**SPELMAN COLLEGE**

**Investigating the Interconnection Between Cellular Aging and Network Robustness**

**A thesis submitted in partial satisfaction of the requirements for the Ethel Waddell Githii Honors Program**

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**ABSTRACT**

Cellular aging, a pleiotropic trait, is influenced by many components of gene networks. 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. Here, we aim to dissect the interconnection of network robustness and life-history traits in *Saccharomyces* *Cerevisiae*. We evaluated the causal interactions of network connectivity, coefficient of variations of gene expression, evolutionary distance, fitness, morphological plasticity, and replicative life span using partial regressions. The results of our study showed significant correlations between replicative lifespan and several robustness proxies. Specifically, replicative lifespan is 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.

# INTRODUCTION AND BACKGROUND

The concept and definition of cellular aging has been a highly debated topic for several decades [[1-5](#_ENREF_1)]. Although many strides have been made towards understanding cellular aging, it is clear that the detailed mechanism of aging on a molecular level 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* is a unicellular organisms and has proven to be an excellent model organism for the study of cellular aging [[5](#_ENREF_5), [6](#_ENREF_6)]. *S. cerevisiae* yeast cells asymmetrical division and have the ability to adapt to severe environmental changes in order to maintain growth and function. Thus, they tend to live to different ages despite their genotypic similarities . It has been determined that these yeast cells share a similar complex internal cell structure to higher-level eukaryotes such as plants and animals, and thus exhibit similar molecular mechanisms of aging. Cellular aging in *Saccharomyces 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 this yeast lifespan measurement was more easily available to us.

Although several hundreds of genes in yeast have been found to effect cellular aging, none of these genes suggest a mechanism that is directly linked to aging. The factors that have previously shown direct effects on RLS include the silent information regulator 2 (Sir2) protein and calorie restriction (CR). Sir2 effects aging due to the “toxic” accumulation of extra chromosomal rDNA circles (ERCs) in the nucleus of a mother cell that can lead to the replicative aging of yeast [[7](#_ENREF_7)]. The deletion of Sir2 increases ERC formation and can thus significantly shortening 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 (**citation?)**.

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](#_ENREF_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](#_ENREF_8)]. Several models of cellular aging have been hypothesized in attempts to accurately define cellular aging, for instance the two-parameter Gompertz model.

Where m is the mortality rate, m0 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 rate 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 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 in this study included: 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.

# MATERIALS AND METHODS

## *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](#_ENREF_9)], protein complexes [[12](#_ENREF_12), [13](#_ENREF_13)], and genetic interactions [[14](#_ENREF_14)]. Evolutionary distance data was given by Dr. Hong Qin. 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.

## *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 in this study: 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 was 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 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 (Ka) 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 (DO I NEED TO GO INTO THE SPECIFICS OF THESE MULTIPLE LINEAR REGRESSION COMBOS???).

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 1/CV, and replicative lifespan and the square root of 1/CV. Multiple regression was also conducted between replicative lifespan and the square root of 1/CV and the number of protein interactions (Biological Implications of the Weibull and Gompertz Models of Aging) ?????? Weibull aging occurs in homogenous systems like machinery, and Gompertz aging occurs in heterogeneous systems like organisms.

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 these 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. DO I NEED TO ELABORATE ON THESE MULTIPLE REGRESSION VARIABLE COMBINATIONS?? (You probably need some to explain the causal diagrams)

# RESULTS

## *Experimental Design*

There were six different parameters considered in this study: Replicative lifespan, fitness, evolutionary distance, morphological plasticity, the number of protein interactions, and the number 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. 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 study in addition to the incorporation of the additional variable influenced the configuration of the alternative hypothesis for this study shown in Figure 1.

## *Growth fitness vs. Lifespan Regression Analysis*

Based on the 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 RLS. 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 mean and standard deviation of the growth fitness data were calculated in order to calculate the coefficient of variation (CV) for fitness plasticity. Linear regression analysis was performed between RLS and one over the CV, and also between RLS and the square root of one over the CV. Neither of these yielded significant results with p-values of 0.4141 and 0.3958 respectively. CV is inversely correlated to robustness. Ricklef and Scheuerlein 2002 argued that rate of aging is proportional to square root of the Gompergz coefficient [[15](#_ENREF_15)]. Therefore, a multiple linear regression was run to test the correlation between RLS and the square root of one over the CV combined with the number of protein interactions. This also did not yield a statistically significant p-value (0.2423).

## *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. Clearly these p-values are larger than .05 and thus no correlation was found between evolutionary distance robustness and replicative lifespan.

*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.

## *Morphological Plasticity vs. Lifespan Regression Analysis*

Morphological plasticity was analyzed due to its inverse relationship with robustness. Standard deviation values of the deletion mutants and lifespan data were compared. The linear regression between replicative lifespan and morphological plasticity exhibited a statistically significant correlation (p-value = 1.349 x 10-5 and R2 = 0.03431). A scatter plot of this result was generated in order to further illustrate the negative correlation.

In addition to examining the standard deviation values of the deletion growth mutants to serve as a proxy for morphological plasticity, linear regression analysis was also performed between RLS and the square root of the calculated standard deviation values, and between RLS and one divided by the square root of the standard deviation values. Both of these variables exhibited significant correlations to RLS with p-values of 1.8 x 10-5 and 2.844 x 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 R2 value and thus served as the variable for morphological plasticity robustness 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 mean (p-value = 2.2 x 10-16, R2 = 0.5191).

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

## *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 x 10-16, 0.0003917, and 1.08 x 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, R2 = 0.02824), degree of protein interactions and morphological plasticity (p-value = 0.0004796, R2 = 0.03021), and degree of genetic interactions and morphological plasticity (p-value = 6.429 x 10-11, R2 = 0.084).

## *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, R2 = 0.0112), RLS did not exhibit a significant correlation with fitness combined with the number of genetic interactions (p-value = 0.1753, R2 = 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, R2 = 0.009782). The remaining multiple linear regression combinations performed between RLS and the other robustness proxies are shown in table 6 in the “Tables and Figures” section. 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 x 10-7, R2 = 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 x 10-6, R2 = 0.1149).

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 x 10-5, R2 = 0.03431), and growth fitness robustness exhibited a significant correlation to replicative lifespan (p-value = 0.01488, R2 = 0.01106). Further, it was found that growth fitness was morphological plasticity were significantly correlated to each other (p-value =1.08 x 10-13, R2 = 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 x 10-5, R2=0.04244), but analyzing the individual p-values revealed that while morphological plasticity exhibited a highly significant individual p-value of 2.58 x 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.

# DISCUSSION

## *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. Although both p-values suggest significant correlations (p-values < 0.05), morphological plasticity robustness exhibited a larger adjusted R2 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.

## *Importance of Study and Future Experimentation*

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**TABLES AND FIGURES**

Figure 1. Alternative Hypothesis

Morphological Plasticity

Evolutionary Distance

Genetic Interactions

Protein Interactions

Fitness

(YPE)

Replicative Lifespan

Table 1. Correlation between growth fitness media and replicative lifespan

|  |  |  |  |
| --- | --- | --- | --- |
| Comparison | Medium | Multiple R2 | 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 |

Table 2. Correlation between evolutionary distance and replicative lifespan

|  |  |  |  |
| --- | --- | --- | --- |
| Species 1 | Species 2 | Multiple R2 | 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 |

Figure 2. Scatter plot of the significant negative correlation between replicative lifespan and morphology plasticity (p-value = 1.349 x 10-5, R-squared = 0.03431).

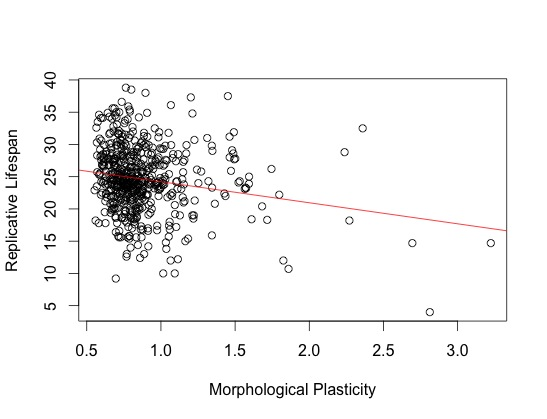


Figure 3.

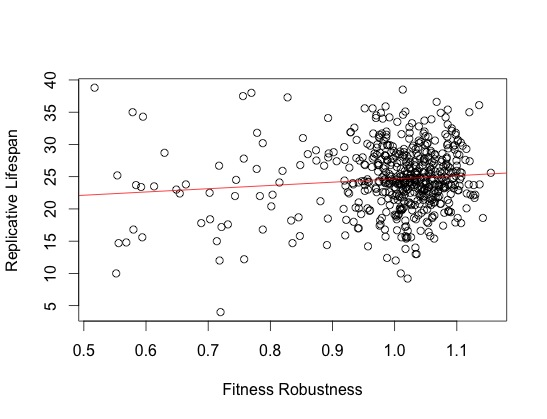


Table 3.

|  |  |  |  |
| --- | --- | --- | --- |
| Lifespan | Robustness Proxy | Multiple R-squared | p-value |
| RLS | YPE | 0.01106 | 0.01488 |
| RLS | Ka | 0.002773 | 0.3758 |
| RLS | pDegree | 0.004564 | 0.1759 |
| RLS | gDegree | 0.0006784 | 0.5641 |
| RLS | scmdstddev | 0.03431 | 1.349e-05 |

Table 4.

|  |  |  |  |
| --- | --- | --- | --- |
| Robustness Proxies | | R2 value | p-value |
| Fitness (YPE) | Number of genetic interactions | 0.1363 | 2.2 x 10-16 |
| Fitness (YPE) | Number of protein interactions | 0.0316 | 0.0003917 |
| Fitness (YPE) | Morphological Plasticity | 0.09902 | 1.08 x 10-13 |

Table 5.

Table 6.

|  |  |  |  |
| --- | --- | --- | --- |
| Factors in Multiple Regression | | R2 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 |

Table 7.

|  |  |  |  |
| --- | --- | --- | --- |
| Factors in Multiple Regression | | R2 value | p-value |
| Replicative Lifespan | * Morphological Plasticity (2.58 x 10-5) * Fitness (0.383) | 0.04244 | 1.045 x 10-5 |
| Morphological Plasticity | * Fitness (1.10 x 10-12) * Replicative Lifespan (2.58 x 10-5) | 0.1287 | <2.2 x 10-16 |
| Fitness | * Morphological Plasticity (1.1 x 1012) * Replicative Lifespan (0.383) | 0.1003 | 7.177 x 10-13 |

Figure 4. Results

Fitness

(YPE)

Morphological Plasticity

Evolutionary Distance

Genetic Interactions

Protein Interactions

Replicative Lifespan