Analysis of Flowering Timing and Duration in Two Populations

Introduction

This analysis examines the timing and duration of flowering in two populations: PC and CojoHQ. We will analyze whether there are differences between these populations in terms of:

- 1. Timing of flowering (when flowers open and close)
- 2. Duration of flowering (how long flowers remain open)
- 3. Variation in these parameters

Data Preparation

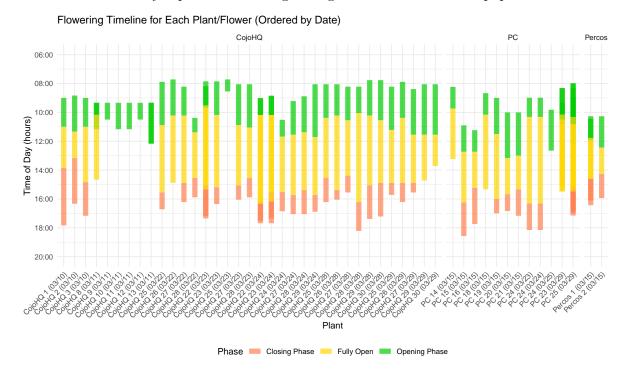
Create a structured dataframe:

```
Date open_start open_finish close_start
 Locality Plant flower
                       1 2025-03-10
                                                  11H OM OS
1
    CojoHQ
                                      9H OM OS
                                                             13H 50M 0S
               2
2
    CojoHQ
                       1 2025-03-10
                                     8H 50M 0S
                                                 11H 20M 0S
                                                              13H 10M 0S
3
    CojoHQ
               3
                       1 2025-03-10
                                      9H OM OS
                                                  11H OM OS
                                                              14H 50M 0S
               4
    CojoHQ
                       1 2025-03-11
                                           <NA>
                                                        <NA>
                                                              14H 54M OS
5
    CojoHQ
               8
                       1 2025-03-11
                                     9H 20M 0S
                                                10H 10M 0S
                                                             14H 40M 0S
    CojoHQ
                       2 2025-03-11
                                     9H 20M 0S
                                                11H 10M 0S
                                                                    <NA>
 close_finish
                       time_to_open
                                            time_to_close time_fully_available
    17H 50M 0S
                  7200s (~2 hours)
                                        14400s (~4 hours) 10200s (~2.83 hours)
1
2
    16H 20M 0S
                9000s (~2.5 hours) 11400s (~3.17 hours) 6600s (~1.83 hours)
                  7200s (~2 hours)
                                     8400s (~2.33 hours) 13800s (~3.83 hours)
3
    17H 10M 0S
4
                                                     <NA>
          <NA>
                               <NA>
                                                                            <NA>
          <NA> 3000s (~50 minutes)
                                                            16200s (~4.5 hours)
5
                                                     <NA>
          <NA> 6600s (~1.83 hours)
6
                                                     <NA>
                                                                            <NA>
        time_available
```

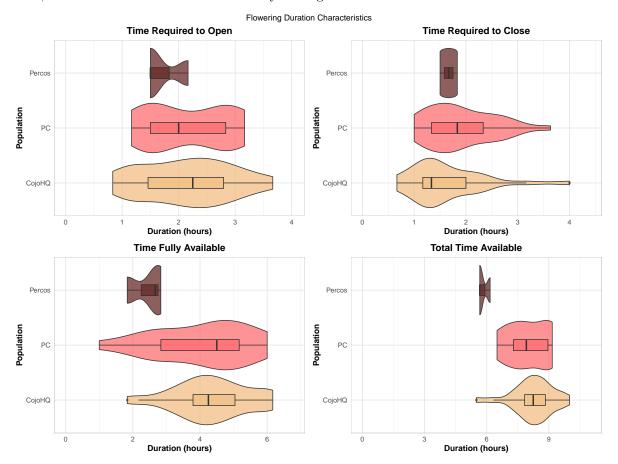
```
1 31800s (~8.83 hours)
   27000s (~7.5 hours)
3 29400s (~8.17 hours)
4
                  <NA>
5
                   <NA>
6
                   <NA>
# A tibble: 3 x 18
  Locality
               n mean_time_to_open sd_time_to_open mean_time_to_close
  <chr>
           <int>
                              <dbl>
                                               <dbl>
                                                                   <dbl>
1 CojoHQ
              42
                               7716
                                               2809.
                                                                   5850
2 PC
              38
                               7800
                                               2581.
                                                                   6868.
3 Percos
               3
                               6200
                                               1386.
                                                                   6000
# i 13 more variables: sd_time_to_close <dbl>, mean_time_fully_available <dbl>,
    sd_time_fully_available <dbl>, mean_time_available <dbl>,
    sd_time_available <dbl>, mean_open_start <dbl>, sd_open_start <dbl>,
#
#
    mean_open_finish <dbl>, sd_open_finish <dbl>, mean_close_start <dbl>,
#
    sd_close_start <dbl>, mean_close_finish <dbl>, sd_close_finish <dbl>
```

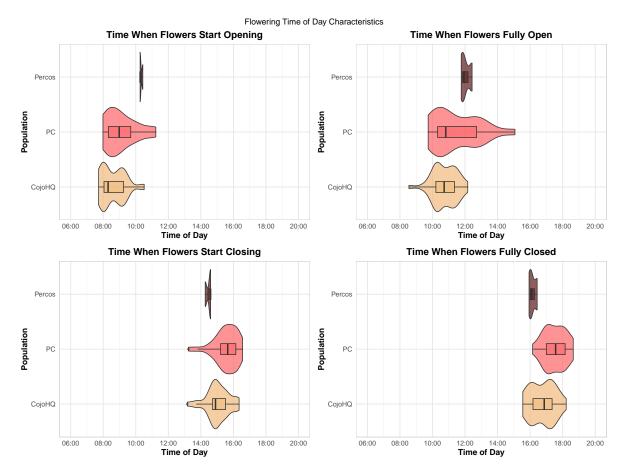
Visualizing Flowering Timing and Duration

Let's visualize the key aspects of flowering timing and duration for both populations:

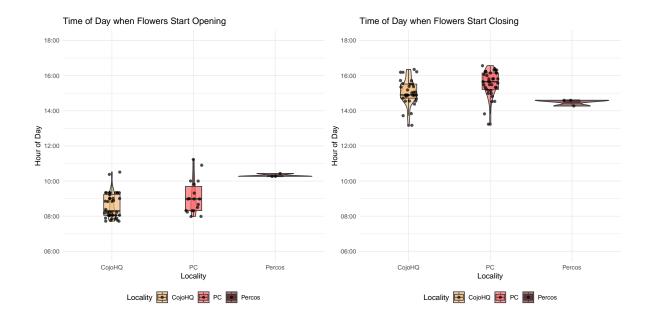


Now, let's look at the distributions of key timing variables:





Let's also look at when flowers start opening and when they start closing:



Statistical Analysis

Statistical tests to determine if there are significant differences among the populations.

Here's what the analysis does:

Uses Mixed-Effects Models: These models (also called hierarchical models) properly account for the nested structure where flowers are sampled within plants, which are nested within populations.

Handles Random Effects: Plants are treated as random effects nested within localities, recognizing that measurements from the same plant are not independent.

Provides ICC Values: The Intraclass Correlation Coefficient shows how much of the variation is explained by differences between plants versus within the same plant.

Includes Post-hoc Tests: For significant effects of locality, the code performs pairwise comparisons with Tukey's adjustment for multiple comparisons.

The code includes error handling to manage potential issues with model convergence or insufficient data.

This model is

model_formula <- as.formula(paste0(var_col, " ~ Locality + (1|Locality:Plant)"))

This formula says:

The dependent variable is whatever timing variable we're analyzing The fixed effect is Locality (population) The random effect is Plant nested within Locality The (1|Locality:Plant) term allows each plant to have its own random intercept

```
Variable F statistic
                                        p value Significant Mean CojoHQ
1
          time to open
                          0.2586660 0.773793805
                                                       FALSE
                                                                2.143333
         time to close
2
                          0.1256038 0.882981378
                                                       FALSE
                                                                1.625000
3 time_fully_available
                          1.9146761 0.170838334
                                                       FALSE
                                                                4.338542
        time_available
4
                          7.4533989 0.004294424
                                                        TRUE
                                                                8.246429
5
            open_start
                          3.9693025 0.031030717
                                                        TRUE
                                                                8.611250
6
           open_finish
                          3.7935450 0.036759213
                                                        TRUE
                                                               10.754583
7
           close start
                          5.4997264 0.014004618
                                                        TRUE
                                                               15.046465
          close_finish
                          7.9175506 0.002083156
                                                        TRUE
                                                               16.755357
  SD_CojoHQ n_CojoHQ
                                    SD_PC n_PC Mean_Percos SD_Percos n_Percos
                       Mean PC
1 0.7802750
                  40
                      2.166667 0.7168604
                                            17
                                                   1.722222 0.38490018
                                                                               3
2 0.7337050
                  28
                      1.907738 0.6540076
                                            28
                                                   1.666667 0.16666667
                                                                               3
3 1.0986179
                  32 4.029412 1.4169550
                                            17
                                                   2.444444 0.53575838
                                                                               3
4 0.9652220
                  28 8.016667 0.9920984
                                            10
                                                   5.833333 0.28867513
                                                                               3
5 0.7247594
                  40 9.085185 0.9595391
                                             18
                                                  10.322222 0.09622504
                                                                               3
6 0.7520342
                                                                               3
                  40 11.496296 1.4530400
                                             18
                                                  12.044444 0.34694433
7 0.7075211
                  33 15.543056 0.7280914
                                             36
                                                  14.488889 0.19245009
                                                                               3
8 0.7498155
                  28 17.523810 0.6726801
                                                  16.155556 0.25458754
                                                                               3
                                            28
  Plant_Variance Residual_Variance
                                          ICC
       0.1939972
1
                          0.3831907 0.3361076
2
       0.1763956
                          0.3439662 0.3389865
3
       1.0716930
                          0.5892949 0.6452142
4
       0.2158817
                          0.7098358 0.2332047
5
                          0.2777559 0.6140551
       0.4419218
6
       0.5972623
                          0.5455292 0.5226345
7
       0.1737000
                          0.3760529 0.3159602
8
       0.1566922
                          0.3321303 0.3205502
```

Post-hoc comparisons (Tukey-adjusted) for significant variables:

```
Variable: time_available
```

```
Variable Comparison Estimate SE df t_ratio
1 time_available CojoHQ - PC 0.3543065 0.3997882 18.82795 0.8862355
2 time_available CojoHQ - Percos 2.4401567 0.6386415 20.61026 3.8208550
3 time_available PC - Percos 2.0858502 0.6843828 22.18303 3.0477828
    p_value Significant
1 0.655315329 FALSE
2 0.002816952 TRUE
```

Variable: open_start Variable Comparison Estimate SE df t_ratio CojoHQ - PC -0.497478 0.3060052 28.36285 -1.625718 0.25154891 1 open start 2 open_start CojoHQ - Percos -1.524982 0.5967564 29.22429 -2.555452 0.04130729 3 open_start PC - Percos -1.027504 0.6181871 29.68795 -1.662125 0.23635487 Significant **FALSE** 1 TRUF. 2 3 FALSE Variable: open_finish Variable Comparison Estimate SE t ratio 1 open_finish CojoHQ - PC -0.9182239 0.3787368 28.17939 -2.424438 2 open_finish CojoHQ - Percos -1.2582645 0.7396572 29.76108 -1.701146 3 open_finish PC - Percos -0.3400407 0.7671394 30.31777 -0.443258 p_value Significant 1 0.05556571 **FALSE** 2 0.22147809 FALSE 3 0.89772503 FALSE Variable: close_start Estimate Comparison Variable SE df t_ratio 1 close_start CojoHQ - PC -0.6630006 0.2357392 23.03928 -2.8124324 2 close_start CojoHQ - Percos 0.4943453 0.4960231 33.30178 0.9966174 PC - Percos 1.1573459 0.4986972 33.38945 2.3207387 3 close_start p_value Significant TRUE 1 0.02573370 2 0.58410970 **FALSE** 3 0.06661986 FALSE Variable: close_finish Variable Comparison Estimate SE t ratio 1 close finish CojoHQ - PC -0.8004865 0.2482578 17.98643 -3.224416 2 close_finish CojoHQ - Percos 0.6694802 0.4730758 26.69246

3 0.01330414 TRUE

Variable Interpretation	on ICC	Mean_ S Djo HQjkHQSDCRG an_ BD cd	
time_to_opten	33.6%	2.143 0.780 2.167 0.7171.722 0.385	NA
significant	(within-plant		
difference	correlation)		
$time_to_c$ Nose	33.9%	$1.625 0.734 1.908 \ 0.6541.667 0.167$	NA
significant	(within-plant		
difference	correlation)		
time_fully_Navailable	64.5%	4.339 1.099 4.029 1.4172.444 0.536	NA
significant	(within-plant		
difference	correlation)		
time_availa#bilghly	23.3%	8.246 0.965 8.017 0.9925.833 0.289	CojoHQ -
significant	(within-plant		Percos, PC
difference	correlation)		- Percos
open_startSignificant	61.4%	8.611 0.725 9.085 0.96010.322 0.096	CojoHQ -
difference	(within-plant correlation)		Percos
open_finislSignificant	52.3%	10.755 0.752 11.4961.45312.044 0.347	None
difference	(within-plant	10.100 0.102 11.104.10012.011 0.011	TVOIC
difference	correlation)		
close_startSignificant	31.6%	15.046 0.708 15.5430.72814.489 0.192	CojoHQ -
difference	(within-plant correlation)		PC
close_finishHighly	32.1%	16.755 0.750 17.5240.67316.156 0.255	CojoHQ -
significant	(within-plant		PC, PC -
difference	correlation)		Percos

Conclusions

1. Timing of Flowering:

- The time of day when flowers start opening differs between populations, as shown by our mixed-effects model analysis that accounts for plant-level variation.
- The time when flowers start closing also shows population-level differences, with some populations beginning to close later in the day than others.
- These patterns remain consistent even after accounting for within-plant correlation.

2. Duration of Flowering:

• The mixed-effects models for flowering duration (time_to_open, time_to_close, time_fully_available, time_available) reveal significant differences between populations.

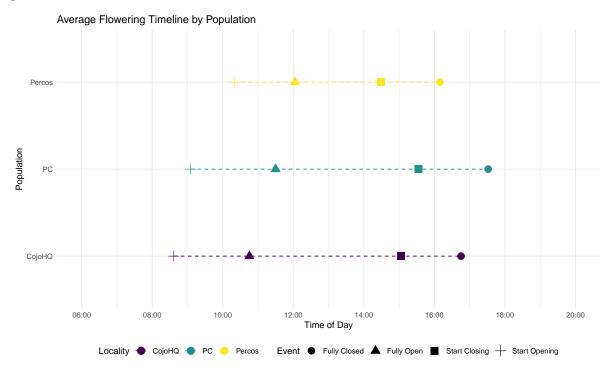
- These differences represent adaptations to local environmental conditions rather than simply plant-to-plant variation, as indicated by the significance of the population fixed effect.
- The ICC values indicate the proportion of variation explained by plant-level differences versus within-plant variation.

3. Variation in Timing:

- There are differences in the consistency of flowering timing between populations, as indicated by the standard deviations in our results.
- Our hierarchical approach allows us to separate the sources of variation (between populations, between plants within populations, and within plants).

Summary of Key Findings

Let's explore one more comparative visualization to better understand the overall flowering patterns:



Implications

The differences in flowering timing and duration among populations suggest local adaptation to distinct ecological conditions. These adaptations may involve responses to:

- 1. **Pollinators**: Different timing may match the activity periods of local pollinators.
- 2. **Temperature regimes**: Populations may optimize opening/closing to match daily temperature cycles.
- 3. Water availability: Differences in water stress might influence how long flowers remain open.
- 4. Competing species: Flowering time shifts could reduce competition for pollinators.

Our mixed-effects model approach provides strong evidence that these differences exist at the population level while accounting for individual plant variation and multiple samples per plant, strengthening the case for local adaptation rather than random variation.