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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 prelimary 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(ggridges)
library(scales)
library(khroma)
```

Load the data and trim all the unecessay 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 (artifiacts 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.

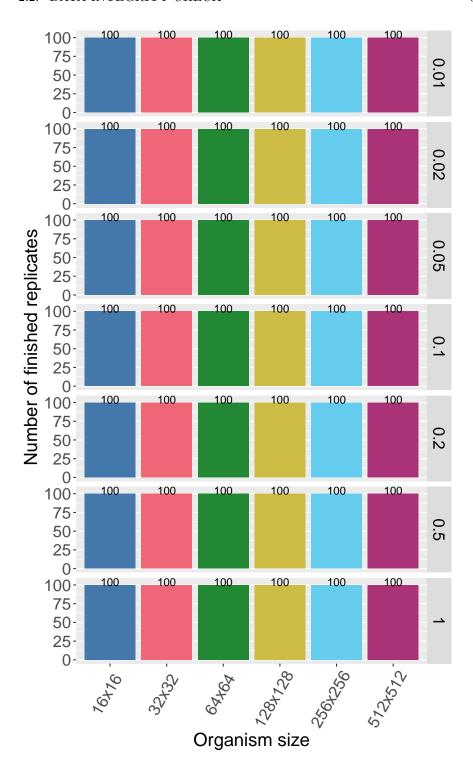
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, CELLMUT)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n
```

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$MCSIZE)
df2\size_factor = factor(df2\size_str, levels = c('16x16', '32x32', '64x64', '128x128'
df2\$size_factor_reversed = factor(df2\$size_str, levels = rev(c('16x16', '32x32', '64x6e'))
df2$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',
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.
# 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

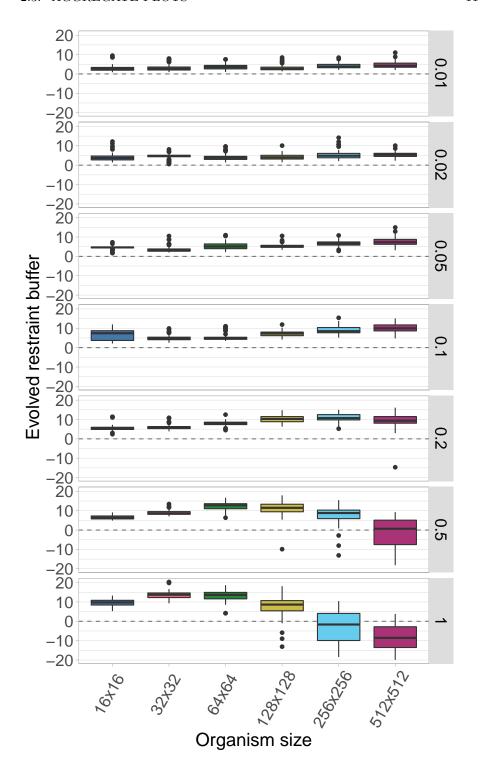
Now we plot the number of finished replicates for each treatment to make sure all data are present. Each row shows a different somatic mutation rate. Each bar/color shows a different organism size.



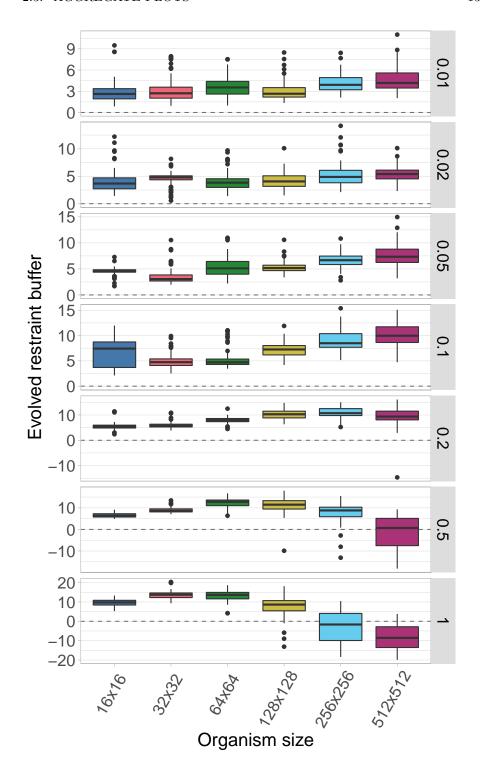
2.3 Aggregate plots

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.

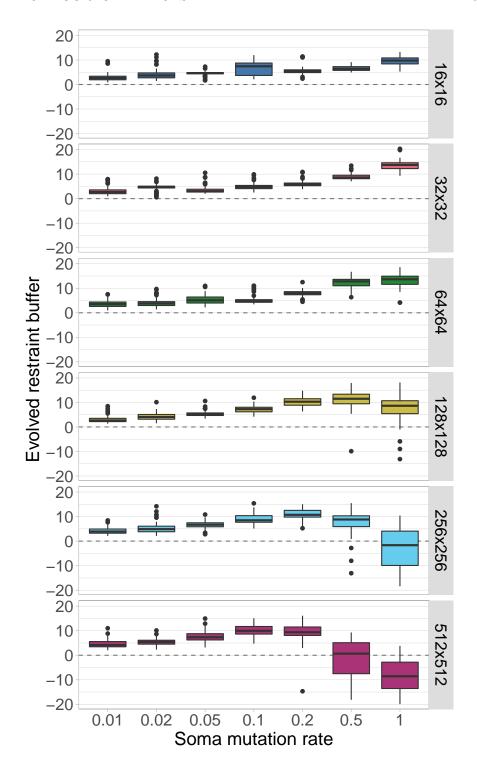


Here we plot the same data, only we allow the y-axis to vary between rows.

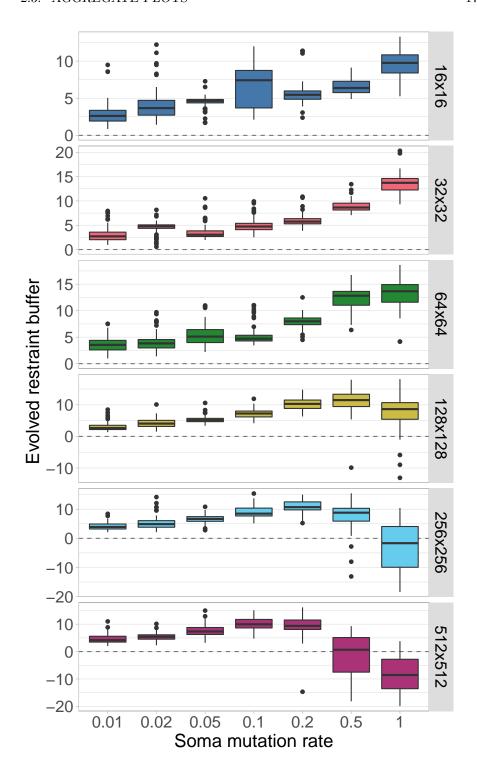


2.3.2 Facet by organism size

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.



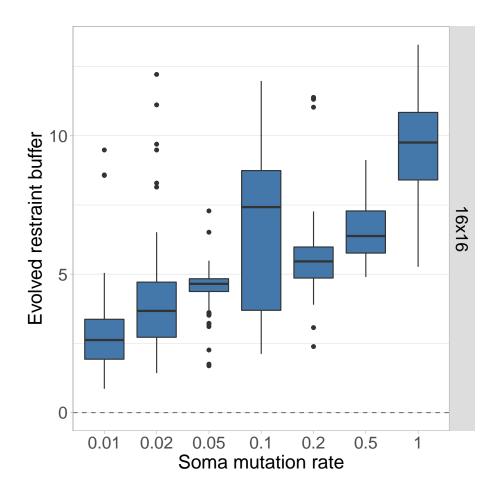
Again, we replot the same data but allow the y-axis to vary between rows.



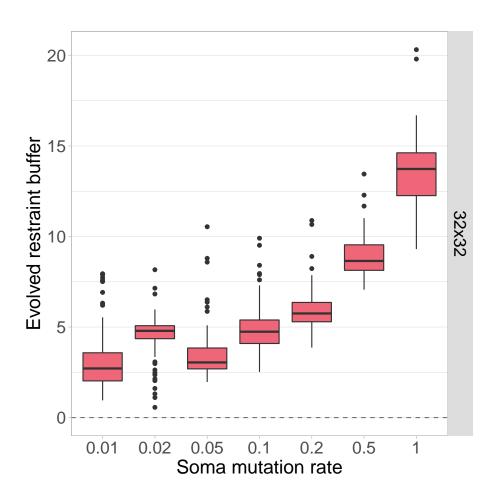
2.4 Single organism size plots

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

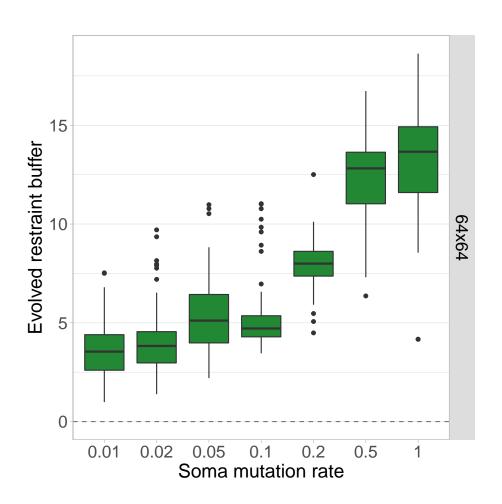
2.4.1 Organism size 16x16



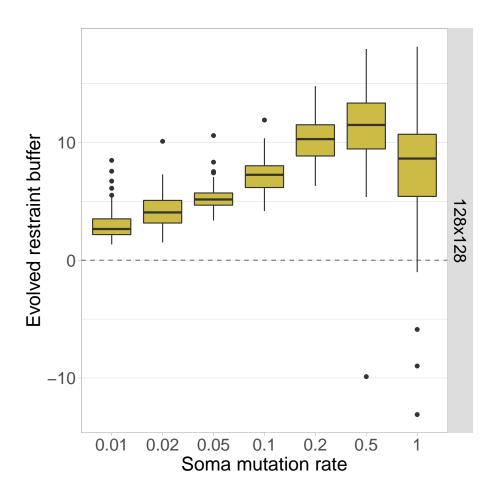
$2.4.2 \quad {\rm Organism \ size} \ 32x32$



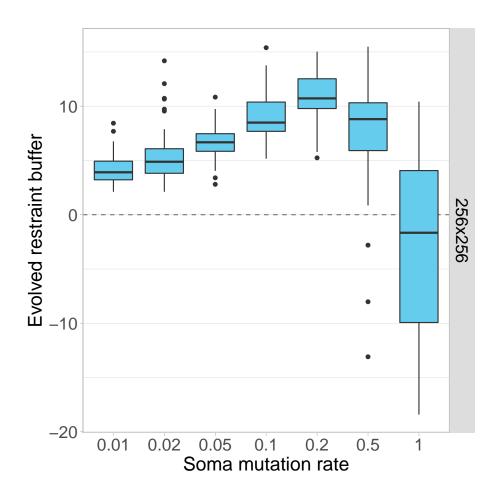
2.4.3 Organism size 64x64



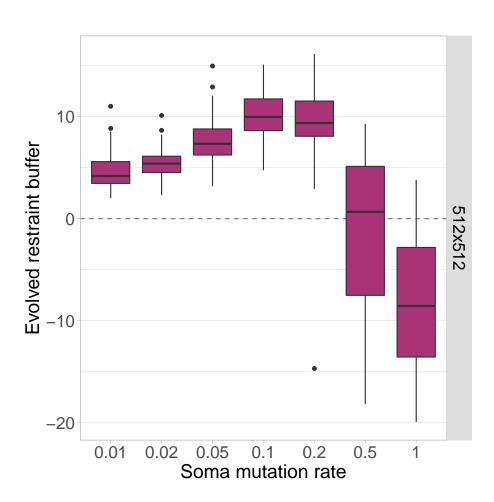
$2.4.4 \quad {\rm Organism~size}~128 {\rm x} 128$



$2.4.5 \quad {\rm Organism~size}~256 {\rm x} 256$



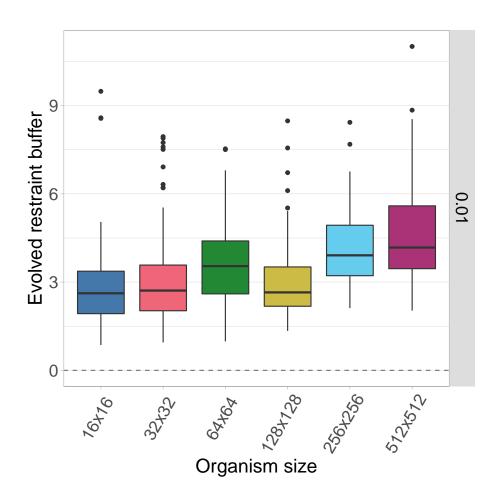
$2.4.6 \quad \text{Organism size } 512\text{x}512$



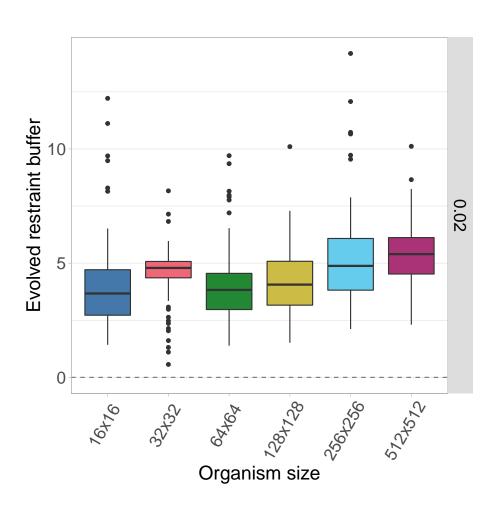
2.5 Single somatic mutation rate plots

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

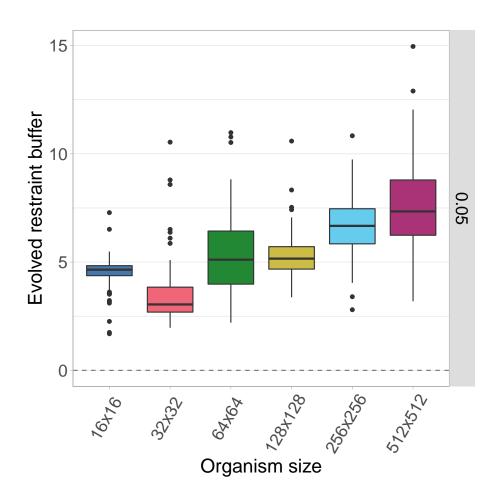
2.5.1 Somatic mut. rate 0.01



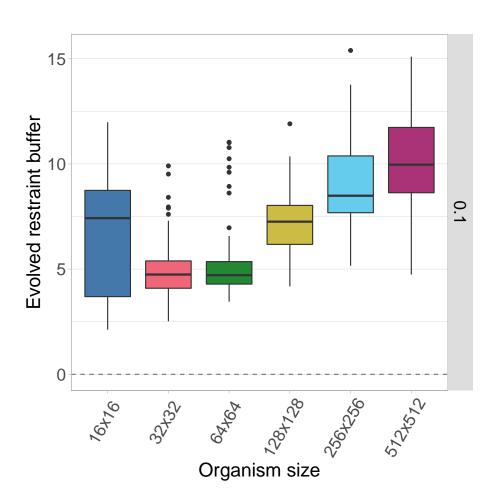
2.5.2 Somatic mut. rate 0.02



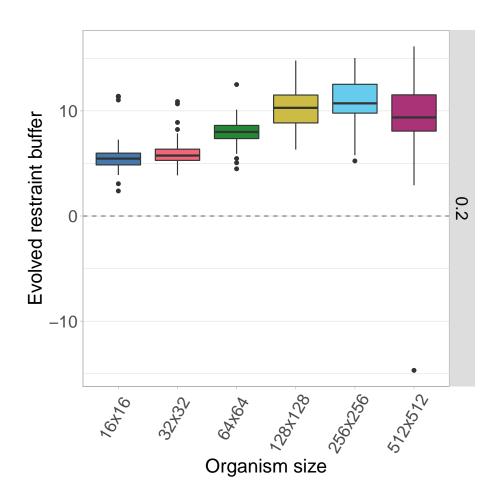
2.5.3 Somatic mut. rate 0.05



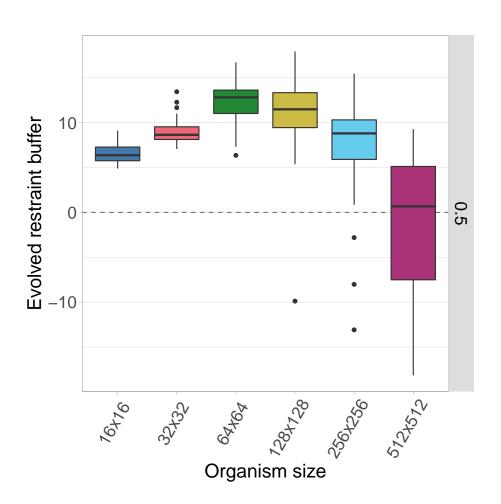
2.5.4 Somatic mut. rate 0.1



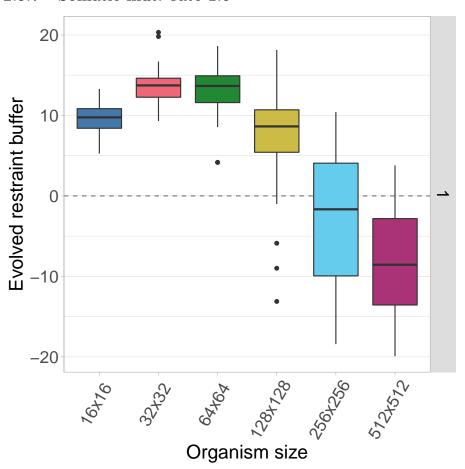
2.5.5 Somatic mut. rate 0.2



2.5.6 Somatic mut. rate 0.5







2.6 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 perfrm 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,]
```

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```
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</pre>
  print(df_kruskal)
##
     soma_mut_rate
                        p_value chi_squared df less_0.01
## 1
              0.01 2.661659e-25
                                    125.0566
                                              5
                                                     TRUE
                                                     TRUE
## 2
              0.02 4.808020e-19
                                     95.4471
                                              5
## 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
pariwise Wilcoxon tests on each to see which pais 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$CELLMUT == mut_rate,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('mut_rate', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw', 'W'
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test$MCSIZE == size_a,)$restraint_value, df_test[df_test$MCSIZE
      df_wilcox[nrow(df_wilcox) + 1,] = c(mut_rate, size_a, size_b, 0, res$p.value, as.numeric(re
  df_wilcox$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
                         256
                                   2.034819e-12 1.453442e-13 1974.5
                                                                          TRUE
                   16
## 5
          0.01
                   16
                         512
                                   4.368517e-15 2.912344e-16 1653.0
                                                                          TRUE
                                   1.074876e-02 1.535537e-03 3703.0
## 6
          0.01
                   32
                          64
                                                                         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
          0.01
                    32
## 9
                          512
                                    1.748038e-11 1.456698e-12 2102.5
                                                                            TRUE
## 10
          0.01
                    64
                          128
                                    1.074876e-02 1.601365e-03 6292.0
                                                                           FALSE
                                    1.397091e-02 2.794183e-03 3776.0
## 11
          0.01
                    64
                          256
                                                                           FALSE
## 12
          0.01
                    64
                          512
                                    7.748038e-05 8.608931e-06 3178.5
                                                                            TRUE
## 13
          0.01
                   128
                                    3.676583e-09 3.342348e-10 2428.5
                                                                            TRUE
                          256
## 14
          0.01
                   128
                          512
                                    2.110112e-12 1.623163e-13 1980.5
                                                                            TRUE
                   256
                                    2.266729e-01 5.666822e-02 4219.5
## 15
          0.01
                          512
                                                                           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
                          128
## 3
          0.02
                    16
                                    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
                                    1.240992e-05 1.240992e-06 6985.0
                                                                            TRUE
                           64
## 7
          0.02
                    32
                          128
                                    2.150816e-02 3.584693e-03 6192.5
                                                                           FALSE
## 8
          0.02
                                    3.993493e-01 9.983733e-02 4326.0
                    32
                          256
                                                                           FALSE
## 9
                                    1.117168e-04 1.396459e-05 3221.5
          0.02
                    32
                          512
                                                                            TRUE
                                    4.025666e-01 2.012833e-01 4476.5
## 10
          0.02
                    64
                          128
                                                                           FALSE
                                    5.648464e-06 5.134967e-07 2944.5
## 11
          0.02
                    64
                          256
                                                                            TRUE
          0.02
                                    6.120346e-11 4.371676e-12 2165.5
## 12
                    64
                          512
                                                                            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
          0.02
                   256
                                    3.993493e-01 1.013587e-01 4329.0
## 15
                          512
                                                                           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
                                    2.819711e-09 5.639421e-10 2462.0
                                                                            TRUE
                    16
                          128
## 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
                                    9.865308e-14 1.409330e-14 1850.0
                                                                            TRUE
                           64
                                    9.672216e-22 8.792924e-23
## 7
          0.05
                    32
                          128
                                                               978.5
                                                                            TRUE
## 8
          0.05
                                    4.456762e-26 3.183402e-27
                    32
                          256
                                                                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
                                    4.409184e-09 1.102296e-09 2505.5
                    64
                          256
                                                                            TRUE
## 12
          0.05
                                    1.967988e-13 3.279979e-14 1894.5
                    64
                          512
                                                                            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
           0.1
## 1
                    16
                           32
                                    3.903716e-03 9.759291e-04 6350.0
                                                                            TRIIF
```

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```
## 2
           0.1
                    16
                           64
                                    9.815188e-02 3.271729e-02 5874.5
                                                                            FALSE
## 3
                                    6.061146e-01 3.140880e-01 4587.5
           0.1
                    16
                          128
                                                                            FALSE
## 4
                    16
                          256
                                    3.278276e-08 5.463793e-09 2612.5
                                                                             TRUE
           0.1
## 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
                                    6.950798e-29 4.964856e-30
           0.1
                    32
                          256
                                                                 340.0
                                                                             TRUE
## 9
           0.1
                    32
                          512
                                    1.934395e-30 1.289597e-31
                                                                 211.5
                                                                             TRUE
                                    2.239733e-18 2.488592e-19 1320.5
## 10
           0.1
                    64
                          128
                                                                             TRUE
## 11
                          256
                                    1.194130e-25 9.951080e-27
           0.1
                    64
                                                                 619.5
                                                                             TRUE
## 12
           0.1
                    64
                          512
                                    1.966283e-27 1.512525e-28
                                                                 463.5
                                                                             TRUE
## 13
           0.1
                          256
                                    8.038941e-11 1.148420e-11 2222.0
                                                                             TRUE
                   128
## 14
           0.1
                   128
                          512
                                    1.880691e-21 1.709719e-22 1006.0
                                                                             TRUE
## 15
                   256
                                    3.931365e-04 7.862729e-05 3383.5
           0.1
                          512
                                                                             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
                                    5.255254e-22 7.507505e-23
                                                                 972.0
                           64
                                                                             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
           0.2
## 10
                    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
                                    6.935985e-03 2.311995e-03 3752.5
           0.2
                   128
                          256
                                                                             TRUE
## 14
           0.2
                   128
                          512
                                    1.987108e-01 1.987108e-01 5526.5
                                                                            FALSE
## 15
                   256
                                    3.309684e-04 8.274210e-05 6611.5
                                                                             TRUE
           0.2
                          512
##
   [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
                                    1.212403e-25 1.212403e-26
                           32
                                                                 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
                                    3.854284e-24 4.282538e-25 764.5
## 6
           0.5
                    32
                           64
                                                                             TRUE
                                    6.344735e-14 1.268947e-14 1844.5
## 7
           0.5
                    32
                          128
                                                                             TRUE
## 8
           0.5
                          256
                                    6.346151e-01 6.346151e-01 5195.0
                    32
                                                                            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
                          256
                                    6.907801e-20 9.868288e-21 8822.0
                    64
                                                                             TRUE
## 12
           0.5
                    64
                          512
                                    9.160009e-33 6.106673e-34 9971.0
                                                                             TRUE
## 13
                                    4.999760e-11 1.249940e-11 7773.0
           0.5
                   128
                          256
                                                                             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]	"Somatio	c mutati	ion rate	: 1"			
##		mut_rate	size_a	size_b	<pre>p_value_corrected</pre>	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 prelimary experiments we conducted to find the default parameters for Primordium. We varied the mutation rate, the probability that an offspring experineces 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(ggridges)
library(scales)
library(khroma)
```

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

df2 = df[!is.na(df\$MCSIZE) & df\$generation == 10000,]

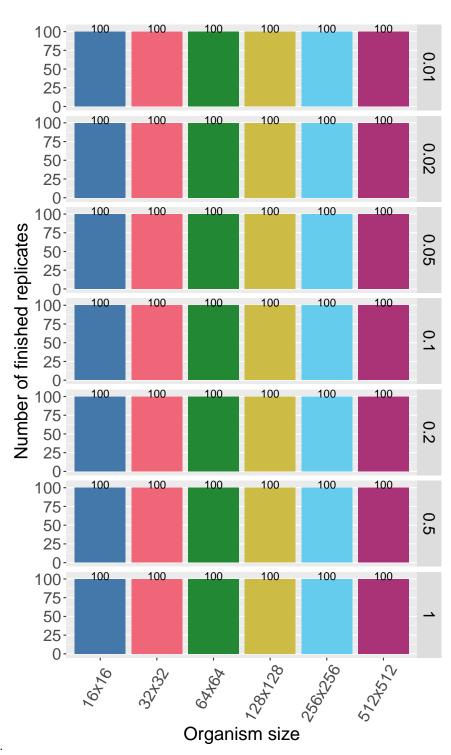
Ignore data for size 8x8 and 1024x1024

```
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$MCSIZE)
df2\size_factor = factor(df2\size_str, levels = c('16x16', '32x32', '64x64', '128x128'
df2\$size_factor_reversed = factor(df2\$size_str, levels = rev(c('16x16', '32x32', '64x6'))
df2$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',
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,
# 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
```

3.1 Data integrity check

text_minor_size = 16
boxplot_color = '#9ecae1'

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

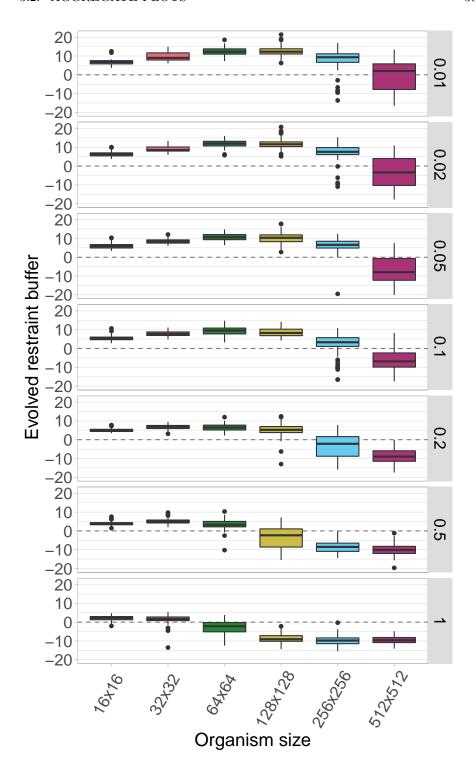


shows a different organism size.

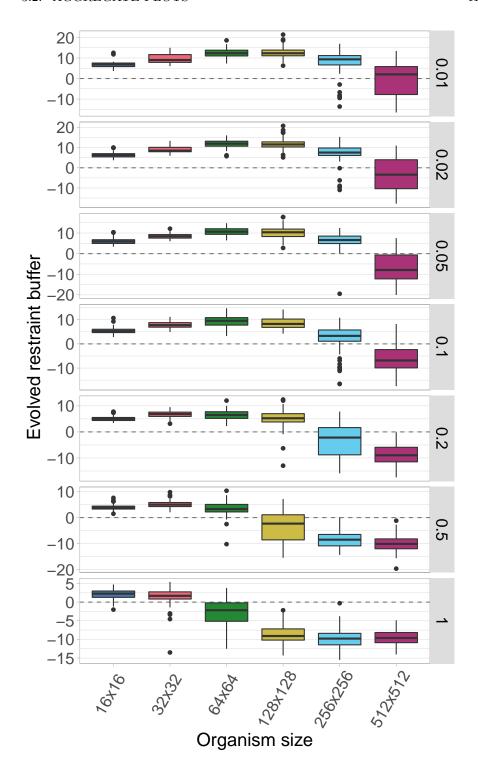
3.2 Aggregate plots

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.

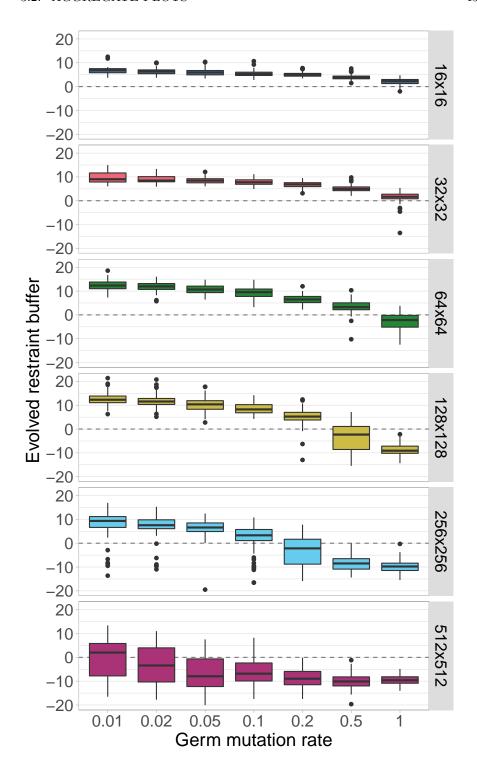


Here is the same data, plotted identically other than now each row can have a different y-axis.

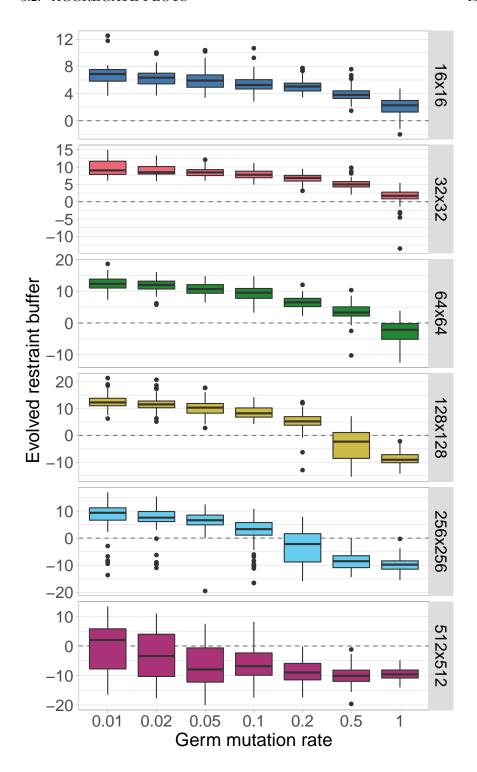


3.2.2 Facet by organism size

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.



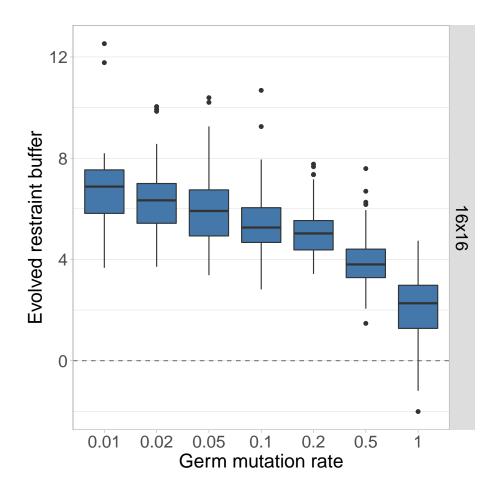
Again, we plot the same data again, but now the y-axis can change between rows.



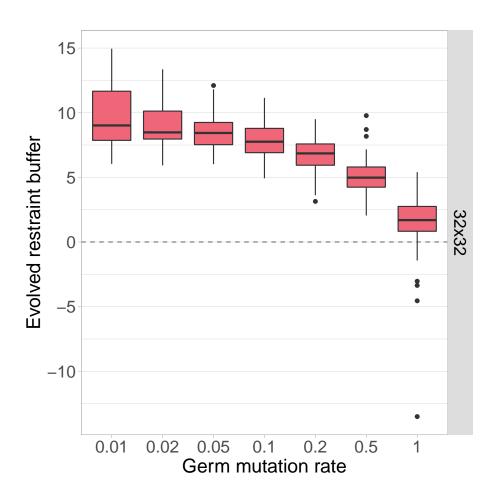
3.3 Single organism size plots

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

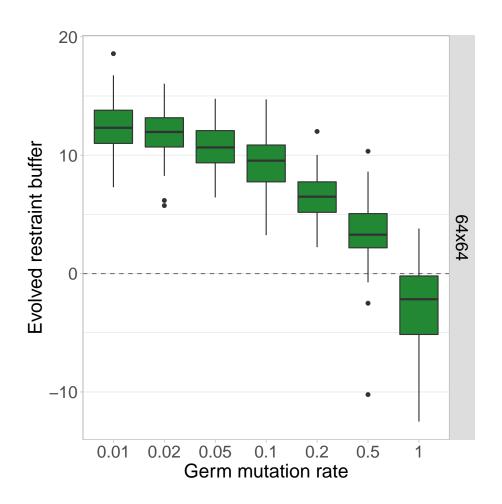
3.3.1 Organism size 16x16



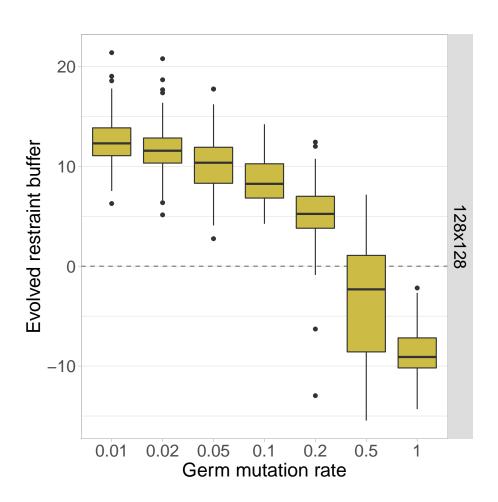
$3.3.2 \quad {\rm Organism \ size} \ 32x32$



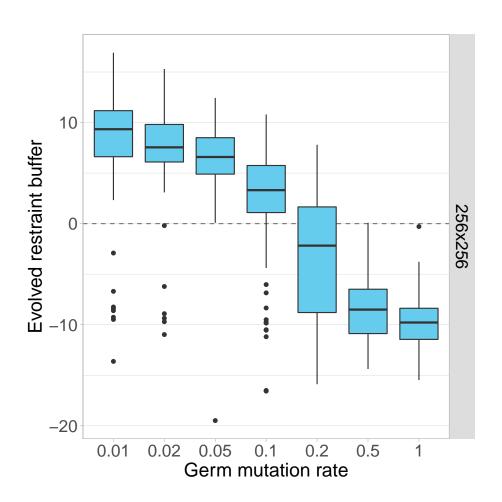
3.3.3 Organism size 64x64



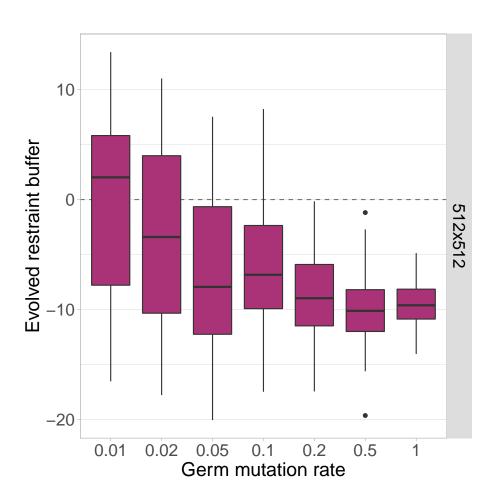
$3.3.4\quad {\rm Organism~size~} 128{\rm x}128$



$3.3.5 \quad \text{Organism size } 256\text{x}256$



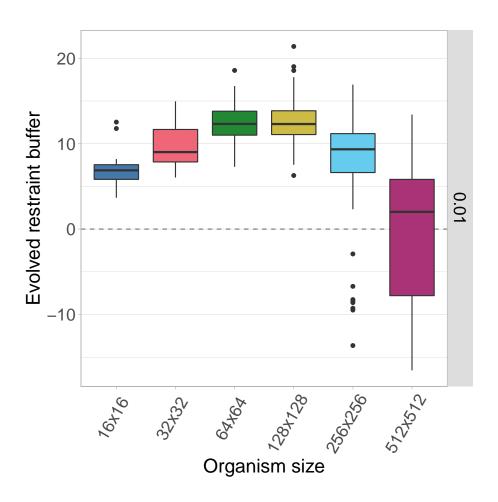
3.3.6 Organism size 512x512



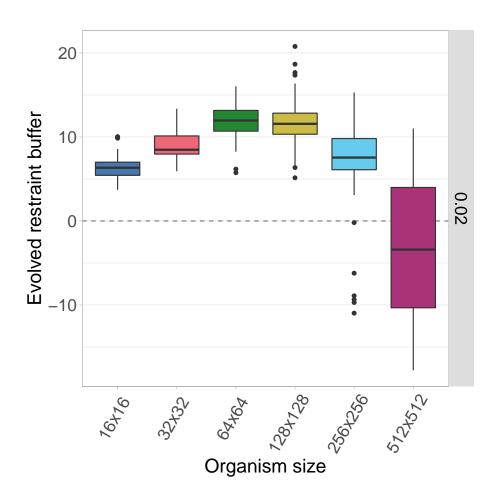
3.4 Single organism size plots

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

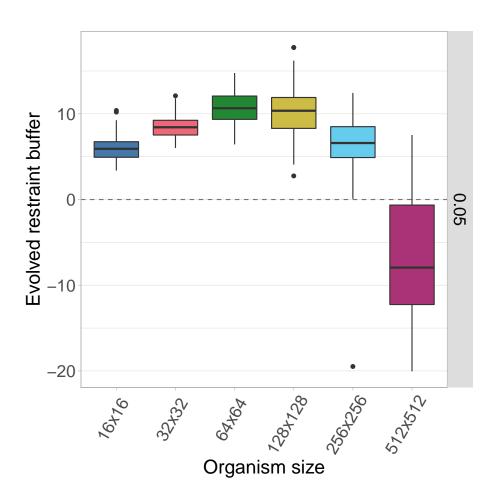
3.4.1 Germ mut. rate 0.01



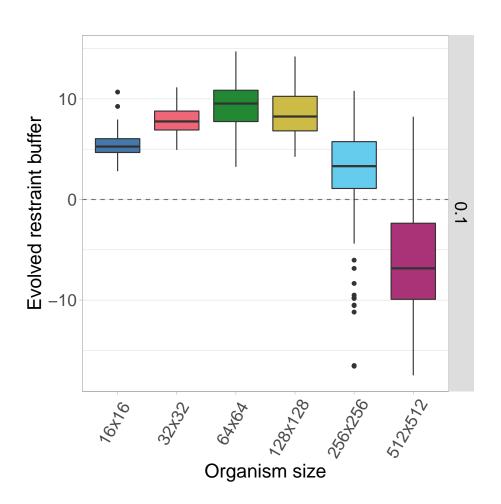
3.4.2 Germ mut. rate 0.02



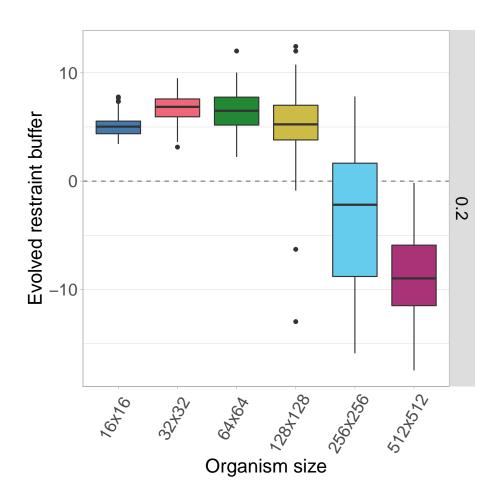
3.4.3 Germ mut. rate 0.05



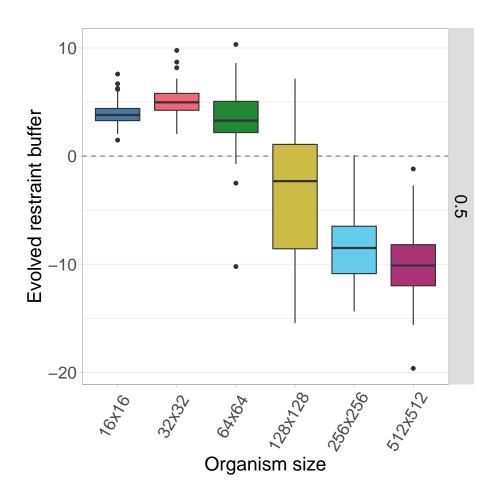
3.4.4 Germ mut. rate 0.1



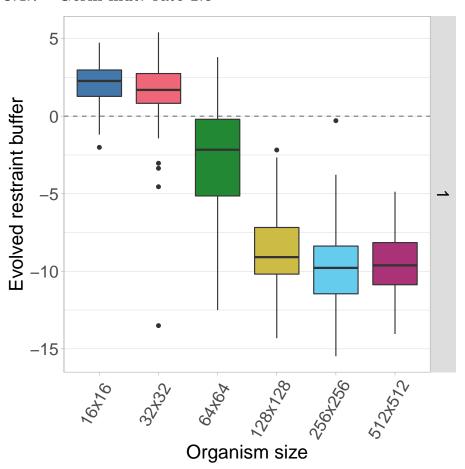
3.4.5 Germ mut. rate 0.2



3.4.6 Germ mut. rate 0.5







3.5 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 perfrm 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,]
```

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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</pre>
  print(df_kruskal)
##
     germ_mut_rate
                        p_value chi_squared df less_0.01
## 1
              0.01 9.191452e-79
                                    374.5160
                                              5
                                                     TRUE
                                    389.2251 5
                                                     TRUE
## 2
              0.02 6.227269e-82
## 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
pariwise Wilcoxon tests on each to see which pais 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$MUT == mut_rate,]
  df_wilcox = data.frame(data = matrix(nrow = 0, ncol = 6))
  colnames(df_wilcox) = c('germ_mut_rate', 'size_a', 'size_b', 'p_value_corrected', 'p_value_raw'
  for(size_idx_a in 1:(length(size_vec) - 1)){
    size_a = size_vec[size_idx_a]
    for(size_idx_b in (size_idx_a + 1):length(size_vec)){
      size_b = size_vec[size_idx_b]
      res = wilcox.test(df_test$MCSIZE == size_a,)$restraint_value, df_test[df_test$MCSIZE
      df_wilcox[nrow(df_wilcox) + 1,] = c(mut_rate, size_a, size_b, 0, res$p.value, as.numeric(re
  df_wilcox$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
               0.01
## 6
                        32
                               64
                                        4.401194e-15 5.501492e-16 1684.5
                                                                               TRUE
```

1

```
## 7
               0.01
                         32
                                128
                                         1.423615e-13 2.847230e-14 1887.0
                                                                                  TRUE
                         32
                                         2.438849e-01 1.219425e-01 5633.5
## 8
                0.01
                                256
                                                                                 FALSE
## 9
                         32
                                512
                                         5.140604e-27 4.673276e-28 9495.0
                                                                                  TRUE
                0.01
## 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
                        128
                                         5.020912e-13 1.255228e-13 8033.5
                0.01
                                256
                                                                                  TRUE
## 14
               0.01
                        128
                                512
                                         1.990837e-31 1.422026e-32 9864.5
                                                                                  TRUE
                        256
                                         1.882508e-17 2.091675e-18 8582.5
## 15
                0.01
                                512
                                                                                  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
                                                                                  TRUE
                                 64
                                         5.800574e-18 8.286535e-19 1375.0
## 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
                        256
                                         4.866893e-20 5.407659e-21 8848.0
## 15
                0.02
                                512
                                                                                  TRUE
##
   [1]
       "Germ mutation rate: 0.05"
##
      germ mut rate size a size b p value corrected p value raw
                                                                          W less 0.01
                         16
## 1
               0.05
                                 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
                                                                                  TRUE
                         16
                                128
                                         3.321119e-21 4.151399e-22 1043.0
## 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
                                         3.387540e-14 5.645899e-15 1802.5
                                                                                  TRUE
                                 64
## 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
                        128
                                         2.838701e-12 5.677403e-13 7950.5
                0.05
                                256
                                                                                  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
```

2.006447e-24 2.229385e-25 739.0

TRIIE

16

32

0.1

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```
## 2
                 0.1
                         16
                                 64
                                         2.197505e-25 1.997732e-26 646.0
                                                                                  TRUE
                                         3.982057e-19 6.636762e-20 1261.5
                                                                                  TRUE
## 3
                 0.1
                         16
                                128
## 4
                 0.1
                                256
                                         2.853915e-06 9.513050e-07 7006.5
                                                                                  TRUE
                         16
## 5
                 0.1
                         16
                                512
                                         1.146029e-26 9.550238e-28 9468.5
                                                                                  TRUE
                                         6.866683e-07 1.716671e-07 2860.0
## 6
                 0.1
                         32
                                64
                                                                                 TRUE
##
  7
                 0.1
                         32
                                128
                                         5.714627e-02 5.714627e-02 4221.0
                                                                                 FALSE
## 8
                         32
                                         7.451552e-21 9.314440e-22 8923.0
                                                                                 TRUE
                 0.1
                                256
## 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
                                256
                                         6.893655e-25 6.893655e-26 9306.5
                 0.1
                         64
                                                                                  TRUE
## 12
                 0.1
                         64
                                512
                                         4.295636e-32 2.863757e-33 9919.0
                                                                                 TRUE
## 13
                 0.1
                                256
                                         9.294756e-21 1.327822e-21 8908.0
                                                                                  TRUE
                        128
                                         2.475810e-31 1.904469e-32 9854.5
## 14
                 0.1
                        128
                                512
                                                                                  TRUE
##
                        256
                                         7.440793e-16 1.488159e-16 8380.0
  15
                 0.1
                                512
                                                                                  TRUE
   [1] "Germ mutation rate: 0.2"
##
      germ_mut_rate size_a size_b p_value_corrected p_value_raw
                                                                           W less_0.01
##
                 0.2
                                 32
                                         6.711164e-17 9.587377e-18
                                                                                   TRUE
   1
                         16
                                                                      1488.5
## 2
                 0.2
                         16
                                 64
                                         2.652853e-08 5.305706e-09
                                                                      2610.5
                                                                                   TRUE
                 0.2
## 3
                         16
                                128
                                         5.723537e-01 4.033561e-01
                                                                      4657.5
                                                                                  FALSE
## 4
                 0.2
                                256
                                         9.414689e-28 1.046077e-28
                                                                      9550.0
                                                                                   TRUE
                         16
## 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
                                                                                  FALSE
                                                                      5437.0
## 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
                                         3.256203e-24 4.070254e-25
                 0.2
                        128
                                256
                                                                      9237.5
                                                                                   TRUE
## 14
                 0.2
                        128
                                512
                                         1.052551e-31 8.771259e-33
                                                                      9881.0
                                                                                   TRUE
## 15
                 0.2
                        256
                                         1.558734e-09 2.597889e-10
                                                                      7587.5
                                                                                   TRUE
                                512
##
   [1] "Germ mutation rate: 0.5"
##
      germ_mut_rate size_a size_b p_value_corrected p_value_raw
                                                                           W less_0.01
## 1
                                         3.627488e-11 7.254975e-12
                                                                                   TRUE
                 0.5
                         16
                                 32
                                                                      2195.0
## 2
                 0.5
                         16
                                 64
                                         1.774145e-01 1.774145e-01
                                                                      5552.5
                                                                                  FALSE
                 0.5
                                         9.003159e-21 1.125395e-21
## 3
                         16
                                128
                                                                      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
                                         1.574642e-07 5.248808e-08
## 6
                 0.5
                         32
                                 64
                                                                     7228.0
                                                                                   TRUE
                                         3.547680e-25 3.941867e-26
## 7
                 0.5
                         32
                                128
                                                                      9328.0
                                                                                   TRUE
## 8
                 0.5
                         32
                                         3.840402e-33 2.560701e-34 10000.0
                                256
                                                                                   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
                                         2.868826e-09 7.172065e-10 7522.5
                 0.5
                        128
                                256
                                                                                   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 mutatio	on rate:	: 1"				
##		<pre>germ_mut_rate</pre>	size_a	size_b	<pre>p_value_corrected</pre>	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

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.

4.1 Data cleaning

Load necessary libraries

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

Load the data and trim all the unecessay 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_lend
df = rbind(df, read.csv('../experiments/2021_02_27__genome_length/evolution/data/scraped_evolution
df = rbind(df, read.csv('../experiments/2021_02_2
```

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

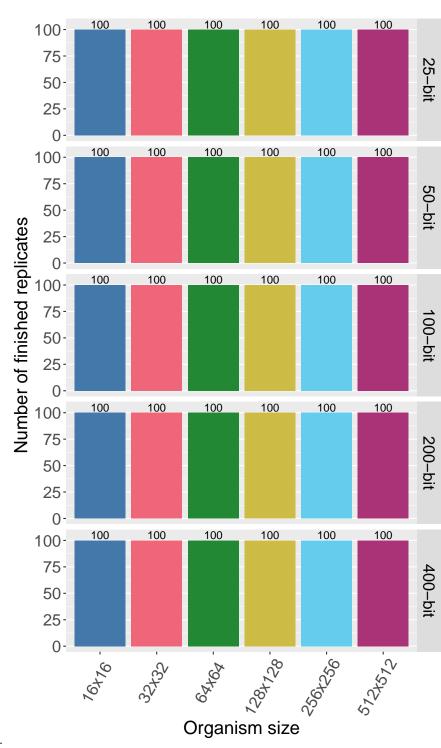
```
# Group the data by size and summarize
data_grouped = dplyr::group_by(df2, MCSIZE, LENGTH)
data_summary = dplyr::summarize(data_grouped, mean_ones = mean(ave_ones), n = dplyr::n
```

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'
df2\$size_factor_reversed = factor(df2\$size_str, levels = rev(c('16x16', '32x32', '64x6'))
df2$length_str = paste0(df2$LENGTH, '-bit')
df2$length_factor = factor(df2$length_str, levels = c('25-bit', '50-bit', '100-bit', '
data_summary$size_str = paste0(data_summary$MCSIZE, 'x', data_summary$MCSIZE)
data_summary$size_factor = factor(data_summary$size_str, levels = c('16x16', '32x32',
data_summary$length_str = pasteO(data_summary$LENGTH, '-bit')
data_summary$length_factor = factor(data_summary$length_str, levels = c('25-bit', '50-'
# 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
```

4.2 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

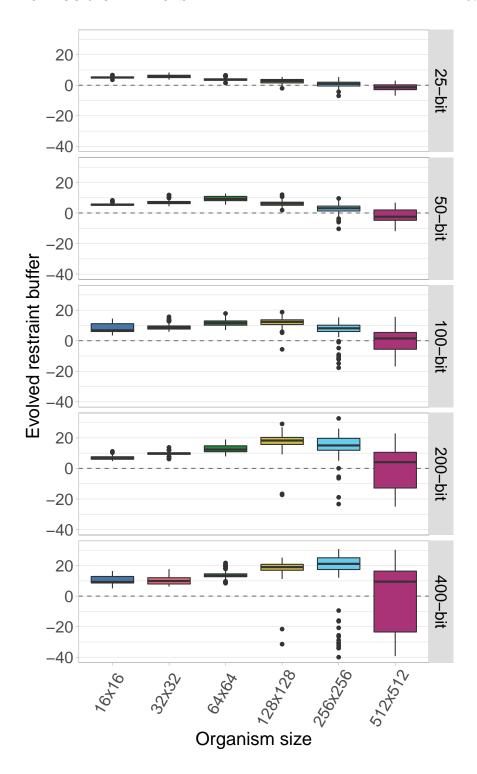


bar/color shows a different organism size.

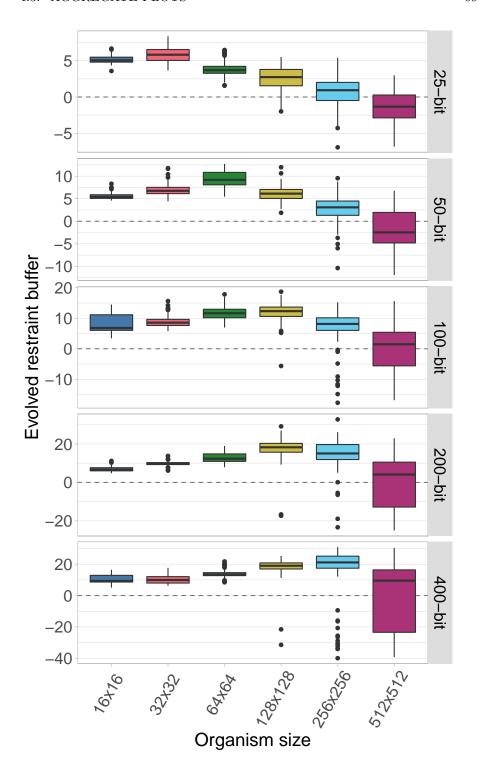
4.3 Aggregate plots

4.3.1 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.

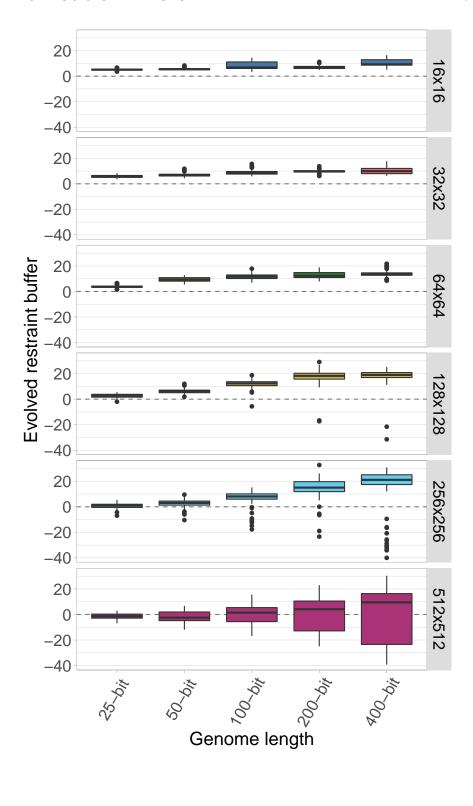


Here we plot the same data, only we allow the y-axis to vary between rows.

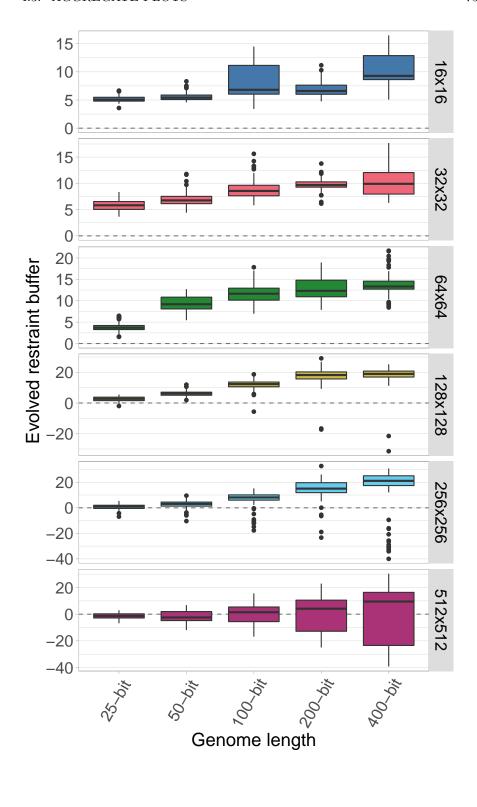


4.3.2 Facet by organism size

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



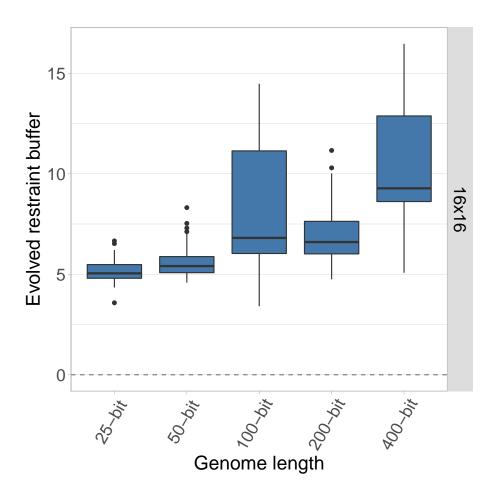
Here is the identical plot but now we allow the y-axis to vary between the rows.



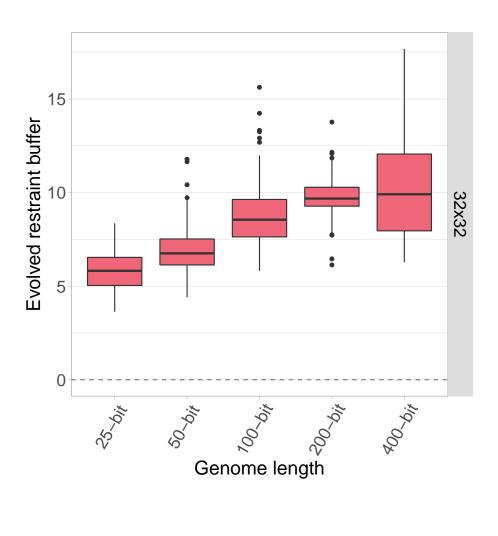
4.4 Single organism size plots

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

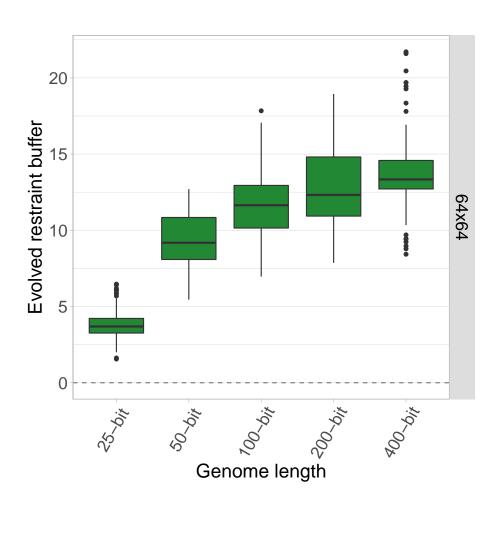
4.4.1 Organism size 16x16



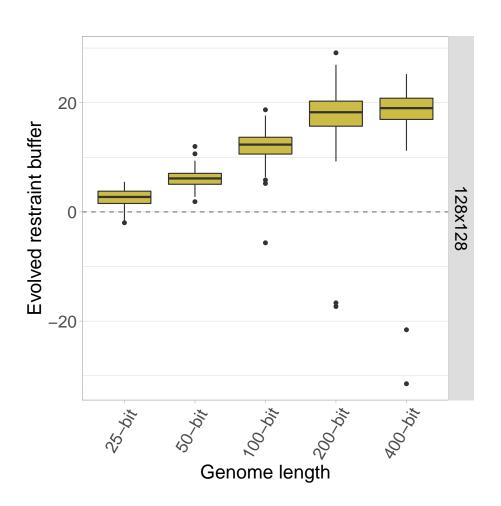
$4.4.2 \quad {\rm Organism \ size} \ 32x32$



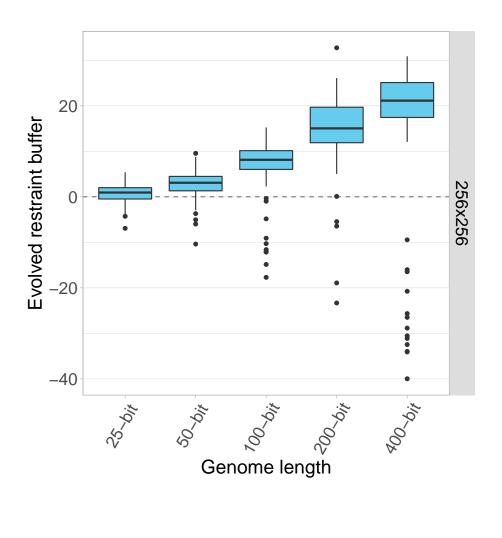
4.4.3 Organism size 64x64



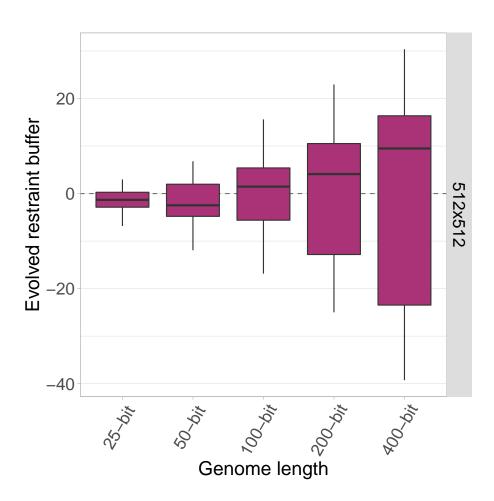
4.4.4 Organism size 128x128



4.4.5 Organism size 256×256



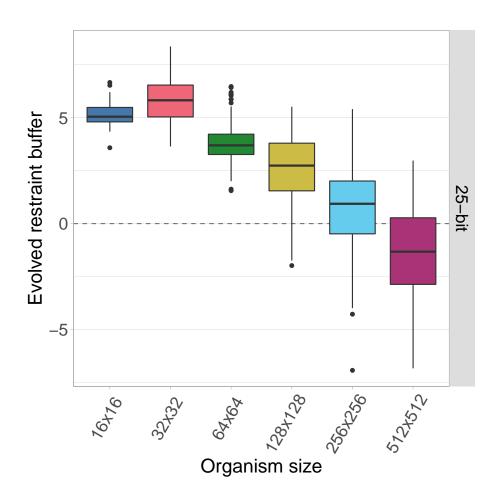
4.4.6 Organism size 512x512



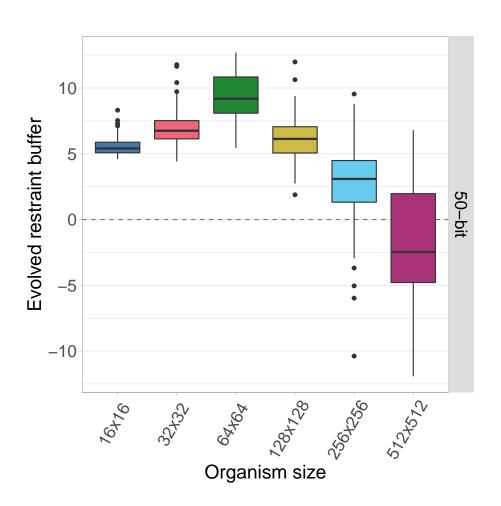
4.5 Single genome length plots

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

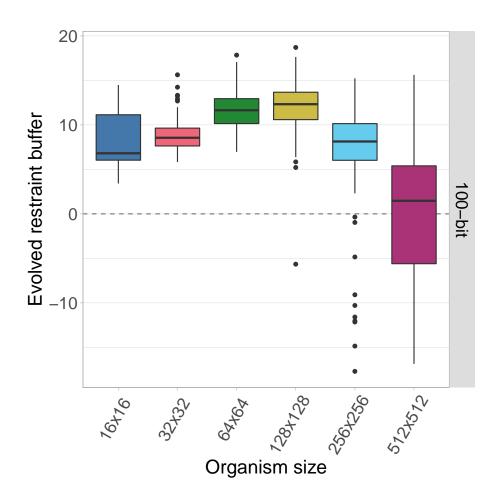
4.5.1 25-bit genomes



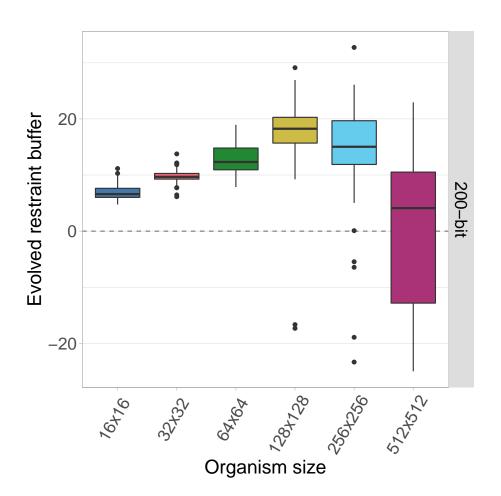
4.5.2 50-bit genomes

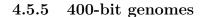


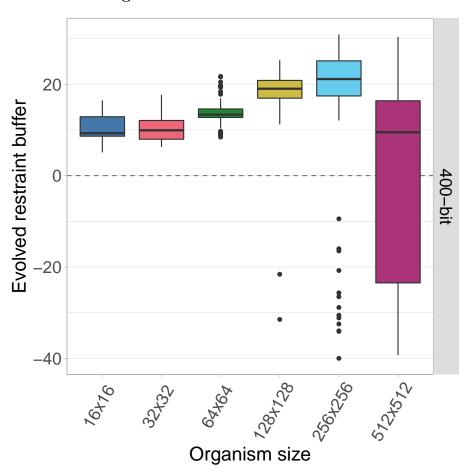
4.5.3 100-bit genomes



4.5.4 200-bit genomes







4.6 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 perfrm 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){
```

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```
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$statistic)[]
  }
  df_kruskal$less_0.01 = df_kruskal$p_value < 0.01</pre>
  print(df_kruskal)
##
     genome_length
                        p_value chi_squared df less_0.01
## 1
                25 1.508889e-97
                                    461.6473 5
                                                      TRUE
## 2
                                    411.5159
                                              5
                                                      TRUE
                50 9.772852e-87
## 3
               100 7.491319e-60
                                    286.6294
                                              5
                                                      TRUE
## 4
               200 1.626963e-75
                                    359.4358 5
                                                      TRUE
## 5
               400 2.857912e-49
                                    237.3380 5
                                                      TRUE
We see that significant variation exists within each genome length, so we perform
pariwise Wilcoxon tests on each to see which pais of sizes are significantly
different.
size_vec = c(16, 32, 64, 128, 256, 512)
length_vec = c(25, 50, 100, 200, 400)
for(genome_length in length_vec){
  df_test = df2[df2$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$MCSIZE == size_a,)$restraint_value, df_test[df_test$MCSIZE
      df_wilcox[nrow(df_wilcox) + 1,] = c(genome_length, size_a, size_b, 0, res$p.value, as.nume)
    }
  }
  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</pre>
  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
                                        3.83888e-33 2.559259e-34 10000.0
                                                                                TRUE
## 6
                 25
                        32
                               64
                                        1.447210e-22 2.412016e-23 9074.5
                                                                               TRUE
                                        1.283820e-27 1.283820e-28 9542.5
## 7
                 25
                        32
                              128
                                                                                TRUE
```

}

```
## 8
                  25
                          32
                                256
                                          2.711275e-32 2.259396e-33
                                                                       9927.0
                                                                                    TRUE
## 9
                  25
                          32
                                512
                                          3.83888e-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
                  25
## 14
                         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
                                          7.415731e-04 3.707866e-04 6457.5
                                                                                   TRUE
                                128
## 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
                         128
                                          4.777498e-28 4.343180e-29 9582.0
                  50
                                512
                                                                                   TRUE
## 15
                  50
                         256
                                512
                                          3.236734e-12 1.078911e-12 7914.5
                                                                                   TRUE
##
       "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
                                          3.952168e-13 7.904337e-14 1941.5
                 100
                          16
                                 64
                                                                                   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
                                128
## 7
                          32
                                          3.085548e-17 3.085548e-18 1435.5
                                                                                   TRUE
                 100
## 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
                         128
                                          9.444159e-28 6.745828e-29 9566.0
                 100
                                512
                                                                                   TRUE
## 15
                 100
                         256
                                512
                                          2.634367e-13 4.390611e-14 8090.0
                                                                                   TRUE
##
       "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
                                         7.523609e-32 5.015739e-33
                                                                                   TRUE
                 200
                          16
                                 64
                                                                       100.0
```

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##	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 lengt	th: 400'	ı				
##		genome_length	size_a	size_b	<pre>p_value_corrected</pre>	p_value_raw	W	less_0.01
##	1	400	16	32	5.405382e-01	5.348472e-01	5254.5	FALSE
## ##	_	400 400	16 16	32 64		5.348472e-01 3.472338e-15		FALSE TRUE
	2				3.472338e-14		1777.5	
##	2	400	16	64	3.472338e-14 4.072814e-28	3.472338e-15	1777.5 401.0	TRUE
##	2 3 4	400 400	16 16	64 128	3.472338e-14 4.072814e-28 1.163125e-16	3.472338e-15 2.715209e-29	1777.5 401.0 1489.0	TRUE TRUE
## ## ##	2 3 4 5	400 400 400	16 16 16	64 128 256	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01	3.472338e-15 2.715209e-29 9.692706e-18	1777.5 401.0 1489.0 5549.0	TRUE TRUE TRUE
## ## ## ##	2 3 4 5 6	400 400 400 400	16 16 16 16	64 128 256 512	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01	1777.5 401.0 1489.0 5549.0 2070.5	TRUE TRUE TRUE FALSE
## ## ## ##	2 3 4 5 6 7	400 400 400 400 400	16 16 16 16 32	64 128 256 512 64	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13	1777.5 401.0 1489.0 5549.0 2070.5 535.5	TRUE TRUE TRUE FALSE TRUE
## ## ## ## ##	2 3 4 5 6 7 8	400 400 400 400 400 400	16 16 16 16 32 32	64 128 256 512 64 128	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0	TRUE TRUE TRUE FALSE TRUE TRUE
## ## ## ## ## ##	2 3 4 5 6 7 8	400 400 400 400 400 400 400	16 16 16 16 32 32 32	64 128 256 512 64 128 256	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16 5.405382e-01	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27 1.988815e-17	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0 5546.0	TRUE TRUE TRUE FALSE TRUE TRUE TRUE
## ## ## ## ## ##	2 3 4 5 6 7 8 9	400 400 400 400 400 400 400	16 16 16 16 32 32 32 32	64 128 256 512 64 128 256 512	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16 5.405382e-01 2.993077e-18	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27 1.988815e-17 1.825748e-01	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0 5546.0 1317.0	TRUE TRUE TRUE FALSE TRUE TRUE FALSE
## ## ## ## ## ## ##	2 3 4 5 6 7 8 9	400 400 400 400 400 400 400 400	16 16 16 16 32 32 32 32 64	64 128 256 512 64 128 256 512 128	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16 5.405382e-01 2.993077e-18 3.197608e-12	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27 1.988815e-17 1.825748e-01 2.302367e-19	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0 5546.0 1317.0 2030.0	TRUE TRUE TRUE FALSE TRUE TRUE TRUE FALSE TRUE
## ## ## ## ## ## ##	2 3 4 5 6 7 8 9 10	400 400 400 400 400 400 400 400 400	16 16 16 16 32 32 32 32 64 64	64 128 256 512 64 128 256 512 128 256	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16 5.405382e-01 2.993077e-18 3.197608e-12 8.293488e-05	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27 1.988815e-17 1.825748e-01 2.302367e-19 3.997010e-13	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0 5546.0 1317.0 2030.0 6763.0	TRUE TRUE FALSE TRUE TRUE TRUE TRUE FALSE TRUE TRUE
## ## ## ## ## ## ## ##	2 3 4 5 6 7 8 9 10 11 12	400 400 400 400 400 400 400 400 400 400	16 16 16 16 32 32 32 32 64 64 64	64 128 256 512 64 128 256 512 128 256 512	3.472338e-14 4.072814e-28 1.163125e-16 5.405382e-01 5.784416e-12 1.489024e-26 2.187697e-16 5.405382e-01 2.993077e-18 3.197608e-12 8.293488e-05 1.019174e-02	3.472338e-15 2.715209e-29 9.692706e-18 1.801794e-01 8.263451e-13 1.063588e-27 1.988815e-17 1.825748e-01 2.302367e-19 3.997010e-13 1.658698e-05	1777.5 401.0 1489.0 5549.0 2070.5 535.5 1523.0 5546.0 1317.0 2030.0 6763.0 3764.5	TRUE TRUE FALSE TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE

Chapter 5

Size 1024x1024 organisms

TBD

Chapter 6

Test of timing sample counts

TBD

Chapter 7

Interactive web app

TBD