

DEPI Day 3 Epistasis

Abigail Seeger

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Load Necessary Packages

```
library(stringr)
library(stringi)
library(dplyr)
library(viridis)
library(ggplot2)
library(extrafont)
library(ggthemes)
library(lemon)
```

Load in Cleaned Data

```
depi_data <- read.table("C:/Users/Owner/Documents/Research/Shiu_Lab/Shiu_Lab_R/Data/Clean_DEPI_Data.csv",
  sep = ",", header = TRUE)
```

Functions

```
add_number <- function(data_frame) {  
  ### First, if the genotype is Col0 (only  
  ### genotype with length 4), assign 0 as  
  ### number Else, assign number as genotype  
  ### with 'mpk' removed Example: mpk1 will  
  ### be 1, mpk1-17 will be 1-17  
  data_frame <- data_frame %>% mutate(number = ifelse(genotype !=  
    "Col0", (stri_sub(genotype, 4, length(genotype))),  
    0))  
  ### Next, for all double mutants, replace  
  ### '-' with '0' Example: 1-17 becomes 1017  
  data_frame$number <- as.numeric(gsub("-",  
    "0", data_frame$number))  
  ### Almost there! There's a problem with  
  ### two single digit double mutants We need  
  ### a four digit number to sort correctly  
  ### Example: mpk1-3 -> 1-3 -> 103, but we  
  ### need it to be 1003 to sort correctly  
  data_frame$number[data_frame$number ==  
    "103"] <- "1003"  
  data_frame$number[data_frame$number ==  
    "506"] <- "5006"  
  data_frame$number[data_frame$number ==  
    "608"] <- "6008"  
  data_frame$number[data_frame$number ==  
    "609"] <- "6009"  
  ### Convert number to a numeric in order  
  ### to sort  
  data_frame$number <- as.numeric(data_frame$number)  
  data_frame <- data_frame %>% arrange(number)  
  data_frame <- data_frame %>% mutate(number_2 = number)  
  data_frame$number_2[nchar(data_frame$number_2) ==  
    4] <- 0  
  data_frame$number_2[nchar(data_frame$number_2) ==  
    5] <- 0  
  return(data_frame)  
}
```

Note that this function uses the normalized value, instead of the measured value.

```
cell_371_data <- function(data_frame) {  
  
  npq_phi2 <- data_frame %>% filter(measurement %in%  
    c("npq", "phi2")) %>% group_by(time_point,  
    measurement) %>% mutate_each(funs(./median(.[genotype ==  
    "Col0"])), normalized_value) %>%  
    group_by(time_point, measurement,  
    genotype) %>% mutate(log2_fold = log2(median(normalized_value)))
```

```

start_end <- unique((data_frame %>% group_by(day) %>%
  filter(time_point %in% c(min(time_point),
    max(time_point))))$time_point)

leaf_area <- data_frame %>% filter(measurement ==
  "leafarea") %>% filter(time_point %in%
  start_end) %>% group_by(time_point,
  measurement) %>% mutate_each(funs(./median(.[genotype ==
  "Col0"])), measured_value) %>% group_by(time_point,
  measurement, genotype) %>% mutate(log2_fold = log2(median(measured_value)))

out <- rbind(npq_phi2, leaf_area) %>%
  group_by(genotype, time_point, measurement)

return(as.data.frame(out))
}

```

Selection Coefficient Calculations

```

selectionCoef <- data.frame(genotype = rep(NA,
  0), SelectionCoefficient = rep(NA, 0),
  Experiment = rep(NA, 0), Measurement = rep(NA,
  0))

for (e in c("Dec", "Jan", "Feb")) {
  for (m in c("phi2", "leafarea", "phi2")) {
    temp_data <- depi_data %>% filter(month ==
      e, measurement == m)
    temp_nrow <- 38 * length(unique(temp_data$time_point))
    for (i in unique(temp_data$time_point)) ### Create an empty data frame to fill with
    ### the information and calculations:
      selectionCoefTmp <- data.frame(genotype = rep(NA,
        temp_nrow), SelectionCoefficient = rep(NA,
        temp_nrow), Experiment = rep(NA,
        temp_nrow), Measurement = rep(NA,
        temp_nrow), Time_Point = rep(NA,
        temp_nrow))
      count <- 1
      for (g in unique(temp_data$genotype)) {
        fm <- mean(filter(temp_data,
          genotype == g, month == e,
          measurement == m, time_point ==
            i)$normalized_value)
        fwt <- mean(filter(temp_data,
          genotype == "Col0", month ==
            e, measurement == m, time_point ==
            i)$normalized_value)
        TempSelectionCoef <- (fm - fwt)/fwt
        selectionCoefTmp[count, 1] <- g
        selectionCoefTmp[count, 2] <- TempSelectionCoef
        selectionCoefTmp[count, 3] <- e
      }
    }
  }
}

```

```

        selectionCoefTmp[count, 4] <- m
        selectionCoefTmp[count, 5] <- i
        count <- count + 1
    }
    selectionCoef <- rbind(selectionCoef,
        selectionCoefTmp)
}

selectionCoef <- selectionCoef %>% arrange(Measurement,
    genotype)

```

Epistasis Calculations

```

all_double_mutants = list()
for (gen in unique(depi_data$genotype)) {
    if (str_detect(gen, "-") == T) {
        all_double_mutants = c(all_double_mutants,
            gen)
    }
}

### Epistasis Calculations: Initialize an
### empty data frame to populate with
### information:
geneticInteractions <- data.frame(genotype = rep(NA,
    0), MutantA = rep(NA, 0), MutantB = rep(NA,
    0), AdditiveEpistasis = rep(NA, 0), ProportionalEpistasis = rep(NA,
    0), Experiment = rep(NA, 0), Measurement = rep(NA,
    0), Time_Point = rep(NA, 0))

### Loop through each experiment and
### measurement:
for (e in c("Dec", "Jan", "Feb")) {
    for (m in c("phi2", "leafarea", "npq")) {
        temp_data <- depi_data %>% filter(month ==
            e, measurement == m)
        temp_nrow <- 25 * length(unique(temp_data$time_point))
        for (i in unique(temp_data$time_point)) {
            ### Filter to each specific experiment and
            ### measurement
            tempData <- filter(depi_data,
                month == e, measurement ==
                m, time_point == i)
            ### Create an empty data frame to fill with
            ### the information and calculations:
            geneticInteractionsTmp <- data.frame(genotype = rep(NA,
                temp_nrow), MutantA = rep(NA,
                temp_nrow), MutantB = rep(NA,
                temp_nrow), AdditiveEpistasis = rep(NA,
                temp_nrow), ProportionalEpistasis = rep(NA,

```

```

temp_nrow), Experiment = rep(NA,
temp_nrow), Measurement = rep(NA,
temp_nrow), Time_Point = rep(NA,
temp_nrow))
### Initialize a row count to use to
### populate the data frame
rowCount <- 1
### For each of the double mutants:
for (dm in unlist(all_double_mutants)) {
  ### Extract the single mutants from the
  ### double mutant
  ma <- unlist(strsplit(dm,
    "-"))[1]
  mb <- paste("mpk", unlist(strsplit(dm,
    "-"))[2], sep = "")
  ### Calculate the fitness of the dm, ma,
  ### mb, and wt
  fdm <- mean(filter(tempData,
    genotype == dm)$normalized_value)
  fwt <- mean(filter(tempData,
    genotype == "Col0")$normalized_value)
  fma <- mean(filter(tempData,
    genotype == ma)$normalized_value)
  fmb <- mean(filter(tempData,
    genotype == mb)$normalized_value)
  ### Calculate Additive and Proportional
  ### Epistasis
  AddEp <- fdm + fwt - (fma +
    fmb)
  PropEp <- log((fdm * fwt)/(fma *
    fmb))
  ### Populate the data frame with this
  ### information:
  geneticInteractionsTmp[rowCount,
    1] <- dm
  geneticInteractionsTmp[rowCount,
    2] <- ma
  geneticInteractionsTmp[rowCount,
    3] <- mb
  geneticInteractionsTmp[rowCount,
    4] <- AddEp
  geneticInteractionsTmp[rowCount,
    5] <- PropEp
  geneticInteractionsTmp[rowCount,
    6] <- e
  geneticInteractionsTmp[rowCount,
    7] <- m
  geneticInteractionsTmp[rowCount,
    8] <- i
  rowCount <- rowCount + 1
}
### Add the rows of the temporary genetic
### interaction information to the main

```

```

    ### data frame
    geneticInteractions <- rbind(geneticInteractions,
                                geneticInteractionsTmp)
  }
}

```

Selection Coefficient Visualizations

First, include a column of “number” and “number_2”

```

selectionCoef <- add_number(selectionCoef)
selectionCoef$genotype <- reorder(selectionCoef$genotype,
                                  desc(selectionCoef$number))
selectionCoef <- add_day_col(selectionCoef)

```

```

temp_plot <- selectionCoef %>% filter(Experiment ==
  "Jan", Measurement == "phi2")

temp_upper_bound <- round(max(temp_plot$SelectionCoefficient) +
  0.05, 2)
temp_lower_bound <- round(min(temp_plot$SelectionCoefficient) -
  0.05, 2)

ggplot(data = temp_plot, aes(x = Time_Point,
  y = genotype, fill = SelectionCoefficient)) +
  labs(fill = "Selection Coefficient",
    x = "Hours", y = NULL, title = "January Day 3 Selection Coefficients") +
  geom_tile(width = 10, height = 10) +
  facet_grid(genotype ~ day, scales = "free",
    switch = "y") + theme_tufte(base_family = "Calibri",
    base_size = 50) + theme(strip.background.y = element_blank(),
    strip.text.y = element_blank(), axis.title.x = element_blank(),
    axis.text.x = element_blank(), axis.ticks.x = element_blank(),
    panel.spacing = unit(0, "lines")) + scale_fill_gradient2(low = "blue",
    high = "red", mid = "white", midpoint = 0,
    limits = c(temp_lower_bound, temp_upper_bound),
    breaks = c(temp_lower_bound, 0, temp_upper_bound),
    labels = c(as.character(temp_lower_bound),
    "0", as.character(temp_upper_bound)))

```

January Day 3 Selection Coefficients



Genetic Interactions Visualizations

```
geneticInteractions <- add_number(geneticInteractions)
geneticInteractions$genotype <- reorder(geneticInteractions$genotype,
  desc(geneticInteractions$number))
```

```
temp_plot <- geneticInteractions %>% filter(month ==
  "Jan", measurement == "phi2")
ggplot(data = cell_371_phi2_jan, aes(x = time_point,
  y = genotype, fill = log2_fold)) + labs(fill = "Log 2 Fold Change",
  x = "Hours", y = NULL, title = "January: Phi2 Log 2 Fold Change") +
```

```

geom_tile(width = 10, height = 10) +
facet_grid(genotype ~ day, scales = "free",
  switch = "y") + theme_tufte(base_family = "Calibri",
base_size = 50) + theme(strip.background.y = element_blank(),
strip.text.y = element_blank(), axis.title.x = element_blank(),
axis.text.x = element_blank(), axis.ticks.x = element_blank(),
panel.spacing = unit(0, "lines")) + scale_fill_gradient2(low = "blue",
high = "red", mid = "white", midpoint = 0,
limits = c(, 0.8), breaks = c(-0.7, 0,
0.8), labels = c("-0.7", "0", "0.8"))

```