

# TRhizo-localAdaptation

## Cockerham's Test (Global Alternate)

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## Load Packages & Data

```
## Load the tidyverse
library(tidyverse)

## Packages for analysis
library(broom)
library(lme4)
library(lmerTest)

## Read in data
biomass.data <- read_rds(file = "data/cleaned_biomass_data.rds")
nodule.data <- read_rds(file = "data/cleaned_nodule_data.rds")

## Recode microbiome levels
biomass.data <- biomass.data %>%
  mutate(Microbiome = if_else(biomass.data$Microbiome == "Local", "Local", "Nonlocal"))
nodule.data <- nodule.data %>%
  mutate(Microbiome = if_else(nodule.data$Microbiome == "Local", "Local", "Nonlocal"))
```

# Quantify Genetic Variation

## Data Management

```
## Aboveground biomass data
# Local
aboveground.biomass.local.data <- biomass.data %>%
  select(Population, Microbiome, Block, Aboveground_Biomass) %>%
  filter(Microbiome == "Local")
# Nonlocal
aboveground.biomass.nonlocal.data <- biomass.data %>%
  select(Population, Microbiome, Block, Aboveground_Biomass) %>%
  filter(Microbiome != "Local")

## Nodule density
# Local / Ambient N
nodule.density.local.ambient.N.data <- nodule.data %>%
  filter(Nitrogen == "Ambient_N") %>%
  select(Population, Microbiome, Block, Nodule_Density) %>%
  filter(Microbiome == "Local")
# Nonlocal / Ambient N
nodule.density.nonlocal.ambient.N.data <- nodule.data %>%
  filter(Nitrogen == "Ambient_N") %>%
  select(Population, Microbiome, Block, Nodule_Density) %>%
  filter(Microbiome != "Local")

# Local / N Addition
nodule.density.local.N.addition.data <- nodule.data %>%
  filter(Nitrogen == "N_Addition") %>%
  select(Population, Microbiome, Block, Nodule_Density) %>%
  filter(Microbiome == "Local")
# Nonlocal / N Addition
nodule.density.nonlocal.N.addition.data <- nodule.data %>%
  filter(Nitrogen == "N_Addition") %>%
  select(Population, Microbiome, Block, Nodule_Density) %>%
  filter(Microbiome != "Local")

## Fixing nodule density data
# Local
fixing.nodule.density.local.data <- nodule.data %>%
  select(Population, Microbiome, Block, Fixing_Nodule_Density) %>%
  filter(Microbiome == "Local")
# Nonlocal
fixing.nodule.density.nonlocal.data <- nodule.data %>%
  select(Population, Microbiome, Block, Fixing_Nodule_Density) %>%
  filter(Microbiome != "Local")
```

## Aboveground Biomass

```
## Aboveground biomass
# Local
aboveground.biomass.local.Vg.LMM <- lmer(
  sqrt(Aboveground_Biomass) ~ Block + (1 | Population),
  data = aboveground.biomass.local.data,
  REML = TRUE
)

aboveground.biomass.local.Vg.df <- data.frame(VarCorr(aboveground.biomass.local.Vg.LMM)) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Local") %>%
  add_column(Nitrogen = "Combined")

# Nonlocal
aboveground.biomass.nonlocal.Vg.LMM <- lmer(
  sqrt(Aboveground_Biomass) ~ Block + (1 | Population),
  data = aboveground.biomass.nonlocal.data,
  REML = TRUE
)

aboveground.biomass.nonlocal.Vg.df <- data.frame(VarCorr(aboveground.biomass.nonlocal.Vg.LMM)) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Nonlocal") %>%
  add_column(Nitrogen = "Combined")
```

## Nodule Density

```
## Nodule density
# Ambient N / Local
nodule.density.local.ambient.N.Vg.LMM <- lmer(
  log(Nodule_Density + 1) ~ Block + (1 | Population),
  data = nodule.density.local.ambient.N.data,
  REML = TRUE
)

nodule.density.local.ambient.N.Vg.df <- data.frame(
  VarCorr(nodule.density.local.ambient.N.Vg.LMM)
) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Local") %>%
  add_column(Nitrogen = "Ambient_N")

# Ambient N / Nonlocal
nodule.density.nonlocal.ambient.N.Vg.LMM <- lmer(
  log(Nodule_Density + 1) ~ Block + (1 | Population),
  data = nodule.density.nonlocal.ambient.N.data,
  REML = TRUE
)

nodule.density.nonlocal.ambient.N.Vg.df <- data.frame(
  VarCorr(nodule.density.nonlocal.ambient.N.Vg.LMM)
) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Nonlocal") %>%
  add_column(Nitrogen = "Ambient_N")

# N Addition / Local
nodule.density.local.N.addition.Vg.LMM <- lmer(
  log(Nodule_Density + 1) ~ Block + (1 | Population),
  data = nodule.density.local.N.addition.data,
  REML = TRUE
)

nodule.density.local.N.addition.Vg.df <- data.frame(
  VarCorr(nodule.density.local.N.addition.Vg.LMM)
) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Local") %>%
  add_column(Nitrogen = "N_Addition")

# N Addition / Nonlocal
nodule.density.nonlocal.N.addition.Vg.LMM <- lmer(
  log(Nodule_Density + 1) ~ Block + (1 | Population),
  data = nodule.density.nonlocal.N.addition.data,
  REML = TRUE
)

nodule.density.nonlocal.N.addition.Vg.df <- data.frame(
```

```
VarCorr(nodule.density.nonlocal.N.addition.Vg.LMM)
) %>%
filter(grp == "Population") %>%
add_column(Microbiome = "Nonlocal") %>%
add_column(Nitrogen = "N_Addition")
```

## Fixing Nodule Density

```
## Fixing nodule density
# Local
fixing.nodule.density.local.Vg.LMM <- lmer(
  log(Fixing_Nodule_Density + 1) ~ Block + (1 | Population),
  data = fixing.nodule.density.local.data,
  REML = TRUE
)

fixing.nodule.density.local.Vg.df <- data.frame(VarCorr(fixing.nodule.density.local.Vg.LMM)) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Local") %>%
  add_column(Nitrogen = "Combined")

# Nonlocal
fixing.nodule.density.nonlocal.Vg.LMM <- lmer(
  log(Fixing_Nodule_Density + 1) ~ Block + (1 | Population),
  data = fixing.nodule.density.nonlocal.data,
  REML = TRUE
)

fixing.nodule.density.nonlocal.Vg.df <- data.frame(VarCorr(fixing.nodule.density.nonlocal.Vg.LMM)) %>%
  filter(grp == "Population") %>%
  add_column(Microbiome = "Nonlocal") %>%
  add_column(Nitrogen = "Combined")
```

## Export Genetic Variation Data

```
## Combine and export data for management in Excel in prep for Cockerham's test
# Bind aboveground biomass data
aboveground.biomass.combined.Vg.data <- bind_rows(
  aboveground.biomass.local.Vg.df,
  aboveground.biomass.nonlocal.Vg.df
)

# Bind nodule density data
nodule.density.combined.Vg.data <- bind_rows(
  nodule.density.local.ambient.N.Vg.df,
  nodule.density.nonlocal.ambient.N.Vg.df,
  nodule.density.local.N.addition.Vg.df,
  nodule.density.nonlocal.N.addition.Vg.df
)

# Bind fixing nodule density data
fixing.nodule.density.combined.Vg.data <- bind_rows(
  fixing.nodule.density.local.Vg.df,
  fixing.nodule.density.nonlocal.Vg.df
)

## Bind all data together
combined.Vg.data <- bind_rows(
  aboveground.biomass.combined.Vg.data,
  nodule.density.combined.Vg.data,
  fixing.nodule.density.combined.Vg.data
) %>%
  add_column(Trait = c(
    rep("Aboveground_Biomass", 2),
    rep("Nodule_Density", 4),
    rep("Fixing_Nodule_Density", 2)
  )) %>%
  select(Trait, Microbiome:Nitrogen, vcov) %>%
  rename(Vg = vcov)

## Export data to prepare for Cockerham's test
write_csv(combined.Vg.data, file = "data/cockerham_data_global_uncleaned.csv")
```



# Genetic Correlations

## Data Management

```
## Format data to calculate correlations
full.data <- nodule.data %>%
  full_join(
    biomass.data %>% select(UID, Aboveground_Biomass),
    by = c("UID")
  ) %>%
  select(
    Population:Nitrogen, Aboveground_Biomass, Nodule_Density, Fixing_Nodule_Density
  ) %>%
  drop_na() %>%
  select(
    Population, Microbiome, Nitrogen,
    Aboveground_Biomass:Fixing_Nodule_Density
  )

## Local by Nonlocal data (aboveground biomass and fixing nodule density)
local.by.nonlocal.data <- full.data %>%
  group_by(Population, Microbiome) %>%
  select(-Nodule_Density) %>%
  summarise(
    AG_Biomass = mean(Aboveground_Biomass),
    Fix_Nod_Density = mean(Fixing_Nodule_Density),
    .groups = "keep"
  ) %>%
  ungroup() %>%
  drop_na() %>%
  pivot_wider(names_from = Microbiome, values_from = AG_Biomass:Fix_Nod_Density)

## Nodule density data (N-treatment specific)
# Local by Nonlocal data
nodule.density.local.by.nonlocal.data <- full.data %>%
  group_by(Population, Microbiome, Nitrogen) %>%
  summarise(
    Nod_Density = mean(Nodule_Density),
    .groups = "keep"
  ) %>%
  ungroup() %>%
  pivot_wider(names_from = Microbiome:Nitrogen, values_from = Nod_Density) %>%
  drop_na()
```

## Correlations

### Aboveground Biomass & Fixing Nodule Density

Table 1: Fitness correlations between local and nonlocal-nonlocal for aboveground biomass and fixing nodule density.

	AG_Biomass_Local	AG_Biomass_Nonlocal	Fix_Nod_Density_Local	Fix_Nod_Density_Nonlocal
AG_Biomass_Local	1.000	0.604	0.135	0.282
AG_Biomass_Nonlocal	0.604	1.000	0.207	0.280
Fix_Nod_Density_Local	0.135	0.207	1.000	0.359
Fix_Nod_Density_Nonlocal	0.282	0.280	0.359	1.000

## Nodule Density

Table 2: Fitness correlations between local and nonlocal-nonlocal by nitrogen treatment for nodule density.

	Local_Ambient_N	Local_N_Addition	Nonlocal_Ambient_N	Nonlocal_N_Addition
Local_Ambient_N	1.000	0.127	0.184	0.006
Local_N_Addition	0.127	1.000	0.512	0.373
Nonlocal_Ambient_N	0.184	0.512	1.000	0.276
Nonlocal_N_Addition	0.006	0.373	0.276	1.000

## Cockerham's Test

```
## Cockerham data
cockerham.global.data <- read_csv(
  "data/cockerham_data_global_cleaned.csv",
  col_types = c("ffnnn"),
  show_col_types = FALSE
)
```

## Set Functions

```
## Function for imperfect correlation (page 88, Cockerham 1963)
imperfect_correlation <- function(Vg1, Vg2, Rg) {
  out <- 2 * sqrt(Vg1) * sqrt(Vg2) * (1 - Rg)

  return(out)
}

## Function for heterogeneous variances (page 88, Cockerham 1963)
heterogeneous_variances <- function(Vg1, Vg2) {
  out <- ((sqrt(Vg1) - sqrt(Vg2))^2)

  return(out)
}

## Calculate imperfect correlation values for each row
cockerham.global.data$Crossing <- sapply(1:nrow(cockerham.global.data), FUN = function(r) {
  Vg1 <- cockerham.global.data$Vg1[r]
  Vg2 <- cockerham.global.data$Vg2[r]
  Rg <- cockerham.global.data$Rg[r]

  crossing.value <- imperfect_correlation(Vg1, Vg2, Rg)

  return(crossing.value)
})

## Calculate heterogeneous variance values for each row
cockerham.global.data$Heterogeneous_Variations <- sapply(1:nrow(cockerham.global.data), FUN = function(r) {
  Vg1 <- cockerham.global.data$Vg1[r]
  Vg2 <- cockerham.global.data$Vg2[r]

  heterogeneous.variance.value <- heterogeneous_variances(Vg1, Vg2)

  return(heterogeneous.variance.value)
})
```

## Calculations for Cockerham's Test

### Aboveground Biomass

```
## Group by trait and calculate sum of imperfect correlation values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
aboveground.biomass.crossing <- cockerham.global.data %>%
  filter(Trait == "Aboveground_Biomass") %>%
  summarise(Crossing_Sum = sum(Crossing) / (2 * (2 - 1)))

## Group by trait and calculate sum of heterogeneous variances values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
aboveground.biomass.heterogeneous.variance <- cockerham.global.data %>%
  filter(Trait == "Aboveground_Biomass") %>%
  summarise(Hetero_Variance_Sum = sum(Heterogeneous_Variations) / (2 * (2 - 1)))

## Make tibble for aboveground biomass calculation
aboveground.biomass.cockerham.global.data <- tibble(
  Trait = "Aboveground_Biomass",
  aboveground.biomass.crossing,
  aboveground.biomass.heterogeneous.variance
) %>%
  mutate(Total_Variance = Crossing_Sum + Hetero_Variance_Sum) %>%
  mutate(Percent_Crossing = 100 * Crossing_Sum / Total_Variance)
```

## Nodule Density

```
## Group by trait and calculate sum of imperfect correlation values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
nodule.density.crossing <- cockerham.global.data %>%
  filter(Trait == "Nodule_Density") %>%
  summarise(Crossing_Sum = sum(Crossing) / (4 * (4 - 1)))

## Group by trait and calculate sum of heterogeneous variances values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
nodule.density.heterogeneous.variance <- cockerham.global.data %>%
  filter(Trait == "Nodule_Density") %>%
  summarise(Hetero_Variance_Sum = sum(Heterogeneous_Variances) / (4 * (4 - 1)))

## Make tibble for Nodule Density calculation
nodule.density.cockerham.global.data <- tibble(
  Trait = "Nodule_Density",
  nodule.density.crossing,
  nodule.density.heterogeneous.variance
) %>%
  mutate(Total_Variance = Crossing_Sum + Hetero_Variance_Sum) %>%
  mutate(Percent_Crossing = 100 * Crossing_Sum / Total_Variance)
```

## Fixing Nodule Density

```
## Group by trait and calculate sum of imperfect correlation values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
fixing.nodule.density.crossing <- cockerham.global.data %>%
  filter(Trait == "Fixing_Nodule_Density") %>%
  summarise(Crossing_Sum = sum(Crossing) / (2 * (2 - 1)))

## Group by trait and calculate sum of heterogeneous variances values
# Divide by number of environments*(number of environments - 1) as per Cockerham 1963
fixing.nodule.density.heterogeneous.variance <- cockerham.global.data %>%
  filter(Trait == "Fixing_Nodule_Density") %>%
  summarise(Hetero_Variance_Sum = sum(Heterogeneous_Variations) / (2 * (2 - 1)))

## Make tibble for Nodule Density calculation
fixing.nodule.density.cockerham.global.data <- tibble(
  Trait = "Fixing_Nodule_Density",
  fixing.nodule.density.crossing,
  fixing.nodule.density.heterogeneous.variance
) %>%
  mutate(Total_Variance = Crossing_Sum + Hetero_Variance_Sum) %>%
  mutate(Percent_Crossing = 100 * Crossing_Sum / Total_Variance)
```

## Results of Cockerham's Tests

Table 3: Results of Cockerham's test for aboveground biomass, nodule density, and fixing nodule density.

Trait	Crossing_Sum	Hetero_Variance_Sum	Total_Variance	Percent_Crossing
Aboveground_Biomass	0.00162	4e-05	0.00166	97.71120
Nodule_Density	0.00111	4e-05	0.00115	96.61462
Fixing_Nodule_Density	0.00106	6e-05	0.00112	95.01701



## R Session Information

Table 4: Packages required for data management and analysis.

Package	Loaded Version	Date
broom	1.0.5	2023-06-09
dplyr	1.1.2	2023-04-20
forcats	1.0.0	2023-01-29
ggplot2	3.4.2	2023-04-03
kableExtra	1.3.4	2021-02-20
knitr	1.43	2023-05-25
lme4	1.1-34	2023-07-04
lmerTest	3.1-3	2020-10-23
lubridate	1.9.2	2023-02-10
Matrix	1.6-0	2023-07-08
purrr	1.0.1	2023-01-10
readr	2.1.4	2023-02-10
stringr	1.5.0	2022-12-02
tibble	3.2.1	2023-03-20
tidyr	1.3.0	2023-01-24
tidyverse	2.0.0	2023-02-22