

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(google sheets4)
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''.
```

Full Spectrum Analyses

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 <- 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 <- 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)

# change column names
colnames(bract_spec) <- gsub("-", "x", colnames(bract_spec))
colnames(petal_spec) <- gsub("-", "x", colnames(petal_spec))
colnames(labellum_spec) <- gsub("-", "x", colnames(labellum_spec))
```

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 <- gsub("\\([0-9]+\\)", "", names(bract_spec))[-1]
petal_samples <- gsub("\\([0-9]+\\)", "", names(petal_spec))[-1]
labellum_samples <- gsub("\\([0-9]+\\)", "", names(labellum_spec))[-1]

# Verify sample counts
table(bract_samples)

## bract_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##         2         3         1         2         1         2         2         1         3         2         2
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##         2         2         2         1         3         2         3         2         4         2         2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##         2         2         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 39x92
##         2         2         2         2         1         2         3         1         1         2         2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
```

```
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x264 62x265 62x267 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      1      2      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      1      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
table(petal_samples)
```

```
## petal_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##      2      2      1      2      1      2      2      1      3      2      2
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##      2      2      2      1      3      2      3      2      4      2      2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##      2      2      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 39x92
##      2      2      2      2      1      2      3      1      1      2      2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      2      1      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      1      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
```

```
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
table(labellum_samples)
```

```
## labellum_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##      2      2      1      2      1      2      2      1      3      2      2
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##      2      2      2      1      3      2      3      2      4      2      2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##      2      2      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 39x92
##      2      2      2      2      1      2      3      1      1      2      2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      2      1      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      2      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
# Aggregate spectra by sample names using mean
bract_spec_avg <- aggspec(bract_spec, by = bract_samples, FUN = mean)
petal_spec_avg <- aggspec(petal_spec, by = petal_samples, FUN = mean)
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 = "admin")
```

```
## processing options applied:
```

```
## Negative value correction: added min to all reflectance
```

```
petal_spec_avg <- procspec(petal_spec_avg, fixneg = "admin")
```

```
## processing options applied:
```

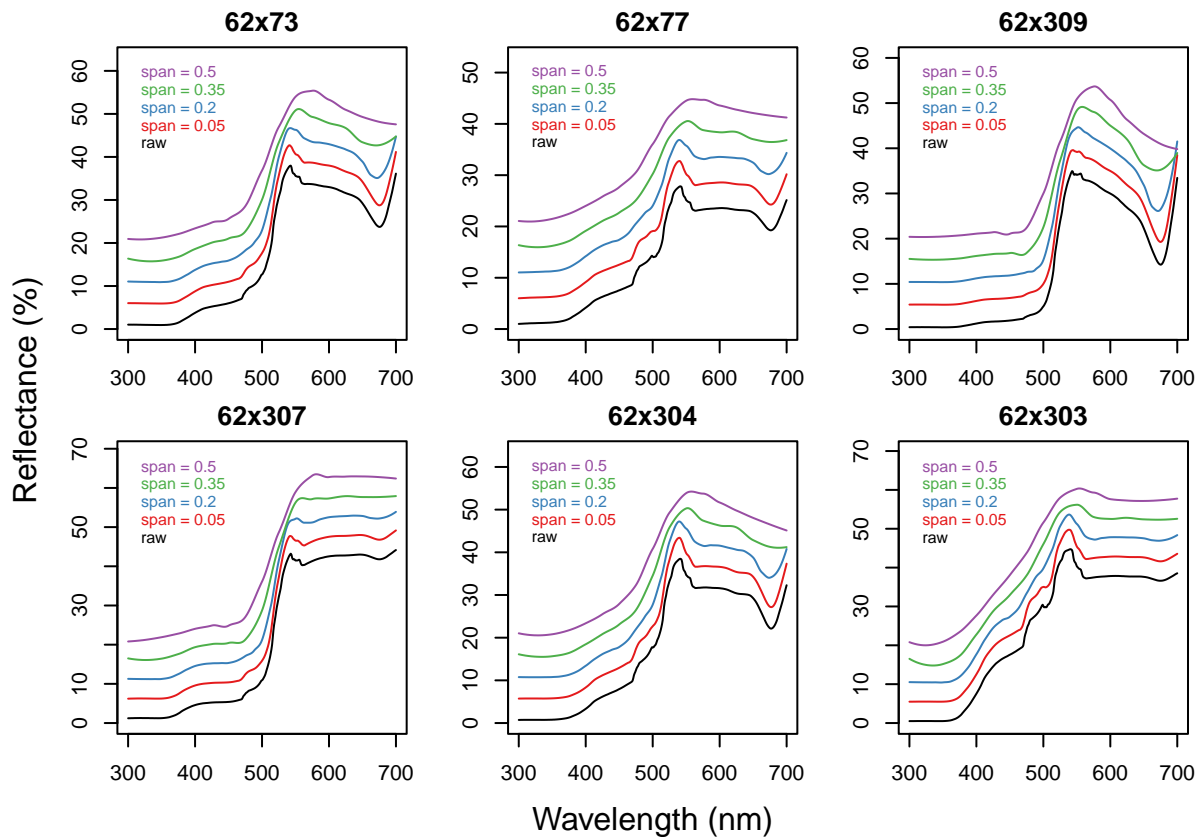
```
## Negative value correction: added min to all reflectance
labellum_spec_avg <- procspec(labellum_spec_avg, fixneg = "admin")
```

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

Determine Smoothing Parameter

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

```
# Plot to determine suitable smoothing span
plotsmooth(bract_spec_avg[,1:7],
  minsmooth = 0.05,
  maxsmooth = 0.5,
  curves = 4,
  ask = FALSE)
```



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
```

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
## 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
```

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")
```

Bract Spectra Plot

```
# Order samples to highlight specific ones
order_spec_bract <- c(
  which(names(bract_spec_scaleminmax) == "125"),
  which(names(bract_spec_scaleminmax) == "126"),
  which(names(bract_spec_scaleminmax) == "BRAC")
)

# Reorder columns to place highlighted samples at the end
columns_bract <- 1:ncol(bract_spec_scaleminmax)
order_spec_bract <- c(columns_bract[columns_bract %notin% order_spec_bract], order_spec_bract)

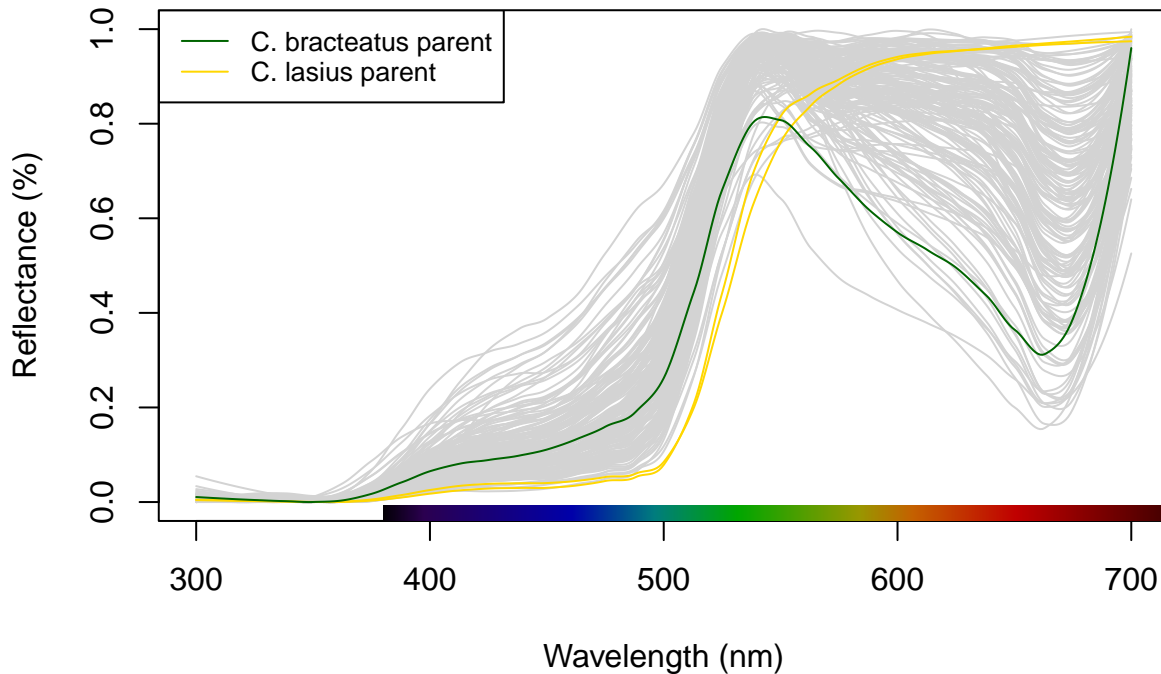
# Plot the spectra
```



```
plot(bract_spec_scaleminmax[order_spec_bract], type = "o",
     col = highlight_colors, main = "Bract Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")

# Add a legend
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
      col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

Bract Spectra



Petal Spectra Plot

```
# Order samples to highlight specific ones
order_spec_petal <- c(
  which(names(petal_spec_scaleminmax) == "125"),
  which(names(petal_spec_scaleminmax) == "126"),
  which(names(petal_spec_scaleminmax) == "BRAC")
)

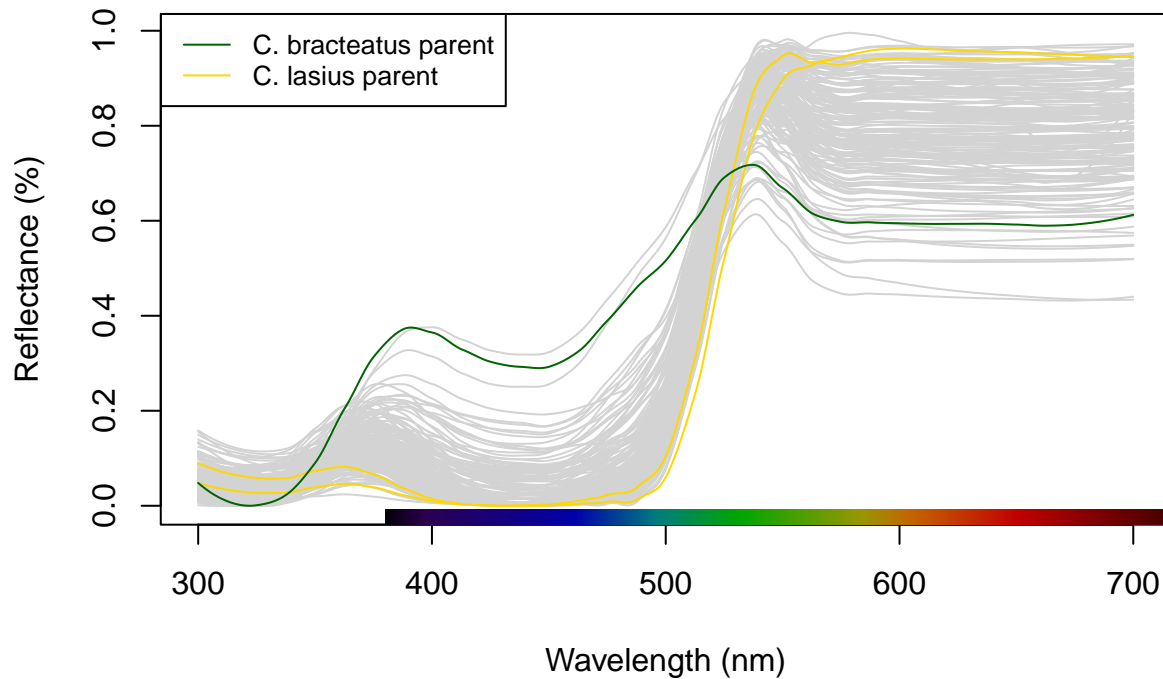
# Reorder columns to place highlighted samples at the end
columns_petal <- 1:ncol(petal_spec_scaleminmax)
order_spec_petal <- c(columns_petal[columns_petal %notin% order_spec_petal], order_spec_petal)

# Plot the spectra
plot(petal_spec_scaleminmax[order_spec_petal], type = "o",
     col = highlight_colors, main = "Petal Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")

# Add a legend
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
```

```
col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

Petal Spectra



Labellum Spectra Plot

```
# Order samples to highlight specific ones
```

```
order_spec_labellum <- c(
  which(names(labellum_spec_scaleminmax) == "125"),
  which(names(labellum_spec_scaleminmax) == "126"),
  which(names(labellum_spec_scaleminmax) == "BRAC")
)
```

```
# Reorder columns to place highlighted samples at the end
```

```
columns_labellum <- 1:ncol(labellum_spec_scaleminmax)
```

```
order_spec_labellum <- c(columns_labellum[columns_labellum %notin% order_spec_labellum], order_spec_labellum)
```

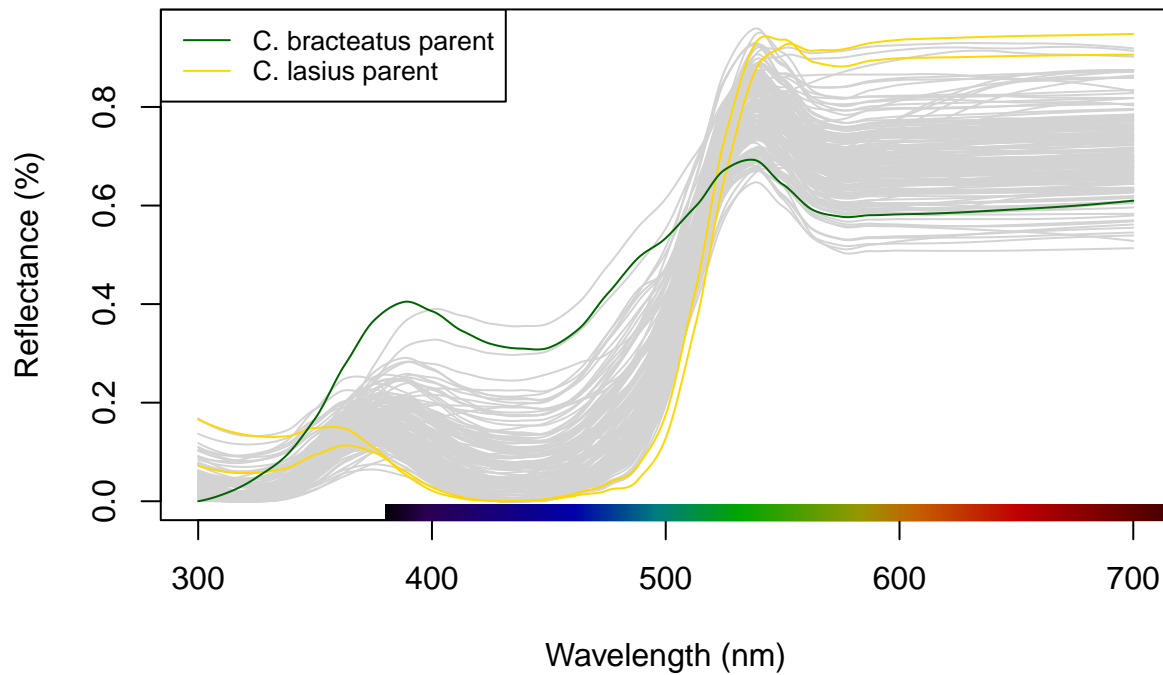
```
# Plot the spectra
```

```
plot(labellum_spec_scaleminmax[order_spec_labellum], type = "o",
     col = highlight_colors, main = "Labellum Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")
```

```
# Add a legend
```

```
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
     col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

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 = 10)
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
## 2
```

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: Peak 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)
```

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
## 2

```

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)

```

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
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_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
## 2

```

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)

```

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))

```

Subset and Rename Columns

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

```

# Subset relevant columns based on analysis needs
summary_bract <- summary_bract[, c("S1U", "S1V", "S1B", "S1G", "S1R", "S5", "S9", "H4")]
summary_petal <- summary_petal[, c("B3", "S1U", "S1V", "S1B", "S1Y", "S1R", "S5", "S9", "H4")]
summary_labellum <- summary_labellum[, c("B3", "S1B", "S1Y", "S1R", "S5", "S6", "S9", "H3", "H4")]

# Rename columns to include tissue type
colnames(summary_bract) <- c("S1U_bract", "S1V_bract", "S1B_bract", "S1G_bract",
                             "S1R_bract", "S5_bract", "S9_bract", "H4_bract")
colnames(summary_petal) <- c("B3_petal", "S1U_petal", "S1V_petal", "S1B_petal",
                             "S1Y_petal", "S1R_petal", "S5_petal", "S9_petal", "H4_petal")
colnames(summary_labellum) <- c("B3_labellum", "S1B_labellum", "S1Y_labellum",
                                "S1R_labellum", "S5_labellum", "S6_labellum",
                                "S9_labellum", "H3_labellum", "H4_labellum")

```

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")
```

UV Analyses

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 <- as.rspec(bract, lim = c(300, 400), whichwl = 1)

## The spectral data contain 456 negative value(s),
## which may produce unexpected results if used in models.
## Consider using procspec() to correct them.

petal_spec <- as.rspec(petal, lim = c(300, 400), whichwl = 1)

## The spectral data contain 86 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, 400), whichwl = 1)

# change column names
colnames(bract_spec) <- gsub("-", "x", colnames(bract_spec))
colnames(petal_spec) <- gsub("-", "x", colnames(petal_spec))
colnames(labellum_spec) <- gsub("-", "x", colnames(labellum_spec))
```

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 <- gsub("\\([0-9]+\\)", "", names(bract_spec))[-1]
petal_samples <- gsub("\\([0-9]+\\)", "", names(petal_spec))[-1]
labellum_samples <- gsub("\\([0-9]+\\)", "", names(labellum_spec))[-1]

# Verify sample counts
table(bract_samples)

## bract_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##         2         3         1         2         1         2         2         1         3         2         2
```



```
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##      2      2      2      1      3      2      3      2      4      2      2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##      2      2      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 39x92
##      2      2      2      2      1      2      3      1      1      2      2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x264 62x265 62x267 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      1      2      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      1      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
table(petal_samples)
```

```
## petal_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##      2      2      1      2      1      2      2      1      3      2      2
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##      2      2      2      1      3      2      3      2      4      2      2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##      2      2      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 39x92
##      2      2      2      2      1      2      3      1      1      2      2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
```

```
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      2      1      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      1      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
table(labellum_samples)
```

```
## labellum_samples
##      125      126      39 39x10 39x109 39x110 39x115 39x116 39x117 39x12 39x122
##      2      2      1      2      1      2      2      1      3      2      2
## 39x123 39x125 39x126 39x13 39x130 39x136 39x14 39x15 39x16 39x17 39x2
##      2      2      2      1      3      2      3      2      4      2      2
## 39x21 39x23 39x25 39x27 39x34 39x39 39x4 39x40 39x41 39x44 39x46
##      2      2      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 39x92
##      2      2      2      2      1      2      3      1      1      2      2
## 39x93 39x95 39x96 39x98 62x10 62x103 62x105 62x109 62x116 62x119 62x122
##      2      2      2      2      1      2      2      1      1      2      1
## 62x125 62x128 62x129 62x13 62x130 62x131 62x134 62x135 62x136 62x137 62x138
##      1      1      2      2      1      2      1      2      2      1      1
## 62x139 62x14 62x140 62x143 62x144 62x147 62x151 62x152 62x153 62x154 62x157
##      1      1      2      1      2      1      1      3      2      2      2
## 62x158 62x16 62x162 62x167 62x168 62x169 62x17 62x172 62x173 62x175 62x18
##      2      2      2      2      1      1      2      2      2      2      1
## 62x182 62x185 62x188 62x189 62x19 62x190 62x197 62x200 62x206 62x21 62x214
##      1      2      1      1      2      2      2      2      2      2      1
## 62x218 62x219 62x22 62x220 62x225 62x233 62x234 62x240 62x251 62x252 62x258
##      2      2      2      1      2      1      1      2      2      1      1
## 62x261 62x262 62x264 62x265 62x268 62x276 62x282 62x293 62x296 62x300 62x302
##      2      2      2      1      2      1      1      2      1      1      1
## 62x303 62x304 62x305 62x306 62x307 62x308 62x309 62x310 62x313 62x315 62x322
##      2      1      1      1      2      1      2      1      1      1      1
## 62x324 62x327 62x332 62x337 62x338 62x351 62x355 62x359 62x363 62x370 62x5
##      1      1      2      2      2      1      2      2      2      1      1
## 62x57 62x58 62x59 62x60 62x63 62x65 62x66 62x69 62x73 62x74 62x75
##      2      2      2      1      1      1      1      2      2      1      1
## 62x77 62x83 62x87 62x91 62x92 62x94 62x96 BRAC
##      1      2      1      2      2      1      1      2
```

```
# Aggregate spectra by sample names using mean
```

```
bract_spec_avg <- aggspec(bract_spec, by = bract_samples, FUN = mean)
petal_spec_avg <- aggspec(petal_spec, by = petal_samples, FUN = mean)
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 = "admin")

## processing options applied:
## Negative value correction: added min to all reflectance
petal_spec_avg <- procspec(petal_spec_avg, fixneg = "admin")

## processing options applied:
## Negative value correction: added min to all reflectance
labellum_spec_avg <- procspec(labellum_spec_avg, fixneg = "admin")

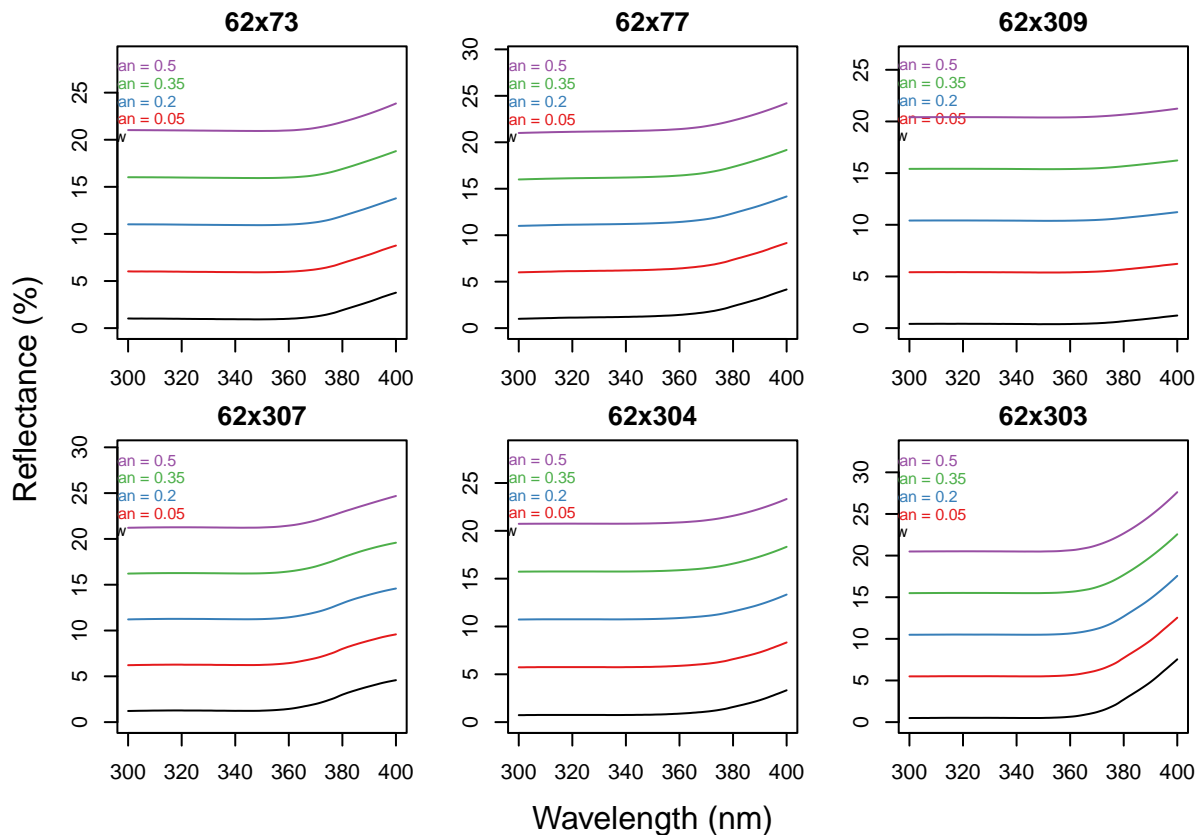
## processing options applied:
## Negative value correction: added min to all reflectance
```

Determine Smoothing Parameter

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

```
# Plot to determine suitable smoothing span
plotsmooth(bract_spec_avg[,1:7],
           minsmooth = 0.05,
           maxsmooth = 0.5,
           curves = 4,
           ask = FALSE)

## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
## Warning in simpleLoess(y, x, w, span, degree = degree, parametric = FALSE, :
## k-d tree limited by memory. ncmax= 200
```



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
```

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
```

```
## 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
```

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")
```

Bract Spectra Plot

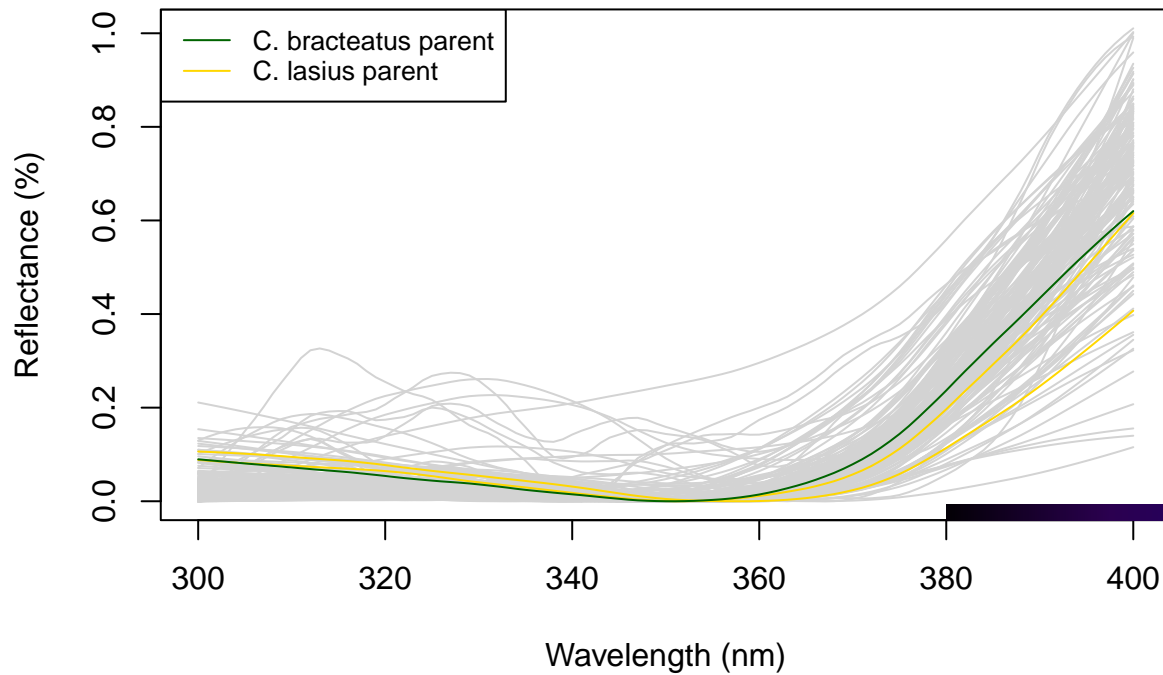
```
# Order samples to highlight specific ones
order_spec_bract <- c(
  which(names(bract_spec_scaleminmax) == "125"),
  which(names(bract_spec_scaleminmax) == "126"),
  which(names(bract_spec_scaleminmax) == "BRAC")
)

# Reorder columns to place highlighted samples at the end
columns_bract <- 1:ncol(bract_spec_scaleminmax)
order_spec_bract <- c(columns_bract[columns_bract %notin% order_spec_bract], order_spec_bract)

# Plot the spectra
plot(bract_spec_scaleminmax[order_spec_bract], type = "o",
     col = highlight_colors, main = "Bract Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")

# Add a legend
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
     col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

Bract Spectra



Petal Spectra Plot

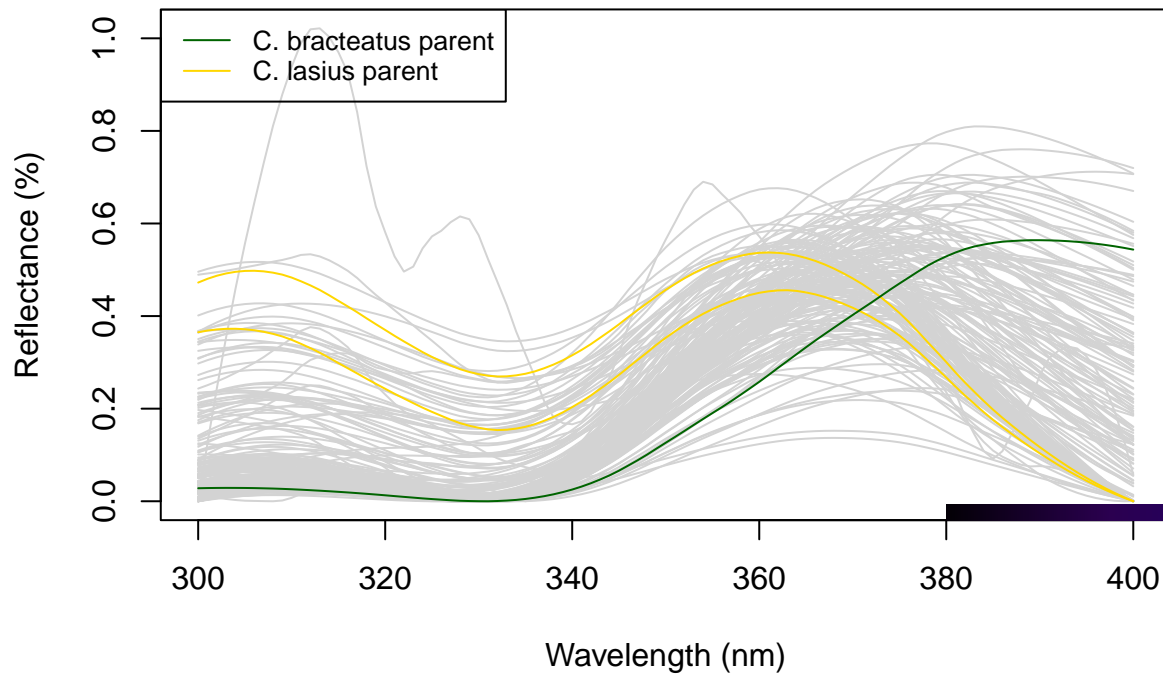
```
# Order samples to highlight specific ones
order_spec_petal <- c(
  which(names(petal_spec_scaleminmax) == "125"),
  which(names(petal_spec_scaleminmax) == "126"),
  which(names(petal_spec_scaleminmax) == "BRAC")
)

# Reorder columns to place highlighted samples at the end
columns_petal <- 1:ncol(petal_spec_scaleminmax)
order_spec_petal <- c(columns_petal[columns_petal %notin% order_spec_petal], order_spec_petal)

# Plot the spectra
plot(petal_spec_scaleminmax[order_spec_petal], type = "o",
     col = highlight_colors, main = "Petal Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")

# Add a legend
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
     col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

Petal Spectra



Labellum Spectra Plot

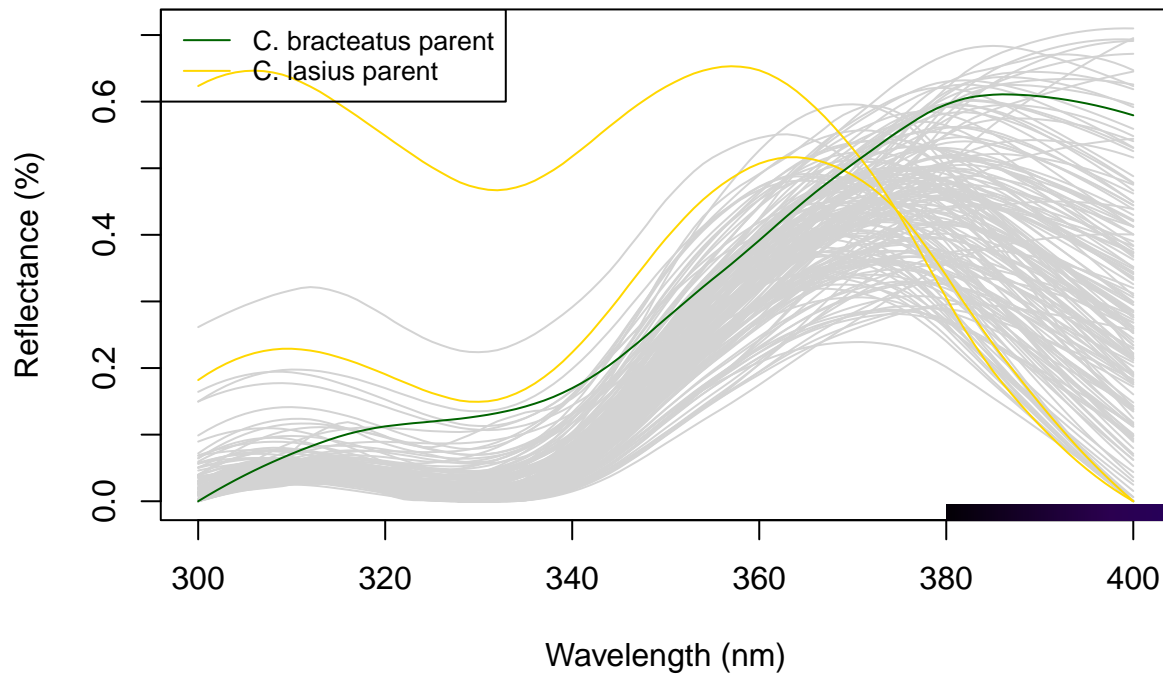
```
# Order samples to highlight specific ones
order_spec_labellum <- c(
  which(names(labellum_spec_scaleminmax) == "125"),
  which(names(labellum_spec_scaleminmax) == "126"),
  which(names(labellum_spec_scaleminmax) == "BRAC")
)

# Reorder columns to place highlighted samples at the end
columns_labellum <- 1:ncol(labellum_spec_scaleminmax)
order_spec_labellum <- c(columns_labellum[columns_labellum %notin% order_spec_labellum], order_spec_labellum)

# Plot the spectra
plot(labellum_spec_scaleminmax[order_spec_labellum], type = "o",
     col = highlight_colors, main = "Labellum Spectra",
     xlab = "Wavelength (nm)", ylab = "Reflectance (%)")

# Add a legend
legend("topleft", legend = c("C. bracteatus parent", "C. lasius parent"),
     col = c("darkgreen", "gold1"), lty = 1, cex = 0.8)
```

Labellum Spectra



Combine and Save Spectral Plots with Highlighted Parents

```
# Save combined spectra plots with highlighted parental samples
pdf("./results/figures/Combined_UVspectra_Bract_Petal_Labellum_Cbracteatus_Clasius_hybrids.pdf", width = 10, height = 10)
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
## 2
```


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: Peak 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_UV <- summary(bract_spec_scalemymax)

## Warning: cannot calculate violet chroma; wavelength below 415 nm
## Warning: cannot calculate blue chroma; wavelength range not between 400 and 510
## nm
## Warning: cannot calculate green chroma; wavelength range not between 510 and
## 605 nm
## Warning: cannot calculate yellow chroma; wavelength range not between 550 and
## 625 nm
## Warning: cannot calculate red chroma; wavelength range not between 605 and 700
## nm

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

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

Plot Bract Spectral Descriptors Histograms

```
pdf("./results/figures/bract_UV_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_UV)) {

  if (!any(!is.na(summary_bract_UV[, i]))) next # Skip columns that are entirely NA

  hist(summary_bract_UV[, i],
        xlab = colnames(summary_bract_UV)[i],
```

```

    main = colnames(summary_bract_UV)[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
## 2

```

Petal Summary

```

# Calculate summary statistics for petal
summary_petal_UV <- summary(petal_spec_scalemimax)

## Warning: cannot calculate violet chroma; wavelength below 415 nm
## Warning: cannot calculate blue chroma; wavelength range not between 400 and 510
## nm
## Warning: cannot calculate green chroma; wavelength range not between 510 and
## 605 nm
## Warning: cannot calculate yellow chroma; wavelength range not between 550 and
## 625 nm
## Warning: cannot calculate red chroma; wavelength range not between 605 and 700
## nm

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

# Extract metrics for specific samples
petal125 <- round(summary_petal_UV["125", ], 3)
petal126 <- round(summary_petal_UV["126", ], 3)

```

```
petalBRAC <- round(summary_petal_UV["BRAC", ], 3)
```

Plot Petal Spectral Descriptors Histograms

```
pdf("./results/figures/petal_UV_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_petal_UV)) {

  if (!any(!is.na(summary_petal_UV[, i]))) next # Skip columns that are entirely NA

  hist(summary_petal_UV[, i],
        xlab = colnames(summary_petal_UV)[i],
        main = colnames(summary_petal_UV)[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
## 2
```

Labellum Summary

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

```
## Warning: cannot calculate violet chroma; wavelength below 415 nm
## Warning: cannot calculate blue chroma; wavelength range not between 400 and 510
## nm
## Warning: cannot calculate green chroma; wavelength range not between 510 and
## 605 nm
## Warning: cannot calculate yellow chroma; wavelength range not between 550 and
## 625 nm
## Warning: cannot calculate red chroma; wavelength range not between 605 and 700
## nm
```

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

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

Plot Labellum Spectral Descriptors Histograms

```
pdf("./results/figures/labellum_UV_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_UV)) {

  if (!any(!is.na(summary_labellum_UV[, i]))) next # Skip columns that are entirely NA

  hist(summary_labellum_UV[, i],
        xlab = colnames(summary_labellum_UV)[i],
        main = colnames(summary_labellum_UV)[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_UV) <- gsub("x", "_", rownames(summary_bract_UV))
rownames(summary_petal_UV) <- gsub("x", "_", rownames(summary_petal_UV))
rownames(summary_labellum_UV) <- gsub("x", "_", rownames(summary_labellum_UV))

```

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_petal_UV <- summary_petal_UV %>% rownames_to_column(var = "id")
summary_labellum_UV <- summary_labellum_UV %>% rownames_to_column(var = "id")

```

Subset and Rename Columns

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

```

# Subset relevant columns based on analysis needs
summary_petal_UV <- summary_petal_UV[, c("id", "H1")]
summary_labellum_UV <- summary_labellum_UV[, c("id", "H1")]

# Rename columns to include tissue type
colnames(summary_petal_UV) <- c("id", "H1_UV_petal")
colnames(summary_labellum_UV) <- c("id", "H1_UV_labellum")

```

Merge Summary Dataframes

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

```

# Merge all summaries by 'id'
joined_df <- summary_bract %>%
  full_join(summary_petal, by = "id") %>%
  full_join(summary_labellum, by = "id") %>%
  full_join(summary_petal_UV, by = "id") %>%
  full_join(summary_labellum_UV, by = "id")

```

Export Summary Descriptors to CSV

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

```
# Write the combined summary dataframe to a CSV file  
write.csv(joined_df, "./results/processed_data/spectral_shape_descriptors.csv",  
          quote = FALSE, row.names = FALSE)
```