

The Evolution of Cellular Restraint in Multicellular Organisms

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Contents

1	Introduction	5
2	Somatic Mutation Rate Sweep	7
2.1	Data cleaning	7
2.2	Data integrity check	8
2.3	Aggregate plots	10
2.4	Single organism size plots	18
2.5	Single somatic mutation rate plots	23
2.6	Statistics	30
3	Germ Mutation Rate Sweep	35
3.1	Data integrity check	36
3.2	Aggregate plots	38
3.3	Single organism size plots	46
3.4	Single organism size plots	51
3.5	Statistics	58
4	Genome Length Sweep	63
5	Size 1024x1024 organisms	65
6	Test of timing sample counts	67
7	Interactive web app	69

Chapter 1

Introduction

This document serves as the supplemental material for our ALife 2021 conference submission “The Evolution of Cellular Restraint in Multicellular Organisms”.

The document is split into sections. Each section can be accessed via the navigation bar on the left side of the screen. Sections mostly correspond to experiments (some that were discussed at length in the paper, others that were not).

Chapter 2

Somatic Mutation Rate Sweep

This experiment was one of the preliminary experiments we conducted to find the default parameters for Primordium. Here, we vary the somatic mutation rate, the probability that a cell replication will result in the offspring cell having a different restraint value from its parent.

We settled on a somatic mutation rate of 0.5 (*i.e.*, each cell replication has a 50% chance of mutation).

The configuration script and data for the experiment can be found under 2021_02_27__soma_mut_fin/ in the experiments directory of the git repository.

2.1 Data cleaning

Load necessary libraries

```
library(dplyr)
library(ggplot2)
library(ggribes)
library(scales)
library(khroma)
```

Load the data and trim all the unnecessary bits (*e.g.*, we initially ran sizes 8x8, 1024x1024 but cut them from the paper to make plots easier to read).

```
# Load the data
df = read.csv('../experiments/2021_02_27__soma_mut_fin/evolution/data/scraped_evolution_data.csv')
# Trim off NAs (artifacts of how we scraped the data) and trim to only have gen 10,000
```

```
df2 = df[!is.na(df$MCSIZE) & df$generation == 10000,]
# Ignore data for size 8x8 and 1024x1024
df2 = df2[df2$MCSIZE != 8 & df2$MCSIZE != 1024,]
```

We group and summarize the data to make to ensure all replicates are present.

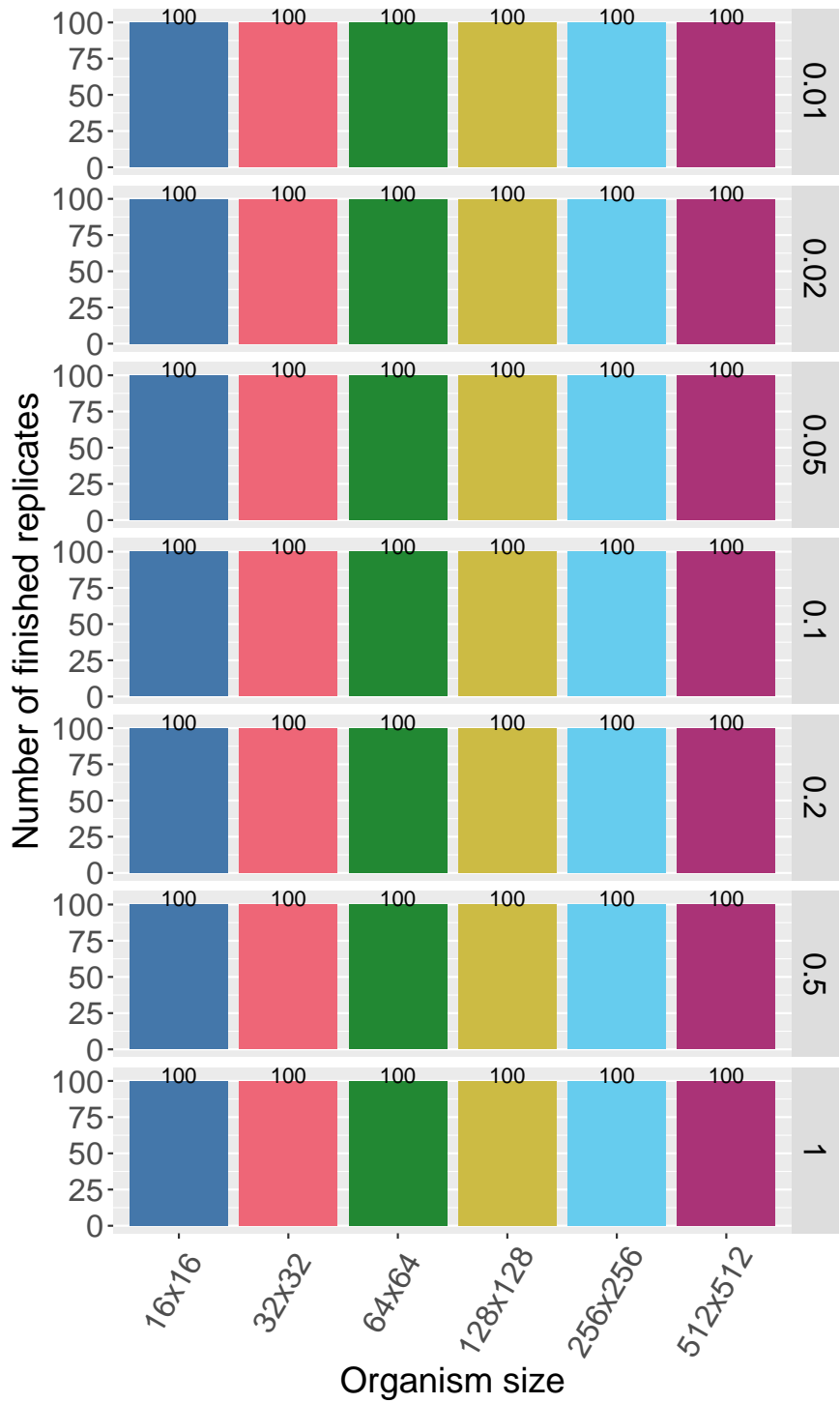
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, CELLMUT)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n
```

We clean the data and create a few helper variables to make plotting easier.

```
# Calculate restraint value (x - 60 because genome length is 100 here)
df2$restraint_value = df2$ave_ones - 60
# Make a nice, clean factor for size
df2$size_str = paste0(df2$MCSIZE, 'x', df2$MCSIZE)
df2$size_factor = factor(df2$size_str, levels = c('16x16', '32x32', '64x64', '128x128',
df2$size_factor_reversed = factor(df2$size_str, levels = rev(c('16x16', '32x32', '64x64', '128x128',
df2$soma_mut_str = paste('soma CELLMUT', df2$CELLMUT)
df2$mut_factor = factor(df2$CELLMUT, levels = c(0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0))
data_summary$size_str = paste0(data_summary$MCSIZE, 'x', data_summary$MCSIZE)
data_summary$size_factor = factor(data_summary$size_str, levels = c('16x16', '32x32', '64x64', '128x128',
data_summary$soma_mut_str = paste('soma CELLMUT', data_summary$CELLMUT)
data_summary$mut_factor = factor(data_summary$CELLMUT, levels = c(0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.0))
# Create a map of colors we'll use to plot the different organism sizes
color_vec = as.character(khroma::color('bright')(7))
color_map = c(
  '16x16' = color_vec[1],
  '32x32' = color_vec[2],
  '64x64' = color_vec[3],
  '128x128' = color_vec[4],
  '256x256' = color_vec[5],
  '512x512' = color_vec[6],
  '1024x1024' = color_vec[7]
)
# Set the sizes for text in plots
text_major_size = 18
text_minor_size = 16
```

2.2 Data integrity check

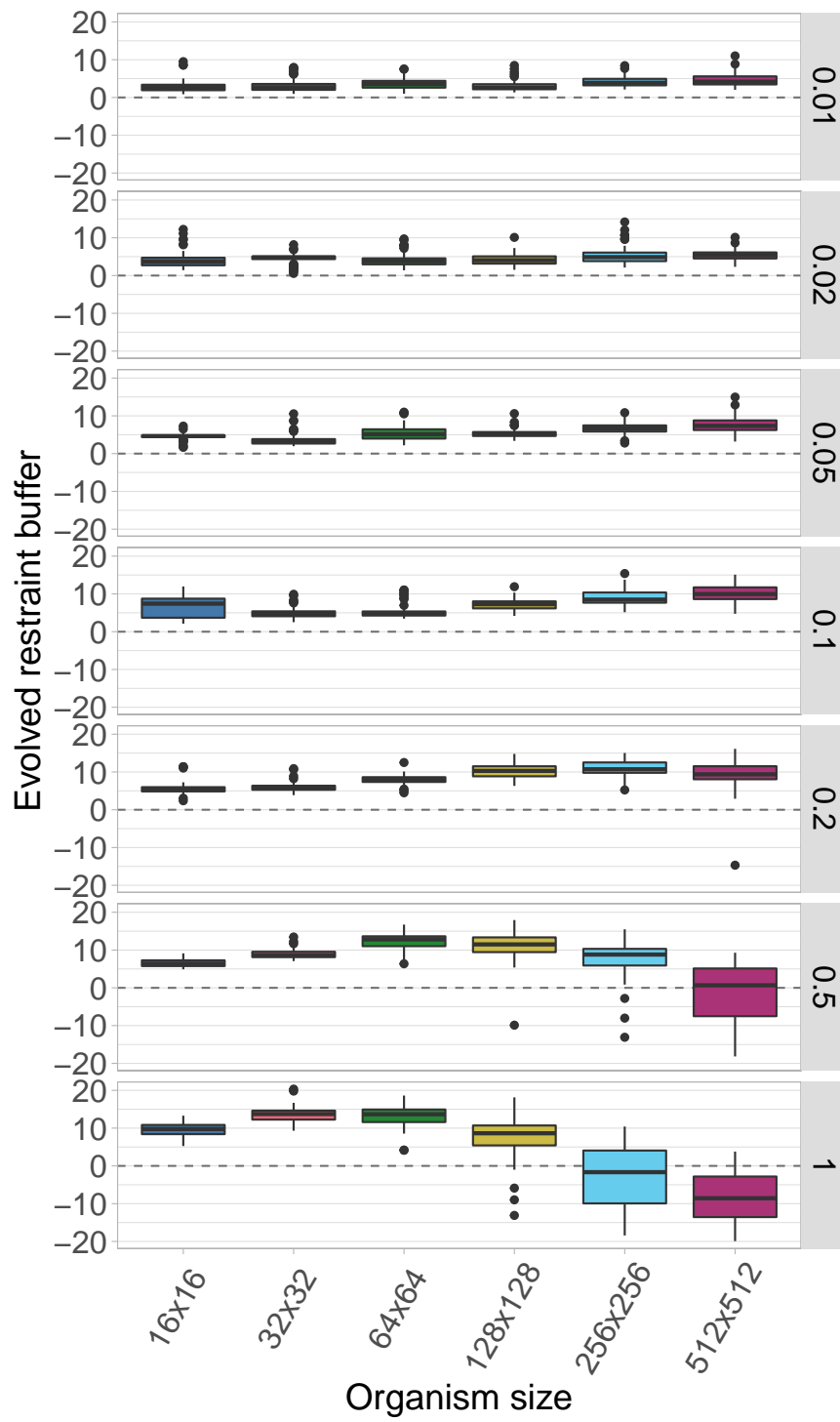
Now we plot the number of finished replicates for each treatment to make sure all data are present. Each row shows a different somatic mutation rate. Each bar/color shows a different organism size.



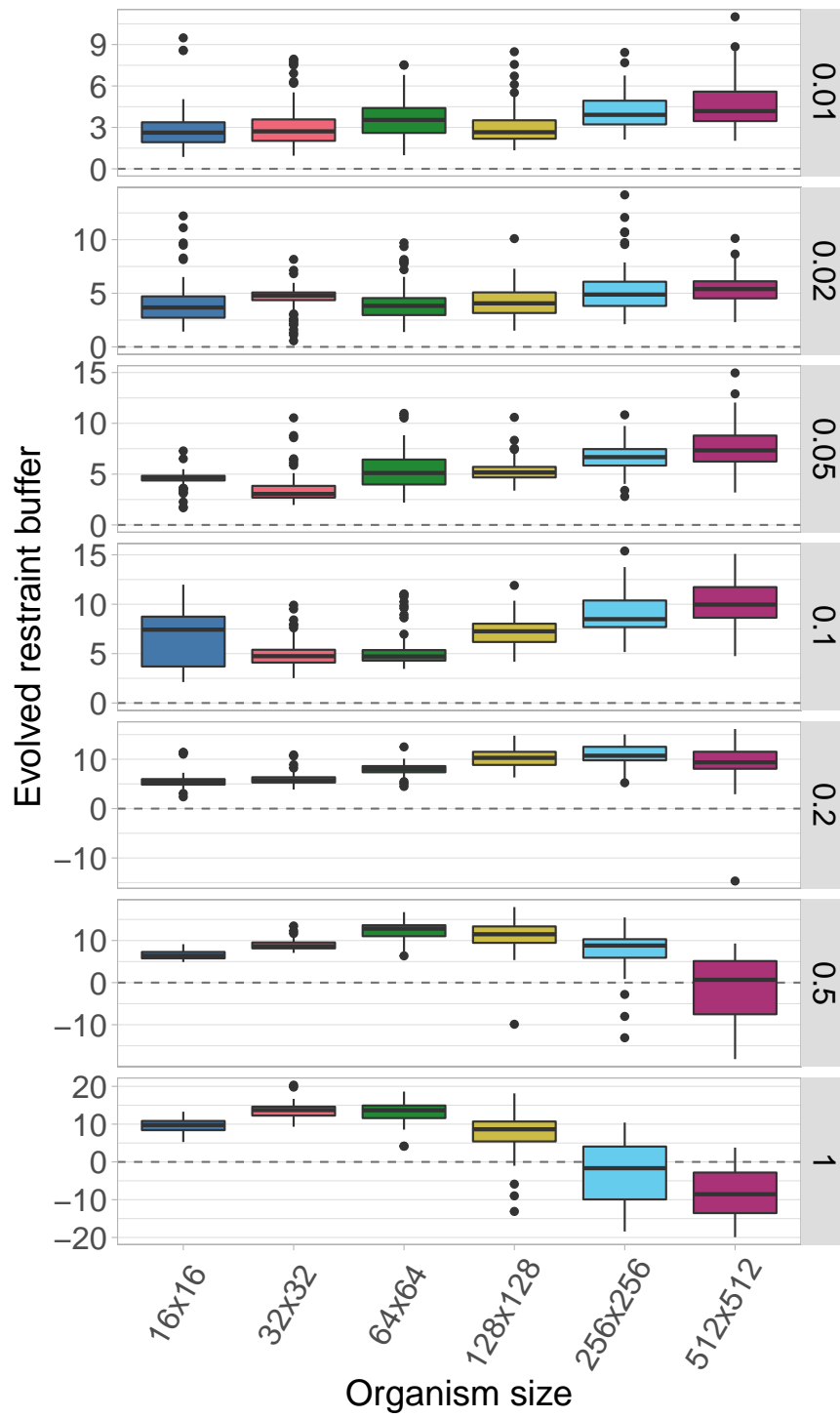
2.3 Aggregate plots

2.3.1 Facet by somatic mutation rate

Here we plot all the data at once. Each row showing a different somatic mutation rate and each boxplot shows a given organism size.

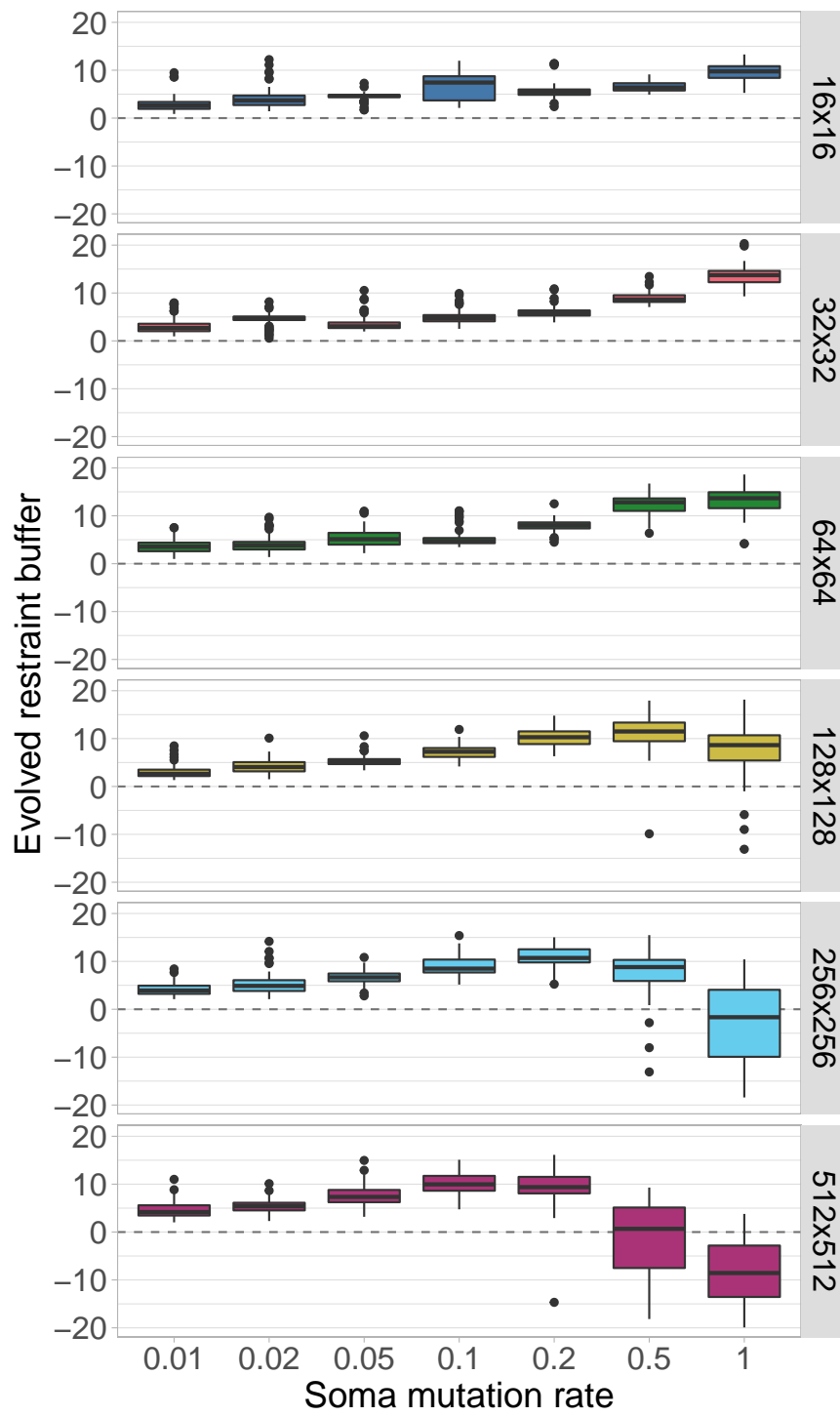


Here we plot the same data, only we allow the y-axis to vary between rows.

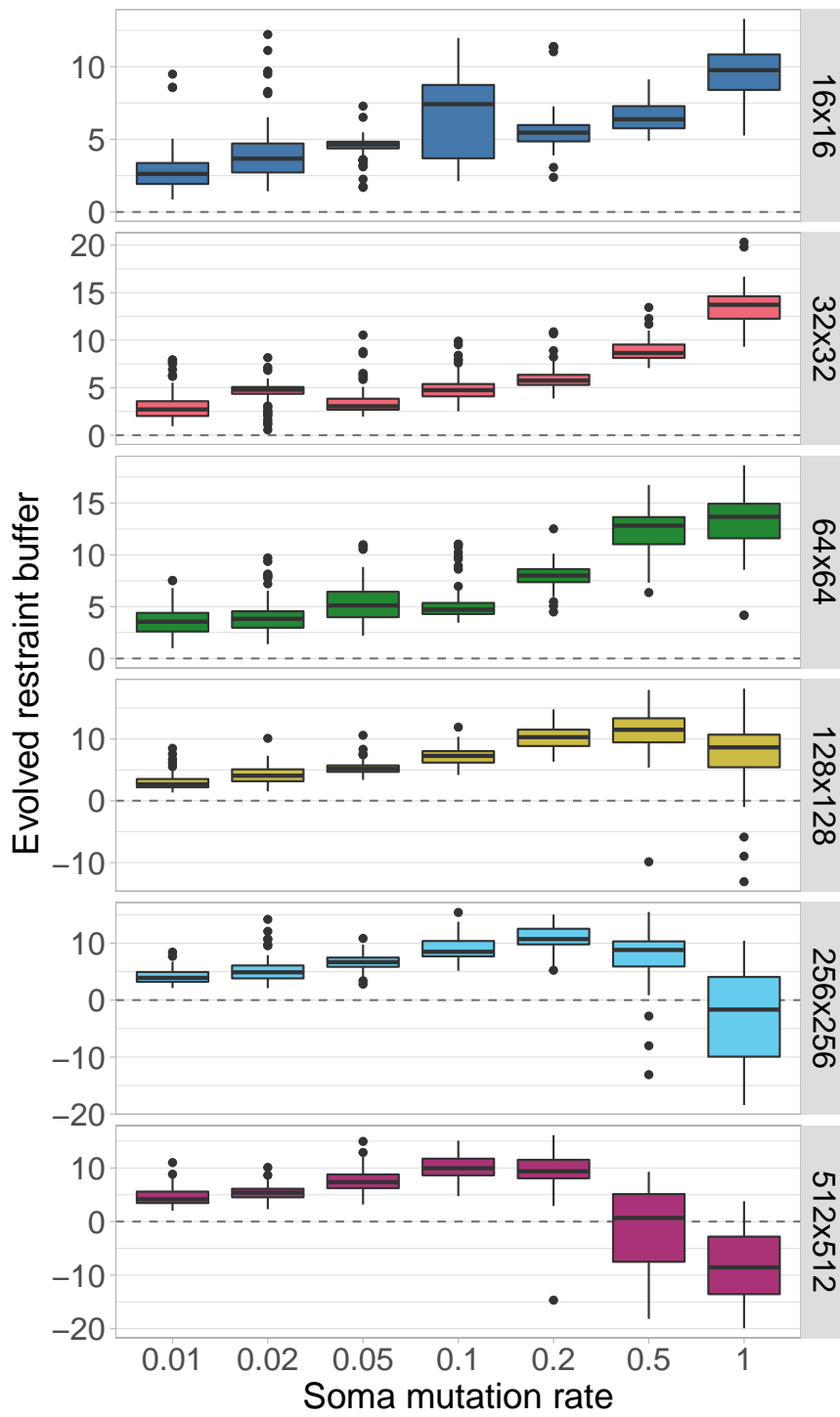


2.3.2 Facet by organism size

Next, we plot the same data, but this time each row corresponds to a certain organism size while somatic mutation rate changes along the x-axis.



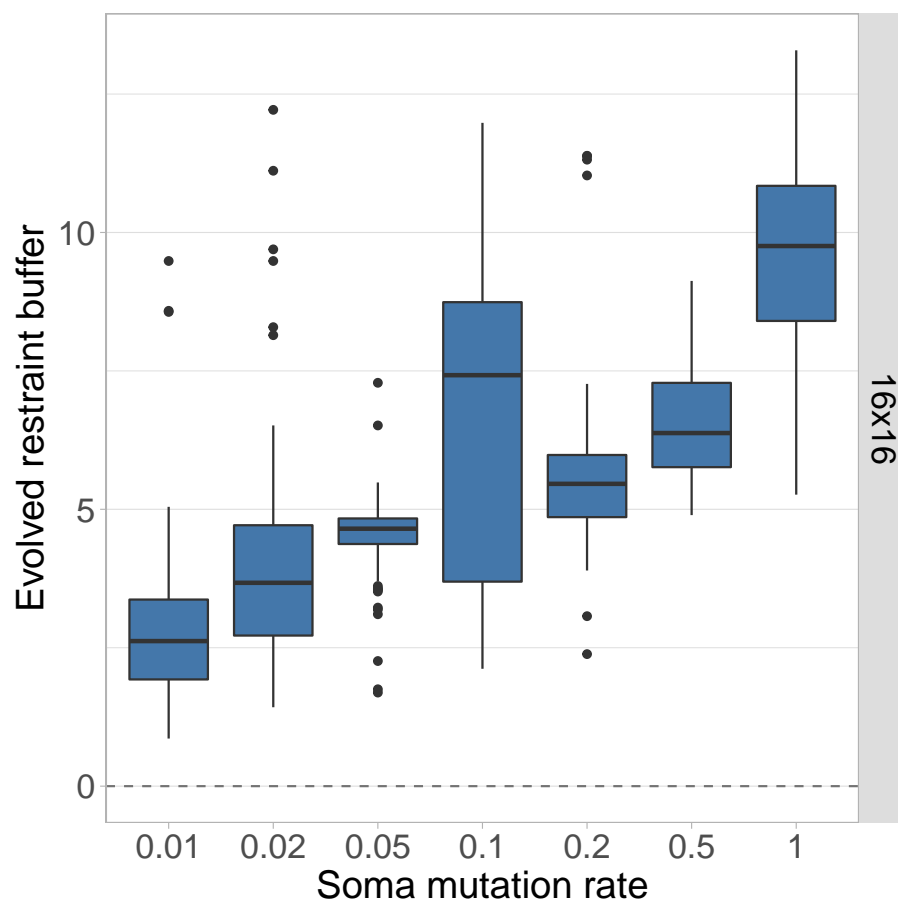
Again, we replot the same data but allow the y-axis to vary between rows.



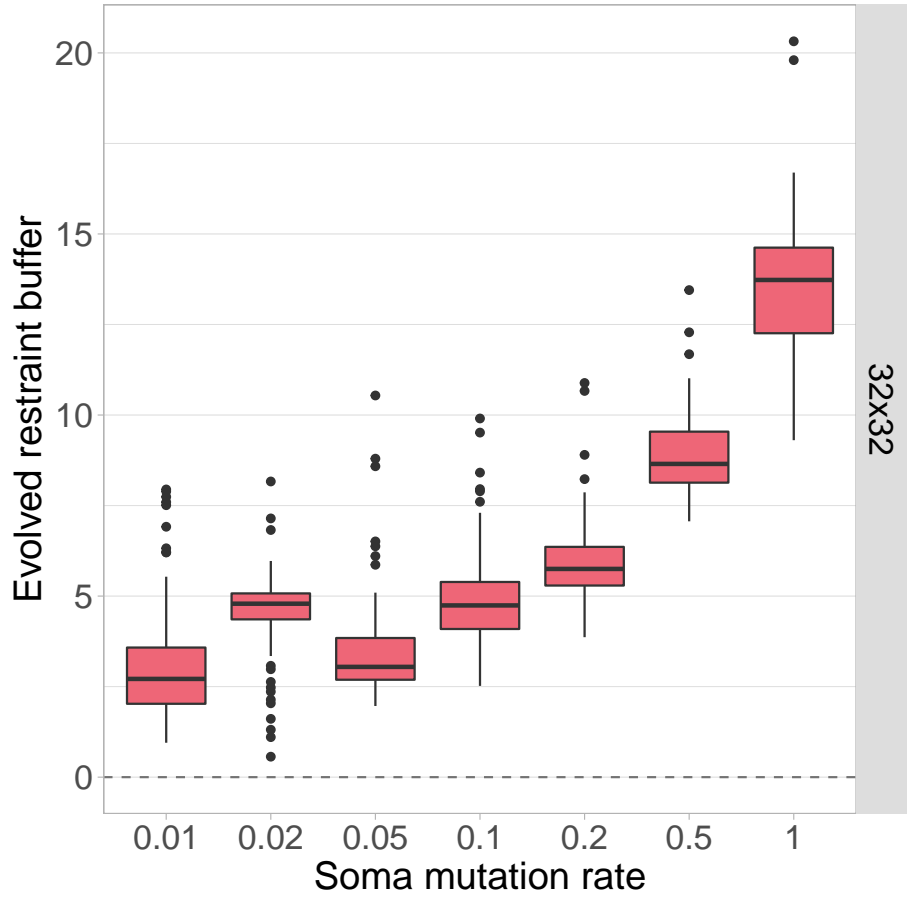
2.4 Single organism size plots

Here we plot each organism size independently, with the somatic mutation rate on the x-axis.

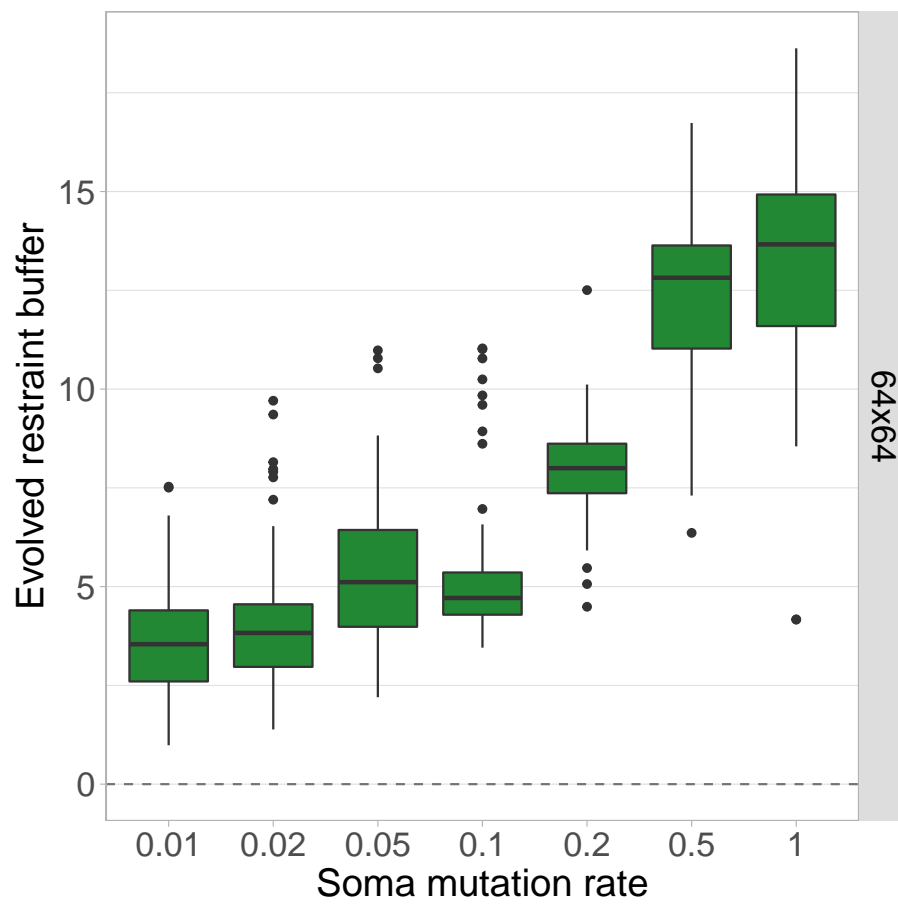
2.4.1 Organism size 16x16



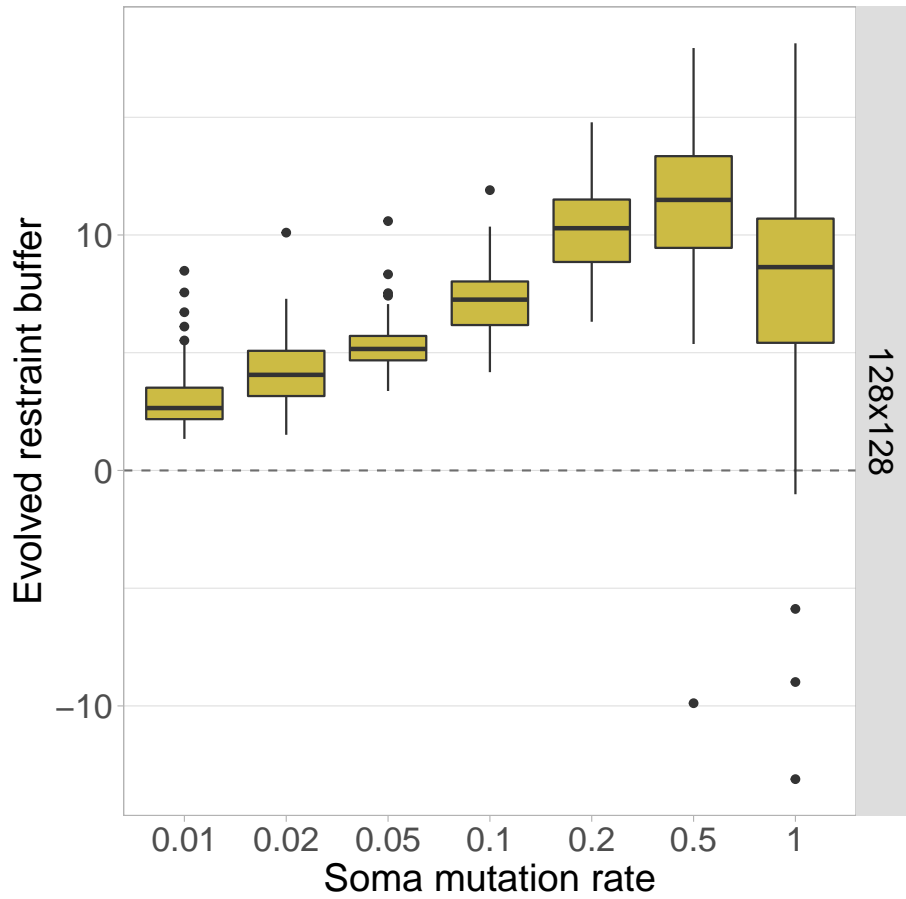
2.4.2 Organism size 32x32



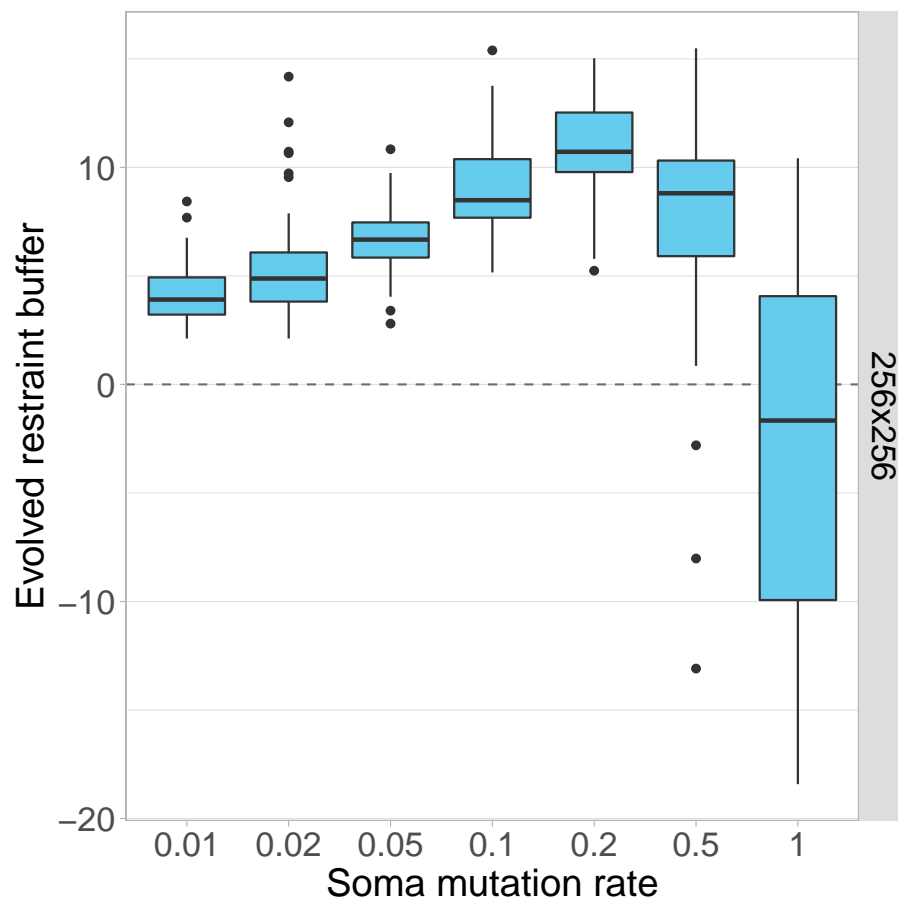
2.4.3 Organism size 64x64



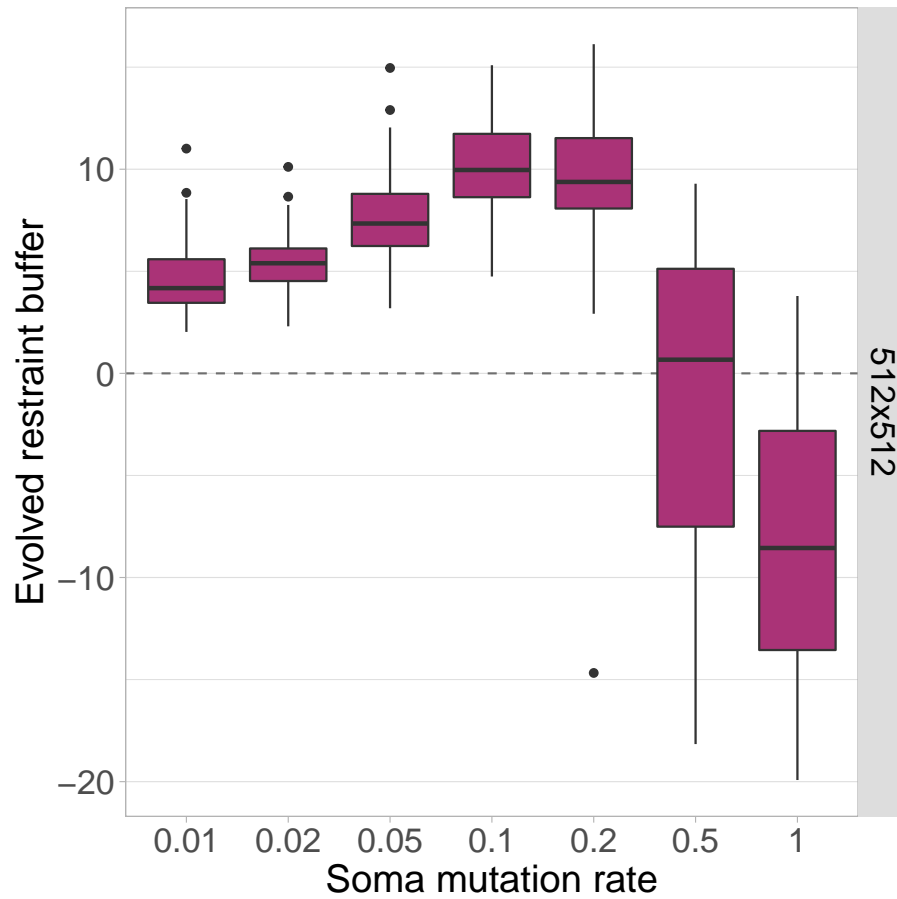
2.4.4 Organism size 128x128



2.4.5 Organism size 256x256



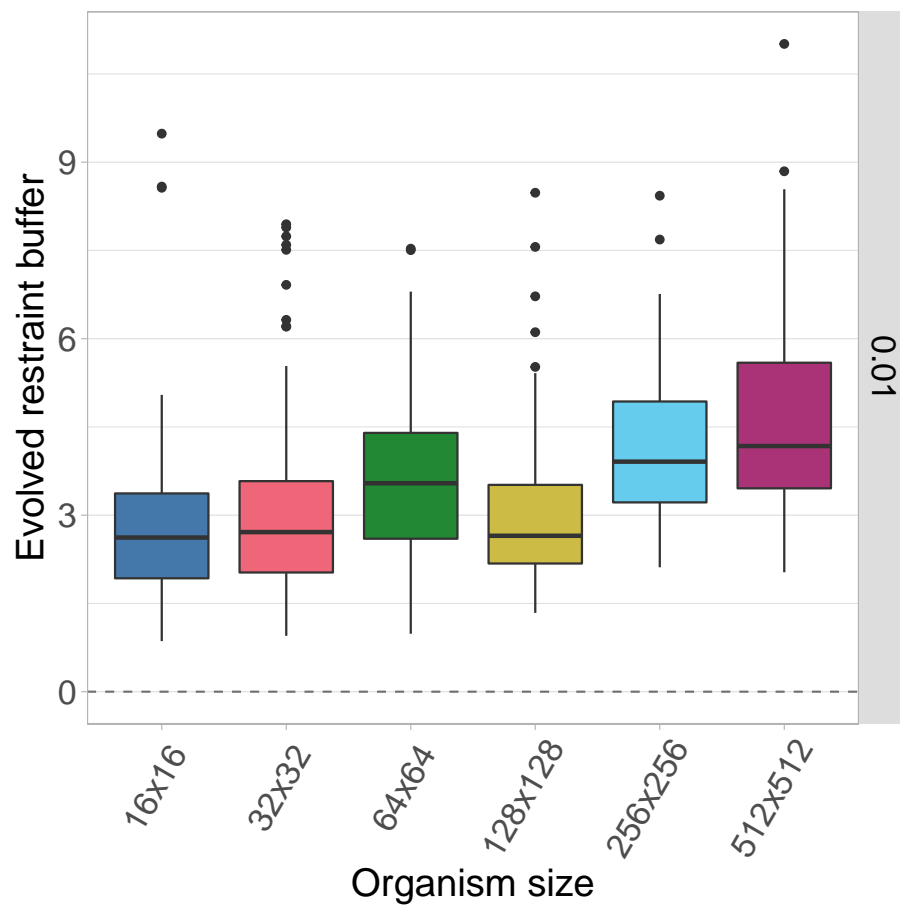
2.4.6 Organism size 512x512



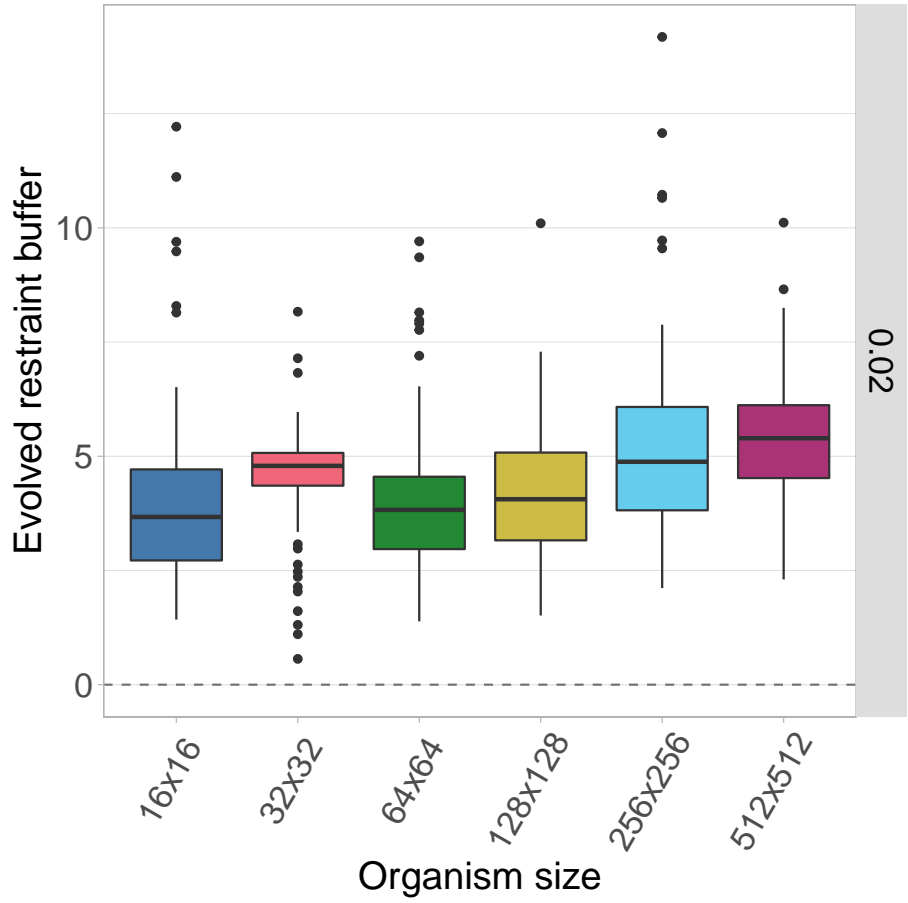
2.5 Single somatic mutation rate plots

Here we plot each somatic mutation rate independently, with organism size varying on the x-axis.

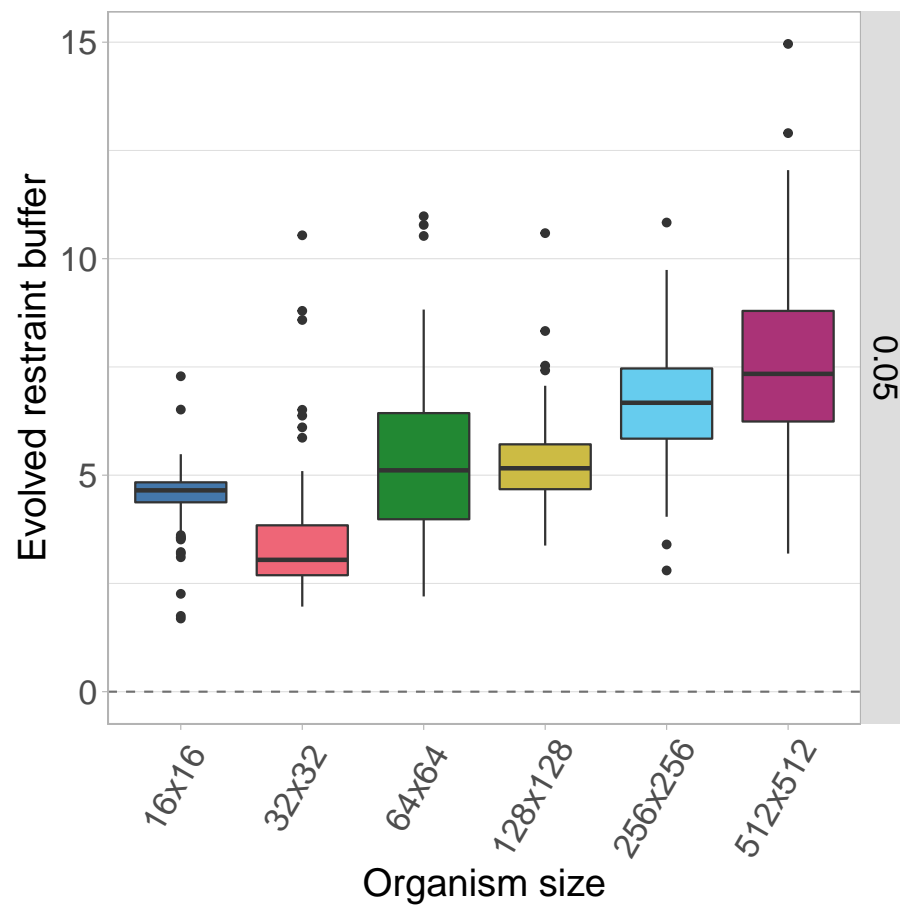
2.5.1 Somatic mut. rate 0.01



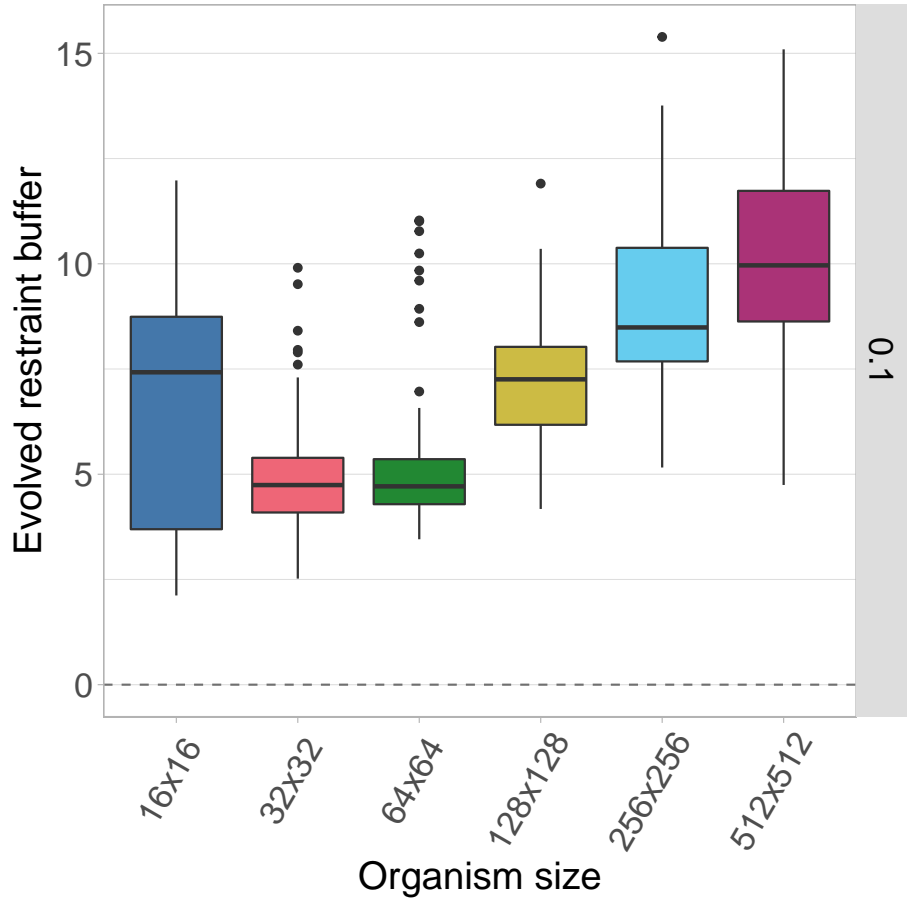
2.5.2 Somatic mut. rate 0.02



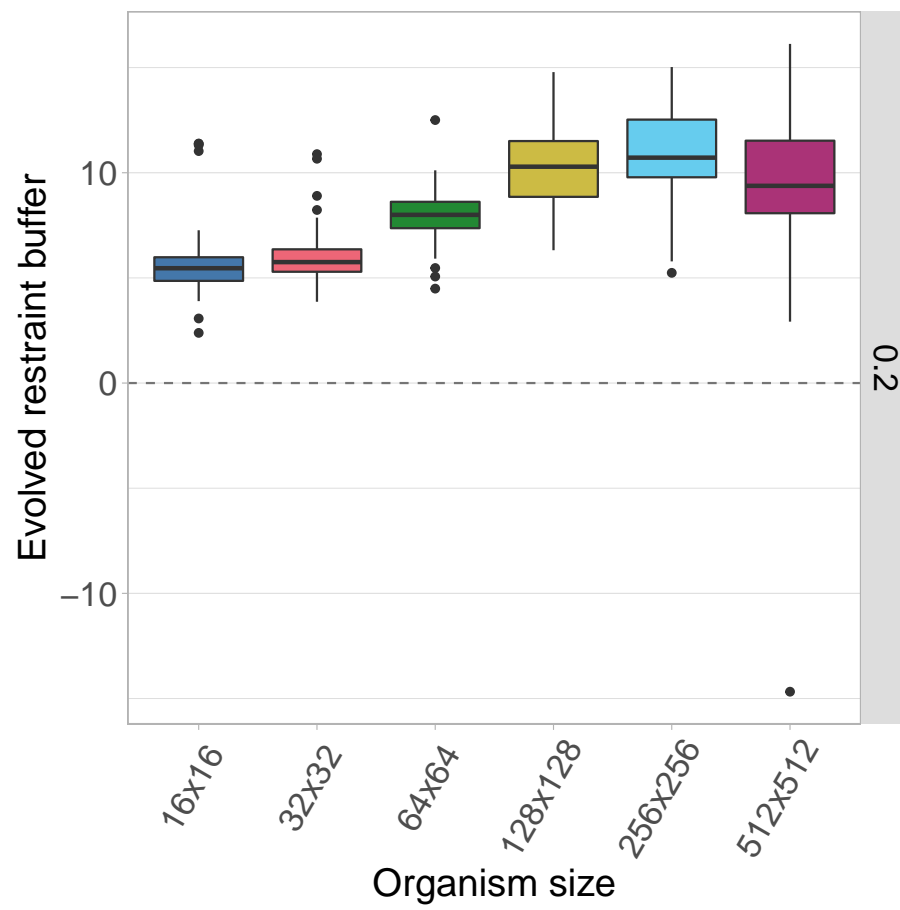
2.5.3 Somatic mut. rate 0.05



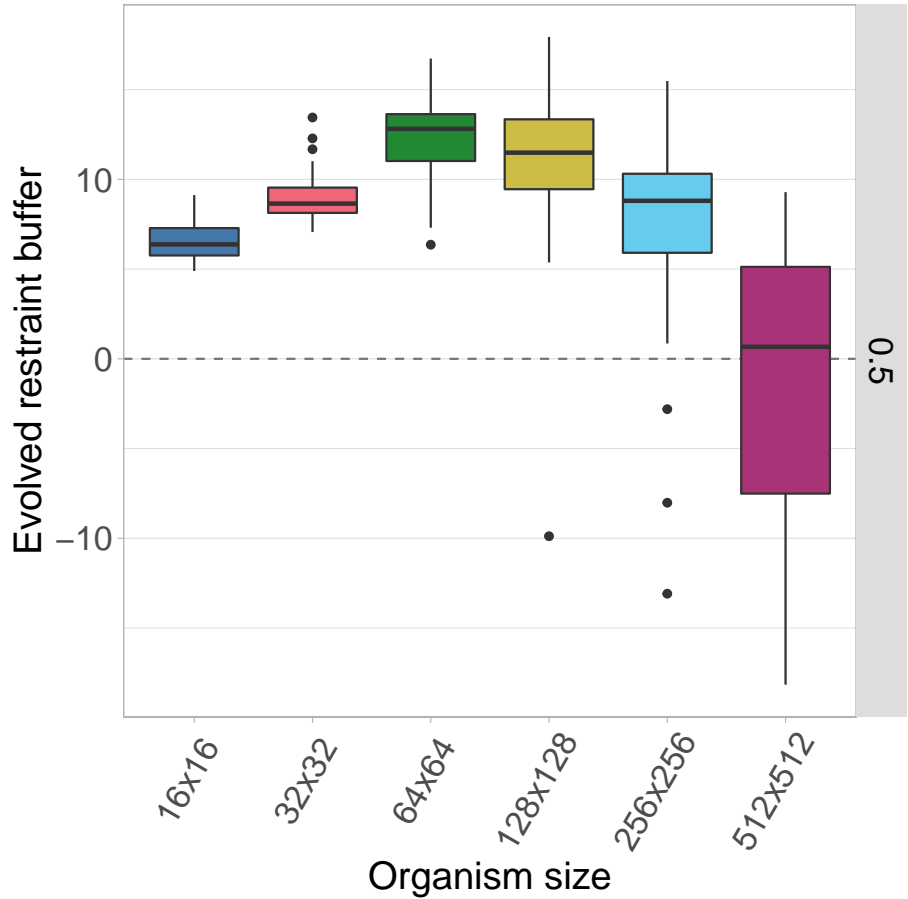
2.5.4 Somatic mut. rate 0.1



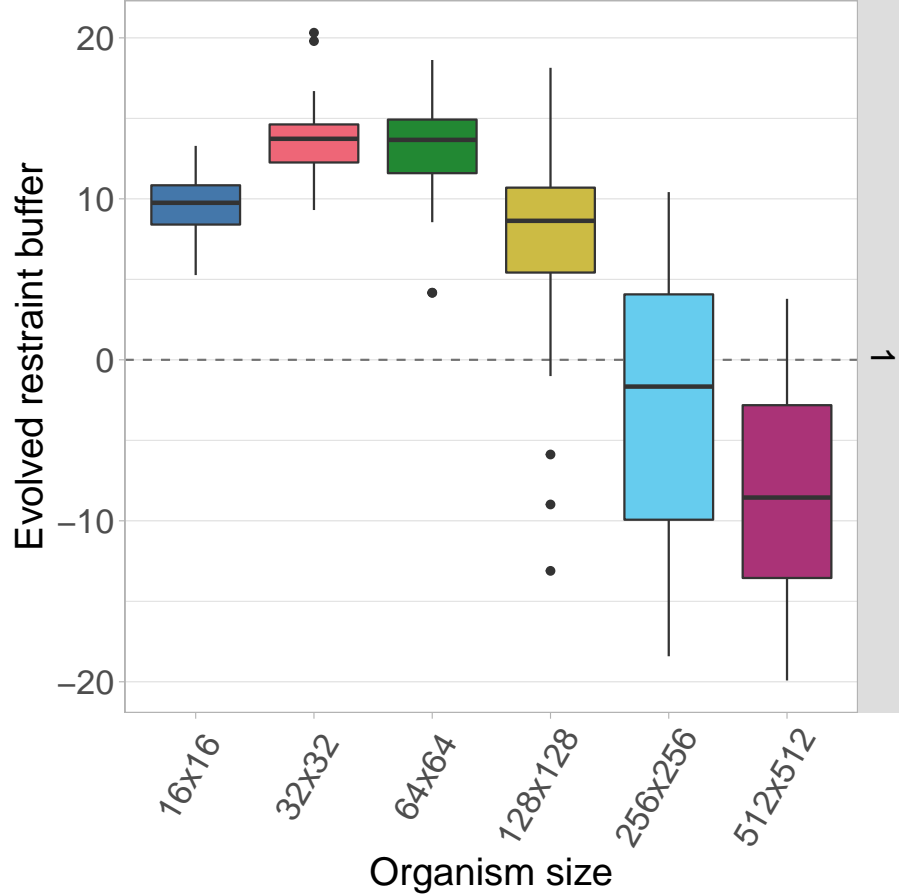
2.5.5 Somatic mut. rate 0.2



2.5.6 Somatic mut. rate 0.5



2.5.7 Somatic mut. rate 1.0



2.6 Statistics

Since organism size is our main point of comparison, we calculate stats for each somatic mutation rate.

First, we perform a Kruskal-Wallis test across all organism sizes to indicate if variance exists at that mutation rate. If variance exists, we then perform a pairwise Wilcoxon Rank-Sum test to show which pairs of organism sizes significantly differ. Finally, we perform Bonferroni-Holm corrections for multiple comparisons.

```
mut_vec = c(0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1)
df_kruskal = data.frame(data = matrix(nrow = 0, ncol = 4))
colnames(df_kruskal) = c('soma_mut_rate', 'p_value', 'chi_squared', 'df')
for(mut_rate in mut_vec){
  df_test = df2[df2$CELLMUT == mut_rate,]
```

```

    res = kruskal.test(df_test$restraint_value ~ df_test$MCSIZE, df_test)
    df_kruskal[nrow(df_kruskal) + 1,] = c(mut_rate, res$p.value, as.numeric(res$statistic)[1], as
  }
  df_kruskal$less_0.01 = df_kruskal$p_value < 0.01
  print(df_kruskal)

```

```

##   soma_mut_rate      p_value chi_squared df less_0.01
## 1          0.01 2.661659e-25    125.0566 5      TRUE
## 2          0.02 4.808020e-19     95.4471 5      TRUE
## 3          0.05 1.142677e-63    304.3847 5      TRUE
## 4          0.10 3.945761e-64    306.5323 5      TRUE
## 5          0.20 4.924029e-79    375.7743 5      TRUE
## 6          0.50 5.011460e-85    403.5832 5      TRUE
## 7          1.00 5.474947e-99    468.3229 5      TRUE

```

We see that significant variation exists within each mutation rate, so we perform pairwise Wilcoxon tests on each to see which pairs of sizes are significantly different.

```

size_vec = c(16, 32, 64, 128, 256, 512)
mut_vec = c(0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1)
for(mut_rate in mut_vec){
  df_test = df2[df2$CELLMUT == mut_rate,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('mut_rate', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw', 'W')
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test[df_test$MCSIZE == size_a,]$restraint_value, df_test[df_test$MCSIZE == size_b,]$restraint_value)
      df_wilcox[nrow(df_wilcox) + 1,] = c(mut_rate, size_a, size_b, 0, res$p.value, as.numeric(res$statistic)[1], res$W)
    }
  }
  df_wilcox$p_value_corrected = p.adjust(df_wilcox$p_value_raw, method = 'holm')
  df_wilcox$less_0.01 = df_wilcox$p_value_corrected < 0.01
  print(paste0('Somatic mutation rate: ', mut_rate))
  print(df_wilcox)
}

```

```

## [1] "Somatic mutation rate: 0.01"
##   mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1     0.01    16    32      9.390497e-01 4.695249e-01 4703.5    FALSE
## 2     0.01    16    64      2.988154e-04 3.735192e-05 3312.0     TRUE
## 3     0.01    16   128      7.079843e-01 2.359948e-01 4514.5    FALSE
## 4     0.01    16   256      2.034819e-12 1.453442e-13 1974.5     TRUE
## 5     0.01    16   512      4.368517e-15 2.912344e-16 1653.0     TRUE
## 6     0.01    32    64      1.074876e-02 1.535537e-03 3703.0    FALSE

```

```

## 7      0.01      32      128      9.390497e-01 7.176323e-01 4851.5      FALSE
## 8      0.01      32      256      8.111610e-09 8.111610e-10 2485.5      TRUE
## 9      0.01      32      512      1.748038e-11 1.456698e-12 2102.5      TRUE
## 10     0.01      64      128      1.074876e-02 1.601365e-03 6292.0      FALSE
## 11     0.01      64      256      1.397091e-02 2.794183e-03 3776.0      FALSE
## 12     0.01      64      512      7.748038e-05 8.608931e-06 3178.5      TRUE
## 13     0.01     128     256      3.676583e-09 3.342348e-10 2428.5      TRUE
## 14     0.01     128     512      2.110112e-12 1.623163e-13 1980.5      TRUE
## 15     0.01     256     512      2.266729e-01 5.666822e-02 4219.5      FALSE
## [1] "Somatic mutation rate: 0.02"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.02      16      32      3.611494e-05 4.012771e-06 3112.5      TRUE
## 2      0.02      16      64      4.740405e-01 4.740405e-01 4706.5      FALSE
## 3      0.02      16     128      2.648393e-01 5.296786e-02 4207.5      FALSE
## 4      0.02      16     256      6.698428e-07 5.582024e-08 2776.5      TRUE
## 5      0.02      16     512      4.142268e-11 2.761512e-12 2139.0      TRUE
## 6      0.02      32      64      1.240992e-05 1.240992e-06 6985.0      TRUE
## 7      0.02      32     128      2.150816e-02 3.584693e-03 6192.5      FALSE
## 8      0.02      32     256      3.993493e-01 9.983733e-02 4326.0      FALSE
## 9      0.02      32     512      1.117168e-04 1.396459e-05 3221.5      TRUE
## 10     0.02      64     128      4.025666e-01 2.012833e-01 4476.5      FALSE
## 11     0.02      64     256      5.648464e-06 5.134967e-07 2944.5      TRUE
## 12     0.02      64     512      6.120346e-11 4.371676e-12 2165.5      TRUE
## 13     0.02     128     256      3.129242e-04 4.470345e-05 3329.0      TRUE
## 14     0.02     128     512      1.760116e-08 1.353935e-09 2519.0      TRUE
## 15     0.02     256     512      3.993493e-01 1.013587e-01 4329.0      FALSE
## [1] "Somatic mutation rate: 0.05"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.05      16      32      8.163575e-15 9.070638e-16 8290.5      TRUE
## 2      0.05      16      64      1.254683e-03 4.182276e-04 3555.5      TRUE
## 3      0.05      16     128      2.819711e-09 5.639421e-10 2462.0      TRUE
## 4      0.05      16     256      1.007639e-23 8.396990e-25 791.0      TRUE
## 5      0.05      16     512      3.169326e-24 2.437943e-25 742.5      TRUE
## 6      0.05      32      64      9.865308e-14 1.409330e-14 1850.0      TRUE
## 7      0.05      32     128      9.672216e-22 8.792924e-23 978.5      TRUE
## 8      0.05      32     256      4.456762e-26 3.183402e-27 576.5      TRUE
## 9      0.05      32     512      1.225797e-27 8.171978e-29 441.0      TRUE
## 10     0.05      64     128      9.619980e-01 9.619980e-01 4980.0      FALSE
## 11     0.05      64     256      4.409184e-09 1.102296e-09 2505.5      TRUE
## 12     0.05      64     512      1.967988e-13 3.279979e-14 1894.5      TRUE
## 13     0.05     128     256      3.061979e-14 3.827473e-15 1782.5      TRUE
## 14     0.05     128     512      4.080298e-17 4.080298e-18 1448.5      TRUE
## 15     0.05     256     512      2.648877e-03 1.324439e-03 3685.5      TRUE
## [1] "Somatic mutation rate: 0.1"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.1       16      32      3.903716e-03 9.759291e-04 6350.0      TRUE

```



```

## 2      0.1      16      64      9.815188e-02 3.271729e-02 5874.5      FALSE
## 3      0.1      16     128      6.061146e-01 3.140880e-01 4587.5      FALSE
## 4      0.1      16     256      3.278276e-08 5.463793e-09 2612.5      TRUE
## 5      0.1      16     512      9.506115e-18 1.188264e-18 1391.5      TRUE
## 6      0.1      32      64      6.061146e-01 3.030573e-01 4578.0      FALSE
## 7      0.1      32     128      8.673971e-21 8.673971e-22 1074.0      TRUE
## 8      0.1      32     256      6.950798e-29 4.964856e-30 340.0      TRUE
## 9      0.1      32     512      1.934395e-30 1.289597e-31 211.5      TRUE
## 10     0.1      64     128      2.239733e-18 2.488592e-19 1320.5      TRUE
## 11     0.1      64     256      1.194130e-25 9.951080e-27 619.5      TRUE
## 12     0.1      64     512      1.966283e-27 1.512525e-28 463.5      TRUE
## 13     0.1     128     256      8.038941e-11 1.148420e-11 2222.0      TRUE
## 14     0.1     128     512      1.880691e-21 1.709719e-22 1006.0      TRUE
## 15     0.1     256     512      3.931365e-04 7.862729e-05 3383.5      TRUE
## [1] "Somatic mutation rate: 0.2"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.2      16      32      1.077048e-02 5.385238e-03 3860.5      FALSE
## 2      0.2      16      64      6.720281e-24 8.400351e-25 791.0      TRUE
## 3      0.2      16     128      1.215721e-28 1.013101e-29 365.5      TRUE
## 4      0.2      16     256      4.359012e-29 3.353086e-30 326.0      TRUE
## 5      0.2      16     512      3.611807e-25 3.283461e-26 665.0      TRUE
## 6      0.2      32      64      5.255254e-22 7.507505e-23 972.0      TRUE
## 7      0.2      32     128      3.542154e-29 2.530110e-30 316.0      TRUE
## 8      0.2      32     256      3.153758e-30 2.102505e-31 228.5      TRUE
## 9      0.2      32     512      1.346976e-24 1.496640e-25 723.5      TRUE
## 10     0.2      64     128      1.237545e-13 2.062574e-14 1870.0      TRUE
## 11     0.2      64     256      6.129521e-25 6.129521e-26 689.0      TRUE
## 12     0.2      64     512      1.436552e-07 2.873105e-08 2728.5      TRUE
## 13     0.2     128     256      6.935985e-03 2.311995e-03 3752.5      TRUE
## 14     0.2     128     512      1.987108e-01 1.987108e-01 5526.5      FALSE
## 15     0.2     256     512      3.309684e-04 8.274210e-05 6611.5      TRUE
## [1] "Somatic mutation rate: 0.5"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.5      16      32      1.212403e-25 1.212403e-26 627.0      TRUE
## 2      0.5      16      64      1.029212e-31 7.351512e-33 113.0      TRUE
## 3      0.5      16     128      1.432034e-27 1.301849e-28 458.0      TRUE
## 4      0.5      16     256      3.887685e-06 1.295895e-06 3018.5      TRUE
## 5      0.5      16     512      1.499786e-19 2.499644e-20 8781.5      TRUE
## 6      0.5      32      64      3.854284e-24 4.282538e-25 764.5      TRUE
## 7      0.5      32     128      6.344735e-14 1.268947e-14 1844.5      TRUE
## 8      0.5      32     256      6.346151e-01 6.346151e-01 5195.0      FALSE
## 9      0.5      32     512      3.036159e-31 2.335507e-32 9847.5      TRUE
## 10     0.5      64     128      9.397051e-03 4.698526e-03 6157.5      TRUE
## 11     0.5      64     256      6.907801e-20 9.868288e-21 8822.0      TRUE
## 12     0.5      64     512      9.160009e-33 6.106673e-34 9971.0      TRUE
## 13     0.5     128     256      4.999760e-11 1.249940e-11 7773.0      TRUE

```

```

## 14      0.5    128    512      6.054856e-31 5.045714e-32 9821.0      TRUE
## 15      0.5    256    512      4.216225e-21 5.270281e-22 8947.0      TRUE
## [1] "Somatic mutation rate: 1"
##      mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1         1      16      32      2.812620e-27 3.515774e-28    494.5      TRUE
## 2         1      16      64      5.606003e-22 9.343338e-23    981.0      TRUE
## 3         1      16     128      2.202125e-02 1.101063e-02   6041.0     FALSE
## 4         1      16     256      4.073858e-28 4.526509e-29   9580.5      TRUE
## 5         1      16     512      3.841268e-33 2.561566e-34  10000.0      TRUE
## 6         1     32      64      7.619035e-01 7.619035e-01   5124.5     FALSE
## 7         1     32     128      2.931097e-22 4.187282e-23   9052.0      TRUE
## 8         1     32     256      3.841268e-33 2.976903e-34   9995.0      TRUE
## 9         1     32     512      3.841268e-33 2.561711e-34  10000.0      TRUE
## 10        1     64     128      1.456083e-19 3.640207e-20   8765.0      TRUE
## 11        1     64     256      2.413338e-32 2.193944e-33   9928.0      TRUE
## 12        1     64     512      3.841268e-33 2.560845e-34  10000.0      TRUE
## 13        1    128     256      1.180975e-20 2.361951e-21   8883.5      TRUE
## 14        1    128     512      1.253447e-30 1.253447e-31   9789.5      TRUE
## 15        1    256     512      6.072904e-07 2.024301e-07   7127.5      TRUE

```

Chapter 3

Germ Mutation Rate Sweep

This experiment was one of the preliminary experiments we conducted to find the default parameters for Primordium. We varied the mutation rate, the probability that an offspring experiences a mutation to its restraint buffer during organism reproduction.

The final default germ mutation rate was 0.02 (*i.e.*, each organism reproduction has a 2% chance of mutation).

The configuration script and data for the experiment can be found under 2021_02_16__germ_mut_fin/ in the experiments directory of the git repository.

Load necessary libraries

```
library(dplyr)
library(ggplot2)
library(ggthemes)
library(scales)
library(khroma)
```

Load the data and trim all the unnecessary bits (*e.g.*, we initially ran sizes 8x8, 1024x1024 but cut them from the paper to make plots easier to read).

```
# Load the data
df = read.csv('../experiments/2021_02_16__germ_mut_fin/evolution/data/scraped_evolution_data.csv')
#df = read.csv('/research/rogue_cell/Primordium/experiments/2021_02_16__germ_mut_fin/evolution/d
# Trim off NAs (artifacts of how we scraped the data) and trim to only have gen 10,000
cat(colnames(df), '\n')
```

```
## X generation ave_ones ave_repro_time min_ones max_ones var_ones rep_id MCSIZE COST GENS MUT PC
df2 = df[!is.na(df$MCSIZE) & df$generation == 10000,]
# Ignore data for size 8x8 and 1024x1024
```

```
df2 = df2[df2$MCSIZE != 8 & df2$MCSIZE != 1024,]
```

We group and summarize the data to make to ensure all replicates are present.

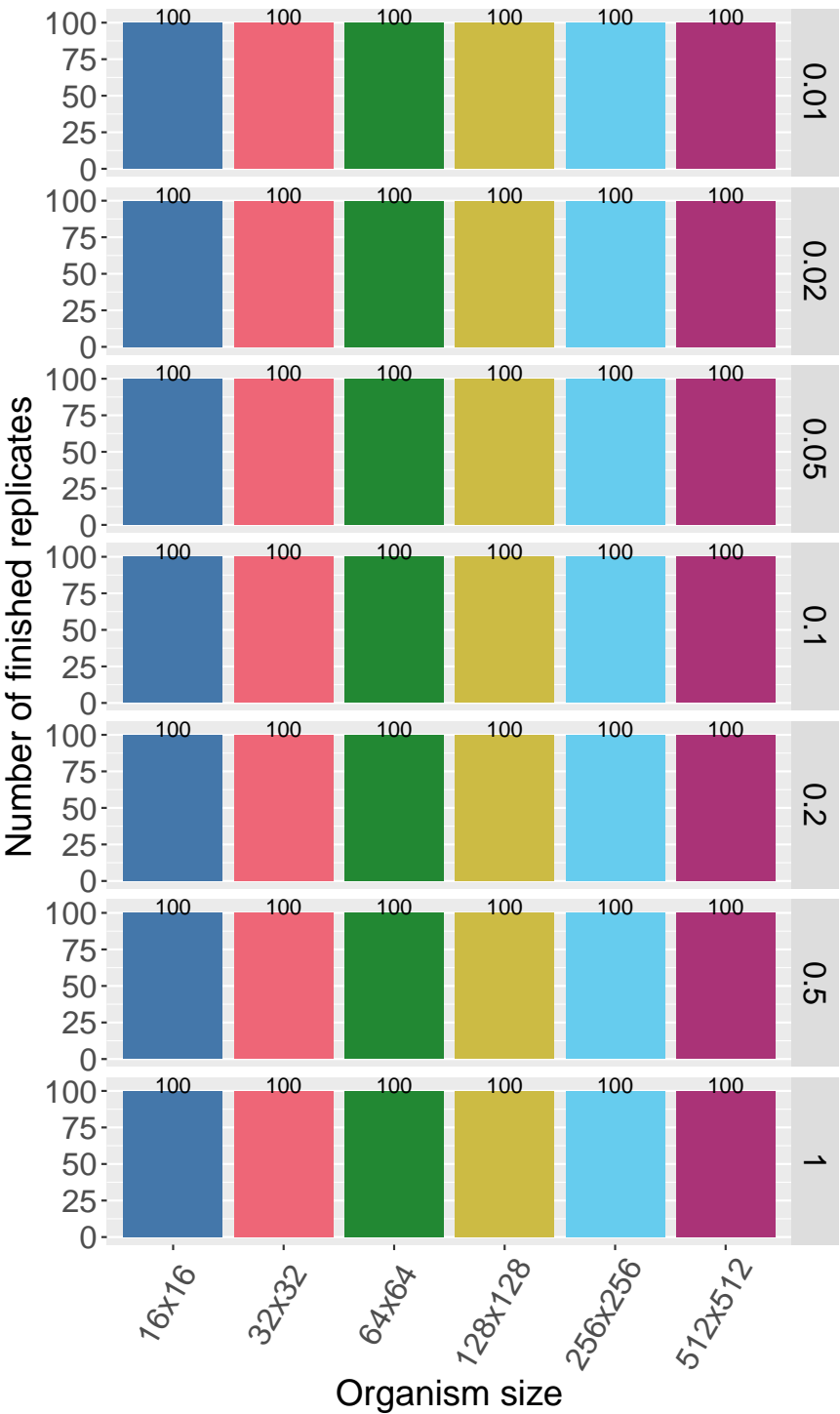
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, MUT)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n())
```

Further cleaning of the data plus adding some variables to make plotting easier.

```
# Calculate restraint value (x - 60 because genome length is 100 here)
df2$restraint_value = df2$ave_ones - 60
# Make a nice, clean factor for size
df2$size_str = paste0(df2$MCSIZE, 'x', df2$MCSIZE)
df2$size_factor = factor(df2$size_str, levels = c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
df2$size_factor_reversed = factor(df2$size_str, levels = rev(c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024')))
df2$germ_mut_str = paste('GERM MUT', df2$MUT)
df2$mut_factor = factor(df2$MUT, levels = c(0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00))
data_summary$size_str = paste0(data_summary$MCSIZE, 'x', data_summary$MCSIZE)
data_summary$size_factor = factor(data_summary$size_str, levels = c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
data_summary$germ_mut_str = paste('GERM MUT', data_summary$MUT)
data_summary$mut_factor = factor(data_summary$MUT, levels = c(0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00))
# Create a map of colors we'll use to plot the different organism sizes
color_vec = as.character(khroma::color('bright')(7))
color_map = c(
  '16x16' = color_vec[1],
  '32x32' = color_vec[2],
  '64x64' = color_vec[3],
  '128x128' = color_vec[4],
  '256x256' = color_vec[5],
  '512x512' = color_vec[6],
  '1024x1024' = color_vec[7]
)
# Set the sizes for text in plots
text_major_size = 18
text_minor_size = 16
boxplot_color = '#9ecae1'
```

3.1 Data integrity check

Now we plot the number of finished replicates for each treatment to make sure all data are present. Each row shows a different germ mutation rate. Each bar/color

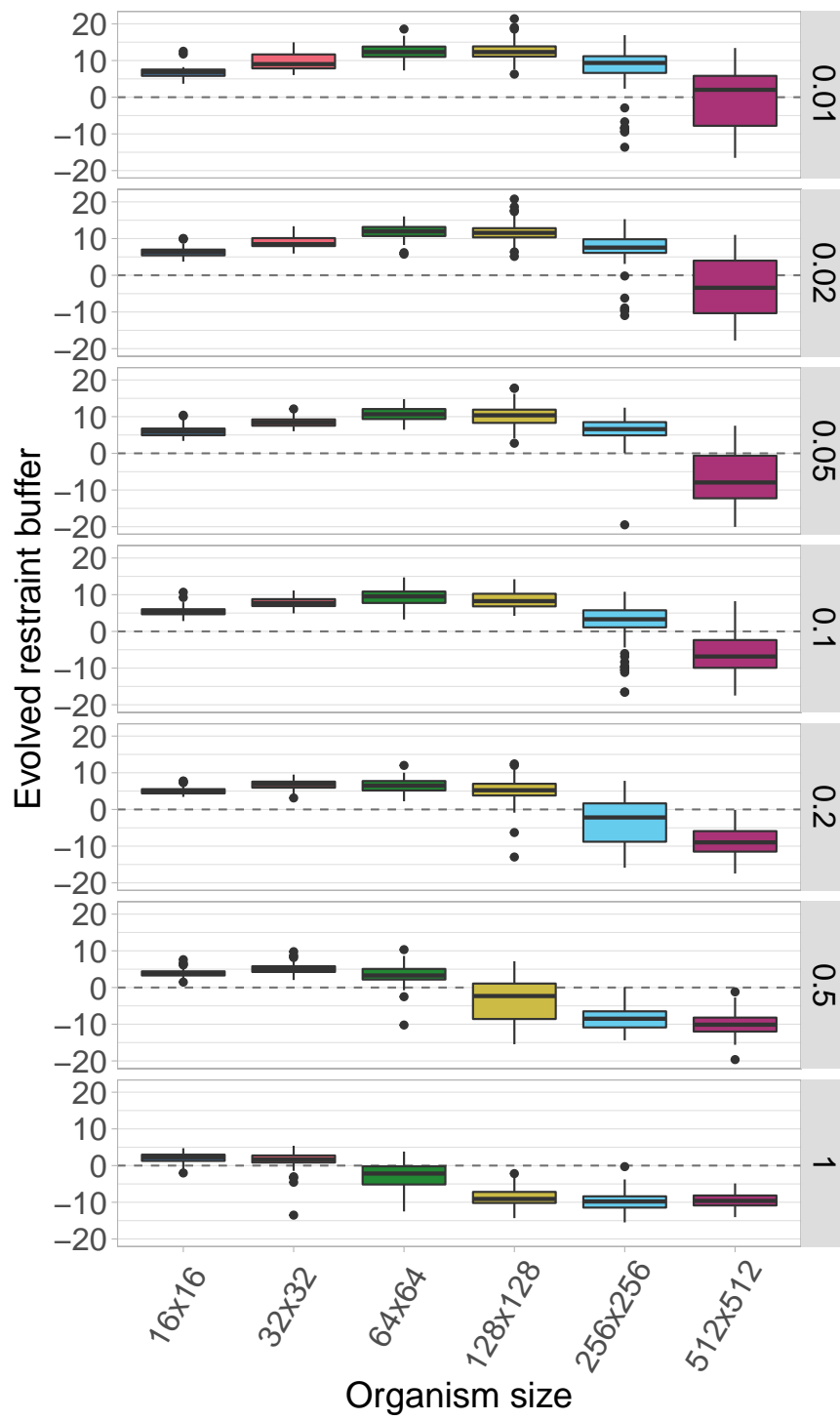


shows a different organism size.

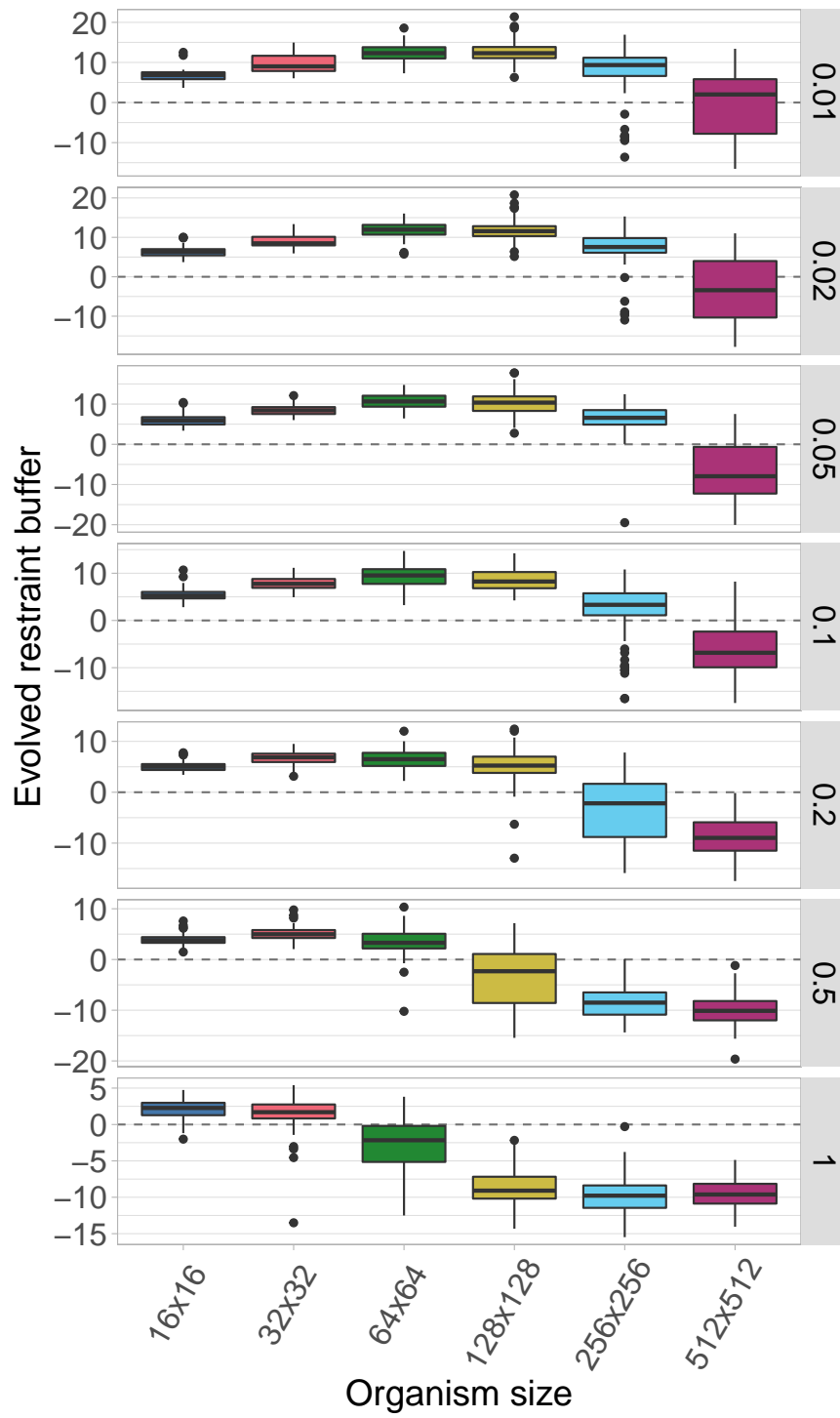
3.2 Aggregate plots

3.2.1 Facet by germ mutation rate

Here we plot all the data at once. Each row shows a different germ mutation rate and each boxplot shows a given organism size.

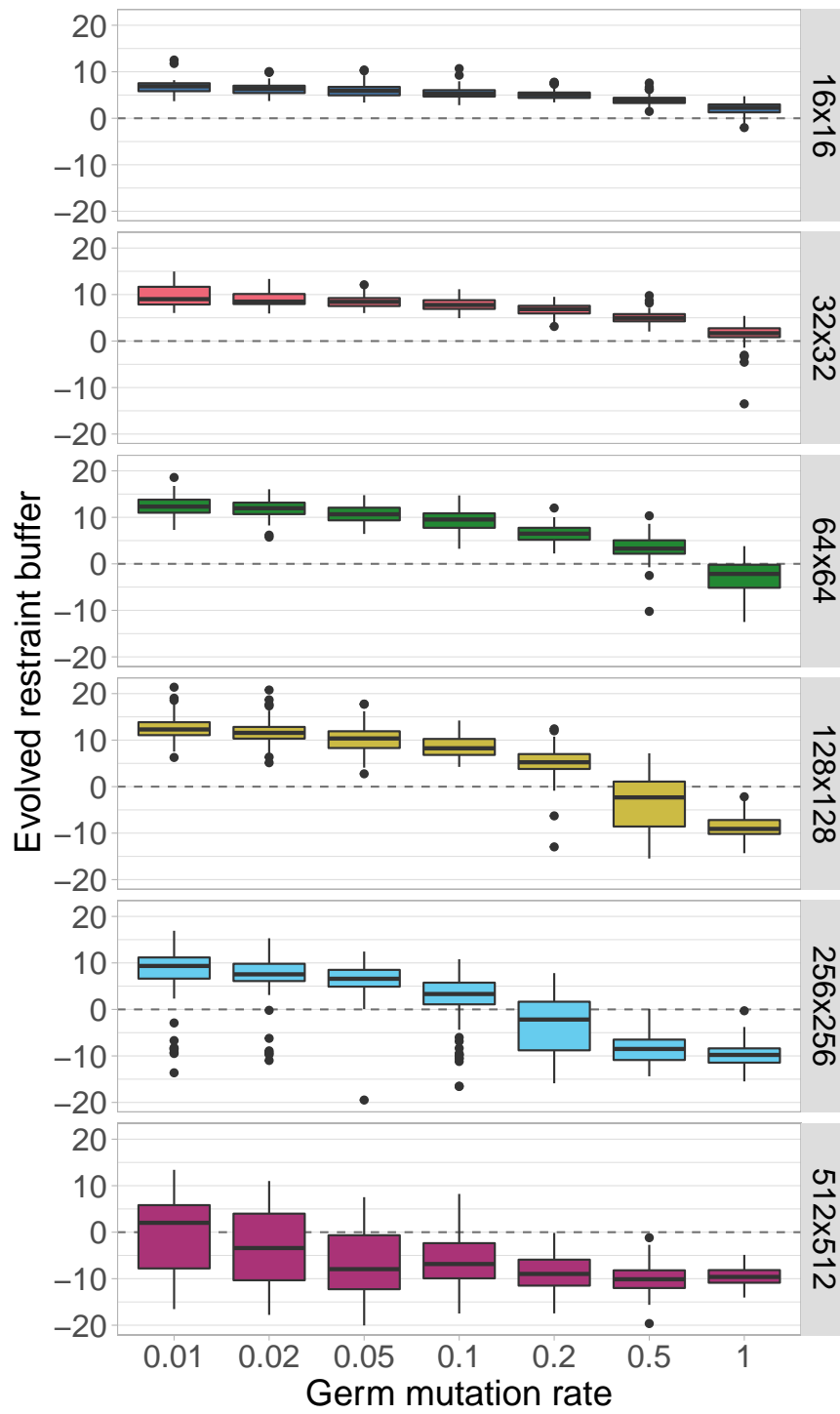


Here is the same data, plotted identically other than now each row can have a different y-axis.

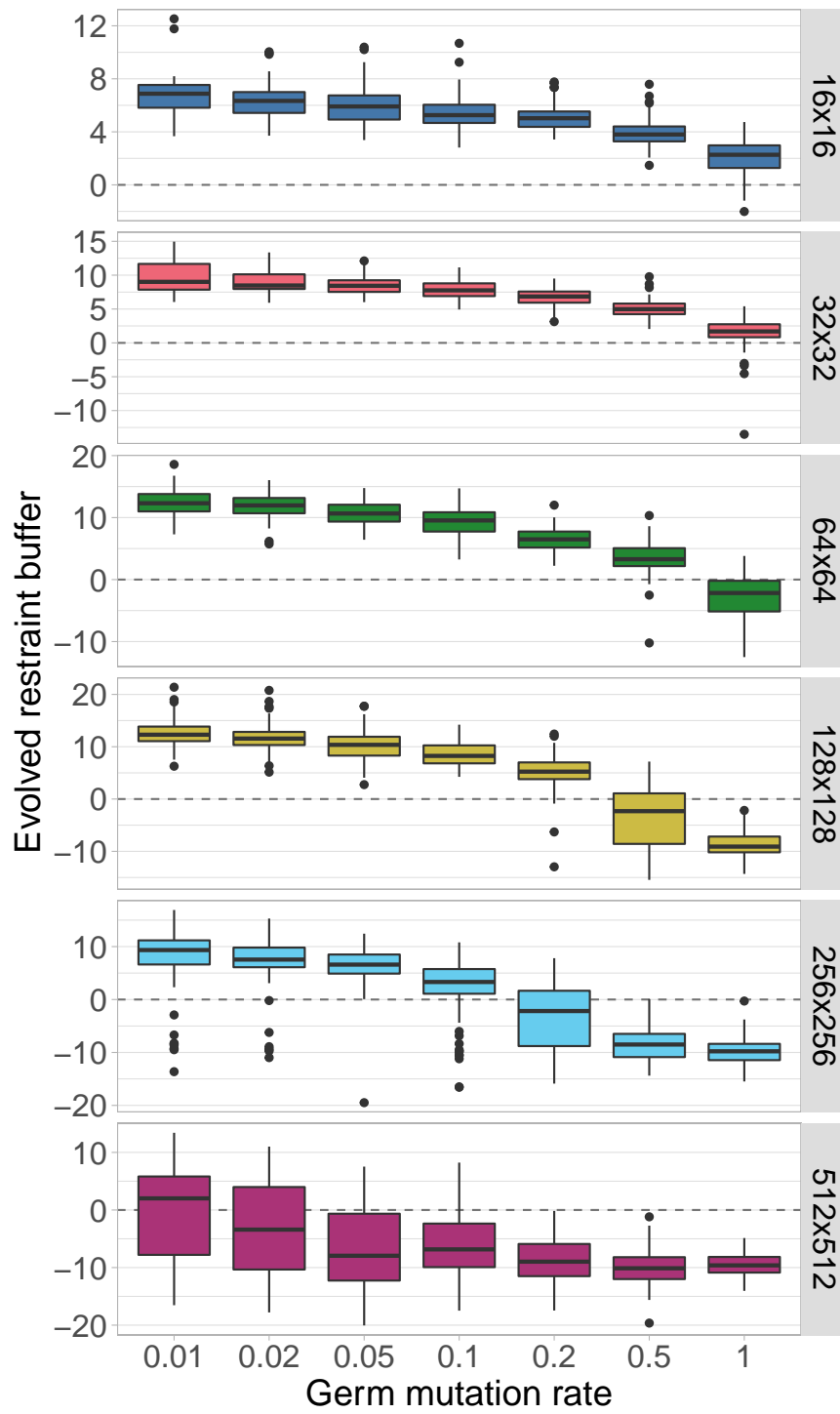


3.2.2 Facet by organism size

Next, we plot the same data, but this time each row corresponds to a certain organism size while germ mutation rate varies along the x-axis.



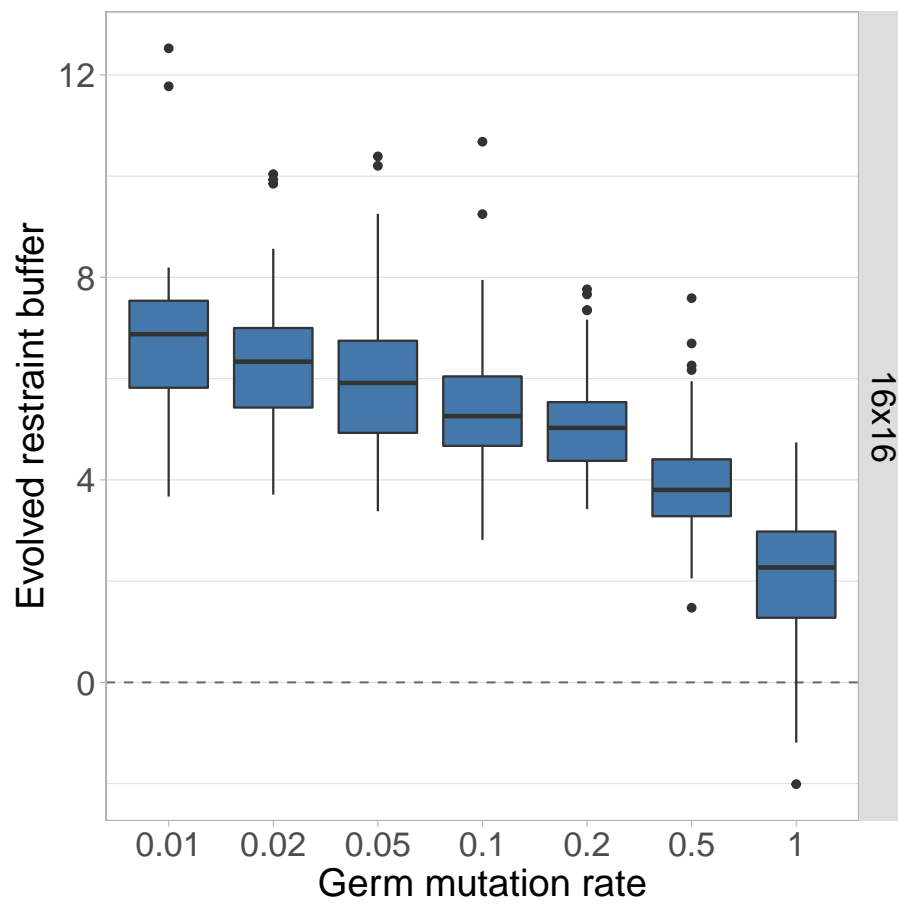
Again, we plot the same data again, but now the y-axis can change between rows.



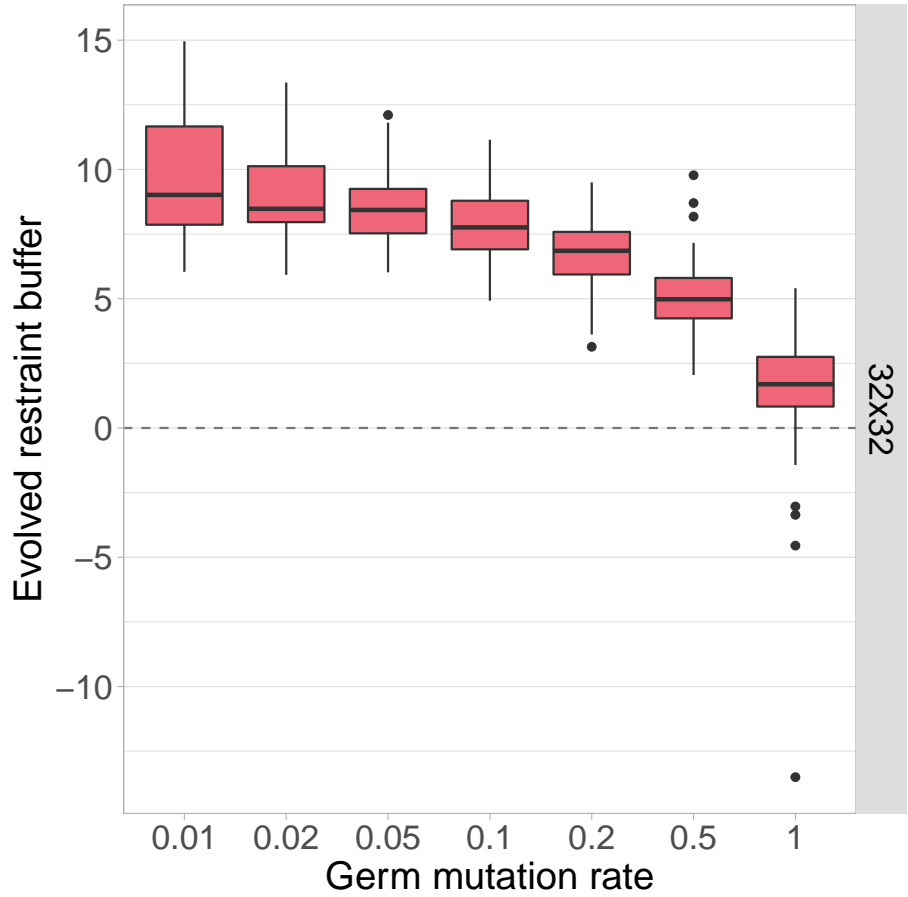
3.3 Single organism size plots

Here we plot each organism size independently, with the germ mutation rate on the x-axis.

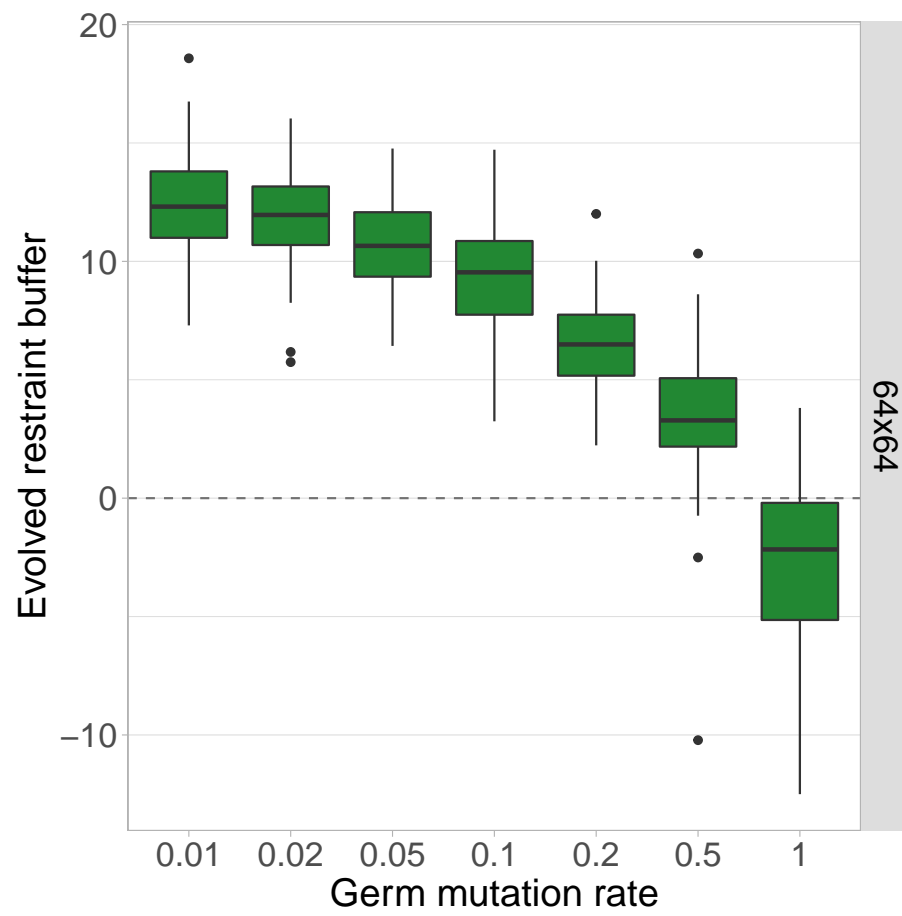
3.3.1 Organism size 16x16



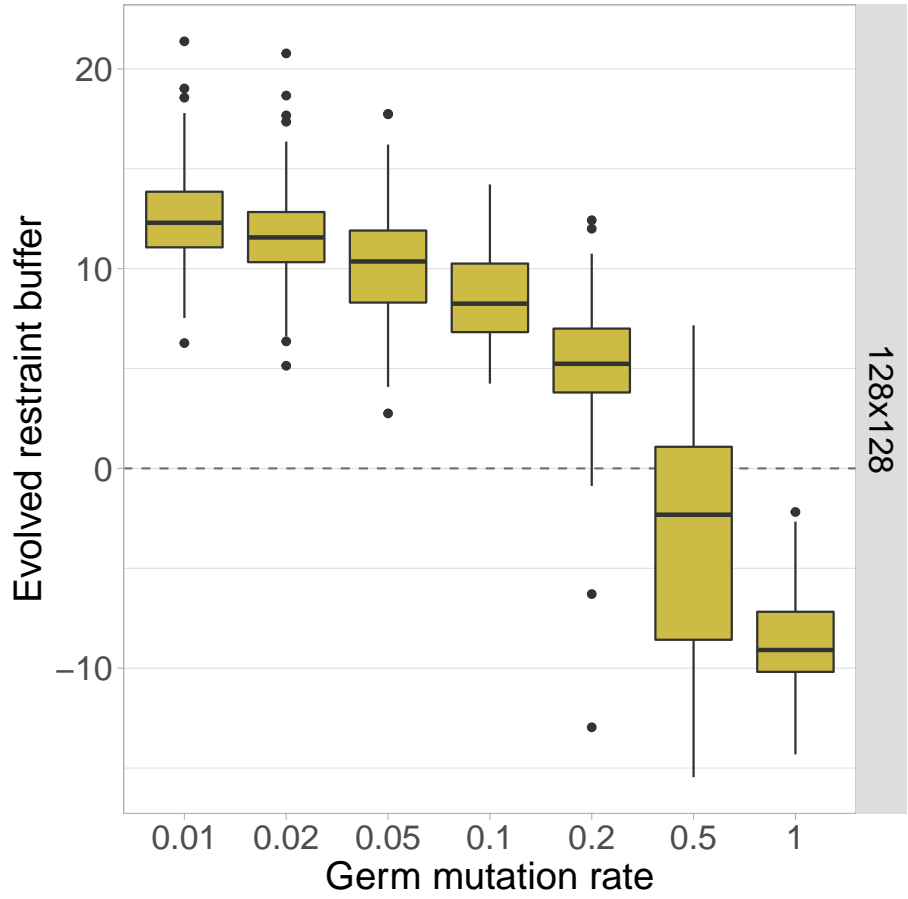
3.3.2 Organism size 32x32



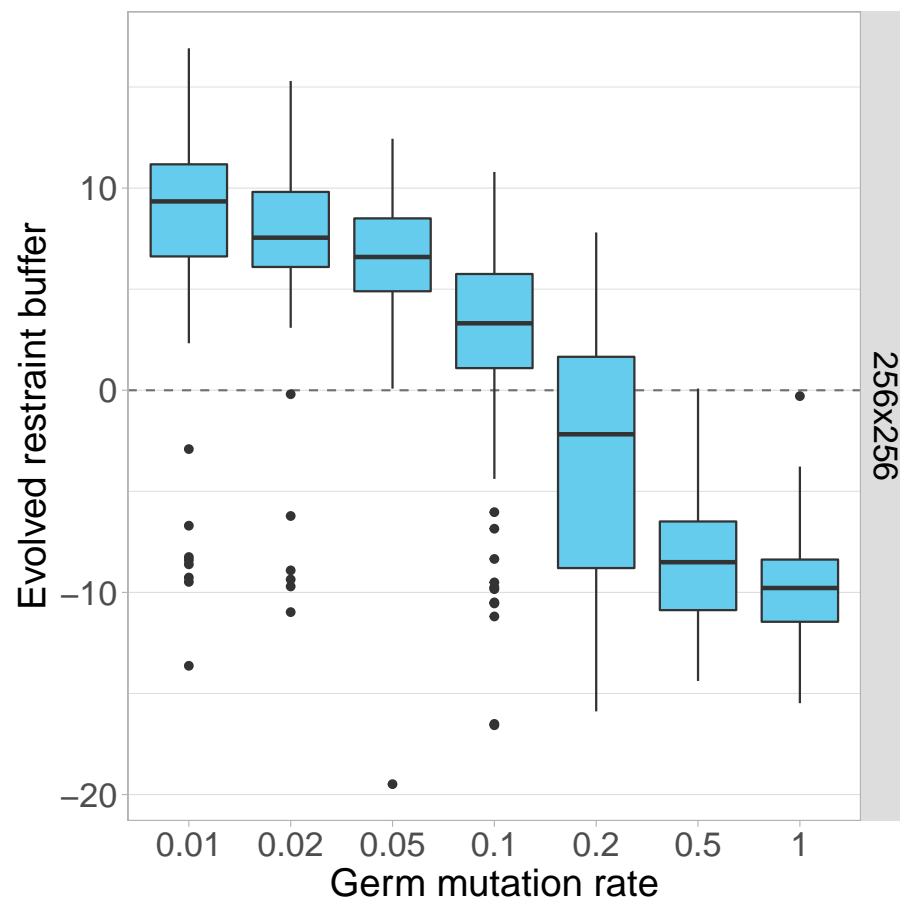
3.3.3 Organism size 64x64



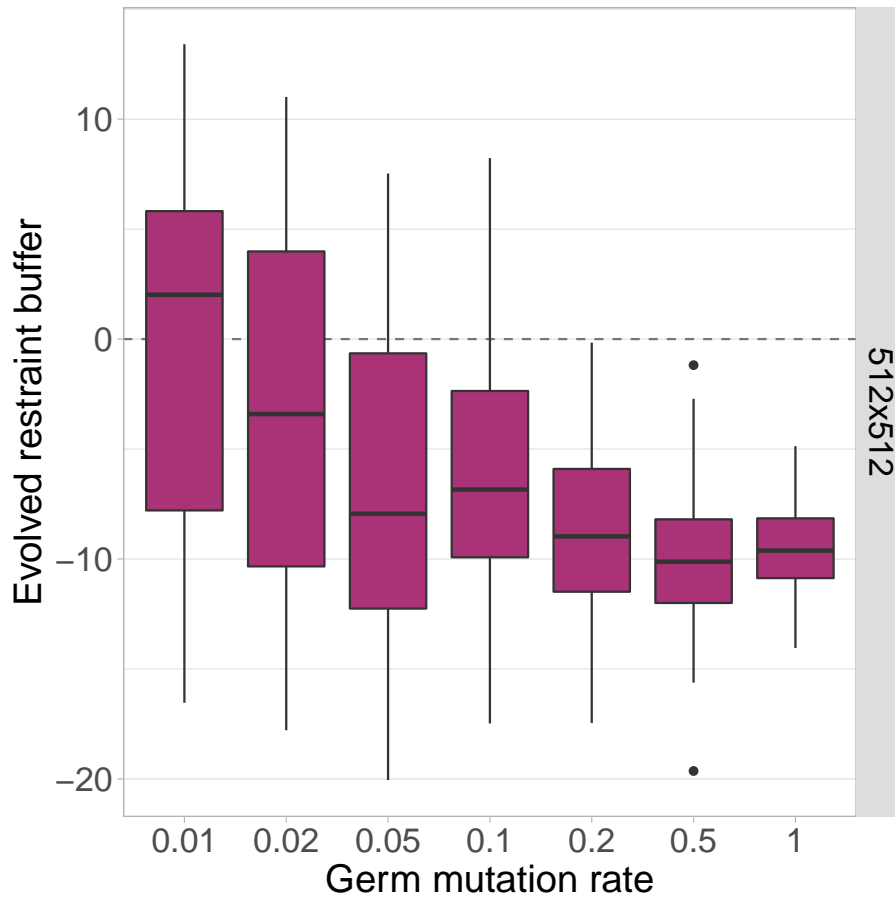
3.3.4 Organism size 128x128



3.3.5 Organism size 256x256



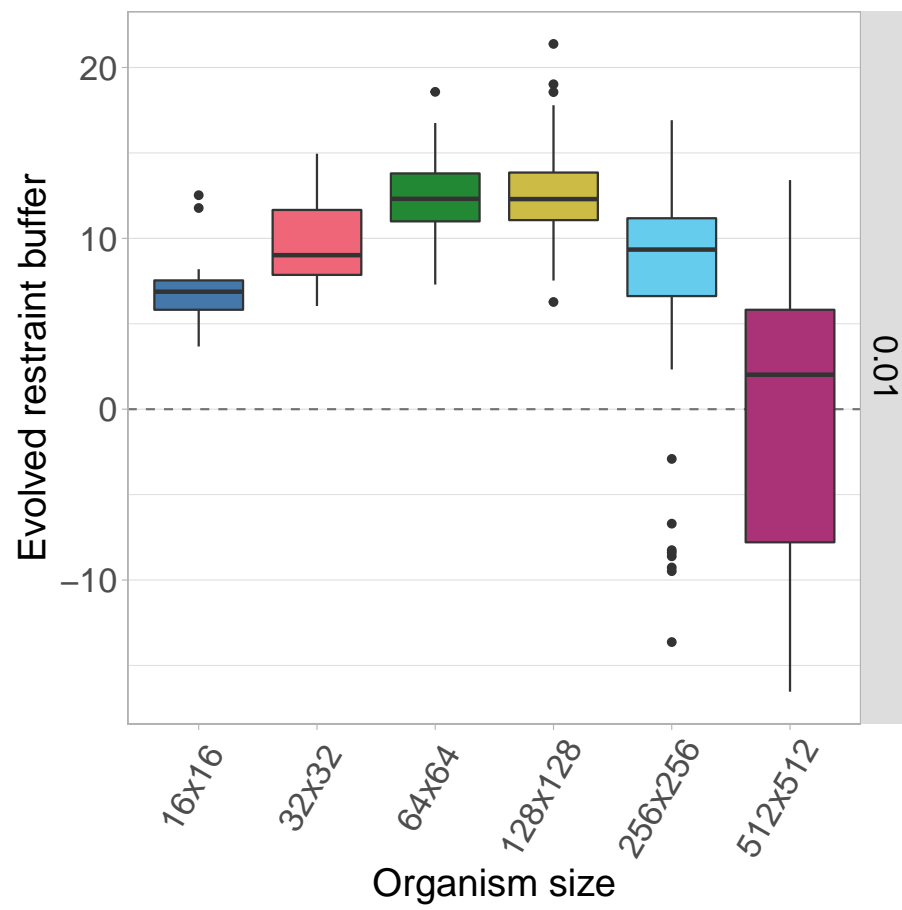
3.3.6 Organism size 512x512



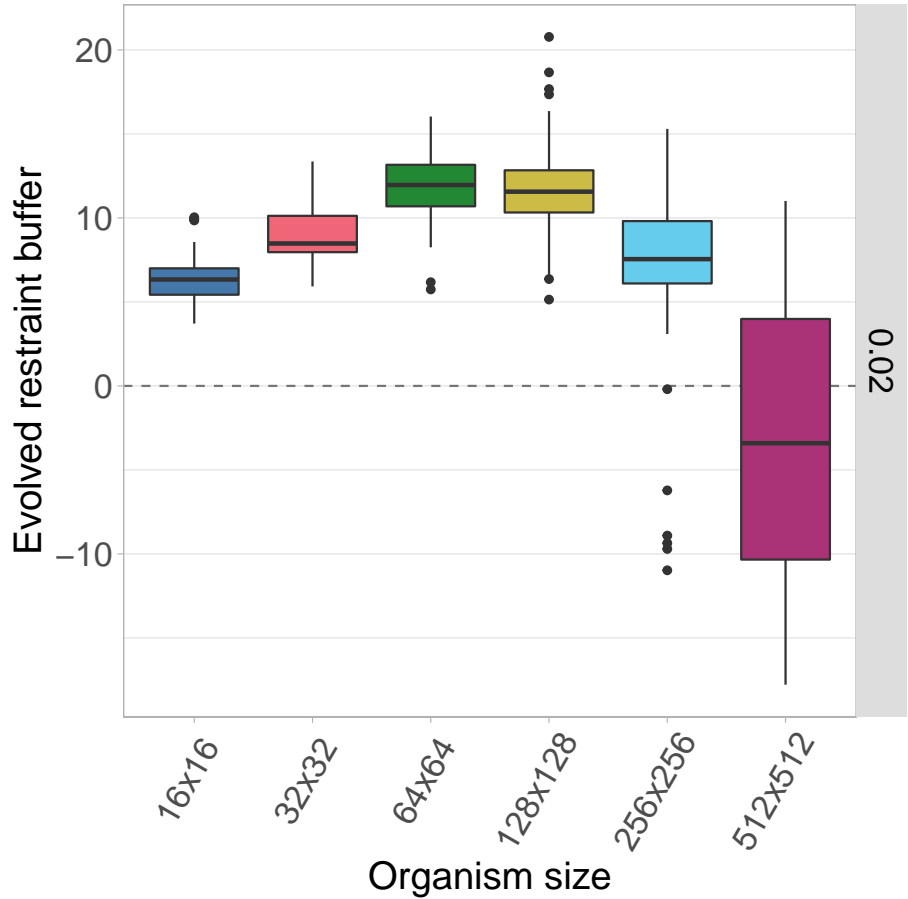
3.4 Single organism size plots

Similarly, here we plot each germ mutation rate independently, with the organism size on the x-axis.

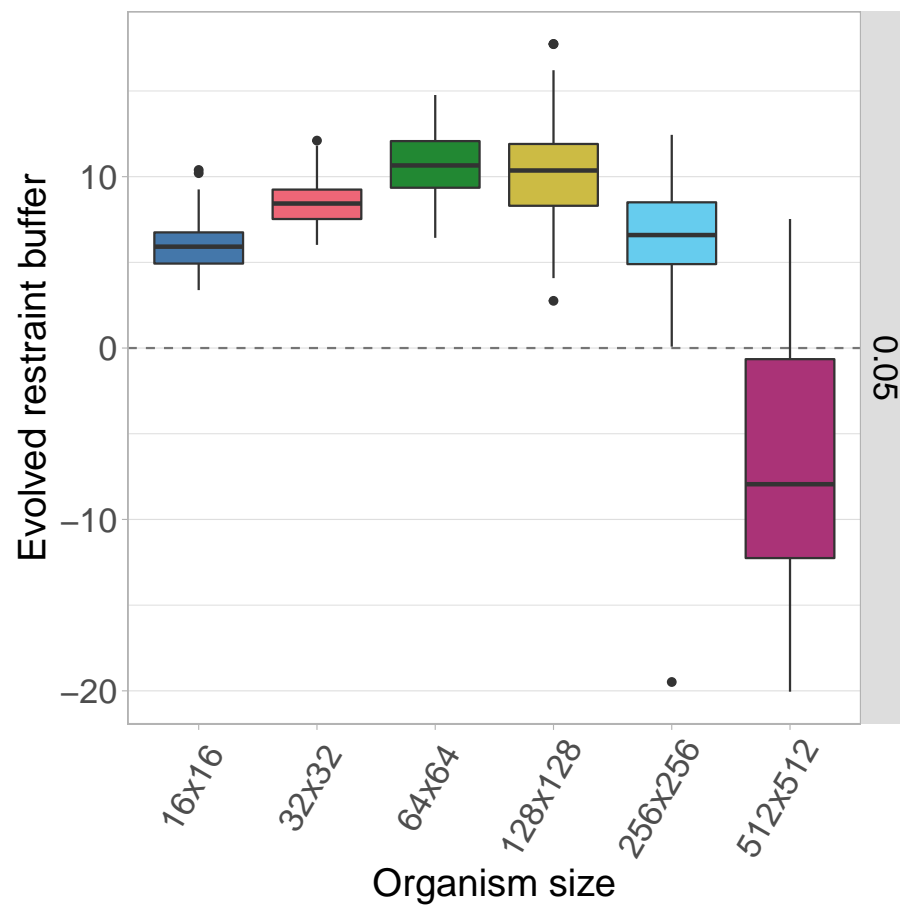
3.4.1 Germ mut. rate 0.01



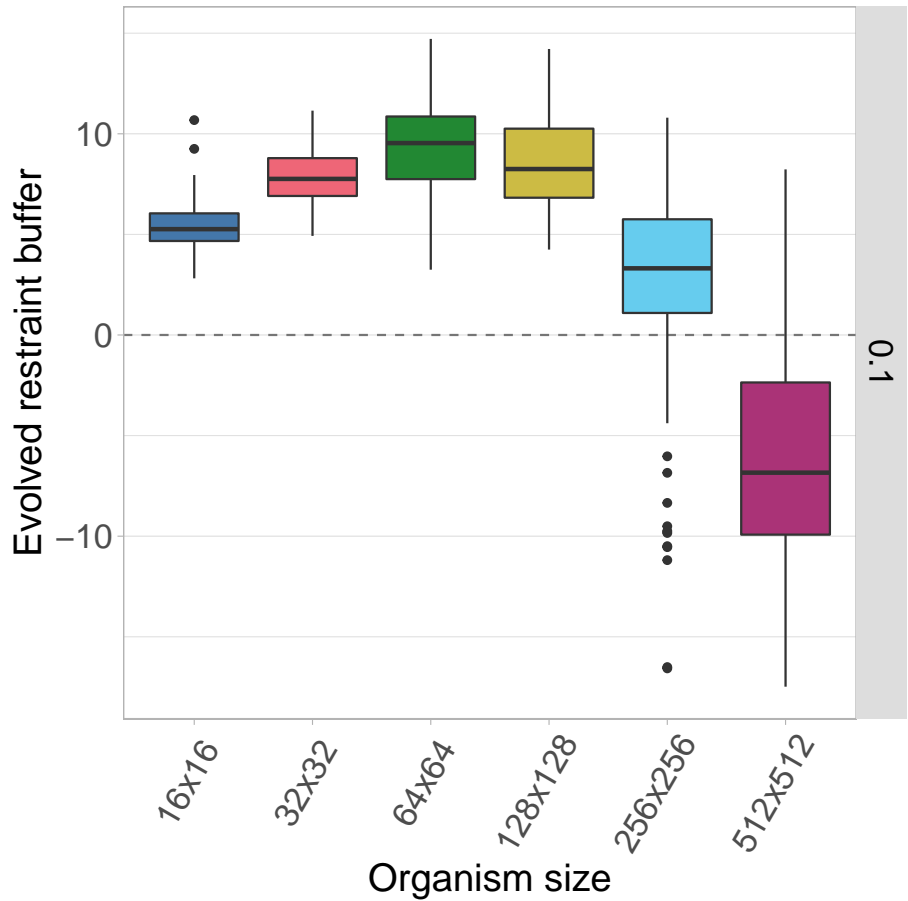
3.4.2 Germ mut. rate 0.02



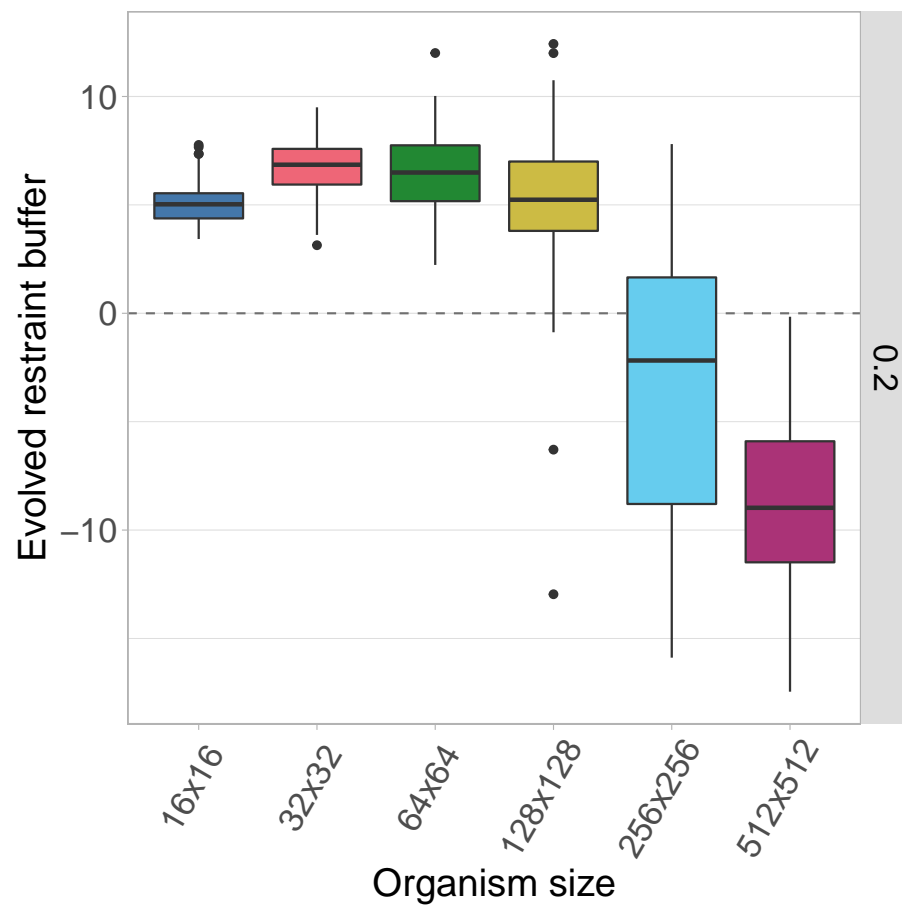
3.4.3 Germ mut. rate 0.05



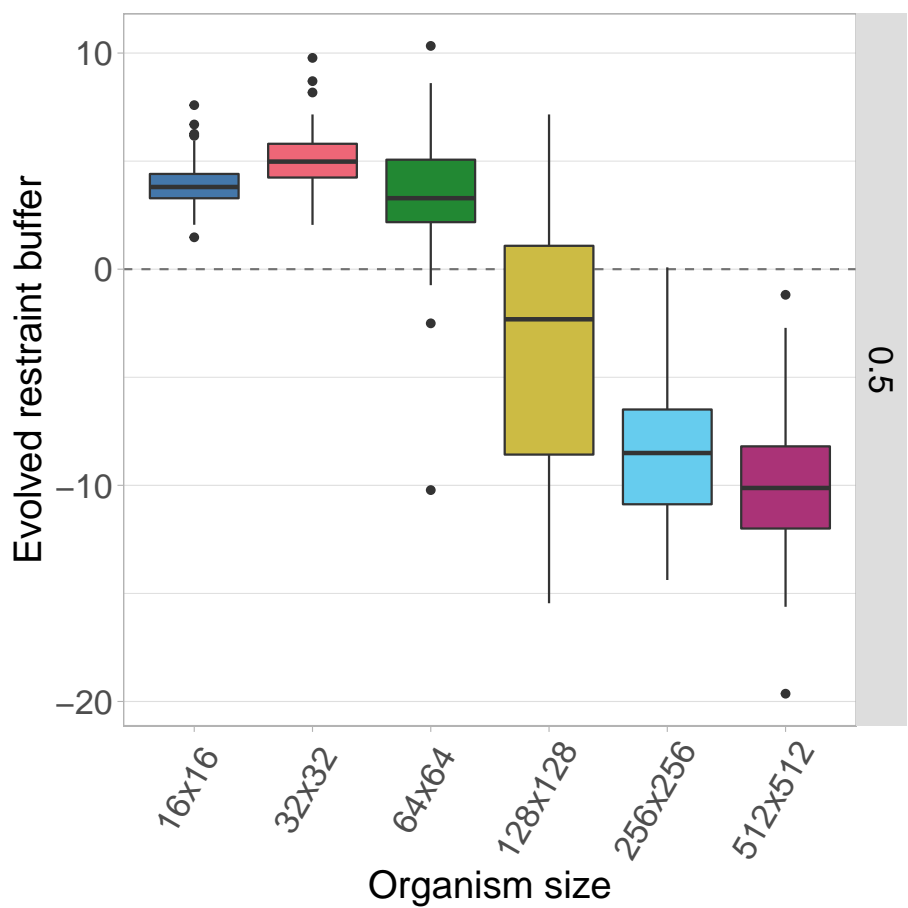
3.4.4 Germ mut. rate 0.1



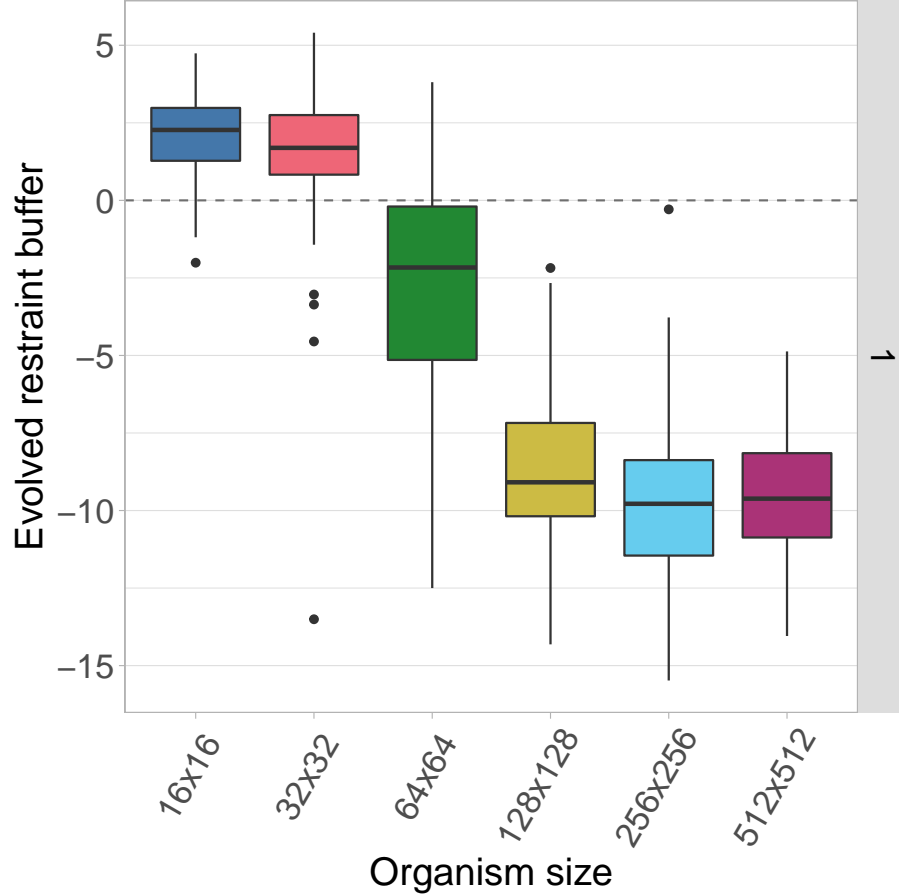
3.4.5 Germ mut. rate 0.2



3.4.6 Germ mut. rate 0.5



3.4.7 Germ mut. rate 1.0



3.5 Statistics

Since organism size is our main point of comparison, we calculate stats for each germ mutation rate.

First, we perform a Kruskal-Wallis test across all organism sizes to indicate if variance exists at that mutation rate. If variance exists, we then perform a pairwise Wilcoxon Rank-Sum test to show which pairs of organism sizes significantly differ. Finally, we perform Bonferroni-Holm corrections for multiple comparisons.

```
mut_vec = c(0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1)
df_kruskal = data.frame(data = matrix(nrow = 0, ncol = 4))
colnames(df_kruskal) = c('germ_mut_rate', 'p_value', 'chi_squared', 'df')
for(mut_rate in mut_vec){
  df_test = df2[df2$MUT == mut_rate,]
```

```

res = kruskal.test(df_test$restraint_value ~ df_test$MCSIZE, df_test)
df_kruskal[nrow(df_kruskal) + 1,] = c(mut_rate, res$p.value, as.numeric(res$statistic)[1], as
}
df_kruskal$less_0.01 = df_kruskal$p_value < 0.01
print(df_kruskal)

```

```

##   germ_mut_rate      p_value chi_squared df less_0.01
## 1          0.01 9.191452e-79    374.5160 5      TRUE
## 2          0.02 6.227269e-82    389.2251 5      TRUE
## 3          0.05 1.934895e-82    391.5809 5      TRUE
## 4          0.10 1.983976e-83    396.1708 5      TRUE
## 5          0.20 3.180895e-85    404.4991 5      TRUE
## 6          0.50 4.313881e-91    431.7152 5      TRUE
## 7          1.00 2.144229e-92    437.7600 5      TRUE

```

We see that significant variation exists within each mutation rate, so we perform pairwise Wilcoxon tests on each to see which pairs of sizes are significantly different.

```

size_vec = c(16, 32, 64, 128, 256, 512)
mut_vec = c(0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1)
for(mut_rate in mut_vec){
  df_test = df2[df2$MUT == mut_rate,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('germ_mut_rate', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw')
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test[df_test$MCSIZE == size_a,]$restraint_value, df_test[df_test$MCSIZE == size_b,]$restraint_value)
      df_wilcox[nrow(df_wilcox) + 1,] = c(mut_rate, size_a, size_b, 0, res$p.value, as.numeric(res$statistic)[1])
    }
  }
  df_wilcox$p_value_corrected = p.adjust(df_wilcox$p_value_raw, method = 'holm')
  df_wilcox$less_0.01 = df_wilcox$p_value_corrected < 0.01
  print(paste0('Germ mutation rate: ', mut_rate))
  print(df_wilcox)
}

```

```

## [1] "Germ mutation rate: 0.01"
##   germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1          0.01    16    32    1.161192e-21 1.161192e-22   990.0      TRUE
## 2          0.01    16    64    1.990837e-31 1.484433e-32   137.0      TRUE
## 3          0.01    16   128    2.032847e-30 1.694039e-31   221.0      TRUE
## 4          0.01    16   256    1.721090e-07 5.736966e-08  2778.5      TRUE
## 5          0.01    16   512    1.237738e-13 2.062896e-14  8130.0      TRUE
## 6          0.01    32    64    4.401194e-15 5.501492e-16  1684.5      TRUE

```

```

## 7      0.01      32      128      1.423615e-13 2.847230e-14 1887.0      TRUE
## 8      0.01      32      256      2.438849e-01 1.219425e-01 5633.5     FALSE
## 9      0.01      32      512      5.140604e-27 4.673276e-28 9495.0     TRUE
## 10     0.01      64      128      9.221418e-01 9.221418e-01 5040.5     FALSE
## 11     0.01      64      256      4.110744e-14 5.872491e-15 8195.5     TRUE
## 12     0.01      64      512      6.122051e-32 4.081368e-33 9907.0     TRUE
## 13     0.01     128      256      5.020912e-13 1.255228e-13 8033.5     TRUE
## 14     0.01     128      512      1.990837e-31 1.422026e-32 9864.5     TRUE
## 15     0.01     256      512      1.882508e-17 2.091675e-18 8582.5     TRUE
## [1] "Germ mutation rate: 0.02"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.02      16      32      5.385908e-24 5.385908e-25  773.5      TRUE
## 2      0.02      16      64      3.620092e-31 2.585780e-32  156.0      TRUE
## 3      0.02      16     128      5.876058e-29 4.896715e-30   339.5      TRUE
## 4      0.02      16     256      7.355430e-06 2.451810e-06 3071.0      TRUE
## 5      0.02      16     512      2.935849e-18 3.669812e-19 8662.0      TRUE
## 6      0.02      32      64      5.800574e-18 8.286535e-19 1375.0      TRUE
## 7      0.02      32     128      4.715120e-12 1.178780e-12 2090.5      TRUE
## 8      0.02      32     256      4.080762e-04 2.040381e-04 6520.5      TRUE
## 9      0.02      32     512      6.645814e-27 6.041649e-28 9485.5      TRUE
## 10     0.02      64     128      2.889472e-01 2.889472e-01 5434.5     FALSE
## 11     0.02      64     256      2.039804e-17 3.399674e-18 8560.0      TRUE
## 12     0.02      64     512      4.109271e-32 2.739514e-33 9920.5      TRUE
## 13     0.02     128     256      2.514342e-14 5.028683e-15 8203.5      TRUE
## 14     0.02     128     512      4.123142e-31 3.171647e-32 9837.0      TRUE
## 15     0.02     256     512      4.866893e-20 5.407659e-21 8848.0      TRUE
## [1] "Germ mutation rate: 0.05"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.05      16      32      1.591362e-24 1.768180e-25  730.0      TRUE
## 2      0.05      16      64      1.063762e-30 8.864684e-32   198.5      TRUE
## 3      0.05      16     128      3.321119e-21 4.151399e-22 1043.0      TRUE
## 4      0.05      16     256      2.538532e-02 1.269266e-02 3979.5     FALSE
## 5      0.05      16     512      1.050337e-26 9.548517e-28 9468.5      TRUE
## 6      0.05      32      64      3.387540e-14 5.645899e-15 1802.5      TRUE
## 7      0.05      32     128      1.306936e-05 4.356453e-06 3119.5      TRUE
## 8      0.05      32     256      1.528740e-07 3.821850e-08 7251.0      TRUE
## 9      0.05      32     512      3.162116e-32 2.258654e-33 9927.0      TRUE
## 10     0.05      64     128      1.546546e-01 1.546546e-01 5583.0     FALSE
## 11     0.05      64     256      4.390965e-19 6.272808e-20 8741.0      TRUE
## 12     0.05      64     512      6.208612e-33 4.139074e-34 9984.0      TRUE
## 13     0.05     128     256      2.838701e-12 5.677403e-13 7950.5      TRUE
## 14     0.05     128     512      9.845016e-32 7.573090e-33 9886.0      TRUE
## 15     0.05     256     512      3.142822e-26 3.142822e-27 9424.0      TRUE
## [1] "Germ mutation rate: 0.1"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.1       16      32      2.006447e-24 2.229385e-25  739.0      TRUE

```

```

## 2      0.1      16      64      2.197505e-25 1.997732e-26 646.0      TRUE
## 3      0.1      16     128      3.982057e-19 6.636762e-20 1261.5     TRUE
## 4      0.1      16     256      2.853915e-06 9.513050e-07 7006.5     TRUE
## 5      0.1      16     512      1.146029e-26 9.550238e-28 9468.5     TRUE
## 6      0.1      32      64      6.866683e-07 1.716671e-07 2860.0     TRUE
## 7      0.1      32     128      5.714627e-02 5.714627e-02 4221.0     FALSE
## 8      0.1      32     256      7.451552e-21 9.314440e-22 8923.0     TRUE
## 9      0.1      32     512      1.091653e-31 7.797522e-33 9885.0     TRUE
## 10     0.1      64     128      2.618271e-02 1.309135e-02 6016.0     FALSE
## 11     0.1      64     256      6.893655e-25 6.893655e-26 9306.5     TRUE
## 12     0.1      64     512      4.295636e-32 2.863757e-33 9919.0     TRUE
## 13     0.1     128     256      9.294756e-21 1.327822e-21 8908.0     TRUE
## 14     0.1     128     512      2.475810e-31 1.904469e-32 9854.5     TRUE
## 15     0.1     256     512      7.440793e-16 1.488159e-16 8380.0     TRUE
## [1] "Germ mutation rate: 0.2"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.2      16      32      6.711164e-17 9.587377e-18 1488.5     TRUE
## 2      0.2      16      64      2.652853e-08 5.305706e-09 2610.5     TRUE
## 3      0.2      16     128      5.723537e-01 4.033561e-01 4657.5     FALSE
## 4      0.2      16     256      9.414689e-28 1.046077e-28 9550.0     TRUE
## 5      0.2      16     512      3.841700e-33 2.561134e-34 10000.0    TRUE
## 6      0.2      32      64      5.723537e-01 2.861769e-01 5437.0     FALSE
## 7      0.2      32     128      2.713788e-06 6.784470e-07 7033.5     TRUE
## 8      0.2      32     256      2.557355e-30 2.324869e-31 9768.0     TRUE
## 9      0.2      32     512      3.841700e-33 2.561422e-34 10000.0    TRUE
## 10     0.2      64     128      8.967634e-04 2.989211e-04 6480.5     TRUE
## 11     0.2      64     256      3.597156e-29 3.597156e-30 9671.5     TRUE
## 12     0.2      64     512      3.841700e-33 2.561422e-34 10000.0    TRUE
## 13     0.2     128     256      3.256203e-24 4.070254e-25 9237.5     TRUE
## 14     0.2     128     512      1.052551e-31 8.771259e-33 9881.0     TRUE
## 15     0.2     256     512      1.558734e-09 2.597889e-10 7587.5     TRUE
## [1] "Germ mutation rate: 0.5"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      0.5      16      32      3.627488e-11 7.254975e-12 2195.0     TRUE
## 2      0.5      16      64      1.774145e-01 1.774145e-01 5552.5     FALSE
## 3      0.5      16     128      9.003159e-21 1.125395e-21 8915.0     TRUE
## 4      0.5      16     256      3.840402e-33 2.560268e-34 10000.0    TRUE
## 5      0.5      16     512      3.840402e-33 2.560412e-34 10000.0    TRUE
## 6      0.5      32      64      1.574642e-07 5.248808e-08 7228.0     TRUE
## 7      0.5      32     128      3.547680e-25 3.941867e-26 9328.0     TRUE
## 8      0.5      32     256      3.840402e-33 2.560701e-34 10000.0    TRUE
## 9      0.5      32     512      3.840402e-33 2.560845e-34 10000.0    TRUE
## 10     0.5      64     128      4.292292e-17 6.131846e-18 8532.5     TRUE
## 11     0.5      64     256      3.128938e-32 3.128938e-33 9916.0     TRUE
## 12     0.5      64     512      1.333109e-32 1.211917e-33 9948.0     TRUE
## 13     0.5     128     256      2.868826e-09 7.172065e-10 7522.5     TRUE

```

```

## 14      0.5    128    512      9.393819e-14 1.565636e-14 8144.5      TRUE
## 15      0.5    256    512      3.381624e-03 1.690812e-03 6285.5      TRUE
## [1] "Germ mutation rate: 1"
##      germ_mut_rate size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      1         16     32      1.080497e-01 5.402483e-02 5789.0      FALSE
## 2      1         16     64      2.560330e-24 2.844811e-25 9251.5      TRUE
## 3      1         16    128      3.840402e-33 2.560268e-34 10000.0     TRUE
## 4      1         16    256      3.840402e-33 2.887894e-34 9996.0      TRUE
## 5      1         16    512      3.840402e-33 2.560412e-34 10000.0     TRUE
## 6      1         32     64      1.004804e-19 1.674674e-20 8799.0      TRUE
## 7      1         32    128      8.265949e-32 8.265949e-33 9883.0      TRUE
## 8      1         32    256      7.190219e-32 6.536563e-33 9891.0      TRUE
## 9      1         32    512      5.842919e-32 4.869099e-33 9901.0      TRUE
## 10     1         64    128      1.007238e-18 2.014476e-19 8689.0      TRUE
## 11     1         64    256      7.963405e-23 1.137629e-23 9105.0      TRUE
## 12     1         64    512      5.680932e-23 7.101164e-24 9124.0      TRUE
## 13     1        128    256      1.357430e-02 3.393576e-03 6199.5     FALSE
## 14     1        128    512      3.704384e-02 1.234795e-02 6024.5     FALSE
## 15     1        256    512      4.892624e-01 4.892624e-01 4716.5     FALSE

```

Chapter 4

Genome Length Sweep

TBD

Chapter 5

Size 1024x1024 organisms

TBD

Chapter 6

Test of timing sample counts

TBD

Chapter 7

Interactive web app

TBD