

The Evolution of Cellular Restraint in Multicellular Organisms

Katherine G. Skocelas, Austin J. Ferguson, Clifford Bohm, Katherine Perry, Rosemary Adaji, Ch

2021-03-18

Contents

1	Introduction	5
1.1	Overview	5
1.2	Interactive web app	5
2	Baseline: Varying organism size	7
2.1	Data cleaning	7
2.2	Data integrity check	8
2.3	Aggregate plots	9
2.4	Statistics	12
3	Somatic Mutation Rate Sweep	15
3.1	Data cleaning	15
3.2	Data integrity check	16
3.3	Aggregate plots	18
3.4	Single organism size plots	26
3.5	Single somatic mutation rate plots	31
3.6	Statistics	38
4	Germ Mutation Rate Sweep	43
4.1	Data integrity check	44
4.2	Aggregate plots	46
4.3	Single organism size plots	54
4.4	Single organism size plots	59
4.5	Statistics	66
5	Genome Length Sweep	71
5.1	Data cleaning	71
5.2	Data integrity check	72
5.3	Aggregate plots	74
5.4	Single organism size plots	82
5.5	Single genome length plots	87
5.6	Statistics	92
6	Timing sample count experiment	97

6.1	Data cleaning	97
6.2	Data integrity check	98
6.3	Plot	100
6.4	Statistics	100

Chapter 1

Introduction

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

1.1 Overview

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). As such, most sections follow the same formula: setup, plots, and then statistics.

1.2 Interactive web app

To build intuition on how organisms fill, we created an interactive web app. The web app is available here: <http://fergusonaj.com/webapps/primordium/>

Chapter 2

Baseline: Varying organism size

Here we show all the data for the baseline experiment, where we vary organism size but otherwise all parameters are set to the defaults.

For this experiment (with all default parameters), we also ran size 8x8 and 1024x1024 organisms. In the paper, however, we only included sizes from 16x16 to 512x512. Size 8x8 organisms are quick to run, but these smaller organisms see the most noise in the fitness data. Conversely, size 1024x1024 organisms take so long to run that it was impractical to run them for each experiment.

Here, we show these results for the baseline experiment, including this additional sizes. The configuration script and data for the experiment can be found under 2021_02_26__org_sizes/ in the experiments directory of the git repository.

2.1 Data cleaning

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_26__org_sizes/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,]
```

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

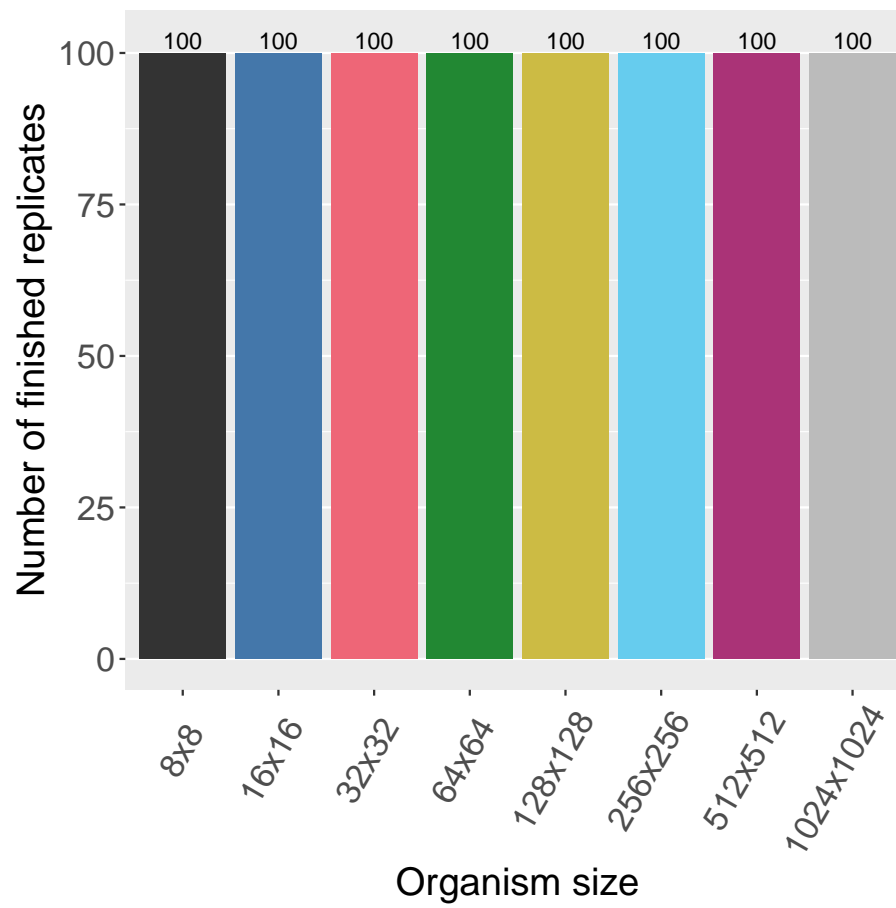
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n())
```

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$size_str, levels = c('8x8', '16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
df2$size_factor_reversed = factor(df2$size_str, levels = rev(c('8x8', '16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024')))
data_summary$size_str = paste0(data_summary$MCSIZE, 'x', data_summary$MCSIZE)
data_summary$size_factor = factor(data_summary$size_str, levels = c('8x8', '16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
# 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(
  '8x8' = '#333333',
  '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

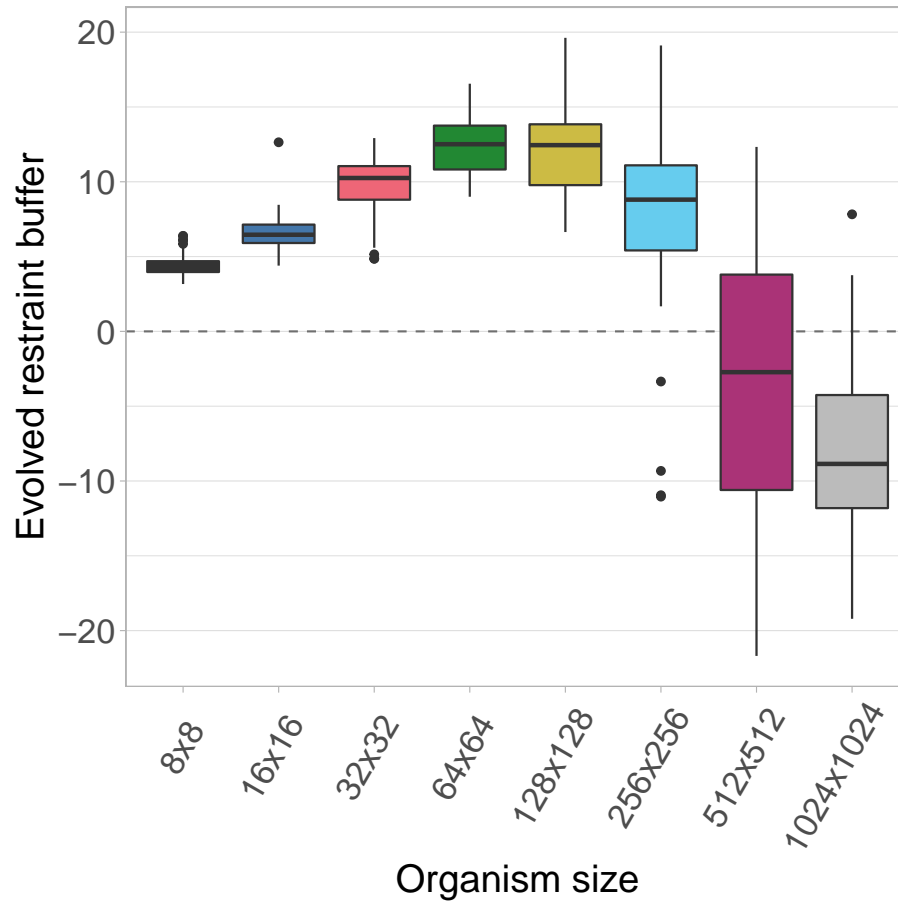
Now we plot the number of finished replicates for each treatment to make sure all data are present. Each bar/color shows a different organism size.



2.3 Aggregate plots

Here we plot all the data at once.

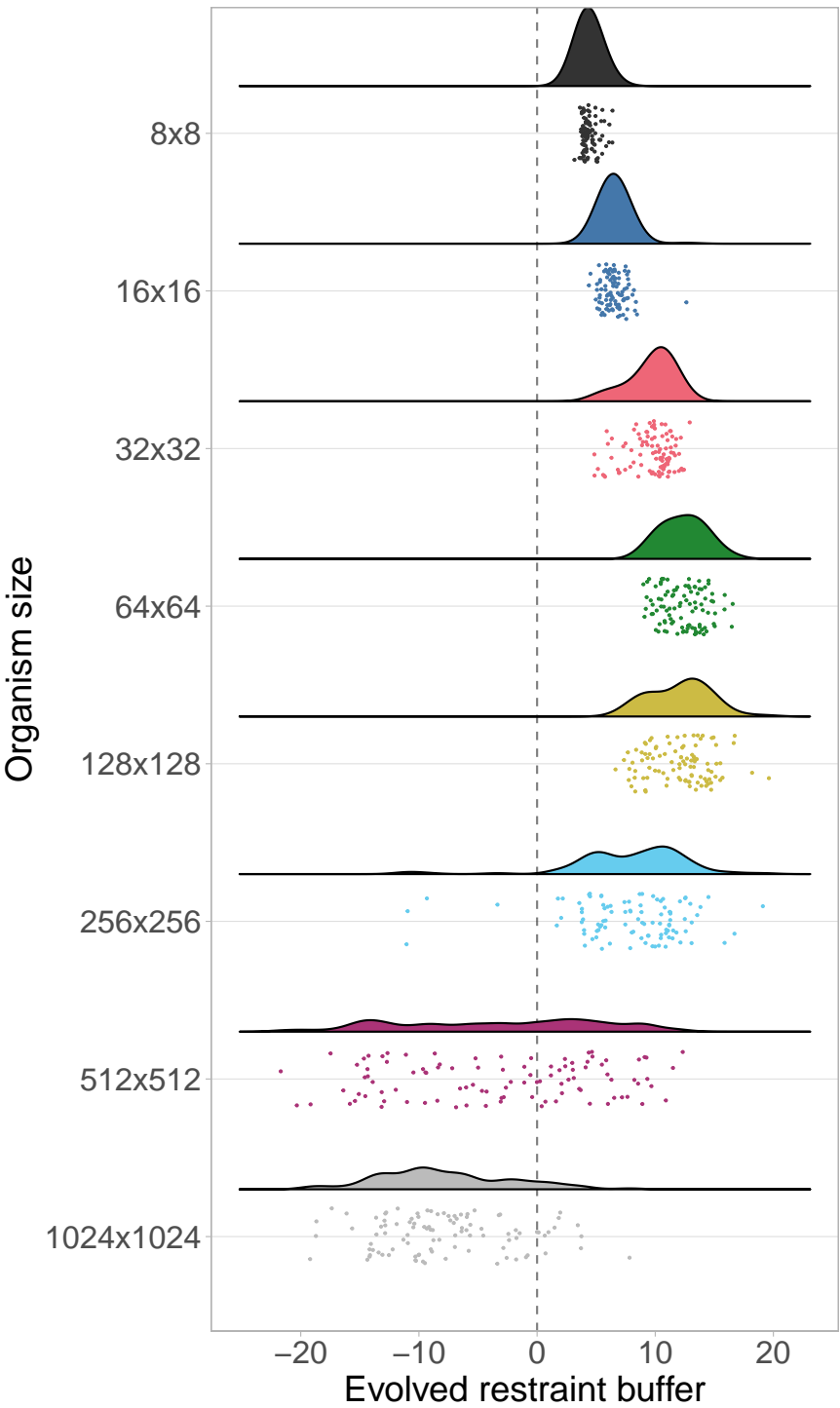
2.3.1 Boxplots



2.3.2 Raincloud plots

We can plot the same data via raincloud plots.

```
## Picking joint bandwidth of 1.16
```



2.4 Statistics

First, we perform a Kruskal-Wallis test across all organism sizes to indicate if variance exists. 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.

```
res = kruskal.test(df2$restraint_value ~ df2$MCSIZE, df2)
df_kruskal = data.frame(data = matrix(nrow = 0, ncol = 3))
colnames(df_kruskal) = c('p_value', 'chi_squared', 'df')
df_kruskal[nrow(df_kruskal) + 1,] = c(res$p.value, as.numeric(res$statistic)[1], as.numeric(res$df))
df_kruskal$less_0.01 = df_kruskal$p_value < 0.01
print(df_kruskal)
```

```
##           p_value chi_squared df less_0.01
## 1 1.506351e-127    610.2553  7      TRUE
```

We see that significant variation exists, 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)
df_test = df2
df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 5))
colnames(df_wilcox) = c('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(size_a, size_b, 0, res$p.value, as.numeric(res$statistic))
  }
}
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(df_wilcox)
```

```
##    size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      16     32  4.406735e-21 4.406735e-22 1045.5      TRUE
## 2      16     64  1.790650e-32 1.193767e-33   51.5      TRUE
## 3      16    128  2.585339e-31 1.988723e-32  147.0      TRUE
## 4      16    256  1.864978e-03 6.216595e-04 3599.0      TRUE
## 5      16    512  3.596138e-17 4.495172e-18 8547.0      TRUE
## 6      32     64  2.103060e-15 3.004372e-16 1654.5      TRUE
## 7      32    128  1.857809e-09 4.644523e-10 2449.5      TRUE
## 8      32    256  8.472946e-03 4.236473e-03 6171.0      TRUE
## 9      32    512  1.338207e-26 1.216552e-27 9459.5      TRUE
## 10     64    128  4.429461e-01 4.429461e-01 5314.5     FALSE
## 11     64    256  2.515682e-15 4.192803e-16 8329.0      TRUE
```

##	12	64	512	1.552625e-31	1.109018e-32	9873.0	TRUE
##	13	128	256	4.763656e-12	9.527311e-13	7921.5	TRUE
##	14	128	512	3.610598e-30	3.008832e-31	9759.0	TRUE
##	15	256	512	7.155324e-19	7.950361e-20	8730.5	TRUE

Chapter 3

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.

3.1 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.

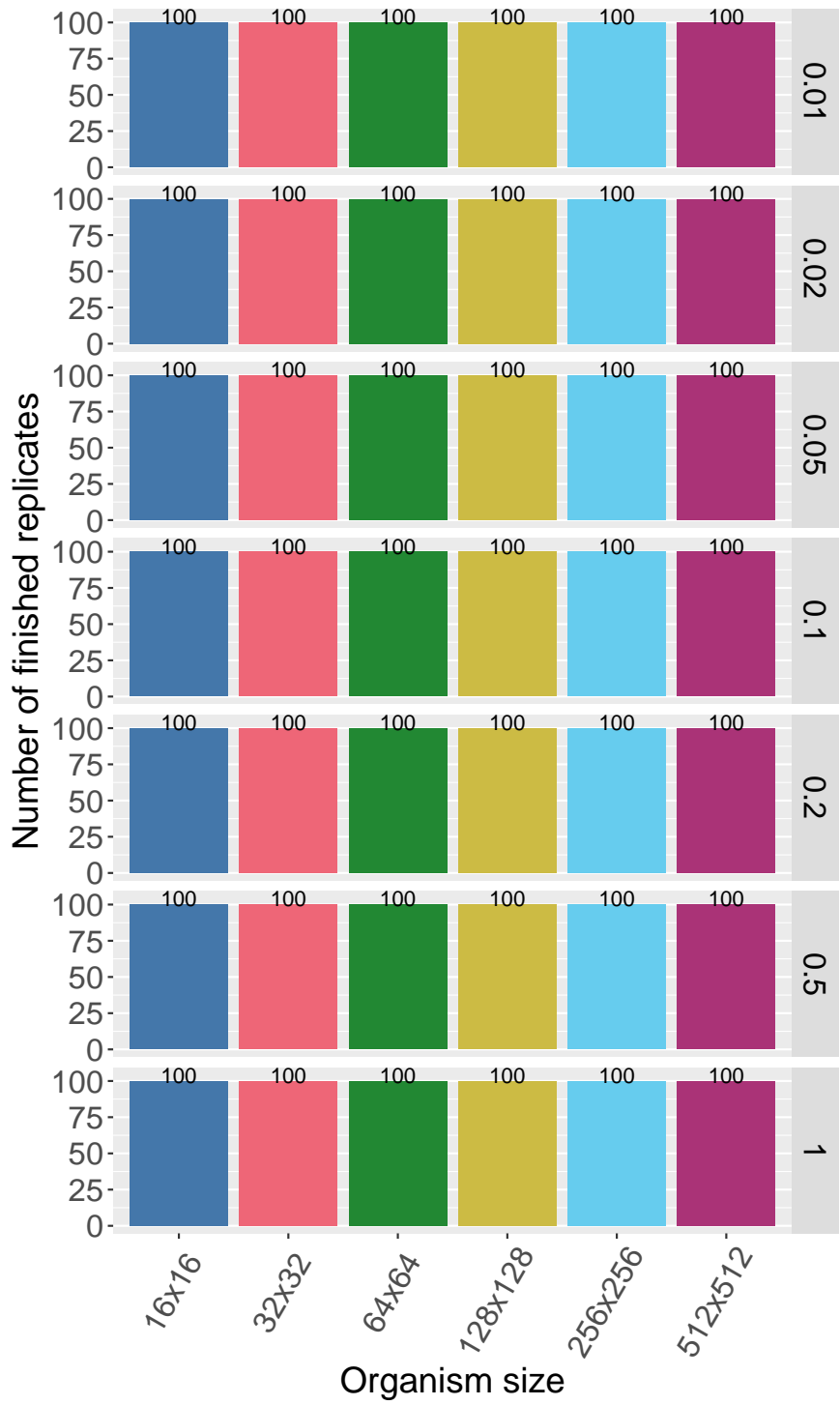
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, CELLMUT)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n)
```

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$size_str, levels = c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
df2$size_factor_reversed = factor(df2$size_str, levels = rev(c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024')))
df2$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', '256x256', '512x512', '1024x1024'))
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
```

3.2 Data integrity check

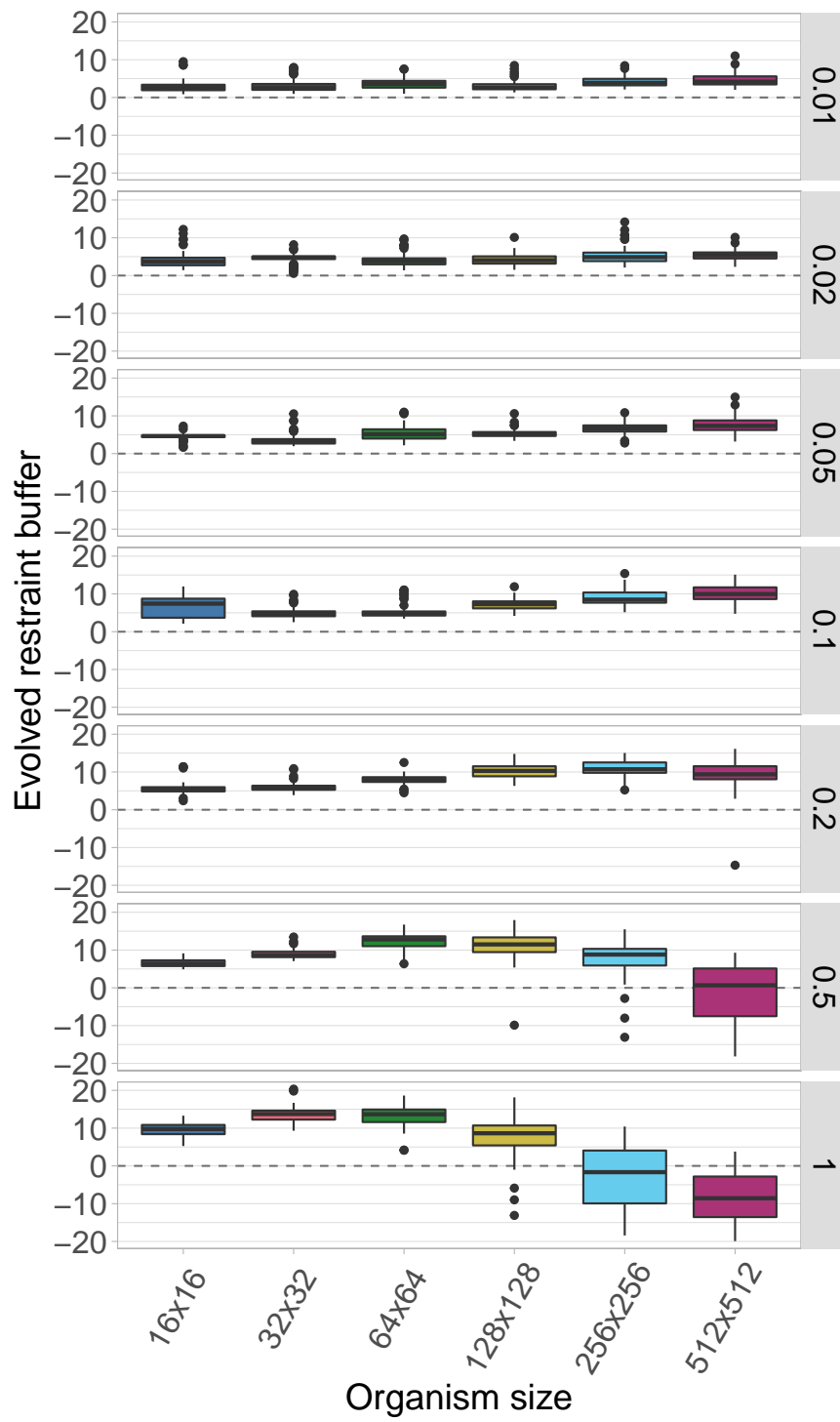
Now we plot the number of finished replicates for each treatment to make sure all data are present. Each row shows a different somatic mutation rate. Each bar/color shows a different organism size.



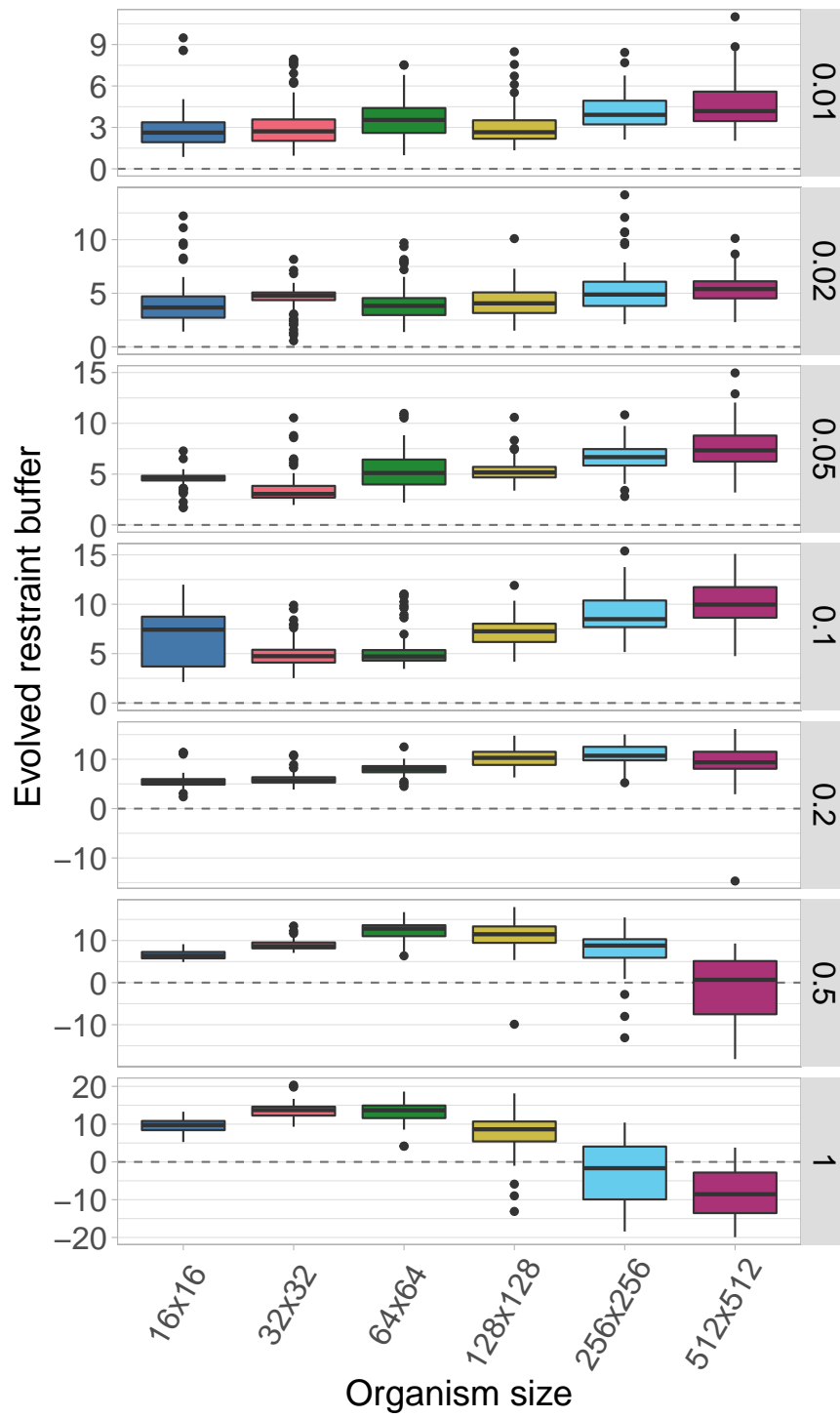
3.3 Aggregate plots

3.3.1 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.

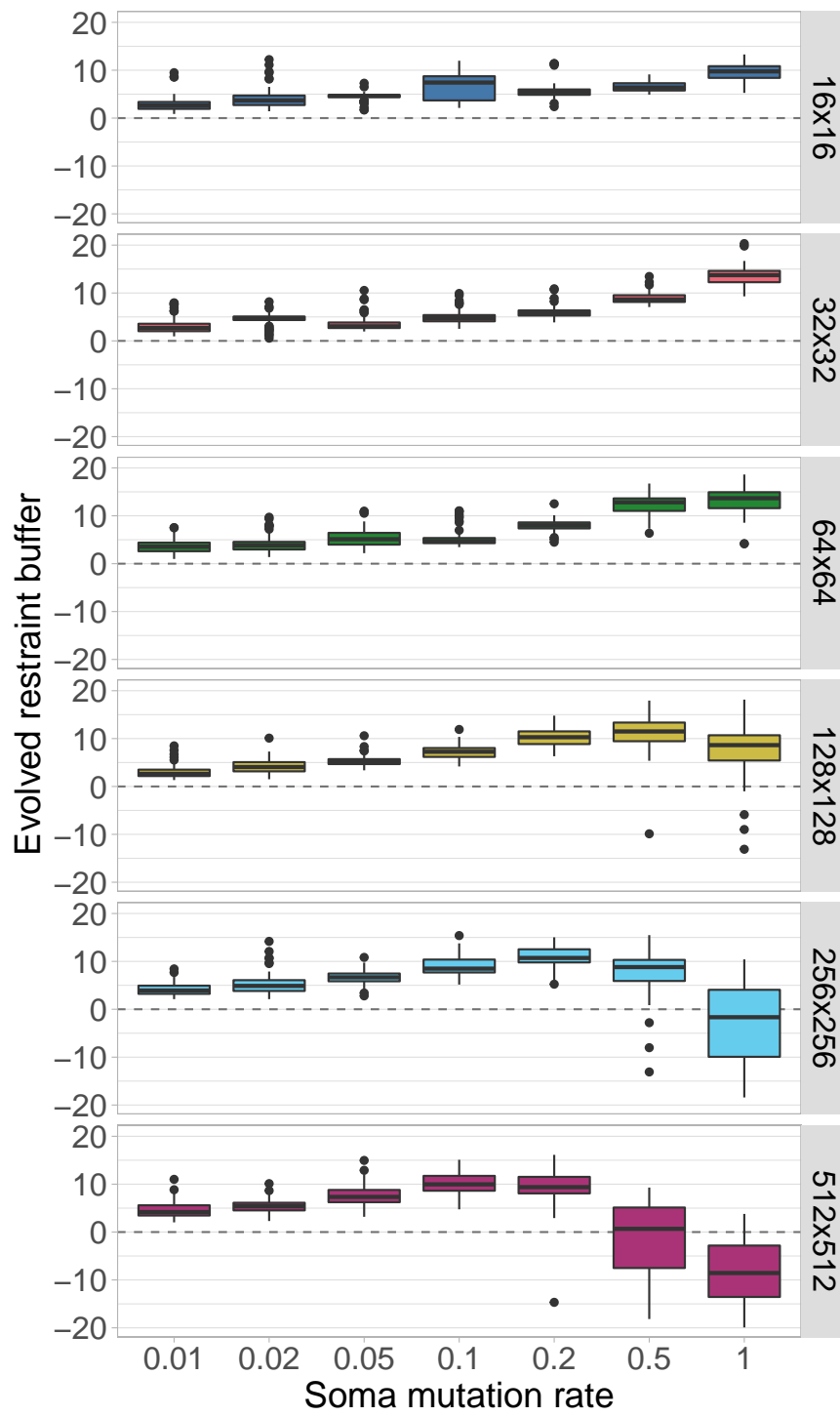


Here we plot the same data, only we allow the y-axis to vary between rows.

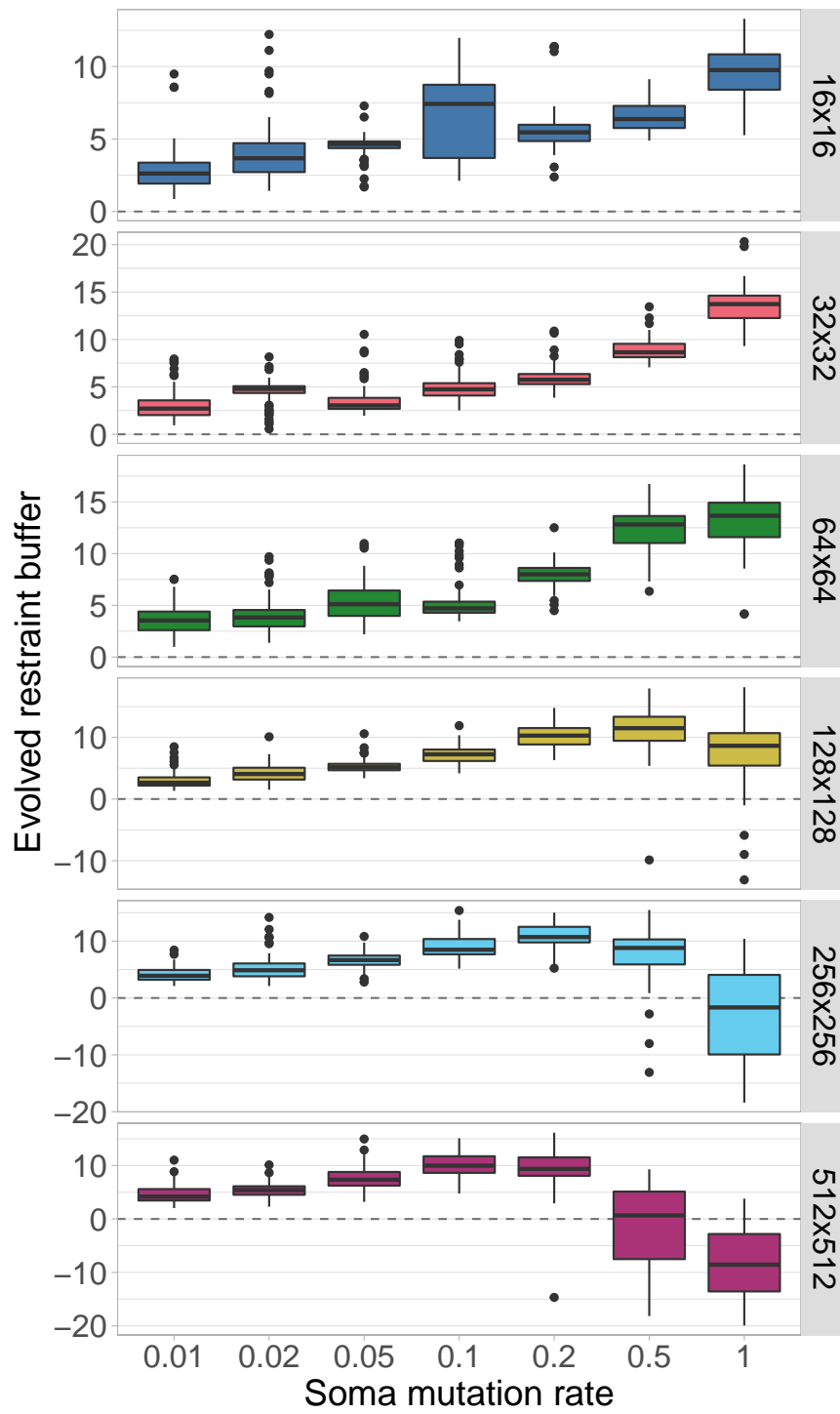


3.3.2 Facet by organism size

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.



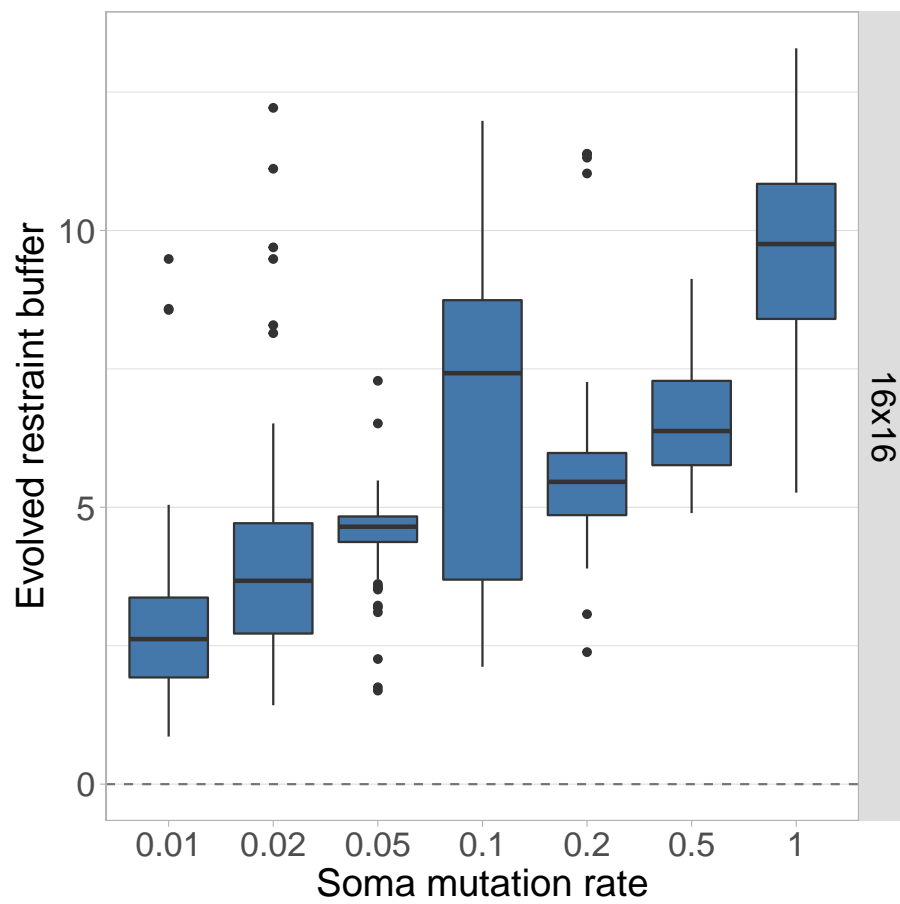
Again, we replot the same data but allow the y-axis to vary between rows.



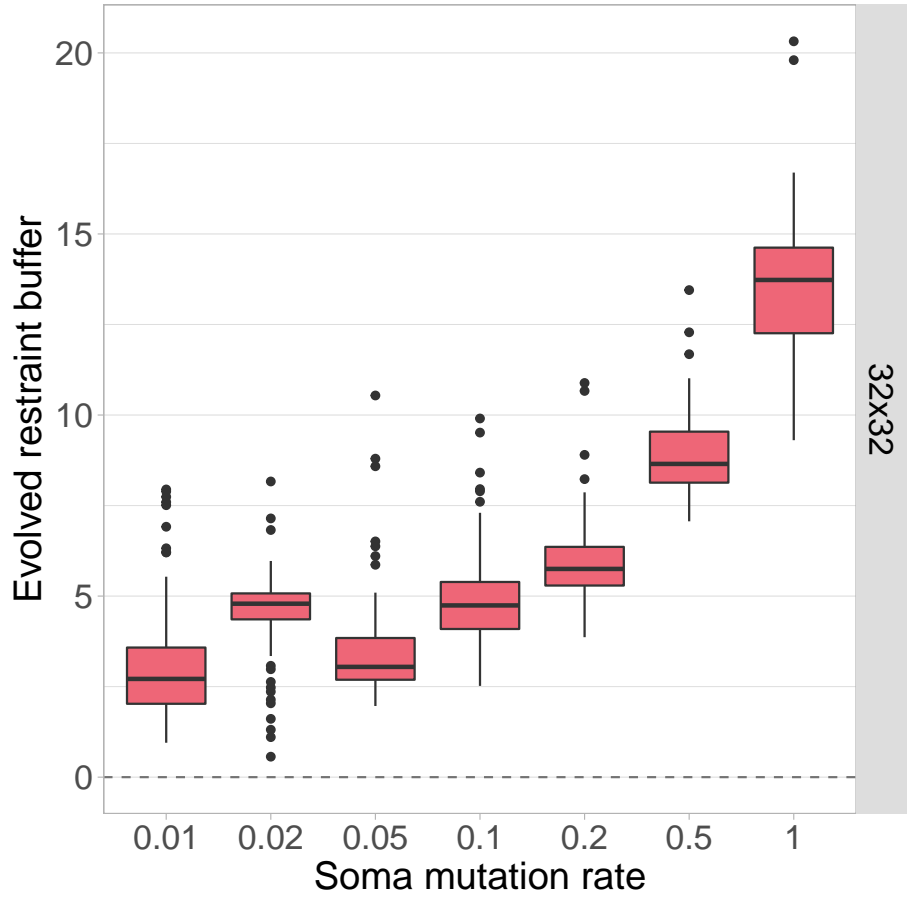
3.4 Single organism size plots

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

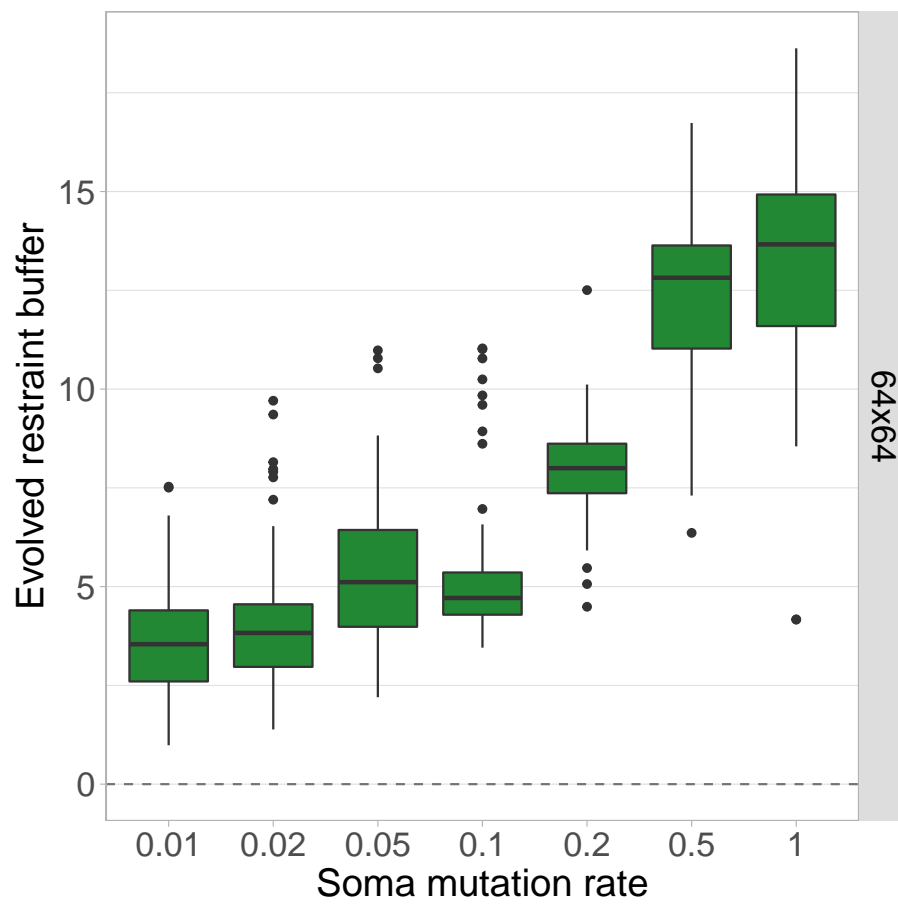
3.4.1 Organism size 16x16



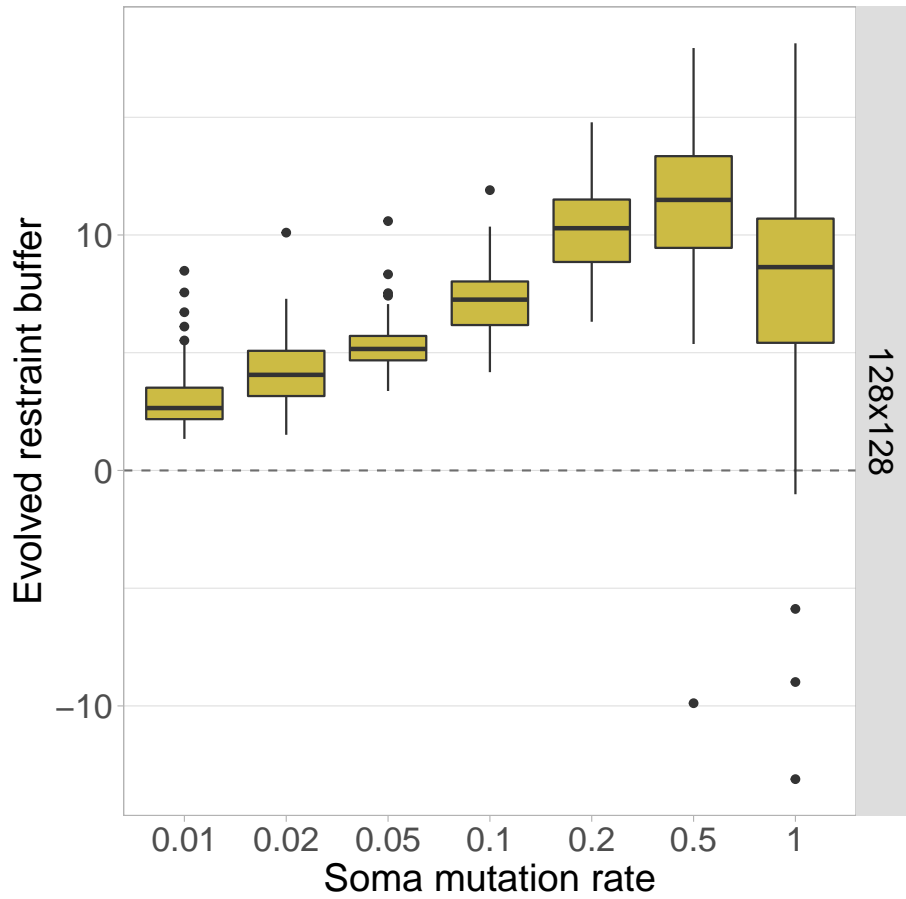
3.4.2 Organism size 32x32



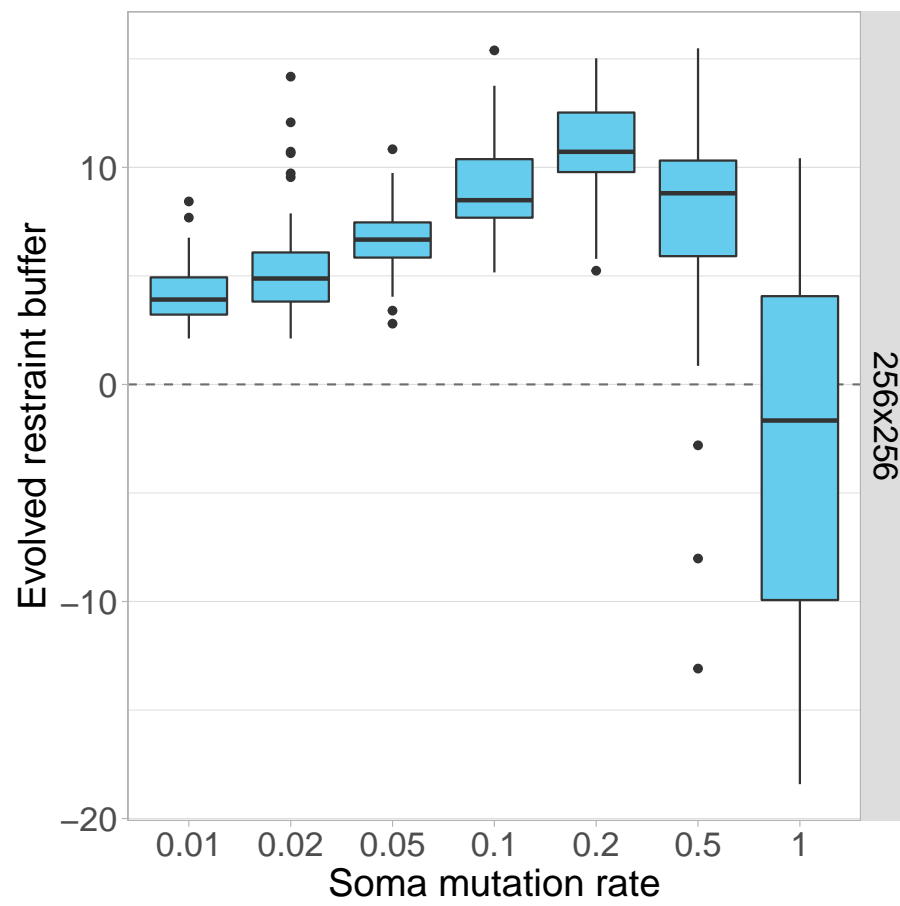
3.4.3 Organism size 64x64



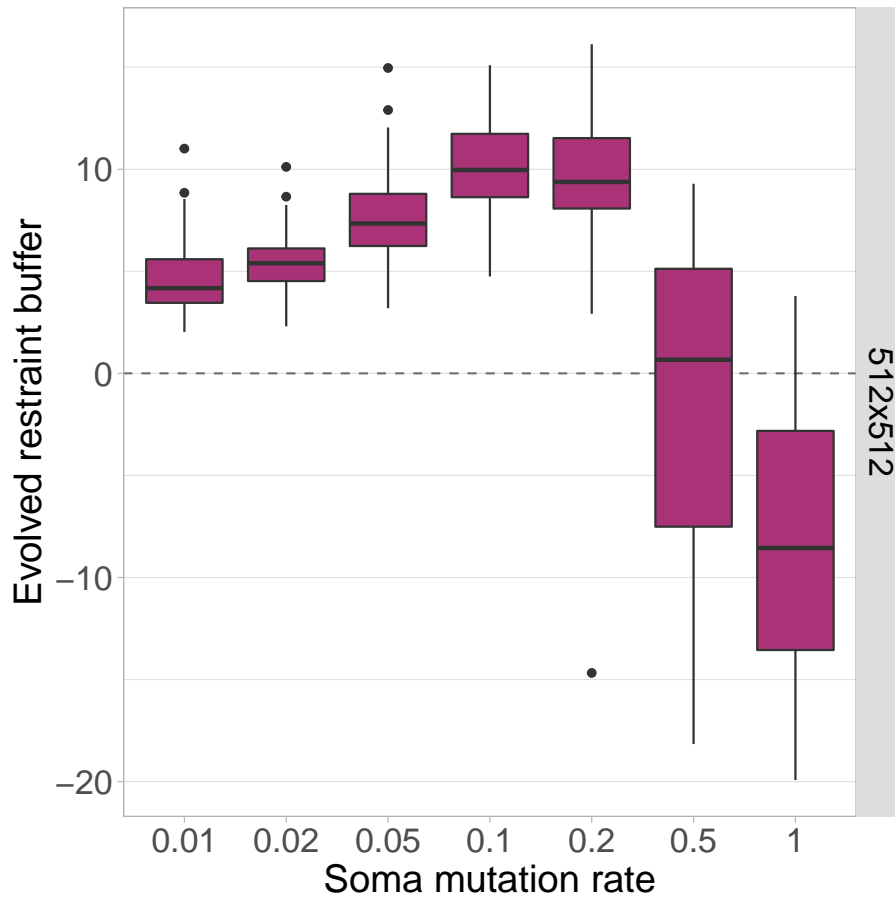
3.4.4 Organism size 128x128



3.4.5 Organism size 256x256



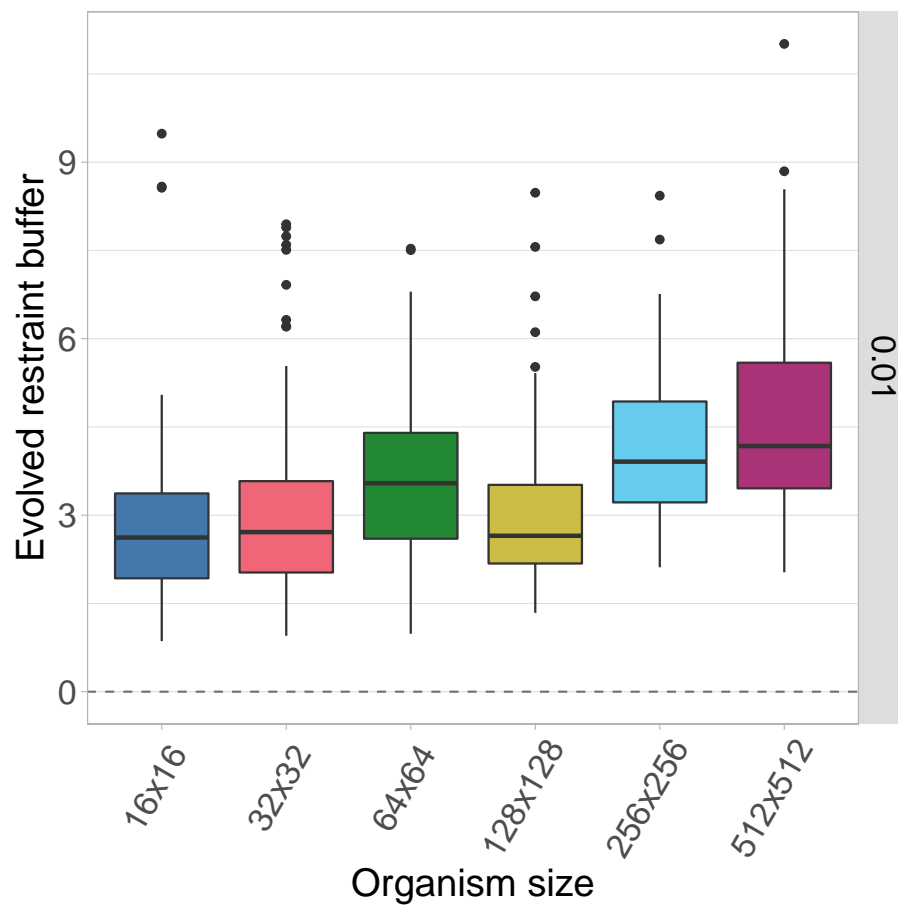
3.4.6 Organism size 512x512



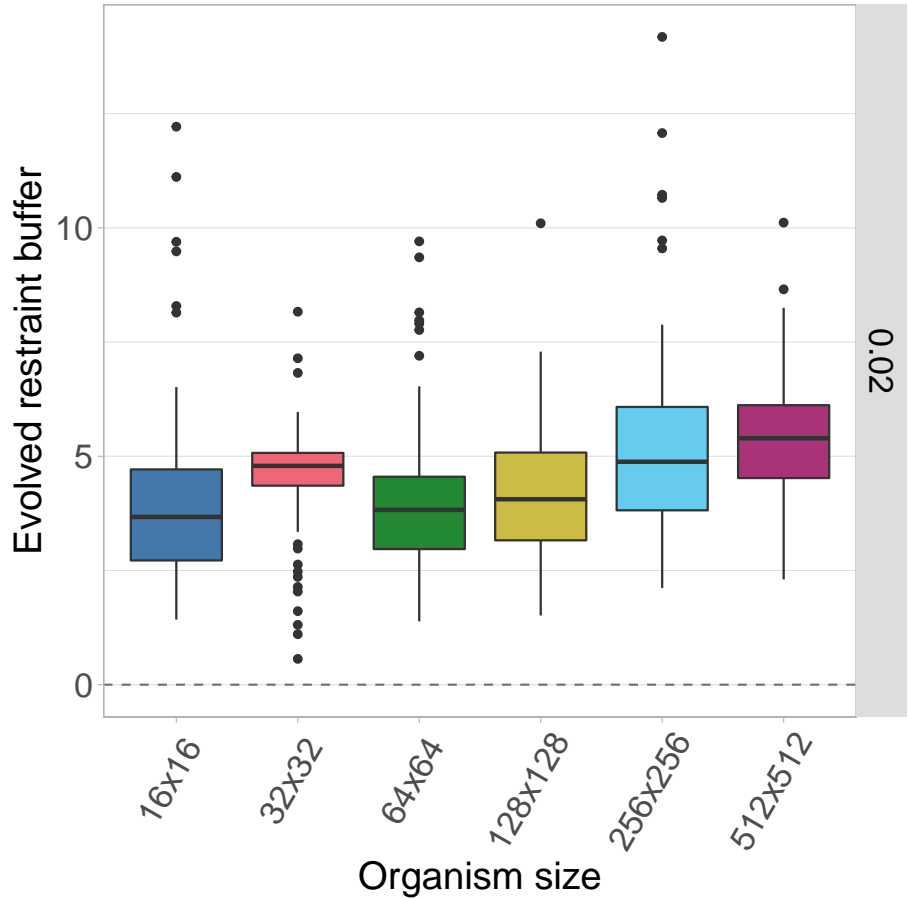
3.5 Single somatic mutation rate plots

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

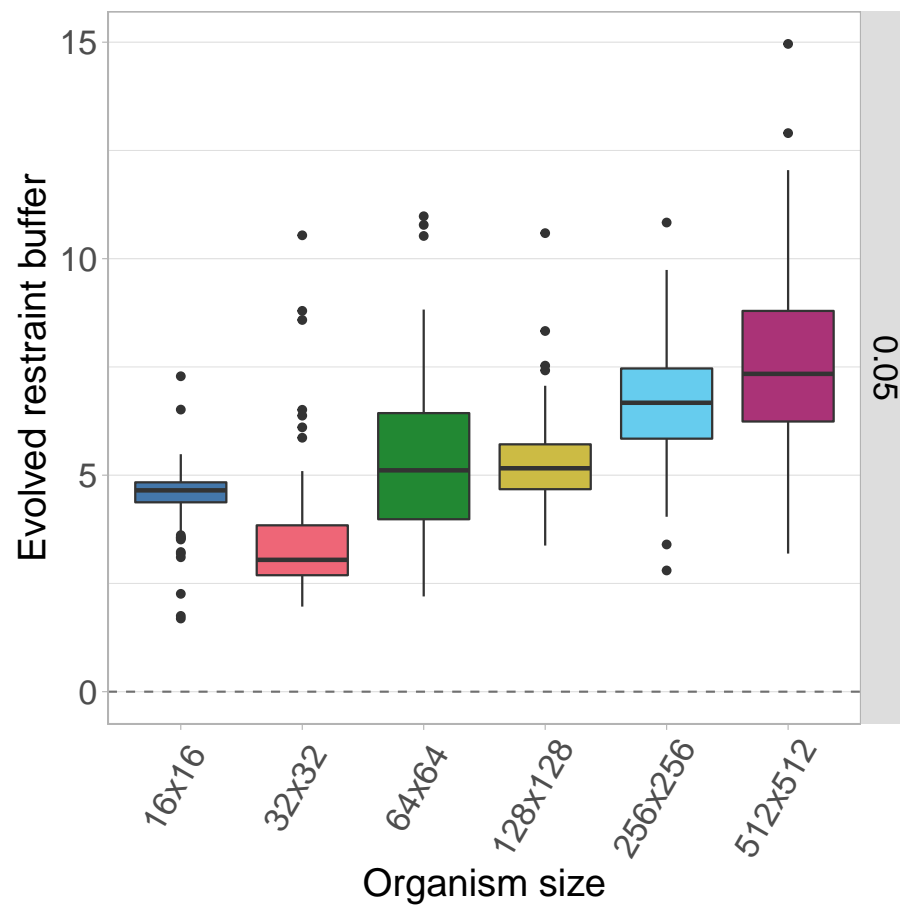
3.5.1 Somatic mut. rate 0.01



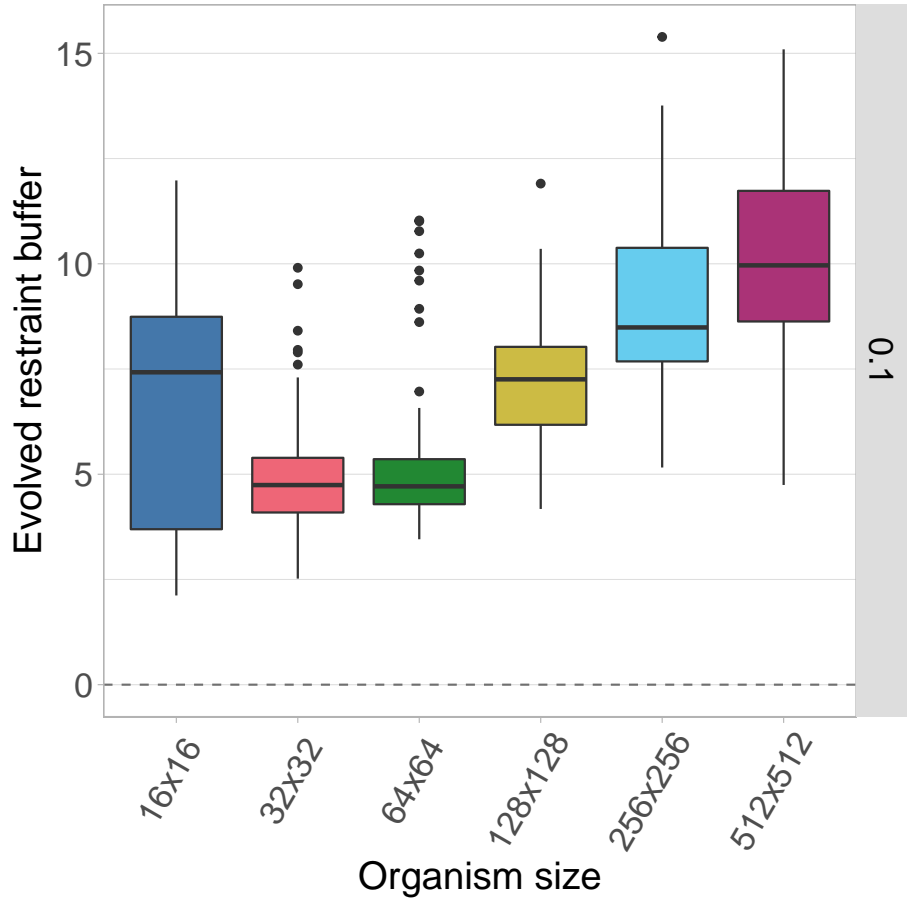
3.5.2 Somatic mut. rate 0.02



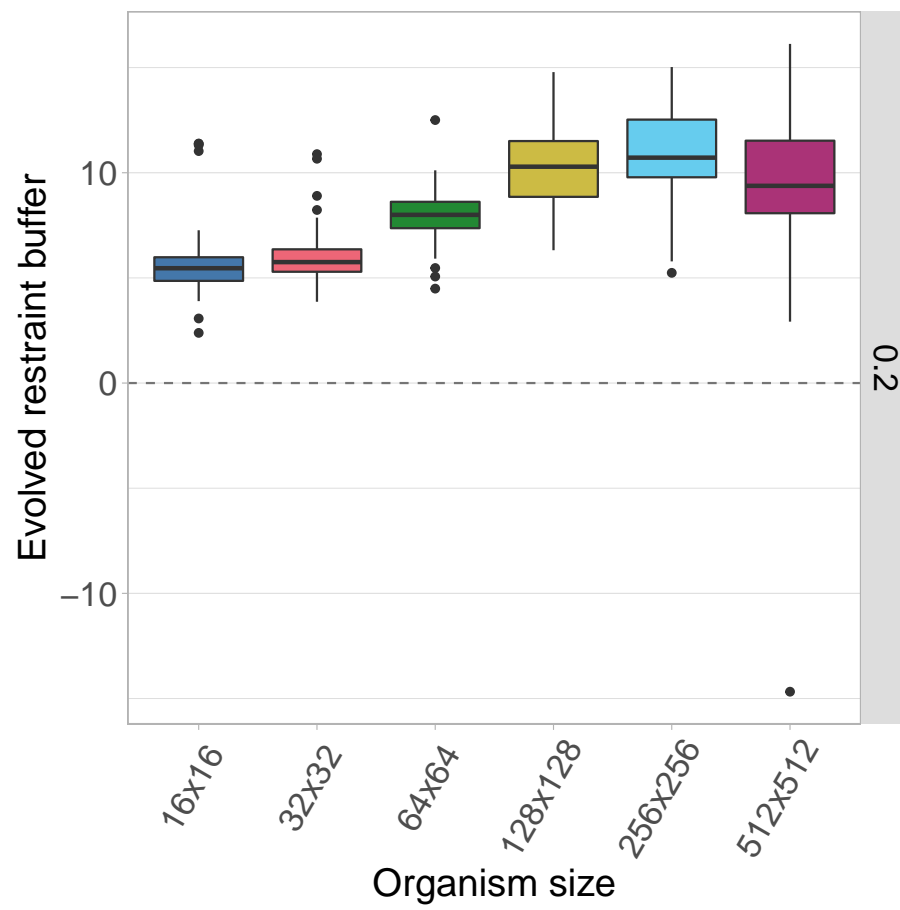
3.5.3 Somatic mut. rate 0.05



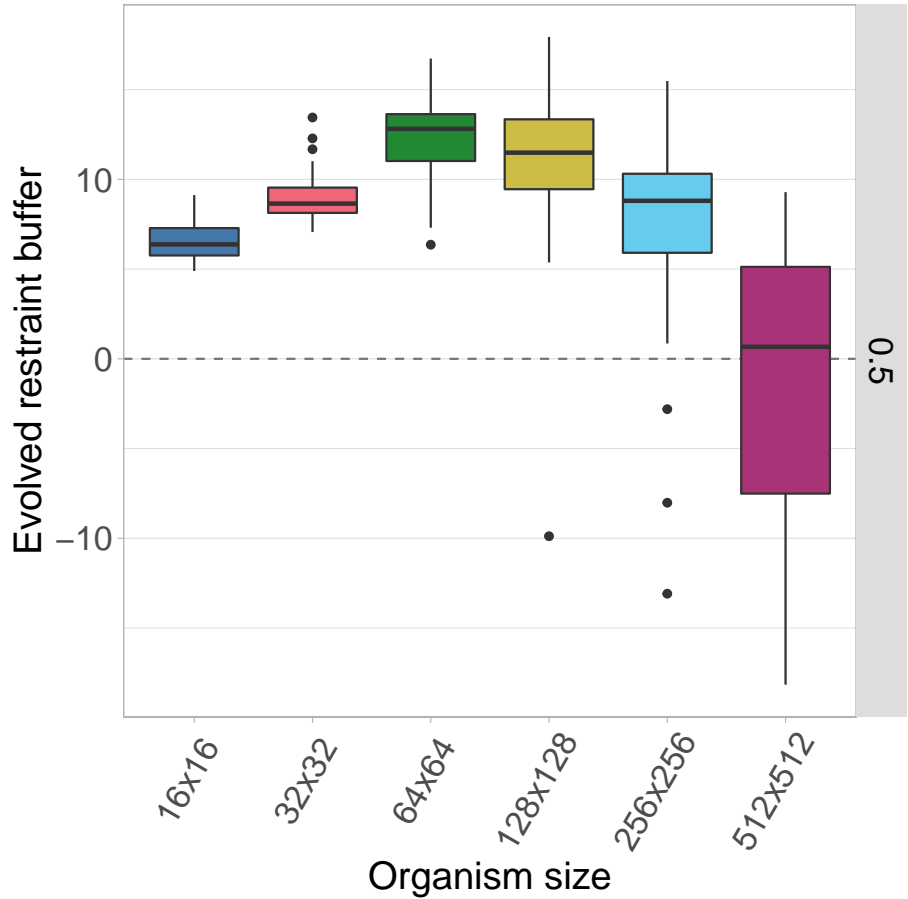
3.5.4 Somatic mut. rate 0.1



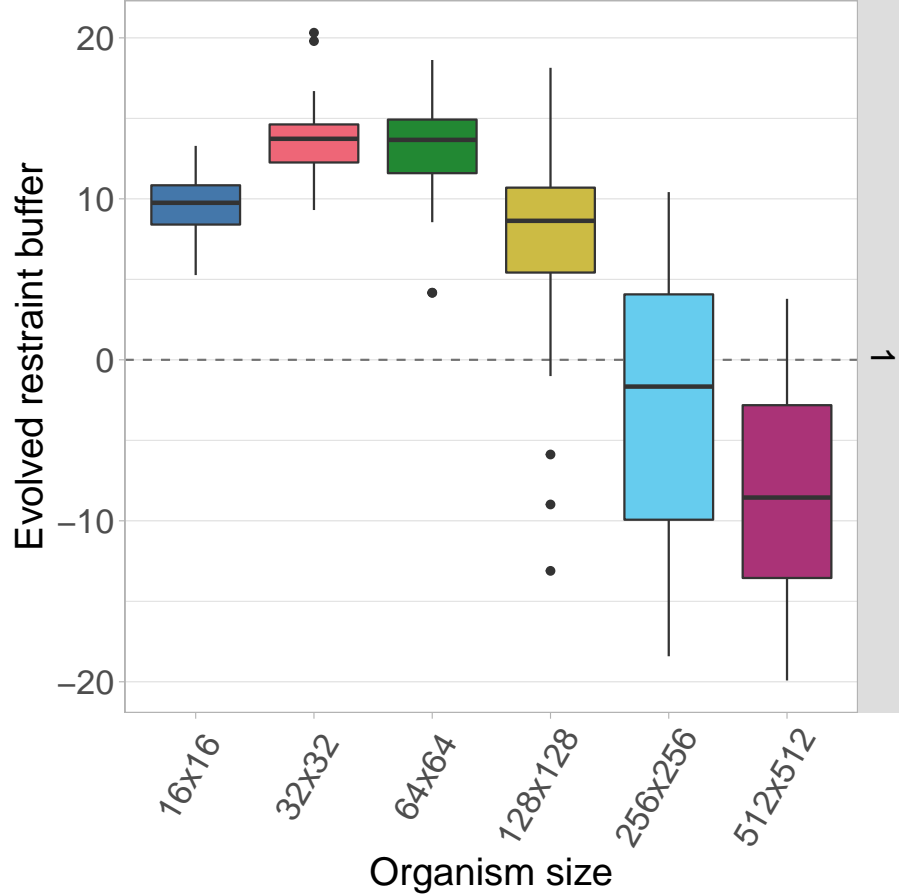
3.5.5 Somatic mut. rate 0.2



3.5.6 Somatic mut. rate 0.5



3.5.7 Somatic mut. rate 1.0



3.6 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$statistic))
    }
  }
  df_wilcox$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 4

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(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_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.

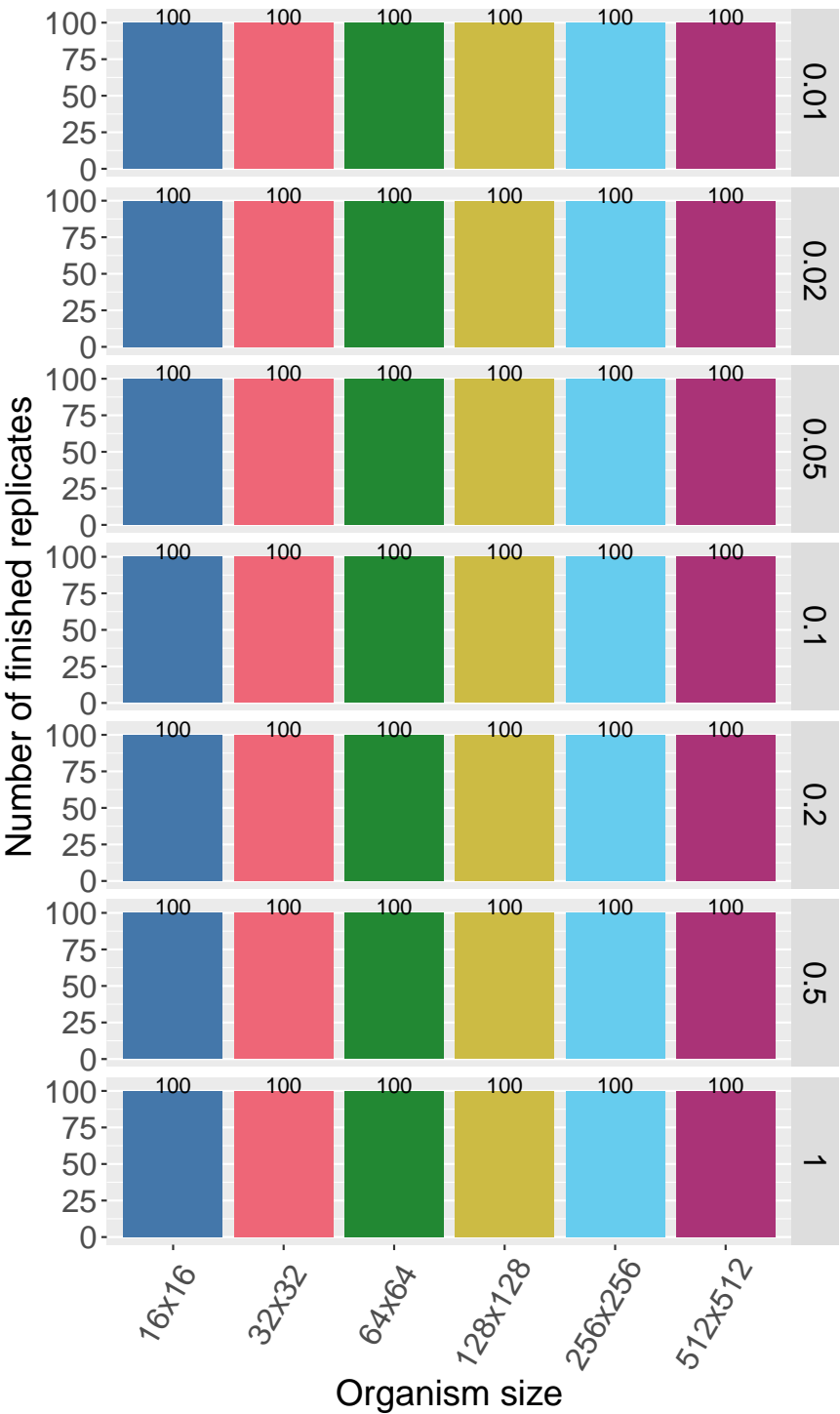
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, MUT)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n
```

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'
```

4.1 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

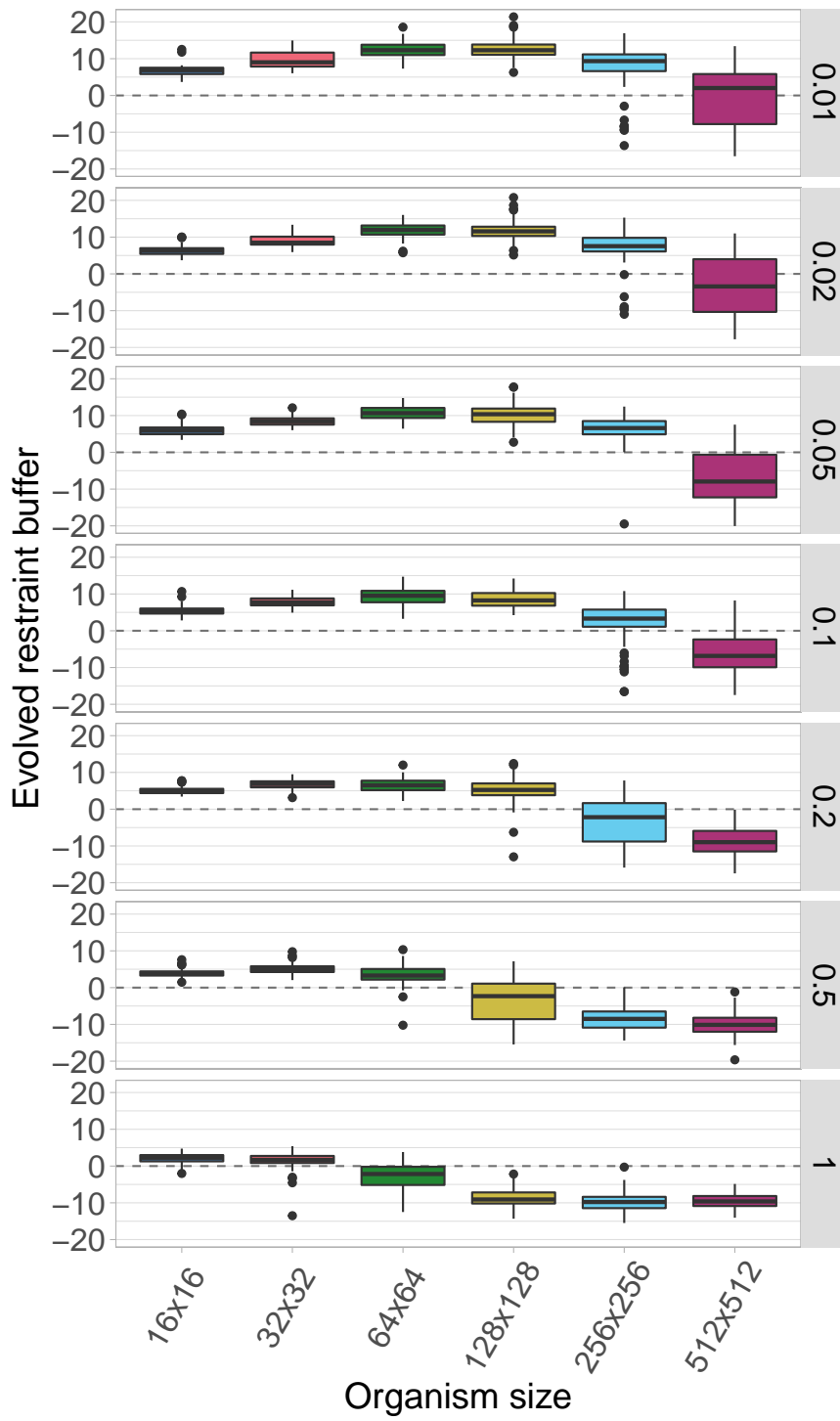


shows a different organism size.

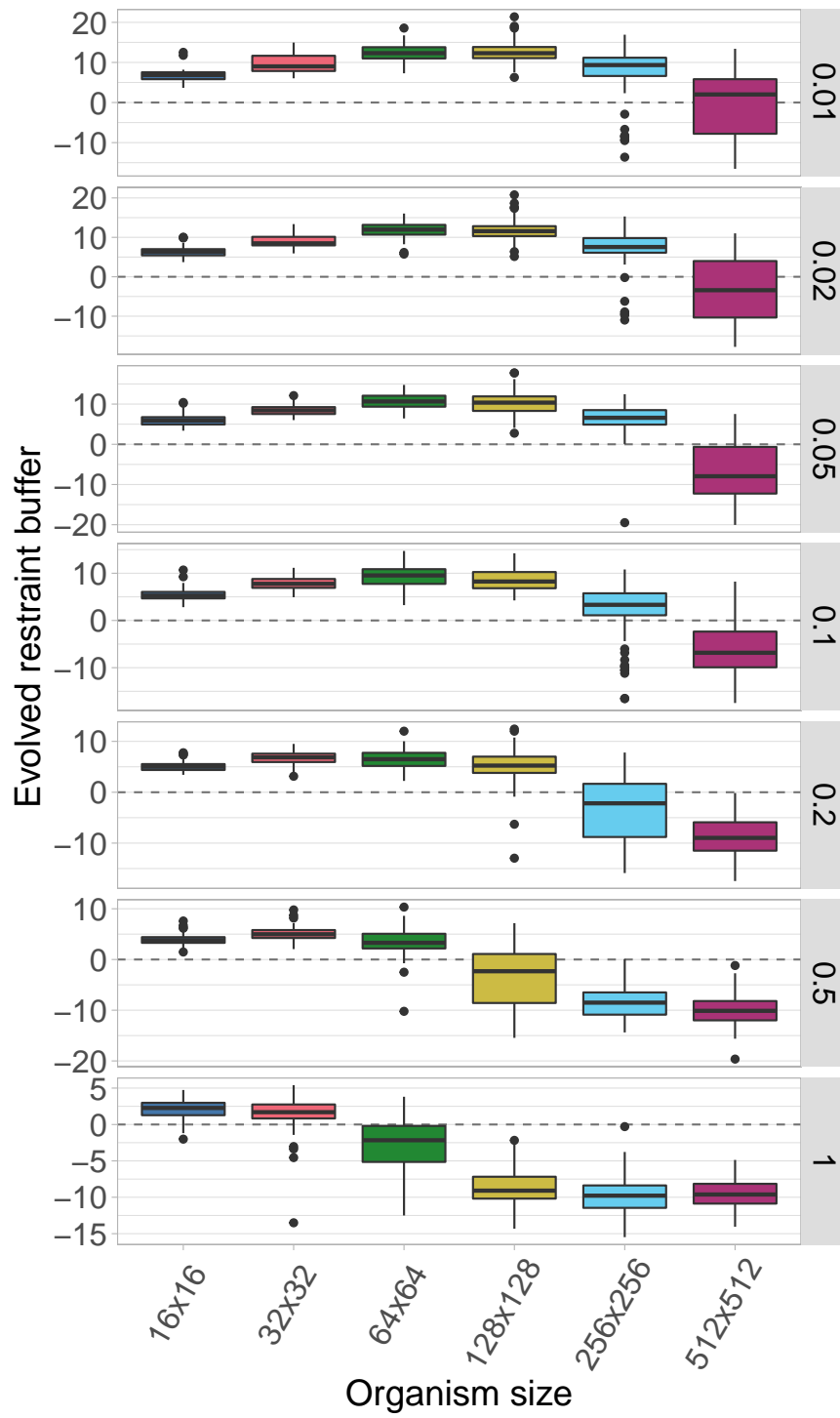
4.2 Aggregate plots

4.2.1 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.

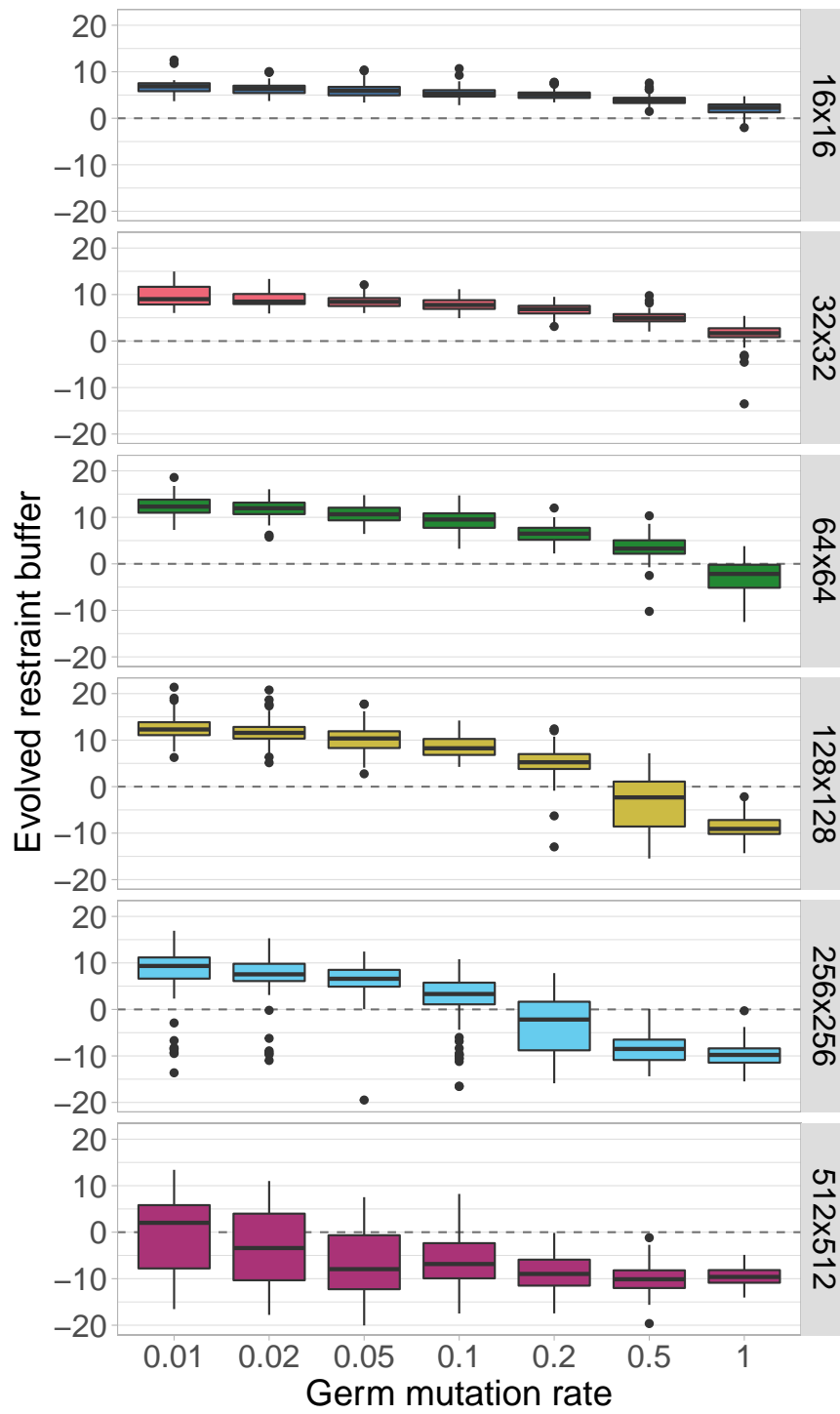


Here is the same data, plotted identically other than now each row can have a different y-axis.

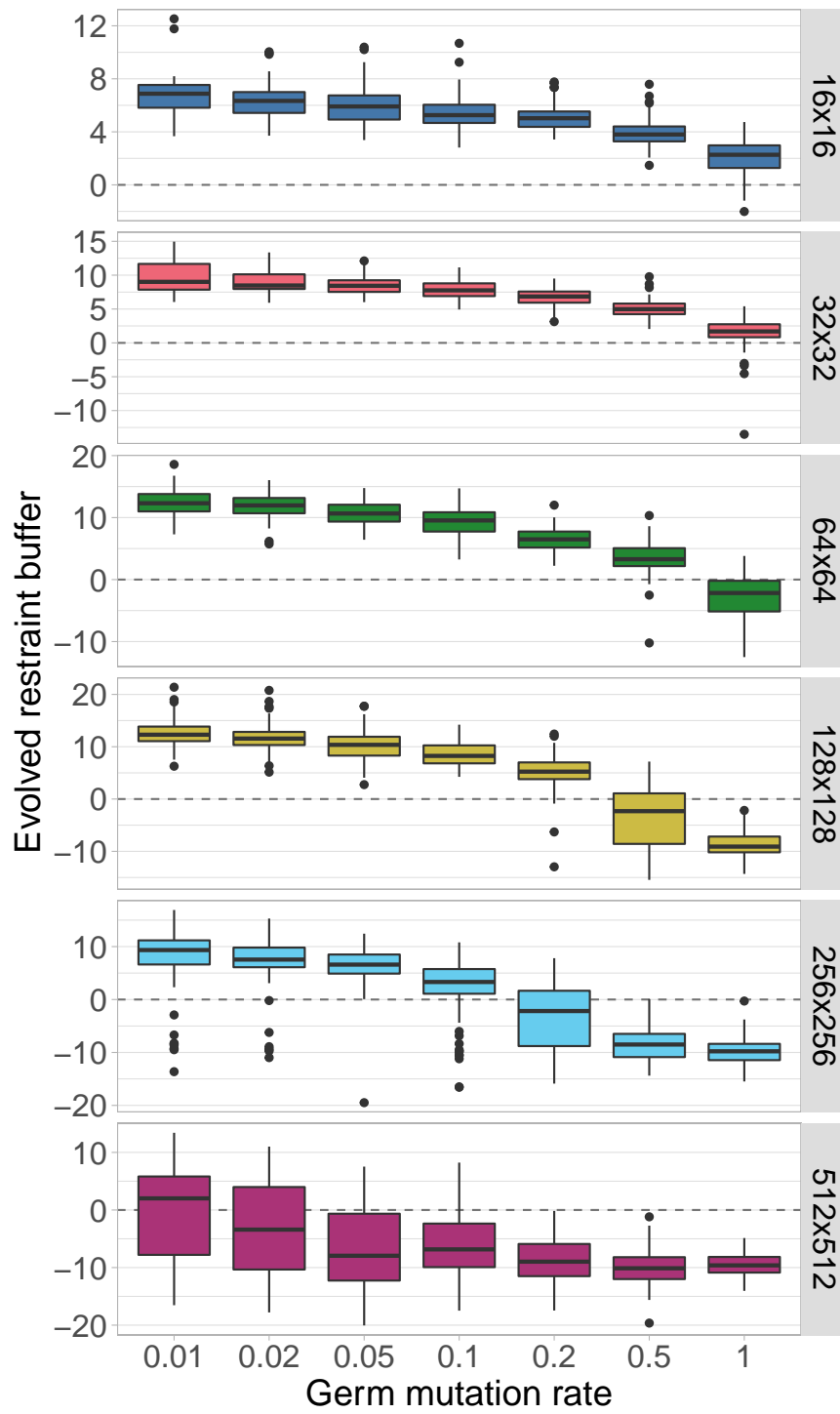


4.2.2 Facet by organism size

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.



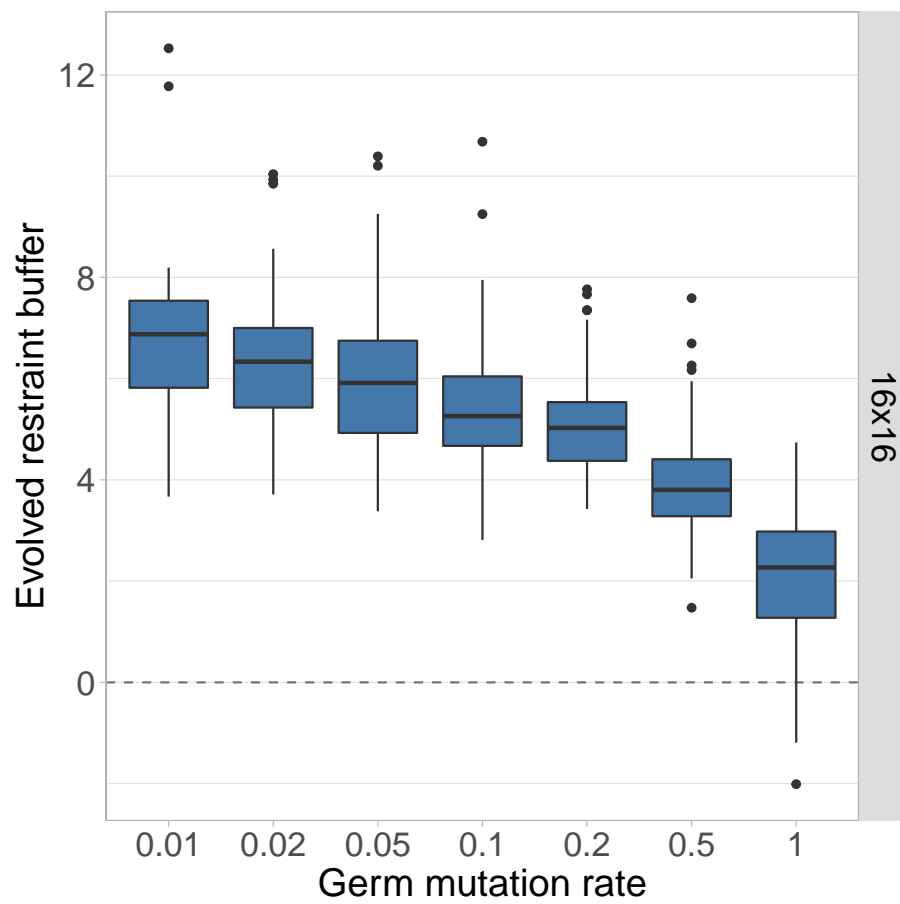
Again, we plot the same data again, but now the y-axis can change between rows.



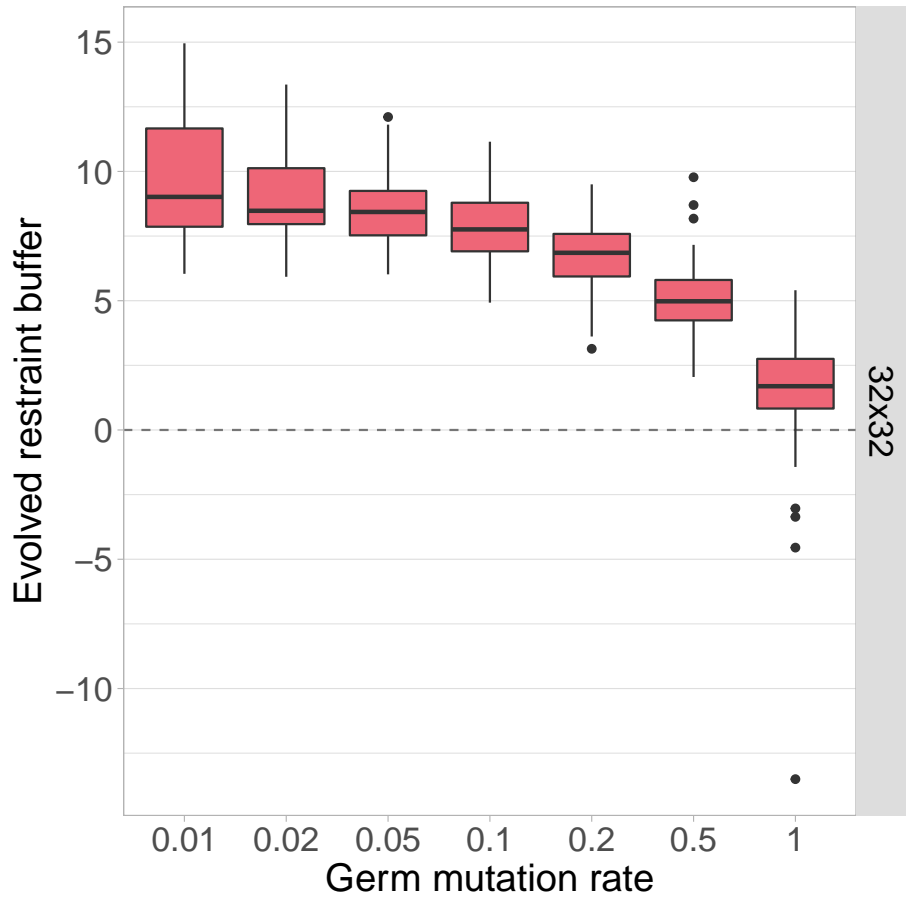
4.3 Single organism size plots

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

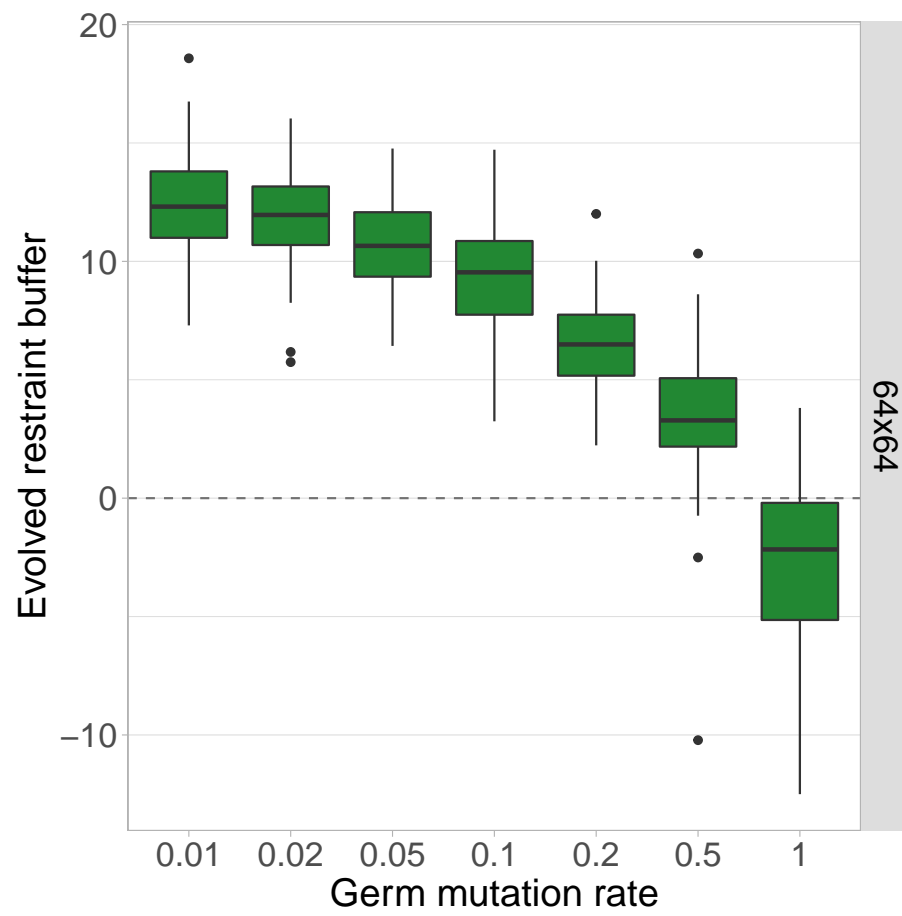
4.3.1 Organism size 16x16



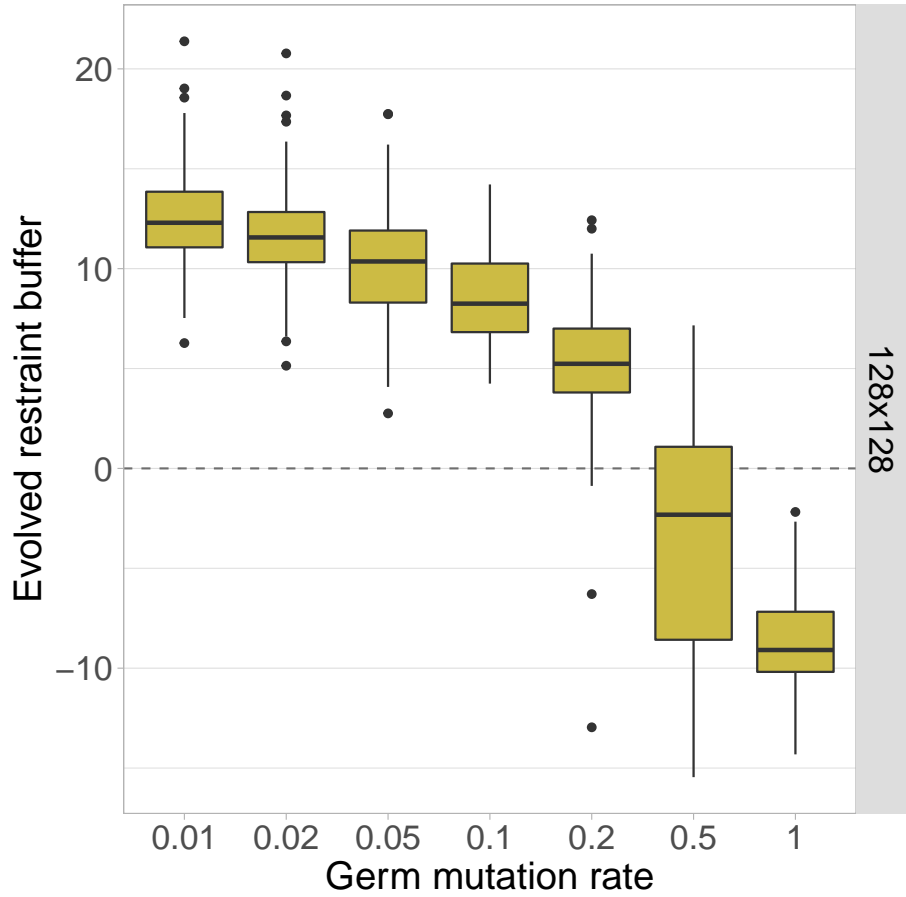
4.3.2 Organism size 32x32



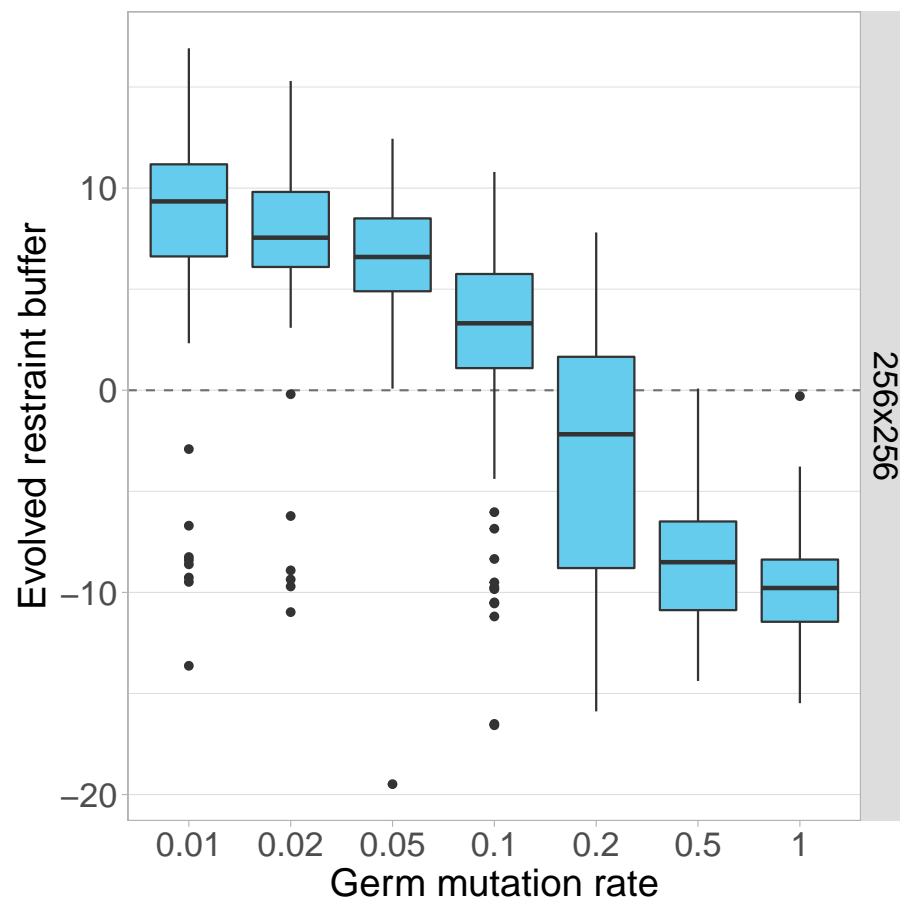
4.3.3 Organism size 64x64



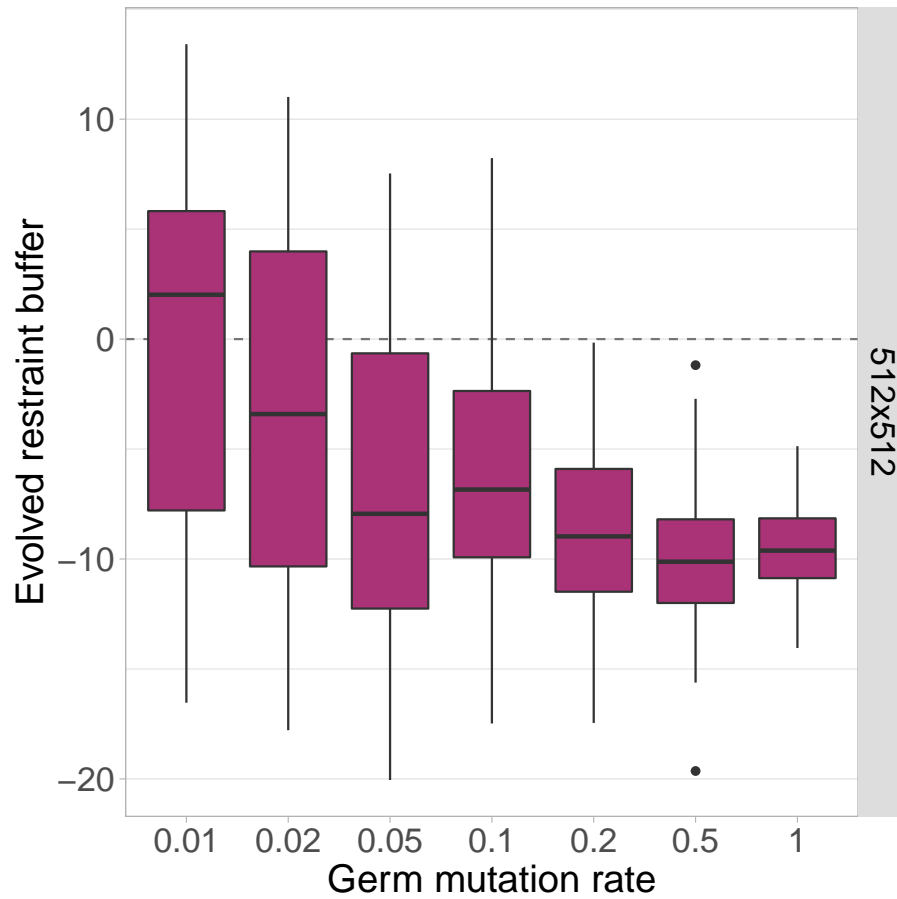
4.3.4 Organism size 128x128



4.3.5 Organism size 256x256



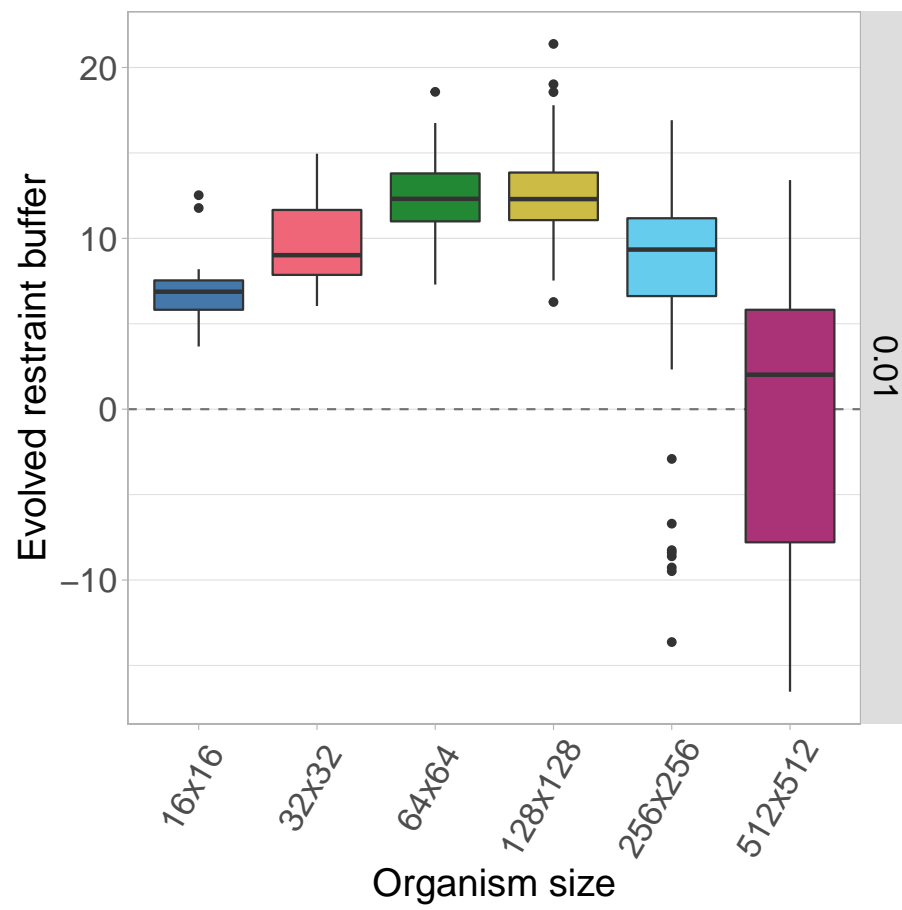
4.3.6 Organism size 512x512



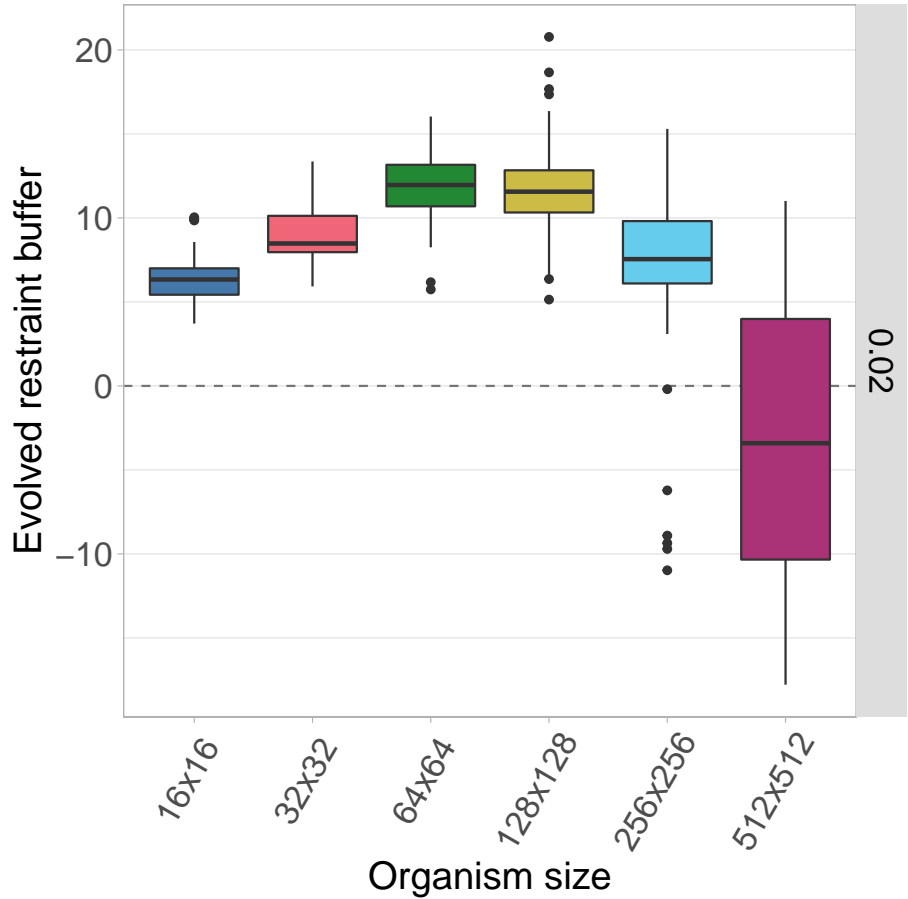
4.4 Single organism size plots

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

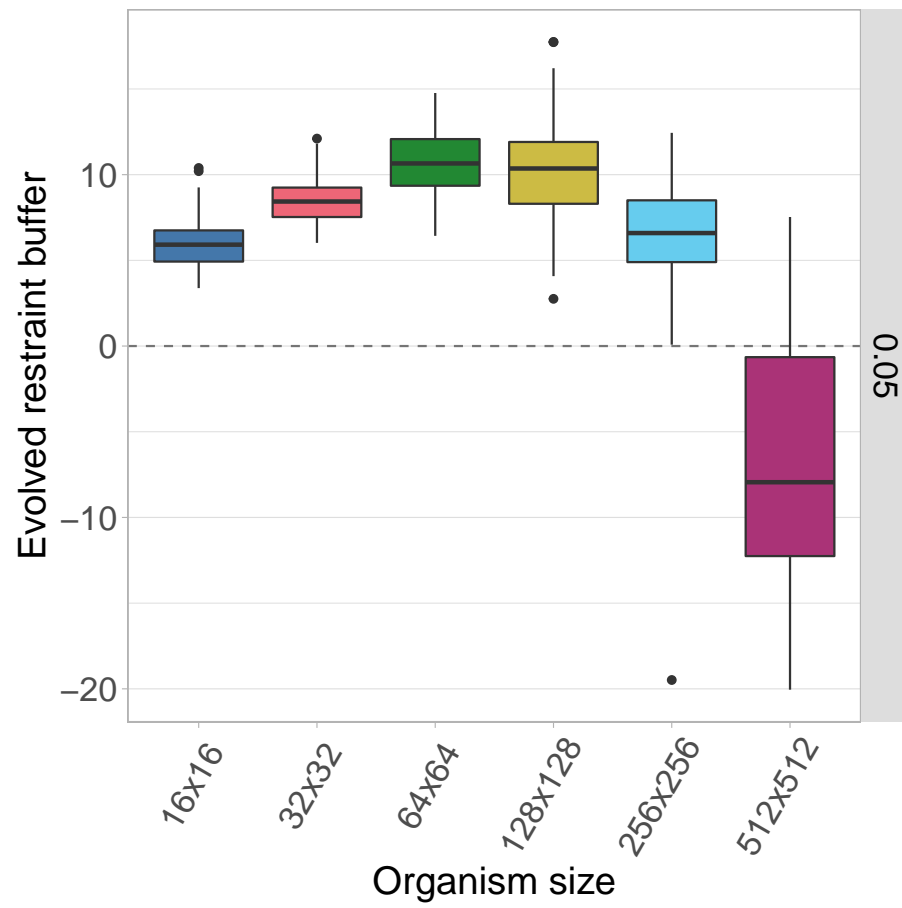
4.4.1 Germ mut. rate 0.01



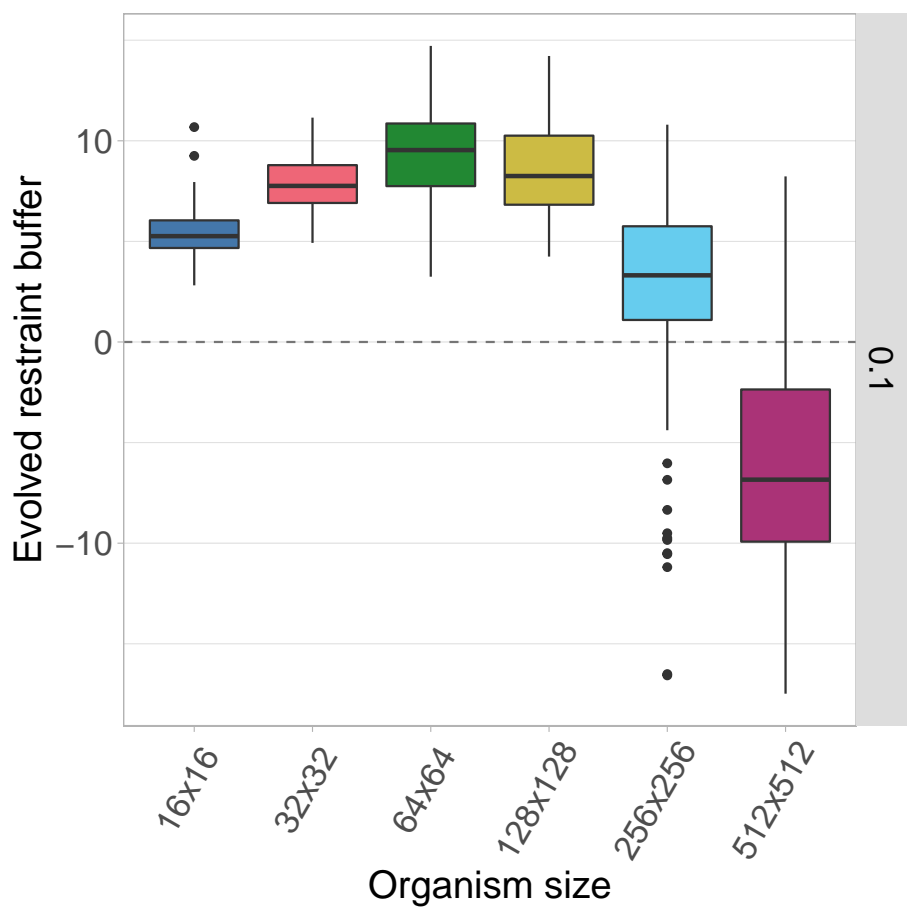
4.4.2 Germ mut. rate 0.02



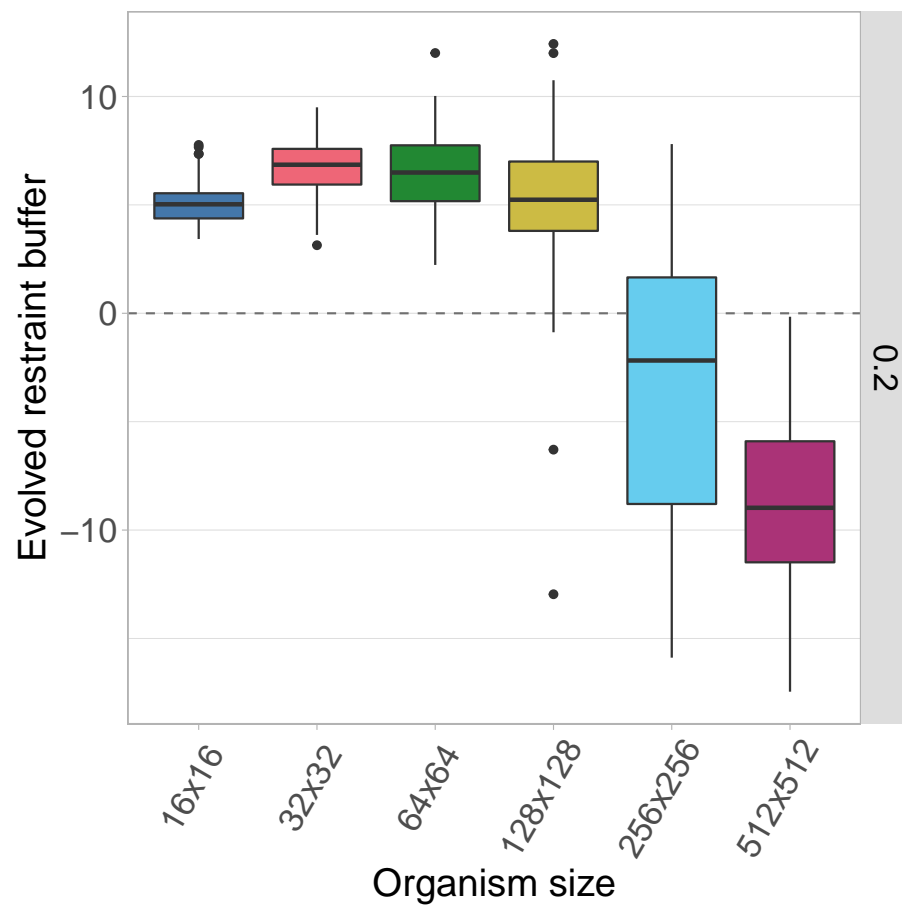
4.4.3 Germ mut. rate 0.05



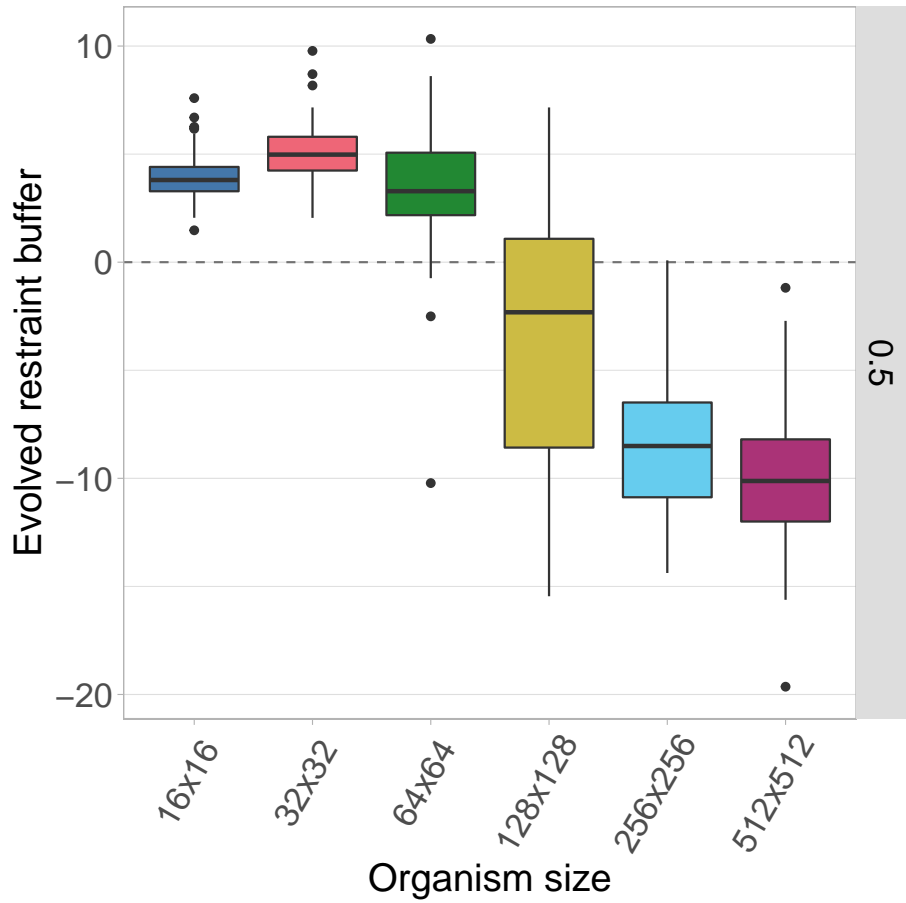
4.4.4 Germ mut. rate 0.1



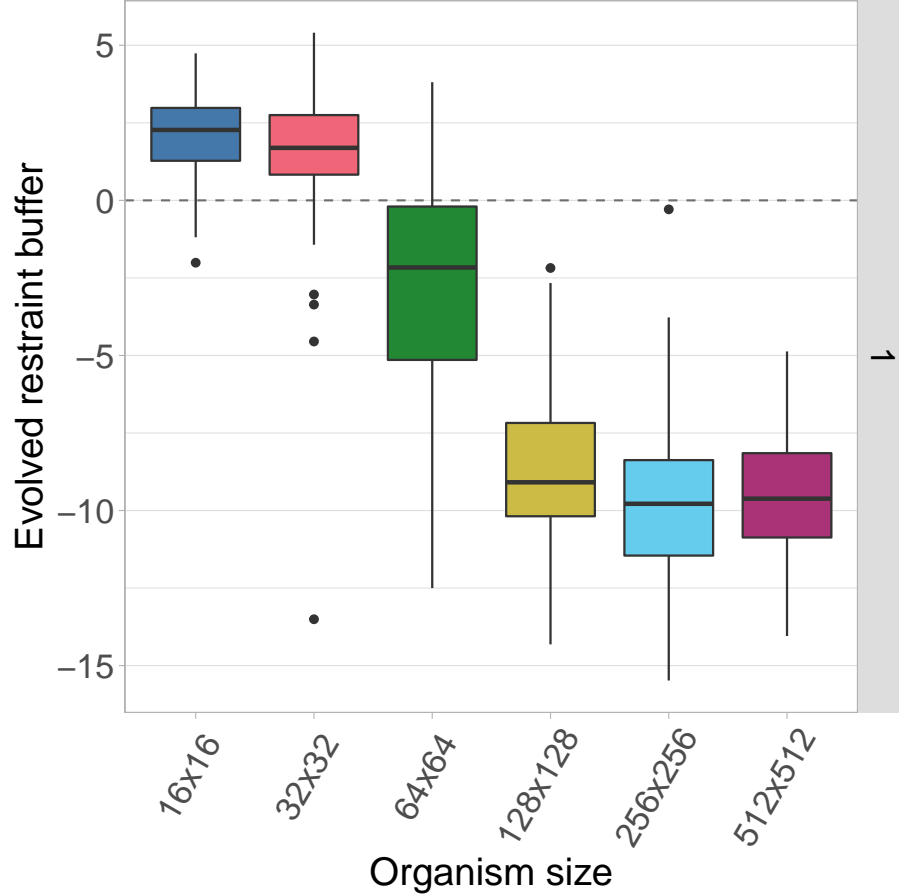
4.4.5 Germ mut. rate 0.2



4.4.6 Germ mut. rate 0.5



4.4.7 Germ mut. rate 1.0



4.5 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.numeric(res$std.error)[1])
  }
  df_kruskal$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$MUT == mut_rate,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('germ_mut_rate', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw', 'W')
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test[df_test$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$statistic)[1], as.numeric(res$std.error)[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 5

Genome Length Sweep

By default, all genomes are bitstrings with 100 bits. Here, we look into the effects of varying this genome length (we use values 25, 50, 100, 200, and 400 bits).

The configuration script and data for the experiment can be found under `2021_02_27__genome_length/` in the experiments directory of the git repository.

5.1 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__genome_length/evolution/data/scraped_evolution_data_len
df = rbind(df, read.csv('../experiments/2021_02_27__genome_length/evolution/data/scraped_evolutio
df = rbind(df, read.csv('../experiments/2021_02_27__genome_length/evolution/data/scraped_evolutio
df = rbind(df, read.csv('../experiments/2021_02_27__genome_length/evolution/data/scraped_evolutio
df = rbind(df, read.csv('../experiments/2021_02_27__genome_length/evolution/data/scraped_evolutio
# 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.

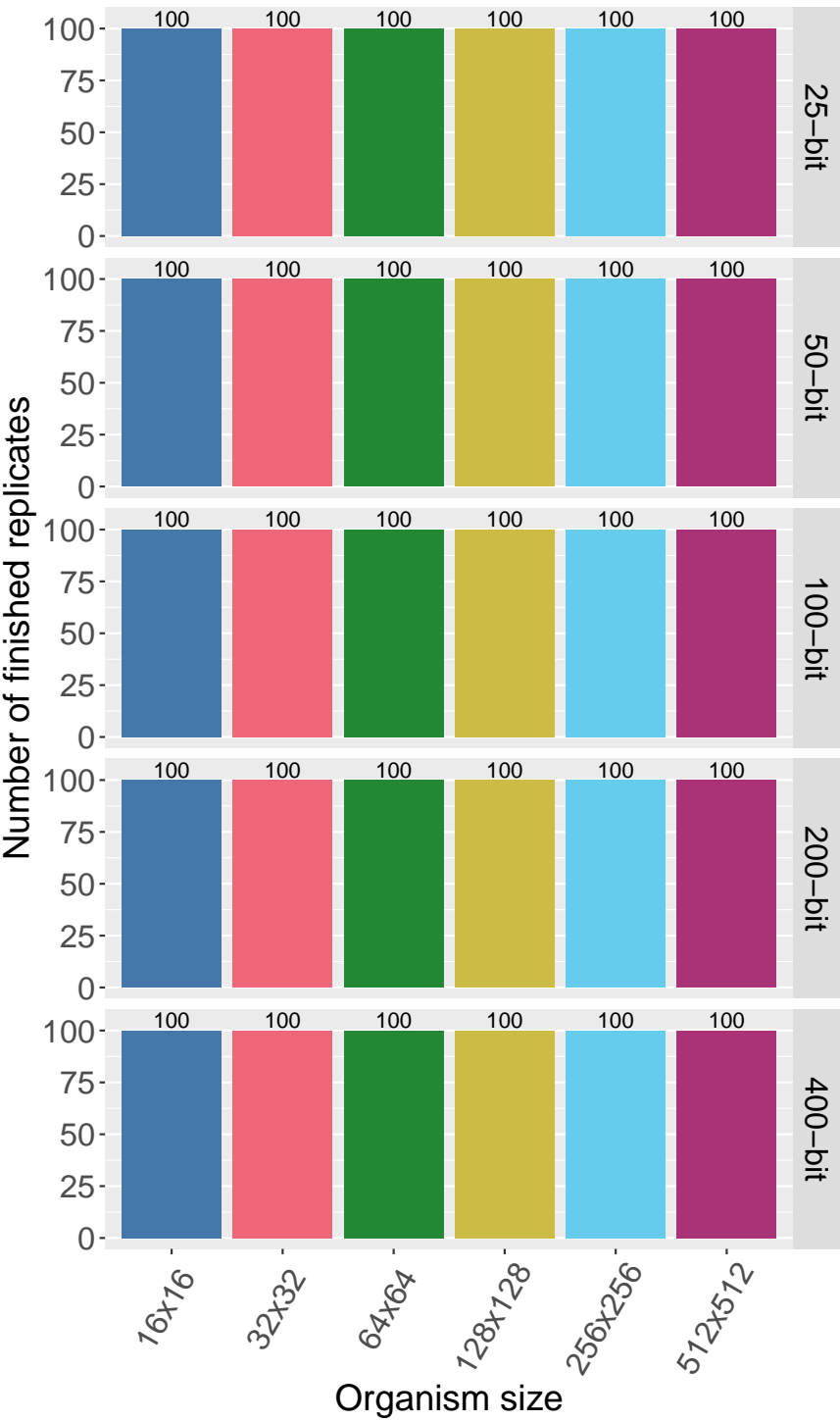
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, LENGTH)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n
```

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

```
## Set variables to make plotting easier
# Calculate restraint value (x - 60% of the genome length)
df2$restraint_value = df2$ave_ones - (df2$LENGTH * 0.6)
# Make a nice, clean factor for size
df2$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$length_str = paste0(df2$LENGTH, '-bit')
df2$length_factor = factor(df2$length_str, levels = c('25-bit', '50-bit', '100-bit', '200-bit', '400-bit', '800-bit', '1600-bit'))
data_summary$size_str = paste0(data_summary$MCSIZE, 'x', data_summary$MCSIZE)
data_summary$size_factor = factor(data_summary$size_str, levels = c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
data_summary$length_str = paste0(data_summary$LENGTH, '-bit')
data_summary$length_factor = factor(data_summary$length_str, levels = c('25-bit', '50-bit', '100-bit', '200-bit', '400-bit', '800-bit', '1600-bit'))
# 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
```

5.2 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 genome length (in bits). Each

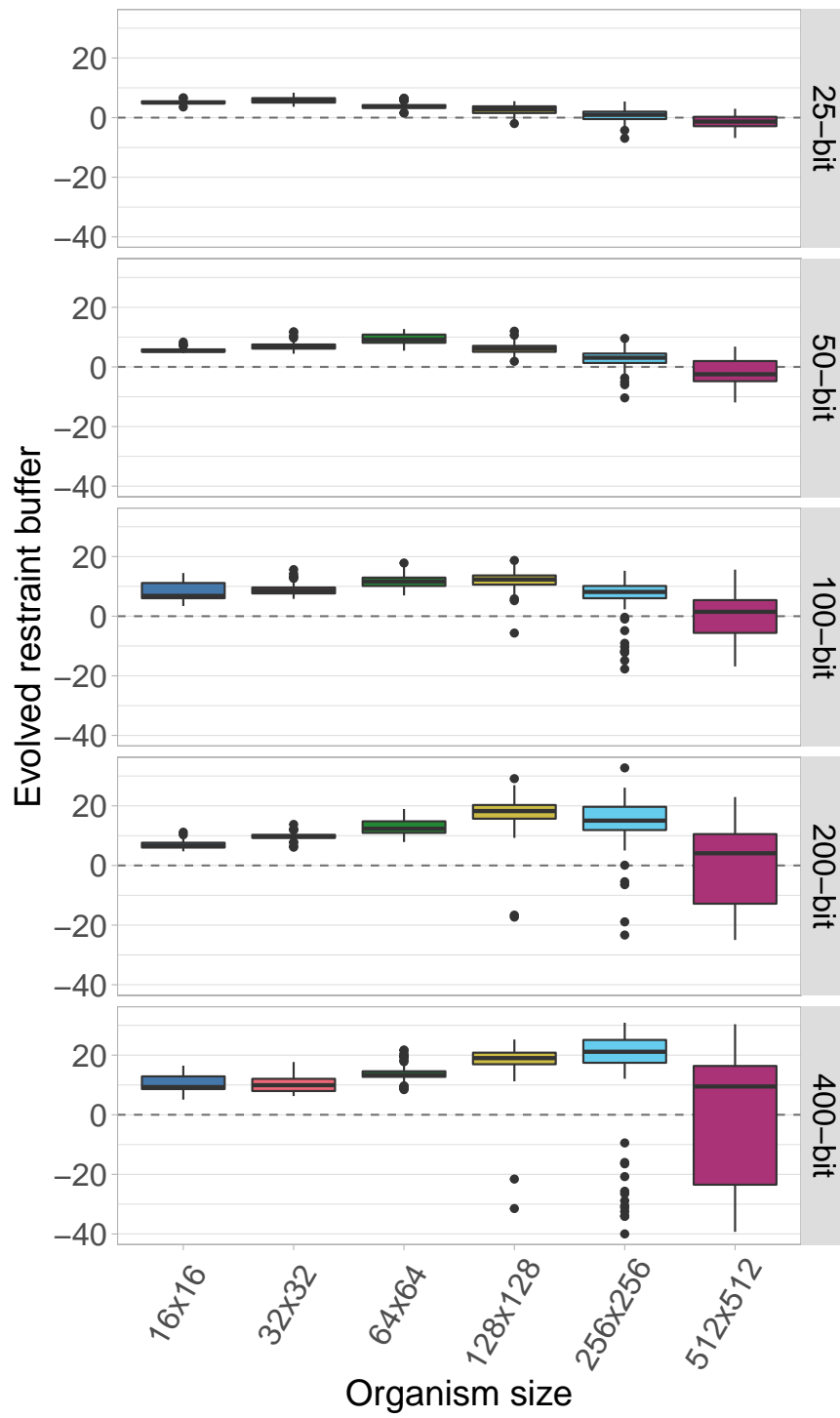


bar/color shows a different organism size.

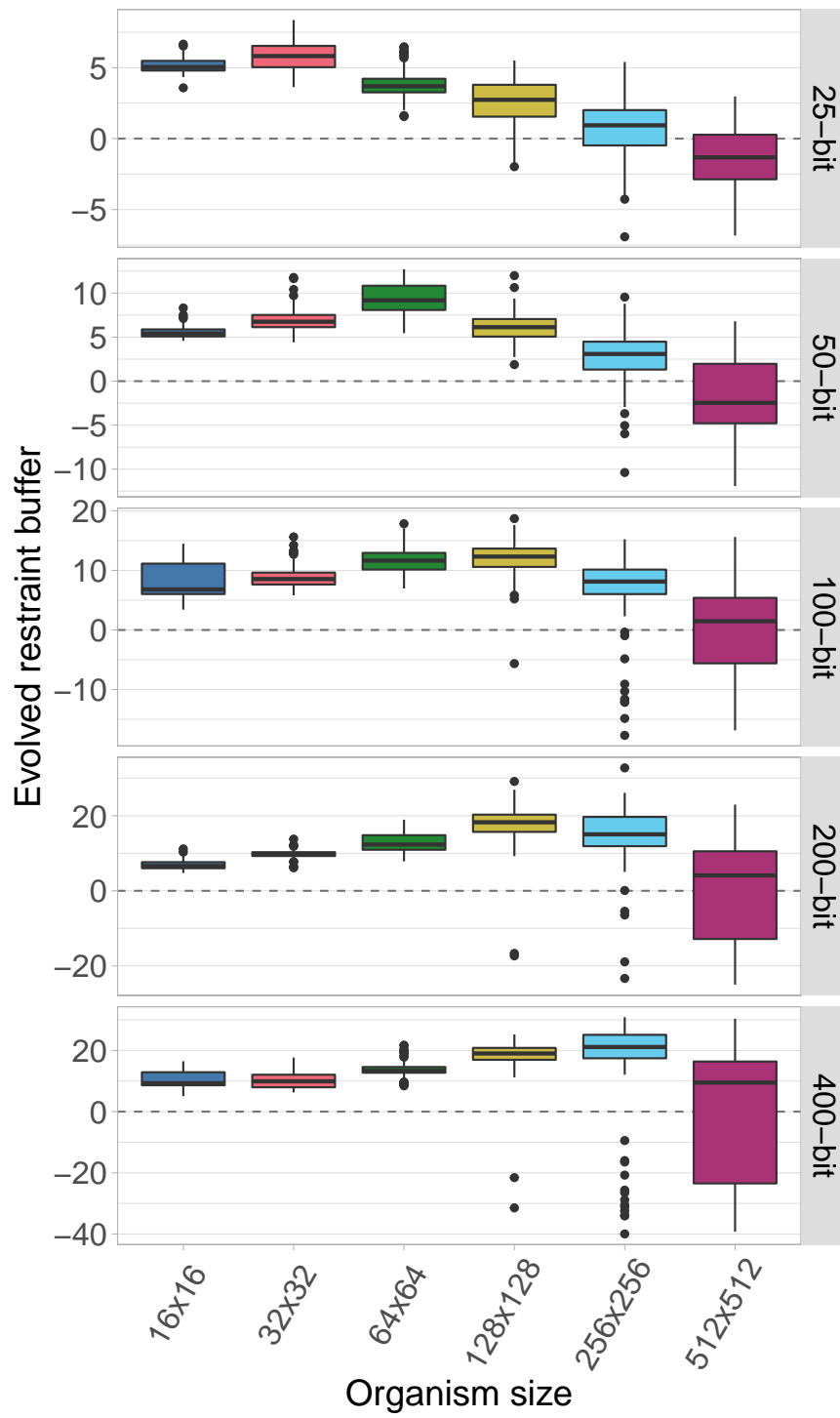
5.3 Aggregate plots

5.3.1 Facet by genome length

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

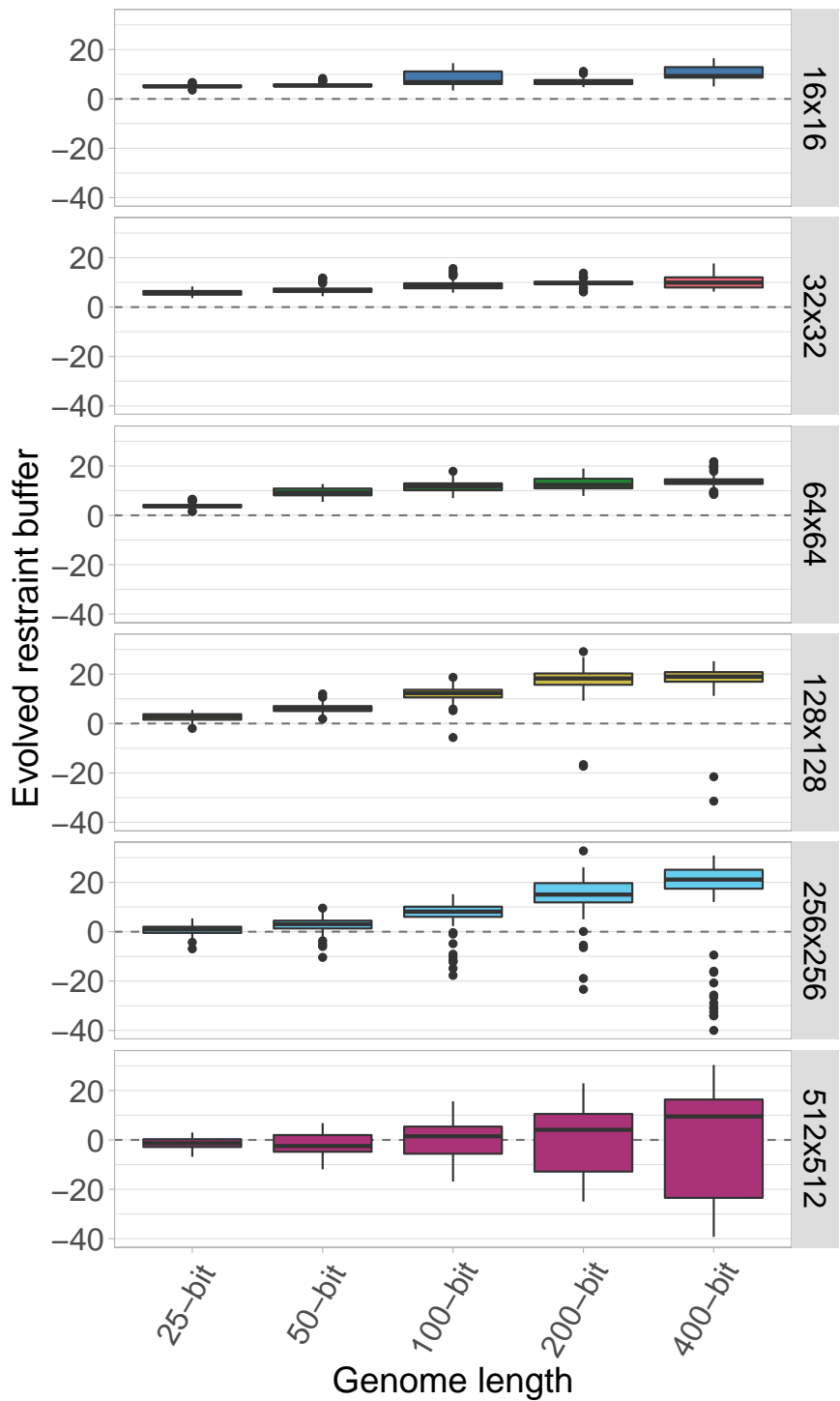


Here we plot the same data, only we allow the y-axis to vary between rows.

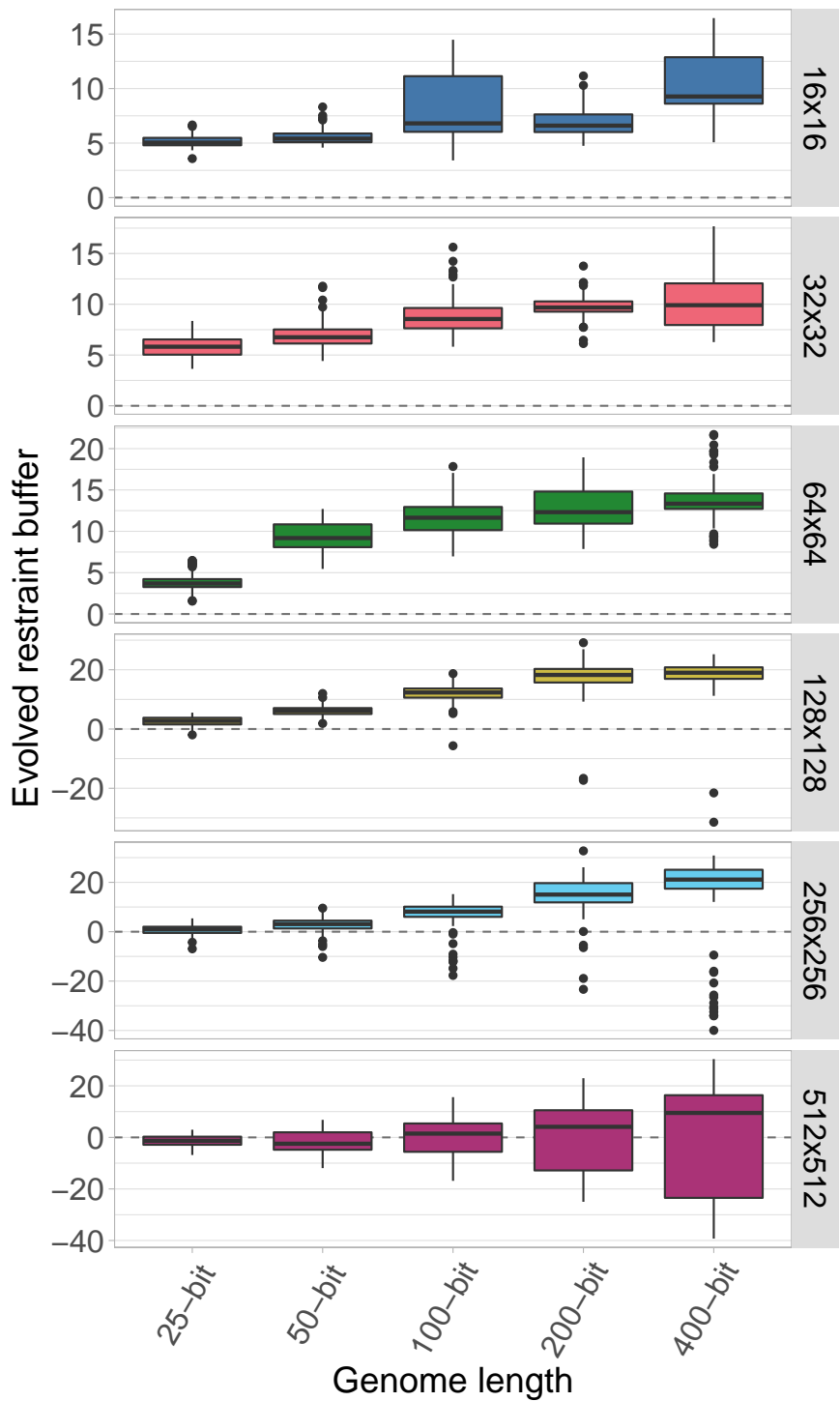


5.3.2 Facet by organism size

Here we plot the same data again, only now each row shows an organisms size while genome length varies on the x-axis.



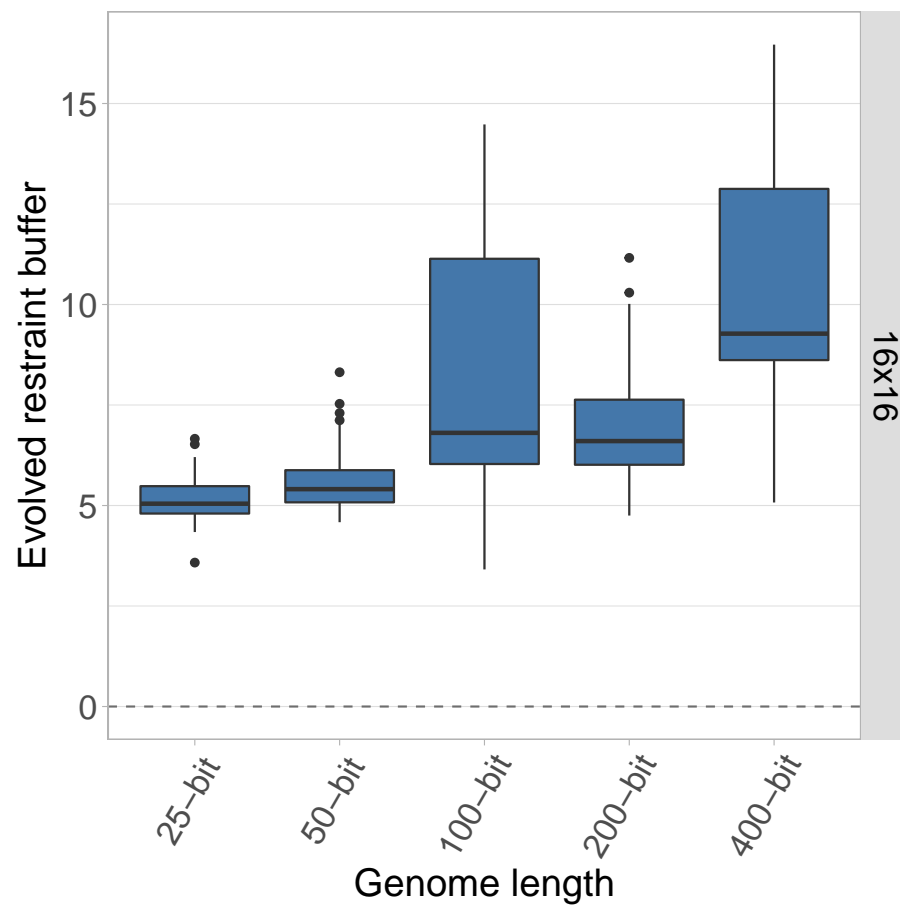
Here is the identical plot but now we allow the y-axis to vary between the rows.



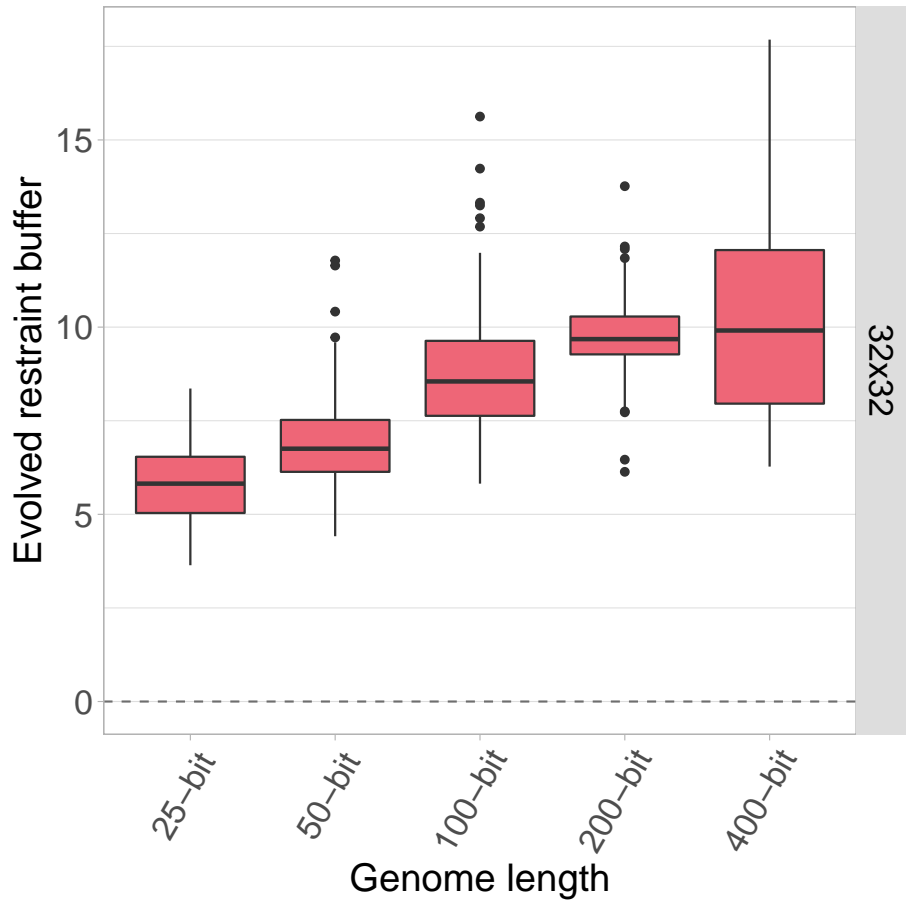
5.4 Single organism size plots

Here we plot each organism size independently, with the genome length on the x-axis.

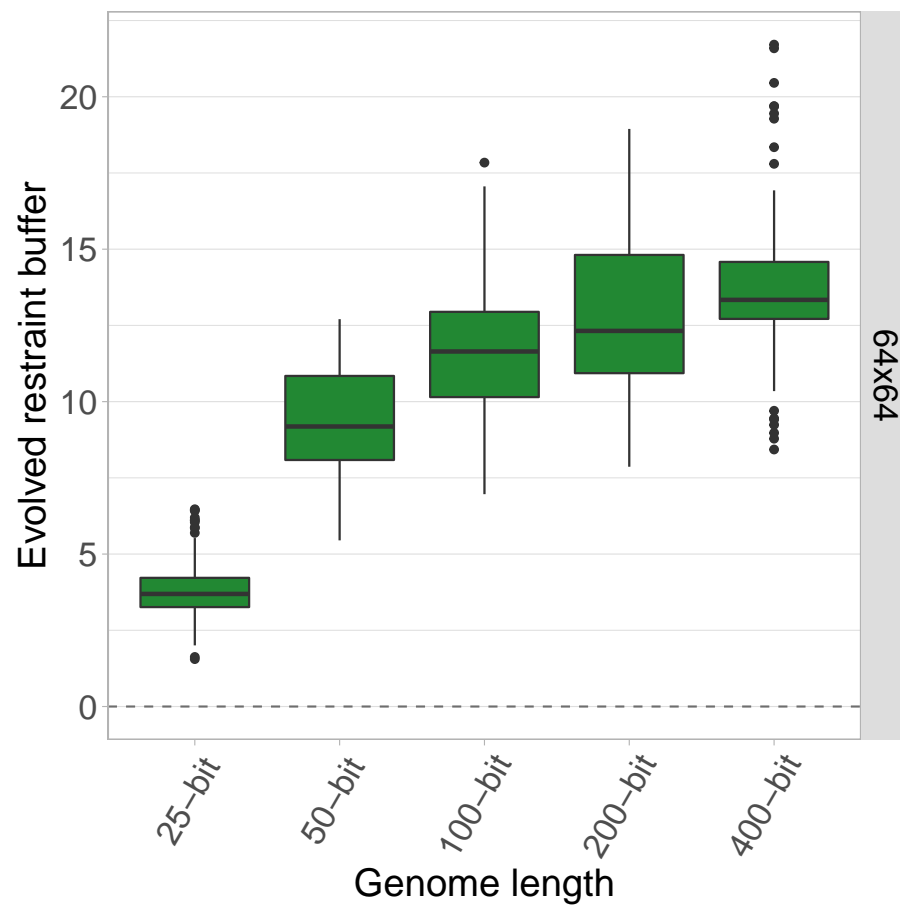
5.4.1 Organism size 16x16



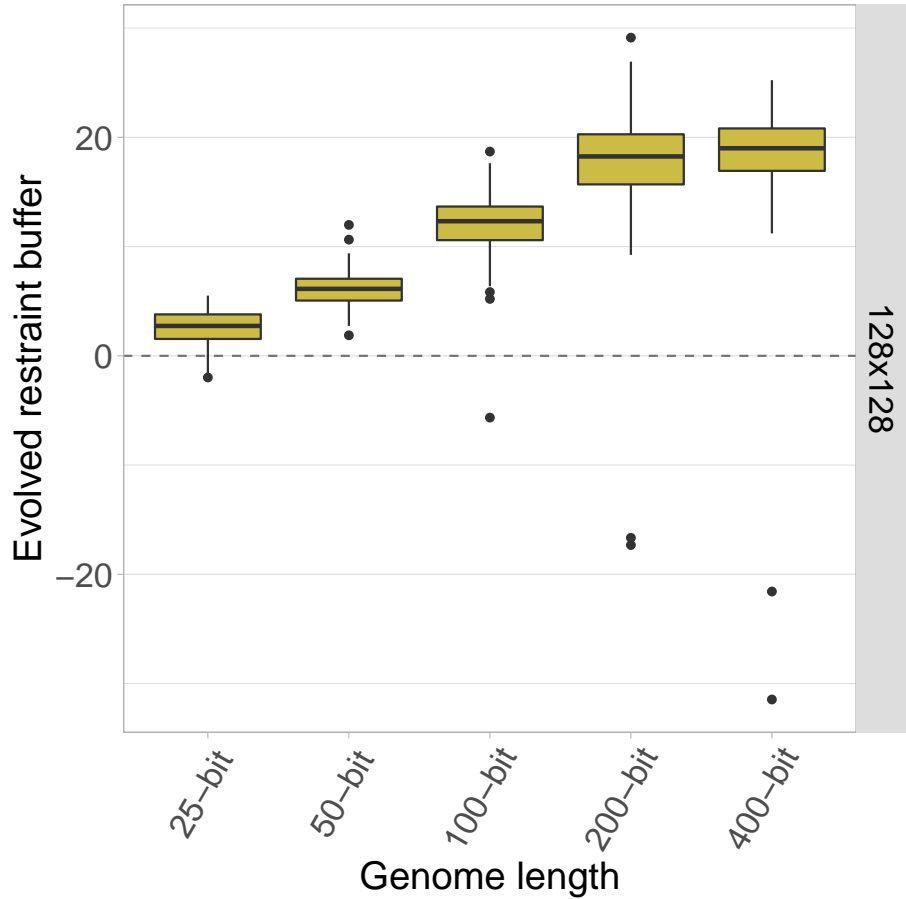
5.4.2 Organism size 32x32



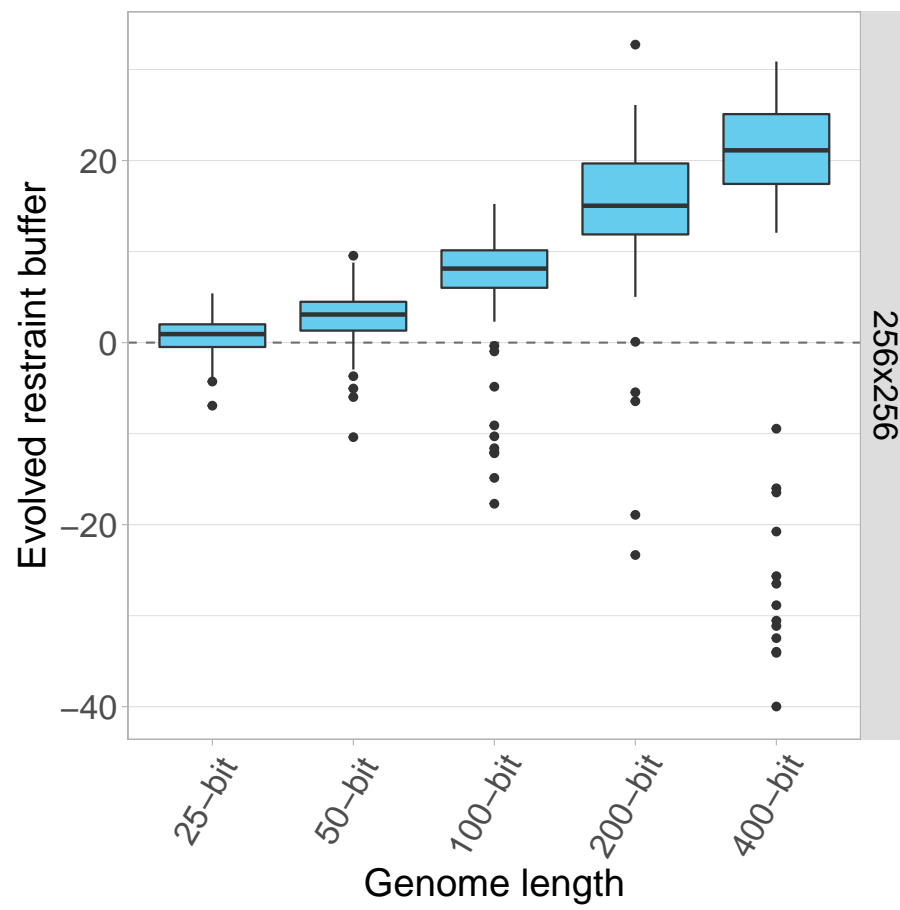
5.4.3 Organism size 64x64



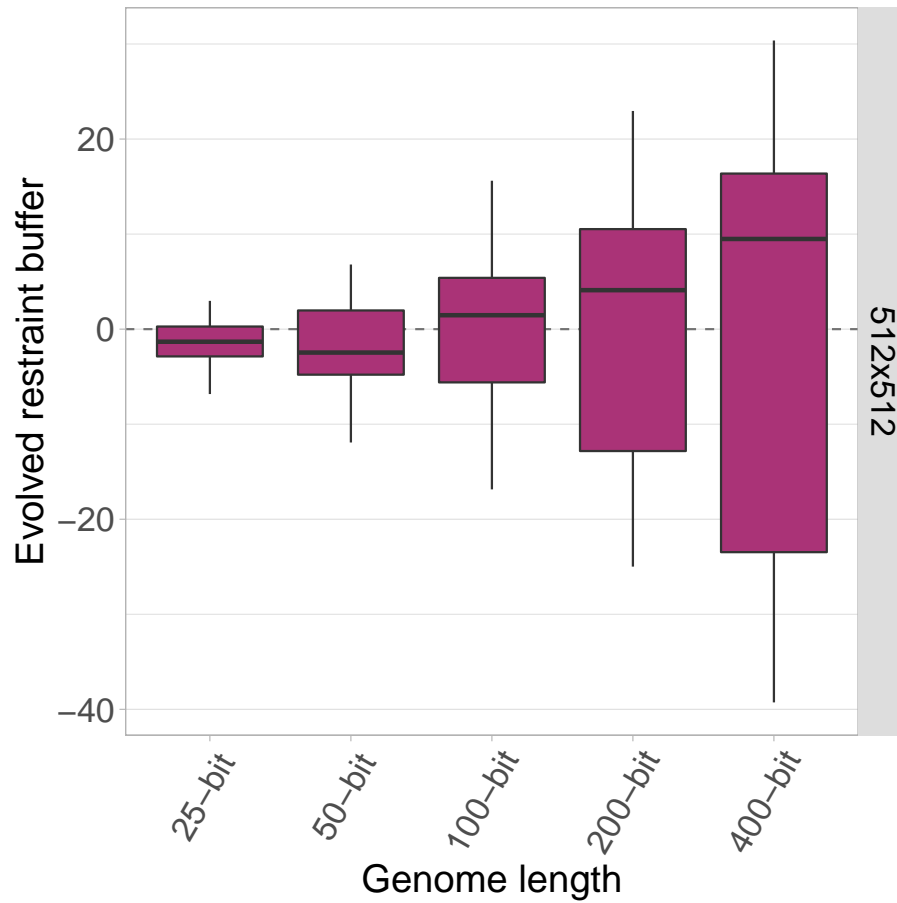
5.4.4 Organism size 128x128



5.4.5 Organism size 256x256



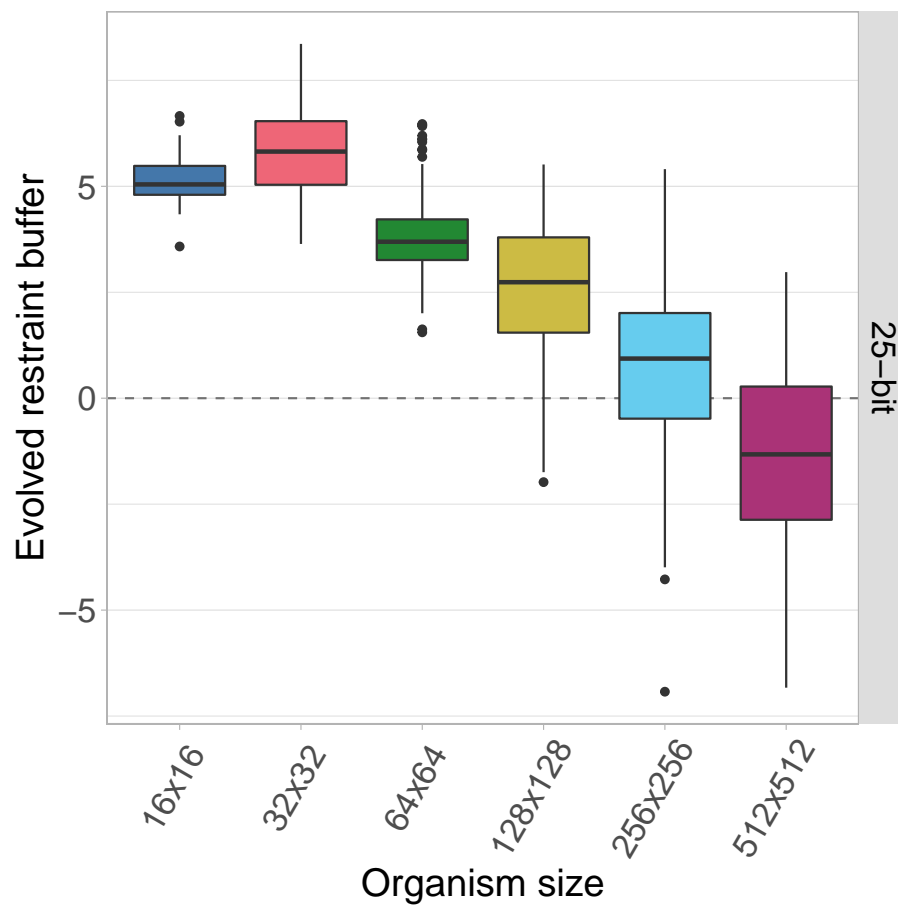
5.4.6 Organism size 512x512



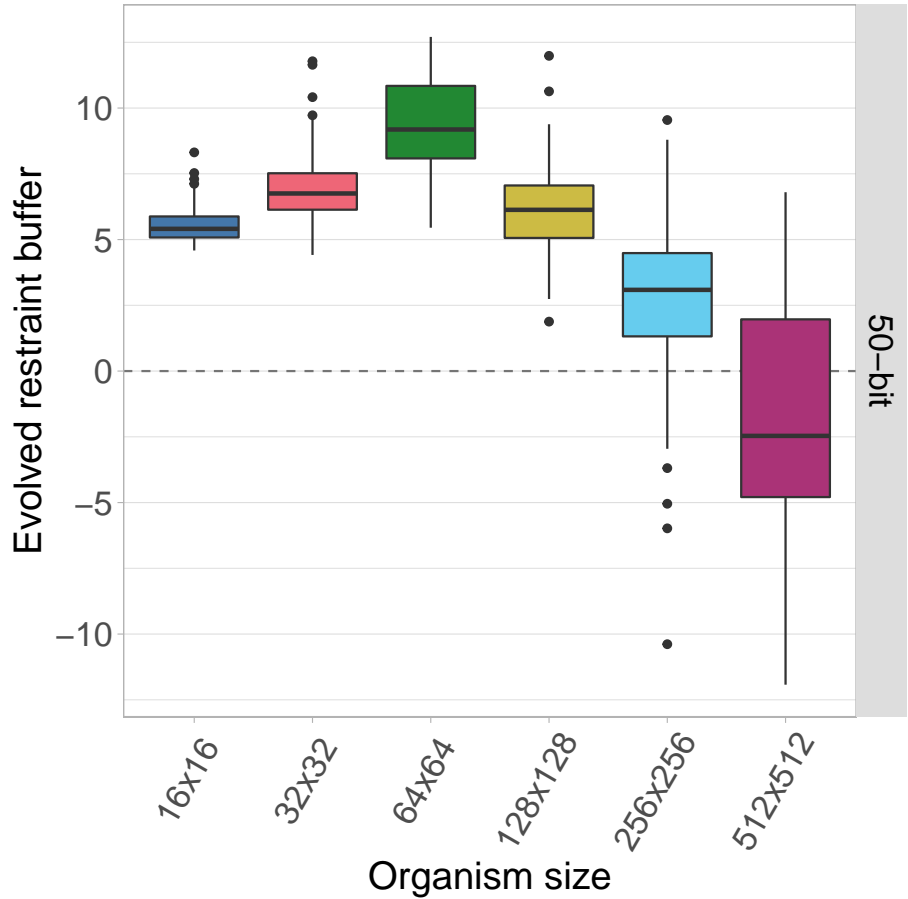
5.5 Single genome length plots

Here we plot each genome length independently, with the organism size on the x-axis.

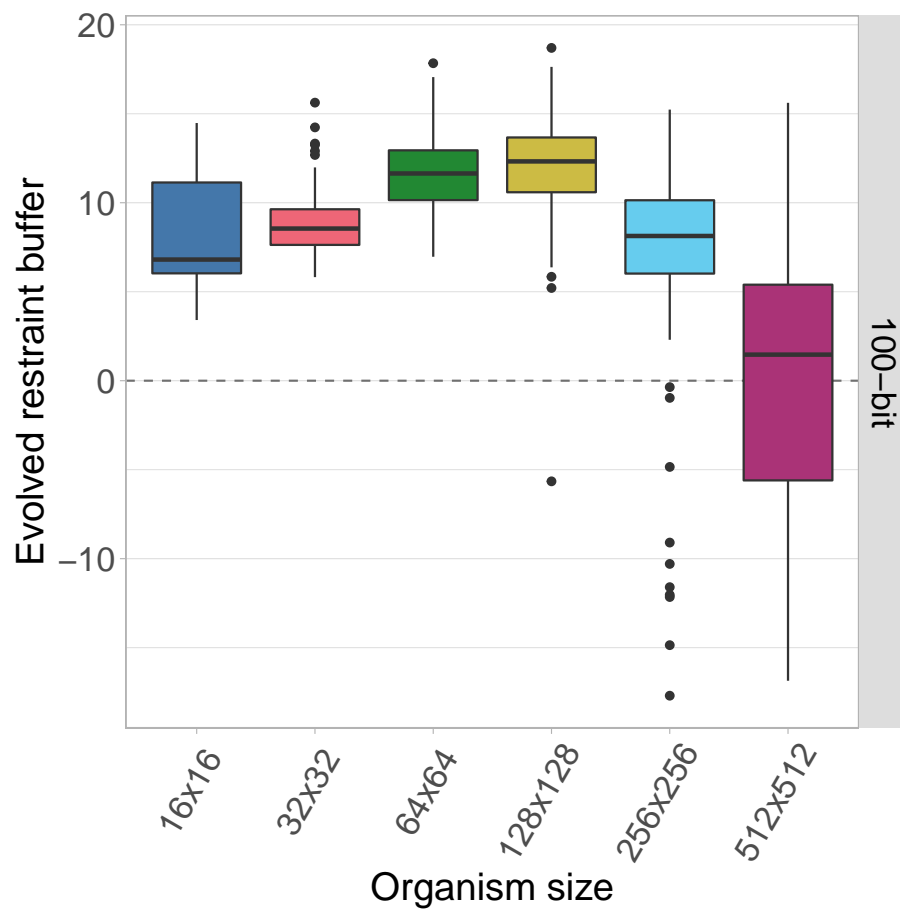
5.5.1 25-bit genomes



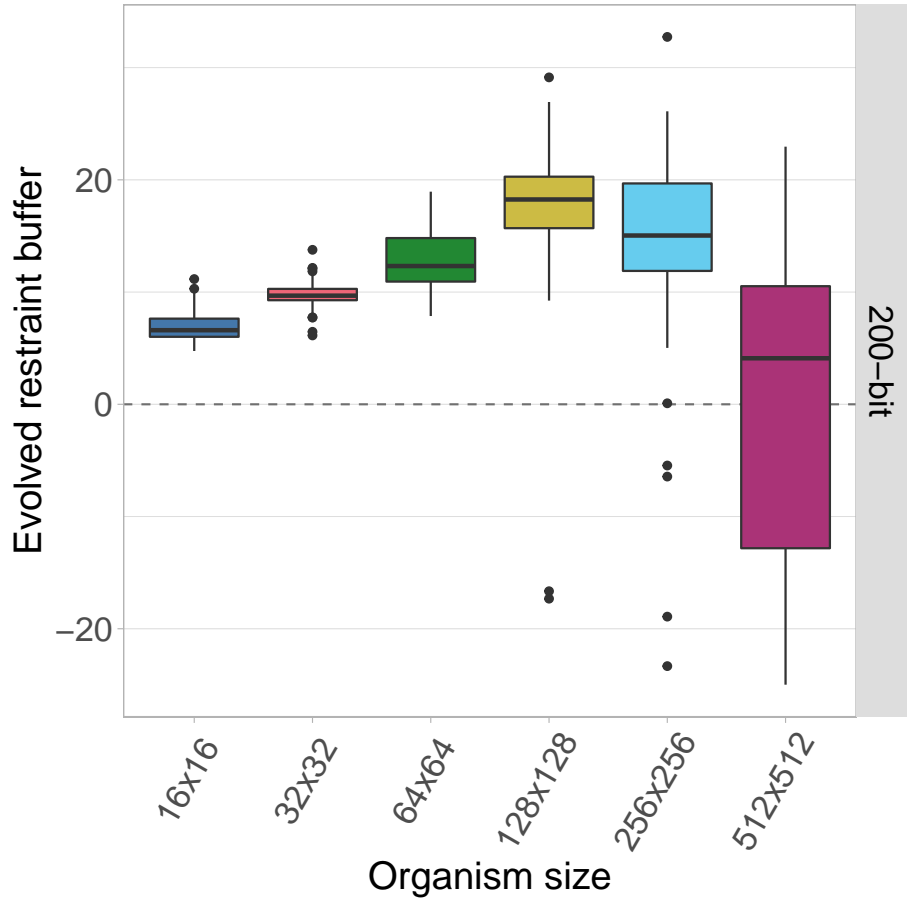
5.5.2 50-bit genomes



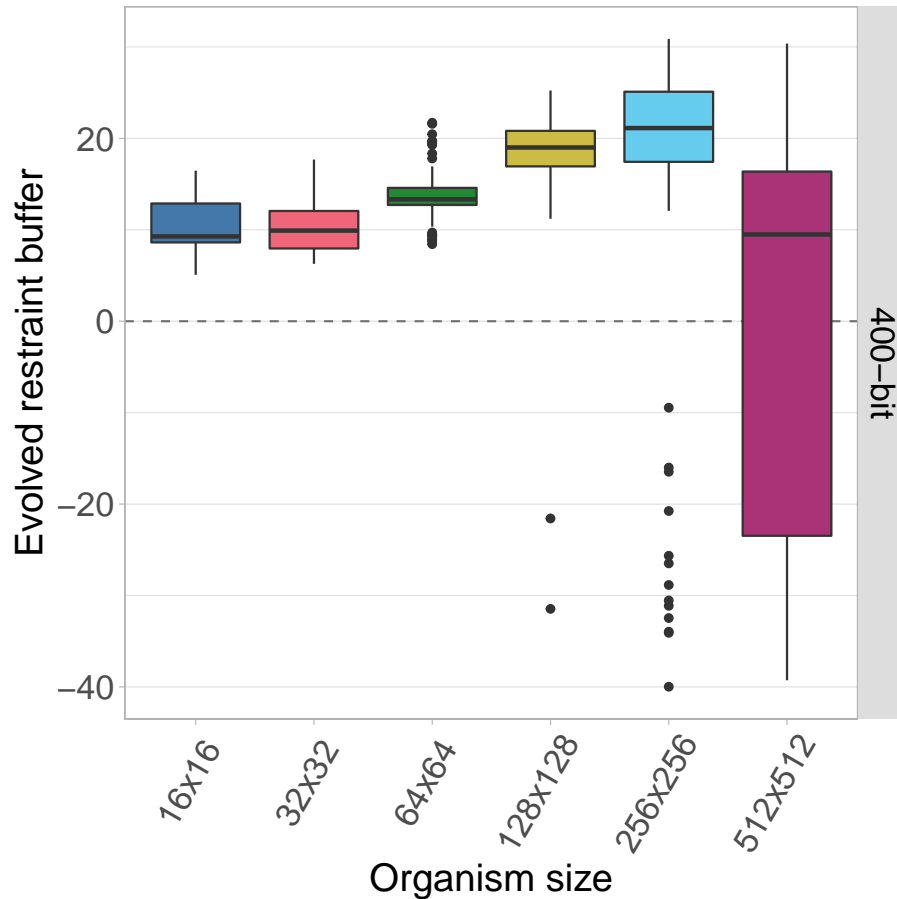
5.5.3 100-bit genomes



5.5.4 200-bit genomes



5.5.5 400-bit genomes



5.6 Statistics

Since organism size is our main point of comparison, we calculate stats for each genome length.

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.

```
length_vec = c(25, 50, 100, 200, 400)
df_kruskal = data.frame(data = matrix(nrow = 0, ncol = 4))
colnames(df_kruskal) = c('genome_length', 'p_value', 'chi_squared', 'df')
for(genome_length in length_vec){
```

```

df_test = df2[df2$LENGTH == genome_length,]
res = kruskal.test(df_test$restraint_value ~ df_test$MCSIZE, df_test)
df_kruskal[nrow(df_kruskal) + 1,] = c(genome_length, res$p.value, as.numeric(res$statistic)[1])
}
df_kruskal$less_0.01 = df_kruskal$p_value < 0.01
print(df_kruskal)

```

```

## genome_length      p_value chi_squared df less_0.01
## 1             25 1.508889e-97    461.6473 5      TRUE
## 2             50 9.772852e-87    411.5159 5      TRUE
## 3            100 7.491319e-60    286.6294 5      TRUE
## 4            200 1.626963e-75    359.4358 5      TRUE
## 5            400 2.857912e-49    237.3380 5      TRUE

```

We see that significant variation exists within each genome length, 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)
length_vec = c(25, 50, 100, 200, 400)
for(genome_length in length_vec){
  df_test = df2[df2$LENGTH == genome_length,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('genome_length', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw')
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test[df_test$MCSIZE == size_a,]$restraint_value, df_test[df_test$MCSIZE == size_b,]$restraint_value)
      df_wilcox[nrow(df_wilcox) + 1,] = c(genome_length, size_a, size_b, 0, res$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('Genome length: ', genome_length))
  print(df_wilcox)
}

```

```

## [1] "Genome length: 25"
## genome_length size_a size_b p_value_corrected p_value_raw W less_0.01
## 1             25    16    32    2.337475e-07 2.337475e-07 2883.5    TRUE
## 2             25    16    64    6.069986e-18 1.213997e-18 8607.5    TRUE
## 3             25    16   128    1.663209e-24 2.376012e-25 9258.5    TRUE
## 4             25    16   256    6.203828e-32 5.639844e-33 9896.0    TRUE
## 5             25    16   512    3.838888e-33 2.559259e-34 10000.0    TRUE
## 6             25    32    64    1.447210e-22 2.412016e-23 9074.5    TRUE
## 7             25    32   128    1.283820e-27 1.283820e-28 9542.5    TRUE

```

```

## 8          25      32      256      2.711275e-32 2.259396e-33 9927.0      TRUE
## 9          25      32      512      3.838888e-33 2.560557e-34 10000.0     TRUE
## 10         25      64      128      1.187343e-07 5.936715e-08 7219.0      TRUE
## 11         25      64      256      2.378014e-26 2.642238e-27 9430.5      TRUE
## 12         25      64      512      1.298354e-32 9.987336e-34 9954.5      TRUE
## 13         25     128      256      1.433015e-10 3.582536e-11 7710.0      TRUE
## 14         25     128      512      7.524923e-25 9.406154e-26 9294.5      TRUE
## 15         25     256      512      4.120336e-10 1.373445e-10 7627.5      TRUE
## [1] "Genome length: 50"
##      genome_length size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1          50      16      32      2.588989e-15 5.177978e-16 1681.5      TRUE
## 2          50      16      64      2.694326e-30 2.072558e-31 228.0      TRUE
## 3          50      16     128      3.224011e-03 3.224011e-03 3794.0      TRUE
## 4          50      16     256      4.089878e-15 1.022470e-15 8284.5      TRUE
## 5          50      16     512      1.182991e-27 1.182991e-28 9545.5      TRUE
## 6          50      32      64      1.797027e-17 2.567181e-18 1427.0      TRUE
## 7          50      32     128      7.415731e-04 3.707866e-04 6457.5      TRUE
## 8          50      32     256      1.165570e-21 1.295078e-22 9005.5      TRUE
## 9          50      32     512      4.567933e-31 3.262810e-32 9836.0      TRUE
## 10         50      64     128      2.265727e-21 2.832159e-22 8973.0      TRUE
## 11         50      64     256      8.866606e-30 7.388839e-31 9727.5      TRUE
## 12         50      64     512      7.213069e-33 4.808713e-34 9979.0      TRUE
## 13         50     128     256      4.869262e-16 8.115436e-17 8409.5      TRUE
## 14         50     128     512      4.777498e-28 4.343180e-29 9582.0      TRUE
## 15         50     256     512      3.236734e-12 1.078911e-12 7914.5      TRUE
## [1] "Genome length: 100"
##      genome_length size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1          100      16      32      7.697389e-02 1.924347e-02 4041.5     FALSE
## 2          100      16      64      3.952168e-13 7.904337e-14 1941.5      TRUE
## 3          100      16     128      2.398968e-14 2.998710e-15 1770.0      TRUE
## 4          100      16     256      4.158441e-01 4.158441e-01 5333.5     FALSE
## 5          100      16     512      5.614119e-18 4.678432e-19 8651.0      TRUE
## 6          100      32      64      1.034976e-13 1.478537e-14 1852.5      TRUE
## 7          100      32     128      3.085548e-17 3.085548e-18 1435.5      TRUE
## 8          100      32     256      1.117541e-01 3.725137e-02 5853.0     FALSE
## 9          100      32     512      2.483010e-22 1.910008e-23 9084.0      TRUE
## 10         100      64     128      1.117541e-01 4.890986e-02 4193.5     FALSE
## 11         100      64     256      5.002561e-15 5.558401e-16 8315.0      TRUE
## 12         100      64     512      5.082595e-28 3.388396e-29 9591.0      TRUE
## 13         100     128     256      1.590814e-17 1.446195e-18 8599.5      TRUE
## 14         100     128     512      9.444159e-28 6.745828e-29 9566.0      TRUE
## 15         100     256     512      2.634367e-13 4.390611e-14 8090.0      TRUE
## [1] "Genome length: 200"
##      genome_length size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1          200      16      32      4.663546e-26 3.886289e-27 584.0      TRUE
## 2          200      16      64      7.523609e-32 5.015739e-33 100.0      TRUE

```

```

## 3      200      16      128      2.146093e-30 1.532923e-31 217.5      TRUE
## 4      200      16      256      1.997886e-24 1.911300e-25 733.0      TRUE
## 5      200      16      512      9.462181e-03 9.462181e-03 6062.5     TRUE
## 6      200      32      64       1.344008e-20 1.493343e-21 1097.0     TRUE
## 7      200      32      128      5.645064e-28 4.342357e-29 418.0      TRUE
## 8      200      32      256      1.309572e-19 1.636965e-20 1200.0     TRUE
## 9      200      32      512      2.440723e-07 6.101808e-08 7217.0     TRUE
## 10     200      64      128      1.166719e-18 1.666742e-19 1302.5     TRUE
## 11     200      64      256      9.151807e-05 3.050602e-05 3293.0     TRUE
## 12     200      64      512      6.237644e-15 1.247529e-15 8274.5     TRUE
## 13     200     128      256      9.982635e-05 4.991318e-05 6660.5     TRUE
## 14     200     128      512      1.997886e-24 1.816260e-25 9269.0     TRUE
## 15     200     256      512      1.717006e-17 2.861676e-18 8568.0     TRUE
## [1] "Genome length: 400"
##      genome_length size_a size_b p_value_corrected p_value_raw      W less_0.01
## 1      400      16      32      5.405382e-01 5.348472e-01 5254.5     FALSE
## 2      400      16      64      3.472338e-14 3.472338e-15 1777.5     TRUE
## 3      400      16      128     4.072814e-28 2.715209e-29 401.0      TRUE
## 4      400      16      256     1.163125e-16 9.692706e-18 1489.0     TRUE
## 5      400      16      512     5.405382e-01 1.801794e-01 5549.0     FALSE
## 6      400      32      64      5.784416e-12 8.263451e-13 2070.5     TRUE
## 7      400      32      128     1.489024e-26 1.063588e-27 535.5      TRUE
## 8      400      32      256     2.187697e-16 1.988815e-17 1523.0     TRUE
## 9      400      32      512     5.405382e-01 1.825748e-01 5546.0     FALSE
## 10     400      64      128     2.993077e-18 2.302367e-19 1317.0     TRUE
## 11     400      64      256     3.197608e-12 3.997010e-13 2030.0     TRUE
## 12     400      64      512     8.293488e-05 1.658698e-05 6763.0     TRUE
## 13     400     128      256     1.019174e-02 2.547935e-03 3764.5     FALSE
## 14     400     128      512     9.137039e-13 1.015227e-13 8045.0     TRUE
## 15     400     256      512     6.084030e-12 1.014005e-12 7918.0     TRUE

```


Chapter 6

Timing sample count experiment

By default, we calculated 100 timing samples for each combination of organism size and restraint buffer value to use for fitness in Primordium (a fresh batch for each experiment). With this experiment we showed that increasing this number to 10,000 has no qualitative difference on results. This was done by replicating the baseline experiment using 10,000 samples and comparing the results to a fresh run with 100 samples.

6.1 Data cleaning

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_24__finite_10k_samples/evolution/data/scraped_evolution_data.csv')
df = rbind(df, read.csv('../experiments/2021_02_24__finite_10k_samples/evolution/data/scraped_evolution_data.csv'))
df = rbind(df, read.csv('../experiments/2021_02_24__finite_10k_samples/evolution/data/scraped_evolution_data.csv'))
df$LENGTH = 100
df = rbind(df, read.csv('../experiments/2021_02_24__finite_10k_samples/evolution/data/scraped_evolution_data.csv'))
df = rbind(df, read.csv('../experiments/2021_02_24__finite_10k_samples/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.

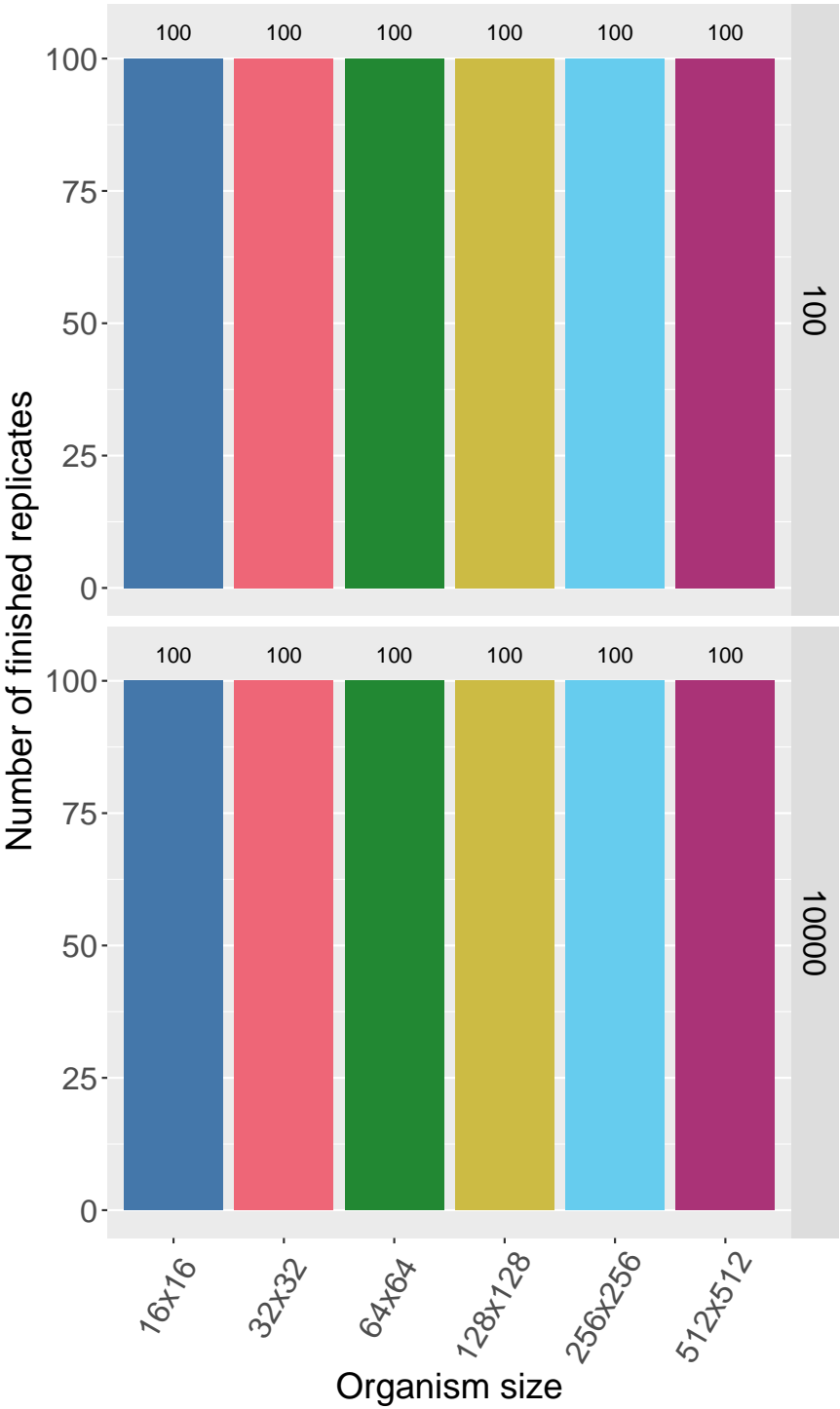
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, SAMPLES)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n)
```

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$size_str, levels = c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024'))
df2$size_factor_reversed = factor(df2$size_str, levels = rev(c('16x16', '32x32', '64x64', '128x128', '256x256', '512x512', '1024x1024')))
data_summary$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'))
# 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
```

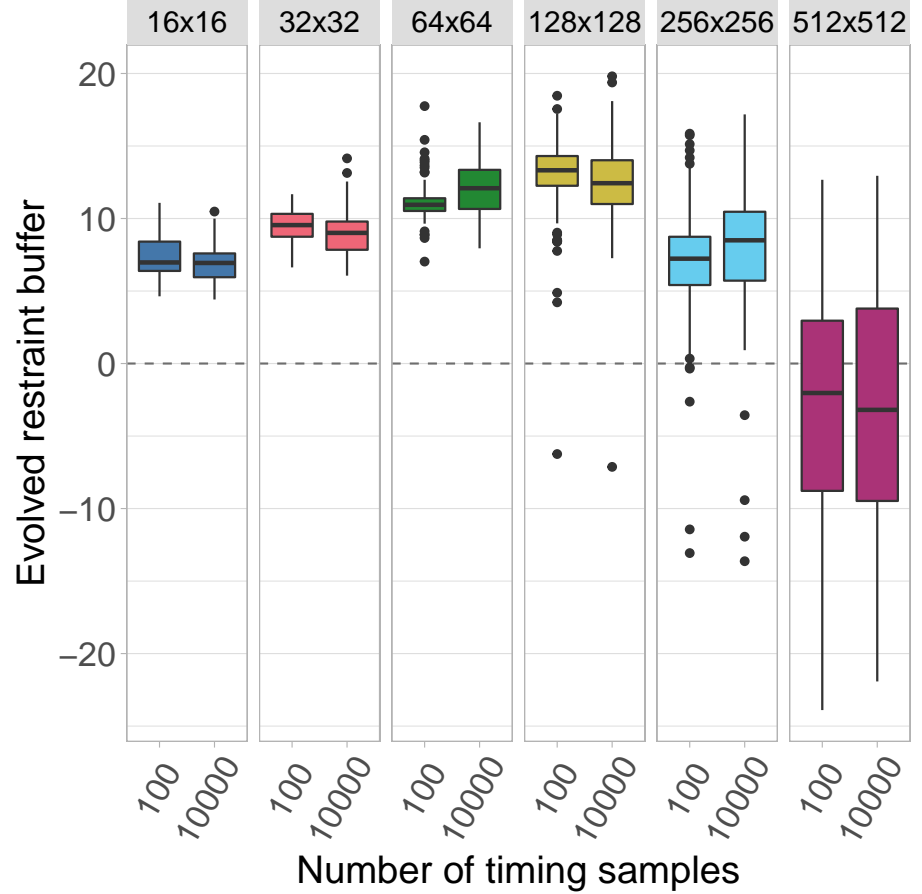
6.2 Data integrity check

Now we plot the number of finished replicates for each treatment to make sure all data are present. Rows show the number of samples used for fitness. Each bar/color shows a different organism size.



6.3 Plot

Here we plot all the data. The figure is split into 6 subplots, each showing a different organism size. Inside each subplot, the number of timing samples is shown on



the x-axis.

6.4 Statistics

The plot shows that the general trend, that the evolved restraint buffer initially increases with organism but then decreases, holds true at both sample counts. Further, we see that the evolved buffer values are fairly consistent between the two sample counts.

While we concluded this was sufficient evidence to use only 100 samples (10,000 is intractable to run for multiple experiments), we include the statistics here. Since we treat each organism size as a group, we simply take a Wilcoxon Rank-Sum test between 100 samples and 10,000 samples

##	org_size	p_value	W	less_0.01
## 1	16	4.243294e-02	5831.0	FALSE
## 2	32	3.489808e-04	6464.0	TRUE
## 3	64	4.913265e-05	3338.0	TRUE
## 4	128	3.021256e-02	5887.5	FALSE
## 5	256	2.561216e-02	4086.0	FALSE
## 6	512	9.066359e-01	5048.5	FALSE