FQ2

2025-11-07

```
library(lme4)
library(tibble)
library(dplyr)
library(tidyverse)
library(ggthemes)
library(ggrepel)
library(lfmm)
library(RSpectra)
load("pl_lt_t.lmer.RData")
load("growth_light_time.lmer.RData")
source("manhattan_plot.R")
```

manhattan plot for 2022

```
pheno_22 <- ranef(pl_lt_t.lmer)$pop %>%
  as_tibble(rownames = "pop",.name_repair = "unique")%>%
  rename(blup_intercept = `(Intercept)...1`,
         blup_light = `mean_light_ly_day2`) %>%
  mutate(model = "Y2022")
```

```
## New names:
## * `(Intercept)` -> `(Intercept)...1`
## * `(Intercept)` -> `(Intercept)...3`
```

```
geno_22 <- read.delim("Data/merged_maf_common_UCD2022.tsv", header = TRUE)

Y <- as.matrix(geno_22[,-1])
Y <- t(Y)
X <- matrix(scale(pheno_22$blup_light), ncol = 1)
rownames(X) <- pheno_22$pop
colnames(X) <- "blup_light"
common <- intersect(rownames(X), rownames(Y))
Y <- Y[common,]
X <- X[common, , drop = FALSE]
mod.lfmm <- lfmm_ridge(Y = Y,
                       X = X,
                       K = 2)

 pv <- lfmm_test(Y = Y,
                 X = X,
                 lfmm = mod.lfmm,
                 calibrate = "gif")
pvalues <- pv$calibrated.pvalue
```

```
plot_data = tibble(loci = geno_22[,1], p_val = pv$pvalue[,1], calibrated.pvalue = pv$calibrated.pvalue[
separate_wider_delim(loci, delim = ":", names = c("chr", "bp")) |>
filter(str_detect(chr, "Chr")) |>
mutate(p.bonf = p.adjust(p_val, method = "bonferroni"),
bp = as.numeric(bp))


manhattan_22 = plot_manhattan(plot_data,
                          chr_col = "chr",
                          pos_col = "bp",
                          value_col = "calibrated.pvalue",
                          transform = "neglog10",
                          title = "Light association",
                          highlight_top_n = 20)
```

import X and Y data in WL2_2023

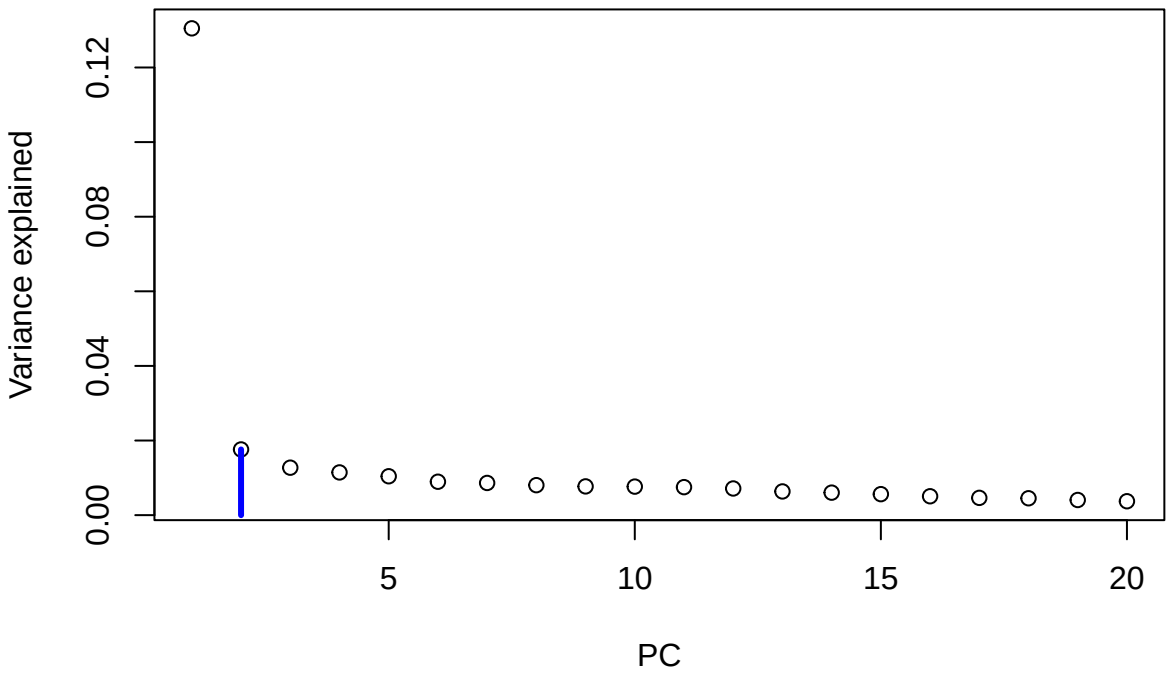
```
pheno_23 <- ranef(growth_light_time.lmer)$parent_pop %>%
  as_tibble(rownames = "parent_pop", .name_repair = "unique") %>%
  rename(blup_intercept = `(Intercept)...1`,
         blup_light = `weekly_avg_SlrW2`) %>%
  mutate(model = "Y2023")
```

```
## New names:
## * `(Intercept)` -> `(Intercept)...1`
## * `(Intercept)` -> `(Intercept)...3`
```

```
geno_23 <- read.delim("Data/merged_maf_common_WL2_2023.tsv", header = TRUE)
```

Perform PCA and find the number of latent factors

```
Y_23 <- as.matrix(geno_23[,-1])
pc_23 <- prcomp(Y_23)
plot(pc_23$sdev[1:20]^2, xlab = 'PC', ylab = "Variance explained")
points(2,pc_23$sdev[2]^2, type = "h", lwd = 3, col = "blue")
```



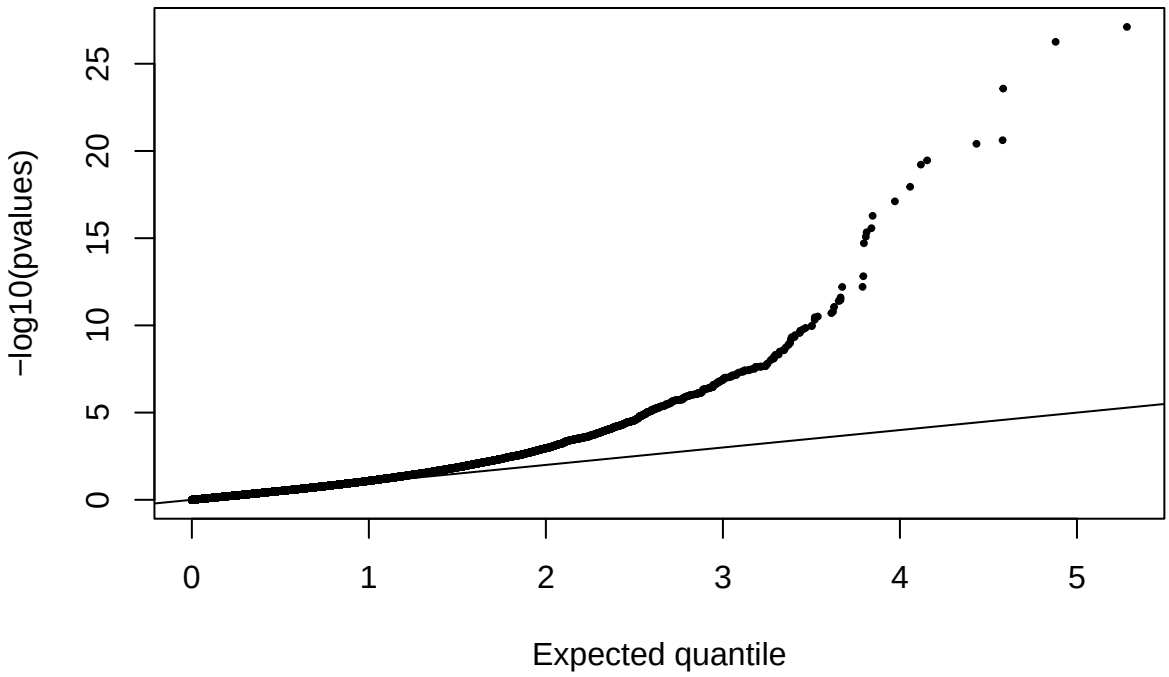
Fit the lfmm model and estimate the p value

```
#process the data
Y_23 <- as.matrix(geno_23[,-1])
Y_23 <- t(Y_23)
X_23 <- matrix(scale(pheno_23$blup_light), ncol = 1)
rownames(X_23) <- pheno_23$parent_pop
colnames(X_23) <- "blup_light"
common <- intersect(rownames(X_23), rownames(Y_23))
Y_23 <- Y_23[common,]
X_23 <- X_23[common, , drop = FALSE]

#fit the model
mod.lfmm_23 <- lfmm_ridge(Y = Y_23,
                          X = X_23,
                          K = 2)
pv_23 <- lfmm_test(Y = Y_23,
                   X = X_23,
                   lfmm = mod.lfmm_23,
                   calibrate = "gif")
pvalues_23 <- pv_23$calibrated.pvalue

#QQ plot
qqplot(rexp(length(pvalues), rate = log(10)),
       -log10(pvalues), xlab = "Expected quantile",
       pch = 19, cex = .4)
```

```
abline(0,1)
```

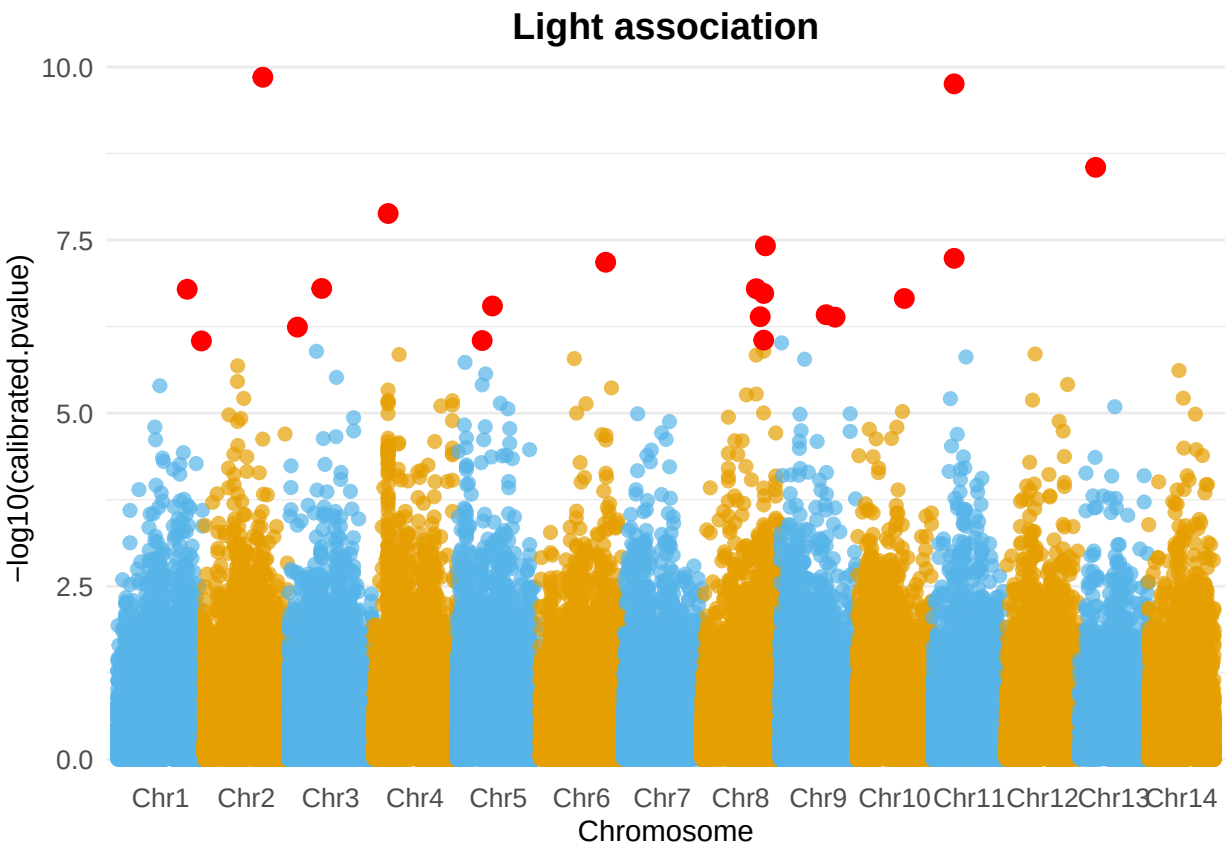


Create a data frame for plotting the manhattan plot.

```
plot_data_23 = tibble(loci = geno_23[,1], p_val = pv_23$pvalue[,1], calibrated.pvalue = pv_23$calibrate
separate_wider_delim(loci, delim = ":", names = c("chr", "bp")) |>
filter(str_detect(chr, "Chr")) |>
mutate(p.bonf = p.adjust(p_val, method = "bonferroni"),
bp = as.numeric(bp))


manhattan_23 = plot_manhattan(plot_data_23,
                          chr_col = "chr",
                          pos_col = "bp",
                          value_col = "calibrated.pvalue",
                          transform = "neglog10",
                          title = "Light association",
                          highlight_top_n = 20)

print(manhattan_23)
```




```
# Save plot
ggsave("light_interaction23.png", manhattan_23, width = 12, height = 6, dpi = 300)
```

Manhattan plot side by side

```
library(cowplot)
```

```
##
## Attaching package: 'cowplot'

## The following object is masked from 'package:ggthemes':
##
##     theme_map

## The following object is masked from 'package:lubridate':
##
##     stamp
```

```
manhattan_tot <- plot_grid(manhattan_22, manhattan_23, ncol = 2, labels = c("2022", "2023"), rel_widths
```

list out top 20 SNPs for both the gardens

```
top20_UCD = plot_data |>
slice_min(calibrated.pvalue, n = 20) |>
select(seqid = chr, bp, p_val, calibrated.pvalue, p.bonf)
write_tsv(top20_UCD, "top20_UCD_loc.tsv")

top20_WL2 = plot_data_23 |>
slice_min(calibrated.pvalue, n = 20) |>
select(seqid = chr, bp, p_val, calibrated.pvalue, p.bonf)
write_tsv(top20_WL2, "top20_WL2_loc.tsv")
```

```
geno_table_23 = Y_23
colnames(geno_table_23) = geno_23[, 1]

freq_plot_data_23 = as.data.frame(X_23) |>
rownames_to_column(var = "Population") |>
mutate(loci1 = as.numeric(geno_table_23[, "Chr2:18026903"]),
loci2 = as.numeric(geno_table_23[, "Chr11:6546598"]),
loci3 = as.numeric(geno_table_23[, "Chr13:4965604"])) |>
pivot_longer(cols = loci1:loci3, names_to = "loci", values_to = "frequency")

freq_plot_data_23 |>
ggplot(aes(x = blup_light, y = frequency, label = Population)) +
geom_point(size = 3) +
geom_smooth(method = "lm", colour = "red") +
geom_text_repel(size = 6) +
facet_wrap(~loci, nrow = 1, scales = "free_y") +
theme_minimal()+
labs(x = "BLUP light", y = "Allele frequency")
```

```
## `geom_smooth()` using formula = 'y ~ x'

## Warning: The following aesthetics were dropped during statistical transformation: label.
## i This can happen when ggplot fails to infer the correct grouping structure in the data.
## i Did you forget to specify a `group` aesthetic or to convert a numerical variable into a factor?
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## The following aesthetics were dropped during statistical transformation: label.
## i This can happen when ggplot fails to infer the correct grouping structure in the data.
## i Did you forget to specify a `group` aesthetic or to convert a numerical variable into a factor?

## Warning: ggrepel: 9 unlabeled data points (too many overlaps). Consider increasing max.overlaps

## Warning: ggrepel: 6 unlabeled data points (too many overlaps). Consider increasing max.overlaps

## Warning: ggrepel: 5 unlabeled data points (too many overlaps). Consider increasing max.overlaps
```

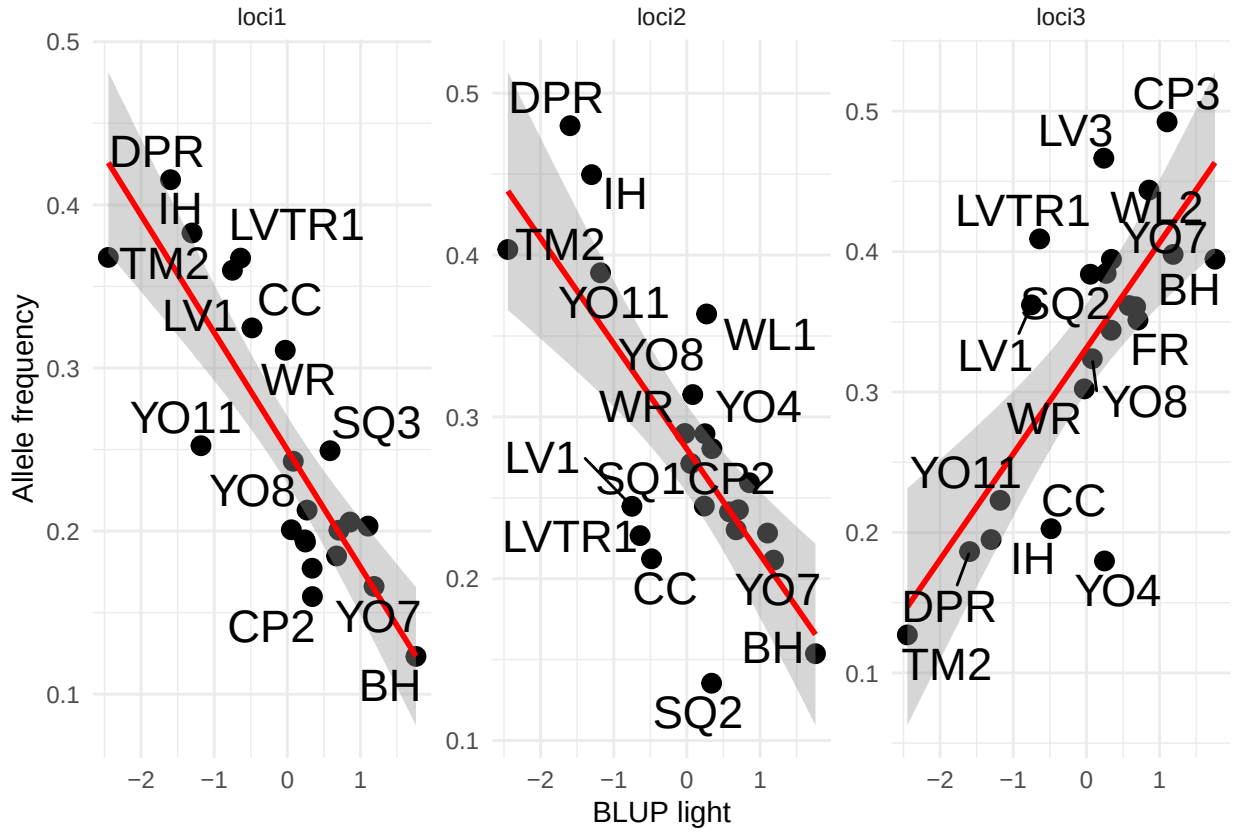


```
genes = read_tsv("Data/gene_description.tsv")
```

```
## Rows: 34643 Columns: 6
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (4): seqid, strand, gene_id, description
## dbl (2): start, end
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
window_size = 10000
```

```
genes_near_snps_UCD = top20_UCD |>
  left_join(genes, by = "seqid", relationship = "many-to-many") |>
  mutate(
    # Calculate distances
    dist_to_start = bp - start,
    dist_to_end = bp - end,

    # Check if SNP is within gene body
    in_gene = bp >= start & bp <= end,

    # Check if SNP is within window (upstream or downstream)
    in_window = (bp >= (start - window_size) & bp <= (end + window_size))
```

```
  ) |>
  filter(in_window) |>
  mutate(
    # Categorize position
    position = case_when(
      in_gene ~ "in_gene",
      bp < start ~ "upstream",
      bp > end ~ "downstream"
    ),
    distance = case_when(
      in_gene ~ 0,
      bp < start ~ start - bp,
      bp > end ~ bp - end
    )
  ) |>
  arrange(seqid, bp, distance)

write_tsv(genes_near_snps_UCD, "UCD_2022_genes.tsv")
genes_near_snps_UCD
```

```
genes_near_snps_WL2 = top20_WL2 |>
  left_join(genes, by = "seqid", relationship = "many-to-many") |>
  mutate(
    # Calculate distances
    dist_to_start = bp - start,
    dist_to_end = bp - end,

    # Check if SNP is within gene body
    in_gene = bp >= start & bp <= end,

    # Check if SNP is within window (upstream or downstream)
    in_window = (bp >= (start - window_size) & bp <= (end + window_size))
  ) |>
  filter(in_window) |>
  mutate(
    # Categorize position
    position = case_when(
      in_gene ~ "in_gene",
      bp < start ~ "upstream",
      bp > end ~ "downstream"
    ),
    distance = case_when(
      in_gene ~ 0,
      bp < start ~ start - bp,
      bp > end ~ bp - end
    )
  ) |>
  arrange(seqid, bp, distance)

write_tsv(genes_near_snps_WL2, "WL2_2023_genes.tsv")
genes_near_snps_WL2
```