

One-way row column design

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2023-11-16

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

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```
# (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
```

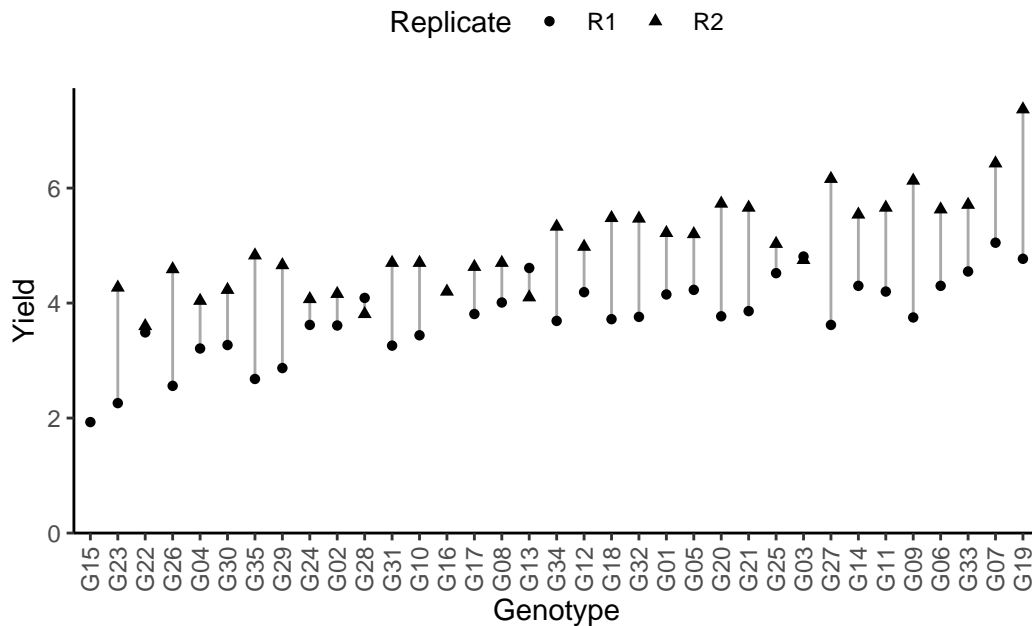
Additionally, we can decide to plot our data.

```

# 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

R1							R2						
G17	G09	G03	G34	G13	G35	G01	G01	G27	G16	G29	G14	G28	G22
G24	G25	G05	G32	G02	G27	G08	G33	G09	G17	G18	G32		G02
G22	G11	G19	G26	G29	G15	G23	G11	G07	G26	G05	G35	G10	G30
G10	G14		G21	G31	G06	G18	G24	G21	G12	G04	G23	G13	G03
G20	G04	G33	G28	G07	G12	G30	G31	G19	G25	G34	G20	G08	G06

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.

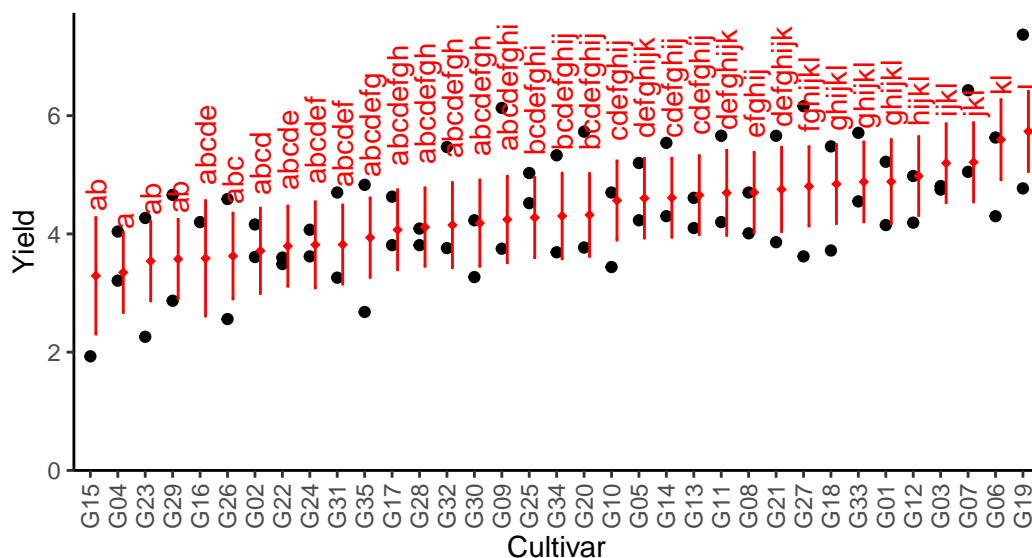
```
# reorder genotype factor levels according to adjusted mean
my_caption <- "Black dots represent raw data. Red diamonds and error bars represent adjust"
```

```

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 (mean¹) 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 .

¹In 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>.