Color Analysis of Floral Tissues

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Introduction

This analysis explores the color properties of different floral tissues — bract, petal, and labellum — using spectral data sourced from Google Sheets. The analysis includes data loading, quality control, spectral processing, visualization, and the computation of summary descriptors.

Setup

Load Required Libraries

```
## libraries
library(googlesheets4)
library(cowplot)
library(dplyr)
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##
       filter, lag
## The following objects are masked from 'package:base':
##
       intersect, setdiff, setequal, union
##
library(tibble)
library(knitr)
library(rmarkdown)
library(pavo)
```

Set working directory

```
# Set the root directory to the project root
knitr::opts_knit$set(root.dir = normalizePath("../"))
```

Define Custom Operators

```
# Define a 'not in' operator
`%notin%` = Negate(`%in%`)
```

Authenticate Google Sheets Access

Authenticate access to Google Sheets using your email. Ensure that the email has the necessary permissions to access the sheets.

```
# Authenticate with Google Sheets
gs4_auth(email = "kuckele@ucsc.edu")
```

Quality Control

Load Spectral Data from Google Sheets

We load the spectral data for bract, petal, and labellum from the specified Google Sheets.

```
# Specify the Google Sheets ID
sheet_id <- "12NWtlqKbLPAxU-rVBTqMQjY_6jH1DulVvNHS_QXAvFI"

# Load data for different floral tissues
bract <- read_sheet(ss = sheet_id, sheet = "Bract")

## v Reading from "Color".

## v Range ''Bract''.

petal <- read_sheet(ss = sheet_id, sheet = "Petal")

## v Reading from "Color".

## v Range ''Petal''.

labellum <- read_sheet(ss = sheet_id, sheet = "Labellum")

## v Reading from "Color".

## v Range ''Labellum''.</pre>
```

Spectral Data Processing

Convert Data to rspec Objects

The spectral data is converted into rspec objects using the pavo package for further analysis.

```
# Set seed for reproducibility
set.seed(1612217)
# Convert datasets to rspec objects with wavelength limits
bract\_spec \leftarrow as.rspec(bract, lim = c(300, 700), whichwl = 1)
## The spectral data contain 555 negative value(s),
## which may produce unexpected results if used in models.
## Consider using procspec() to correct them.
petal_spec \leftarrow as.rspec(petal, lim = c(300, 700), whichwl = 1)
## The spectral data contain 272 negative value(s),
## which may produce unexpected results if used in models.
## Consider using procspec() to correct them.
labellum_spec <- as.rspec(labellum, lim = c(300, 700), whichwl = 1)</pre>
# change column names
colnames(bract_spec) <- gsub("-", "x", colnames(bract_spec))</pre>
colnames(petal_spec) <- gsub("-", "x", colnames(petal_spec))</pre>
colnames(labellum_spec) <- gsub("-", "x", colnames(labellum_spec))</pre>
```

Note: The conversion may produce warnings about negative values in spectral data, which are addressed in subsequent steps.

Average the Spectra

Aggregate the spectral data by sample names, averaging replicates.

```
# Extract sample names by removing trailing numbers in parentheses
bract_samples \leftarrow gsub("\\([0-9]+\\)$", "", names(bract_spec))[-1]
petal_samples <- gsub("\\([0-9]+\\)$", "", names(petal_spec))[-1]</pre>
labellum_samples <- gsub("\\([0-9]+\\)$", "", names(labellum_spec))[-1]
# Verify sample counts
table(bract_samples)
## bract_samples
##
      125
                      39
                           39x10 39x109 39x110 39x115 39x116 39x117
                                                                        39x12 39x122
##
                                                                            2
                       1
                               2
                                       1
                                              2
                                                      2
                                                              1
                                                                     3
##
  39x123 39x125 39x126
                           39x13 39x130 39x136
                                                 39x14
                                                         39x15
                                                                 39x16
                                                                        39x17
                                                                                 39x2
                       2
                                              2
                                                      3
                                                             2
                                                                     4
##
        2
                2
                               1
                                       3
           39x23
                   39x25
                           39x27
                                  39x34
                                                                 39x41
                                                                                39x46
##
    39x21
                                          39x39
                                                  39x4
                                                         39x40
                                                                        39x44
                2
                       2
                               2
                                       2
                                              2
                                                      2
                                                             2
                                                                     2
                                                                            2
##
##
    39x49
           39x50
                   39x51
                           39x55
                                  39x56
                                          39x57
                                                  39x6
                                                         39x60
                                                                39x65
                                                                        39x67
                                                                                39x68
                       2
##
        2
                2
                               2
                                       2
                                              2
                                                      2
                                                             2
                                                                     2
                                                                            2
                                                                                    1
##
    39x75
           39x77
                   39x78
                           39x79
                                   39x8
                                          39x81
                                                 39x82
                                                         39x86
                                                                39x87
                                                                        39x89
                       2
                               2
                                              2
##
                2
                                       1
                                                      3
                                                             1
                                                                     1
    39x93
##
           39x95
                   39x96
                           39x98
                                  62x10 62x103 62x105 62x109 62x116 62x119 62x122
##
                2
                       2
                               2
                                       1
                                              2
                                                      2
                                                             1
  62x125 62x128 62x129
                          62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##
##
                       2
                               2
                                              2
                                                      1
                                                             2
        1
                1
                                      1
           62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##
  62x139
##
                       2
                               1
                                       2
                                              1
                                                      1
                                                             3
           62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175
##
  62x158
                                                                               62x18
                       2
                               2
                                              1
                                                      2
                                       1
## 62x182 62x185 62x188 62x189
                                  62x19 62x190 62x197 62x200 62x206
                                                                        62x21 62x214
                                       2
                                              2
                                                      2
                                                             2
##
                       1
                               1
                   62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
## 62x218 62x219
##
        2
                2
                       2
                               1
                                       2
                                              1
                                                      1
                                                             2
                                                                     2
                                                                            1
## 62x261 62x264 62x265 62x267 62x268 62x276 62x282 62x293 62x296 62x300 62x302
                2
                       1
                               2
                                       2
                                              1
                                                      1
                                                             2
                                                                     1
##
  62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##
                       1
                               1
                                       2
                                              1
                                                      2
                                                             1
                1
                                                                     1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370
                                                                                 62x5
##
                       2
                                       2
                                                      2
                                                             2
                                                                     2
        1
                1
                               1
                                              1
                                                                            1
##
    62x57
           62x58
                   62x59
                           62x60
                                  62x63
                                          62x65
                                                 62x66
                                                         62x69
                                                                62x73
                                                                        62x74
                                                                                62x75
##
        2
                2
                       2
                               1
                                       1
                                              1
                                                      1
                                                             2
                                                                     2
                                                                            1
##
    62x77
           62x83
                   62x87
                           62x91
                                  62x92
                                          62x94
                                                 62x96
                                                          BRAC
                               2
                                       2
##
                2
                       1
                                              1
                                                      1
table(petal_samples)
## petal_samples
##
      125
             126
                           39x10 39x109 39x110 39x115 39x116 39x117
                                                                        39x12 39x122
                      39
                2
                       1
                               2
                                      1
                                              2
                                                      2
                                                             1
                                                                     3
                                                                            2
  39x123 39x125 39x126
                           39x13 39x130 39x136
                                                 39x14
                                                         39x15
                                                                39x16
                                                                        39x17
                                                                                 39x2
##
##
        2
                2
                       2
                               1
                                       3
                                              2
                                                      3
                                                             2
                                                                     4
                                                                            2
                   39x25
                                                                                39x46
##
    39x21
           39x23
                           39x27
                                  39x34
                                          39x39
                                                  39x4
                                                         39x40
                                                                39x41
                                                                        39x44
##
        2
                2
                       2
                               2
                                       2
                                              2
                                                      2
                                                             2
                                                                     2
                                                                            2
                                                                                    2
                          39x55 39x56 39x57
    39x49
           39x50
                   39x51
                                                  39x6
                                                        39x60 39x65
                                                                        39x67
                                                                                39x68
```

2 2 2 2 2 2 39x75 39x77 39x78 39x79 39x8 39x81 39x82 39x86 39x87 39x89 39x92 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122 ## ## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138 2 1 ## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157 1 1 ## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18 ## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214 ## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258 ## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302 ## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322 ## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 ## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75 ## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC ##

table(labellum_samples)

labellum_samples ## ## .3 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46 ## 39x49 39x50 39x51 39x55 39x56 39x57 39x6 39x60 39x65 39x67 ## ## 39x75 39x77 39x78 39x79 ## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122 ## ## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138 ## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157 ## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18 ## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214 ## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258 ## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302 ## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322

```
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370
                                                                            62x5
##
                      2
                             2
                                    2
                                           1
                                                  2
                                                         2
                                                                2
                                                                               1
        1
              1
                                                                       1
##
   62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69
                                                            62x73 62x74 62x75
##
                      2
                                                  1
                                                         2
                                                                2
                                                                               1
                             1
                                    1
                                           1
                                                                       1
   62x77
##
          62x83
                  62x87
                         62x91
                                62x92
                                       62x94
                                              62x96
                                                      BRAC
##
                             2
                                    2
                      1
# Aggregate spectra by sample names using mean
bract_spec_avg <- aggspec(bract_spec, by = bract_samples, FUN = mean)</pre>
petal_spec_avg <- aggspec(petal_spec, by = petal_samples, FUN = mean)</pre>
labellum_spec_avg <- aggspec(labellum_spec, by = labellum_samples, FUN = mean)
```

Fix Negative Reflectance Values

Negative reflectance values are corrected by adding the minimum reflectance.

```
# Fix negative values by adding the minimum reflectance
bract_spec_avg <- procspec(bract_spec_avg, fixneg = "addmin")

## processing options applied:
## Negative value correction: added min to all reflectance

petal_spec_avg <- procspec(petal_spec_avg, fixneg = "addmin")

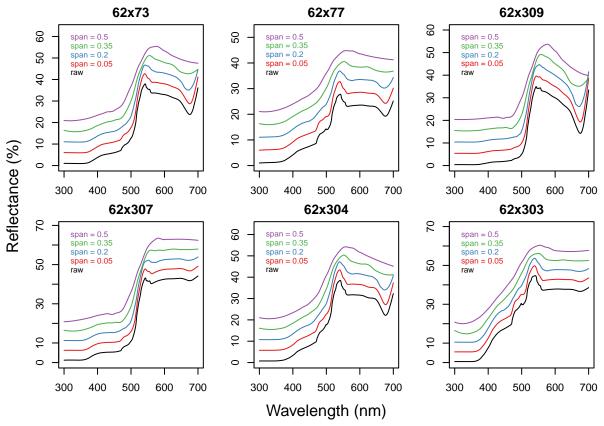
## processing options applied:
## Negative value correction: added min to all reflectance

labellum_spec_avg <- procspec(labellum_spec_avg, fixneg = "addmin")

## processing options applied:
## Negative value correction: added min to all reflectance</pre>
```

Determine Smoothing Parameter

Use plotsmooth to visualize and decide on an appropriate smoothing span.



Choose a span (e.g., 0.2) based on the plot to balance smoothness and data fidelity.

Smooth the Spectral Data

Apply smoothing to the spectral data using the chosen span.

```
# Apply smoothing with span = 0.2
bract_spec_sm <- procspec(bract_spec_avg, opt = "smooth", span = 0.2)

## processing options applied:
## smoothing spectra with a span of 0.2

petal_spec_sm <- procspec(petal_spec_avg, opt = "smooth", span = 0.2)

## processing options applied:
## smoothing spectra with a span of 0.2

labellum_spec_sm <- procspec(labellum_spec_avg, opt = "smooth", span = 0.2)

## processing options applied:
## smoothing spectra with a span of 0.2</pre>
```

Scale the Spectral Data

Scale the spectral data to different reference points for comparative analysis.

```
# Scale spectra to both minimum and maximum reflectance
bract_spec_scaleminmax <- procspec(bract_spec_sm, opt = c("min", "max"))
## processing options applied:
## Scaling spectra to a minimum value of zero</pre>
```

```
## Scaling spectra to a maximum value of 1

petal_spec_scaleminmax <- procspec(petal_spec_sm, opt = c("min", "max"))

## processing options applied:
## Scaling spectra to a minimum value of zero
## Scaling spectra to a maximum value of 1

labellum_spec_scaleminmax <- procspec(labellum_spec_sm, opt = c("min", "max"))

## processing options applied:
## Scaling spectra to a minimum value of zero
## Scaling spectra to a maximum value of 1</pre>
```

Plot Processed Spectra

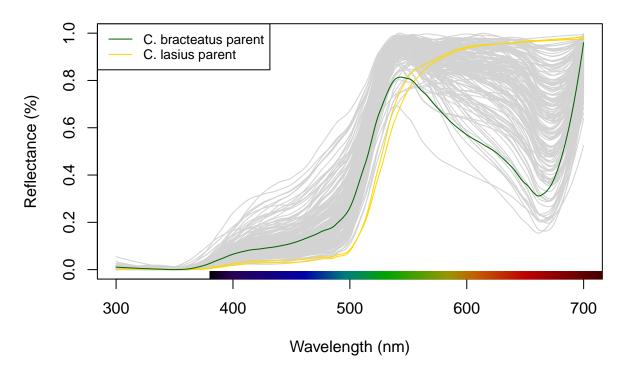
Visualize the processed spectral data for each floral tissue, highlighting specific samples.

Define Common Plotting Parameters

```
# Define a color palette for highlighting specific samples
highlight_colors <- c(rep("lightgrey", 170), "gold1", "gold1", "darkgreen")</pre>
```

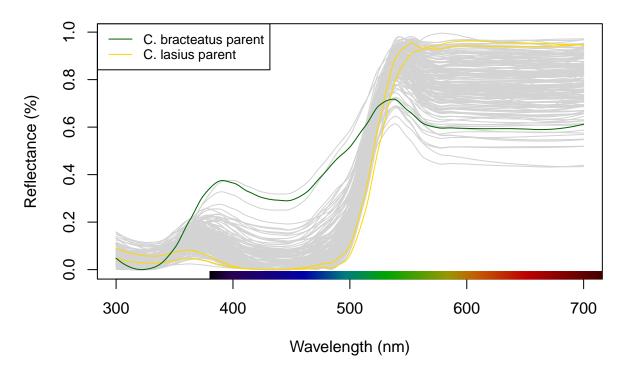
Bract Spectra Plot

Bract Spectra



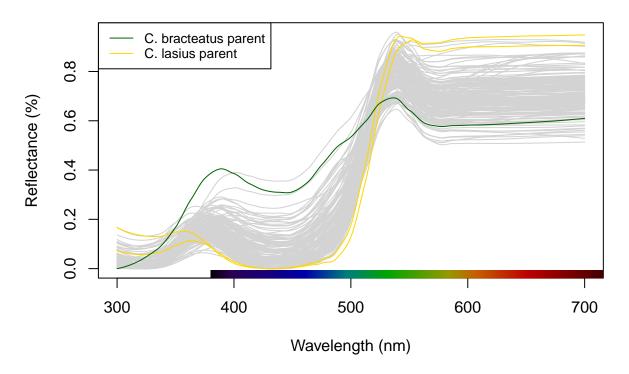
Petal Spectra Plot

Petal Spectra



Labellum Spectra Plot

Labellum Spectra



Combine and Save Spectral Plots with Highlighted Parents

##

```
# Save combined spectra plots with highlighted parental samples
pdf("./results/figures/Combined_Spectra_Bract_Petal_Labellum_Cbracteatus_Clasius_hybrids.pdf", width = .
par(mfrow = c(1, 3))
# Increase left margin to provide more space for y-axis labels
par(mar = c(5, 5, 4, 2) + 0.1) # c(bottom, left, top, right)
plot(bract_spec_scaleminmax[order_spec_bract], type = "o", col = highlight_colors,
     main = "Bract spectra", xlab = "Wavelength (nm)", ylab = "Reflectance (%)",
     cex.main = 3, cex.lab = 2.5, cex.axis = 2, lwd = 2)
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
       col = c("darkgreen", "gold1"), lty = 1, cex = 2.5, bty = "n")
plot(petal_spec_scaleminmax[order_spec_petal], type = "o", col = highlight_colors,
     main = "Petal spectra", xlab = "Wavelength (nm)", ylab = "Reflectance (%)",
     cex.main = 3, cex.lab = 2.5, cex.axis = 2, lwd = 2)
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
       col = c("darkgreen", "gold1"), lty = 1, cex = 2.5, bty = "n")
plot(labellum_spec_scaleminmax[order_spec_labellum], type = "o", col = highlight_colors,
     main = "Labellum spectra", xlab = "Wavelength (nm)", ylab = "Reflectance (%)",
     cex.main = 3, cex.lab = 2.5, cex.axis = 2, lwd = 2)
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
       col = c("darkgreen", "gold1"), lty = 1, cex = 2.5, bty = "n")
dev.off()
## pdf
```

Spectral Descriptors

We compute various spectral descriptors to quantify the color properties of the floral tissues.

Define spectral descriptors

B1: Total brightness B2: Mean brightness B3: Intensity (Rmax) S1U to S1R: Relative contributions of UV, Violet, Blue, Green, Yellow, and Red spectral ranges to total brightness S2: Spectral saturation (Rmax/Rmin) S3: Chroma S4: Spectral purity S5: Chroma S6: Contrast (Rmax - Rmin) S7: Spectral saturation S8: Chroma ((Rmax - Rmin)/B2) S9: Carotenoid chroma ((R700 - R450)/R700) S10: Peaky chroma H1 to H5: Hue metrics (e.g., peak wavelength) Note: Some metrics may be sensitive to spectral noise.

Calculate Spectral Descriptors

Bract

```
# Calculate spectral descriptors for bract
summary_bract <- summary(bract_spec_scaleminmax)

# Remove S2 due to infinite values
summary_bract <- summary_bract %>% select(-S2)

# Extract metrics for parents
bract125 <- round(summary_bract["125", ], 3)
bract126 <- round(summary_bract["126", ], 3)
bractBRAC <- round(summary_bract["BRAC", ], 3)</pre>
```

Plot Bract Spectral Descriptors Histograms

```
pdf("./results/figures/bract_descriptors_histograms.pdf", width = 14, height = 10)
# Set up plotting area: 4 rows x 6 columns for histograms
par(mfrow = c(4, 6),
                           # 4 rows, 6 columns
   mar = c(5, 5, 4, 2) + 0.1, # Margins for each plot: bottom, left, top, right
    oma = c(0, 0, 0, 5)) # Outer margins: bottom, left, top, right
# Loop through each metric and plot histogram with parental lines
for (i in 1:ncol(summary bract)) {
 hist(summary bract[, i],
       xlab = colnames(summary_bract)[i],
      main = colnames(summary_bract)[i],
      col = "lightgrey",
      border = "white")
  # Add vertical lines for parental samples
  abline(v = bract125[i], col = 'gold1', lwd = 3)
                                                    # C. lasius parent
 abline(v = bract126[i], col = 'gold1', lwd = 3) # C. lasius parent
  abline(v = bractBRAC[i], col = 'darkgreen', lwd = 3) # C. bracteatus parent
}
# Allow drawing in the outer margin
par(xpd = TRUE)
plot.new()
```

```
# Add a shared legend in the outer right margin
legend("topright",
      inset = c(0, 0), # Adjusts the position of the legend
      legend = c("C. lasius parent", "C. bracteatus parent"),
      col = c("gold1", "darkgreen"),
      lty = 1,
                            # Line type: solid
      lwd = 3,
                           # Line width
      cex = 1.5,
                           # Text size
      bty = "n")
                           # No box around the legend
# Close the PDF device to save the file
dev.off()
## pdf
##
```

Petal Summary

```
# Calculate summary statistics for petal
summary_petal <- summary(petal_spec_scaleminmax)

# Remove S2 due to infinite values
summary_petal <- summary_petal %>% select(-S2)

# Extract metrics for specific samples
petal125 <- round(summary_petal["125", ], 3)
petal126 <- round(summary_petal["126", ], 3)
petalBRAC <- round(summary_petal["BRAC", ], 3)</pre>
```

Plot Petal Spectral Descriptors Histograms

```
pdf("./results/figures/petal_descriptors_histograms.pdf", width = 14, height = 10)
# Set up plotting area: 4 rows x 6 columns for histograms
                       # 4 rows, 6 columns
par(mfrow = c(4, 6),
    mar = c(5, 5, 4, 2) + 0.1, # Margins for each plot: bottom, left, top, right
    oma = c(0, 0, 0, 5)) # Outer margins: bottom, left, top, right
# Loop through each metric and plot histogram with parental lines
for (i in 1:ncol(summary_petal)) {
  hist(summary_petal[, i],
       xlab = colnames(summary_petal)[i],
       main = colnames(summary_petal)[i],
       col = "lightgrey",
       border = "white")
  # Add vertical lines for parental samples
  abline(v = petal125[i], col = 'gold1', lwd = 3)  # C. lasius parent
abline(v = petal126[i], col = 'gold1', lwd = 3)  # C. lasius parent
  abline(v = petalBRAC[i], col = 'darkgreen', lwd = 3) # C. bracteatus parent
# Allow drawing in the outer margin
```

```
par(xpd = TRUE)
plot.new()
# Add a shared legend in the outer right margin
legend("topright",
      inset = c(0, 0), # Adjusts the position of the legend
      legend = c("C. lasius parent", "C. bracteatus parent"),
      col = c("gold1", "darkgreen"),
      lty = 1,
                            # Line type: solid
      lwd = 3,
                           # Line width
      cex = 1.5,
                           # Text size
      bty = "n")
                           # No box around the legend
# Close the PDF device to save the file
dev.off()
## pdf
##
```

Labellum Summary

```
# Calculate summary statistics for labellum
summary_labellum <- summary(labellum_spec_scaleminmax)

# Remove S2 due to infinite values
summary_labellum <- summary_labellum %>% select(-S2)

# Extract metrics for specific samples
labellum125 <- round(summary_labellum["125", ], 3)
labellum126 <- round(summary_labellum["126", ], 3)
labellumBRAC <- round(summary_labellum["BRAC", ], 3)</pre>
```

Plot Labellum Spectral Descriptors Histograms

```
pdf("./results/figures/labellum_descriptors_histograms.pdf", width = 14, height = 10)
# Set up plotting area: 4 rows x 6 columns for histograms
par(mfrow = c(4, 6),
                       # 4 rows, 6 columns
   mar = c(5, 5, 4, 2) + 0.1, # Margins for each plot: bottom, left, top, right
   oma = c(0, 0, 0, 5)) # Outer margins: bottom, left, top, right
# Loop through each metric and plot histogram with parental lines
for (i in 1:ncol(summary_labellum)) {
 hist(summary_labellum[, i],
      xlab = colnames(summary_labellum)[i],
      main = colnames(summary_labellum)[i],
      col = "lightgrey",
      border = "white")
 # Add vertical lines for parental samples
 abline(v = labellum125[i], col = 'gold1', lwd = 3) # C. lasius parent
 abline(v = labellum126[i], col = 'gold1', lwd = 3) # C. lasius parent
```

```
abline(v = labellumBRAC[i], col = 'darkgreen', lwd = 3) # C. bracteatus parent
}
# Allow drawing in the outer margin
par(xpd = TRUE)
plot.new()
# Add a shared legend in the outer right margin
legend("topright",
      inset = c(0, 0), # Adjusts the position of the legend
      legend = c("C. lasius parent", "C. bracteatus parent"),
       col = c("gold1", "darkgreen"),
      lty = 1,
                            # Line type: solid
      lwd = 3,
                            # Line width
       cex = 1.5,
                            # Text size
      bty = "n")
                            # No box around the legend
# Close the PDF device to save the file
dev.off()
## pdf
## 2
```

Data Formatting and Export

Reformat Rownames

Replace 'x' with ' ' in row names for consistency.

```
# Replace 'x' with '_' in row names
rownames(summary_bract) <- gsub("x", "_", rownames(summary_bract))
rownames(summary_petal) <- gsub("x", "_", rownames(summary_petal))
rownames(summary_labellum) <- gsub("x", "_", rownames(summary_labellum))</pre>
```

Subset and Rename Columns

Select relevant metrics and rename columns to indicate their corresponding floral tissue.

Convert Rownames to a Column

Add the row names as a new column id to facilitate merging.

```
# Convert row names to a column named 'id'
summary_bract <- summary_bract %>% rownames_to_column(var = "id")
summary_petal <- summary_petal %>% rownames_to_column(var = "id")
summary_labellum <- summary_labellum %>% rownames_to_column(var = "id")
```

Merge Summary Dataframes

Combine the summary dataframes for bract, petal, and labellum into a single dataframe.

```
# Merge bract and petal summaries by 'id'
joined_df <- summary_bract %>%
full_join(summary_petal, by = "id") %>%
full_join(summary_labellum, by = "id")
```

Export Summary Descriptors to CSV

Save the combined summary descriptors to a CSV file for further analysis or reporting.