth fitness.

LIST OF TABLES AND FIGURES

CHAPTER 1. INTRODUCTION AND BACKGROUND

The concept and definition of cellular aging has been a highly debated topic for several decades [1-5]. Although many strides have been made towards understanding the intricacies of cellular aging, it is clear that on a molecular level the detailed mechanism is far from understood. For the purpose of this study, aging will be defined as the loss of function that is generally accompanied by decreasing fertility and increasing mortality with advancing age.

Saccharomyces cerevisiae yeast cells are unicellular organisms that have proven to be excellent models for the study of cellular aging [5, 6]. It has been determined that *S. cerevisiae* yeast cells share a similar, complex, internal cell structure to higher-level eukaryotes such as animals (specifically humans), and thus exhibit similar molecular mechanisms of aging. Similar to human cells, yeast cells contain compartmentalized organelles like the nucleus and mitochondria, in addition to the fact that several human genes have been found to have equivalents in yeast. *S. cerevisiae* yeast cells exhibit asymmetrical division and have the ability to adapt to severe environmental changes in order to maintain growth and function. Thus, they tend to express differing degrees of aging despite their genotypic similarities. Cellular aging in *S. cerevisiae* is most commonly measured in two ways: replicative lifespan (RLS), and chronological lifespan (CLS). Replicative lifespan is defined as the number of daughter cells created by mother cells before they senesce and cease to divide. Chronological lifespan measures how long a cell can survive in an arrested non-dividing state. In this study, cellular aging was measured based on replicative lifespan because more information about this yeast life span measurement was available.

Although several hundreds of genes in yeast have been found to affect cellular aging, none of these suggest a mechanism that is directly linked to the aging process. The gene factors that have previously shown direct effects on RLS include the silent information regulator 2 (Sir2) protein and calorie restriction (CR). Sir2 is hypothesized to be a key factor in an organism's response to stress, and affects aging due to the "toxic" accumulation of extra chromosomal rDNA circles (ERCs) in the nucleus of a mother cell that can lead to yeast cell senescence[7]. The deletion of Sir2 increases ERC formation and can thus significantly shortening replicative lifespan. Conversely, it is hypothesized that an over expression of Sir2 will significantly increase life span. In addition to the Sir2 protein, calorie restriction has been found to have an effect on RLS. CR has been found to extend both RLS and CLS, and can be achieved by decreasing the glucose levels in the culture medium. The molecular mechanism for this phenomenon is unclear, but proteins, Mdh1 and Aat1, have been identified as factors that affect calorie restriction.

It has been found that genotypically homogeneous yeast cells from the same colony will live to different ages under identical environmental circumstances, suggesting that aging is a largely stochastic process [8]. Despite this, there exist universal characteristics of aging at the demographic level (Strehler-Mildvan correlation), suggesting a common principle in the stochastic processes of aging [8]. Several models of cellular aging have been hypothesized in attempts to accurately define the process; for instance the two-parameter Gompertz model.

$$m = -\frac{1}{s}\frac{ds}{dt} = m_0\, e^{Gt} \qquad Eq.1$$

Where m is the mortality rate, m_0 is the initial mortality rate, s is the survival fraction of a population (i.e. viability), t is time, and the Gompertz coefficient G,

determines the acceleration of mortality rate over time and is therefore a parameter for aging. The Gompertz model ties to the Strehler-Mildvan correlation because it observes a negative correlation between G and the natural log of the initial mortality rate (this correlation was first observed in humans with the Strehler-Mildvan correlation). This correlation implies that there could exist an underlying model to determine cellular aging.

Previous research has provided evidence that cellular aging is an emergent property of gene networks, which allow for the communication of molecules inside the cell (Qin, manuscript in preparation). These gene networks are made up of a supply of DNA segments that interact with one another, but the level of gene expression varies depending on the type of cell and the environment. Based on earlier studies, it is evident that these gene networks allow the cell to adapt and to survive, and thus depicts the robustness of the cell.

In this study, we investigate the interconnection between cellular robustness and cellular aging in the yeast *Saccharomyces Cerevisiae*. Cellular robustness is defined as the ability of a cell to maintain homeostasis throughout genetic, environmental, or stochastic perturbations, such as temperature, time, and cellular damage. Previous research has hypothesized that cells with greater robustness experience a longer lifespan and that phenotypic capacitors influence robustness (Qin, manuscript in preparation). Since cellular aging is defined as the deterioration of cellular functions, it follows that as a cell's network robustness decreases it will be less able to adapt against external perturbations, causing a depletion of functionality of the protein activities (aging). Specifically, the Gompertz model predicts a positive correlation between cellular aging and cellular robustness. Thus, leading to the formulation of our hypothesis in this study

that replicative lifespan in *S. Cerevisiae* will be directly correlated to robustness and thus to several different proxies of robustness. The robustness proxies that we investigated include: the number of protein interactions, the number of genetic interactions, evolutionary distance, fitness, and morphological plasticity. These robustness factors were selected because data in these areas was most easily accessible. This study examined each robustness proxy to determine the relationship to replicative lifespan using R statistical software. We examined the relationships between each individual robustness proxy and RLS, the robustness proxies to each other, and multiple combinations of the robustness proxies to RLS.

CHAPTER 2. MATERIALS AND METHODS

2.1 Data Source

RLS data for 564 different *Saccharomyces Cerevisiae* yeast gene deletion mutants was obtained from the Kaeberlein group. Growth fitness measures in various conditions were obtained from Steinmetz, et.al. 2002 and Deutschbauer, et.al. 2005. Several network datasets were used including protein-protein interactions from DIP, BioGRID, and BIND [9-11], protein complexes [12, 13], and genetic interactions [14]. Dr. Hong Qin provided evolutionary distance data. Yeast deletion mutation with known effects on morphology is available at the *Saccharomyces Cerevisiae* Morphological Database (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). SCMD provides a list of 501 morphological parameters in four groups: cell shapes, bud sizes, nucleus locations, and actin localizations (Ohya, 2005 #534) and the analyzed data for morphological plasticity came from this database. The variance, standard deviation, and coefficient of variation of the morphological plasticity data set were calculated because they are proportional to the robustness of the cell.

2.2 Statistical Analysis

Each analysis and numerical calculation was performed using R 2.15.1 and an open source software called R-studio 0.97.332. This software was used to perform linear and multiple regression analysis on the different variable factors that comprise robustness and cellular aging. Multiple R-squared values and p-values were analyzed to determine significant correlations between replicative lifespan and several proxies of cellular robustness. In this study, p-values smaller than 0.05 were considered statistically significant. Six different parameters were analyzed: replicative lifespan, number of

protein interactions, number of genetic interactions, fitness, morphological plasticity, and evolutionary distance. Firstly, the data sets containing replicative lifespan, fitness growth, and evolutionary distance tables were read into a working directory in R-Studio. The data tables containing information on genetic pairs and protein interaction pairs were also read into the working directory and the degree of protein and genetic interactions were calculated. The cellular morphology mutant table was read into the working directory, and the data was normalized. The standard deviation, mean, and coefficient of variation were then calculated by row on the morphology data in order to represent the cell morphological plasticity proxy of robustness.

Linear regression analysis was then conducted on each of the robustness proxies with replicative lifespan, beginning with cellular growth fitness. The fitness robustness data contained results for the growth of S. Cerevisiae in five different growth mediums. Those mediums were YPD (1% Bacto-peptone (Difco), 2% yeast extract and 2% glucose), YPDGE (0.1% glucose, 3% glycerol, and 2% ethanol), YPG (3% glycerol), YPE (2% ethanol), and YPL (2% lactate) (Steinmetz et. al. 2002). Linear regression analysis was conducted between each growth medium and replicative lifespan in order to determine which growth medium was most informative and should be used for further experimentation. The strongest correlation was determined based on the smallest p-values and the most informative growth medium was determined by the largest R-squared value. A scatter plot of the results of this regression analysis was generated.

Linear regression was then performed between evolutionary distance and replicative lifespan. Evolutionary distance was incorporated into a column in the replicative lifespan table. The evolutionary distance between genes in S. Cerevisiae and

homologs in S. Paradoxus, S. Mikatae, and S. Bayanus were used as predictors of robustness in these analyses. Different evolutionary distance values were analyzed for each homolog and then linear regression analysis was performed and plotted. The analysis of the resulting p-values determined whether or not there existed a correlation between evolutionary distance and replicative lifespan.

Similarly, the number of connecting degrees for protein and genetic interactions was incorporated into a column in the replicative lifespan table. First, the frequency of protein and gene interactions was summarized from the list of protein pairs that associate and the list of genetic pairs that associate. This frequency data was then matched with the lifespan data to perform linear regression. Scatter plots of these results were also created. Multiple regression analysis was also conducted between various combinations of replicative lifespan, growth fitness (YPE), number of protein interactions, and number of genetic interactions.

The mean, standard deviation, and coefficient of variation (CV) of the fitness data were calculated to assess the fitness plasticity. This calculated coefficient of variation for fitness plasticity was incorporated into a column of the lifespan table and linear regression was performed to assess the correlation between replicative lifespan and the reciprocal of CV, and replicative lifespan and the square root of the reciprocal of CV. Multiple regression was also conducted between replicative lifespan and the square root of the reciprocal of CV and the number of protein interactions [15].

The calculated mean, standard deviation, and coefficient of variation for the morphology data were incorporated into the lifespan data table as columns. Linear regression analysis was performed between replicative lifespan and standard deviation,

replicative lifespan and the square root of 1 over standard deviation, replicative lifespan and the square root of standard deviation, replicative lifespan and the calculated mean, and replicative lifespan and the coefficient of variation. Regression analysis was also performed between the mean and standard deviation of the morphology data. Several of the results were plotted.

Multiple linear regression analysis was then performed between Replicative lifespan and several of the robustness proxies that were incorporated into the lifespan data table. In addition, multiple regression analysis was performed between cellular growth fitness and several of the robustness proxies.

CHAPTER 3. RESULTS

3.1 Experimental Design

There were six different parameters considered in this study: Replicative lifespan, fitness, evolutionary distance, morphological plasticity, the degree of protein interactions, and the degree of genetic interactions. Theoretically, there were 15 different correlations that could possibly exist between these parameters. Based on earlier experimentation, our hypothesis predicted the correlations shown in Figure 1. Specifically, previous research indicated that there exist significant correlations between morphological plasticity and growth fitness to replicative lifespan (Matheson, Morrison, Levy). In addition, previous research has shown that there also exist correlations between evolutionary distance and protein interactions, protein interactions and morphological plasticity, and morphological plasticity and fitness (Montgomery, Payton). In addition to these past findings, this study incorporated the number of genetic interactions as a proxy of robustness, which was not a factor in the previous studies. The results of these studies in addition to the incorporation of the additional variable influenced the configuration of the alternative hypothesis for this study shown in Figure 1.

3.2 Growth fitness vs. Lifespan Regression Analysis

Firstly, the growth fitness robustness variable was analyzed with cellular aging to determine which growth medium yielded the most informative data for the analysis. Based on the calculated p-values, the growth measures in the YPE, and YPL growth mediums demonstrated the strongest correlation to RLS (p-value < 0.05). Out of these two growth mediums, the multiple R-squared values were calculated and analyzed to determine which was most informative to cellular aging. YPE exhibited the largest R-

squared value (0.01106), and thus proved to be the most informative. Therefore, the fitness robust data for the yeast cells in the growth medium YPE was used for the remainder of the study. The results of this analysis are shown in Table 1 and the correlation between YPE and RLS is shown in the scatter plot in Figure 3.

The mean and standard deviation of the growth fitness data were calculated in order to determine the coefficient of variation (CV) for fitness plasticity. Linear regression analysis was performed between RLS and the reciprocal of CV, and also between RLS and the square root of the reciprocal of CV. Neither of these yielded significant results with p-values of 0.4141 and 0.3958 respectively. In addition, a multiple linear regression was run to test the correlation between RLS and the square root of the reciprocal of CV combined with the number of protein interactions. This test was run due to the fact that CV is inversely correlated to robustness and Ricklef and Scheuerlein argued that the rate of aging is proportional to the square root of the Gompertz coefficient [15]. The results of this multiple regression did not yield a statistically significant p-value (0.2423).

3.3 Evolutionary Distance vs. Lifespan Regression Analysis

Evolutionary distance robustness showed no correlation to replicative lifespan. For each homolog, S. Paradoxus, S. Mikatae, and S. Bayanus, linear regression analysis between the calculated evolutionary distances with S. Cerevisiae produced p-values that were not statistically significant. S. Paradoxus gave a p-value of 0.3758, S. Mikatae gave a p-value of 0.2109, and S. Bayanus gave a p-value of 0.3175, as shown in Table 2. Clearly these p-values are larger than .05 and thus no significant correlation was found between evolutionary distance robustness and replicative lifespan. Given that none of the

homologs proved to be more informative or significant over the others, S. Paradoxus was chosen to represent evolutionary distance robustness for the remainder of the study.

3.4 Protein and Genetic Interactions vs. Lifespan Regression Analysis

The number of protein interactions and the number of genetic interactions did not exhibit a statistically significant correlation to replicative lifespan. The degree of protein interactions yielded an insignificant p-value of 0.1759 and the degree of genetic interactions yielded an insignificant p-value of 0.5641.

3.5 Morphological Plasticity vs. Lifespan Regression Analysis

Morphological plasticity was analyzed due to its inverse relationship with robustness. Standard deviation values of the deletion mutants were calculated from the SCMD to represent morphological plasticity data and were compared to replicative lifespan data. The linear regression between replicative lifespan and morphological plasticity exhibited a statistically significant correlation (p-value = 1.349×10^{-5} and $R^2 = 0.03431$). A scatter plot of this result was generated in order to further illustrate the negative correlation and can be found in Appendix A (Figure 2).

In addition to examining the standard deviation values of the deletion growth mutants to serve as a proxy for morphological plasticity, the square root of the calculated standard deviation values in addition to the reciprocal of the square root of the standard deviation values were calculated to serve as proxies for morphological plasticity. Linear regression analysis was performed between RLS and both of these variables. Both the square root of the standard deviation and its reciprocal exhibited significant correlations to RLS with p-values of 1.8×10^{-5} and 2.844×10^{-5} respectively. Further, the mean values

of the deletion mutants also proved to have a significant correlation with RLS (p-value = 0.00188). Of these possible proxies for morphological plasticity, the calculated standard deviation showed the smallest p-value and the largest R^2 value and thus served as the variable most informative for morphological plasticity robustness and was used for the remainder of the study.

The coefficient of variation (standard deviation divided by the mean) for the morphology data was also calculated and linear regression performed with RLS. There was not a significant correlation between the coefficient of variation and RLS, but there was a strong correlation between the morphology standard deviation and the morphology mean (p-value = 2.2×10^{-16}, $R^2 = 0.5191$). It is unclear if this strong correlation between the standard deviation and the mean could have an effect on the correlation observed between the coefficient of variation and RLS.

The results of each linear regression analysis conducted between the robustness proxies and replicative lifespan are shown in Table 3.

3.6 Correlations between robustness proxies

In addition to performing linear regression analysis to determine if each of the robustness proxies were correlated to replicative lifespan, linear regression analysis was also performed to determine if the robustness proxies were correlated to each other. This analysis revealed that the number of genetic interactions, the number of protein interactions, and morphological plasticity robustness are significantly related to growth fitness robustness (YPE), with p-values of 2.2×10^{-16}, 0.0003917, and 1.08×10^{-13} respectively.

Further, it was found that there was a significant correlation between evolutionary distance and degree of protein interactions (p-value = 0.01196, $R^2 = 0.02824$), morphological plasticity and degree of protein interactions (p-value = 0.0004796, $R^2 = 0.03021$), and morphological plasticity and degree of genetic interactions (p-value = 6.429×10^{-11}, $R^2 = 0.084$). The results of these linear regressions are shown in Table 4.

3.7 Multiple Regression Analysis

Multiple regression analysis was performed between replicative lifespan and several of the robustness proxies. Specifically, RLS did not exhibit a significant correlation with fitness combined with the number of protein interactions (p-value = 0.1106, $R^2 = 0.0112$), RLS did not exhibit a significant correlation with fitness combined with the number of genetic interactions (p-value = 0.1753, $R^2 = 0.007244$), and lastly RLS did not exhibit a significant correlation with fitness combined with the number protein interactions and the number of genetic interactions (p-value = 0.3371, $R^2 = 0.009782$). The remaining multiple linear regression combinations performed between RLS and the other robustness proxies are shown in Table 6. None of these multiple linear regression combinations yielded statistically significant correlations (p-values > 0.05).

Also, multiple regression analysis was performed between cellular growth fitness and several proxies of robustness. Specifically, multiple regression analysis was performed between growth fitness robustness (YPE) and evolutionary distance, combined with the number of genetic interactions, combined with morphological plasticity robustness. The p-value for this regression analysis proved to be statistically significant (p-value = 4.084×10^{-7}, $R^2 = 0.1231$). Similarly, multiple regression analysis was performed between growth fitness robustness (YPE) and evolutionary distance, combined

with the number of protein interactions, combined with morphological plasticity robustness. The p-value for this regression analysis was also statistically significant (p-value = 8.773×10^{-6}, $R^2 = 0.1149$). These results are shown in Table 5.

Lastly, multiple regression analysis was performed between replicative lifespan, morphological plasticity, and growth fitness (YPE). This analysis was done because previous research showed these robustness proxies to have the strongest relationships with replicative lifespan. As previously discussed, when examined individually, morphological plasticity exhibited a significant strong correlation with replicative lifespan (p-value = 1.349×10^{-5}, $R^2 = 0.03431$), and growth fitness robustness exhibited a significant correlation to replicative lifespan (p-value = 0.01488, $R^2 = 0.01106$). Further, it was found that growth fitness and morphological plasticity were significantly correlated to each other as shown in Figure 4 (p-value =1.08×10^{-13}, $R^2 = 0.09902$). When multiple linear regression analysis was performed between replicative lifespan and morphological plasticity combined with growth fitness (YPE), the correlation was statistically significant (p-value = 1.045×10^{-5}, R^2=0.04244), but analyzing the individual p-values revealed that while morphological plasticity exhibited a highly significant individual p-value of 2.58×10^{-5}, growth fitness exhibited a p-value of 0.383, which is no longer significant. The results multiple regression analysis is shown in Table 7.

CHAPTER 4. DISCUSSION

4.1 Correlations

Based on the computational method used in this study, we were able to obtain substantial genetic evidence that fitness robustness and morphological plasticity robustness are directly related to RLS. Firstly, the growth fitness in ethanol medium (YPE) showed the strongest correlation with lifespan. This result suggests that respiratory metabolism is most informative to lifespan in yeast cells. Secondly, although both fitness and morphological plasticity p-values suggest significant correlations (p-values < 0.05), morphological plasticity robustness exhibited a larger adjusted R^2 value compared to growth fitness suggesting that there is a stronger correlation between morphological plasticity and RLS. Furthermore, based on the calculated p-values, morphological plasticity and growth fitness are also correlated although the causal parameter is presently unknown. This positive correlation suggests that a cell's fitness or ability to grow and survive relative to other cells is possibly related to the cell's ability to change its morphology, or shape, in response to various environmental factors. Based on this information, it can be hypothesized that cells that are better able to adapt and change as needed have a greater ability to reproduce and avoid being damaged or destroyed by external factors.

In addition, the results of the multiple regression analysis between RLS and growth fitness combined with morphological plasticity exhibited a significant correlation, but examination of the individual p-values showed that while morphological plasticity maintained a significant correlation, the correlation between growth fitness and RLS was no longer significant. This implies that growth fitness is not directly correlated with RLS,

but rather correlated with RLS through its correlation to morphological plasticity. Again the causality of this indirect correlation is presently unknown.

The coefficient of variation (CV) of the SCMD morphological plasticity data was calculated by dividing the calculated mean by the calculated standard deviation. Interestingly, after performing linear regression analysis, the CV did not show a significant correlation to replicative lifespan ($p = 0.3542$). Further, it was shown that there exists a significant correlation between SCMD mean and SCMD standard deviation ($p < 2.2x10^{-16}$). It is possible that the correlation of these two parameters may have offset each other in the calculation of the CV, thus skewing the results of the linear regression. Due to this, the calculated standard deviation served as the robustness proxy for morphological plasticity and exhibited a significant negative correlation to replicative lifespan. By definition, morphological plasticity and cellular robustness have an inverse relationship because morphological plasticity describes a cell's ability to change in response to external perturbations, while robustness refers to cells that are able to withstand these perturbations. Thus this negative correlation between RLS and morphological plasticity supports our hypothesis that cellular robustness is directly correlated to replicative lifespan or cellular aging.

In conclusion, based on the results of both the linear and multiple regression analysis, the verified correlations between replicative lifespan and the various robustness proxies are illustrated in Figure 5.

4.2 Importance of Study and Future Experimentation

This study underlines the advancing attempts to understand why aging occurs in cells and organisms. The usage of the yeast *S. Cerevisiae* as our model in this study, gave

us the ability to compare our results to aging in higher organisms. It is hoped that with advances in technology and scientific understanding we will be able to learn more about aging specifically as it relates to humans from studies such as this one.

Future studies should be conducted involving yeast cells that are grown and manipulated in the lab rather than using data obtained from outside sources. This would allow direct observation of the different aging processes occurring within the yeast cells. This observation could provide the opportunity to discern specifically how the yeast cells respond to environmental perturbations and thus provide deeper insight into robustness in general and the various proxies used to quantify robustness. Further, observing the aging process could not only identify the correlations between robustness proxies and cellular aging, but also give greater insight into why the correlations between certain proxies exist.

In addition, an alternative study that investigates chronological lifespan (CLS) in addition to replicative lifespan (RLS) could give further insight into the interconnection between robustness and aging. Using both definitions of aging could provide greater knowledge of the aging process in yeast cells. Further, providing a larger scope for the definition of cellular aging and analyzing the differences and similarities of correlations between CLS, RLS, and the various robustness proxies could better solidify the results of this study and provide more understanding of the correlations.

The indirect correlation that was observed between growth fitness robustness (YPE) and replicative lifespan via morphological plasticity robustness should be further examined. This correlation could provide very insightful information about the ways in which cells grow and adapt to their environment in relation to other cells as aging occurs.

Further study could provide insight into why fitness is only indirectly correlated with replicative lifespan and assess the true importance of morphological plasticity to cellular aging and other robustness proxies.

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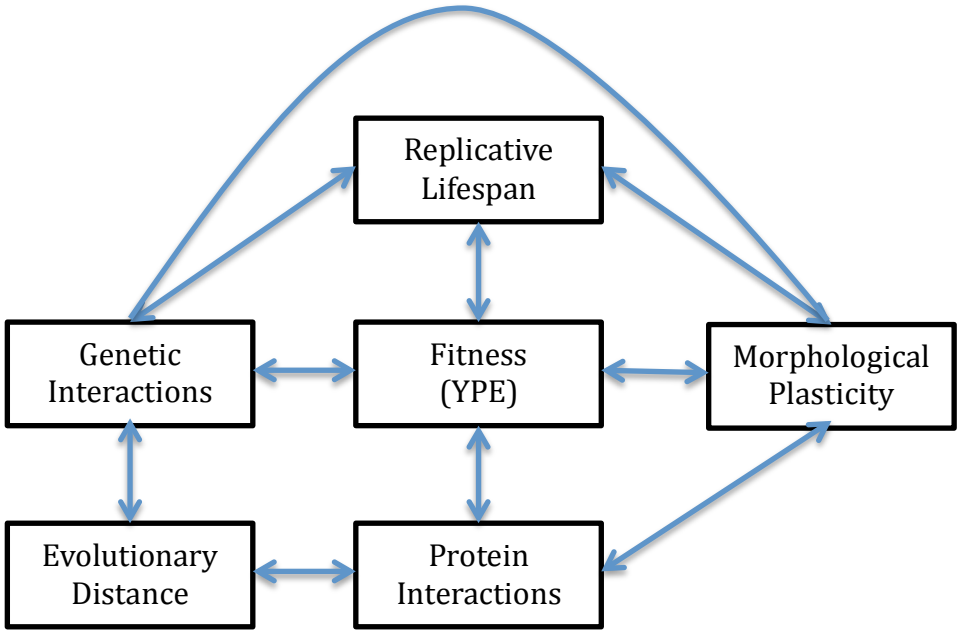
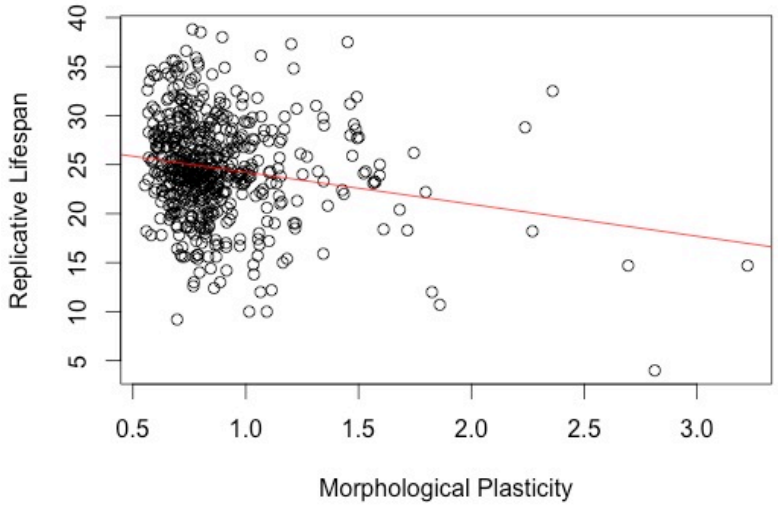
APPENDIX A: Figures

Figure 1. Alternative Hypothesis



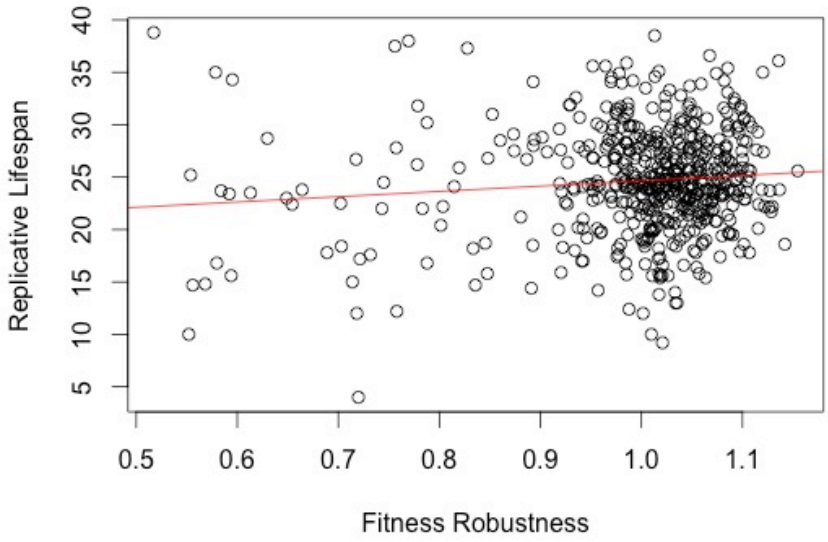
Hypothesis shows possible connections between robustness proxies and replicative lifespan.

Figure 2. Scatter plot of Replicative lifespan vs. Morphological plasticity



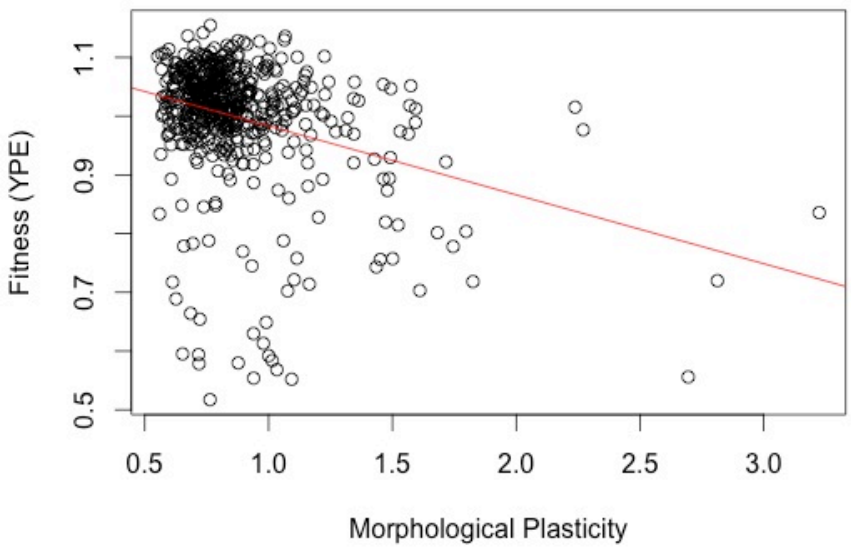
Plot shows significant negative correlation between replicative lifespan and morphology plasticity (p-value = 1.349×10^{-5}, R-squared = 0.03431).

Figure 3. Scatter plot of Replicative Lifespan vs. Fitness Robustness



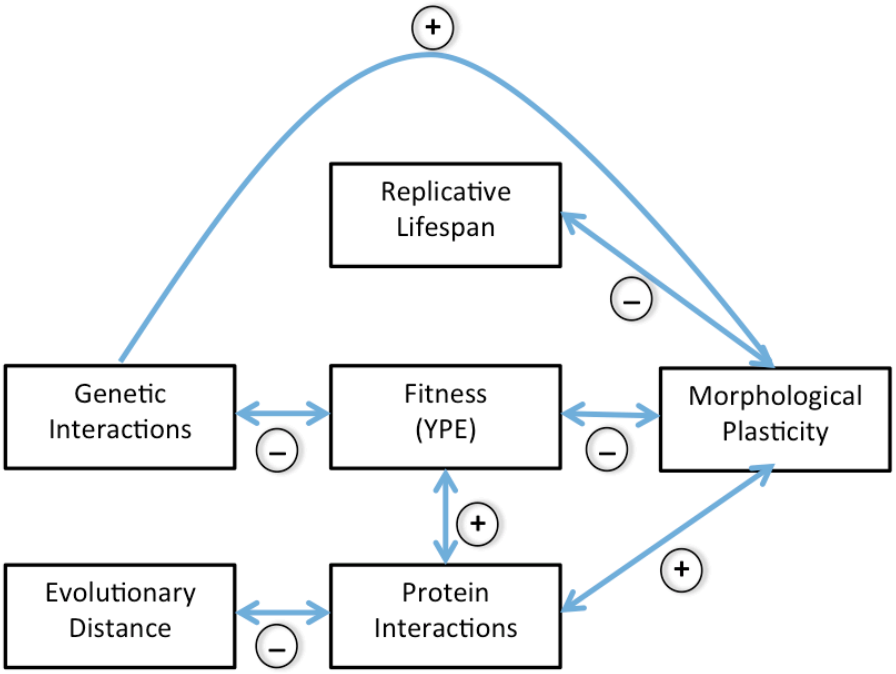
Plot shows significant positive correlation with a p-value of 0.01488 and an R-squared of 0.01106.

Figure 4. Scatter plot of Fitness Robustness vs. Morphological Plasticity



Plot shows the significant negative relationship between morphological plasticity and fitness robustness (YPE) (p-value = 1.08×10^{-13}, $R^2 = 0.09902$).

Figure 5. Results



APPENDIX B: Tables

Table 1. Relationship Between Fitness Robustness and Cellular Aging

Comparison	Medium	Multiple R^2	p-value
RLS	YPD	0.001457	0.3778
RLS	YPDGE	0.001226	0.4186
RLS	YPG	0.006863	0.05527
RLS	YPE	0.01106	0.01488
RLS	YPL	0.00782	0.04071

YPE and YPL exhibit significant p-values. YPE exhibits smallest p-value and highest R-squared value.

Table 2. Relationship Between Evolutionary Distance and Cellular Aging

Species 1	Species 2	Multiple R^2	p-value
S. Cerevisiae	S. Paradoxus	0.002773	0.3758
S. Cerevisiae	S. Mikatae	0.007775	0.2109
S. Cerevisiae	S. Bayanus	0.004759	0.3175

All results are statistically insignificant, therefore no correlation is observed.

Table 3. Linear Correlations Between Cellular Aging and Robustness Proxies

Lifespan	Robustness Proxy	Multiple R-squared	p-value
RLS	Fitness	0.01106	0.01488
RLS	Evolutionary Distance	0.002773	0.3758
RLS	Protein Interactions	0.004564	0.1759
RLS	Genetic Interactions	0.0006784	0.5641
RLS	Morphological Plasticity	0.03431	1.349e-05

Fitness and morphological plasticity p-values are statistically significant

Table 4. Linear Correlations Between Robustness Proxies

Robustness Proxy	Robustness Proxy	Multiple R-squared	p-value
Evolutionary Distance	Degree of Protein Interactions	0.02824	0.01196
Morphological Plasticity	Degree of Protein Interactions	0.03021	0.0004796
Morphological Plasticity	Degree of Genetic Interactions	0.084	6.429×10^{-11}
Fitness	Degree of Genetic Interactions	0.1363	$<2.2 \times 10^{-16}$
Fitness	Degree of Protein Interactions	0.0316	0.0003917
Fitness	Morphological Plasticity	0.09902	1.08×10^{-13}

Table 5. Multiple Regression Analysis of Cellular Fitness

Factors in Multiple Regression		R^2 value	p-value
Fitness	• Evolutionary Distance (0.066324) • Number of Protein Interactions (0.072944) • Morphological Plasticity (0.000433)	0.1149	8.773×10^{-6}
Fitness	• Evolutionary Distance (0.738861) • Number of Genetic Interactions (0.000348) • Morphological Plasticity (0.000353)	0.1231	4.084×10^{-7}

Cellular fitness (YPE) significantly correlated to the number of gene and protein interactions, and morphological plasticity.

Table 6. Multiple Regression Analysis of Cellular Aging

Factors in Multiple Regression		R^2 value	p-value
RLS	• Number of Protein Interactions (0.308*) • Fitness (0.104)	0.0112	0.1106
RLS	• Number of Genetic Interactions (0.9471) • Fitness (0.0789)	0.007244	0.1753
RLS	• Number of Genetic Interactions (0.632) • Number of Protein Interactions (0.248) • Fitness (0.407)	0.009782	0.3371
RLS	• Fitness (0.92647) • Evolutionary Distance (0.37269) • Number of Genetic Interactions (0.38392) • Morphological Plasticity (0.00617)	0.03494	0.06671
RLS	• Fitness (0.107) • Evolutionary Distance (0.855) • Number of Protein Interactions (0.575) • Morphological Plasticity (0.135)	0.02141	0.3273

*Individual p-values are shown in parentheses.

Table 7. Multiple Regression Analysis

Factors in Multiple Regression		R^2 value	p-value
Replicative Lifespan	• Morphological Plasticity (2.58×10^{-5}) • Fitness (0.383)	0.04244	1.045×10^{-5}
Morphological Plasticity	• Fitness (1.10×10^{-12}) • Replicative Lifespan (2.58×10^{-5})	0.1287	$<2.2 \times 10^{-16}$
Fitness	• Morphological Plasticity (1.1×10^{12}) • Replicative Lifespan (0.383)	0.1003	7.177×10^{-13}

Insignificant p-values show fitness and replicative lifespan are not directly correlated