BumblebeeRobbing

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Behavioral assays done on bumblebees at Rocky Mountains Biology Laboratory (RMBL) field season 2023. Observations were carried out by Nick Dabagia and Daniel Souto for Corydalis and Mertensia species. Y-Tube assays carried out by Oriana Gutierrez in 2024.

Methods:

Refer to Souto-Vilarós et al. 'Yeast volatiles promote larceny in bumble bee behavior.'

Data analysis:

The dataset is arranged as date, plant species, bee individual, bee species, whether or not a choice was made, stalk number, treatment, whether or not the bee tried to legitimately visit the flower, whether it robbed the flower, time to rob, time feeding, flower number (total for that bout) and additional notes.

Note that not all packages are used in the current analysis

We need to wrangle the data a bit. Namely: Filter the data to include only cases where a flower was robbed (column CHOICE, "Yes"), Remove an outlier which was inactive on a flwoer over 3 minutes. Make sure all data is numeric and there are no missing data.

This final data set includes: 57 total successful robber trials (out of 116 bee trials transcribed) 42 Corydalis 2° robbers (22 bifarius, 16 flavifrons, 4 mixtus) and 15 Mertensia 2° robbers (all flavifrons).

Bombus mixtus removed from final analysis due to low sample size.

```
robbed_flowers <- visitation %>%
  filter(robbed == "yes") %>% #note that this will reduce total number of bees which made a choice sinc
  filter(species != "mixtus")%>%
  filter(timetorob !=154.7)

robbed_flowers <- robbed_flowers %>%
  mutate(
    timetorob = as.numeric(timetorob),
    timefeeding = as.numeric(timefeeding)
) %>%
  filter(!is.na(timetorob) & !is.na(timefeeding))

robbed_flowers <- robbed_flowers %>%
  mutate(trytolegit_binary = ifelse(trytolegit == "yes", 1, 0))

#How many bees per bee species per flower species in the original and the filtered datasets?

visitation %>% group_by(sample, plantsp, species) %>% count()
```

A tibble: 120 x 4

```
## # Groups:
               sample, plantsp, species [120]
##
      sample
                           species
                 plantsp
                           <chr>>
##
      <chr>
                 <chr>
##
  1 CORYBIF001 corydalis bifarius
                                        5
##
    2 CORYBIF002 corydalis bifarius
                                        1
  3 CORYBIF003 corydalis bifarius
  4 CORYBIF004 corydalis bifarius
## 5 CORYBIF005 corydalis bifarius
                                        1
  6 CORYBIF006 corydalis bifarius
                                       11
  7 CORYBIF007 corydalis bifarius
                                       10
## 8 CORYBIF008 corydalis bifarius
                                       10
## 9 CORYBIF009 corydalis bifarius
                                        1
## 10 CORYBIF010 corydalis bifarius
                                        1
## # i 110 more rows
robbed flowers %>% group by(sample, plantsp, species, bee) %>% count()
## # A tibble: 49 x 5
             sample, plantsp, species, bee [49]
## # Groups:
##
      sample
                 plantsp
                           species
                                      bee
##
      <chr>
                 <chr>
                           <chr>>
                                    <int> <int>
   1 CORYBIF001 corydalis bifarius
                                        3
                                              3
  2 CORYBIF004 corydalis bifarius
##
                                             15
## 3 CORYBIF006 corydalis bifarius
                                        7
                                             11
## 4 CORYBIF007 corydalis bifarius
                                             10
## 5 CORYBIF008 corydalis bifarius
                                        2
                                              9
## 6 CORYBIF011 corydalis bifarius
                                        7
                                             12
## 7 CORYBIF012 corydalis bifarius
                                        8
                                             19
## 8 CORYBIF014 corydalis bifarius
                                        5
                                             20
## 9 CORYBIF017 corydalis bifarius
                                              9
                                       11
## 10 CORYBIF019 corydalis bifarius
                                              6
## # i 39 more rows
```

Simple summary statistics for time to rob (mean, SE) broken down by bumblebee species and plant sp. Note that time to rob is longer for control flowers, but also SE higher for controls. Consistent response for Mreu plants. Tactic switch, I just converted into a binary column yes = 1, no = 0. Feeding time is higher in both treatments, with high SE but consistent throughout.

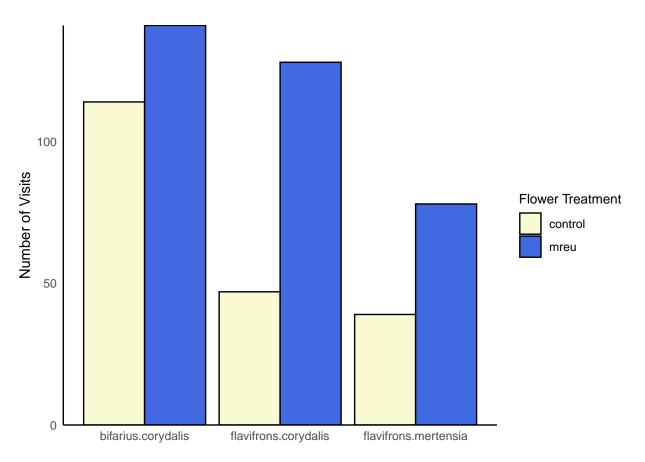
```
summary_stats <- robbed_flowers %>%
group_by(species, treatment, plantsp) %>%
summarize(
    Mean_TimeToRob = mean(timetorob, na.rm = TRUE),
    SE_TimeToRob = sd(timetorob, na.rm = TRUE) / sqrt(n()),
    Mean_TimeFeeding = mean(timefeeding, na.rm = TRUE),
    SE_TimeFeeding = sd(timetorob, na.rm = TRUE) / sqrt(n()),
    Mean_tacticswitch = mean(trytolegit_binary, na.rm = TRUE),
    SE_tacticswitch = sd(trytolegit_binary, na.rm = TRUE) / sqrt(n())
) %>%
arrange(plantsp, treatment)
```

```
## 'summarise()' has grouped output by 'species', 'treatment'. You can override
## using the '.groups' argument.
```

```
print(summary_stats)
## # A tibble: 6 x 9
## # Groups:
               species, treatment [4]
                treatment plantsp
                                    Mean TimeToRob SE TimeToRob Mean TimeFeeding
     species
     <chr>>
                <chr>
                          <chr>>
                                              <dbl>
                                                           <dbl>
                                               5.42
                                                           0.637
                                                                              7.76
## 1 bifarius
                control
                          corydalis
## 2 flavifrons control
                          corydalis
                                               5.24
                                                           0.672
                                                                              8.04
                                                                              7.09
## 3 bifarius mreu
                          corydalis
                                               4.16
                                                           0.343
## 4 flavifrons mreu
                          corydalis
                                               3.20
                                                           0.272
                                                                              8.04
                                               7.12
                                                                              9.25
## 5 flavifrons control
                                                           1.08
                          mertensia
## 6 flavifrons mreu
                          mertensia
                                               3.97
                                                           0.426
                                                                              8.06
## # i 3 more variables: SE_TimeFeeding <dbl>, Mean_tacticswitch <dbl>,
       SE_tacticswitch <dbl>
write.table(summary_stats, "results/summary_feeding_stats.csv", row.names = FALSE)
# Summarize the data to calculate the number of visits
summary data <- robbed flowers %>%
  group_by(treatment, species, plantsp) %>%
  summarize(
    number_of_visits = n(), # Count the number of visits
    .groups = "drop"
# Calculate the mean, stdev, and se for each treatment group (control vs mreu)
visit_summary <- summary_data %>%
  group_by(species, plantsp) %>%
  summarize(
    mean_visits_control = mean(number_of_visits[treatment == "control"], na.rm = TRUE),
    mean_visits_treated = mean(number_of_visits[treatment == "mreu"], na.rm = TRUE),
    stdev_diff = sd(number_of_visits[treatment == "mreu"] - number_of_visits[treatment == "control"], n
    se_diff = stdev_diff / sqrt(n()),
    .groups = "drop"
  )
# View the resulting summary table
print(visit_summary)
## # A tibble: 3 x 6
     species
                plantsp mean_visits_control mean_visits_treated stdev_diff se_diff
                                                                                <dbl>
##
     <chr>>
                <chr>>
                                        <dbl>
                                                            <dbl>
                                                                        <dbl>
## 1 bifarius
                                                                                   NA
                corydal~
                                          114
                                                              141
                                                                           NA
## 2 flavifrons corvdal~
                                           47
                                                              128
                                                                           NA
                                                                                   NA
## 3 flavifrons mertens~
                                           39
                                                               78
                                                                           NA
                                                                                   NA
# Perform t-tests for each species and plantsp combination
t_test_results <- robbed_flowers %>%
  group_by(species, plantsp) %>%
  summarize(
    t test = list(
      t.test(
```

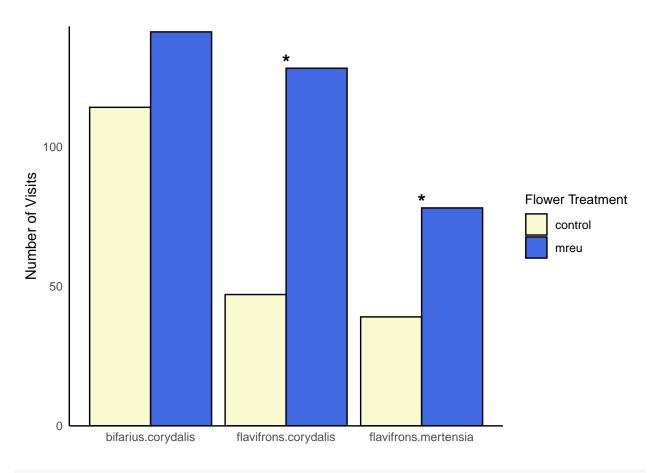
```
x = treatment == "mreu", # Logical vector for mreu
       y = treatment == "control" # Logical vector for control
   ),
    .groups = "drop"
# Extract p-values and t-statistics from the t-test results
t_test_summary <- t_test_results %>%
  mutate(
   p_value = sapply(t_test, function(x) x$p.value), # Extract p-value
    statistic = sapply(t_test, function(x) x$statistic) # Extract t-statistic
  )
# Select only necessary columns
t_test_summary <- t_test_summary %>%
 dplyr::select(species, plantsp, p_value, statistic)
# View the summarized results
print(t_test_summary)
## # A tibble: 3 x 4
##
    species
               plantsp
                          p_value statistic
##
     <chr>>
                <chr>
                            <dbl>
                                      <dbl>
## 1 bifarius corydalis 1.68e- 2
                                       2.40
## 2 flavifrons corydalis 5.52e-20
                                       9.74
## 3 flavifrons mertensia 1.77e- 7
                                       5.39
# Extract p-values, t-statistics, and degrees of freedom from the t-test results
t_test_summary <- t_test_results %>%
 mutate(
   p_value = sapply(t_test, function(x) x$p.value),
                                                         # Extract p-value
   statistic = sapply(t_test, function(x) x$statistic), # Extract t-statistic
   df = sapply(t_test, function(x) x$parameter)
                                                         # Extract degrees of freedom
  )
# Explicitly use dplyr::select()
t_test_summary <- t_test_summary %>%
 dplyr::select(species, plantsp, p_value, statistic, df)
# View the summarized results
print(t_test_summary)
## # A tibble: 3 x 5
##
     species
               plantsp
                          p_value statistic
     <chr>
               <chr>
                            <dbl>
                                      <dbl> <dbl>
## 1 bifarius corydalis 1.68e- 2
                                       2.40 508
## 2 flavifrons corydalis 5.52e-20
                                       9.74
                                              348
## 3 flavifrons mertensia 1.77e- 7
                                       5.39
                                              232
# Create a histogram
ggplot(robbed_flowers, aes(x = interaction(species, plantsp), fill = treatment)) +
```

```
geom_bar(position = "dodge", color = "black") +
  labs(x = NULL,
    y = "Number of Visits",
   fill = "Flower Treatment"
  ) +
  scale_fill_manual(
    values = c("mreu" = "#4169E1",
               "control" = "#FAFAD2")
  )+
  scale_y_continuous(expand = c(0, 0)) + # Ensures bars rest on the x-axis line
  theme_minimal() +
  theme(
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    axis.line = element_line(color = "black"),
    axis.line.x = element_line(),
    axis.line.y = element_line(),
    legend.title = element_text(size = 10),
    legend.text = element_text(size = 9)
  ) -> plot
plot
```



```
ggsave("results/bee_visits_histogram.pdf", plot = plot, width = 8, height = 6)
```

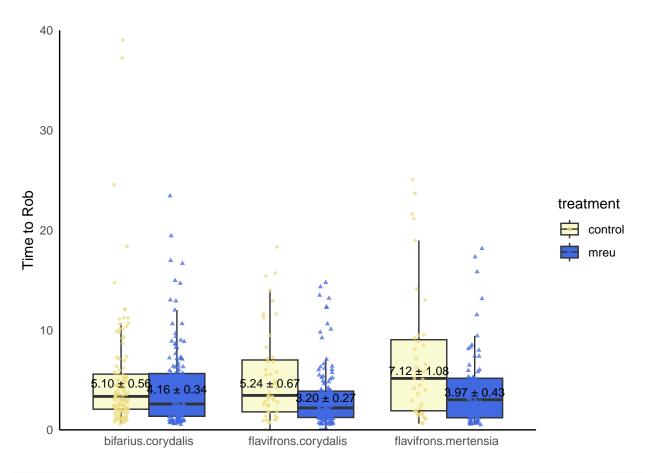
```
summary_df <- robbed_flowers %>%
  group_by(group = interaction(species, plantsp), treatment) %>%
  summarise(count = n(), .groups = "drop") %>%
  group_by(group) %>%
 summarise(
   \max_{y} = \max(\text{count}),
   x_pos = as.numeric(factor(group)), # for proper placement on x axis
   .groups = "drop"
  ) %>%
 mutate(label = "*")
## Warning: Returning more (or less) than 1 row per 'summarise()' group was deprecated in
## dplyr 1.1.0.
## i Please use 'reframe()' instead.
## i When switching from 'summarise()' to 'reframe()', remember that 'reframe()'
## always returns an ungrouped data frame and adjust accordingly.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
ggplot(robbed_flowers, aes(x = interaction(species, plantsp), fill = treatment)) +
  geom_bar(position = position_dodge(width = 0.9), color = "black") +
  geom text(
   data = summary_df,
   aes(x = group, y = max_y + 2, label = label),
   inherit.aes = FALSE,
   size = 6
  ) +
  labs(x = NULL,
       y = "Number of Visits",
       fill = "Flower Treatment") +
  scale_fill_manual(
   values = c("mreu" = "#4169E1", "control" = "#FAFAD2")
  scale_y_continuous(expand = c(0, 0)) + # Ensures bars rest on the x-axis line
  theme_minimal() +
  theme(
   panel.grid.major = element_blank(),
   panel.grid.minor = element blank(),
   axis.line = element line(color = "black"),
   axis.line.x = element_line(),
   axis.line.y = element_line(),
   legend.title = element_text(size = 10),
   legend.text = element_text(size = 9)
  ) -> plot2
plot2
```



ggsave("results/bee_visits_histogram.pdf", plot = plot2, width = 8, height = 6)

```
mean_se <- function(x) {</pre>
  m <- mean(x, na.rm = TRUE)</pre>
  se <- sd(x, na.rm = TRUE) / sqrt(length(na.omit(x)))</pre>
  return(paste0(round(m, 2), " ± ", round(se, 2)))
}
timetorob_box_species <- ggplot(robbed_flowers, aes(x = interaction(species, plantsp), y = timetorob, f</pre>
  geom_boxplot(outlier.shape = NA, position = position_dodge(width = 0.75)) +
  geom_point(position = position_jitterdodge(jitter.width = 0.1, dodge.width = 0.75),
             aes(shape = treatment, color = treatment),
             size = 1, alpha = 0.75) +
  stat_summary(
    fun.data = function(x) {
      m \leftarrow mean(x)
      se <- sd(x) / sqrt(length(x))</pre>
      data.frame(y = m + se * -1.5, label = sprintf("%.2f ± %.2f", m, se))
    },
    geom = "text",
    position = position_dodge(width = 0.75),
    size = 3,
    vjust = 0,
    color = "black"
  ) +
```

```
scale_fill_manual(
   values = c("mreu" = "#4169E1",
               "control" = "#FAFAD2")
 )+
  scale_color_manual(
   values = c("mreu" = "#4169E1",
              "control" = "#EEDD82")
  )+
 labs(
   #title = "Time to rob by bee species and treatment",
   x = NULL
   y = "Time to Rob"
  ) +
  #scale_fill_discrete(name = "Treatment") +
  #scale_color_discrete(name = "Treatment") +
  scale_shape_manual(name = "Treatment", values = c(16, 17), guide = "none") +
  scale_y_continuous(expand = c(0, 0), limits = c(0, 40)) +
   #title = "Time to rob by bee species and treatment",
   x = NULL
   y = "Time to Rob"
  ) +
  theme_minimal() +
 theme(
   panel.grid.major = element_blank(),
   panel.grid.minor = element_blank(),
   axis.line = element_line(color = "black"),
   axis.line.x = element_line(),
   axis.line.y = element_line(),
   )
timetorob <- timetorob_box_species +</pre>
  coord_cartesian(ylim = c(0, 40))
timetorob
## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_boxplot()').
## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_summary()').
## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom point()').
```



ggsave("results/timetorob_pub.pdf", plot = timetorob, width = 8, height = 6)

```
## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_boxplot()').

## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_summary()').

## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom_point()').
```

ANOVA time to rob on bee species (1) and plant species (2) - in both cases treatment is highly significant, but interaction between either plant or bee species and treatment is not. Suggests instead that only the nectar treatment significantly different

```
anova_result <- aov(timetorob ~ species + treatment, data = robbed_flowers)
summary(anova_result)</pre>
```

```
##
                Df Sum Sq Mean Sq F value
                                            Pr(>F)
                       29
                             29.2
                                    1.249
                                             0.264
## species
## treatment
                 1
                      455
                            455.1
                                   19.479 1.23e-05 ***
## Residuals
                             23.4
               544
                    12711
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

```
anova_result_plant <- aov(timetorob ~ plantsp + treatment, data = robbed_flowers)
summary(anova_result_plant)</pre>
```

```
Df Sum Sq Mean Sq F value
                                           Pr(>F)
                                   1.918
                                            0.167
## plantsp
                      45
                            44.6
                           491.4 21.115 5.38e-06 ***
## treatment
                1
                     491
                            23.3
## Residuals
              544 12659
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
```

alternative hypothesis: true location shift is not equal to 0

To make sure this significance holds, I did wilcox test only on the mean time to rob. Again, significantly different ($p = \langle 0.05 \rangle$

```
treatment_control <- subset(robbed_flowers, treatment == "control") #control subset
treatment_mreu <- subset(robbed_flowers, treatment == "mreu") #inoculated flowers subset

# Perform the Wilcoxon rank-sum test
wilcox_test_result <- wilcox.test(treatment_control$timetorob, treatment_mreu$timetorob) #wilcox test
# Print the test result
print(wilcox_test_result) #wilcox result - highly significant difference of mean time to rob between tr

##
## Wilcoxon rank sum test with continuity correction
##
## data: treatment_control$timetorob and treatment_mreu$timetorob
## W = 42406, p-value = 1.502e-05</pre>
```

We do the same analysis for feedint time and show that there is no significant difference between treatments on the feeding time of bees. It seems that yeast does not have a significant flavor(?) difference or not not different enough or nasty enough for bees to care. They spend the same amount of time feeding on sterile or yeast innoculated nectar

```
feedingtime box species <- ggplot(robbed flowers, aes(x = interaction(species, plantsp), y = timefeedin
  geom_boxplot(outlier.shape = NA, position = position_dodge(width = 0.75)) +
  geom_point(position = position_jitterdodge(jitter.width = 0.1, dodge.width = 0.75),
             aes(shape = treatment, color = treatment),
             size = 1, alpha = 0.75) +
  stat_summary(
    fun.data = function(x) {
      m \leftarrow mean(x)
      se <- sd(x) / sqrt(length(x))</pre>
      data.frame(y = m + se * -.5, label = sprintf("\%.2f \pm \%.2f", m, se))
    },
    geom = "text",
    position = position_dodge(width = 0.75),
    size = 3,
    vjust = 0,
    color = "black"
  ) +
  scale fill manual(
```

```
values = c("mreu" = "#4169E1",
              "control" = "#FAFAD2")
 )+
 scale_color_manual(
   values = c("mreu" = "#4169E1",
              "control" = "#EEDD82")
 )+
 labs(
   title = "Feeding time by bee species and treatment",
   x = NULL
   y = "Time to Rob"
 ) +
  #scale_fill_discrete(name = "Treatment") +
  #scale_color_discrete(name = "Treatment") +
  scale_shape_manual(name = "Treatment", values = c(16, 17), guide = "none") +
  scale_y_continuous(expand = c(0, 0)) + # Ensures bars rest on the x-axis line
  theme_minimal() +
 theme(
   panel.grid.major = element_blank(),
   panel.grid.minor = element_blank(),
   axis.line = element_line(color = "black"),
   axis.line.x = element_line(),
   axis.line.y = element_line(),
   legend.title = element_text(size = 10),
   legend.text = element_text(size = 9)
 )
feedingtime_box_species
```

Feeding time by bee species and treatment 40 treatment control mreu

 $8.04 \pm 1.48.04 \pm 0.66$

Time to Rob

##

20

 $.76 \pm 0.81$

 $.09 \pm 0.5$

Wilcoxon rank sum test with continuity correction

data: treatment_control\$timefeeding and treatment_mreu\$timefeeding

```
bifarius.corydalis
                                    flavifrons.corydalis
                                                          flavifrons.mertensia
ggsave("results/feeding_pub.pdf", plot = feedingtime_box_species, width = 8, height = 6)
ANOVA and wilcox test confirm. No difference on time spent feeding depending on treatment of nectar.
anova_result_feeding <- aov(timefeeding ~ species + treatment, data = robbed_flowers)</pre>
# Print the ANOVA table
summary(anova_result_feeding)
##
                 Df Sum Sq Mean Sq F value Pr(>F)
                        91
                                      1.518 0.218
## species
                                      0.771 0.380
                              46.07
## treatment
                        46
                  1
## Residuals
                544
                    32510
                              59.76
# Perform the Wilcoxon rank-sum test
wilcox_test_result_feeding <- wilcox.test(treatment_control$timefeeding, treatment_mreu$timefeeding)</pre>
# Print the test result
print(wilcox_test_result_feeding)
##
```

.25 ± 1.628.06 ± 0.73

```
## W = 34333, p-value = 0.8367 ## alternative hypothesis: true location shift is not equal to 0
```

This included all plant species combined. Below I break down everything per plant species (Corydalis and Mertensia)

First subset data

```
corydalis <- subset(robbed_flowers, plantsp == "corydalis")
mertensia <- subset(robbed_flowers, plantsp == "mertensia")</pre>
```

Since for Corydalis we have 2 bee species, I still did ANOVA. For Mertensia, there is only one bee species so makes more sense to do wilcox. In the case of Corydalis, the two bee species take different times to find the robbing hole (p = 0.03), while time to find the hole for different treatments is stil highly significant (p = 0.001). We could do different analysis for each bee species, if worth it.

```
anova_result_robbing_corydalis <- aov(timetorob ~ species + treatment, data = corydalis)
# Print the ANOVA table
summary(anova_result_robbing_corydalis)</pre>
```

```
##
                Df Sum Sq Mean Sq F value Pr(>F)
## species
                1
                       98
                            98.34
                                    4.309 0.0385 *
                           230.28 10.091 0.0016 **
## treatment
                      230
                1
## Residuals
               427
                     9744
                            22.82
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
anova result feeding corydalis <- aov(timefeeding ~ species + treatment, data = corydalis)
# Print the ANOVA table
summary(anova result feeding corydalis)
```

```
##
                Df Sum Sq Mean Sq F value Pr(>F)
                                    0.733 0.392
## species
                 1
                       44
                            43.61
## treatment
                 1
                       19
                            18.60
                                    0.313 0.576
## Residuals
               427
                    25394
                            59.47
```

For Corydalis, significant difference both for species and treatment, but not the interaction. So, bee species show difference in the time it takes to find a robbing hole (p = 0.03), and significantly different according to treatment (p = 0.001).

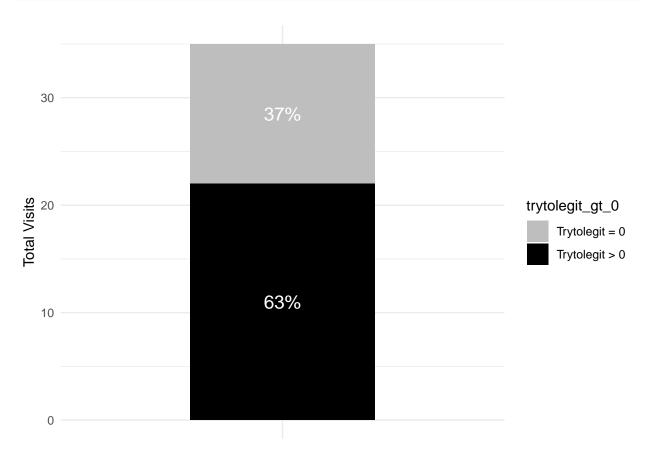
Wilcox test for mertenisa, time to rob significantly different between treatments (P = 0.006)

```
wilcox_mertensia_timetorob <- wilcox.test(timetorob ~ treatment, data = mertensia)
wilcox_mertensia_timetorob</pre>
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: timetorob by treatment
## W = 1988, p-value = 0.006991
## alternative hypothesis: true location shift is not equal to 0
```

```
legit_summary <- corydalis %>%
  group_by(unique_name) %>%
  summarise(trytolegit_binary = sum(trytolegit_binary))
print(legit_summary)
## # A tibble: 35 x 2
##
                                     trytolegit_binary
      unique_name
##
      <chr>>
                                                 <dbl>
## 1 Aug-01_corydalis_3_bifarius
## 2 Aug-01_corydalis_7_bifarius
## 3 Aug-01_corydalis_8_bifarius
                                                     2
## 4 Aug-01_corydalis_9_flavifrons
                                                    11
## 5 Aug-02_corydalis_12_flavifrons
                                                     2
## 6 Aug-02_corydalis_1_flavifrons
## 7 Aug-02_corydalis_2_bifarius
                                                    1
                                                    2
## 8 Aug-02_corydalis_7_bifarius
## 9 Aug-02_corydalis_8_bifarius
                                                     2
## 10 Aug-03_corydalis_11_bifarius
## # i 25 more rows
visit_summary <- corydalis %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
visit_summary
## # A tibble: 35 x 3
##
      unique_name
                                     total_visits legit_visits
      <chr>
                                            <int>
                                                         <int>
##
## 1 Aug-01_corydalis_3_bifarius
                                              15
                                                             2
## 2 Aug-01_corydalis_7_bifarius
                                               11
                                                             1
## 3 Aug-01_corydalis_8_bifarius
                                              10
                                                             2
## 4 Aug-01_corydalis_9_flavifrons
                                              15
                                                            11
## 5 Aug-02_corydalis_12_flavifrons
                                              15
                                                             2
## 6 Aug-02_corydalis_1_flavifrons
                                              1
                                                             0
                                              9
                                                             1
## 7 Aug-02_corydalis_2_bifarius
## 8 Aug-02_corydalis_7_bifarius
                                             12
                                                             2
                                                             2
## 9 Aug-02_corydalis_8_bifarius
                                             19
## 10 Aug-03_corydalis_11_bifarius
## # i 25 more rows
y_tube <- read.csv("data/y_tube_ori_2025.csv", header = T)</pre>
table(y_tube$Initial_Choice)
##
## C T
## 9 13
```

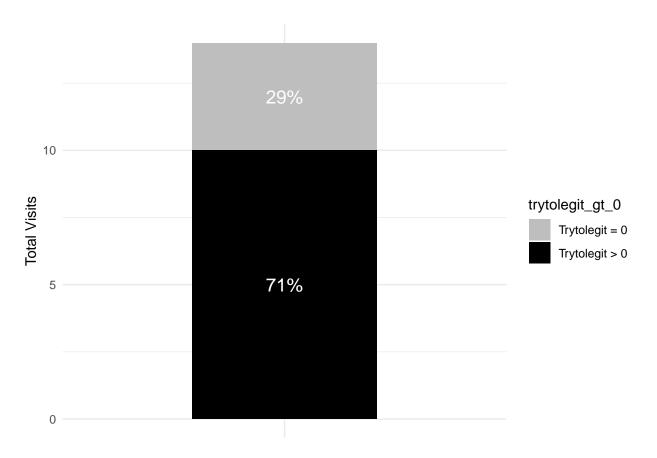
```
chisq.test(table(y_tube$Initial_Choice))
##
##
   Chi-squared test for given probabilities
## data: table(y_tube$Initial_Choice)
## X-squared = 0.72727, df = 1, p-value = 0.3938
t <- t.test(y_tube$Yeast_time, y_tube$Control_time, paired = TRUE, alternative = "greater")
##
## Paired t-test
##
## data: y_tube$Yeast_time and y_tube$Control_time
## t = 2.4177, df = 21, p-value = 0.0124
## alternative hypothesis: true mean difference is greater than 0
## 95 percent confidence interval:
## 12.99907
                  Tnf
## sample estimates:
## mean difference
          45.09091
corydalis_bifarius <- subset(corydalis, species == "bifarius")</pre>
corydalis_flavifrons <- subset(corydalis, species == "flavifrons")</pre>
summary_corydalis <- corydalis %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
# Create a new column indicating whether trytolegit_binary > 0
legit_summary <- legit_summary %>%
  mutate(trytolegit_gt_0 = ifelse(trytolegit_binary > 0, "Trytolegit > 0", "Trytolegit = 0"))
# Summarize the data by the new column
summary_data <- legit_summary %>%
  group_by(trytolegit_gt_0) %>%
 summarise(total_visits = n())
# Calculate total visits
total_visits <- sum(summary_data$total_visits)</pre>
# Calculate percentage of visits for each category
summary_data <- summary_data %>%
 mutate(percentage = total_visits / sum(total_visits))
# Plot the barplot
ggplot(summary_data, aes(x = "", y = total_visits, fill = trytolegit_gt_0)) +
  geom bar(stat = "identity", width = 0.5) +
 geom_text(aes(label = scales::percent(percentage)),
```



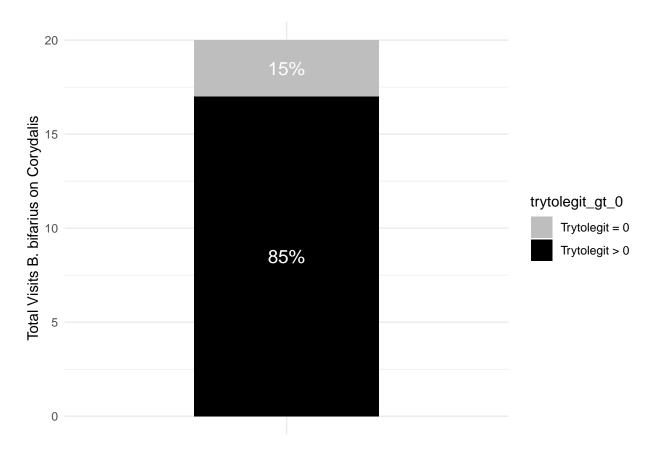
```
legit_summary_mert <- mertensia %>%
  group_by(unique_name) %>%
  summarise(trytolegit_binary = sum(trytolegit_binary))
print(legit_summary_mert)
```

```
## # A tibble: 14 x 2
##
                                     trytolegit_binary
      unique_name
      <chr>
                                                 <dbl>
##
## 1 Aug-10_mertensia_10_flavifrons
                                                     4
## 2 Aug-10_mertensia_3_flavifrons
                                                     1
## 3 Aug-10_mertensia_7_flavifrons
                                                     4
## 4 Aug-10_mertensia_8_flavifrons
                                                     3
## 5 Aug-11_mertensia_1_flavifrons
                                                     5
## 6 Aug-11_mertensia_3_flavifrons
                                                     2
## 7 Aug-14_mertensia_10_flavifrons
                                                     0
## 8 Aug-14_mertensia_1_flavifrons
                                                     2
## 9 Aug-14_mertensia_2_flavifrons
                                                     1
```

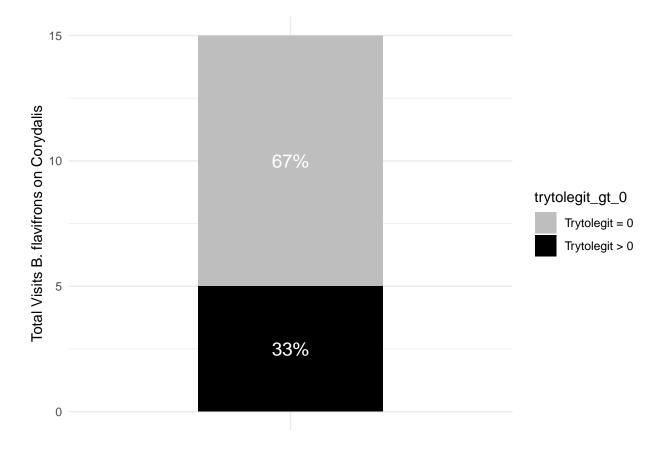
```
## 10 Aug-15_mertensia_1_flavifrons
## 11 Aug-15_mertensia_2_flavifrons
                                                     0
## 12 Aug-15 mertensia 4 flavifrons
                                                     5
## 13 Aug-15_mertensia_6_flavifrons
                                                     2
## 14 Aug-15_mertensia_8_flavifrons
visit_summary_mert <- mertensia %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
visit_summary_mert
## # A tibble: 14 x 3
##
     unique_name
                                     total_visits legit_visits
##
      <chr>
                                            <int>
                                                         <int>
## 1 Aug-10_mertensia_10_flavifrons
                                               14
## 2 Aug-10 mertensia 3 flavifrons
                                               1
## 3 Aug-10 mertensia 7 flavifrons
                                               23
                                                             4
## 4 Aug-10_mertensia_8_flavifrons
                                               18
                                                             3
                                               9
                                                             5
## 5 Aug-11_mertensia_1_flavifrons
## 6 Aug-11_mertensia_3_flavifrons
                                                             2
## 7 Aug-14_mertensia_10_flavifrons
                                                             0
                                               1
## 8 Aug-14_mertensia_1_flavifrons
                                               13
                                                             2
                                               2
## 9 Aug-14_mertensia_2_flavifrons
                                                             1
## 10 Aug-15_mertensia_1_flavifrons
                                               9
                                                             0
## 11 Aug-15_mertensia_2_flavifrons
                                               4
                                                             0
## 12 Aug-15_mertensia_4_flavifrons
                                               10
                                                             5
                                                             2
## 13 Aug-15 mertensia 6 flavifrons
                                               3
## 14 Aug-15_mertensia_8_flavifrons
summary_mertensia <- mertensia %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
# Create a new column indicating whether trytolegit_binary > 0
legit summary mert <- legit summary mert %>%
 mutate(trytolegit_gt_0 = ifelse(trytolegit_binary > 0, "Trytolegit > 0", "Trytolegit = 0"))
# Summarize the data by the new column
summary_data_mert <- legit_summary_mert %>%
  group_by(trytolegit_gt_0) %>%
  summarise(total visits = n())
# Calculate total visits
total_visits_mert <- sum(summary_data_mert$total_visits)</pre>
# Calculate percentage of visits for each category
summary_data_mert <- summary_data_mert %>%
  mutate(percentage = total_visits / sum(total_visits))
```



```
# Create a new column indicating whether trytolegit_binary > 0
legit_summary_corybif <- legit_summary_corybif %>%
 mutate(trytolegit gt 0 = ifelse(trytolegit binary > 0, "Trytolegit > 0", "Trytolegit = 0"))
# Summarize the data by the new column
summary_data_corybif <- legit_summary_corybif %>%
  group_by(trytolegit_gt_0) %>%
  summarise(total_visits = n())
# Calculate total visits
total_visits_corybif <- sum(summary_data_corybif$total_visits)</pre>
# Calculate percentage of visits for each category
summary_data_corybif <- summary_data_corybif %>%
 mutate(percentage = total_visits / sum(total_visits))
# Plot the barplot
ggplot(summary_data_corybif, aes(x = "", y = total_visits, fill = trytolegit_gt_0)) +
  geom_bar(stat = "identity", width = 0.5) +
  geom_text(aes(label = scales::percent(percentage)),
            position = position_stack(vjust = 0.5),
            color = "white", size = 5) +
 labs(x = NULL, y = "Total Visits B. bifarius on Corydalis") +
  scale_fill_manual(values = c("Trytolegit = 0" = "gray", "Trytolegit > 0" = "black")) +
  theme_minimal() +
 theme(axis.text.x=element_blank()) # Hide x-axis label
```



```
summary_cory_flav <- corydalis_flavifrons %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
legit_summary_coryflav <- corydalis_flavifrons %>%
  group_by(unique_name) %>%
  summarise(trytolegit_binary = sum(trytolegit_binary))
visit_summary_coryflav <- corydalis_flavifrons %>%
  group_by(unique_name) %>%
  summarise(total_visits = n(),
            legit_visits = sum(trytolegit == 'yes'))
# Create a new column indicating whether trytolegit_binary > 0
legit_summary_coryflav <- legit_summary_coryflav %>%
  mutate(trytolegit_gt_0 = ifelse(trytolegit_binary > 0, "Trytolegit > 0", "Trytolegit = 0"))
# Summarize the data by the new column
summary_data_coryflav <- legit_summary_coryflav %>%
  group_by(trytolegit_gt_0) %>%
  summarise(total_visits = n())
# Calculate total visits
total_visits_coryflav <- sum(summary_data_coryflav$total_visits)</pre>
```



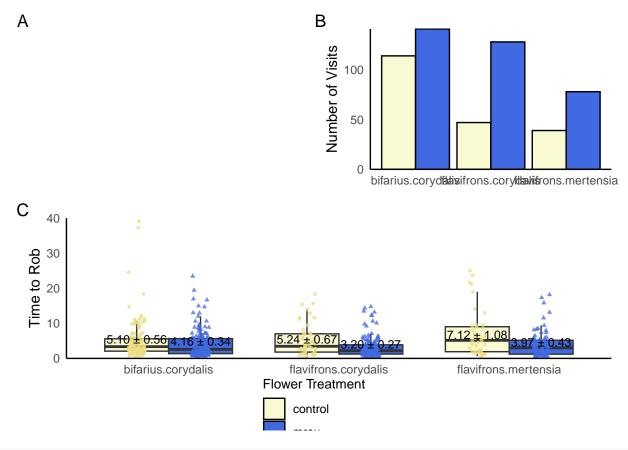
library(patchwork)

```
## Warning: package 'patchwork' was built under R version 4.4.3
##
## Attaching package: 'patchwork'
## The following object is masked from 'package:MASS':
##
## area
```

```
## Warning: package 'cowplot' was built under R version 4.4.3
##
## Attaching package: 'cowplot'
## The following object is masked from 'package:patchwork':
##
       align_plots
## The following object is masked from 'package:lubridate':
##
##
       stamp
blank_plot <- ggplot()+</pre>
  theme_void()+
 theme(panel.background = element_rect(fill = "white", color = NA))
timetorob_legend <- timetorob + theme(legend.position = "none")</pre>
plot_legend <- plot + theme(legend.position = "none")</pre>
combined_plot <- (blank_plot | plot_legend) / timetorob_legend +</pre>
plot_layout(widths = c(1,9), heights = c(1,1))+
 plot_annotation(tag_levels = "A")
# Extract legend from plot1
legend <- cowplot::get_legend(</pre>
  plot + theme(legend.position = "right")
## Warning in get_plot_component(plot, "guide-box"): Multiple components found;
## returning the first one. To return all, use 'return_all = TRUE'.
# Use cowplot to assemble final plot with legend below
final_plot <- cowplot::plot_grid(</pre>
  combined_plot,
 legend,
 ncol = 1,
 rel_heights = c(1, 0.1)
## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_boxplot()').
## Warning: Removed 1 row containing non-finite outside the scale range
## ('stat_summary()').
## Warning: Removed 1 row containing missing values or values outside the scale range
## ('geom_point()').
```

library(cowplot)

final_plot



ggsave("results/combined_plot.pdf", final_plot, width = 7, height = 8, units = "in")