Supplementary Text S5: On overclustering correction

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Contents

1 Overview

Here, we will verify whether the *dispersal* term of the orthogroup scores really penalizes overclustering. For that, we ran OrthoFinder (Emms and Kelly 2019) one more time using the previously described Brassicaceae data set, but now with an Markov inflation parameter (*mcl*) of 5. An *mcl* of 5 is usually considered too large, so we would expect orthogroup scores to be lower than, for instance, runs with *mcl* = 3. Our goal here is to verify if our hypothesis is true.

Loading required packages:

```
set.seed(123) # for reproducibility

library(here)
library(cogeqc)
library(ggpubr)
library(rstatix)
library(patchwork)
library(tidyverse)

source(here("code", "utils.R"))
```

2 Data acquisition

We ran OrthoFinder with the following code:

```
# Run OrthoFinder - default DIAMOND mode, mcl = 5
orthofinder -f data -S diamond -I 5 -o products/result_files/default_5 -og
```

Now, we will load our data to the R session as a list of **cogeqc**-friendly orthogroup data frames.

```
# Extract tar.xz file
tarfile <- here("products", "result_files", "Orthogroups.tar.xz")
outdir <- tempdir()

system2("tar", args = c("-xf", tarfile, "--directory", outdir))

# Get path to OrthoFinder output
og_files <- list.files(
    path = outdir,
    pattern = "Orthogroups.*", full.names = TRUE
)

# Remove files for the ultrasensitive DIAMOND mode and add mcl=5
og_files <- c(
    og_files[c(2, 1, 3, 4)],
    here("products", "result_files", "Orthogroups_default_5.tsv.gz")
)
```

```
# Read and parse files
ogs <- lapply(og_files, function(x) {
    og <- read_orthogroups(x)
    og <- og %>%
        mutate(Species = stringr::str_replace_all(Species, "\\.", "")) %>%
        mutate(Gene = str_replace_all(
            Gene, c(
                "\\.[0-9]$" = "",
                "\\.[0-9]\\.p$" = "",
                "\\.t[0-9]$" = "",
                "\\.g$" = ""
            )
        ))
    return(og)
})
names(ogs) <- c("1", "1.5", "2", "3", "5")
```

Next, we will load InterPro annotation from PLAZA 5.0 (Van Bel et al. 2022).

```
# Define function to read functional annotation from PLAZA 5.0
read_annotation <- function(url, cols = c(1, 3)) {
    annot <- readr::read_tsv(url, show_col_types = FALSE, skip = 8) %>%
        select(cols)
    names(annot)[1:2] <- c("Gene", "Annotation")
    return(annot)
}

# Get Interpro annotation
base <- "https://ftp.psb.ugent.be/pub/plaza/plaza_public_dicots_05/InterPro/"
interpro <- list(
    Athaliana = read_annotation(paste0(base, "interpro.ath.csv.gz")),
    Aarabicum = read_annotation(paste0(base, "interpro.aar.csv.gz")),
    Alyrata_cvMN47 = read_annotation(paste0(base, "interpro.aly.csv.gz")),
    Bcarinata_cvzd1 = read_annotation(paste0(base, "interpro.bca.csv.gz")),
    Crubella_cvMonteGargano = read_annotation(paste0(base, "interpro.cru.csv.gz")),
    Chirsuta = read_annotation(paste0(base, "interpro.chi.csv.gz")),
    Sparvula = read_annotation(paste0(base, "interpro.spa.csv.gz"))
)

interpro <- lapply(interpro, as.data.frame)
```

3 Validating the overclustering correction

Now that we have all data we need (orthogroup data frames and domain annotations), let's calculate orthogroup scores. Here, we will use the function `calculate_H_with_terms()` from the file *utils.R*, which contains a slightly modified version of the function `calculate_H()` from **cogeqc**, but instead of updating the uncorrected scores with the corrected scores, it returns the dispersal terms and corrected scores and separate variables.

```
# Calculate orthogroup scores with and without correction for overclustering
og_homogeneity <- Reduce(rbind, lapply(seq_along(ogs), function(x) {

    mode <- names(ogs)[x]
    annotation <- Reduce(rbind, interpro) |> distinct()

    message("Working on ", mode)

    orthogroup_df <- merge(
        ogs[[x]],
        annotation,
        all.x = TRUE
    )

    scores_df <- calculate_H_with_terms(
        orthogroup_df, correct_overclustering = TRUE, update_score = FALSE
    )
    scores_df$Mode <- mode

    return(scores_df)
}))

og_homogeneity$Mode <- factor(
    og_homogeneity$Mode, levels = unique(og_homogeneity$Mode)
)
```

Next, let's visualize orthogroup scores with and without corrections, as well as look at the dispersal terms for each mode.

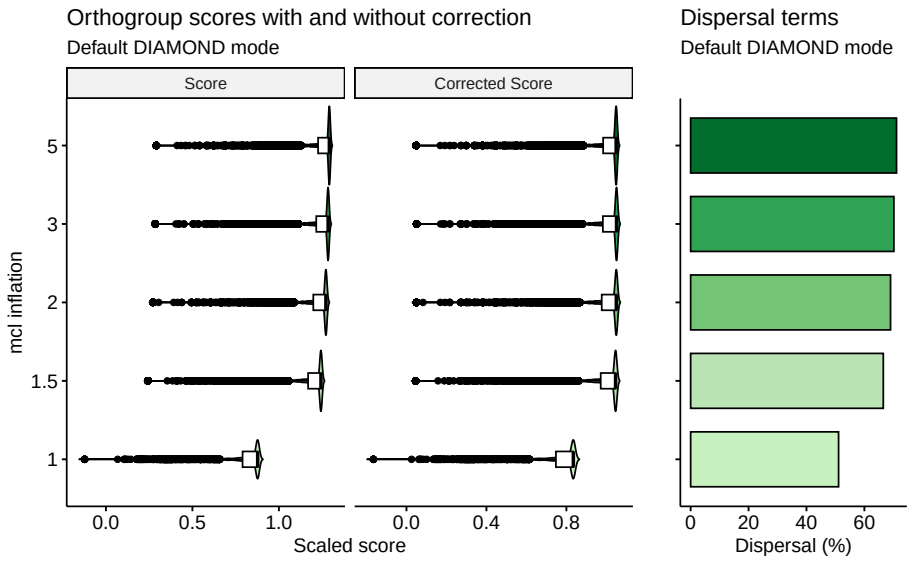
```
# Plot scores
p_scores <- og_homogeneity |>
    mutate(
        Score = (Score - min(Score) / (max(Score) - min(Score))),
        Score_c = (Score_c - min(Score_c) / (max(Score_c) - min(Score_c)))
    ) |>
    dplyr::select(Orthogroup, Score, `Corrected Score` = Score_c, Mode) |>
    pivot_longer(
        !c("Orthogroup", "Mode"),
        names_to = "Measure",
        values_to = "Score"
    ) |>
    mutate(
        Measure = factor(Measure, levels = c("Score", "Corrected Score"))
    ) |>
    ggpubr::ggviolin(
        x = "Mode", y = "Score",
        orientation = "horiz",
        fill = "Mode",
        palette = rev(c("#006D2C", "#31A354", "#74C476", "#BAE4B3", "#c7f2bf")),
        add = "boxplot", add.params = list(fill = "white")
    ) +
```

```
        labs(
            x = "mcl inflation", y = "Scaled score",
            title = "Orthogroup scores with and without correction",
            subtitle = "Default DIAMOND mode"
        ) +
        facet_wrap(~Measure, scales = "free_x", nrow = 1) +
        theme(legend.position = "none")

    # Plot dispersal terms
    p_dispersal <- og_homogeneity |>
        dplyr::select(Mode, Dispersal) |>
        mutate(Dispersal = Dispersal * 100) |>
        dplyr::distinct() |>
        ggpubr::ggbarplot(
            x = "Mode", y = "Dispersal", stat = "identity",
            orientation = "horiz",
            fill = "Mode",
            palette = rev(c("#006D2C", "#31A354", "#74C476", "#BAE4B3", "#c7f2bf")),
        ) +
        labs(
            x = "", y = "Dispersal (%)",
            title = "Dispersal terms",
            subtitle = "Default DIAMOND mode"
        ) +
        theme(
            legend.position = "none",
            axis.text.y = element_blank()
        )

    # Combine plots
    p_combined <- patchwork::wrap_plots(p_scores, p_dispersal, widths = c(2.5, 1))

    p_combined
```



We can see that, without correction (homogeneity only), increasing the value for the *mcl* parameter leads to increasingly larger scores. However, as homogeneity increases, the dispersal also increases. After correcting for dispersal, larger values for the *mcl* parameter do not lead to higher orthogroup scores.

To verify that formally, let's perform a Mann-Whitney U test for differences in orthogroup scores for runs with mcl of 3 and 5 with and without correcting for dispersal.

```
# Without dispersal
compare(og_homogeneity, "Score ~ Mode") |>
    filter(group1 == "3" & group2 == "5")
##   group1 group2    n1    n2 padj_greater padj_less padj_interpretation
## 1      3      5 23849 25595            1         0               less
##     effsize magnitude
## 1 0.3913194  moderate

# With dispersal
compare(og_homogeneity, "Score_c ~ Mode") |>
    filter(group1 == "3" & group2 == "5")
##   group1 group2    n1    n2 padj_greater padj_less padj_interpretation
## 1      3      5 23849 25595            0         1             greater
##     effsize magnitude
## 1 0.3591549  moderate
```

As expected, without correcting for dispersal, using $mcl = 5$ leads to better orthogroup scores than using $mcl = 3$. However, after correction, orthogroup scores for $mcl = 5$ are worse than scores for $mcl = 3$, which is desired.

Session info

This document was created under the following conditions:

```
## - Session info ---------------------------------------------------------------
##  setting  value
##  version  R version 4.3.0 (2023-04-21)
##  os       Ubuntu 20.04.5 LTS
##  system   x86_64, linux-gnu
##  ui       X11
##  language (EN)
##  collate  en_US.UTF-8
##  ctype    en_US.UTF-8
##  tz       Europe/Brussels
##  date     2023-10-06
##  pandoc   3.1.1 @ /usr/lib/rstudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
##
## - Packages -------------------------------------------------------------------
##  package          * version   date (UTC) lib source
##  abind              1.4-5     2016-07-21 [1] CRAN (R 4.3.0)
##  ape                5.7-1     2023-03-13 [1] CRAN (R 4.3.0)
##  aplot              0.1.10    2023-03-08 [1] CRAN (R 4.3.0)
##  backports          1.4.1     2021-12-13 [1] CRAN (R 4.3.0)
```

```
##  beeswarm           0.4.0     2021-06-01 [1] CRAN (R 4.3.0)
##  BiocGenerics       0.46.0    2023-04-25 [1] Bioconductor
##  BiocManager        1.30.21.1 2023-07-18 [1] CRAN (R 4.3.0)
##  BiocStyle        * 2.29.1    2023-08-04 [1] Github (Bioconductor/BiocStyle@7c0e093)
##  Biostrings         2.68.0    2023-04-25 [1] Bioconductor
##  bitops             1.0-7     2021-04-24 [1] CRAN (R 4.3.0)
##  bookdown           0.34      2023-05-09 [1] CRAN (R 4.3.0)
##  broom              1.0.4     2023-03-11 [1] CRAN (R 4.3.0)
##  car                3.1-2     2023-03-30 [1] CRAN (R 4.3.0)
##  carData            3.0-5     2022-01-06 [1] CRAN (R 4.3.0)
##  cli                3.6.1     2023-03-23 [1] CRAN (R 4.3.0)
##  codetools          0.2-19    2023-02-01 [4] CRAN (R 4.2.2)
##  cogeqc           * 1.4.0     2023-04-25 [1] Bioconductor
##  coin               1.4-2     2021-10-08 [1] CRAN (R 4.3.0)
##  colorspace         2.1-0     2023-01-23 [1] CRAN (R 4.3.0)
##  crayon             1.5.2     2022-09-29 [1] CRAN (R 4.3.0)
##  digest             0.6.33    2023-07-07 [1] CRAN (R 4.3.0)
##  dplyr            * 1.1.2     2023-04-20 [1] CRAN (R 4.3.0)
##  evaluate           0.21      2023-05-05 [1] CRAN (R 4.3.0)
##  fansi              1.0.4     2023-01-22 [1] CRAN (R 4.3.0)
##  farver             2.1.1     2022-07-06 [1] CRAN (R 4.3.0)
##  fastmap            1.1.1     2023-02-24 [1] CRAN (R 4.3.0)
##  forcats          * 1.0.0     2023-01-29 [1] CRAN (R 4.3.0)
##  generics           0.1.3     2022-07-05 [1] CRAN (R 4.3.0)
##  GenomeInfoDb       1.36.0    2023-04-25 [1] Bioconductor
##  GenomeInfoDbData   1.2.10    2023-04-28 [1] Bioconductor
##  ggbeeswarm         0.7.2     2023-04-29 [1] CRAN (R 4.3.0)
##  ggfun              0.0.9     2022-11-21 [1] CRAN (R 4.3.0)
##  ggplot2          * 3.4.1     2023-02-10 [1] CRAN (R 4.3.0)
##  ggplotify          0.1.0     2021-09-02 [1] CRAN (R 4.3.0)
##  ggpubr           * 0.6.0     2023-02-10 [1] CRAN (R 4.3.0)
##  ggsignif           0.6.4     2022-10-13 [1] CRAN (R 4.3.0)
##  ggtree             3.8.0     2023-04-25 [1] Bioconductor
##  glue               1.6.2     2022-02-24 [1] CRAN (R 4.3.0)
##  gridGraphics       0.5-1     2020-12-13 [1] CRAN (R 4.3.0)
##  gtable             0.3.3     2023-03-21 [1] CRAN (R 4.3.0)
##  here             * 1.0.1     2020-12-13 [1] CRAN (R 4.3.0)
##  hms                1.1.3     2023-03-21 [1] CRAN (R 4.3.0)
##  htmltools          0.5.5     2023-03-23 [1] CRAN (R 4.3.0)
##  igraph             1.4.2     2023-04-07 [1] CRAN (R 4.3.0)
##  IRanges            2.34.0    2023-04-25 [1] Bioconductor
##  jsonlite           1.8.7     2023-06-29 [1] CRAN (R 4.3.0)
##  knitr              1.43      2023-05-25 [1] CRAN (R 4.3.0)
##  labeling           0.4.2     2020-10-20 [1] CRAN (R 4.3.0)
##  lattice            0.20-45   2021-09-22 [4] CRAN (R 4.2.0)
##  lazyeval           0.2.2     2019-03-15 [1] CRAN (R 4.3.0)
##  libcoin            1.0-9     2021-09-27 [1] CRAN (R 4.3.0)
##  lifecycle          1.0.3     2022-10-07 [1] CRAN (R 4.3.0)
##  lubridate        * 1.9.2     2023-02-10 [1] CRAN (R 4.3.0)
##  magrittr           2.0.3     2022-03-30 [1] CRAN (R 4.3.0)
##  MASS               7.3-58.2  2023-01-23 [4] CRAN (R 4.2.2)
```

```
##  Matrix             1.5-1     2022-09-13 [4] CRAN (R 4.2.1)
##  matrixStats        1.0.0     2023-06-02 [1] CRAN (R 4.3.0)
##  modeltools         0.2-23    2020-03-05 [1] CRAN (R 4.3.0)
##  multcomp           1.4-25    2023-06-20 [1] CRAN (R 4.3.0)
##  munsell            0.5.0     2018-06-12 [1] CRAN (R 4.3.0)
##  mvtnorm            1.1-3     2021-10-08 [1] CRAN (R 4.3.0)
##  nlme               3.1-162   2023-01-31 [4] CRAN (R 4.2.2)
##  patchwork        * 1.1.2     2022-08-19 [1] CRAN (R 4.3.0)
##  pillar             1.9.0     2023-03-22 [1] CRAN (R 4.3.0)
##  pkgconfig          2.0.3     2019-09-22 [1] CRAN (R 4.3.0)
##  plyr               1.8.8     2022-11-11 [1] CRAN (R 4.3.0)
##  purrr            * 1.0.1     2023-01-10 [1] CRAN (R 4.3.0)
##  R6                 2.5.1     2021-08-19 [1] CRAN (R 4.3.0)
##  Rcpp               1.0.10    2023-01-22 [1] CRAN (R 4.3.0)
##  RCurl              1.98-1.12 2023-03-27 [1] CRAN (R 4.3.0)
##  readr            * 2.1.4     2023-02-10 [1] CRAN (R 4.3.0)
##  reshape2           1.4.4     2020-04-09 [1] CRAN (R 4.3.0)
##  rlang              1.1.1     2023-04-28 [1] CRAN (R 4.3.0)
##  rmarkdown          2.23      2023-07-01 [1] CRAN (R 4.3.0)
##  rprojroot          2.0.3     2022-04-02 [1] CRAN (R 4.3.0)
##  rstatix          * 0.7.2     2023-02-01 [1] CRAN (R 4.3.0)
##  rstudioapi         0.14      2022-08-22 [1] CRAN (R 4.3.0)
##  S4Vectors          0.38.0    2023-04-25 [1] Bioconductor
##  sandwich           3.0-2     2022-06-15 [1] CRAN (R 4.3.0)
##  scales             1.2.1     2022-08-20 [1] CRAN (R 4.3.0)
##  sessioninfo        1.2.2     2021-12-06 [1] CRAN (R 4.3.0)
##  stringi            1.7.12    2023-01-11 [1] CRAN (R 4.3.0)
##  stringr          * 1.5.0     2022-12-02 [1] CRAN (R 4.3.0)
##  survival           3.5-3     2023-02-12 [4] CRAN (R 4.2.2)
##  TH.data            1.1-2     2023-04-17 [1] CRAN (R 4.3.0)
##  tibble           * 3.2.1     2023-03-20 [1] CRAN (R 4.3.0)
##  tidyr            * 1.3.0     2023-01-24 [1] CRAN (R 4.3.0)
##  tidyselect         1.2.0     2022-10-10 [1] CRAN (R 4.3.0)
##  tidytree           0.4.2     2022-12-18 [1] CRAN (R 4.3.0)
##  tidyverse        * 2.0.0     2023-02-22 [1] CRAN (R 4.3.0)
##  timechange         0.2.0     2023-01-11 [1] CRAN (R 4.3.0)
##  treeio             1.24.1    2023-05-31 [1] Bioconductor
##  tzdb               0.3.0     2022-03-28 [1] CRAN (R 4.3.0)
##  utf8               1.2.3     2023-01-31 [1] CRAN (R 4.3.0)
##  vctrs              0.6.3     2023-06-14 [1] CRAN (R 4.3.0)
##  vipor              0.4.5     2017-03-22 [1] CRAN (R 4.3.0)
##  withr              2.5.0     2022-03-03 [1] CRAN (R 4.3.0)
##  xfun               0.39      2023-04-20 [1] CRAN (R 4.3.0)
##  XVector            0.40.0    2023-04-25 [1] Bioconductor
##  yaml               2.3.7     2023-01-23 [1] CRAN (R 4.3.0)
##  yulab.utils        0.0.6     2022-12-20 [1] CRAN (R 4.3.0)
##  zlibbioc           1.46.0    2023-04-25 [1] Bioconductor
##  zoo                1.8-12    2023-04-13 [1] CRAN (R 4.3.0)
##
##  [1] /home/faalm/R/x86_64-pc-linux-gnu-library/4.3
##  [2] /usr/local/lib/R/site-library
```

```
##  [3] /usr/lib/R/site-library
##  [4] /usr/lib/R/library
##
## ------------------------------------------------------------------------------
```

References

Emms, David M, and Steven Kelly. 2019. "OrthoFinder: Phylogenetic Orthology Inference for Comparative Genomics." *Genome Biology* 20 (1): 1–14.

Van Bel, Michiel, Francesca Silvestri, Eric M Weitz, Lukasz Kreft, Alexander Botzki, Frederik Coppens, and Klaas Vandepoele. 2022. "PLAZA 5.0: Extending the Scope and Power of Comparative and Functional Genomics in Plants." *Nucleic Acids Research* 50 (D1): D1468–74.