

6. One-way ANOVA in a Row-Column Design

Analysis of Variance (ANOVA); Resolvable Row-Column Design

Dr. Paul Schmidt

To install and load all the packages used in this chapter, run the following code:

```
for (pkg in c("agridat", "desplot", "emmeans", "ggtext", "here", "lme4",
            "lmerTest", "multcomp", "multcompView", "tidyverse")) {
  if (!require(pkg, character.only = TRUE)) install.packages(pkg)
}

library(agridat)
library(desplot)
library(emmeans)
library(ggtext)
library(here)
library(lme4)
library(lmerTest)
library(multcomp)
library(multcompView)
library(tidyverse)
```

Row-Column Designs

In Chapter 3, we encountered the Latin Square design, which controls for two sources of variation (rows and columns) simultaneously. However, the Latin Square has a major limitation: the number of treatments must equal the number of rows and columns. This makes it impractical for experiments with many treatments.

What is a Row-Column Design?

A **resolvable row-column design** extends the Latin Square concept to accommodate more treatments. Like an alpha design, it has complete replicates that are subdivided - but here each replicate is subdivided into both incomplete rows and incomplete columns. This provides double blocking within each replicate.

The key features are:

1. **Two-dimensional blocking:** Each replicate has both row and column structure
2. **Incomplete blocks:** Neither rows nor columns contain all treatments
3. **Resolvability:** Replicates are complete, containing each treatment exactly once
4. **Flexibility:** Can accommodate various numbers of treatments

The advantages include:

1. **Control of two gradients:** Accounts for spatial trends in two directions simultaneously
2. **More treatments than Latin Square:** Not limited to txt arrangements
3. **Increased precision:** Double blocking can substantially reduce experimental error
4. **Practical for field trials:** Matches the rectangular layout of many field experiments

Data

This example considers data published in R. A. Kempton, P. N. Fox, and M. Cerezo [1] 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`) each. Thus, each replicate forms a 5×7 grid with incomplete rows and columns.

Import

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

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

# A tibble: 68 × 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
```

The dataset contains:

- `rep`: Two complete replicates (R1, R2)
- `row`: Row position within replicate (1-5)
- `col`: Column position within replicate (1-7)
- `gen`: 35 genotypes
- `yield`: Crop yield

Note that there are missing values in this dataset - two plots have no yield recorded.

Format

For our analysis, `gen` should be encoded as a factor. We also create factor versions of `row` and `col` for the statistical model:

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

dat

# A tibble: 68 × 7
  rep     row     col gen   yield rowF   colF
  <fct> <int> <int> <fct> <dbl> <fct> <fct>
1 R1      1       1 G20  3.77  1       1
2 R1      1       2 G04  3.21  1       2
```

```

3 R1      1      3 G33    4.55 1      3
4 R1      1      4 G28    4.09 1      4
5 R1      1      5 G07    5.05 1      5
6 R1      1      6 G12    4.19 1      6
7 R1      1      7 G30    3.27 1      7
8 R1      2      1 G10    3.44 2      1
9 R1      2      2 G14    4.3  2      2
10 R1     2      4 G21   3.86 2      4
# i 58 more rows

```

Explore

Let's examine the summary statistics by genotype:

```

dat %>%
  group_by(gen) %>%
  summarise(
    count = n(),
    mean_yield = mean(yield, na.rm = TRUE),
    sd_yield = sd(yield, na.rm = TRUE)
  ) %>%
  arrange(desc(mean_yield))

```

```

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

```

Most genotypes appear twice (once per replicate), but some have only one observation due to missing data. Let's visualize the 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()

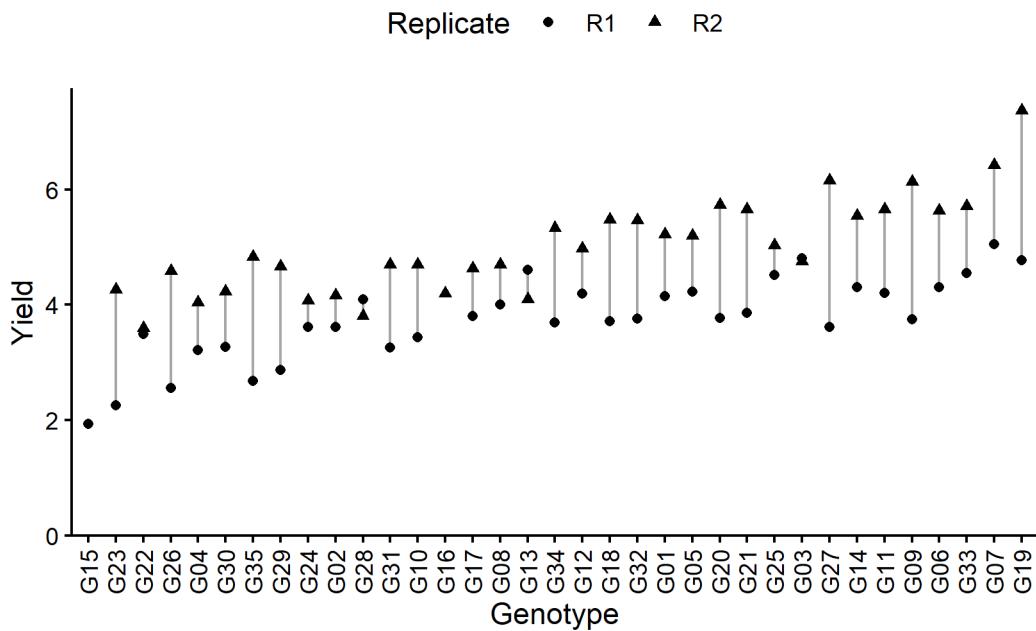
ggplot(data = dat) +
  aes(
    y = yield,
    x = gen,
    shape = rep
  ) +
  geom_line(
    aes(group = gen),
    color = "darkgrey"
  ) +
  geom_point() +
  scale_x_discrete(
    name = "Genotype",
    limits = gen_order
  ) +
  scale_y_continuous(

```

```

  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)
)
)

```



Now let's look at the field layout. Note that the two missing plots will appear as white/empty:

```

desplot(
  data = dat,
  form = gen ~ col + row | rep, # fill color per genotype, panels per replicate
  text = gen,
  cex = 0.7,
  shorten = FALSE,
  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

The black lines show the row and column structure within each replicate. Each genotype appears once per replicate, but in different row-column positions.

Model and ANOVA

Choosing Between Fixed and Random Effects

For a row-column design, the model needs to account for row and column effects within each replicate. We can treat these as either fixed or random effects. Let's compare both approaches:

```
# Rows and columns as fixed effects
mod_fixed <- lm(yield ~ gen + rep + rep:rowF + rep:colF,
                  data = dat)

# Rows and columns as random effects
mod_random <- lmer(yield ~ gen + rep + (1 | rep:rowF) + (1 | rep:colF),
                     data = dat)
```

Now compare the average s.e.d. for genotype comparisons:

```
# s.e.d. for fixed model
sed_fixed <- mod_fixed %>%
  emmeans(pairwise ~ "gen", adjust = "none") %>%
  pluck("contrasts") %>%
  as_tibble() %>%
  pull("SE") %>%
  mean()
```

NOTE: A nesting structure was detected in the fitted model:
 rowF %in% rep, colF %in% rep

```
# s.e.d. for random model
sed_random <- mod_random %>%
  emmeans(pairwise ~ "gen", adjust = "none", lmer.df = "kenward-roger") %>%
  pluck("contrasts") %>%
  as_tibble() %>%
  pull("SE") %>%
  mean()

tibble(
  model = c("Fixed row/col", "Random row/col"),
  mean_sed = c(sed_fixed, sed_random)
)
```

```
# A tibble: 2 × 2
  model      mean_sed
  <chr>      <dbl>
1 Fixed row/col  0.408
2 Random row/col  0.402
```

In this case, the fixed effects model has a slightly smaller s.e.d., so we'll use it for our analysis.

⚠ Model assumptions met?

It is at this point (i.e. after fitting the model and before interpreting the ANOVA) that one should check whether the model assumptions are met. Find out more in Appendix A1: Model Diagnostics.

Conducting the ANOVA

```
ANOVA <- anova(mod_fixed)
ANOVA
```

```
Analysis of Variance Table

Response: yield
            Df  Sum Sq Mean Sq  F value    Pr(>F)
gen          34 32.157  0.9458 10.7456 4.969e-05 ***
rep          1 24.901 24.9014 282.9193 1.042e-09 ***
rep:rowF     8  2.512  0.3140   3.5680  0.023647 *
rep:colF    12  6.327  0.5273   5.9905  0.002067 **
Residuals  12  1.056  0.0880
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The genotype effect is not statistically significant ($p > 0.05$), indicating no strong evidence for differences among genotypes in this trial. However, let's still examine the mean comparisons.

Mean Comparisons

```
mean_comp <- mod_fixed %>%
  emmeans(specs = ~ gen) %>%
  cld(adjust = "none", Letters = letters)

mean_comp
```

gen	emmmean	SE	df	lower.CL	upper.CL	.group
G04	3.48	0.270	12	2.89	4.07	a
G23	3.58	0.270	12	2.99	4.17	ab
G15	3.60	0.442	12	2.64	4.57	abcde
G35	3.63	0.277	12	3.03	4.24	abc
G31	3.79	0.280	12	3.18	4.40	abcd
G02	3.84	0.279	12	3.23	4.45	abcd
G26	3.90	0.291	12	3.26	4.53	abcde
G24	3.90	0.267	12	3.32	4.49	abcd f
G29	3.91	0.276	12	3.31	4.51	abcde
G30	3.99	0.270	12	3.40	4.58	abcdefg
G32	4.12	0.276	12	3.52	4.72	abcdefghijkl
G17	4.14	0.282	12	3.53	4.75	abcdefghijkl
G09	4.15	0.274	12	3.56	4.75	abcdefghijkl
G34	4.20	0.267	12	3.62	4.78	abcdefghijkl
G16	4.23	0.432	12	3.29	5.17	abcdefghijkl
G05	4.25	0.278	12	3.64	4.85	abcdefghijkl
G20	4.25	0.266	12	3.67	4.83	abcdefghijkl
G22	4.27	0.282	12	3.66	4.88	abcdefghijkl
G10	4.36	0.278	12	3.76	4.97	abcdefghijkl
G28	4.37	0.278	12	3.77	4.98	abcdefghijkl
G18	4.48	0.284	12	3.86	5.10	abcdefghijkl
G21	4.57	0.269	12	3.98	5.16	abcdefghijkl
G08	4.58	0.285	12	3.95	5.20	abcdefghijkl
G25	4.59	0.277	12	3.98	5.19	abcdefghijkl
G13	4.73	0.284	12	4.11	5.35	e ghijkl
G27	4.75	0.282	12	4.13	5.36	fghijk
G33	4.76	0.286	12	4.13	5.38	e ghijk
G14	4.79	0.270	12	4.20	5.38	ghijkl
G01	4.88	0.268	12	4.30	5.46	hijkl
G07	4.94	0.270	12	4.35	5.53	hijkl
G11	4.97	0.276	12	4.37	5.57	hijkl
G12	5.13	0.293	12	4.49	5.77	ijkl
G03	5.15	0.281	12	4.54	5.76	jk1
G06	5.53	0.280	12	4.92	6.14	k1
G19	5.60	0.281	12	4.99	6.22	1

Results are averaged over the levels of: colF, rowF, rep
 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.

The compact letter display shows the groupings based on Fisher's LSD test. With 35 genotypes, the letter display becomes complex, but it still provides a concise summary of which genotypes differ significantly.

Visualizing Results

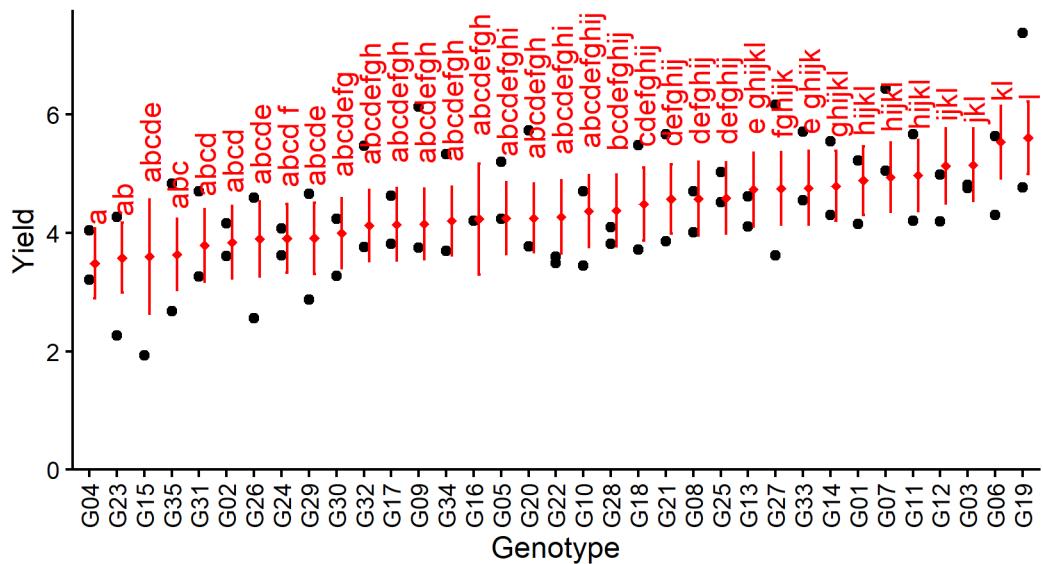
```
my_caption <- "Black dots represent raw data. Red diamonds and error bars represent
adjusted means with 95% confidence limits per genotype. Means followed by a common
letter are not significantly different according to Fisher's LSD test."
my_caption
```

```
ggplot() +
  aes(x = gen) +
```

```

# black dots representing the raw data
geom_point(
  data = dat,
  aes(y = yield)
) +
# red diamonds representing the adjusted means
geom_point(
  data = mean_comp,
  aes(y = emmean),
  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(ymax = lower.CL, ymin = upper.CL),
  color = "red",
  width = 0.1,
  position = position_nudge(x = 0.2)
) +
# red letters
geom_text(
  data = mean_comp,
  aes(y = upper.CL, label = str_trim(.group)),
  color = "red",
  angle = 90,
  hjust = -0.2,
  position = position_nudge(x = 0.2)
) +
scale_x_discrete(
  name = "Genotype",
  limits = as.character(mean_comp$gen)
) +
scale_y_continuous(
  name = "Yield",
  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 genotype. Means followed by a common letter are not significantly different according to Fisher's LSD test.

Bonus: Design Efficiency

Let's assess the efficiency of the row-column design compared to a simple RCBD (ignoring the row and column structure):

```
# s.e.d. squared for RCBD (ignoring row/column structure)
avg_sed_rcbd <- lm(yield ~ gen + rep, data = dat) %>%
  emmeans(pairwise ~ "gen", adjust = "none") %>%
  pluck("contrasts") %>%
  as_tibble() %>%
  pull("SE") %>%
  mean()

# Efficiency
avg_sed_rcbd^2 / sed_fixed^2
```

[1] 1.953932

An efficiency > 1 indicates that the row-column design is more efficient than a simple RCBD, meaning the row and column blocking successfully reduced experimental error.

Wrapping Up

You've now learned how to analyze data from a resolvable row-column design, which provides powerful control over two sources of spatial variation.

Key Takeaways

1. **Row-column designs** control for two sources of variation simultaneously by blocking in both row and column directions within each replicate.
2. **More flexible than Latin Square:** Can accommodate any number of treatments, not limited to $t \times t$ arrangements.
3. **Double blocking** within replicates provides increased precision when spatial trends exist in two directions.
4. **Model choice** between fixed and random row/column effects can be based on which gives smaller average s.e.d.
5. **The model with fixed effects:** `yield ~ gen + rep + rep:rowF + rep:colF` (rows and columns nested within replicates).
6. **Design efficiency** > 1 compared to RCBD confirms the benefit of the additional row-column structure.
7. **Missing data handling:** Row-column designs can still be analyzed when some observations are missing, though precision may be affected.

Design Comparison Summary

Let's summarize the progression of designs covered in this chapter series:

Design	Blocking Structure	Model Formula	Best For
CRD	None	$y \sim trt$	Homogeneous conditions
RCBD	Complete blocks	$y \sim trt + block$	One gradient
Latin Square	Rows + columns (complete)	$y \sim trt + row + col$	Two gradients, few treatments
Alpha Design	Incomplete blocks in reps	$y \sim trt + rep + (1 rep:block)$	Many treatments, one gradient
Augmented	Checks + entries	$y \sim trt + block$	Screening many unreplicated entries
Row-Column	Inc. rows + cols in reps	$y \sim trt + rep + rep:row + rep:col$	Many treatments, two gradients

Bibliography

[1] R. A. Kempton, P. N. Fox, and M. Cerezo, *Statistical Methods for Plant Variety Evaluation*. Springer Netherlands, 1997. doi: 10.1007/978-94-009-1503-9.