One-way row column design

Paul Schmidt

2023-11-16

One-way ANOVA & pairwise comparison post hoc tests in a resolvable row column design.

Table of contents


```
# (install &) load packages
pacman::p_load(
  conflicted,
  desplot,
  emmeans,
  ggtext,
  lme4,
  lmerTest,
  multcomp,
  multcompView,
```

```
  tidyverse)

# handle function conflicts
conflicts_prefer(dplyr::filter)
conflicts_prefer(dplyr::select)
conflicts_prefer(lmerTest::lmer)
```

1 Data

This example is taken from Chapter *"3.10 Analysis of a resolvable row-column design"* of the course material "Mixed models for metric data (3402-451)" by Prof. Dr. Hans-Peter Piepho. It considers data published in Kempton, Fox, and Cerezo (1996) from a yield trial laid out as a resolvable row-column design. The trial had 35 genotypes (`gen`), 2 complete replicates (`rep`) with 5 rows (`row`) and 7 columns (`col`). Thus, a complete replicate is subdivided into incomplete rows and columns.

1.1 Import

The data is available as part of the {agridat} package:

```
dat <- as_tibble(agridat::kempton.rowcol)
dat
```

```
# A tibble: 68 x 5
   rep     row   col gen   yield
   <fct> <int> <int> <fct> <dbl>
 1 R1        1     1 G20    3.77
 2 R1        1     2 G04    3.21
 3 R1        1     3 G33    4.55
 4 R1        1     4 G28    4.09
 5 R1        1     5 G07    5.05
 6 R1        1     6 G12    4.19
 7 R1        1     7 G30    3.27
 8 R1        2     1 G10    3.44
 9 R1        2     2 G14    4.3
10 R1        2     4 G21    3.86
# i 58 more rows
```

1.2 Format

For our analysis, `gen`, `row` and `col` should be encoded as factors. However, the `desplot()` function needs `row` and `col` as formatted as integers. Therefore we create copies of these columns encoded as factors and named `rowF` and `colF`:

```
dat <- dat %>%
  mutate(
    colF = as.factor(col),
    rowF = as.factor(row)
  )
```

1.3 Explore

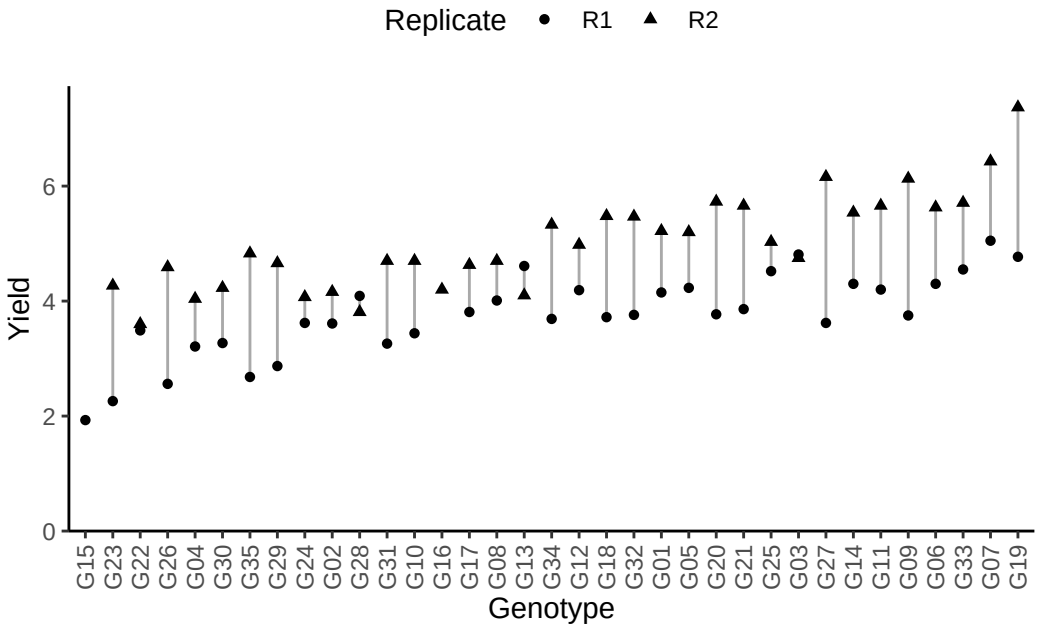
We make use of `dlookr::describe()` to conveniently obtain descriptive summary tables. Here, we get can summarize per block and per cultivar.

```
dat %>%
  group_by(gen) %>%
  dlookr::describe(yield) %>%
  select(2:sd) %>%
  arrange(desc(mean))
```

```
# A tibble: 35 x 5
   gen       n    na  mean     sd
   <fct> <int> <int> <dbl>  <dbl>
 1 G19       2     0  6.07 1.84
 2 G07       2     0  5.74 0.976
 3 G33       2     0  5.13 0.820
 4 G06       2     0  4.96 0.940
 5 G09       2     0  4.94 1.68
 6 G11       2     0  4.93 1.03
 7 G14       2     0  4.92 0.877
 8 G27       2     0  4.89 1.80
 9 G03       2     0  4.78 0.0424
10 G25       2     0  4.78 0.361
# i 25 more rows
```

Additionally, we can decide to plot our data.

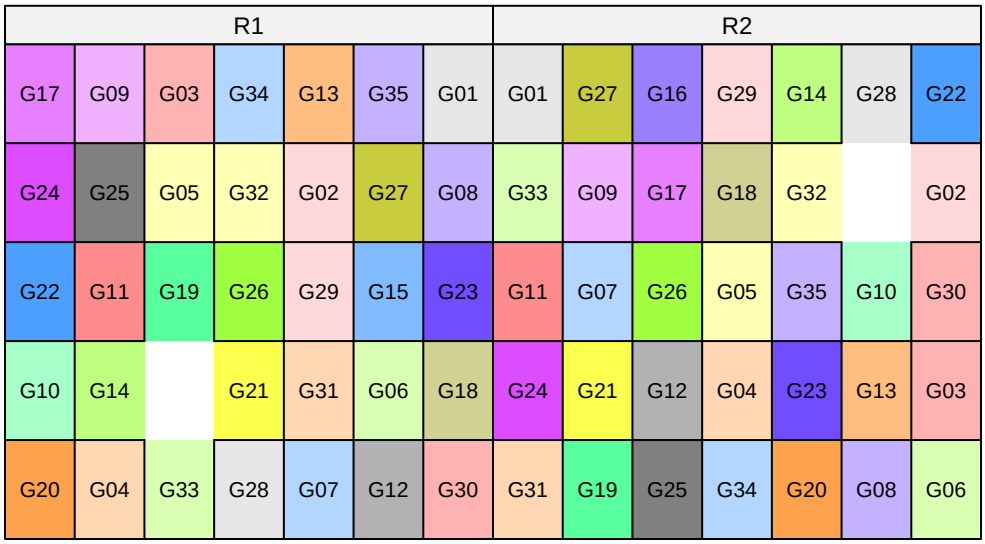
```r
# sort genotypes by mean yield
gen_order <- dat %>%
  group_by(gen) %>%
  summarise(mean = mean(yield, na.rm = TRUE)) %>%
  arrange(mean) %>%
  pull(gen) %>%
  as.character()

ggplot(data = dat) +
  aes(
    y = yield,
    x = gen,
    shape = rep
  ) +
  geom_line(
    aes(group = gen),
    color = "darkgrey"
  ) +
  geom_point() +
  scale_x_discrete(
    name = "Genotype",
    limits = gen_order
  ) +
  scale_y_continuous(
    name = "Yield",
    limits = c(0, NA),
    expand = expansion(mult = c(0, 0.05))
  ) +
  scale_shape_discrete(
    name = "Replicate"
  ) +
  guides(shape = guide_legend(nrow = 1)) +
  theme_classic() +
  theme(
    legend.position = "top",
    axis.text.x = element_text(angle = 90, vjust = 0.5)
  )
```



Finally, since this is an experiment that was laid with a certain experimental design (= a resolvable row column design) - it makes sense to also get a field plan. This can be done via `desplot()` from {desplot}. In this case it is worth noting that there is missing data, as yield values for two plots are not present in the data.

```
desplot(
  data = dat,
  form = gen ~ col + row | rep, # fill color per genotype, headers per replicate
  text = gen,
  cex = 0.7,
  shorten = "no",
  out1 = row, out1.gpar=list(col="black"), # lines between rows
  out2 = col, out2.gpar=list(col="black"), # lines between columns
  main = "Field layout",
  show.key = FALSE
)
```

Field layout



2 Model

Finally, we can decide to fit a linear model with `yield` as the response variable and `gen` as fixed effects, since our goal is to compare them to each other. Since the trial was laid out in rows and columns, we also need `rowF` and `colF` effects in the model, but these can be taken either as a fixed or as random effects. Since our goal is to compare genotypes, we will determine which of the two models we prefer by comparing the average standard error of a difference (s.e.d.) for the comparisons between adjusted genotype means - the lower the s.e.d. the better.

```
# blocks as fixed (linear model)
mod_frc <- lm(yield ~ gen + rep + rowF + colF,
              data = dat)

avg_sed_mod_frc <- mod_frc %>%
  emmeans(pairwise ~ "gen",
          adjust = "none") %>%
  pluck("contrasts") %>% # extract diffs
  as_tibble() %>% # format to table
  pull("SE") %>% # extract s.e.d. column
  mean() # get arithmetic mean
```

```
avg_sed_mod_frc
```

```
[1] 0.4828268
```

```
# blocks as random (linear mixed model)
mod_rrc <- lmer(yield ~ gen + rep + (1 | rowF) + (1 | colF),
                data = dat)

avg_sed_mod_rrc <- mod_rrc %>%
  emmeans(pairwise ~ "gen",
          adjust = "none",
          lmer.df = "kenward-roger") %>%
  pluck("contrasts") %>% # extract diffs
  as_tibble() %>% # format to table
  pull("SE") %>% # extract s.e.d. column
  mean() # get arithmetic mean

avg_sed_mod_rrc
```

```
[1] 0.4834463
```

As a result, we find that the model with fixed row and column effects has the slightly smaller s.e.d. and is therefore more precise in terms of comparing genotypes.

⚠ Model assumptions met? (click to show)

It would be at this moment (i.e. after fitting the model and before running the ANOVA), that you should check whether the model assumptions are met. Find out more in the summary article "Model Diagnostics"

3 ANOVA

Based on our model, we can then conduct an ANOVA:

```
ANOVA <- anova(mod_frc)
ANOVA
```

```
Analysis of Variance Table

Response: yield
          Df Sum Sq Mean Sq  F value    Pr(>F)
gen       34 32.157  0.9458   5.6767 3.505e-05 ***
rep        1 24.901 24.9014 149.4615 2.778e-11 ***
rowF       4  1.436  0.3591   2.1553  0.107861
colF       6  4.794  0.7990   4.7956  0.002873 **
Residuals 22  3.665  0.1666
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Accordingly, the ANOVA's F-test did not find the cultivar effects to be statistically significant ($p < .001^{***}$).

4 Mean comparison

Besides an ANOVA, one may also want to compare adjusted yield means between cultivars via post hoc tests (t-test, Tukey test etc.).

```
mean_comp <- mod_frc %>%
  emmeans(specs = ~ gen) %>% # adj. mean per genotype
  cld(adjust = "none", Letters = letters) # compact letter display (CLD)

mean_comp
```

```
 gen emmean    SE df lower.CL upper.CL .group
 G15   3.29 0.475 22     2.31     4.28  ab
 G04   3.35 0.326 22     2.67     4.03  a
 G23   3.54 0.323 22     2.87     4.21  ab
 G29   3.57 0.323 22     2.90     4.25  ab
 G16   3.59 0.470 22     2.61     4.56  abcde
 G26   3.63 0.350 22     2.90     4.35  abc
 G02   3.71 0.347 22     2.99     4.43  abcd
 G22   3.79 0.327 22     3.12     4.47  abcde
 G24   3.82 0.351 22     3.09     4.54  abcdef
 G31   3.82 0.322 22     3.15     4.49  abcdef
 G35   3.94 0.324 22     3.27     4.61  abcdefg
 G17   4.07 0.326 22     3.39     4.75  abcdefgh
 G28   4.12 0.320 22     3.45     4.78  abcdefgh
```

```
 G32   4.15 0.347 22     3.43     4.87  abcdefgh
 G30   4.18 0.352 22     3.45     4.91  abcdefgh
 G09   4.24 0.352 22     3.51     4.98  abcdefghi
 G25   4.28 0.326 22     3.60     4.95   bcdefghi
 G34   4.30 0.349 22     3.58     5.03   bcdefghij
 G20   4.32 0.337 22     3.62     5.02   bcdefghij
 G10   4.56 0.323 22     3.90     5.23    cdefghij
 G05   4.60 0.325 22     3.93     5.28     defghijk
 G14   4.61 0.322 22     3.94     5.28    cdefghij
 G13   4.66 0.322 22     3.99     5.32    cdefghij
 G11   4.69 0.347 22     3.98     5.41     defghijk
 G08   4.70 0.326 22     4.03     5.38      efghij
 G21   4.75 0.344 22     4.04     5.47     defghijk
 G27   4.80 0.323 22     4.13     5.48       fghijkl
 G18   4.84 0.322 22     4.18     5.51        ghijkl
 G33   4.88 0.325 22     4.21     5.56        ghijkl
 G01   4.88 0.343 22     4.17     5.60        ghijkl
 G12   4.98 0.322 22     4.31     5.65         hijkl
 G03   5.20 0.321 22     4.53     5.86          ijkl
 G07   5.21 0.323 22     4.54     5.88           jkl
 G06   5.59 0.326 22     4.92     6.27            kl
 G19   5.73 0.326 22     5.06     6.41             l

Results are averaged over the levels of: rep, rowF, colF
Confidence level used: 0.95
significance level used: alpha = 0.05
NOTE: If two or more means share the same grouping symbol,
      then we cannot show them to be different.
      But we also did not show them to be the same.
```

It can be seen that the compact letter display is kind of reaching its limit as the way differences are found to be statistically significant here is quite complex.

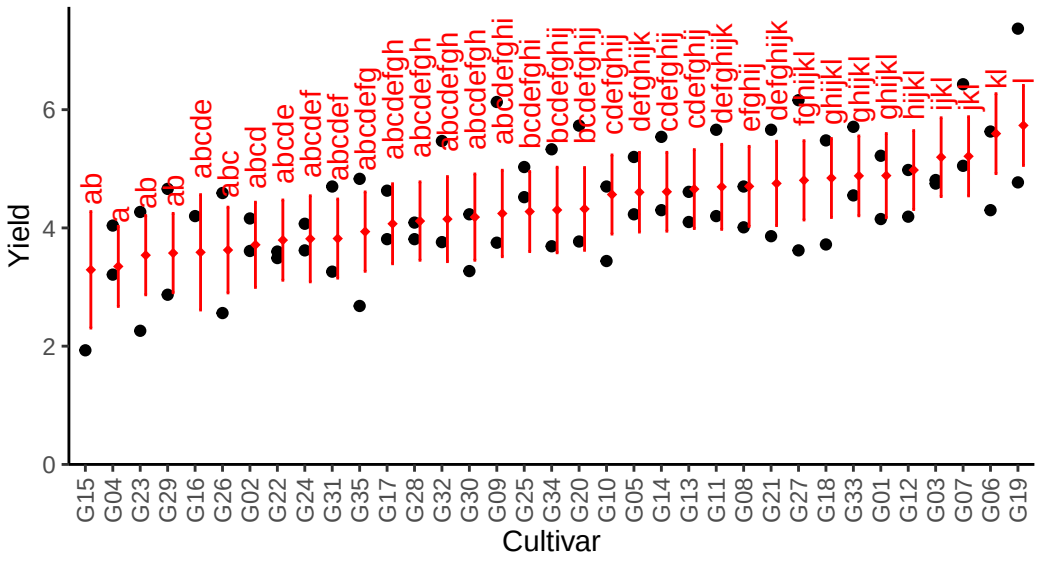
Note that if you would like to see the underlying individual contrasts/differences between adjusted means, simply add `details = TRUE` to the `cld()` statement. Furthermore, check out the Summary Article "Compact Letter Display".

Finally, we can create a plot that displays both the raw data and the results, *i.e.* the comparisons of the adjusted means that are based on the linear model.

```
# reorder genotype factor levels according to adjusted mean
my_caption <- "Black dots represent raw data. Red diamonds and error bars represent adjust
```

```r
ggplot() +
  # green/red dots representing the raw data
  geom_point(
    data = dat,
    aes(y = yield, x = gen)
  ) +
  # red diamonds representing the adjusted means
  geom_point(
    data = mean_comp,
    aes(y = emmean, x = gen),
    shape = 18,
    color = "red",
    position = position_nudge(x = 0.2)
  ) +
  # red error bars representing the confidence limits of the adjusted means
  geom_errorbar(
    data = mean_comp,
    aes(ymin = lower.CL, ymax = upper.CL, x = gen),
    color = "red",
    width = 0.1,
    position = position_nudge(x = 0.2)
  ) +
  # red letters
  geom_text(
    data = mean_comp,
    aes(y = upper.CL, x = gen, label = str_trim(.group)),
    color = "red",
    angle = 90,
    hjust = -0.2,
    position = position_nudge(x = 0.2)
  ) +
  scale_x_discrete(
    name = "Cultivar",
    limits = as.character(mean_comp$gen)
  ) +
  scale_y_continuous(
    name = "Yield",
    # limits = c(0, NA),
    expand = expansion(mult = c(0, 0.05))
  ) +
  coord_cartesian(ylim = c(0, NA)) +
```

```
  labs(caption = my_caption) +
  theme_classic() +
  theme(plot.caption = element_textbox_simple(margin = margin(t = 5)),
        plot.caption.position = "plot",
        axis.text.x = element_text(angle = 90, vjust = 0.5))
```



Black dots represent raw data. Red diamonds and error bars represent adjusted means with 95% confidence limits per cultivar. Means followed by a common letter are not significantly different according to the t−test.

5 Bonus

Here are some other things you would maybe want to look at for the analysis of this dataset.

5.1 Variance components

To extract variance components from our models, we unfortunately need different functions per model since only of of them is a mixed model and we used different functions to fit them.

```
# Residual Variance
summary(mod_frc)$sigma^2
```

```
[1] 0.1666074
```

```
# Both Variance Components
as_tibble(VarCorr(mod_rrc))
```

```
# A tibble: 3 x 5
  grp      var1        var2    vcov sdcor
  <chr>    <chr>       <chr>  <dbl> <dbl>
1 colF     (Intercept) <NA>  0.144  0.380
2 rowF     (Intercept) <NA>  0.0293 0.171
3 Residual <NA>        <NA>  0.167  0.409
```

5.2 Efficiency

The *efficiency of a resolvable design* can be calculated as its mean s.e.d. compared to the (mean[1]) s.e.d. of the analogous RCBD, i.e. leaving out the incomplete block effects within the replicates. Above, we have already calculated the mean s.e.d. of our resolvable design so we can square it and get `avg_sed_mod_frc^2` which is 0.23312. Accordingly, we can fit a model leaving out the incomplete block effects and get the s.e.d. just like before and also square it:

```
avg_sed_mod_RCBD <- lm(yield ~ gen + rep, data = dat) %>%
  emmeans(pairwise ~ "gen",
          adjust = "none",
          lmer.df = "kenward-roger") %>%
  pluck("contrasts") %>% # extract diffs
  as_tibble() %>% # format to table
  pull("SE") %>% # extract s.e.d. column
  mean()

avg_sed_mod_RCBD^2
```

```
[1] 0.3257469
```

Finally, the efficiency of this resolvable design is then

```
avg_sed_mod_RCBD^2 / avg_sed_mod_frc^2
```

```
[1] 1.397325
```

meaning that the resolvable design is more efficient since the efficiency is > 1.

[1] In this scenario, all s.e.d. of the RCBD model would be identical so we don't really need to get the average, but could instead argue that there is only one constant s.e.d.

Kempton, R. A., P. N. Fox, and M. Cerezo. 1996. *Statistical Methods for Plant Variety Evaluation*. Springer Netherlands. https://doi.org/10.1007/978-94-009-1503-9.