Supplemental Material for Adaptive phenotypic plasticity stabilizes evolution in fluctuating environments

Alexander Lalejini, Austin J. Ferguson, Nkrumah A. Grant, and Charles Ofria

2021-07-16

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Chapter 1

Introduction

This is the supplemental material for our work entitled, *Adaptive phenotypic plasticity stabilizes evolution in fluctuating environments.* Preprint forthcoming.

1.1 About our supplemental material

This supplemental material is hosted on GitHub using GitHub pages. The source code and configuration files used to generate this supplemental material can be found in this GitHub repository. We compiled our data analyses and supplemental documentation into this nifty web-accessible book using bookdown.

Our supplemental material includes the following:

- Data availability (Section 2)
- Guide for running our experiments (Section 3)
- Avida instruction set (Section 4)
- Experiment analyses (including source code):
  - Validating the evolution of phenotypic plasticity (Section 5)
  - Effect of adaptive phenotypic plasticity on evolutionary change (Section 6)
    - Results with variable-length genomes (Section 10)
  - Effect of adaptive phenotypic plasticity on the evolution and maintenance of novel traits (Section **??**)
  - Effect of adaptive phenotypic plasticity on the accumulation of deleterious instructions (Section 8)
  - Exploring how regulation is encoded in genomes in Avida (Section 9)

1.2 Contributing authors

- Alexander Lalejini

- Austin J. Ferguson
- Nkrumah A. Grant
- Charles Ofria

1.3 Research overview

Abstract:

Fluctuating environmental conditions are ubiquitous in natural systems, and populations have evolved various strategies to cope with such fluctuations. The particular mechanisms that evolve profoundly influence subsequent evolutionary dynamics. One such mechanism is phenotypic plasticity, which is the ability of a single genotype to produce alternate phenotypes in an environmentally dependent context. Here, we use digital organisms (self-replicating computer programs) to investigate how adaptive phenotypic plasticity alters evolutionary dynamics and influences evolutionary outcomes in cyclically changing environments. Specifically, we examined the evolutionary histories of both plastic populations and non-plastic populations to ask: (1) Does adaptive plasticity promote or constrain evolutionary change? (2) Are plastic populations better able to evolve and then maintain novel traits? And (3), how does adaptive plasticity affect the potential for maladaptive traits to accumulate in evolving genomes? We find that populations with adaptive phenotypic plasticity undergo less evolutionary change than non-plastic populations, which must rely on genetic variation from de novo mutations to continuously readapt to environmental fluctuations. Indeed, the non-plastic populations undergo more frequent selective sweeps and accumulate many more genetic changes. We find that the repeated selected sweeps in non-plastic populations drive the loss of beneficial traits via deleterious hitchhiking, whereas phenotypic plasticity can stabilize populations against environmental fluctuations. This stabilization allows plastic populations to more easily retain novel adaptive traits than their non-plastic counterparts. In general, the evolution of adaptive phenotypic plasticity shifted evolutionary dynamics to be more similar to that of populations evolving in a static environment than to non-plastic populations evolving in an identical fluctuating environment. All natural environments subject populations to some form of change; our findings suggest that the stabilizing effect of phenotypic plasticity plays an important role in subsequent adaptive evolution.

Chapter 2

Data availability

2.1 Source code

The source code for this work is publicly accessible on GitHub: https://github.com/amlalejini/evolutionary-consequences-of-plasticity

2.2 Experimental results

The data from our experiments are available online in our OSF repository (Lalejini and Ferguson, 2021) at https://osf.io/sav2c/.

Chapter 3

Compile and run experiments locally

Here, we provide a brief guide to compiling and running our experiments using our Docker image.

Please file an issue on GitHub if something is unclear or does not work.

3.1 Docker

You can use the Dockerfile in our repository to build a docker image locally, or you can pull the latest docker image from DockerHub using

```
docker pull amlalejini/evolutionary-consequences-of-plasticity
```

This will pull down a docker image with:

- all of the requisite dependencies installed/downloaded
- all experiment source code
- the minimal set of raw data needed to compile the supplemental material
- a build of our supplemental material (which will also run all of our analyses)

To run the container interactively:

```
docker run -it --entrypoint bash amlalejini/evolutionary-consequences-of-plasticity
```

You can exit the container at any point with `ctrl-d`.

Inside the container, you should be able to navigate to `/opt/evolutionary-consequences-of-plasticity`:

```
cd /opt/evolutionary-consequences-of-plasticity
```

To run Avida, you'll need to `cd` into the `avida` directory and run `./build_avida`.

All of the Avida configuration files necessary for re-running our experiments can be found here: https://github.com/amlalejini/evolutionary-consequences-of-plasticity/tree/master/experiments.

For example, the configuration files for our evolutionary change experiment are here: https://github.com/amlalejini/evolutionary-consequences-of-plasticity/tree/master/experiments/2021-02-08-evo-dynamics/hpcc/config.

Chapter 4

Avida instruction set

4.1 Default instructions

We used the following default instructions in all of our experiments:

```
# No-ops
INST nop-A
INST nop-B
INST nop-C

# Flow control operations
INST if-n-equ
INST if-less
INST if-label
INST mov-head
INST jmp-head
INST get-head
INST set-flow

# Single Argument Math
INST shift-r
INST shift-l
INST inc
INST dec
INST push
INST pop
INST swap-stk
INST swap

# Double Argument Math
```

```
INST add
INST sub
INST nand

# Biological Operations
INST h-copy
INST h-alloc
INST h-divide

# I/O and Sensory
INST IO
INST h-search
```

Each of these instructions is described in the Avida documentation.

4.2 Custom instructions

We implemented several custom instructions for this work:

- `INST sense-react-NAND`
  - Provides sensory feedback on whether the NAND Boolean logic task is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a -1 if it is punished, and a 0 if it is neither rewarded nor punished.
- `INST sense-react-NOT`
  - Provides sensory feedback on whether the NOT Boolean logic task is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a -1 if it is punished, and a 0 if it is neither rewarded nor punished.
- `INST sense-react-AND`
  - Provides sensory feedback on whether the AND Boolean logic task is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a -1 if it is punished, and a 0 if it is neither rewarded nor punished.
- `INST sense-react-ORN`
  - Provides sensory feedback on whether the ORN Boolean logic task is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a -1 if it is punished, and a 0 if it is neither rewarded nor punished.
- `INST sense-react-OR`
  - Provides sensory feedback on whether the OR Boolean logic task is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a -1 if it is punished, and a 0 if it is neither rewarded nor punished.
- `INST sense-react-ANDN`
  - Provides sensory feedback on whether the ANDN Boolean logic task

  is currently rewarded or punished by pushing a 1 to the organism's active stack if it is rewarded, a `-1` if it is punished, and a 0 if it is neither rewarded nor punished.

- `INST poison`
  - Each time `poison` is executed, the organism reduces the metabolic rate of the organism by a fixed rate (specified by `POISON_PENALTY` in `avida.cfg`).

Chapter 5

Validation experiment

In this experiment, we validate that (1) we observe the evolution of phenotypic plasticity in a changing environment when digital organisms have access to sensory instructions (capable of differentiating environmental states) and (2) that adaptive phenotypic plasticity does not evolve when populations lack access to sensory instructions.

5.1 Overview

```
total_updates <- 200000
replicates <- 100

all_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")

# Relative location of data.
working_directory <- "experiments/2021-01-07-validation/analysis/" # << For bookdown
# working_directory <- "./"                                            # << For local analysis
```

We evolved populations of digital organisms under four conditions:

1. A fluctuating environment with access to sensory instructions
2. A fluctuating environment without access to sensory instructions (i.e., sensory instructions are no-operations)
3. A constant environment with access to sensory instructions
4. A constant environment without access to sensory instructions

In fluctuating environments, we alternate between rewarding and punishing different sets of computational tasks. In one environment, we reward tasks not, and, or and punish tasks nand, ornot, andnot. In the alternative environment, we reward tasks nand, ornot, andnot and punish tasks not, and, or. In constant environments, we reward all tasks (not, nand, and, ornot, or, andnot).

For each replicate of each condition, we extract the dominant (i.e., most numerous) genotype at the end of the run to analyze further. We expect to observe the evolution of adaptive phenotypic plasticity in only the first experimental condition. In conditions without sensors, plasticity in any form should be unable to evolve.

5.2 Analysis dependencies

Load all required R libraries.

```
library(ggplot2)
library(tidyverse)
library(cowplot)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd9
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

5.3 Setup

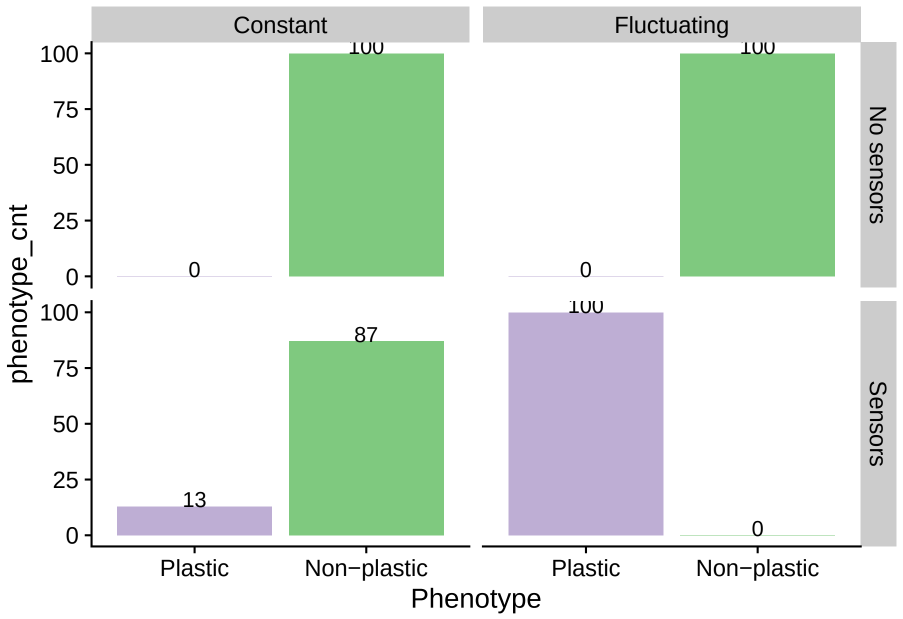
```
data_loc <- paste0(working_directory, "data/aggregate.csv")
data <- read.csv(data_loc, na.strings="NONE")

data$DISABLE_REACTION_SENSORS <- as.factor(data$DISABLE_REACTION_SENSORS)
```

```r
data$chg_env <- as.factor(data$chg_env)
data$dom_plastic_odd_even <- as.factor(data$dom_plastic_odd_even)
data$sensors <- data$DISABLE_REACTION_SENSORS == "0"
data$is_plastic <- data$dom_plastic_odd_even == "True"

env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
}

# Count observed plasticity for each condition (I'm sure there's a 'tidier' way to do this..)
observed_plasticity <- data.frame(
  environment=character(),
  sensors=character(),
  plastic=integer(),
  nonplastic=integer(),
  plastic_adaptive=integer(),
  plastic_optimal=integer(),
  plastic_nonadaptive=integer()
)
for (env_chg in levels(data$chg_env)) {
  for (disabled_sensors in levels(data$DISABLE_REACTION_SENSORS)) {
    cond_data <- filter(data, chg_env == env_chg & data$DISABLE_REACTION_SENSORS == disabled_sens
    environment_label <- env_label_fun(env_chg)
    sensors_label <- sensors_label_fun(disabled_sensors == "0")

    observed_plasticity <- observed_plasticity %>% add_row(
      environment=environment_label,
      sensors=sensors_label,
      plastic=nrow(filter(cond_data, is_plastic==TRUE)),
      nonplastic=nrow(filter(cond_data, is_plastic==FALSE)),
      plastic_adaptive=nrow(filter(cond_data, dom_adaptive_plasticity=="True")),
      plastic_optimal=nrow(filter(cond_data, dom_optimal_plastic=="True")),
      plastic_nonadaptive=nrow(filter(cond_data, is_plastic==TRUE & dom_adaptive_plasticity=="Fal
    )
  }
}

observed_plasticity <- pivot_longer(
  observed_plasticity,
  cols=c("plastic", "plastic_adaptive", "plastic_optimal", "plastic_nonadaptive", "nonp
  names_to="phenotype",
  values_to="phenotype_cnt"
)

####### misc #######
# Configure our default graphing theme
theme_set(theme_cowplot())
```

5.4 Evolution of phenotypic plasticity

For each experimental condition, do we observe the evolution of phenotypic plasticity? To test for phenotypic plasticity, we culture digital organisms in both environments from the fluctuating condition (including organisms evolved in a constant environment). Any plasticity that we observe from digital organisms evolved under constant conditions is cryptic variation (as these organisms were never exposed to these culturing environments).

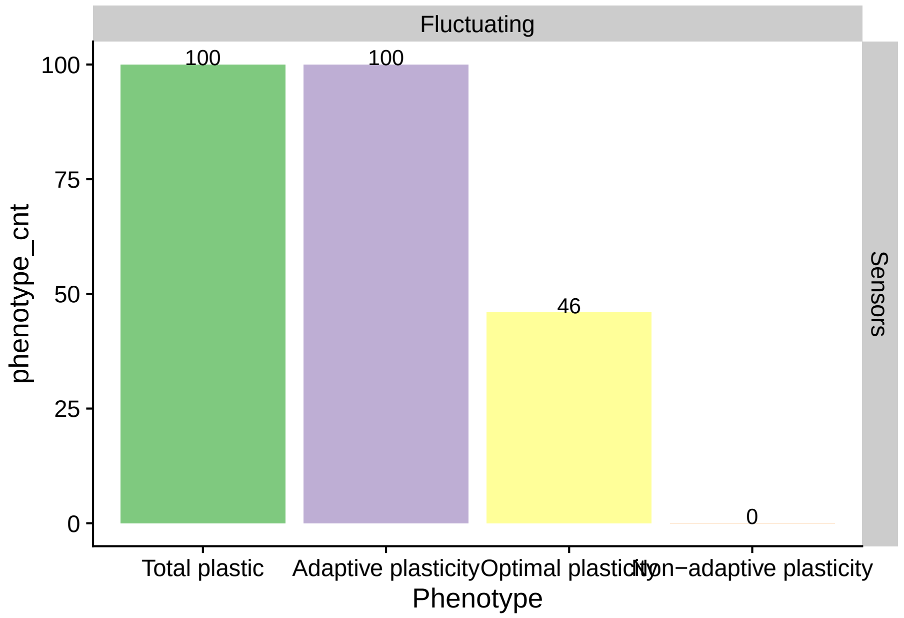
```
ggplot(filter(observed_plasticity, phenotype %in% c("plastic", "nonplastic")), aes(x=pl
  geom_bar(
    stat="identity",
    position=position_dodge(0.9)
  ) +
  geom_text(
    stat="identity",
    mapping=aes(label=phenotype_cnt),
    vjust=0.05
  ) +
  scale_fill_brewer(palette="Accent") +
  scale_x_discrete(
    name="Phenotype",
    limits=c("plastic", "nonplastic"),
    labels=c("Plastic", "Non-plastic")
  ) +
  facet_grid(sensors~environment) +
  theme(
    legend.position="none"
  )
```



Indeed, we do not observe the evolution of phenotypic plasticity in any replicates in which digital organisms do not have access to sensory instructions. We do observe the evolution of plasticity (not necessarily adaptive plasticity) in both constant and fluctuating environments where sensors are enabled.

To what extent is the observed phenotypic plasticity adaptive?

```
ggplot(filter(observed_plasticity, environment=="Fluctuating" & sensors == "Sensors" & phenotype
  geom_bar(
    stat="identity",
    position=position_dodge(0.9)
  ) +
  geom_text(
    stat="identity",
    mapping=aes(label=phenotype_cnt),
    vjust=0.05
  ) +
  scale_fill_brewer(palette="Accent") +
  scale_x_discrete(
    name="Phenotype",
    limits=c("plastic",  "plastic_adaptive", "plastic_optimal", "plastic_nonadaptive"),
    labels=c("Total plastic", "Adaptive plasticity", "Optimal plasticity", "Non-adaptive plastici
  ) +
  facet_grid(sensors~environment) +
  theme(
    legend.position="none"
  )
```


Fluctuating
Sensors
phenotype_cnt
100
75
50
25
0
100
100
46
0
Total plastic
Adaptive plasticity
Optimal plasticity
Non−adaptive plasticity
Phenotype

Chapter 6

Evolutionary change

The effect of adaptive phenotypic plasticity on evolutionary change.

6.1 Overview

```r
total_updates <- 200000
replicates <- 100
alpha <- 0.05

all_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")

# Relative location of data.
working_directory <- "experiments/2021-02-08-evo-dynamics/analysis/" # << For bookdown
# working_directory <- "./"                                              # << For local analysis
```

6.2 Analysis dependencies

Load all required R libraries.

```r
library(ggplot2)
library(rstatix)
library(ggsignif)
library(scales)
library(tidyverse)
library(cowplot)
library(RColorBrewer)
library(Hmisc)
library(boot)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd97
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

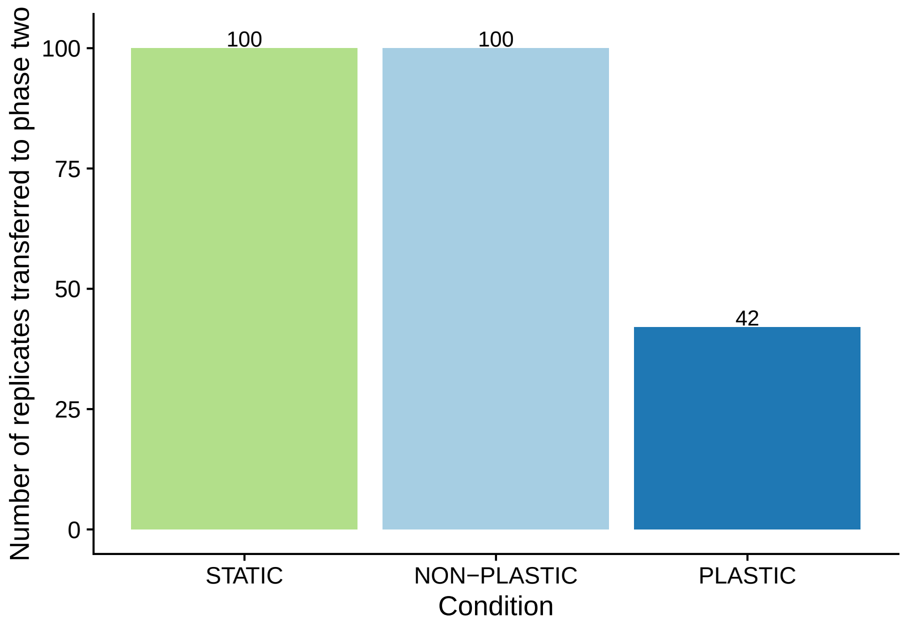
6.3 Setup

```
summary_data_loc <- paste0(working_directory, "data/aggregate.csv")
summary_data <- read.csv(summary_data_loc, na.strings="NONE")

summary_data$DISABLE_REACTION_SENSORS <- as.factor(summary_data$DISABLE_REACTION_SENSOR
summary_data$chg_env <- summary_data$chg_env == "True"
summary_data$dominant_plastic_odd_even <- as.factor(summary_data$dominant_plastic_odd_e
summary_data$sensors <- summary_data$DISABLE_REACTION_SENSORS == "0"
summary_data$is_plastic <- summary_data$dominant_plastic_odd_even == "True"

env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
}

# note that this labeler makes assumptions about how we set up our experiment
condition_label_fun <- function(has_sensors, env_chg) {
  if (has_sensors && env_chg) {
    return("PLASTIC")
  } else if (env_chg) {
    return("NON-PLASTIC")
  } else {
    return("STATIC")
  }
}

summary_data$env_label <- mapply(
  env_label_fun,
  summary_data$chg_env
)
summary_data$sensors_label <- mapply(
  sensors_label_fun,
  summary_data$sensors
)
summary_data$condition <- mapply(
  condition_label_fun,
  summary_data$sensors,
  summary_data$chg_env
)

condition_order = c(
  "STATIC",
  "NON-PLASTIC",
  "PLASTIC"
)
pairwise_comparisons <- list(
  c("STATIC", "NON-PLASTIC"),
  c("STATIC", "PLASTIC"),
  c("PLASTIC", "NON-PLASTIC")
)

p_label <- function(p_value) {
  threshold = 0.0001
  if (p_value < threshold) {
    return(paste0("p < ", threshold))
  } else {
    return(paste0("p = ", p_value))
  }
}

# *really* inefficient way to identify outliers
is_outlier <- function(value, cond, data, column) {
  cond_data <- filter(data, condition==cond)
  q1 <- summary(cond_data[,column])[["1st Qu."]]
  q3 <- summary(cond_data[,column])[["3rd Qu."]]
  H <- 1.5 * IQR(cond_data[,column])
  return( (value < (q1-H)) || (value > (q3+H)) )
}

####### misc #######
# Configure our default graphing theme
theme_set(theme_cowplot())
# Palette
cb_palette <- "Paired"
# Create a directory to store plots
dir.create(paste0(working_directory, "plots"), showWarnings=FALSE)
# Define sample mean function
samplemean <- function(x, d) {
  return(mean(x[d]))
}
```

6.4 The evolution of phenotypic plasticity

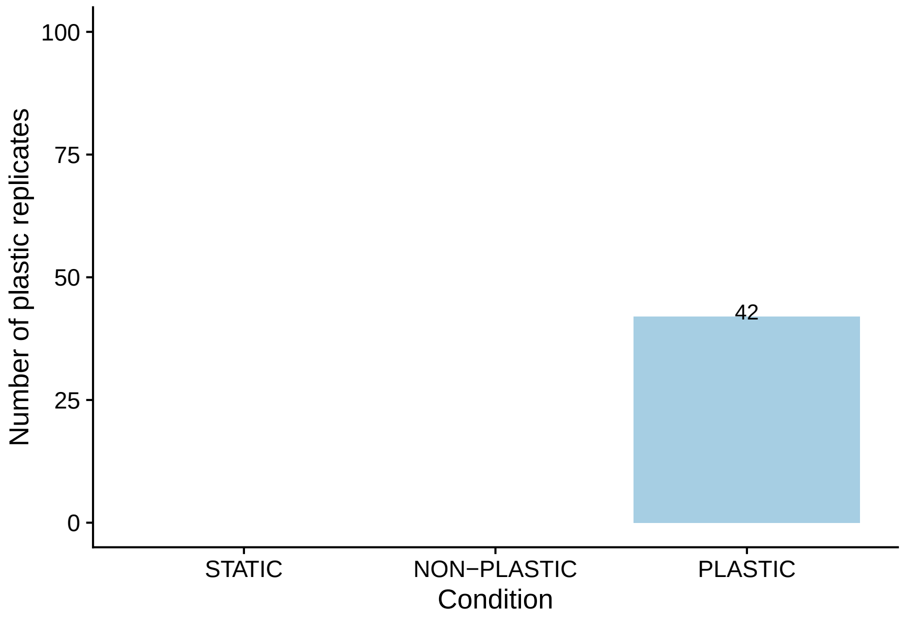
For sensor-enabled populations in fluctuating environments, we only transfered populations containing an optimally plastic genotype to phase-two.

```
summary_data_grouped = dplyr::group_by(summary_data, condition)
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())

ggplot(summary_data_group_counts, aes(x=condition, y=n, fill=condition)) +
  geom_col(position=position_dodge(0.9)) +
  geom_text(aes(label=n, y=n+2)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  ylab("Number of replicates transferred to phase two") +
  theme(
    legend.position="none"
  )
```



We can confirm our expectation that the dominant genotypes in non-plastic conditions are not phenotypically plastic.

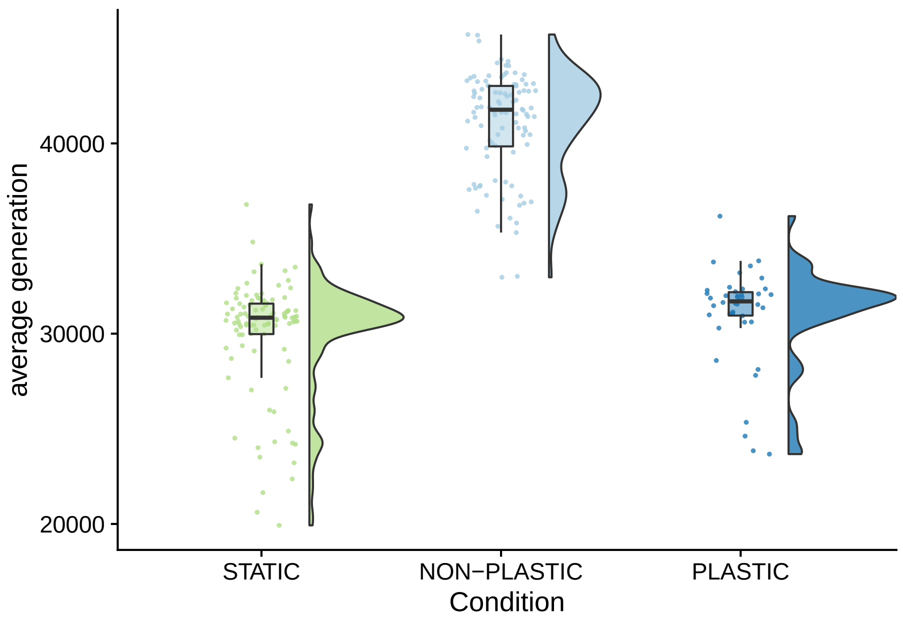
```
summary_data_grouped = dplyr::group_by(summary_data, condition, is_plastic)
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())
ggplot(filter(summary_data_group_counts, is_plastic), aes(x=condition, y=n, fill=condition)) +
  geom_col(
    position=position_dodge(0.9)
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  geom_text(aes(label=n, y=n+1)) +
  ylab("Number of plastic replicates") +
  ylim(0, 100) +
  theme(
    legend.position="none"
  )
```



6.5 Average generation

How many generations elapsed in each of of our treatments?

```
ggplot(summary_data, aes(x=condition, y=time_average_generation, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  ylab("average generation") +
  theme(
    legend.position="none"
  )
```



```
ggsave(paste0(working_directory, "plots/", "average-generation.png"))
```

```
## Saving 6.5 x 4.5 in image
```

```
kruskal.test(
  formula=time_average_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  time_average_generation by condition
## Kruskal-Wallis chi-squared = 177.33, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$time_average_generation,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$time_average_generation and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.004
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$time_average_generation)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$time_average_generation)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$time_average_generation)
  )
)
```

```
## [1] "PLASTIC median: 31697.65; STATIC median: 30839.75; NON-PLASTIC median: 41768.65
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=time_average_generation~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=9982"
## [1] "STATIC<-->PLASTIC: W=2818"
## [1] "PLASTIC<-->NON-PLASTIC: W=4186"
```

```
summary_data %>%
  group_by(condition) %>%
  summarise(mean=mean(time_average_generation),sd=sd(time_average_generation))
```

```
## # A tibble: 3 x 3
##   condition     mean    sd
##   <chr>        <dbl> <dbl>
## 1 NON-PLASTIC 41090. 2702.
## 2 PLASTIC     31016. 2615.
## 3 STATIC      30002. 3011.
```

6.6 Coalescence event count

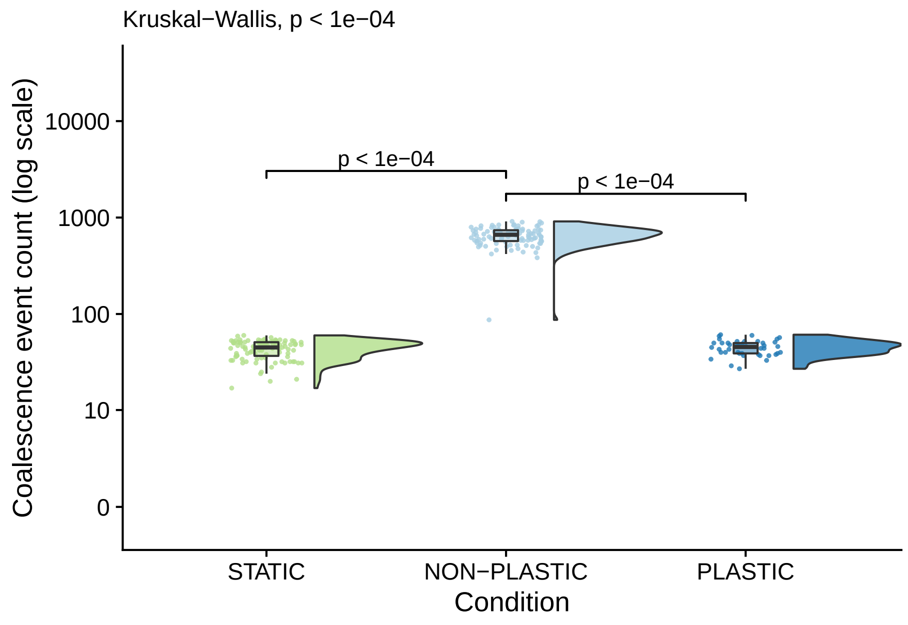
The number of times the most recent common ancestor changes gives us the number of selective sweeps that occur during the experiment.

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(phylo_mrca_changes ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=1)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in
stat.test$manual_position <-   log10(stat.test$y.position) * c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
```

```r
  is_outlier,
  summary_data$phylo_mrca_changes,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="phylo_mrca_changes")
)

coalescence_events_fig <- ggplot(
    summary_data,
    aes(x=condition, y=phylo_mrca_changes,fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order,
    breaks=condition_order
  ) +
  scale_y_continuous(
    name="Coalescence event count (log scale)",
    trans=pseudo_log_trans(sigma = 1, base = 10),
    breaks=c(0, 10, 100, 1000, 10000),
    limits=c(-1, 35000)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=phylo_mrca_changes~condition, data=summary_data)$p.valu
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "selective-sweeps.pdf"),
  width=5,
  height=5
)
coalescence_events_fig
```



```r
kruskal.test(
  formula=phylo_mrca_changes~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  phylo_mrca_changes by condition
## Kruskal-Wallis chi-squared = 175.46, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$phylo_mrca_changes,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$phylo_mrca_changes and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      1
##
## P value adjustment method: bonferroni
```

```r
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$phylo_mrca_changes)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$phylo_mrca_changes)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$phylo_mrca_changes)
  )
)
```

```
## [1] "PLASTIC median: 45.5; STATIC median: 45; NON-PLASTIC median: 663.5"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=phylo_mrca_changes~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

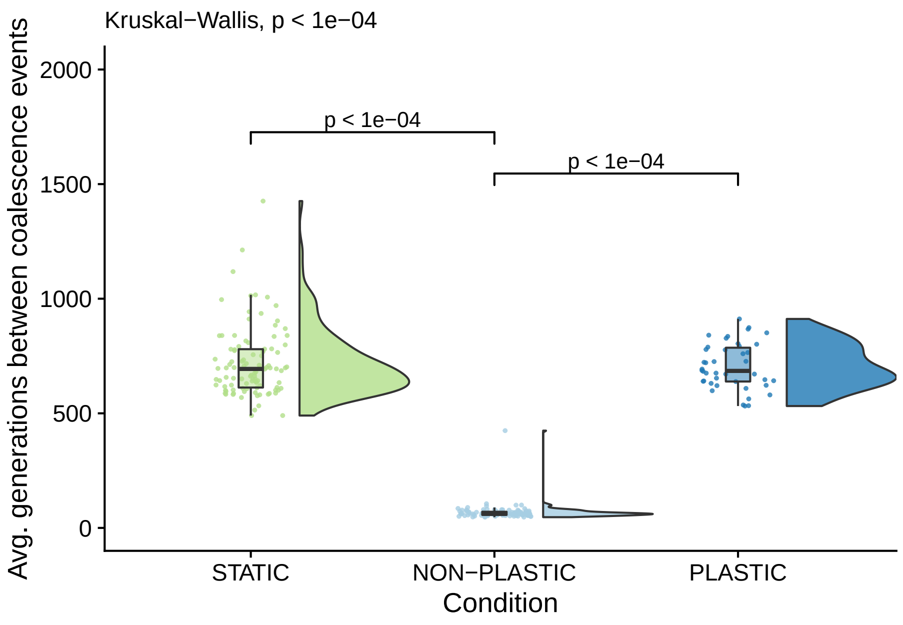
```
## [1] "STATIC<-->NON-PLASTIC: W=10000"
## [1] "STATIC<-->PLASTIC: W=2215"
## [1] "PLASTIC<-->NON-PLASTIC: W=4200"
```

6.6.1 Average number of generations between coalescence events

```
# Compute frequency of coalescence events
summary_data$generations_per_mrca_change <- summary_data$time_average_generation / summary_data$p

# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(generations_per_mrca_change ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition")
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <-  stat.test$y.position
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$generations_per_mrca_change,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="generations_per_mrca_change")
)

coalescence_events_freq_fig <- ggplot(
    summary_data,
    aes(x=condition, y=generations_per_mrca_change, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Avg. generations between coalescence events",
    limits=c(0, 2000),
    breaks=seq(0, 2000, 500)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=generations_per_mrca_change~condition, data=s
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "generations-between-selective-sweeps.png"),
  width=5,
  height=5
)
coalescence_events_freq_fig
```



```
kruskal.test(
  formula=generations_per_mrca_change~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  generations_per_mrca_change by condition
## Kruskal-Wallis chi-squared = 175.33, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$generations_per_mrca_change,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$generations_per_mrca_change and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      1
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$generations_per_mrca_change)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$generations_per_mrca_change)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$generations_per_mrca_change)
  )
)
```

```
## [1] "PLASTIC median: 685.001780758557; STATIC median: 693.676265008576; NON-PLASTIC
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=generations_per_mrca_change~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=0"
## [1] "STATIC<-->PLASTIC: W=2151"
## [1] "PLASTIC<-->NON-PLASTIC: W=0"
```

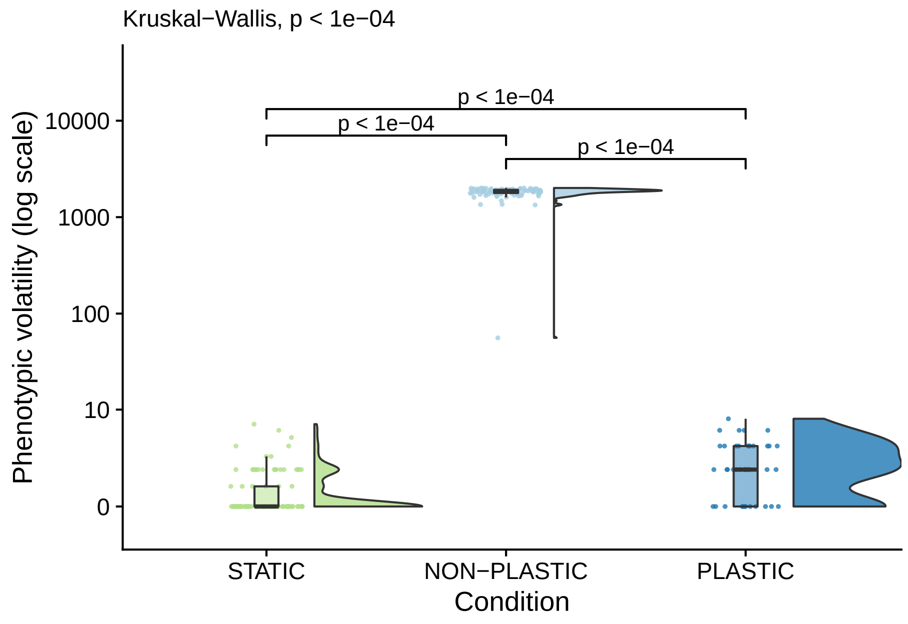
6.7 Phenotypic volatility along the dominant lineage

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_trait_volatility ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition", step.increase=1)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <-  log10(stat.test$y.position) * c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_trait_volatility,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_trait_volatility")
)

phenotypic_volatility_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_trait_volatility, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Phenotypic volatility (log scale)",
    trans=pseudo_log_trans(sigma = 1, base = 10),
    breaks=c(0, 10, 100, 1000, 10000),
    limits=c(-1, 35000)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_trait_volatility~condition,
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj<=alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "phenotypic-volatility.pdf"),
  width=5,
  height=5
)
```

```r
phenotypic_volatility_fig
```



```r
kruskal.test(
  formula=dominant_lineage_trait_volatility~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_trait_volatility by condition
## Kruskal-Wallis chi-squared = 190.78, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_trait_volatility,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_trait_volatility and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     8.7e-07
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_trait_volatility
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_trait_volatility)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_trait_volati
  )
)
```

```
## [1] "PLASTIC median: 2; STATIC median: 0; NON-PLASTIC median: 1868"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

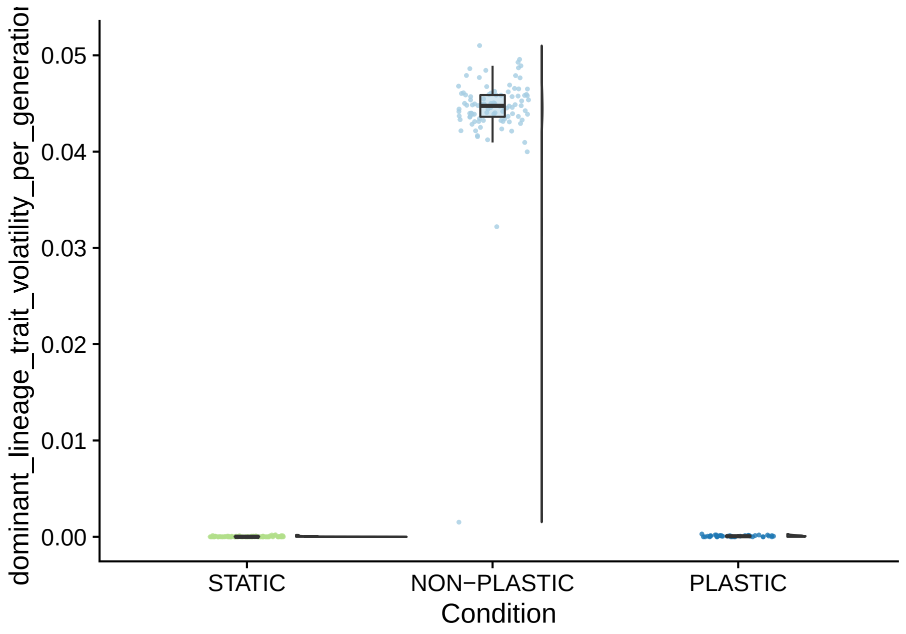
```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_trait_volatility~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=10000"
## [1] "STATIC<-->PLASTIC: W=3116.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=4200"
```

6.7.1 Phenotypic volatility normalized by generations elapsed

```
summary_data$dominant_lineage_trait_volatility_per_generation <- summary_data$dominant_
```

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_trait_volatility_per_generation, fill=co
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



```
kruskal.test(
  formula=dominant_lineage_trait_volatility_per_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_trait_volatility_per_generation by condition
## Kruskal-Wallis chi-squared = 189.62, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_trait_volatility_per_generation,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_trait_volatility_per_generation and summary_da
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     4.2e-06
##
```

```
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_trait_volatility_per_gener
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_trait_volatility_per_genera
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_trait_volatility_per_g
  )
)
```

```
## [1] "PLASTIC median: 6.33339279717772e-05; STATIC median: 0; NON-PLASTIC median: 0.04474401456
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_trait_volatility_per_generation~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=10000"
## [1] "STATIC<-->PLASTIC: W=3061.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=4200"
```

6.8 Phenotypic fidelity

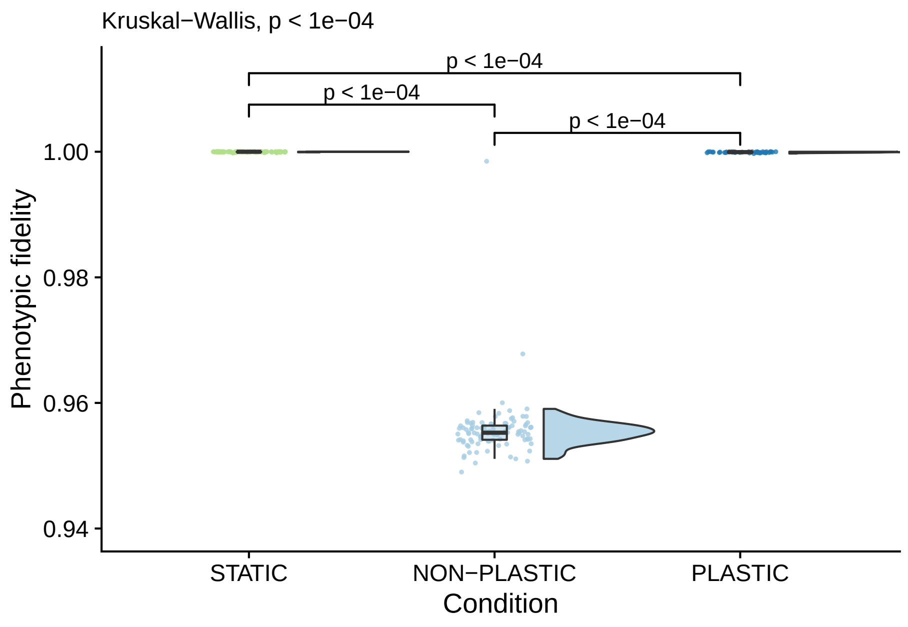
Frequency that an offspring's genotype is identical to a parent genotype (along the dominant lineage).

```
summary_data$dominant_lineage_trait_fidelity <- (summary_data$dominant_generation_born - summary_

# Compute manual labels for geom_signif
```

```
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_trait_fidelity ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=1.5)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <-  stat.test$y.position * c(1.0,1.0,1.0005)
stat.test$label <- mapply(p_label,stat.test$p.adj)


summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_trait_fidelity,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_trait_fidelity")
)


phenotypic_fidelity_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_trait_fidelity, fill=condition)
  ) +
  geom_flat_violin(
    data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Phenotypic fidelity",
    limits=c(0.94, 1.013),
    breaks=c(0.94, 0.96, 0.98, 1.0) #seq(0.94, 1.0, 0.01)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_trait_fidelity~condition, data=summary
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "phenotypic-fidelity.pdf"),
  width=5,
  height=5
)
phenotypic_fidelity_fig
```



```r
kruskal.test(
  formula=dominant_lineage_trait_fidelity~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_trait_fidelity by condition
## Kruskal-Wallis chi-squared = 189.62, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_trait_fidelity,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_trait_fidelity and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     4.2e-06
##
```

```
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_trait_fidelity)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_trait_fidelity)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_trait_fidelity)
  )
)
```

```
## [1] "PLASTIC median: 0.999936666072028; STATIC median: 1; NON-PLASTIC median: 0.95525598543618
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_trait_fidelity~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

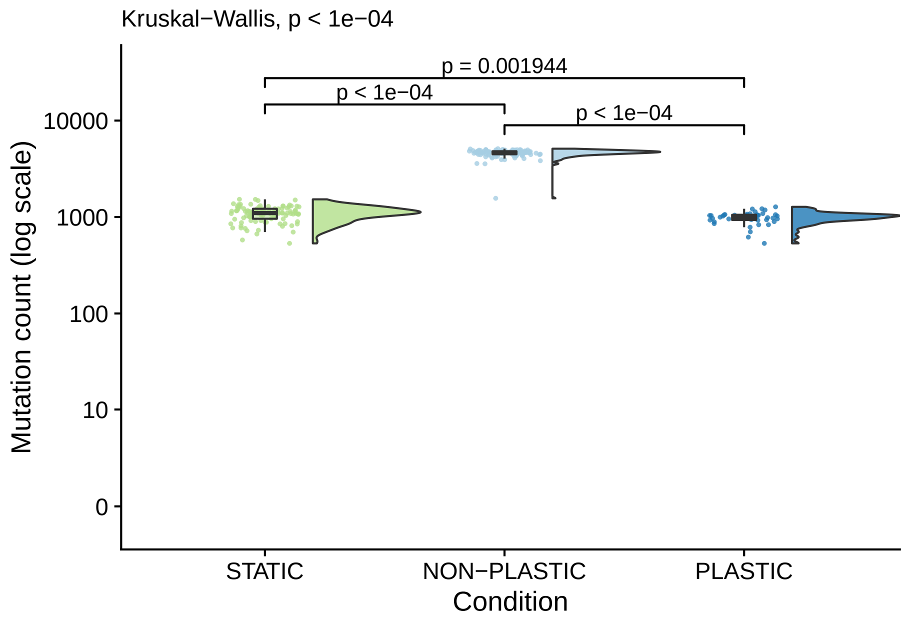
```
## [1] "STATIC<-->NON-PLASTIC: W=0"
## [1] "STATIC<-->PLASTIC: W=1138.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=0"
```

6.9 Mutation count

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_total_mut_cnt ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=1)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <- log10(stat.test$y.position) *  c(1.0,1.0,1.03) # c(1.0,1.
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_total_mut_cnt,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_total_mut_cnt")
)

mutation_count_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_total_mut_cnt, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data, !is_outlier),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Mutation count (log scale)",
    trans=pseudo_log_trans(sigma = 1, base = 10),
    breaks=c(0, 10, 100, 1000, 10000),
    limits=c(-1, 35000)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_total_mut_cnt~condition, data=summary_
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "mutation-accumulation.pdf"),
  width=5,
  height=4
)
mutation_count_fig
```



```
kruskal.test(
  formula=dominant_lineage_total_mut_cnt~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_total_mut_cnt by condition
## Kruskal-Wallis chi-squared = 179.33, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_total_mut_cnt,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_total_mut_cnt and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.0019
##
```

```
## P value adjustment method: bonferroni
```

```r
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_total_mut_cnt)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_total_mut_cnt)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_total_mut_cnt)
  )
)
```

```
## [1] "PLASTIC median: 998.5; STATIC median: 1100; NON-PLASTIC median: 4657.5"
```

```r
print("Wilcox rank sum test statistics:")
```

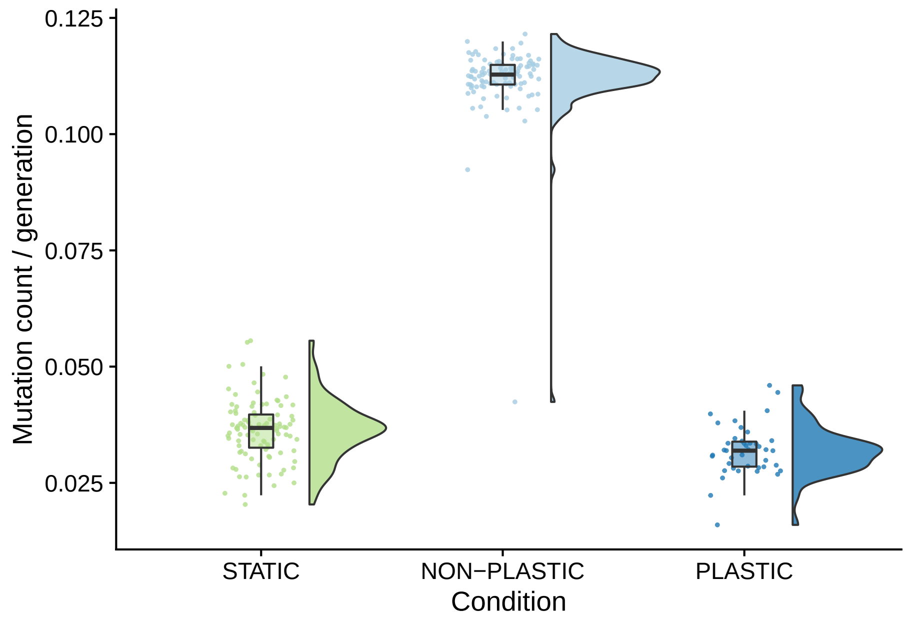
```
## [1] "Wilcox rank sum test statistics:"
```

```r
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_total_mut_cnt~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=10000"
## [1] "STATIC<-->PLASTIC: W=1336.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=4200"
```

6.9.1 Mutation count normalized by generations elapsed

```r
summary_data$mutations_per_generation <- summary_data$dominant_lineage_total_mut_cnt / summary_da

ggplot(summary_data, aes(x=condition, y=mutations_per_generation, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Mutation count / generation") +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



```r
kruskal.test(
  formula=mutations_per_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  mutations_per_generation by condition
## Kruskal-Wallis chi-squared = 180.11, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$mutations_per_generation,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$mutations_per_generation and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      2e-04
##
```

```
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$mutations_per_generation)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$mutations_per_generation)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$mutations_per_generation)
  )
)
```

```
## [1] "PLASTIC median: 0.0319267181456982; STATIC median: 0.0368157192941933; NON-PLAS
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=mutations_per_generation~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=9987"
## [1] "STATIC<-->PLASTIC: W=1206"
## [1] "PLASTIC<-->NON-PLASTIC: W=4198"
```

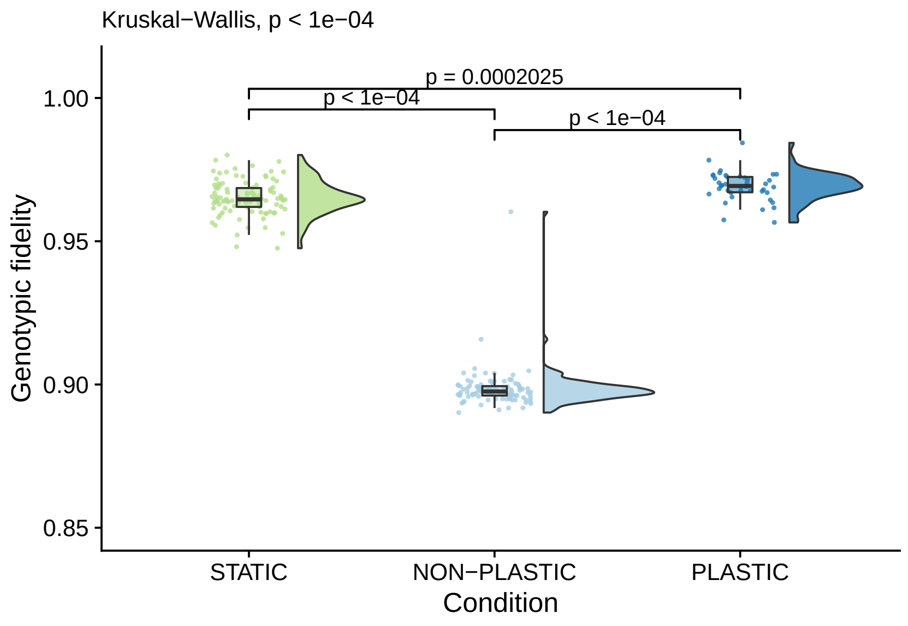
6.10 Genotypic fidelity

The frequency that an offspring's genotype is the same as a parent's genotype.

```
summary_data$dominant_lineage_genotypic_fidelity <- (summary_data$dominant_generation_l

# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_genotypic_fidelity ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=0.2)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <- stat.test$y.position * c(1.0,1.0,1.0)
stat.test$label <- mapply(p_label,stat.test$p.adj)

genotypic_fidelity_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_genotypic_fidelity, fill=condition)
  ) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Genotypic fidelity",
    limits=c(0.85, 1.01),
    breaks=c(0.85, 0.90, 0.95, 1.0) #seq(0.85, 1.0, 0.02)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_genotypic_fidelity~conditio
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
ggsave(
  paste0(working_directory, "plots/", "genotypic-fidelity.pdf"),
  width=5,
  height=4
)
genotypic_fidelity_fig
```



```
kruskal.test(
  formula=dominant_lineage_genotypic_fidelity~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_genotypic_fidelity by condition
## Kruskal-Wallis chi-squared = 179.86, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_genotypic_fidelity,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_genotypic_fidelity and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      2e-04
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_genotypic_fidelity)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_genotypic_fidelity)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_genotypic_fidelity)
  )
)
```

```
## [1] "PLASTIC median: 0.969286906891951; STATIC median: 0.964620594632577; NON-PLASTIC median:
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_genotypic_fidelity~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

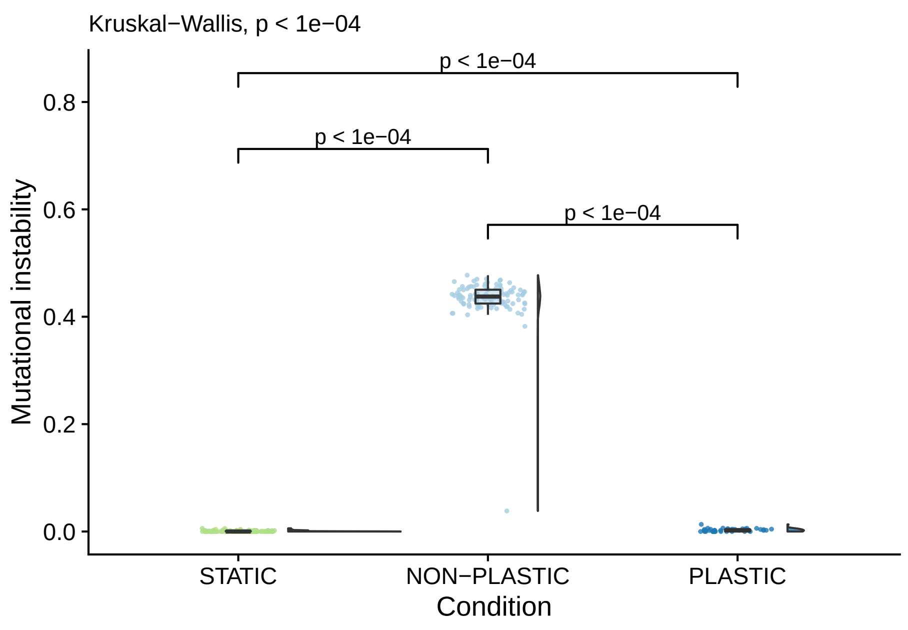
```
## [1] "STATIC<-->NON-PLASTIC: W=18"
## [1] "STATIC<-->PLASTIC: W=2992"
## [1] "PLASTIC<-->NON-PLASTIC: W=2"
```

6.11 Characterizing variation along dominant lineages

6.11.1 Mutational instability

```
summary_data$frac_phenotype_changing_mut_steps <- summary_data$dominant_lineage_num_mu
summary_data$frac_phenotype_stable_mut_steps <- 1 - summary_data$frac_phenotype_changi

# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(frac_phenotype_changing_mut_steps ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=0.2)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <- stat.test$y.position #* c(1.0,1.0,1.0)
stat.test$label <- mapply(p_label,stat.test$p.adj)

ggplot(summary_data, aes(x=condition, y=frac_phenotype_changing_mut_steps, fill=condit
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Mutational instability") +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=frac_phenotype_changing_mut_steps~condition, data=summa
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```



```
ggsave(paste0(working_directory, "plots/", "frac_phenotype_changing_mutational_steps.p
```

```
## Saving 6.5 x 4.5 in image
```

```
kruskal.test(
  formula=frac_phenotype_changing_mut_steps~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_phenotype_changing_mut_steps by condition
## Kruskal-Wallis chi-squared = 191.23, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$frac_phenotype_changing_mut_steps,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$frac_phenotype_changing_mut_steps and summary_data$condition
##
##             NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     2.3e-07
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$frac_phenotype_changing_mut_steps)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$frac_phenotype_changing_mut_steps)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$frac_phenotype_changing_mut_steps)
  )
)
```

```
## [1] "PLASTIC median: 0.00224941742616098; STATIC median: 0; NON-PLASTIC median: 0.437583018324
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=frac_phenotype_changing_mut_steps~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

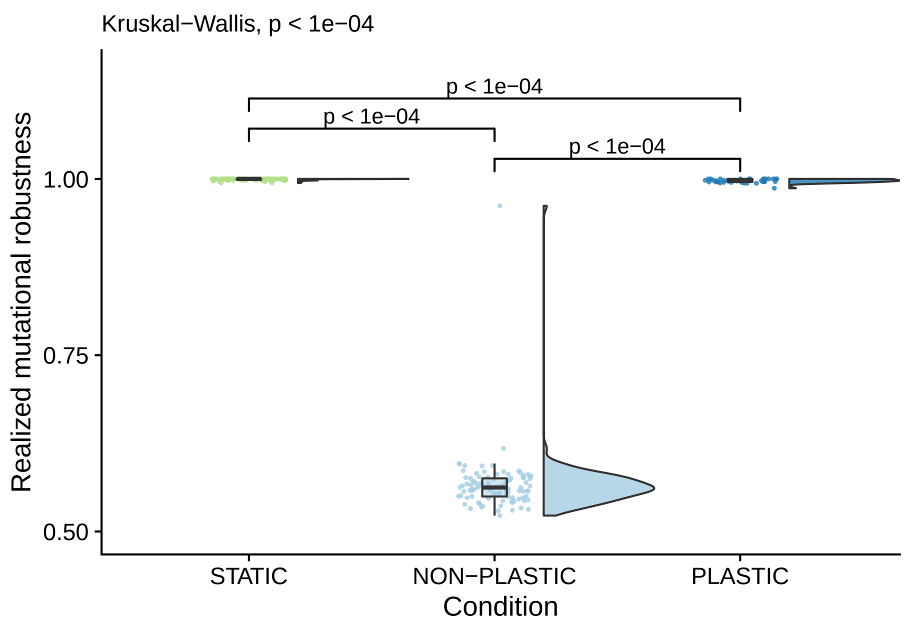
```
## [1] "STATIC<-->NON-PLASTIC: W=10000"
## [1] "STATIC<-->PLASTIC: W=3172"
## [1] "PLASTIC<-->NON-PLASTIC: W=4200"
```

6.11.2 Mutational stability (realized mutational robustness)

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(frac_phenotype_stable_mut_steps ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition",step.increase=0.75)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <- stat.test$y.position #* c(1.0,1.0,1.0)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_trait_volatility,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_trait_volatility")
)

mutational_stability_fig <- ggplot(
    summary_data,
    aes(x=condition, y=frac_phenotype_stable_mut_steps, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_y_continuous(
    name="Realized mutational robustness",
    limits=c(0.5, 1.15),
    breaks=c(0.5, 0.75, 1.0)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=frac_phenotype_stable_mut_steps~condition, data=summary
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
mutational_stability_fig
```



```
kruskal.test(
  formula=frac_phenotype_stable_mut_steps~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_phenotype_stable_mut_steps by condition
## Kruskal-Wallis chi-squared = 191.23, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$frac_phenotype_stable_mut_steps,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$frac_phenotype_stable_mut_steps and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     2.3e-07
##
```

```
## P value adjustment method: bonferroni
```

```r
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$frac_phenotype_stable_mut_steps)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$frac_phenotype_stable_mut_steps)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$frac_phenotype_stable_mut_steps)
  )
)
```

```
## [1] "PLASTIC median: 0.997750582573839; STATIC median: 1; NON-PLASTIC median: 0.56241698167545
```

```r
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```r
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=frac_phenotype_stable_mut_steps~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

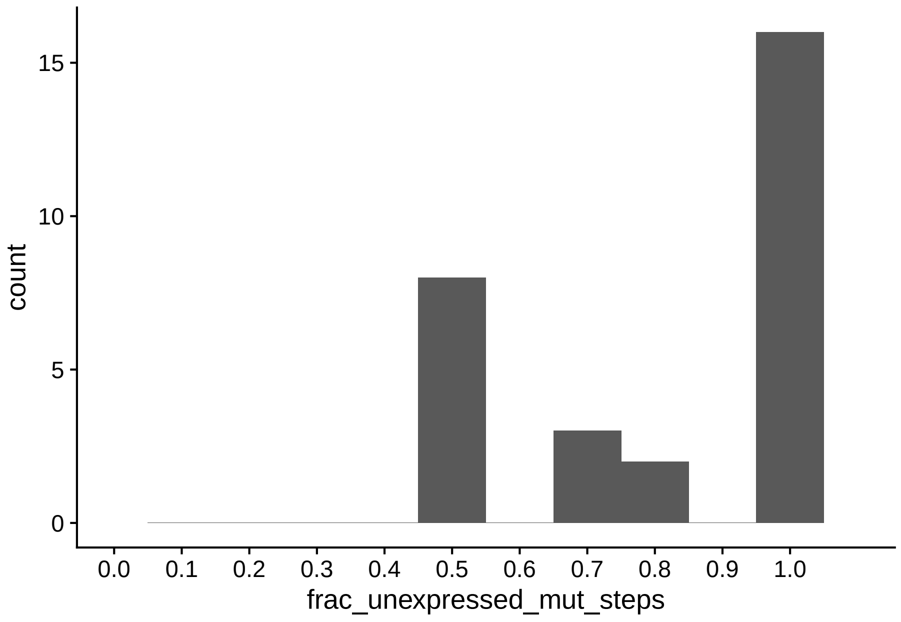
```
## [1] "STATIC<-->NON-PLASTIC: W=0"
## [1] "STATIC<-->PLASTIC: W=1028"
## [1] "PLASTIC<-->NON-PLASTIC: W=0"
```

6.11.3 For PLASTIC populations, what fraction of phenotype-altering mutations occurred in the unexpressed phenotype?

```r
summary_data$frac_unexpressed_mut_steps <- summary_data$dominant_lineage_num_mut_steps_that_chang
summary_data$frac_expressed_mut_steps <- summary_data$dominant_lineage_num_mut_steps_that_change_
```

```
ggplot(filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_that_
  geom_histogram(binwidth=0.1) +
  scale_x_continuous(
    limits=c(0, 1.1),
    breaks=seq(0, 1.0, 0.1)
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Removed 2 rows containing missing values (geom_bar).
```



```
print(paste0("PLASTIC - Mean with bootstrapped 95% CI"))
```

```
## [1] "PLASTIC - Mean with bootstrapped 95% CI"
```

```
bo <- boot(filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_
print(bo)
```

```
##
## ORDINARY NONPARAMETRIC BOOTSTRAP
##
##
## Call:
## boot(data = filter(summary_data, condition == "PLASTIC" & dominant_lineage_num_mut_
##     0)$frac_unexpressed_mut_steps, statistic = samplemean, R = 10000)
```

```
##
##
## Bootstrap Statistics :
##      original        bias    std. error
## t1* 0.8247126 -0.0007856322  0.03986807
```

```
print(boot.ci(bo, conf=0.95, type="perc"))
```

```
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
## Based on 10000 bootstrap replicates
##
## CALL :
## boot.ci(boot.out = bo, conf = 0.95, type = "perc")
##
## Intervals :
## Level     Percentile
## 95%   ( 0.7443,  0.8994 )
## Calculations and Intervals on Original Scale
```

```
plastic_summary_data <- filter(summary_data, condition=="PLASTIC")
aggregate_frac_mut_steps_that_change_unexpressed_phenotype <- sum(plastic_summary_data$dominant_l
sum(plastic_summary_data$dominant_lineage_num_mut_steps_that_change_unexpressed_phenotype)
```

```
## [1] 83
```

```
sum(plastic_summary_data$dominant_lineage_num_mut_steps_that_change_aggregate_phenotype)
```

```
## [1] 102
```

```
aggregate_frac_mut_steps_that_change_unexpressed_phenotype
```

```
## [1] 0.8137255
```

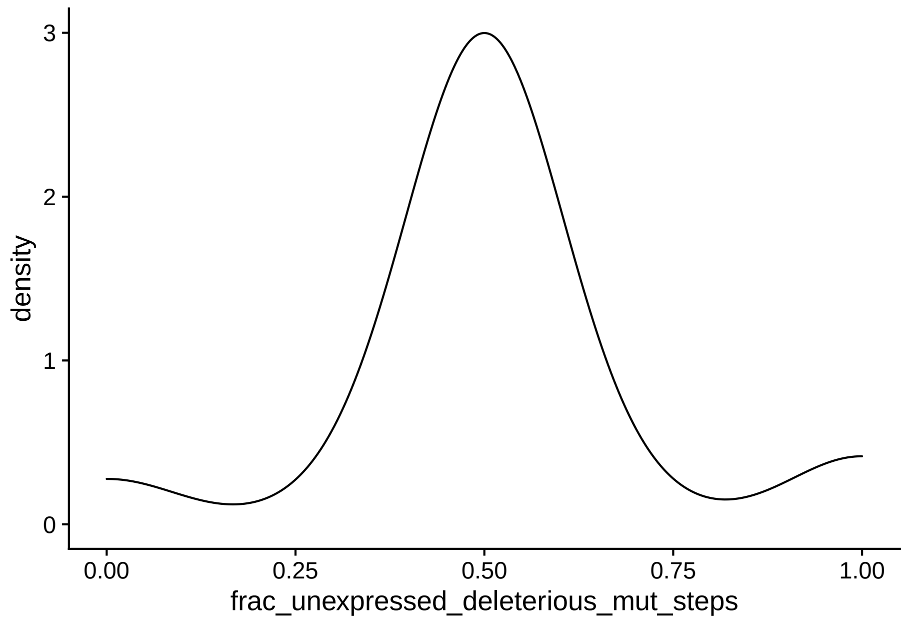
83 / 102 (0.8137255)

6.11.4 For PLASTIC populations, what fraction of mutations that affect the unexpressed phenotype are deleterious versus beneficial?

```
aggregate_frac_unexpressed_deleterious_mut_steps <- sum(plastic_summary_data$dominant_lineage_num
aggregate_frac_unexpressed_beneficial_mut_steps <- sum(plastic_summary_data$dominant_lineage_num_
```

6.11.4.1 Deleterious mutations

```
summary_data$frac_unexpressed_deleterious_mut_steps <- summary_data$dominant_lineage_num_mut_step
ggplot(
  filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_that_change_unexpres
  aes(x=frac_unexpressed_deleterious_mut_steps)
  ) +
  geom_density() +
  theme(
    legend.position="none"
  )
```



```
bo <- boot(filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_
print(bo)
```

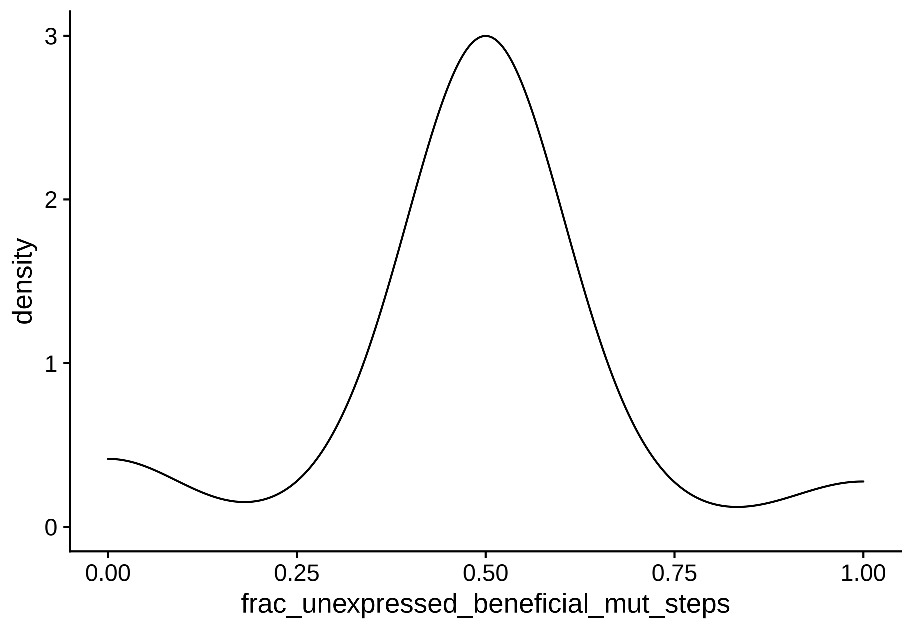
```
##
## ORDINARY NONPARAMETRIC BOOTSTRAP
##
##
## Call:
## boot(data = filter(summary_data, condition == "PLASTIC" & dominant_lineage_num_mut_s
##     0)$frac_unexpressed_deleterious_mut_steps, statistic = samplemean,
##     R = 10000)
##
##
## Bootstrap Statistics :
##      original        bias    std. error
## t1* 0.5172414 -0.0001421839  0.03979612
```

```
print(boot.ci(bo, conf=0.95, type="perc"))
```

```
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
## Based on 10000 bootstrap replicates
##
## CALL :
## boot.ci(boot.out = bo, conf = 0.95, type = "perc")
##
## Intervals :
## Level     Percentile
## 95%   ( 0.4402,  0.5954 )
## Calculations and Intervals on Original Scale
```

6.11.4.2 Beneficial mutations

```
summary_data$frac_unexpressed_beneficial_mut_steps <- summary_data$dominant_lineage_num_mut_steps

ggplot(
  filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_that_change_unexpres
  aes(x=frac_unexpressed_beneficial_mut_steps)
  ) +
  geom_density() +
  theme(
    legend.position="none"
  )
```



```
bo <- boot(filter(summary_data, condition=="PLASTIC" & dominant_lineage_num_mut_steps_that_change
print(bo)
```

```
##
## ORDINARY NONPARAMETRIC BOOTSTRAP
##
##
## Call:
## boot(data = filter(summary_data, condition == "PLASTIC" & dominant_lineage_num_mut_s
##     0)$frac_unexpressed_beneficial_mut_steps, statistic = samplemean,
##     R = 10000)
##
##
## Bootstrap Statistics :
##      original        bias    std. error
## t1* 0.4827586 -0.0002229885  0.03961709
```

```
print(boot.ci(bo, conf=0.95, type="perc"))
```

```
## BOOTSTRAP CONFIDENCE INTERVAL CALCULATIONS
## Based on 10000 bootstrap replicates
##
## CALL :
## boot.ci(boot.out = bo, conf = 0.95, type = "perc")
##
## Intervals :
## Level     Percentile
## 95%   ( 0.4046,  0.5609 )
## Calculations and Intervals on Original Scale
```

6.12 Mutational robustness

Mutational robustness measures the fraction of one-step mutations on a focal genotype that result in a phenotypic change. Here, we calculate the mutational robustness of the representative genotype from each replicate (the most abundant genotype in the final population).

This data is located in a separate .tar.gz file on OSF, so we need to load it and wrangle the data.

```
# Load the data
df_mut = read.csv(paste0(working_directory, 'mutational_robustness/data/aggregated_muta
# Extract the treatment for each line
df_mut$treatment = 'STATIC'
df_mut[df_mut$environment == 'chg-u100',]$treatment = 'PLASTIC'
df_mut[df_mut$environment == 'chg-u100' & df_mut$sensors == F,]$treatment = 'NON-PLASTI
df_mut$treatment_factor = factor(df_mut$treatment, levels = c('STATIC', 'NON-PLASTIC',
# For compatibility with is_outlier above
df_mut$condition = df_mut$treatment_factor
# Calculate robustness (originally calculated as volatility)
```

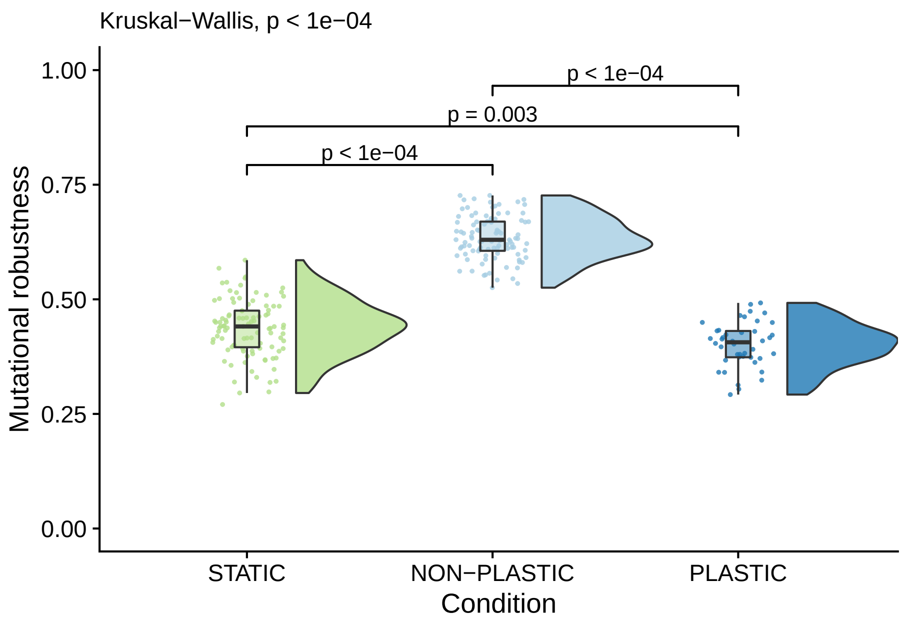
```
df_mut$mutational_robustness = 1 - df_mut$one_step_diff_pheno_frac
```

Now we can plot mutational robustness:

```
# Compute manual labels for geom_signif
stat.test <- df_mut %>%
  wilcox_test(mutational_robustness ~ treatment_factor) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="treatment_factor")
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <-   stat.test$y.position * c(1.05,1.1,1.15)
stat.test$label <- mapply(p_label,stat.test$p.adj)
df_mut$is_outlier <- mapply(
  is_outlier,
  df_mut$mutational_robustness,
  df_mut$treatment_factor,
  MoreArgs=list(data=df_mut, column="mutational_robustness")
)

# Remap colors so that colors map
color_map = c(
  'STATIC' = brewer.pal(3, cb_palette)[3],
  'PLASTIC' = brewer.pal(3, cb_palette)[2],
  'NON-PLASTIC' = brewer.pal(3, cb_palette)[1]
)

# Plot!
mut_robustness_fig <- ggplot(df_mut, aes(x=treatment_factor, y=mutational_robustness, fill=treatm
  geom_flat_violin( data=filter(df_mut,is_outlier==FALSE),
    scale="width", position = position_nudge(x = .2, y = 0), alpha = .8) +
  geom_point(mapping=aes(color=treatment_factor), position = position_jitter(width = .15), size =
  geom_boxplot(width = .1, outlier.shape = NA, alpha = 0.5) +
  scale_x_discrete( name="Condition") +
  scale_y_continuous( name='Mutational robustness', limits=c(0, 1)) +
  scale_fill_manual( values = color_map ) +
  scale_color_manual( values = color_map ) +
  labs( subtitle=paste0( "Kruskal-Wallis, ", p_label(signif(kruskal.test(formula=mutational_robus
  ggsignif::geom_signif( data=filter(stat.test, p.adj <= alpha),  aes(xmin=group1,xmax=group2,ann
  theme( legend.position="none" )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

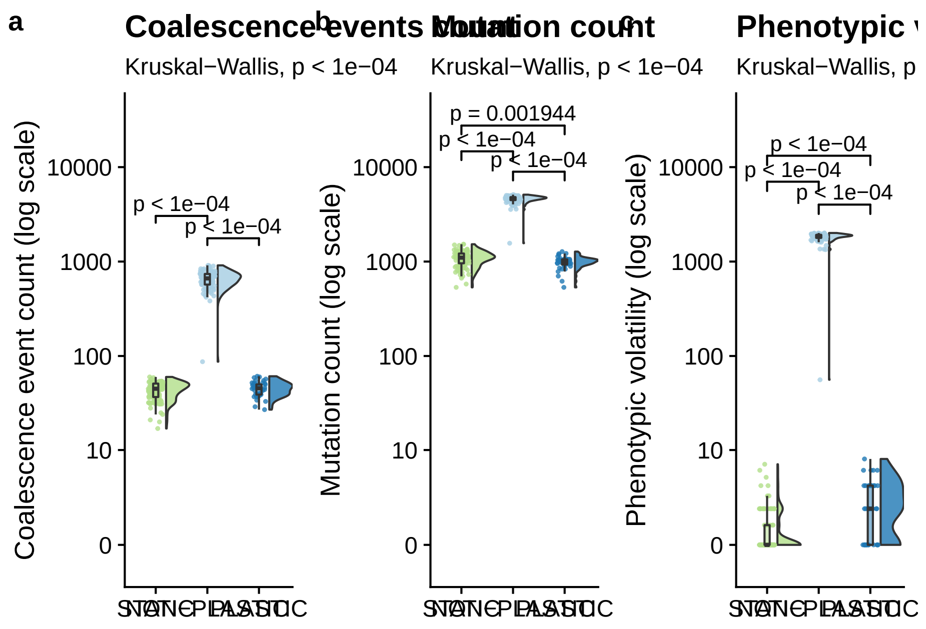
```
mut_robustness_fig
```



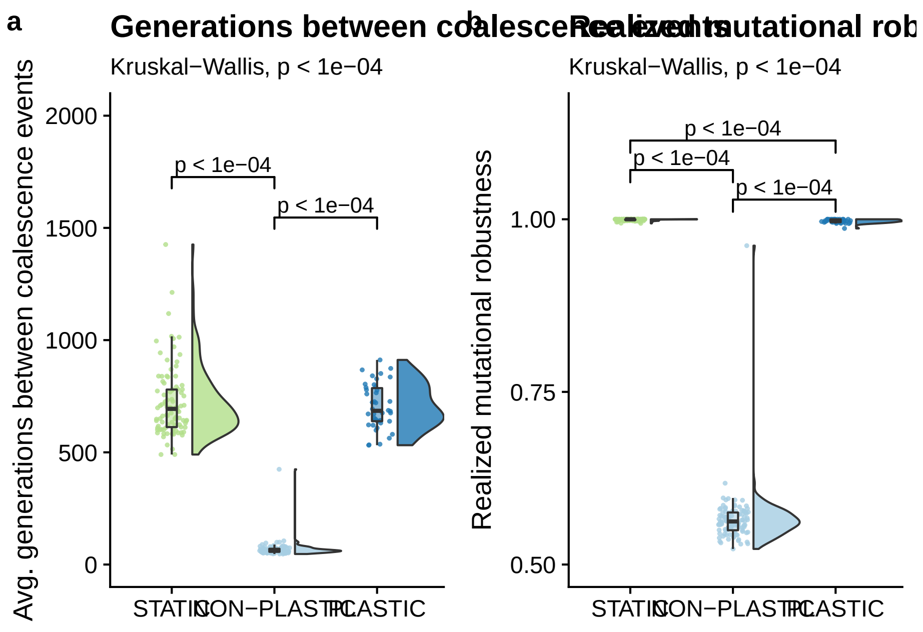
6.13 Manuscript figures

Figures styled for the paper.

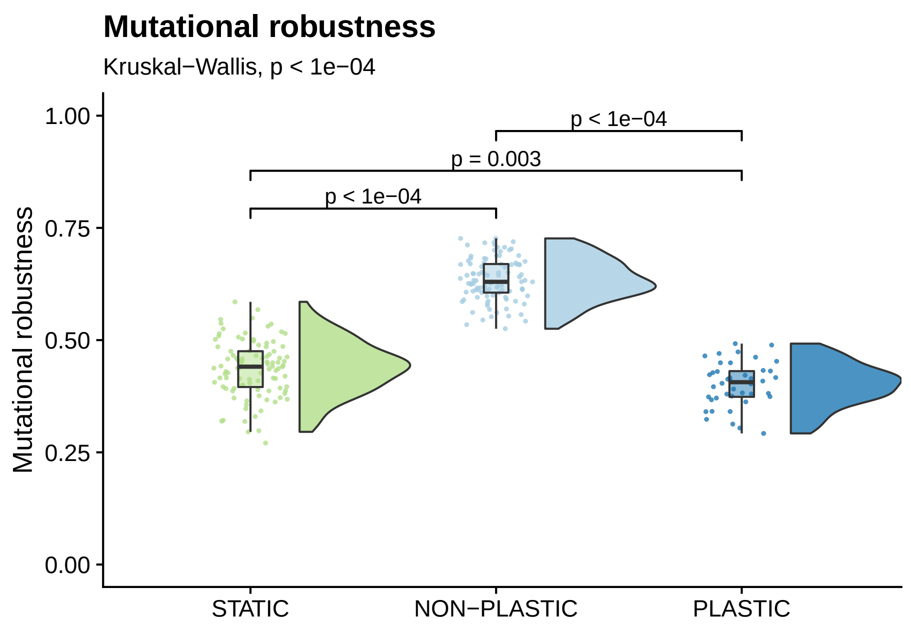
```r
magnitude_grid <- plot_grid(
  coalescence_events_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Coalescence events count"),
  mutation_count_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Mutation count"),
  phenotypic_volatility_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Phenotypic volatility"),
  nrow=1,
  ncol=3,
  align="v",
  labels="auto"
)
magnitude_grid
```



```
pace_grid <- plot_grid(
  coalescence_events_freq_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Generations between coalescence events"),
  mutational_stability_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Realized mutational robustness"),
  nrow=1,
  ncol=2,
  align="v",
  labels="auto"
)
pace_grid
```



```
# Even though mutational robustness is shown by itself, this ensures it is plotted ide
mut_robustness_grid = plot_grid(
  mut_robustness_fig +
    theme(
      legend.position="none",
      axis.title.x=element_blank()
    ) +
    ggtitle("Mutational robustness"),
  nrow=1,
  ncol=1,
  align="v",
  labels=""
)
mut_robustness_grid
```



```
save_plot(
  paste0(working_directory, "plots/", "evolutionary-change-magnitude-panel.pdf"),
  magnitude_grid,
  base_height=6,
  base_asp=3/1
)

save_plot(
  paste0(working_directory, "plots/", "evolutionary-change-pace-panel.pdf"),
  pace_grid,
  base_height=6,
  base_asp=2/1
)

save_plot(
  paste0(working_directory, "plots/", "mutational-robustness.pdf"),
  mut_robustness_grid,
  base_height=6,
  base_asp=1
)
```

Chapter 7

Evolution and maintenance of novel functions

The effect of adaptive phenotypic plasticity on the evolution and maintenance of novel functions.

7.1 Overview

```
total_updates <- 200000
replicates <- 100
alpha <- 0.05

focal_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")
extra_traits <- c(
  "nor","xor","equals",
  "logic_3aa","logic_3ab","logic_3ac",
  "logic_3ad","logic_3ae","logic_3af",
  "logic_3ag","logic_3ah","logic_3ai",
  "logic_3aj","logic_3ak","logic_3al",
  "logic_3am","logic_3an","logic_3ao",
  "logic_3ap","logic_3aq","logic_3ar",
  "logic_3as","logic_3at","logic_3au",
  "logic_3av","logic_3aw","logic_3ax",
  "logic_3ay","logic_3az","logic_3ba",
  "logic_3bb","logic_3bc","logic_3bd",
  "logic_3be","logic_3bf","logic_3bg",
  "logic_3bh","logic_3bi","logic_3bj",
  "logic_3bk","logic_3bl","logic_3bm",
  "logic_3bn","logic_3bo","logic_3bp",
  "logic_3bq","logic_3br","logic_3bs",
  "logic_3bt","logic_3bu","logic_3bv",
  "logic_3bw","logic_3bx","logic_3by",
  "logic_3bz","logic_3ca","logic_3cb",
  "logic_3cc","logic_3cd","logic_3ce",
  "logic_3cf","logic_3cg","logic_3ch",
  "logic_3ci","logic_3cj","logic_3ck",
  "logic_3cl","logic_3cm","logic_3cn",
  "logic_3co","logic_3cp"
)

# Relative location of data.
working_directory <- "experiments/2021-01-31-complex-features/analysis/" # << For book
# working_directory <- "./"
```

7.2 Analysis dependencies

Load all required R libraries.

```
library(ggplot2)
library(rstatix)
library(ggsignif)
library(scales)
library(tidyverse)
library(cowplot)
library(RColorBrewer)
library(Hmisc)
library(boot)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd97
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

7.3 Setup

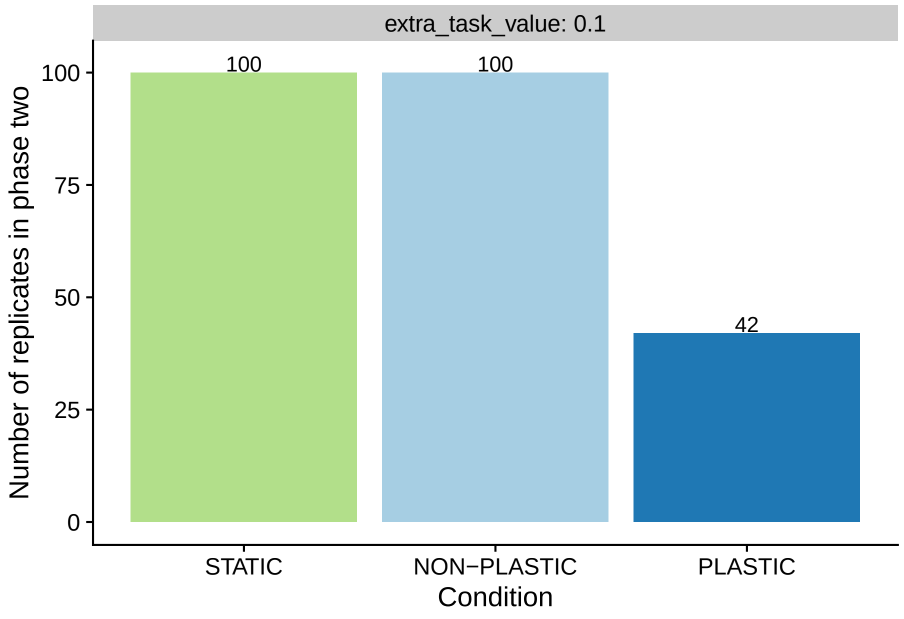
```
####### summary data #######
summary_data_loc <- paste0(working_directory, "data/aggregate.csv")
summary_data <- read.csv(summary_data_loc, na.strings="NONE")

summary_data$DISABLE_REACTION_SENSORS <- as.factor(summary_data$DISABLE_REACTION_SENSORS)
summary_data$chg_env <- summary_data$chg_env == "True"
summary_data$dominant_plastic_odd_even <- as.factor(summary_data$dominant_plastic_odd_even)
summary_data$sensors <- summary_data$DISABLE_REACTION_SENSORS == "0"
summary_data$is_plastic <- summary_data$dominant_plastic_odd_even == "True"
summary_data$extra_task_value <- as.factor(summary_data$extra_task_value)
summary_data <- filter(summary_data, extra_task_value == 0.1)

env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
}

condition_label_fun <- function(has_sensors, env_chg) {
  if (has_sensors && env_chg) {
    return("PLASTIC")
  } else if (env_chg) {
    return("NON-PLASTIC")
  } else {
    return("STATIC")
  }
}

summary_data$env_label <- mapply(
  env_label_fun,
  summary_data$chg_env
)
summary_data$sensors_label <- mapply(
  sensors_label_fun,
  summary_data$sensors
)
summary_data$condition <- mapply(
  condition_label_fun,
  summary_data$sensors,
  summary_data$chg_env
)

condition_order = c(
  "STATIC",
  "NON-PLASTIC",
  "PLASTIC"
)
pairwise_comparisons <- list(
  c("STATIC", "NON-PLASTIC"),
  c("STATIC", "PLASTIC"),
  c("PLASTIC", "NON-PLASTIC")
)

p_label <- function(p_value) {
  threshold = 0.0001
  if (p_value < threshold) {
    return(paste0("p < ", threshold))
  } else {
    return(paste0("p = ", p_value))
  }
}

# *really* inefficient way to identify outliers
is_outlier <- function(value, cond, data, column) {
  cond_data <- filter(data, condition==cond)
  q1 <- summary(cond_data[,column])[["1st Qu."]]
  q3 <- summary(cond_data[,column])[["3rd Qu."]]
  H <- 1.5 * IQR(cond_data[,column])
  return( (value < (q1-H)) || (value > (q3+H)) )
}
```

```
###### time series #####
lineage_time_series_data_loc <- paste0(working_directory, "data/lineage_series.csv")
lineage_time_series_data <- read.csv(lineage_time_series_data_loc)

lineage_time_series_data$DISABLE_REACTION_SENSORS <- as.factor(lineage_time_series_data$DISABLE_R
lineage_time_series_data$chg_env <- lineage_time_series_data$chg_env == "True"
lineage_time_series_data$sensors <- lineage_time_series_data$DISABLE_REACTION_SENSORS == "0"
lineage_time_series_data$extra_task_value <- as.factor(lineage_time_series_data$extra_task_value)

lineage_time_series_data$env_label <- mapply(
  env_label_fun,
  lineage_time_series_data$chg_env
)
lineage_time_series_data$sensors_label <- mapply(
  sensors_label_fun,
  lineage_time_series_data$sensors
)
lineage_time_series_data$condition <- mapply(
  condition_label_fun,
  lineage_time_series_data$sensors,
  lineage_time_series_data$chg_env
)

####### misc #######
# Configure our default graphing theme
theme_set(theme_cowplot())
# Palette
cb_palette <- "Paired"
# Create directory to dump plots
dir.create(paste0(working_directory, "plots"), showWarnings=FALSE)
# Sample mean function
samplemean <- function(x, d) {
  return(mean(x[d]))
}
```

7.4 The evolution of phenotypic plasticity

For sensor-enabled populations in fluctuating environments, we only transfered populations containing an optimally plastic genotype to phase two.

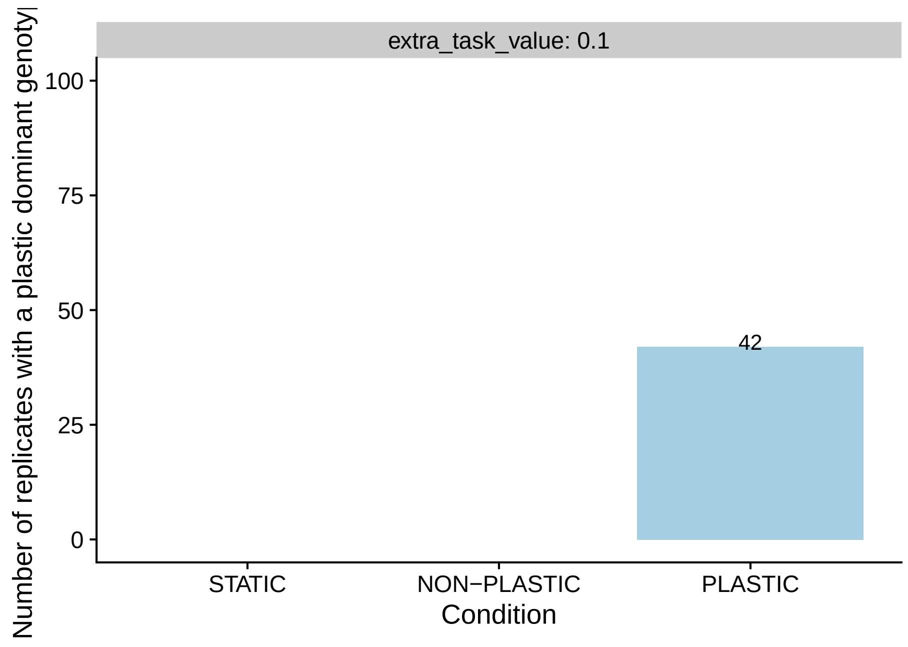
```
summary_data_grouped = dplyr::group_by(summary_data, sensors, env_label, condition, extra_task_va
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())

ggplot(summary_data_group_counts, aes(x=condition, y=n, fill=condition)) +
  geom_col(position=position_dodge(0.9)) +
  geom_text(aes(label=n, y=n+2)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  ylab("Number of replicates in phase two") +
  facet_wrap(~extra_task_value, labeller=label_both) +
  theme(
    legend.position="none"
  )
```



We can confirm our expectation that the dominant genotypes in non-plastic conditions are not phenotypically plastic.

```
summary_data_grouped = dplyr::group_by(summary_data, condition, is_plastic, extra_task
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())
ggplot(filter(summary_data_group_counts, is_plastic), aes(x=condition, y=n, fill=condi
  geom_col(position=position_dodge(0.9)) +
```

```r
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  ylim(0, 100) +
  geom_text(aes(label=n, y=n+1)) +
  ylab("Number of replicates with a plastic dominant genotype") +
  facet_wrap(~extra_task_value, labeller=label_both) +
  theme(
    legend.position="none"
  )
```



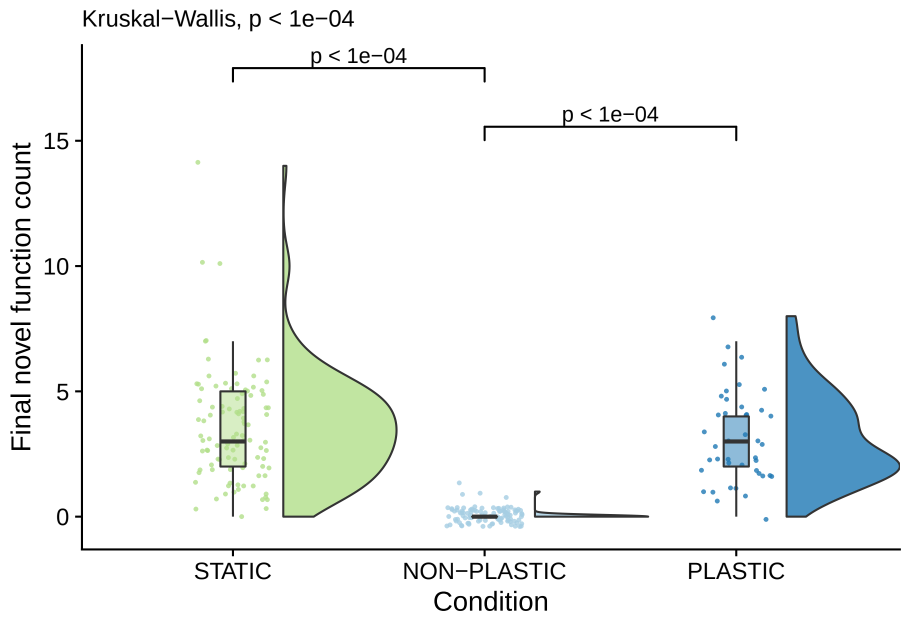
7.5 Final novel function count (dominant genotype)

How many novel functions do final dominant genotypes perform?

```r
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_extra_tasks ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition") # ,step.increase=1
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <- stat.test$y.position #* c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_extra_tasks,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_extra_tasks")
)

final_novel_task_count_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_extra_tasks, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Final novel function count"
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_extra_tasks~condition, data=summary_data)$p.va
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  # coord_flip()
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
final_novel_task_count_fig
```



```r
kruskal.test(
  formula=dominant_extra_tasks~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_extra_tasks by condition
## Kruskal-Wallis chi-squared = 177.17, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$dominant_extra_tasks,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_extra_tasks and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.9
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_extra_tasks)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_extra_tasks)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_extra_tasks)
  )
)
```

```
## [1] "PLASTIC median: 3; STATIC median: 3; NON-PLASTIC median: 0"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

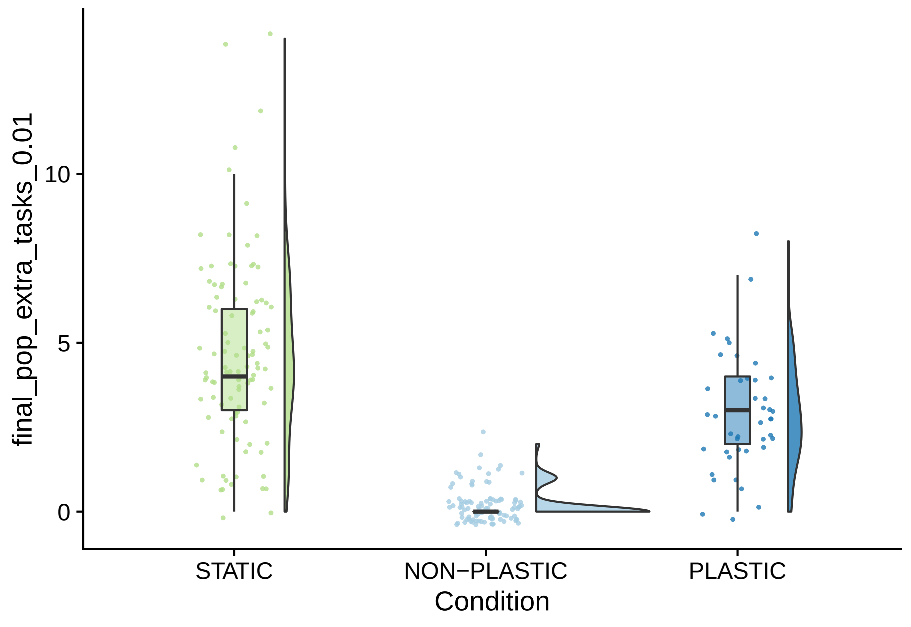
```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_extra_tasks~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=184"
## [1] "STATIC<-->PLASTIC: W=1871"
## [1] "PLASTIC<-->NON-PLASTIC: W=64"
```

7.6 Novel function count (final population)

How many novel functions are performed across the final population (1% of organisms must perform to count)?

```r
ggplot(summary_data, aes(x=condition, y=final_pop_extra_tasks_0.01, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```



```r
kruskal.test(
  formula=final_pop_extra_tasks_0.01~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  final_pop_extra_tasks_0.01 by condition
## Kruskal-Wallis chi-squared = 169.47, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$final_pop_extra_tasks_0.01,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$final_pop_extra_tasks_0.01 and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     0.00016
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$final_pop_extra_tasks_0.01)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$final_pop_extra_tasks_0.01)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$final_pop_extra_tasks_0.01)
  )
)
```

```
## [1] "PLASTIC median: 3; STATIC median: 4; NON-PLASTIC median: 0"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=final_pop_extra_tasks_0.01~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

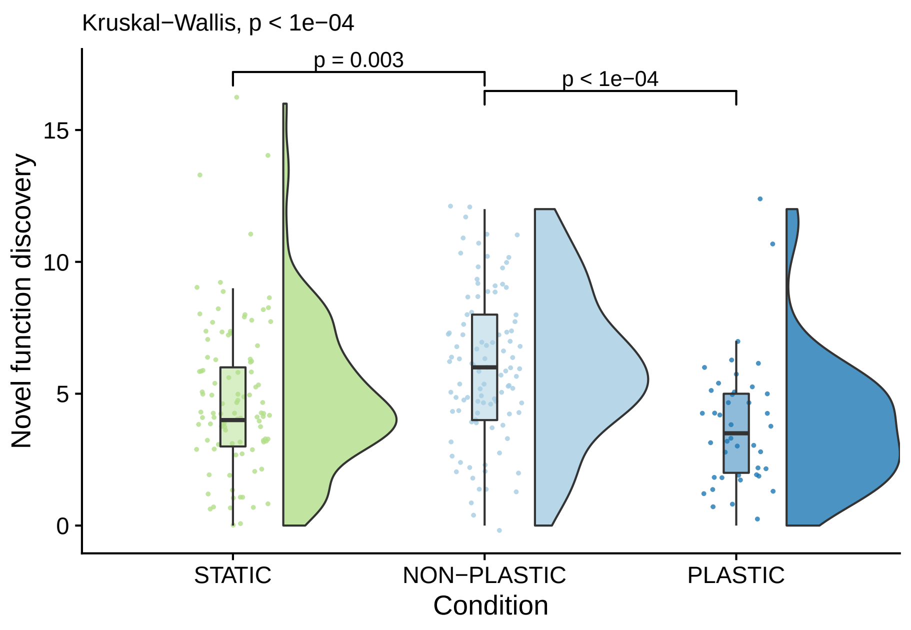
```
## [1] "STATIC<-->NON-PLASTIC: W=227.5"
## [1] "STATIC<-->PLASTIC: W=1203"
## [1] "PLASTIC<-->NON-PLASTIC: W=225.5"
```

7.7 Novel function discovery (lineage)

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_extra_traits_discovered ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition") # ,step.increase=1
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <- stat.test$y.position #* c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_extra_traits_discovered,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_extra_traits_discovered")
)

lineage_novel_task_discovery_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_extra_traits_discovered, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Novel function discovery"
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_extra_traits_discovered~cond
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  # coord_flip()
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
lineage_novel_task_discovery_fig
```



```
kruskal.test(
  formula=dominant_lineage_extra_traits_discovered~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_extra_traits_discovered by condition
## Kruskal-Wallis chi-squared = 24.099, df = 2, p-value = 5.846e-06
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_extra_traits_discovered,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_extra_traits_discovered and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 1.7e-05     -
## STATIC  0.0035      0.0561
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_discovered)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_extra_traits_discovered)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_traits_discovere
  )
)
```

```
## [1] "PLASTIC median: 3.5; STATIC median: 4; NON-PLASTIC median: 6"
```

```
print("Wilcox rank sum test statistics:")
```

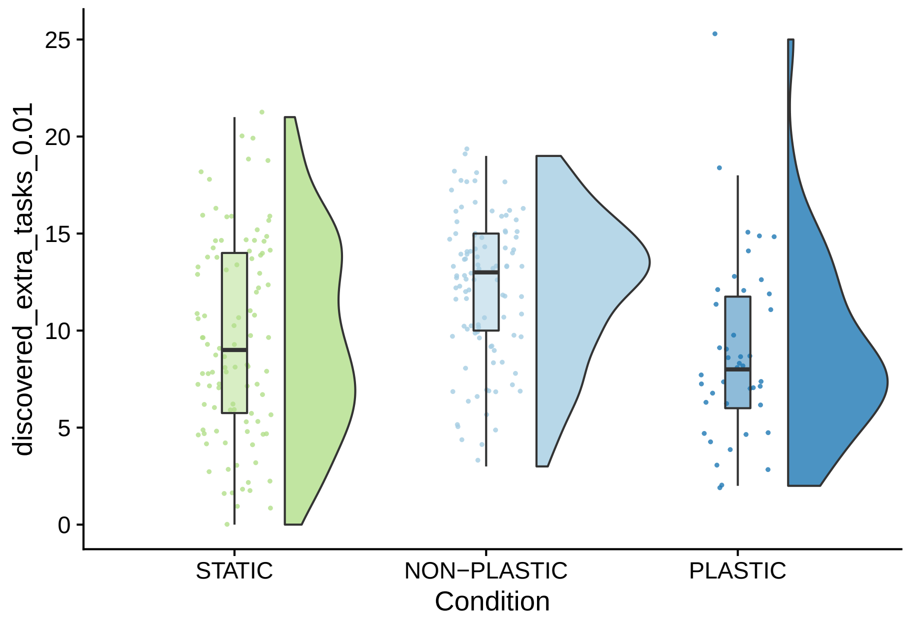
```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_extra_traits_discovered~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=6319.5"
## [1] "STATIC<-->PLASTIC: W=1578"
## [1] "PLASTIC<-->NON-PLASTIC: W=3110.5"
```

7.8 Novel function discovery (population)

```
ggplot(
    summary_data,
    aes(x=condition, y=discovered_extra_tasks_0.01, fill=condition)
  ) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```



```
kruskal.test(
  formula=discovered_extra_tasks_0.01~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  discovered_extra_tasks_0.01 by condition
## Kruskal-Wallis chi-squared = 24.271, df = 2, p-value = 5.365e-06
```

```
pairwise.wilcox.test(
  x=summary_data$discovered_extra_tasks_0.01,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$discovered_extra_tasks_0.01 and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 2.4e-05     -
## STATIC  0.00035     1.00000
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$discovered_extra_tasks_0.01)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$discovered_extra_tasks_0.01)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$discovered_extra_tasks_0.01)
  )
)
```

```
## [1] "PLASTIC median: 8; STATIC median: 9; NON-PLASTIC median: 13"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=discovered_extra_tasks_0.01~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

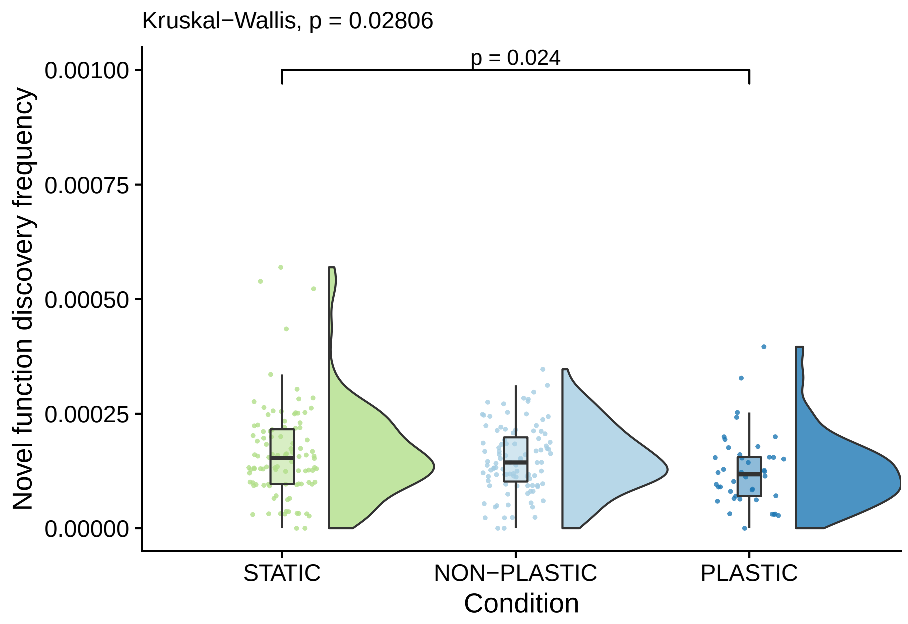
```
## [1] "STATIC<-->NON-PLASTIC: W=6573.5"
## [1] "STATIC<-->PLASTIC: W=1918.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=3096"
```

7.9 Novel function discovery frequency (lineage)

```
summary_data$dominant_lineage_extra_traits_discovered_per_generation <- summary_data$dominant_lin
summary_data$dominant_lineage_extra_traits_generations_per_discovery <- summary_data$dominant_gen

# Compute manual labels for geom_signif
# stat.test <- filter(summary_data, dominant_lineage_extra_traits_discovered > 0) %>%
#   wilcox_test(dominant_lineage_extra_traits_generations_per_discovery ~ condition) %>%
#   adjust_pvalue(method = "bonferroni") %>%
#   add_significance() %>%
#   add_xy_position(x="condition") # ,step.increase=1
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_extra_traits_discovered_per_generation ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition", step.increase=0.0001) # ,step.increase=1
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <- stat.test$y.position #* c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_extra_traits_discovered_per_generation,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_extra_traits_discovered_per_generatio
)

lineage_novel_task_discovery_freq_fig <- ggplot(
    # filter(summary_data, dominant_lineage_extra_traits_discovered > 0),
    summary_data,
    aes(x=condition, y=dominant_lineage_extra_traits_discovered_per_generation, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Novel function discovery frequency") +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_extra_traits_discovered_per
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
lineage_novel_task_discovery_freq_fig
```



```
kruskal.test(
  formula=dominant_lineage_extra_traits_discovered_per_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_extra_traits_discovered_per_generation by condition
## Kruskal-Wallis chi-squared = 7.1465, df = 2, p-value = 0.02806
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_extra_traits_discovered_per_generation,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_extra_traits_discovered_per_generation and summary_data$c
##
##         NON-PLASTIC PLASTIC
## PLASTIC 0.092       -
## STATIC  1.000       0.025
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_dis
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_extra_traits_disc
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_traits
  )
)
```

```
## [1] "PLASTIC median: 0.000117695011124939; STATIC median: 0.00015363220504867; NON-I
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

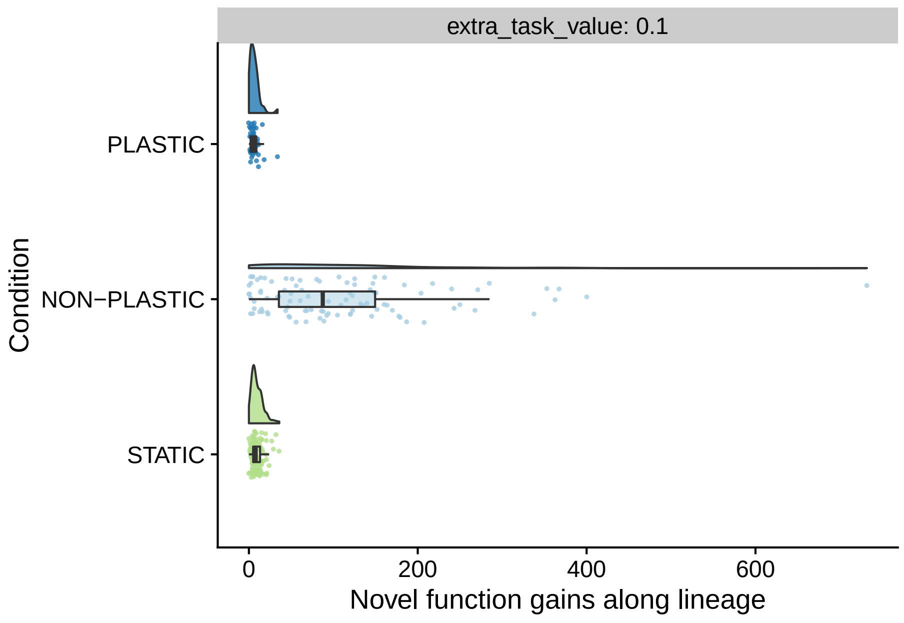
```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_extra_traits_discovered_per_generation~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=4751"
## [1] "STATIC<-->PLASTIC: W=1510.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=2584"
```

7.10 Novel functions gained (lineage)

```
ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_extra_traits_gained, fill=condition)
```

```
  ) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Novel function gains along lineage") +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  coord_flip() +
  facet_wrap(
    ~extra_task_value,
    labeller=label_both
  ) +
  theme(
    legend.position="none"
  )
```



```
ggsave(
  paste0(working_directory, "plots/dominant-lineage-extra-tasks-gained.pdf"),
  width=15,
  height=10
)
```

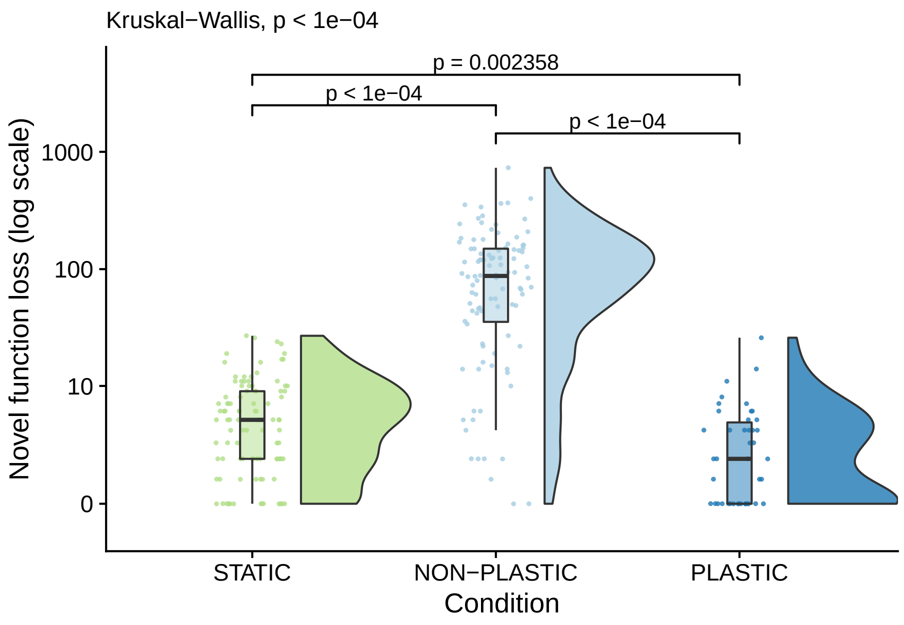
7.11 Novel function loss (lineage)

```
# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_extra_traits_lost ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition", step.increase=1)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <-  log10(stat.test$y.position) * c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_extra_traits_lost,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_extra_traits_lost")
)
```

```
lineage_novel_task_loss_fig <- ggplot(
    summary_data,
    aes(x=condition, y=dominant_lineage_extra_traits_lost, fill=condition)
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Novel function loss (log scale)",
    trans=pseudo_log_trans(sigma=1, base=10),
    breaks=c(0,10,100,1000),
    limits=c(-1,5000)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_extra_traits_lost~condition, data=summ
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj<=alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  # coord_flip()
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
lineage_novel_task_loss_fig
```



```
kruskal.test(
  formula=dominant_lineage_extra_traits_lost~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_extra_traits_lost by condition
## Kruskal-Wallis chi-squared = 129.06, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_extra_traits_lost,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_extra_traits_lost and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 2.7e-16     -
## STATIC  < 2e-16     0.0024
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_lost)
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_extra_traits_lost)
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_traits_lost)
  )
)
```

```
## [1] "PLASTIC median: 2; STATIC median: 5; NON-PLASTIC median: 87.5"
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_extra_traits_lost~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

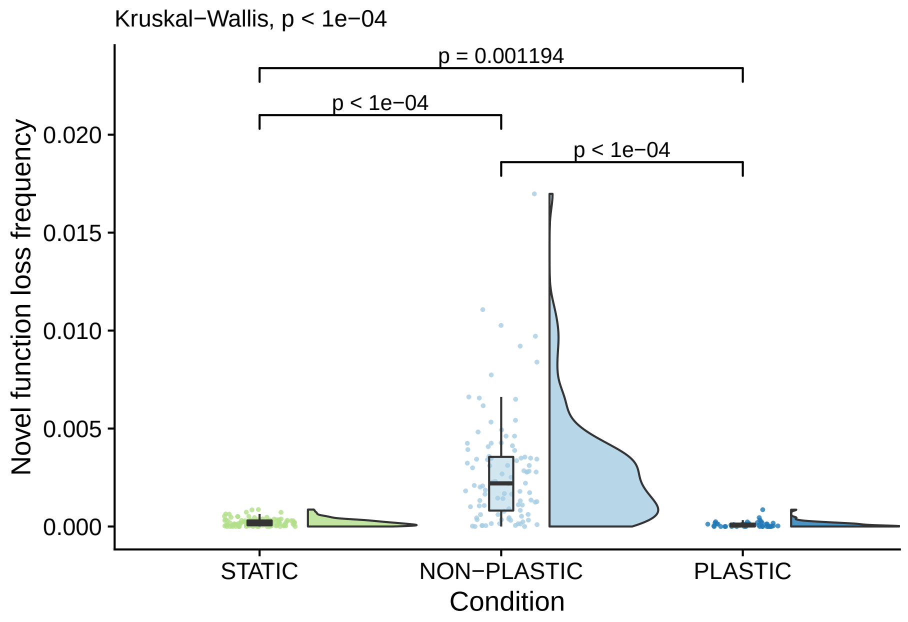
```
## [1] "STATIC<-->NON-PLASTIC: W=9105"
## [1] "STATIC<-->PLASTIC: W=1353.5"
## [1] "PLASTIC<-->NON-PLASTIC: W=3959"
```

7.12 Frequency of novel function loss (lineage)

```
summary_data$dominant_lineage_extra_traits_lost_per_generation <- summary_data$dominant
summary_data$dominant_lineage_extra_traits_generations_per_loss <- summary_data$dominan

# Compute manual labels for geom_signif
stat.test <- summary_data %>%
  wilcox_test(dominant_lineage_extra_traits_lost_per_generation ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition", step.increase=.1)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad beh
stat.test$manual_position <-  stat.test$y.position #* c(1.0,1.0,1.03)
stat.test$label <- mapply(p_label,stat.test$p.adj)

summary_data$is_outlier <- mapply(
  is_outlier,
  summary_data$dominant_lineage_extra_traits_lost_per_generation,
  summary_data$condition,
  MoreArgs=list(data=summary_data, column="dominant_lineage_extra_traits_lost_per_gener
)


lineage_novel_task_loss_freq_fig <- ggplot(
    # filter(summary_data, dominant_lineage_extra_traits_lost > 0),
    summary_data,
    aes(x=condition, y=dominant_lineage_extra_traits_lost_per_generation, fill=conditio
  ) +
  geom_flat_violin(
    # data=filter(summary_data,is_outlier==FALSE),
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Novel function loss frequency") +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_extra_traits_lost_per_generation~condi
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj<=alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
lineage_novel_task_loss_freq_fig
```



```r
kruskal.test(
  formula=dominant_lineage_extra_traits_lost_per_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_extra_traits_lost_per_generation by condition
## Kruskal-Wallis chi-squared = 121.41, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_extra_traits_lost_per_generation,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_extra_traits_lost_per_generation and summary_da
##
##         NON-PLASTIC PLASTIC
## PLASTIC 1.1e-15     -
## STATIC  < 2e-16     0.0012
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_lost_per_gene
  ),
  paste0(
    "STATIC median: ",
    median(filter(summary_data, condition=="STATIC")$dominant_lineage_extra_traits_lost_per_gener
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_traits_lost_per_
  )
)
```

```
## [1] "PLASTIC median: 6.25141973661864e-05; STATIC median: 0.000161396283669756; NON-PLASTIC me
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=dominant_lineage_extra_traits_lost_per_generation~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

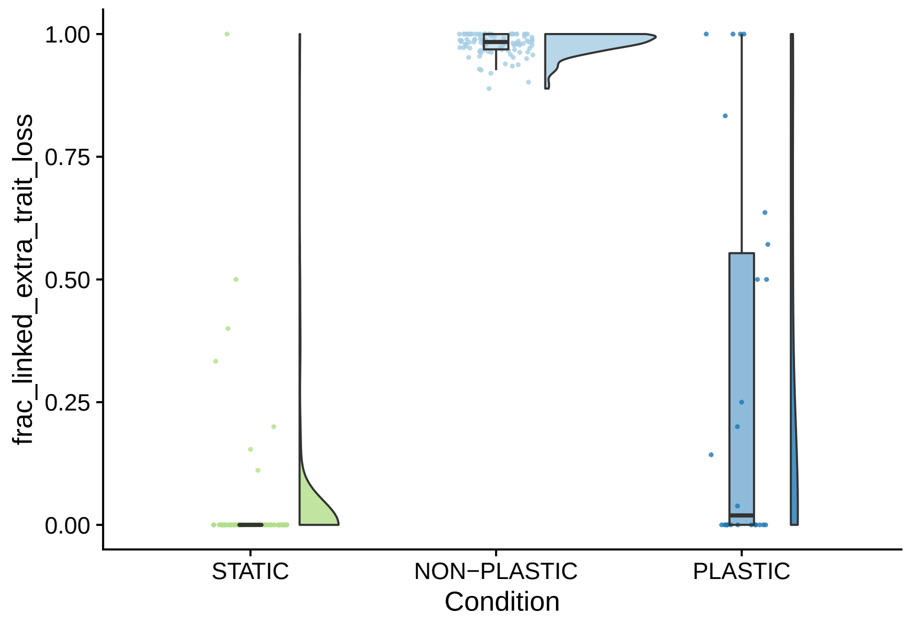
```
## [1] "STATIC<-->NON-PLASTIC: W=8940"
## [1] "STATIC<-->PLASTIC: W=1311"
## [1] "PLASTIC<-->NON-PLASTIC: W=3922"
```

7.13 How many instances of novel function loss co-occurred with changes in base phenotype?

Function loss linked with base function changes.

```
lost_traits_summary_data <- filter(summary_data, extra_task_value==0.1 & dominant_linea
lost_traits_summary_data$frac_linked_extra_trait_loss <- lost_traits_summary_data$domin
```

```
ggplot(lost_traits_summary_data, aes(x=condition, y=frac_linked_extra_trait_loss, fill=
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



```r
kruskal.test(
  formula=frac_linked_extra_trait_loss~condition,
  data=lost_traits_summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_linked_extra_trait_loss by condition
## Kruskal-Wallis chi-squared = 153.68, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=lost_traits_summary_data$frac_linked_extra_trait_loss,
  g=lost_traits_summary_data$condition,
  p.adjust.method="bonferroni",
  conf.int=TRUE,
  conf.level=0.95
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  lost_traits_summary_data$frac_linked_extra_trait_loss and lost_traits_summary_data$cond
##
##         NON-PLASTIC PLASTIC
## PLASTIC 1.9e-08     -
## STATIC  < 2e-16     1.8e-06
##
## P value adjustment method: bonferroni
```

```
paste(
  sep="; ",
  paste0(
    "PLASTIC median: ",
    median(filter(lost_traits_summary_data, condition=="PLASTIC")$frac_linked_extra_tra
  ),
  paste0(
    "STATIC median: ",
    median(filter(lost_traits_summary_data, condition=="STATIC")$frac_linked_extra_tra
  ),
  paste0(
    "NON-PLASTIC median: ",
    median(filter(lost_traits_summary_data, condition=="NON-PLASTIC")$frac_linked_extra
  )
)
```

```
## [1] "PLASTIC median: 0.0192307692307692; STATIC median: 0; NON-PLASTIC median: 0.98
```

```
print("Wilcox rank sum test statistics:")
```

```
## [1] "Wilcox rank sum test statistics:"
```

```
for (pair in pairwise_comparisons) {
  pair_data <- filter(lost_traits_summary_data, condition %in% pair)
  pair_data$condition <- as.factor(pair_data$condition)
  wt <- wilcox.test(
    formula=frac_linked_extra_trait_loss~condition,
    data=pair_data,
    exact=FALSE,
    paired=FALSE
  )
  print(paste0(pair[1], "<-->", pair[2], ": W=",wt$statistic))
}
```

```
## [1] "STATIC<-->NON-PLASTIC: W=8344"
## [1] "STATIC<-->PLASTIC: W=1602"
## [1] "PLASTIC<-->NON-PLASTIC: W=2212"
```

```
sum(filter(lost_traits_summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_
```

```
## [1] 10998
```

```
sum(filter(lost_traits_summary_data, condition=="NON-PLASTIC")$dominant_lineage_extra_
```

```
## [1] 11229
```

```
aggregate_frac_linked_extra_trait_loss_nonplastic <- sum(filter(lost_traits_summary_data, conditi
aggregate_frac_linked_extra_trait_loss_nonplastic
```

```
## [1] 0.9794283
```

```
sum(filter(lost_traits_summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_lost_lin
```

```
## [1] 29
```

```
sum(filter(lost_traits_summary_data, condition=="PLASTIC")$dominant_lineage_extra_traits_lost)
```

```
## [1] 142
```

```
aggregate_frac_linked_extra_trait_loss_plastic <- sum(filter(lost_traits_summary_data, condition=
aggregate_frac_linked_extra_trait_loss_plastic
```

```
## [1] 0.2042254
```

```
sum(filter(lost_traits_summary_data, condition=="STATIC")$dominant_lineage_extra_traits_lost_link
```

```
## [1] 13
```

```
sum(filter(lost_traits_summary_data, condition=="STATIC")$dominant_lineage_extra_traits_lost)
```

```
## [1] 631
```

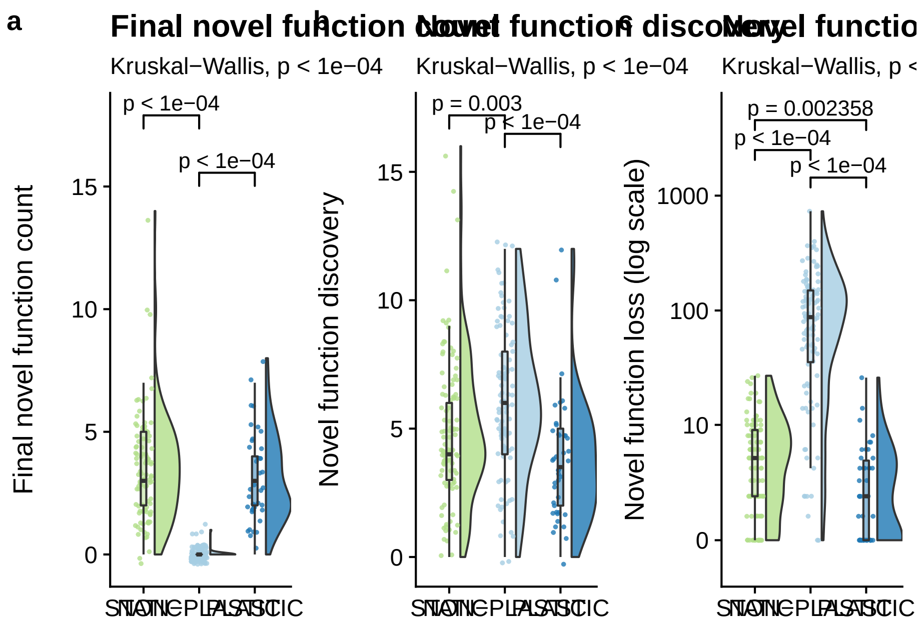
```
aggregate_frac_linked_extra_trait_loss_nonplastic <- sum(filter(lost_traits_summary_data, conditi
aggregate_frac_linked_extra_trait_loss_nonplastic
```

```
## [1] 0.02060222
```

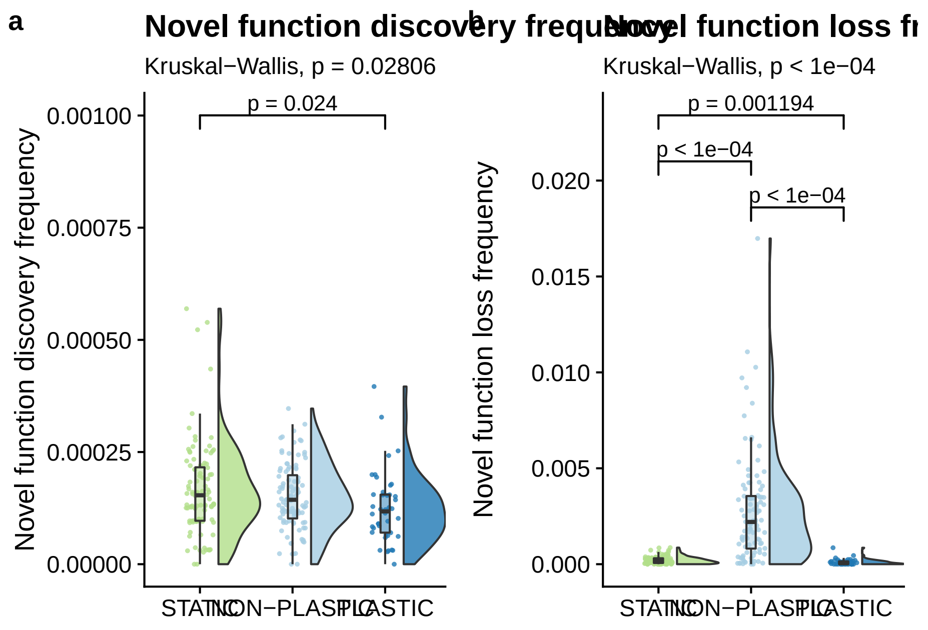
7.14 Manuscript figures

7.15 Combined panel

```
magnitude_grid <- plot_grid(
  final_novel_task_count_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Final novel function count"),
  lineage_novel_task_discovery_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Novel function discovery"),
  lineage_novel_task_loss_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Novel function loss"),
  nrow=1,
  align="v",
  labels="auto"
)
magnitude_grid
```



```
pace_grid <- plot_grid(
  lineage_novel_task_discovery_freq_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Novel function discovery frequency"),
  lineage_novel_task_loss_freq_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Novel function loss frequency"),
  nrow=1,
  align="v",
  labels="auto"
)
pace_grid
```



```r
save_plot(
   paste0(working_directory, "plots/", "complex-traits-magnitude-panel.pdf"),
   magnitude_grid,
   base_height=6,
   base_asp=3/1
)

save_plot(
   paste0(working_directory, "plots/", "complex-traits-pace-panel.pdf"),
   pace_grid,
   base_height=6,
   base_asp=2/1
)
```

Chapter 8

Accumulation of deleterious instructions

The effect of adaptive phenotypic plasticity on the accumulation of deleterious instructions.

8.1 Overview

```
total_updates <- 200000
replicates <- 100
alpha <- 0.05
focal_poison_penalty <- 0.1

focal_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")

# Relative location of data.
working_directory <- "experiments/2021-02-05-hitchhiking/analysis/" # << For bookdown
# working_directory <- "./"
```

8.2 Analysis dependencies

Load all required R libraries.

```
library(RColorBrewer)
library(ggplot2)
library(rstatix)
library(ggsignif)
library(scales)
library(tidyverse)
library(cowplot)
library(Hmisc)
library(boot)
library(fmsb)
library(knitr)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd9
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

8.3 Setup

```
####### summary data #######
summary_data_loc <- paste0(working_directory, "data/aggregate.csv")
summary_data <- read.csv(summary_data_loc, na.strings="NONE")

summary_data$DISABLE_REACTION_SENSORS <- as.factor(summary_data$DISABLE_REACTION_SENSO
summary_data$chg_env <- summary_data$chg_env == "True"
summary_data$dominant_plastic_odd_even <- as.factor(summary_data$dominant_plastic_odd_
summary_data$sensors <- summary_data$DISABLE_REACTION_SENSORS == "0"
summary_data$is_plastic <- summary_data$dominant_plastic_odd_even == "True"
summary_data$POISON_PENALTY <- as.factor(summary_data$POISON_PENALTY)

summary_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation <
```

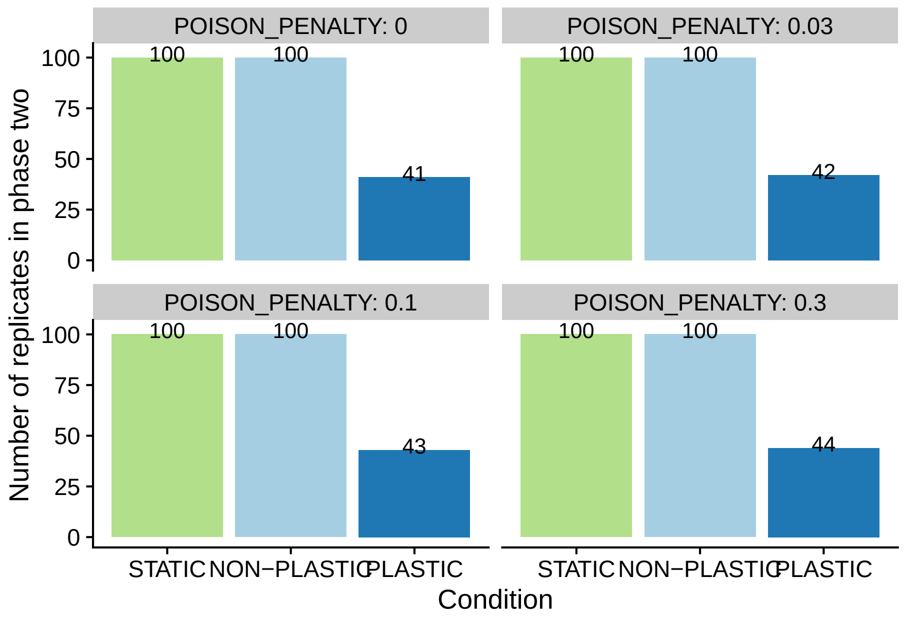
```
summary_data$frac_hitchhiking_linked_trait_change <- summary_data$dominant_lineage_num_times_hitc
summary_data$frac_unexpressed_hitchhiker_inc <- summary_data$dominant_lineage_num_times_hitchhike
summary_data$frac_expressed_hitchiker_inc <- summary_data$dominant_lineage_num_times_hitchhike_ir

env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
}

condition_label_fun <- function(has_sensors, env_chg) {
  if (has_sensors && env_chg) {
    return("PLASTIC")
  } else if (env_chg) {
    return("NON-PLASTIC")
  } else {
    return("STATIC")
  }
}

summary_data$env_label <- mapply(
  env_label_fun,
  summary_data$chg_env
)
summary_data$sensors_label <- mapply(
  sensors_label_fun,
  summary_data$sensors
)
summary_data$condition <- mapply(
  condition_label_fun,
  summary_data$sensors,
  summary_data$chg_env
)

condition_order = c(
  "STATIC",
  "NON-PLASTIC",
  "PLASTIC"
)

pairwise_comparisons <- list(
  c("STATIC", "NON-PLASTIC"),
  c("STATIC", "PLASTIC"),
  c("PLASTIC", "NON-PLASTIC")
)

p_label <- function(p_value) {
  threshold = 0.0001
  if (p_value < threshold) {
    return(paste0("p < ", threshold))
  } else {
    return(paste0("p = ", p_value))
  }
}

poison_penalties <- levels(summary_data$POISON_PENALTY)

###### time series #####
lineage_time_series_data_loc <- paste0(working_directory, "data/lineage_series.csv")
lineage_time_series_data <- read.csv(lineage_time_series_data_loc)

lineage_time_series_data$DISABLE_REACTION_SENSORS <- as.factor(lineage_time_series_data
lineage_time_series_data$chg_env <- lineage_time_series_data$chg_env == "True"
lineage_time_series_data$sensors <- lineage_time_series_data$DISABLE_REACTION_SENSORS =
lineage_time_series_data$POISON_PENALTY <- as.factor(lineage_time_series_data$POISON_V

lineage_time_series_data$env_label <- mapply(
  env_label_fun,
  lineage_time_series_data$chg_env
)
lineage_time_series_data$sensors_label <- mapply(
  sensors_label_fun,
  lineage_time_series_data$sensors
)
lineage_time_series_data$condition <- mapply(
  condition_label_fun,
  lineage_time_series_data$sensors,
  lineage_time_series_data$chg_env
)
```

```r
####### misc #######
# Configure our default graphing theme
focal_summary_data <- filter(summary_data, POISON_PENALTY==focal_poison_penalty)
theme_set(theme_cowplot())
cb_palette <- "Paired"
dir.create(paste0(working_directory, "plots"), showWarnings=FALSE)
samplemean <- function(x, d) {
  return(mean(x[d]))
}
```

8.4 Evolution of phenotypic plasticity

For sensor-enabled populations in fluctuating environments, we only transfered populations containing an optimally plastic genotype to phase-two.

```r
summary_data_grouped = dplyr::group_by(summary_data, sensors, env_label, condition, POISON_PENALT
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())

ggplot(summary_data_group_counts, aes(x=condition, y=n, fill=condition)) +
  geom_col(position=position_dodge(0.9)) +
  geom_text(aes(label=n, y=n+2)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  ylab("Number of replicates in phase two") +
  facet_wrap(~POISON_PENALTY, labeller=label_both) +
  theme(
    legend.position="none"
  )
```

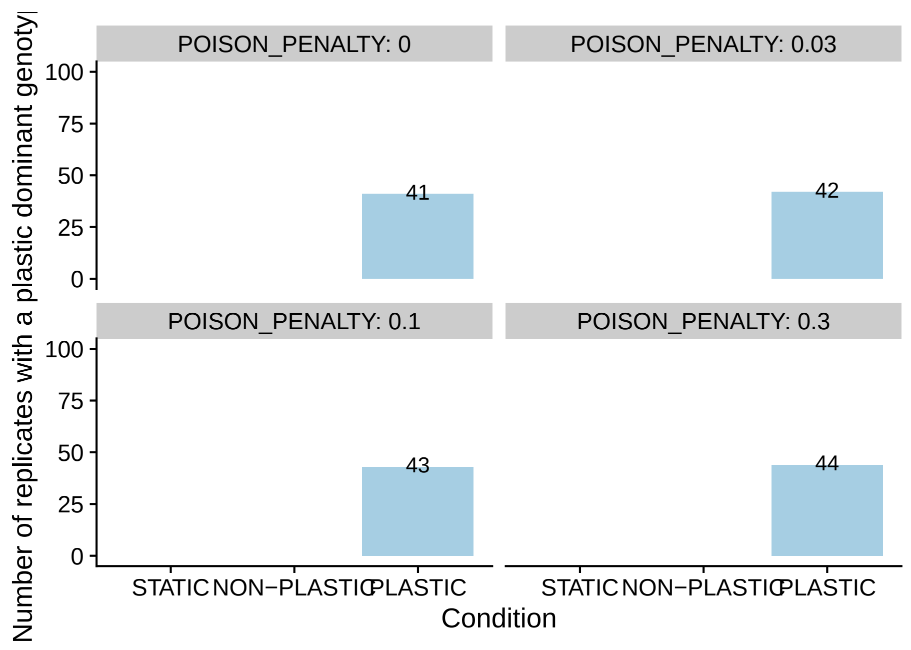


We can confirm our expectation that the dominant genotypes in non-plastic conditions are not phenotypically plastic.

```
summary_data_grouped = dplyr::group_by(summary_data, condition, is_plastic, POISON_PENA
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())
```

```
## `summarise()` has grouped output by 'condition', 'is_plastic'. You can override usin
```

```
ggplot(filter(summary_data_group_counts, is_plastic), aes(x=condition, y=n, fill=condit
  geom_col(position=position_dodge(0.9)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  geom_text(aes(label=n, y=n+1)) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  ylab("Number of replicates with a plastic dominant genotype") +
  ylim(0, 100) +
  facet_wrap(~POISON_PENALTY, labeller=label_both) +
  theme(
    legend.position="none"
  )
```



8.5 Deleterious instruction execution

Note that under the hood, the deleterious instruction is the `poison` instruction in Avida.

8.5.1 Number of replicates where final dominant genotype executes the deleterious instruction

```
for (penalty in poison_penalties) {
  occurrences <- c(
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="NON-PLASTIC" & dominant_tim
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="PLASTIC" & dominant_times_p
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="STATIC" & dominant_times_po
  )
  trials <- c(
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="NON-PLASTIC")$RANDOM_SEED),
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="PLASTIC")$RANDOM_SEED),
    length(filter(summary_data, POISON_PENALTY==penalty & condition=="STATIC" )$RANDOM_SEED)
  )
  names(trials) <- c(
    "NON-PLASTIC",
    "PLASTIC",
    "STATIC"
  )
  names(occurrences) <- c(
    "NON-PLASTIC",
    "PLASTIC",
    "STATIC"
  )
  poison_exec_table <- data.frame(
    executes.poison=occurrences,
    replicates=trials
  )
  cat(paste0("#### Penalty: ", penalty, "\n"))
  cat(print(kable(poison_exec_table)))
  cat("\n")
  ft <- pairwise.fisher.test(x=occurrences, n=trials, p.adjust.method="bonferroni")
  print(ft)
  cat("\n\n")
}
```

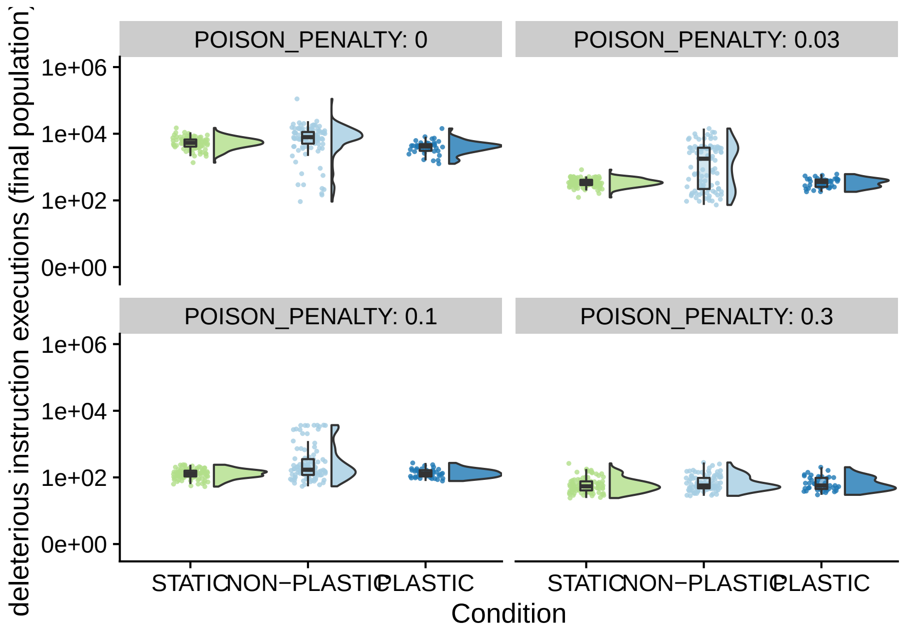
```
## #### Penalty: 0
##
## \begin{tabular}{l|r|r}
## \hline
##   & executes.poison & replicates\\
## \hline
## NON-PLASTIC & 86 & 100\\
## \hline
## PLASTIC & 27 & 41\\
## \hline
## STATIC & 85 & 100\\
## \hline
## \end{tabular}
##
##
##  Pairwise comparisons using Pairwise comparison of proportions (Fisher)
##
## data:  occurrences out of trials
##
##         NON-PLASTIC PLASTIC
## PLASTIC 0.03        -
## STATIC  1.00        0.06
##
## P value adjustment method: bonferroni
##
```

```
##
## #### Penalty: 0.03
##
## \begin{tabular}{l|r|r}
## \hline
##   & executes.poison & replicates\\
## \hline
## NON-PLASTIC & 46 & 100\\
## \hline
## PLASTIC & 1 & 42\\
## \hline
## STATIC & 1 & 100\\
## \hline
## \end{tabular}
##
##
##  Pairwise comparisons using Pairwise comparison of proportions (Fisher)
##
## data:  occurrences out of trials
##
##         NON-PLASTIC PLASTIC
## PLASTIC 1.2e-07     -
## STATIC  2.9e-15     1
##
## P value adjustment method: bonferroni
##
##
## #### Penalty: 0.1
##
## \begin{tabular}{l|r|r}
## \hline
##   & executes.poison & replicates\\
## \hline
## NON-PLASTIC & 14 & 100\\
## \hline
## PLASTIC & 0 & 43\\
## \hline
## STATIC & 0 & 100\\
## \hline
## \end{tabular}
##
##
##  Pairwise comparisons using Pairwise comparison of proportions (Fisher)
##
## data:  occurrences out of trials
##
```

```
##         NON-PLASTIC PLASTIC
## PLASTIC 0.03212     -      
## STATIC  0.00022     1.00000
## 
## P value adjustment method: bonferroni 
## 
## 
## #### Penalty: 0.3
## 
## \begin{tabular}{l|r|r}
## \hline
##   & executes.poison & replicates\\
## \hline
## NON-PLASTIC & 0 & 100\\
## \hline
## PLASTIC & 0 & 44\\
## \hline
## STATIC & 0 & 100\\
## \hline
## \end{tabular}
## 
## 
##  Pairwise comparisons using Pairwise comparison of proportions (Fisher) 
## 
## data:  occurrences out of trials 
## 
##         NON-PLASTIC PLASTIC
## PLASTIC 1           -      
## STATIC  1           1      
## 
## P value adjustment method: bonferroni
```

8.5.2 Deleterious instruction execution (final population)

```r
ggplot(summary_data, aes(x=condition, y=final_population_poison, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_y_continuous(
    name="deleterious instruction executions (final population)",
    trans=pseudo_log_trans(sigma=1,base=10),
    breaks=c(0,100,10000,1000000),
    limits=c(-1,1000000)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  facet_wrap(
    ~POISON_PENALTY,
    labeller=label_both
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```



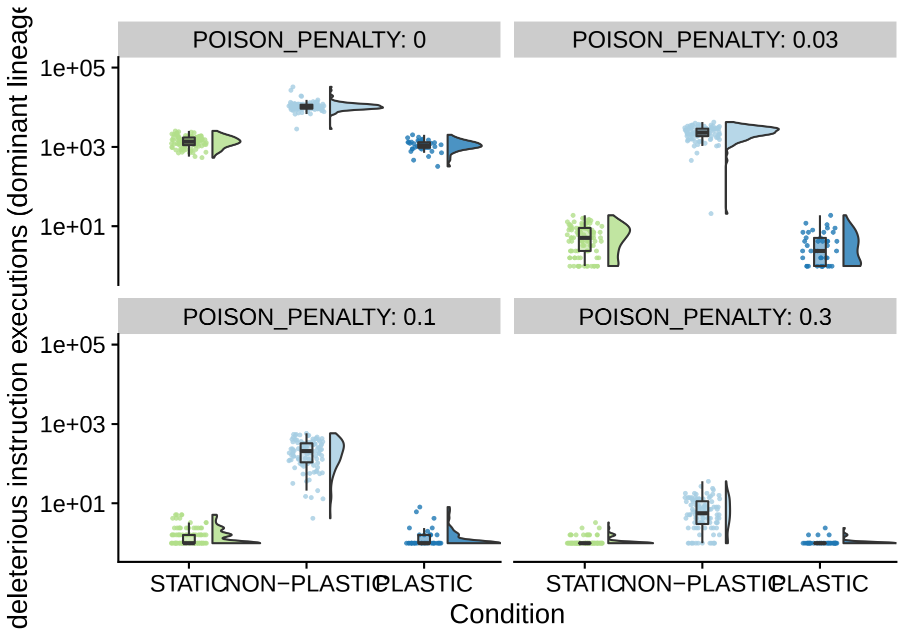
```r
ggsave(
  paste0(working_directory, "plots/final-population-poison-log.pdf"),
  width=15,
  height=10
)
```

```r
for (penalty in poison_penalties) {
  stat_data <- filter(summary_data, POISON_PENALTY==penalty)
  print(
    paste0(
      "PENALTY: ", penalty
    )
  )
  kt <- kruskal.test(
      formula=final_population_poison~condition,
      data=stat_data
    )
  print(
    kt
  )
  if (is.na(kt$p.value)) { next }
  if (kt$p.value > 0.05) { next }
  print(
    pairwise.wilcox.test(
      x=stat_data$final_population_poison,
      g=stat_data$condition,
      p.adjust.method="bonferroni"
    )
  )
}
```

```
## [1] "PENALTY: 0"
##
##  Kruskal-Wallis rank sum test
##
## data:  final_population_poison by condition
## Kruskal-Wallis chi-squared = 43.589, df = 2, p-value = 3.426e-10
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$final_population_poison and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 8.7e-07     -
## STATIC  9.8e-07     0.00074
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.03"
##
##  Kruskal-Wallis rank sum test
##
## data:  final_population_poison by condition
## Kruskal-Wallis chi-squared = 20.74, df = 2, p-value = 3.136e-05
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$final_population_poison and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 0.003       -
## STATIC  1e-04       1.000
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.1"
##
##  Kruskal-Wallis rank sum test
##
## data:  final_population_poison by condition
## Kruskal-Wallis chi-squared = 20.608, df = 2, p-value = 3.35e-05
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$final_population_poison and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 0.0093      -
## STATIC  4.9e-05     1.0000
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.3"
##
##  Kruskal-Wallis rank sum test
##
## data:  final_population_poison by condition
## Kruskal-Wallis chi-squared = 3.3994, df = 2, p-value = 0.1827
```

8.5.3 Cummulative deleterious instruction execution along final dominant lineages

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_times_poison_executed, fill=co
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_y_continuous(
    name="deleterious instruction executions (dominant lineage)",
    trans=pseudo_log_trans(sigma = 1, base = 10),
    breaks=c(10,1000,100000),
    limits=c(-1,100000)
  ) +
  facet_wrap(
    ~POISON_PENALTY,
    labeller=label_both
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



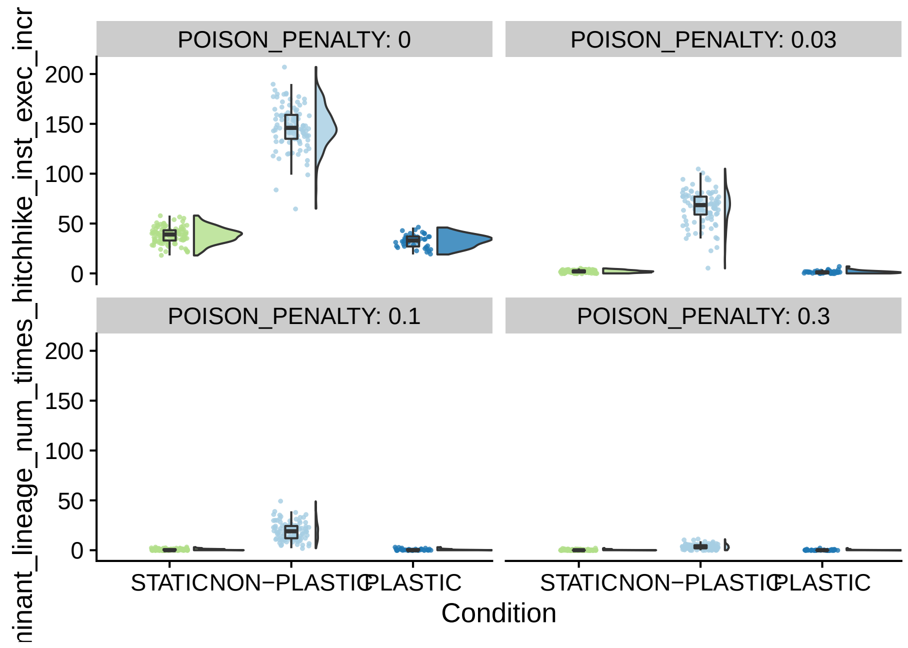
```
ggsave(
  paste0(working_directory, "plots/final-dominant-lineage-poison-log.pdf"),
  width=15,
  height=10
)
```

```
for (penalty in poison_penalties) {
  stat_data <- filter(summary_data, POISON_PENALTY==penalty)
  print(
    paste0(
      "PENALTY: ", penalty
    )
  )
  kt <- kruskal.test(
      formula=dominant_lineage_times_poison_executed~condition,
      data=stat_data
    )
  print(
    kt
  )
  if (is.na(kt$p.value)) { next }
  if (kt$p.value > 0.05) { next }
  print(
    pairwise.wilcox.test(
      x=stat_data$dominant_lineage_times_poison_executed,
      g=stat_data$condition,
      p.adjust.method="bonferroni"
    )
  )
}
```

```
## [1] "PENALTY: 0"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_times_poison_executed by condition
## Kruskal-Wallis chi-squared = 178.84, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_times_poison_executed and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.0018
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.03"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_times_poison_executed by condition
## Kruskal-Wallis chi-squared = 178.62, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_times_poison_executed and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.011
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.1"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_times_poison_executed by condition
## Kruskal-Wallis chi-squared = 184.83, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_times_poison_executed and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.21
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.3"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_times_poison_executed by condition
## Kruskal-Wallis chi-squared = 149.48, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_times_poison_executed and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 4.4e-16     -
## STATIC  < 2e-16     0.84
##
## P value adjustment method: bonferroni
```

8.6 Characterizing mutations that increase deleterious instruction execution

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_num_times_hitchhike_inst_exec
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  facet_wrap(
    ~POISON_PENALTY,
    labeller=label_both
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



```
for (penalty in poison_penalties) {
  stat_data <- filter(summary_data, POISON_PENALTY==penalty)
  print(
    paste0(
      "PENALTY: ", penalty
    )
  )
  kt <- kruskal.test(
      formula=dominant_lineage_num_times_hitchhike_inst_exec_increases~condition,
      data=stat_data
    )
  print(
    kt
  )
  if (is.na(kt$p.value)) { next }
  if (kt$p.value > 0.05) { next }
  print(
    pairwise.wilcox.test(
      x=stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases,
      g=stat_data$condition,
      p.adjust.method="bonferroni"
    )
  )
}
```

```
## [1] "PENALTY: 0"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases by condition
## Kruskal-Wallis chi-squared = 179.79, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases and stat_c
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     0.00046
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.03"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases by condition
## Kruskal-Wallis chi-squared = 179.35, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases and stat_c
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.03
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.1"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases by condition
## Kruskal-Wallis chi-squared = 185.34, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases and stat_c
##
```

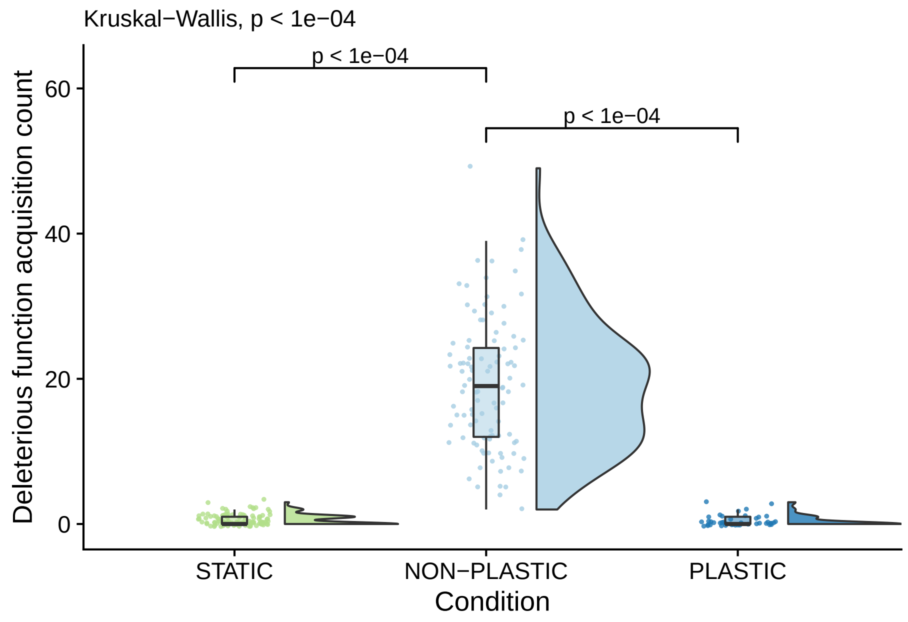
```
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.27
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.3"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases by condition
## Kruskal-Wallis chi-squared = 146.35, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases and stat_data$condit
##
##         NON-PLASTIC PLASTIC
## PLASTIC 7.8e-16     -
## STATIC  < 2e-16     0.86
##
## P value adjustment method: bonferroni
```

Focal figure for the manuscript:

```r
# Compute manual labels for geom_signif
stat.test <- focal_summary_data %>%
  wilcox_test(dominant_lineage_num_times_hitchhike_inst_exec_increases ~ condition) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition")
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <-  stat.test$y.position
stat.test$label <- mapply(p_label,stat.test$p.adj)

poison_increases_fig <- ggplot(
    focal_summary_data,
    aes(x=condition, y=dominant_lineage_num_times_hitchhike_inst_exec_increases, fill=condition)
  ) +
  geom_flat_violin(
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Deleterious function acquisition count",
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip()
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_num_times_hitchhike_inst_exe
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

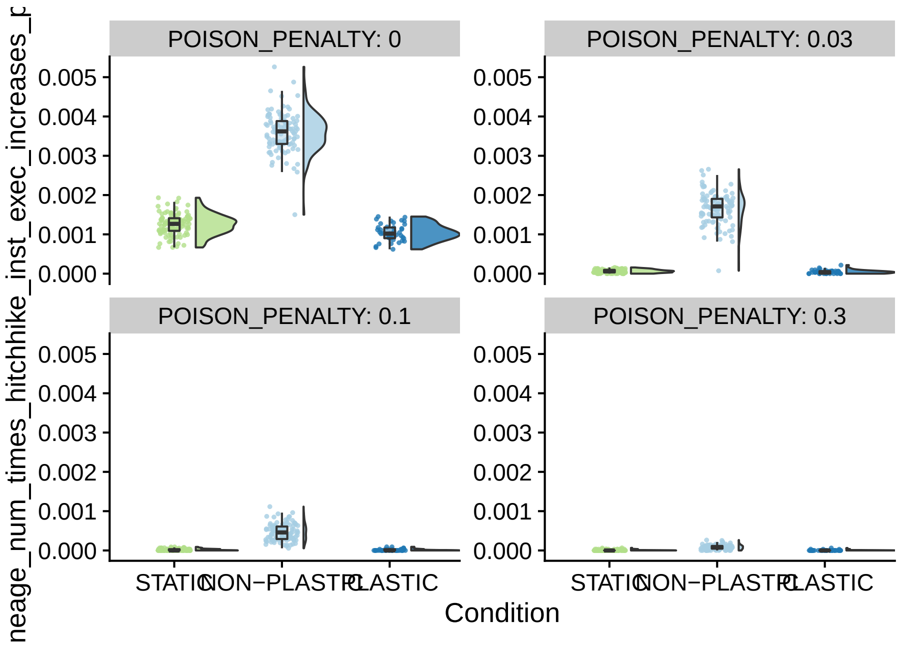
```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
poison_increases_fig
```



8.6.2 Frequency of increases in deleterious instruction execution (lineage)

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_num_times_hitchhike_inst_exec_increases_
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  facet_wrap(
    ~POISON_PENALTY,
    labeller=label_both,
    scales="free_y"
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```



```
ggsave(
  paste0(working_directory, "plots/final-dominant-lineage-poison-increase-per-generatio
  width=15,
  height=10
)
```

```
for (penalty in poison_penalties) {
  stat_data <- filter(summary_data, POISON_PENALTY==penalty)
  print(
    paste0(
      "PENALTY: ", penalty
    )
  )
  kt <- kruskal.test(
      formula=dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation~condition,
      data=stat_data
    )
  print(
    kt
  )
  if (is.na(kt$p.value)) { next }
  if (kt$p.value > 0.05) { next }
  print(
    pairwise.wilcox.test(
      x=stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation,
      g=stat_data$condition,
      p.adjust.method="bonferroni"
    )
  )
}
```

```
## [1] "PENALTY: 0"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation by condition
## Kruskal-Wallis chi-squared = 180.05, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation and s
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     7.8e-05
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.03"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation by condition
## Kruskal-Wallis chi-squared = 176.25, df = 2, p-value < 2.2e-16
##
```

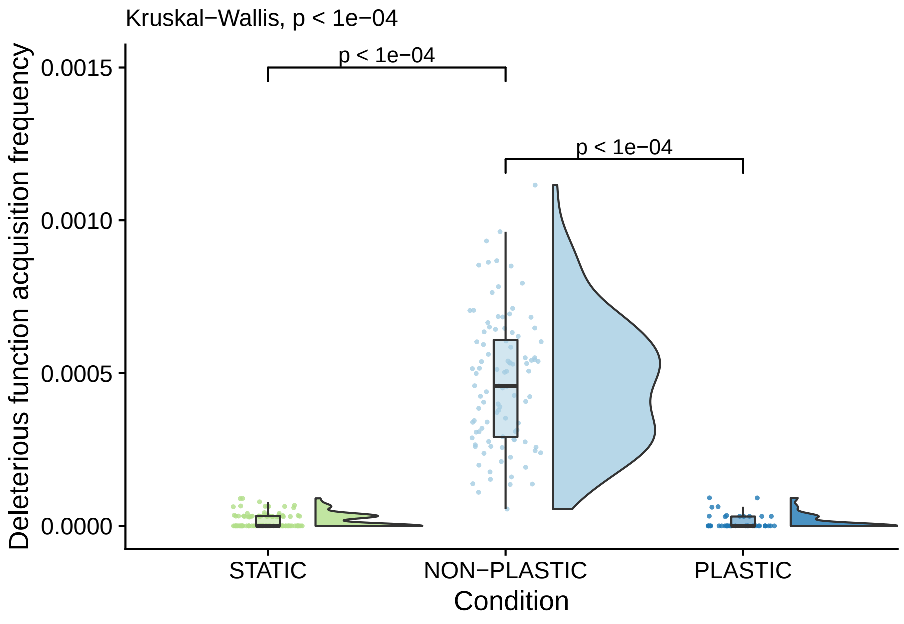
```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_genera
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.019
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.1"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation by co
## Kruskal-Wallis chi-squared = 184.17, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_genera
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.2
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.3"
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation by co
## Kruskal-Wallis chi-squared = 140.99, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$dominant_lineage_num_times_hitchhike_inst_exec_increases_per_genera
##
##         NON-PLASTIC PLASTIC
## PLASTIC 2.2e-15     -
## STATIC  < 2e-16     0.79
##
## P value adjustment method: bonferroni
```

Figure for the manuscript:

```r
# Compute manual labels for geom_signif
stat.test <- focal_summary_data %>%
  wilcox_test(dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation ~ conditior
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition", step.increase=0.2)
# Tweak y.position manually to account for scaled axis (edge case that triggers bad behavior in g
stat.test$manual_position <-  stat.test$y.position
stat.test$label <- mapply(p_label,stat.test$p.adj)

poison_increases_per_gen_fig <- ggplot(
    focal_summary_data,
    aes(x=condition, y=dominant_lineage_num_times_hitchhike_inst_exec_increases_per_generation, f
  ) +
  geom_flat_violin(
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Deleterious function acquisition frequency",
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  # coord_flip()
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=dominant_lineage_num_times_hitchhike_inst_exe
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

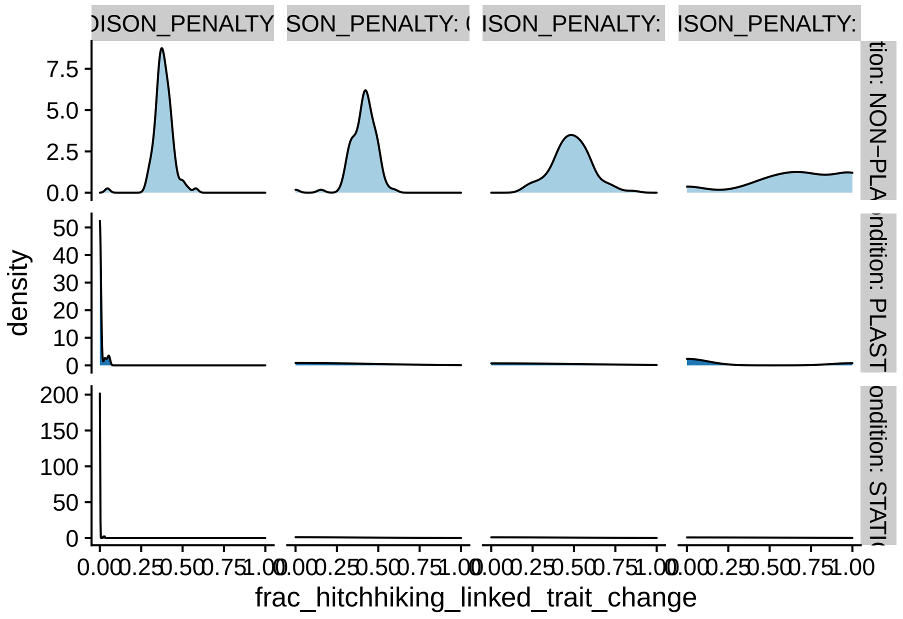
```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
poison_increases_per_gen_fig
```



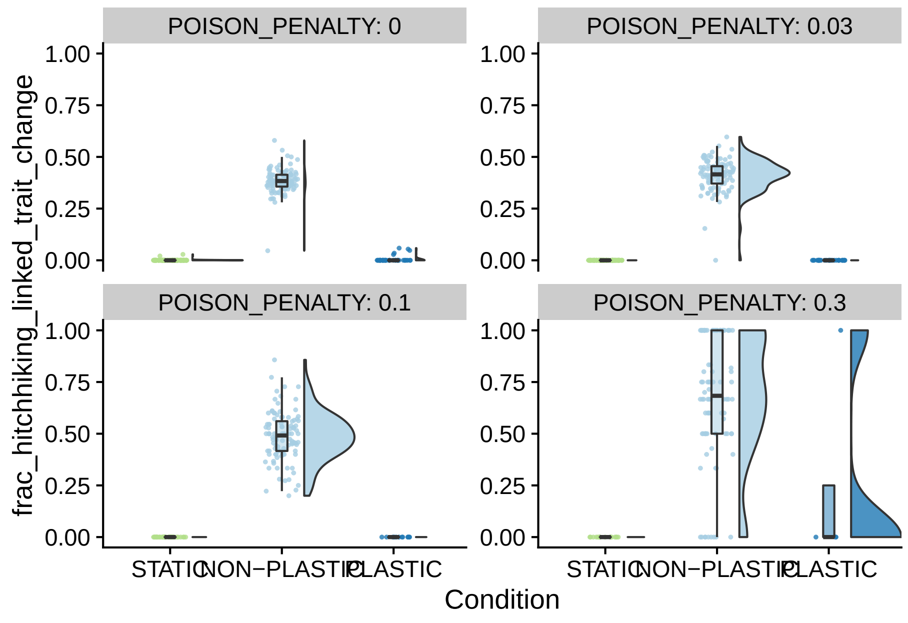
8.6.3 What fraction of mutations that increase deleterious instruction execution co-occur with base trait changes?

```
ggplot(filter(summary_data, dominant_lineage_num_times_hitchhike_inst_exec_increases>0), aes(x=fr
  geom_density() +
  facet_grid(
    condition~POISON_PENALTY,
    labeller=label_both,
    scales="free_y"
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  theme(
    legend.position="none"
  )
```



```
ggsave(
  paste0(working_directory, "plots/dominant-lineage-frac_hitchhiking_linked_trait_change.png"),
  width=15,
  height=10
)
```

```
ggplot(filter(summary_data, dominant_lineage_num_times_hitchhike_inst_exec_increases>0
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  facet_wrap(
    ~POISON_PENALTY,
    labeller=label_both,
    scales="free_y"
  ) +
  # coord_flip() +
  theme(
    legend.position="none"
  )
```



```
for (penalty in poison_penalties) {
  stat_data <- filter(summary_data, POISON_PENALTY==penalty & dominant_lineage_num_times_hitchhik
  print(
    paste0(
      "PENALTY: ", penalty
    )
  )
  kt <- kruskal.test(
      formula=frac_hitchhiking_linked_trait_change~condition,
      data=stat_data
    )
  print(
    kt
  )
  if (is.na(kt$p.value)) { next }
  if (kt$p.value > 0.05) { next }
  print(
    pairwise.wilcox.test(
      x=stat_data$frac_hitchhiking_linked_trait_change,
      g=stat_data$condition,
      p.adjust.method="bonferroni",
      exact=FALSE
    )
  )
}
```

```
## [1] "PENALTY: 0"
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_hitchhiking_linked_trait_change by condition
## Kruskal-Wallis chi-squared = 211.29, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$frac_hitchhiking_linked_trait_change and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.031
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.03"
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_hitchhiking_linked_trait_change by condition
## Kruskal-Wallis chi-squared = 186.88, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$frac_hitchhiking_linked_trait_change and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 2.9e-16     -
## STATIC  < 2e-16     -
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.1"
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_hitchhiking_linked_trait_change by condition
## Kruskal-Wallis chi-squared = 113.72, df = 2, p-value < 2.2e-16
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$frac_hitchhiking_linked_trait_change and stat_data$condition
##
```

```
##         NON-PLASTIC PLASTIC
## PLASTIC 3.3e-08     -
## STATIC  < 2e-16     -
##
## P value adjustment method: bonferroni
## [1] "PENALTY: 0.3"
##
##  Kruskal-Wallis rank sum test
##
## data:  frac_hitchhiking_linked_trait_change by condition
## Kruskal-Wallis chi-squared = 34.791, df = 2, p-value = 2.788e-08
##
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  stat_data$frac_hitchhiking_linked_trait_change and stat_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 0.26        -
## STATIC  2.4e-08     0.18
##
## P value adjustment method: bonferroni
```

```
denom <- sum(filter(summary_data, condition=="NON-PLASTIC" & POISON_PENALTY==0.1)$dominant_lineag
num <- sum(filter(summary_data, condition=="NON-PLASTIC" & POISON_PENALTY==0.1)$dominant_lineage_
paste0("NON-PLASTIC (0.1 penalty): ", num/denom, "(", num, "/", denom, ")")
```

```
## [1] "NON-PLASTIC (0.1 penalty): 0.498956158663883(956/1916)"
```

```
denom <- sum(filter(summary_data, condition=="PLASTIC" & POISON_PENALTY==0.1)$dominant_lineage_nu
num <- sum(filter(summary_data, condition=="PLASTIC" & POISON_PENALTY==0.1)$dominant_lineage_num_
paste0("PLASTIC (0.1 penalty): ", num/denom, " (", num, "/", denom, ")")
```

```
## [1] "PLASTIC (0.1 penalty): 0 (0/18)"
```

```
denom <- sum(filter(summary_data, condition=="STATIC" & POISON_PENALTY==0.1)$dominant_lineage_num
num <- sum(filter(summary_data, condition=="STATIC" & POISON_PENALTY==0.1)$dominant_lineage_num_t
paste0("STATIC (0.1 penalty): ", num/denom, " (", num, "/", denom, ")")
```

```
## [1] "STATIC (0.1 penalty): 0 (0/58)"
```

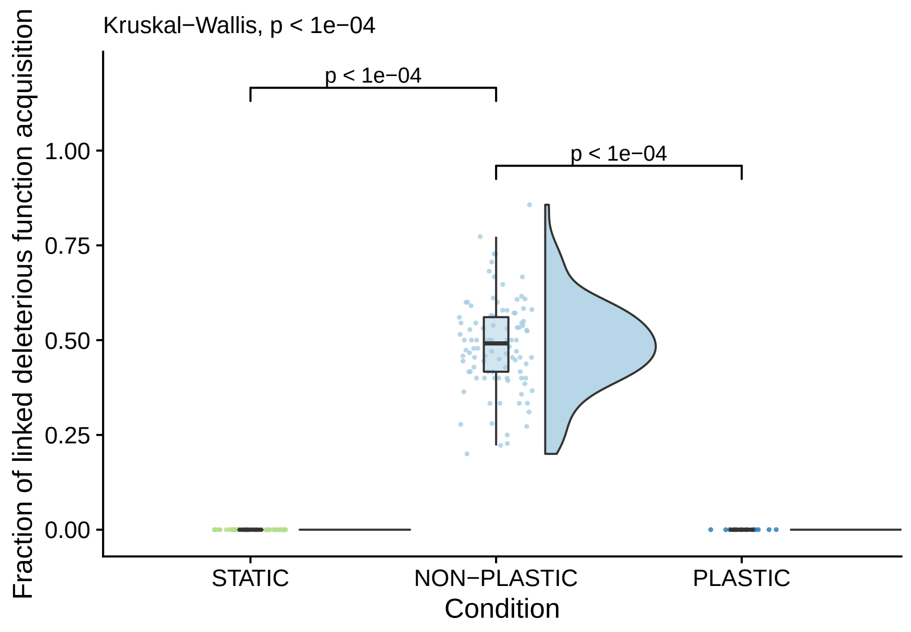
Focal figure for the manuscript:

```
# Compute manual labels for geom_signif
stat.test <-filter(focal_summary_data,dominant_lineage_num_times_hitchhike_inst_exec_increases>0)
  wilcox_test(frac_hitchhiking_linked_trait_change ~ condition, comparisons=list(c("PLASTIC", "NO
  adjust_pvalue(method = "bonferroni") %>%
  add_significance() %>%
  add_xy_position(x="condition")
```

```
# Tweak y.position manually to account for scaled axis (edge case that triggers bad be
stat.test$manual_position <-  stat.test$y.position
stat.test$label <- mapply(p_label,stat.test$p.adj)

linked_trait_change_fig <- ggplot(
    filter(focal_summary_data, dominant_lineage_num_times_hitchhike_inst_exec_increase
    aes(x=condition, y=frac_hitchhiking_linked_trait_change, fill=condition)
  ) +
  geom_flat_violin(
    scale="width",
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order,
    labels=condition_order
  ) +
  scale_y_continuous(
    name="Fraction of linked deleterious function acquisition",
    limits=c(-0.01, 1.2),
    breaks=c(0, 0.25, 0.50, 0.75, 1.0)
  ) +
  scale_fill_brewer(
    palette=cb_palette
  ) +
  scale_color_brewer(
    palette=cb_palette
  ) +
  labs(
    subtitle=paste0(
      "Kruskal-Wallis, ",
      p_label(signif(kruskal.test(formula=frac_hitchhiking_linked_trait_change~conditi
    )
  ) +
  ggsignif::geom_signif(
    data=filter(stat.test, p.adj <= alpha),
    aes(xmin=group1,xmax=group2,annotations=label,y_position=manual_position),
    manual=TRUE,
    inherit.aes=FALSE
  ) +
  theme(
    legend.position="none"
  )
```

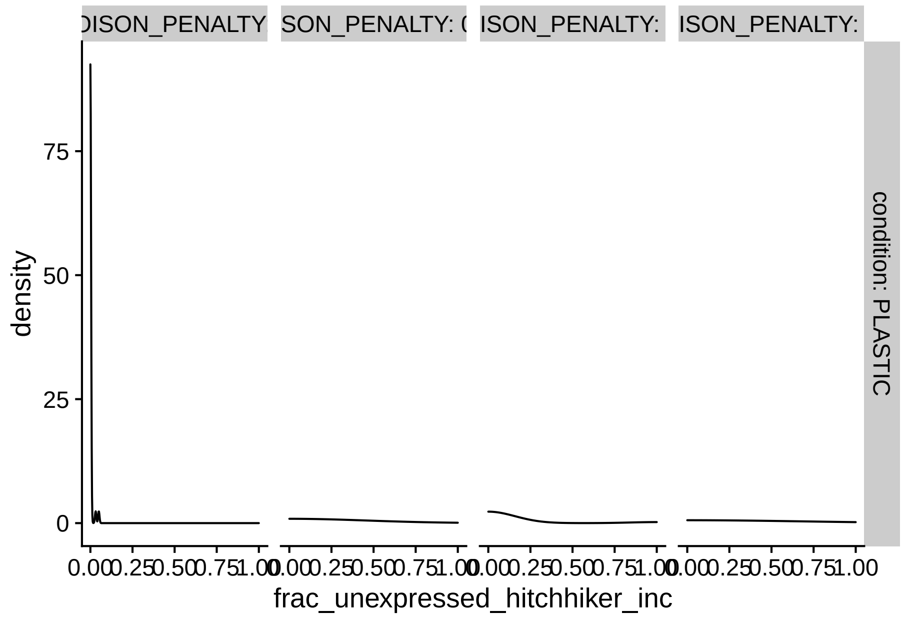
```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```

```
linked_trait_change_fig
```



8.7 What fraction of deleterious execution increases occur in unexpressed phenotype (as cryptic variation)?

```
ggplot(filter(summary_data, dominant_lineage_num_times_hitchhike_inst_exec_increases>0 & conditic
  geom_density() +
  facet_grid(
    condition~POISON_PENALTY,
    labeller=label_both,
    scales="free_y"
  ) +
  theme(
    legend.position="none"
  )
```

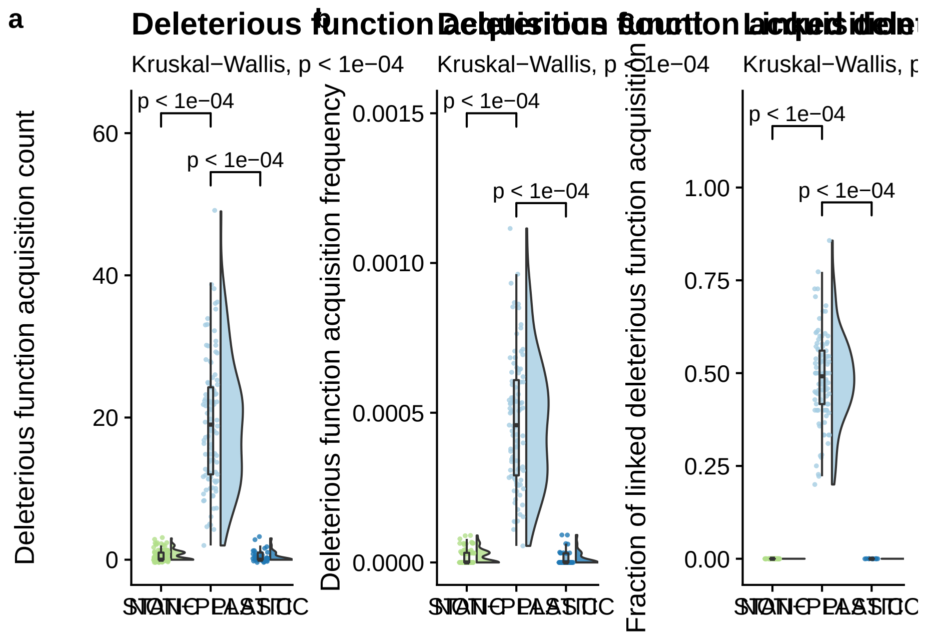


```
denom <- sum(filter(summary_data, condition=="PLASTIC" & POISON_PENALTY==0.1)$dominant_
num <- sum(filter(summary_data, condition=="PLASTIC" & POISON_PENALTY==0.1)$dominant_li
paste0("PLASTIC: ", num/denom, " (", num, "/", denom, ")")
```

```
## [1] "PLASTIC: 0.0555555555555556 (1/18)"
```

8.8 Manuscript figures

```
grid <- plot_grid(
  poison_increases_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Deleterious function acquisition count"),
  poison_increases_per_gen_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Deleterious function acquisition frequency"),
  linked_trait_change_fig +
    theme(
      axis.title.x=element_blank()
    ) +
    ggtitle("Linked deleterious function acquisition"),
  nrow=1,
  align="v",
  labels="auto"
)
save_plot(
   paste0(working_directory, "plots/", "poison-accumulation-panel.pdf"),
   grid,
   base_height=6,
   base_asp=3/1
)
grid
```



Chapter 9

Regulation in Avida

9.1 Overview

```
total_updates <- 200000
replicates <- 100

all_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")

# Relative location of data.
working_directory <- "experiments/2021-02-08-evo-dynamics/analysis/" # << For bookdown
# working_directory <- "./"                                          # << For local analysis
```

9.2 Analysis dependencies

Load all required R libraries.

```
library(ggplot2)
library(tidyverse)
library(cowplot)
library(RColorBrewer)
library(Hmisc)
library(boot)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd97121f7f9ce9
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

9.3 Setup

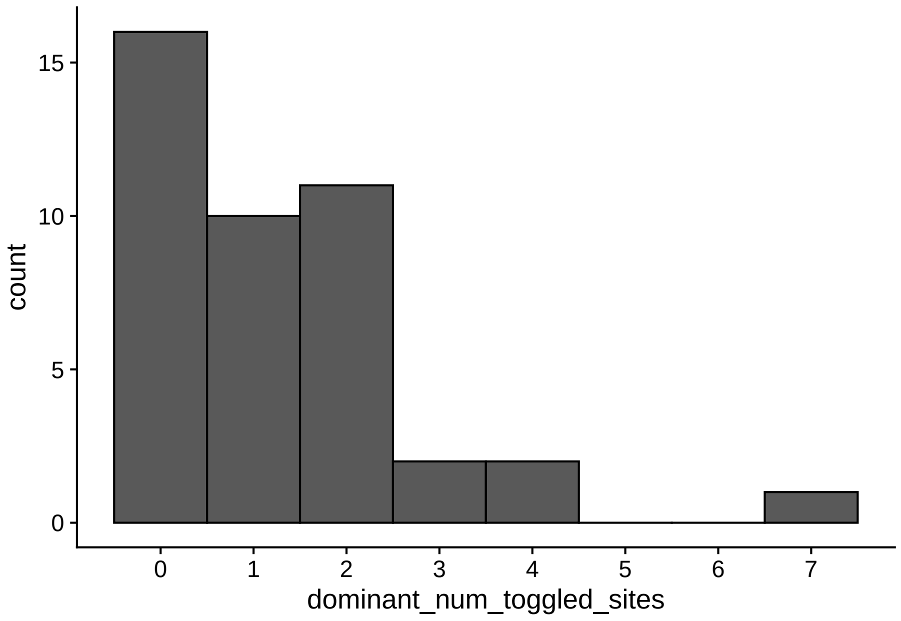
```
trace_summary_data_loc <- paste0(working_directory, "data/trace_summary.csv")
trace_summary_data <- read.csv(trace_summary_data_loc, na.strings="NONE")

trace_summary_data$DISABLE_REACTION_SENSORS <- as.factor(trace_summary_data$DISABLE_REA
trace_summary_data$chg_env <- trace_summary_data$chg_env == "True"
trace_summary_data$sensors <- trace_summary_data$DISABLE_REACTION_SENSORS == "0"


env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
}
```

```
# note that this labeler makes assumptions about how we set up our experiment
condition_label_fun <- function(has_sensors, env_chg) {
  if (has_sensors && env_chg) {
    return("PLASTIC")
  } else if (env_chg) {
    return("NON-PLASTIC")
  } else {
    return("STATIC")
  }
}

trace_summary_data$env_label <- mapply(
  env_label_fun,
  trace_summary_data$chg_env
)
trace_summary_data$sensors_label <- mapply(
  sensors_label_fun,
  trace_summary_data$sensors
)
trace_summary_data$condition <- mapply(
  condition_label_fun,
  trace_summary_data$sensors,
  trace_summary_data$chg_env
)

####### misc #######
# Configure our default graphing theme
theme_set(theme_cowplot())
dir.create(paste0(working_directory, "plots"), showWarnings=FALSE)
```

9.4 How many instructions do plastic genomes toggle depending on environmental context?

```
ggplot(trace_summary_data, aes(x=dominant_num_toggled_sites)) +
    geom_histogram(
      binwidth=1,
      color="black"
    ) +
    scale_fill_brewer(
      palette="Paired"
    ) +
    scale_color_brewer(
      palette="Paired"
```

```
  ) +
  scale_x_continuous(
    breaks=seq(0, max(trace_summary_data$dominant_num_toggled_sites)+1)
  ) +
  theme(
    legend.position="none"
  )
```



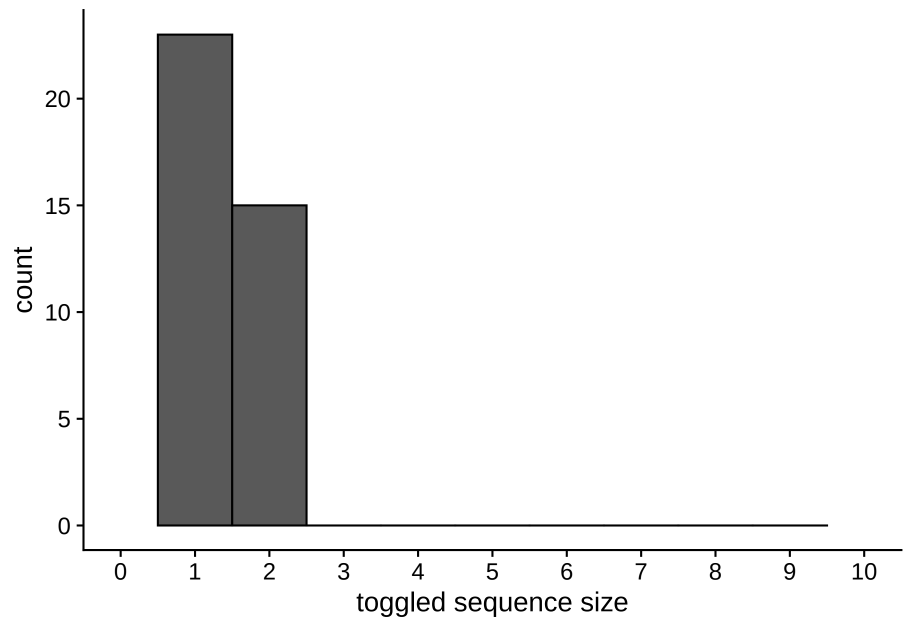
```
ggsave(paste0(working_directory, "plots/", "toggled-sites.png"))
```

```
## Saving 6.5 x 4.5 in image
```

9.5 What is the distrubution of toggled sequence sizes?

```
chunk_sizes <- data.frame(
  size=integer()
)
for (sizes in trace_summary_data$dominant_toggled_chunk_sizes) {
  if (sizes == "") { next }
  sizes <- unlist(lapply(str_split(sizes, ';'), as.integer))
  chunk_sizes <- rbind(chunk_sizes, data.frame(size=c(sizes)))
}
```

```
ggplot(chunk_sizes, aes(x=size)) +
    geom_histogram(
      binwidth=1,
      color="black"
    ) +
    scale_fill_brewer(
      palette="Paired"
    ) +
    scale_color_brewer(
      palette="Paired"
    ) +
    scale_x_continuous(
      name="toggled sequence size",
      breaks=seq(0, 10),
      limits=c(0, 10)
    ) +
    theme(
      legend.position="none"
    )
```



```
ggsave(paste0(working_directory, "plots/", "toggled-chunk-sizes.png"))
```

```
## Saving 6.5 x 4.5 in image
```

Chapter 10

Evolutionary change (variable length genomes)

10.1 Overview

```
total_updates <- 200000
replicates <- 100

all_traits <- c("not","nand","and","ornot","or","andnot")
traits_set_a <- c("not", "and", "or")
traits_set_b <- c("nand", "ornot", "andnot")

# Relative location of data.
working_directory <- "experiments/2021-01-30-evo-dynamics/analysis/" # << For bookdown
# working_directory <- "./"                                            # << For local analysis
```

10.2 Analysis dependencies

Load all required R libraries.

```
library(ggplot2)
library(tidyverse)
library(cowplot)
library(RColorBrewer)
library(Hmisc)
library(boot)
source("https://gist.githubusercontent.com/benmarwick/2a1bb0133ff568cbe28d/raw/fb53bd97121f7f9ce9
```

These analyses were conducted/knitted with the following computing environment:

```
print(version)
```

```
##                _
## platform       x86_64-pc-linux-gnu
## arch           x86_64
## os             linux-gnu
## system         x86_64, linux-gnu
## status
## major          4
## minor          1.0
## year           2021
## month          05
## day            18
## svn rev        80317
## language       R
## version.string R version 4.1.0 (2021-05-18)
## nickname       Camp Pontanezen
```

10.3 Setup

```
summary_data_loc <- paste0(working_directory, "data/aggregate.csv")
summary_data <- read.csv(summary_data_loc, na.strings="NONE")

summary_data$DISABLE_REACTION_SENSORS <- as.factor(summary_data$DISABLE_REACTION_SENSO
summary_data$chg_env <- summary_data$chg_env == "True"
summary_data$dominant_plastic_odd_even <- as.factor(summary_data$dominant_plastic_odd_
summary_data$sensors <- summary_data$DISABLE_REACTION_SENSORS == "0"
summary_data$is_plastic <- summary_data$dominant_plastic_odd_even == "True"

env_label_fun <- function(chg_env) {
  if (chg_env) {
    return("Fluctuating")
  } else {
    return("Constant")
  }
}

sensors_label_fun <- function(has_sensors) {
  if (has_sensors) {
    return("Sensors")
  } else {
    return("No sensors")
  }
```

```r
}

# note that this labeler makes assumptions about how we set up our experiment
condition_label_fun <- function(has_sensors, env_chg) {
  if (has_sensors && env_chg) {
    return("PLASTIC")
  } else if (env_chg) {
    return("NON-PLASTIC")
  } else {
    return("STATIC")
  }
}

summary_data$env_label <- mapply(
  env_label_fun,
  summary_data$chg_env
)
summary_data$sensors_label <- mapply(
  sensors_label_fun,
  summary_data$sensors
)
summary_data$condition <- mapply(
  condition_label_fun,
  summary_data$sensors,
  summary_data$chg_env
)

condition_order = c(
  "STATIC",
  "NON-PLASTIC",
  "PLASTIC"
)

####### misc #######
# Configure our default graphing theme
theme_set(theme_cowplot())
dir.create(paste0(working_directory, "plots"), showWarnings=FALSE)
```

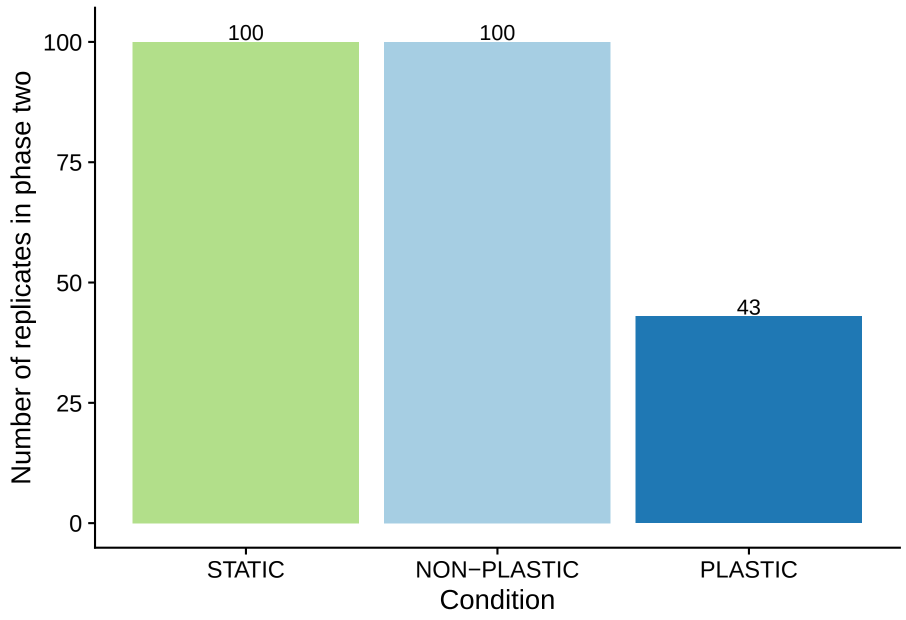
10.4 Evolution of phenotypic plasticity

For sensor-enabled populations in fluctuating environments, we only transfered populations containing an optimally plastic genotype to phase two.

```r
summary_data_grouped = dplyr::group_by(summary_data, sensors, env_label, condition)
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())
```

```
## `summarise()` has grouped output by 'sensors', 'env_label'. You can override using
```

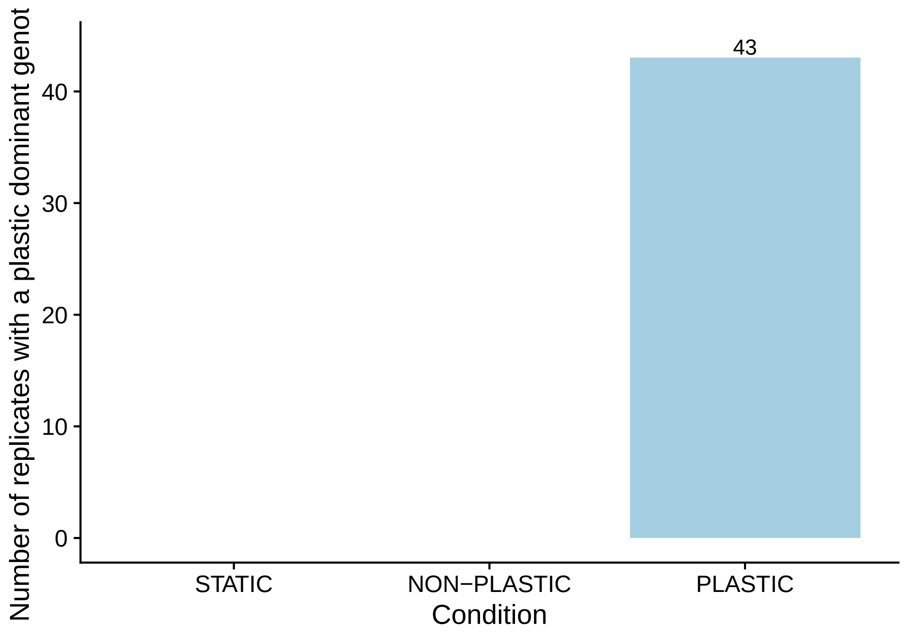
```
ggplot(summary_data_group_counts, aes(x=condition, y=n, fill=condition)) +
  geom_col(position=position_dodge(0.9)) +
  geom_text(aes(label=n, y=n+2)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  ylab("Number of replicates in phase two") +
  theme(
    legend.position="none"
  )
```



We can confirm our expectation that the dominant genotypes in non-plastic conditions are not phenotypically plastic.

```
summary_data_grouped = dplyr::group_by(summary_data, condition, is_plastic)
summary_data_group_counts = dplyr::summarize(summary_data_grouped, n=dplyr::n())
```

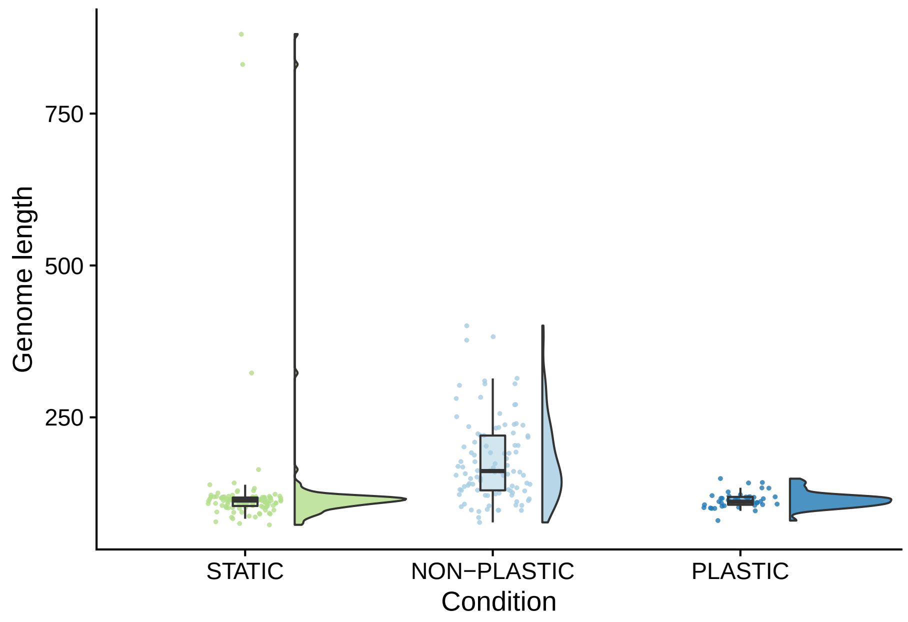
```
ggplot(filter(summary_data_group_counts, is_plastic), aes(x=condition, y=n, fill=condition)) +
  geom_col(position=position_dodge(0.9)) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  geom_text(aes(label=n, y=n+1)) +
  ylab("Number of replicates with a plastic dominant genotype") +
  theme(
    legend.position="none"
  )
```



10.5 Genome length

Single-instruction insertions and deletions were possible for this experiment, so genome size also evolved.

```
ggplot(summary_data, aes(x=condition, y=dominant_genome_length, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  ylab("Genome length") +
  theme(
    legend.position="none"
  )
```



```r
kruskal.test(
  formula=dominant_genome_length~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_genome_length by condition
## Kruskal-Wallis chi-squared = 82.798, df = 2, p-value < 2.2e-16
```

```r
pairwise.wilcox.test(
  x=summary_data$dominant_genome_length,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_genome_length and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC 1.8e-10     -
## STATIC  < 2e-16     1
##
```

```
## P value adjustment method: bonferroni
```

```r
median(filter(summary_data, condition=="PLASTIC")$phylo_mrca_changes)
```

```
## [1] 45
```

```r
median(filter(summary_data, condition=="STATIC")$phylo_mrca_changes)
```

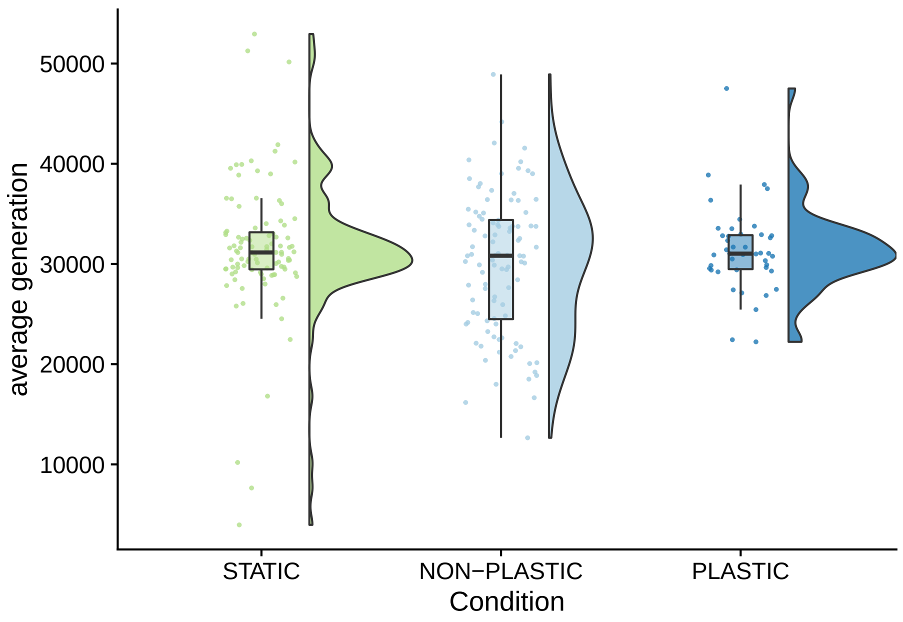
```
## [1] 47
```

```r
median(filter(summary_data, condition=="NON-PLASTIC")$phylo_mrca_changes)
```

```
## [1] 393
```

10.6 Average generation

```r
ggplot(summary_data, aes(x=condition, y=time_average_generation, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  ylab("average generation") +
  theme(
    legend.position="none"
  )
```



```
median(filter(summary_data, condition=="PLASTIC")$time_average_generation)
```

```
## [1] 31028.6
```

```
median(filter(summary_data, condition=="STATIC")$time_average_generation)
```

```
## [1] 31147.5
```

```
median(filter(summary_data, condition=="NON-PLASTIC")$time_average_generation)
```

```
## [1] 30817.95
```

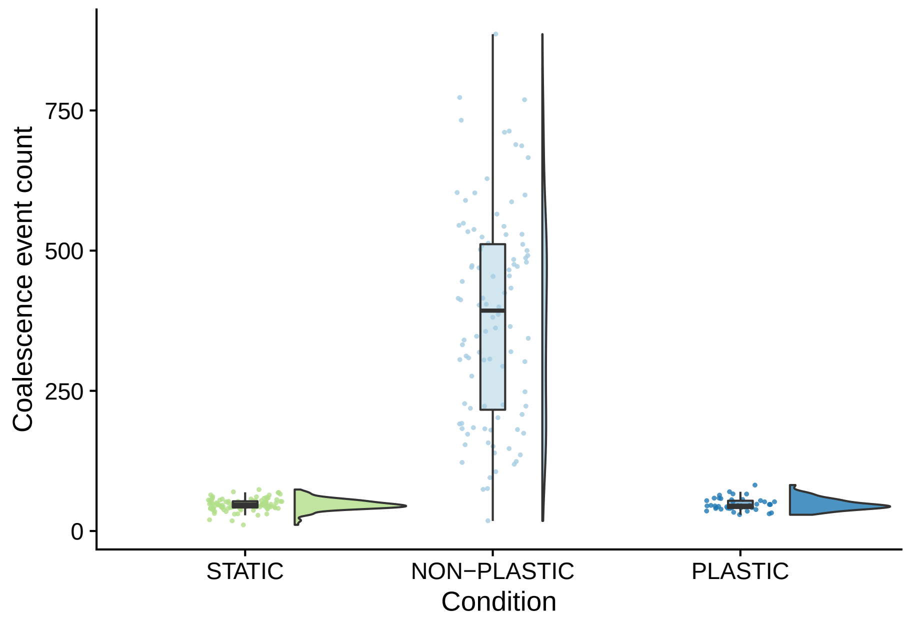
```
kruskal.test(
  formula=time_average_generation~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  time_average_generation by condition
## Kruskal-Wallis chi-squared = 1.3804, df = 2, p-value = 0.5015
```

10.7 Coalescence event count

The number of times the most recent common ancestor changes gives us the number of selective sweeps that occur during the experiment.

```
ggplot(summary_data, aes(x=condition, y=phylo_mrca_changes, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  ylab("Coalescence event count") +
  theme(
    legend.position="none"
  )
```



```r
paste0(
  "PLASTIC: ",
  median(filter(summary_data, condition=="PLASTIC")$phylo_mrca_changes)
)
```

```
## [1] "PLASTIC: 45"
```

```r
paste0(
  "STATIC: ",
  median(filter(summary_data, condition=="STATIC")$phylo_mrca_changes)
)
```

```
## [1] "STATIC: 47"
```

```r
paste0(
  "NON-PLASTIC: ",
  median(filter(summary_data, condition=="NON-PLASTIC")$phylo_mrca_changes)
)
```

```
## [1] "NON-PLASTIC: 393"
```

```r
kruskal.test(
  formula=phylo_mrca_changes~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
```

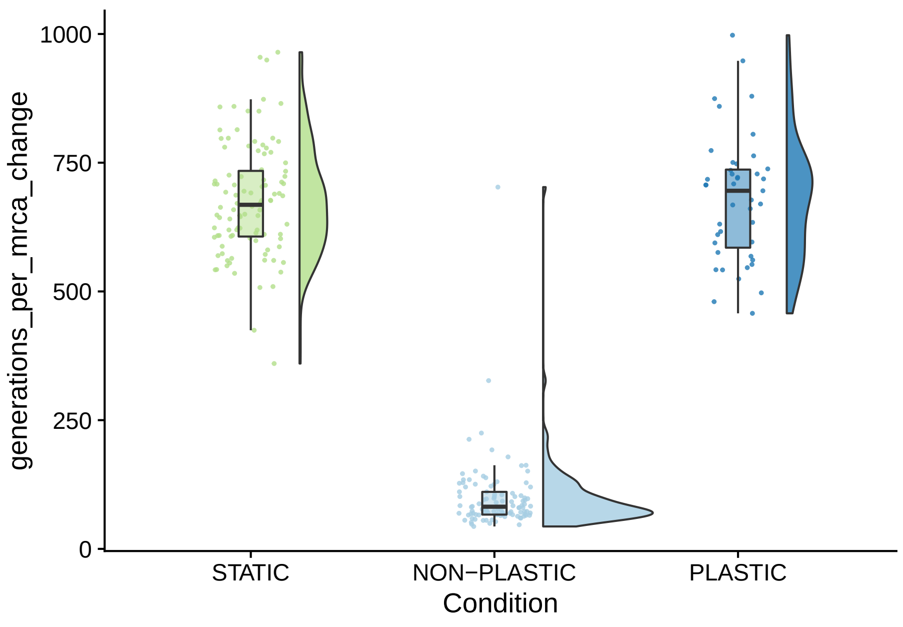
```
##
## data:  phylo_mrca_changes by condition
## Kruskal-Wallis chi-squared = 168.89, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$phylo_mrca_changes,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$phylo_mrca_changes and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      1
##
## P value adjustment method: bonferroni
```

10.7.1 Average number of generations between coalescence events

```
summary_data$generations_per_mrca_change <- summary_data$time_average_generation / summ

ggplot(summary_data, aes(x=condition, y=generations_per_mrca_change, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  theme(
    legend.position="none"
  )
```



```
paste0(
  "PLASTIC: ",
  median(filter(summary_data, condition=="PLASTIC")$generations_per_mrca_change)
)
```

```
## [1] "PLASTIC: 695.504761904762"
```

```
paste0(
  "STATIC: ",
  median(filter(summary_data, condition=="STATIC")$generations_per_mrca_change)
)
```

```
## [1] "STATIC: 668.25523255814"
```

```
paste0(
  "NON-PLASTIC: ",
  median(filter(summary_data, condition=="NON-PLASTIC")$generations_per_mrca_change)
)
```

```
## [1] "NON-PLASTIC: 81.9208459944751"
```

```
kruskal.test(
  formula=generations_per_mrca_change~condition,
  data=summary_data
)
```

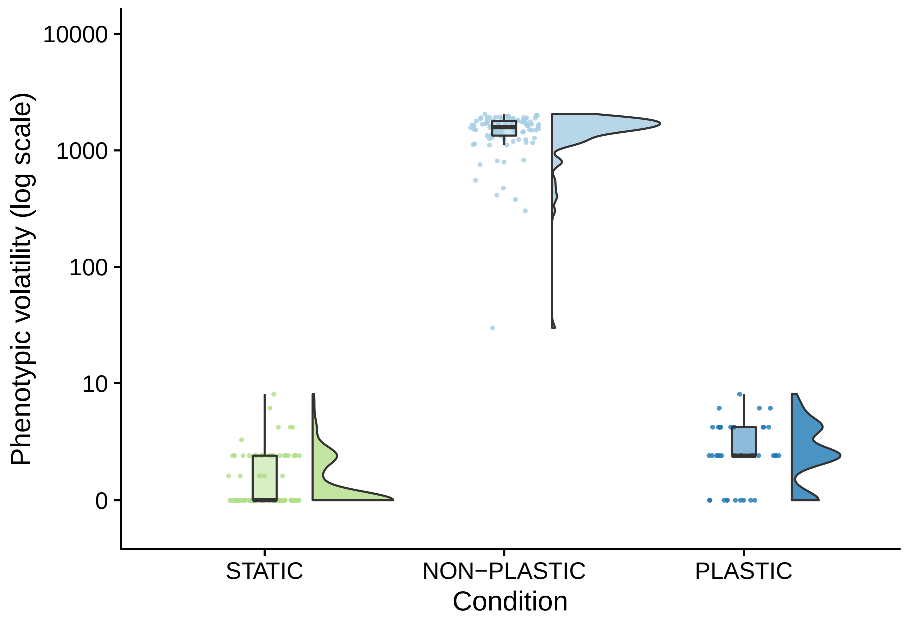
```
##
##  Kruskal-Wallis rank sum test
##
## data:  generations_per_mrca_change by condition
## Kruskal-Wallis chi-squared = 171.73, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$generations_per_mrca_change,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$generations_per_mrca_change and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      1
##
## P value adjustment method: bonferroni
```

10.8 Phenotypic volatility along the dominant lineage

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_trait_volatility, fill=conditi
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_y_continuous(
    name="Phenotypic volatility (log scale)",
    trans="pseudo_log",
    breaks=c(0, 10, 100, 1000, 10000),
    limits=c(-1,10000)
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  theme(
    legend.position="none"
  )
```



```
paste0(
  "PLASTIC: ",
  median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_trait_volatility)
)
```

```
## [1] "PLASTIC: 2"
```

```
paste0(
  "STATIC: ",
  median(filter(summary_data, condition=="STATIC")$dominant_lineage_trait_volatility)
)
```

```
## [1] "STATIC: 0"
```

```
paste0(
  "NON-PLASTIC: ",
  median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_trait_volatili
)
```

```
## [1] "NON-PLASTIC: 1580"
```

```
kruskal.test(
  formula=dominant_lineage_trait_volatility~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
```

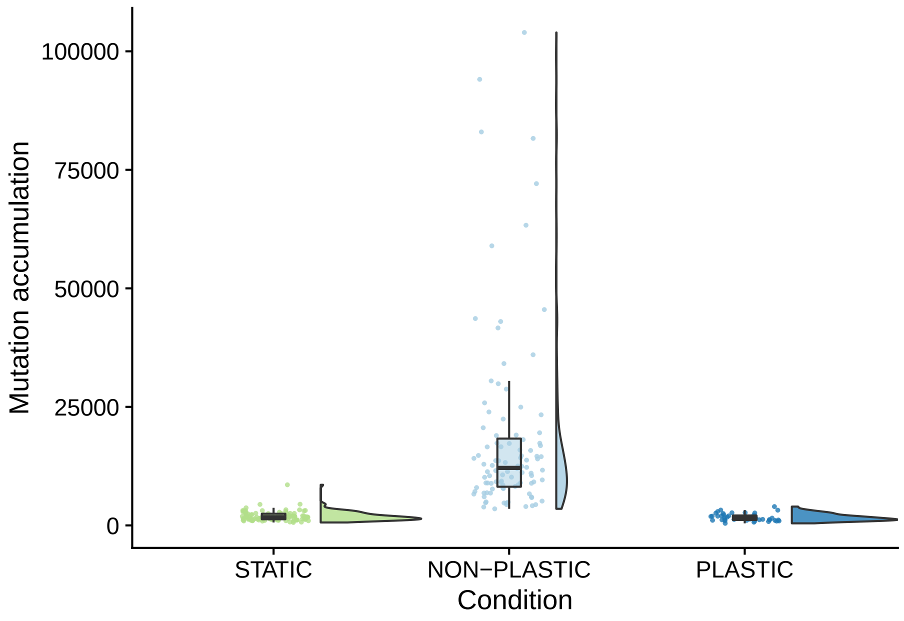
```
##
## data:  dominant_lineage_trait_volatility by condition
## Kruskal-Wallis chi-squared = 191.98, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_trait_volatility,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_trait_volatility and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC < 2e-16     -
## STATIC  < 2e-16     5.2e-08
##
## P value adjustment method: bonferroni
```

10.9 Mutation count (along dominant lineage)

```
ggplot(summary_data, aes(x=condition, y=dominant_lineage_total_mut_cnt, fill=condition)) +
  geom_flat_violin(
    position = position_nudge(x = .2, y = 0),
    alpha = .8
  ) +
  geom_point(
    mapping=aes(color=condition),
    position = position_jitter(width = .15),
    size = .5,
    alpha = 0.8
  ) +
  geom_boxplot(
    width = .1,
    outlier.shape = NA,
    alpha = 0.5
  ) +
  ylab("Mutation accumulation") +
  scale_x_discrete(
    name="Condition",
    limits=condition_order
  ) +
  scale_fill_brewer(
    palette="Paired"
  ) +
  scale_color_brewer(
    palette="Paired"
  ) +
  theme(
    legend.position="none"
  )
```



```
paste0(
  "PLASTIC: ",
  median(filter(summary_data, condition=="PLASTIC")$dominant_lineage_total_mut_cnt)
)
```

```
## [1] "PLASTIC: 1552"
```

```
paste0(
  "STATIC: ",
  median(filter(summary_data, condition=="STATIC")$dominant_lineage_total_mut_cnt)
)
```

```
## [1] "STATIC: 1724.5"
```

```
paste0(
  "NON-PLASTIC: ",
  median(filter(summary_data, condition=="NON-PLASTIC")$dominant_lineage_total_mut_cnt
)
```

```
## [1] "NON-PLASTIC: 12123"
```

```
kruskal.test(
  formula=dominant_lineage_total_mut_cnt~condition,
  data=summary_data
)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  dominant_lineage_total_mut_cnt by condition
## Kruskal-Wallis chi-squared = 174.38, df = 2, p-value < 2.2e-16
```

```
pairwise.wilcox.test(
  x=summary_data$dominant_lineage_total_mut_cnt,
  g=summary_data$condition,
  p.adjust.method="bonferroni",
)
```

```
##
##  Pairwise comparisons using Wilcoxon rank sum test with continuity correction
##
## data:  summary_data$dominant_lineage_total_mut_cnt and summary_data$condition
##
##         NON-PLASTIC PLASTIC
## PLASTIC <2e-16      -
## STATIC  <2e-16      0.57
##
## P value adjustment method: bonferroni
```

Bibliography

Lalejini, A. M. and Ferguson, A. J. (2021). Data for evolutionary consequences of phenotypic plasticity.