

Two-way split-plot design

Paul Schmidt

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Two-way ANOVA & pairwise comparison post hoc tests in a split-plot design.

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

# handle function conflicts
conflicts_prefer(dplyr::filter)
```

```
conflicts_prefer(dplyr::select)
conflicts_prefer(lmerTest::lmer)
```

1 Data

This dataset was originally published in Gomez and Gomez (1984) from a yield (kg/ha) trial with 4 genotypes (**G**) and 6 nitrogen levels (**N**), leading to 24 treatment level combinations. The data set here has 3 complete replicates (**rep**) and is laid out as a split-plot design.

1.1 Import

```
# data is available online:
path <- "https://raw.githubusercontent.com/SchmidtPaul/dsfair_quarto/master/data/Gomez&Gom

dat <- read_csv(path) # use path from above
dat

# A tibble: 72 x 7
  yield    row    col rep  mainplot G      N
  <dbl> <dbl> <dbl> <chr> <chr>    <chr> <chr>
1  4520     4     1 rep1  mp01    Simba Goomba
2  5598     2     2 rep1  mp02    Simba Koopa
3  6192     1     3 rep1  mp03    Simba Toad
4  8542     2     4 rep1  mp04    Simba Peach
5  5806     2     5 rep1  mp05    Simba Diddy
6  7470     1     6 rep1  mp06    Simba Yoshi
7  4034     2     1 rep1  mp01    Nala   Goomba
8  6682     4     2 rep1  mp02    Nala   Koopa
9  6869     3     3 rep1  mp03    Nala   Toad
10 6318     4     4 rep1  mp04    Nala   Peach
# i 62 more rows
```

1.2 Format

Before anything, the columns **rep**, **N** and **G** should be encoded as factors, since R by default encoded them as character.

```
dat <- dat %>%
  mutate(across(c(rep:N), ~ as.factor(.x)))
```

1.3 Explore

We make use of `dlookr::describe()` to conveniently obtain descriptive summary tables. Here, we get can summarize per nitrogen level, per genotype and also per nitrogen-genotype-combination.

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

```
# A tibble: 6 x 5
```

	N	n	na	mean	sd
	<fct>	<int>	<int>	<dbl>	<dbl>
1	Diddy	12	0	5866.	832.
2	Toad	12	0	5864.	1434.
3	Yoshi	12	0	5812	2349.
4	Peach	12	0	5797.	2660.
5	Koopa	12	0	5478.	657.
6	Goomba	12	0	4054.	672.

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

```
# A tibble: 4 x 5
```

	G	n	na	mean	sd
	<fct>	<int>	<int>	<dbl>	<dbl>
1	Simba	18	0	6554.	1475.
2	Nala	18	0	6156.	1078.
3	Timon	18	0	5563.	1269.
4	Pumba	18	0	3642.	1434.

```

dat %>%
  group_by(N, G) %>%
  dlookr::describe(yield) %>%
  select(2:sd) %>%
  arrange(desc(mean)) %>%
  print(n=Inf)

# A tibble: 24 x 6
   N      G      n    na mean    sd
   <fct> <fct> <int> <int> <dbl> <dbl>
1 Peach Simba     3     0 8701.  270.
2 Yoshi Simba     3     0 7563.   86.9
3 Yoshi Nala      3     0 6951.  808.
4 Toad  Nala      3     0 6895.  166.
5 Toad  Simba     3     0 6733.  490.
6 Yoshi Timon     3     0 6687.  496.
7 Peach Nala      3     0 6540.  936.
8 Diddy Simba     3     0 6400.  523.
9 Diddy Nala      3     0 6259.  499.
10 Peach Timon     3     0 6065. 1097.
11 Toad  Timon     3     0 6014.  515.
12 Diddy Timon     3     0 5994.  101.
13 Koopa Nala      3     0 5982.  684.
14 Koopa Simba     3     0 5672.  458.
15 Koopa Timon     3     0 5443.  589.
16 Koopa Pumba     3     0 4816.  506.
17 Diddy Pumba     3     0 4812.  963.
18 Goomba Pumba     3     0 4481.  463.
19 Goomba Nala      3     0 4306.  646.
20 Goomba Simba     3     0 4253.  248.
21 Toad  Pumba     3     0 3816. 1311.
22 Goomba Timon     3     0 3177.  453.
23 Yoshi Pumba     3     0 2047.  703.
24 Peach Pumba     3     0 1881.  407.

```

Additionally, we can decide to plot our data. One way to deal with the combination of two factors would be to use [panels/facets in ggplot2](#).

Note that we here define a custom set of colors for the Nitrogen levels that will be used throughout this chapter.

```

Ncolors <- met.brewer("VanGogh2", 6) %>%
  as.vector() %>%
  set_names(levels(dat$N))

ggplot(data = dat) +
  aes(y = yield, x = N, color = N) +
  facet_wrap(~G, labeller = label_both) +
  stat_summary(
    fun = mean,
    colour = "grey",
    geom = "line",
    linetype = "dotted",
    group = 1
  ) +
  geom_point() +
  scale_x_discrete(
    name = "Nitrogen"
  ) +
  scale_y_continuous(
    name = "Yield",
    limits = c(0, NA),
    expand = expansion(mult = c(0, 0.1))
  ) +
  scale_color_manual(
    values = Ncolors,
    guide = "none"
  ) +
  theme_bw() +
  theme(axis.text.x = element_text(
    angle = 45,
    hjust = 1,
    vjust = 1
  ))

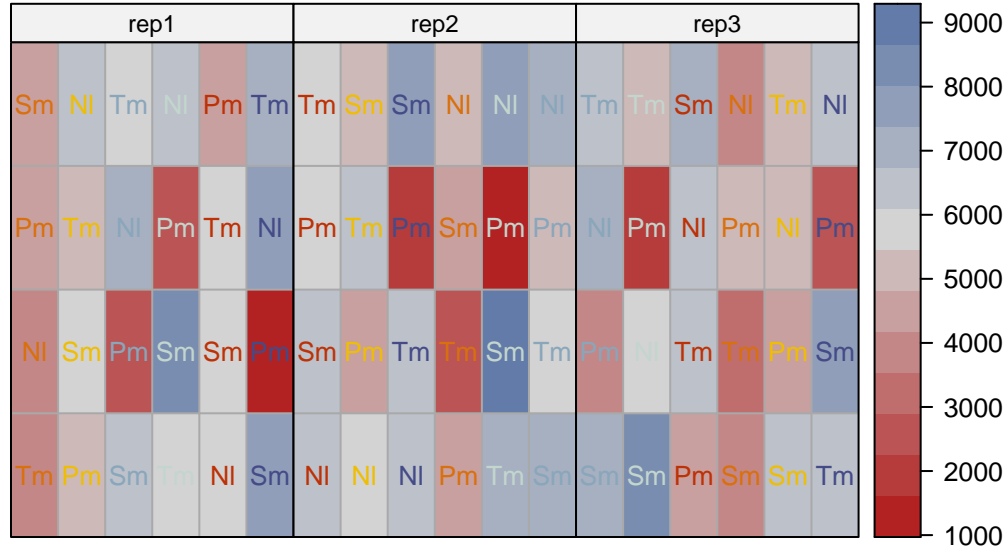
```



Finally, since this is an experiment that was laid with a certain experimental design (= a split-plot design) - it makes sense to also get a field plan. This can be done via `desplot()` from `{desplot}`.

```
desplot(
  data = dat,
  form = rep ~ col + row | rep, # fill color per rep, headers per rep
  col.regions = c("white", "grey95", "grey90"),
  text = G, # genotype names per plot
  cex = 0.8, # genotype names: font size
  shorten = "abb", # genotype names: abbreviate
  col = N, # color of genotype names for each N-level
  col.text = Ncolors, # use custom colors from above
  out1 = col, out1.gpar = list(col = "darkgrey"), # lines between columns
  out2 = row, out2.gpar = list(col = "darkgrey"), # lines between rows
  main = "Field layout", # plot title
  show.key = TRUE, # show legend
  key.cex = 0.7 # legend font size
)
```

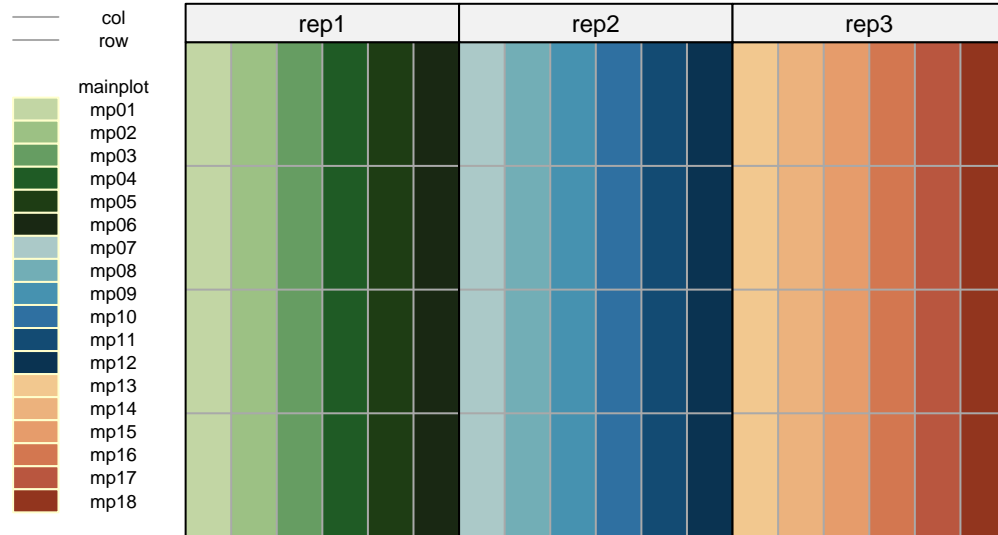

Yield per plot



```
mainplotcolors <- c(met.brewer("VanGogh3", 6),
                    met.brewer("Hokusai2", 6),
                    met.brewer("OKeefe2", 6)) %>%
  as.vector() %>%
  set_names(levels(dat$mainplot))

desplot(
  data = dat,
  form = mainplot ~ col + row | rep, # fill color per rep, headers per rep
  col.regions = mainplotcolors,
  out1 = col, out1.gpar = list(col = "darkgrey"), # lines between columns
  out2 = row, out2.gpar = list(col = "darkgrey"), # lines between rows
  main = "Experimental design focus", # plot title
  show.key = TRUE, # don't show legend
  key.cex = 0.6
)
```


Experimental design focus



2 Model

Finally, we can decide to fit a linear model with yield as the response variable. In this example it makes sense to mentally group the effects in our model as either *design effects* or *treatment effects*. The treatments here are the genotypes G and the nitrogen levels N which we will include in the model as main effects, but also via their interaction effect N:G. Regarding the design, the model needs to contain a block (**rep**) effect representing the three complete blocks. Additionally, there should also be random effects for the 18 mainplots, since they represent additional randomization units.

```
mod <- lmer(yield ~ G + N + G:N +
            rep + (1 | rep:mainplot),
            data = dat)
```

⚠ 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)
ANOVA

Type III Analysis of Variance Table with Satterthwaite's method
      Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
G    89885051 29961684     3    36 85.7416 < 2.2e-16 ***
N    19192886  3838577     5    10 10.9849 0.0008277 ***
rep    683088   341544     2    10  0.9774 0.4095330
G:N  69378044  4625203    15    36 13.2360 2.078e-10 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Accordingly, the ANOVA's F-test found the nitrogen-genotype-interaction 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.). Especially because of the results of this ANOVA, we should compare means for all N:G interactions and **not** for the N and/or G main effects. When doing so, we still have multiple options to choose from. I here decide to compare all genotype means per nitrogen

```
mean_comp <- mod %>%
  emmeans(specs = ~ N|G) %>% # adj. mean per cultivar
  cld(adjust = "Tukey", Letters = letters) # compact letter display (CLD)
```

```
mean_comp
```

```
G = Nala:
  N      emmean SE   df lower.CL upper.CL .group
Goomba  4306 366 41.9    3297    5315    a
Koopa   5982 366 41.9    4973    6991    b
Diddy   6259 366 41.9    5250    7268    b
Peach   6540 366 41.9    5531    7550    b
Toad    6895 366 41.9    5886    7904    b
```

Yoshi	6951	366	41.9	5941	7960	b
-------	------	-----	------	------	------	---

G = Pumba:

N	emmean	SE	df	lower.CL	upper.CL	.group
Peach	1881	366	41.9	871	2890	a
Yoshi	2047	366	41.9	1037	3056	a
Toad	3816	366	41.9	2807	4825	b
Goomba	4481	366	41.9	3472	5491	b
Diddy	4812	366	41.9	3803	5821	b
Koopa	4816	366	41.9	3807	5825	b

G = Simba:

N	emmean	SE	df	lower.CL	upper.CL	.group
Goomba	4253	366	41.9	3243	5262	a
Koopa	5672	366	41.9	4663	6681	ab
Diddy	6400	366	41.9	5391	7409	bc
Toad	6733	366	41.9	5723	7742	bc
Yoshi	7563	366	41.9	6554	8573	cd
Peach	8701	366	41.9	7691	9710	d

G = Timon:

N	emmean	SE	df	lower.CL	upper.CL	.group
Goomba	3177	366	41.9	2168	4187	a
Koopa	5443	366	41.9	4433	6452	b
Diddy	5994	366	41.9	4985	7003	b
Toad	6014	366	41.9	5005	7023	b
Peach	6065	366	41.9	5056	7075	b
Yoshi	6687	366	41.9	5678	7697	b

Results are averaged over the levels of: rep

Degrees-of-freedom method: kenward-roger

Confidence level used: 0.95

Conf-level adjustment: sidak method for 6 estimates

P value adjustment: tukey method for comparing a family of 6 estimates

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.

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.

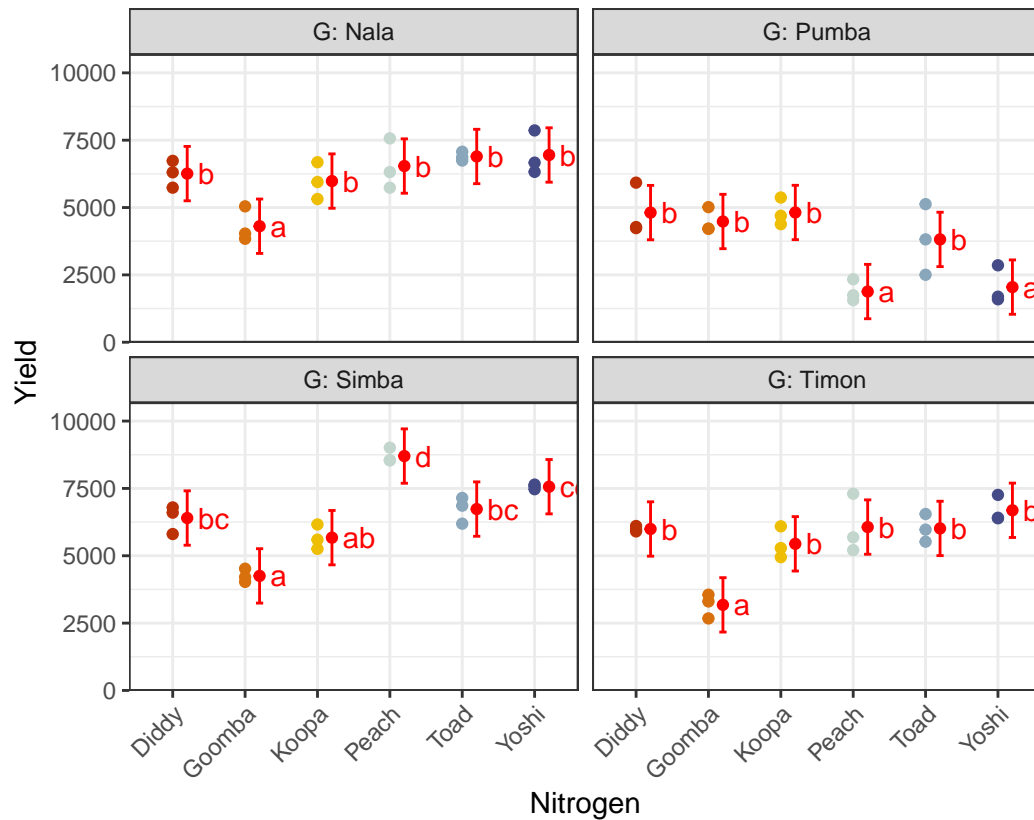
```
my_caption <- "The four facettes represent genotypes Simba, Nala, Timon and Pumba. Black d

ggplot() +
  facet_wrap(~G, labeller = label_both) + # facette per G level
  aes(x = N) +
  # black dots representing the raw data
  geom_point(
    data = dat,
    aes(y = yield, color = N)
  ) +
  # red dots representing the adjusted means
  geom_point(
    data = mean_comp,
    aes(y = emmean),
    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),
    color = "red",
    width = 0.1,
    position = position_nudge(x = 0.2)
  ) +
  # red letters
  geom_text(
    data = mean_comp,
    aes(y = emmean, label = str_trim(.group)),
    color = "red",
    position = position_nudge(x = 0.35),
    hjust = 0
  ) +
  scale_x_discrete(
    name = "Nitrogen"
  ) +
  scale_y_continuous(
    name = "Yield",
    limits = c(0, NA),
```

```

    expand = expansion(mult = c(0, 0.1))
  ) +
  scale_color_manual(
    values = Ncolors,
    guide = "none"
  ) +
  theme_bw() +
  labs(caption = my_caption) +
  theme(
    plot.caption = element_textbox_simple(margin = margin(t = 5)),
    plot.caption.position = "plot",
    axis.text.x = element_text(
      angle = 45,
      hjust = 1,
      vjust = 1
    )
  )
)

```



The four facettes represent genotypes Simba, Nala, Timon and Pumba. Black dots represent raw data. Red dots and error bars represent adjusted means with 95% confidence limits per cultivar. For each genotype separately, means followed by a common letter are not significantly different according to the Tukey-test.

Gomez, Kwanchai A, and Arturo A Gomez. 1984. *Statistical Procedures for Agricultural Research*. 2nd ed. An International Rice Research Institute Book. Nashville, TN: John Wiley & Sons.