ENTMLGY 6707 Entomological Techniques and Data Analysis

Supplemental activity (do not submit): Randomized Complete Block and Latin Squares

1 Introduction

Stratified designs are used to (ideally) increase precision of estimates by experimentally controlling or reducing variation. This tutorial covers how to analyze two types of experimental designs: randomized complete block and Latin squares. The examples include multiple ways of analyzing the same exact data.

Nowadays, some folks use mixed-effects models to analyze stratified designs. Be aware that a fixed-effect only approach might be preferred, given that we assume random effects are normally distriubuted and it is hard to test that assumption when there are only a few levels of a random effect (e.g., 3 blocks).

Either way, you still have to be careful about specifying the random effects correctly. And you will notice that if you do, the sums of squares and F-statistics for each treatment are typically equivalent. There are sometimes differences, but rarely do they influence the overall conclusions.

The following packages are necessary to complete this tutorial.

```
library(car)
library(lme4)
library(lmerTest)
library(tidyverse)
library(agricolae)
library(emmeans)
```

2 Randomized complete block

The data "Trefoil" contains data from seven genetically different populations of birdsfoot trefoil (forage crop) seedlings evaluated for their response to a single application of a herbicide. The experimental unit was a plot containing 6 plants (= sample units) of a chosen population, and there were 8 replicates (blocks) in an RCB layout. The data collected were individual plant fresh weights (in grams) three weeks after the herbicide treatment.

```
trefoil <- read.table("Trefoil.txt", header=T, sep="\t",
                      colClasses = c("factor","factor", "numeric",
                                     "numeric", "numeric", "numeric",
                                     "numeric", "numeric", "numeric"))
head(trefoil)
```

```
##   Rep Sample  Pop1  Pop2  Pop3  Pop4  Pop5  Pop6  Pop7
## 1   1      1 0.060 0.238 0.296 0.246 0.318 0.550 0.321
## 2   1      2 0.243 0.215 0.141 0.484 0.322 0.474 0.516
## 3   1      3 0.142 0.107 0.346 0.359 0.341 0.521 0.640
## 4   1      4 0.213 0.109 0.613 0.173 0.351 0.525 0.559
## 5   1      5 0.055 0.251 0.208 0.144 0.168 0.580 0.364
## 6   1      6 0.038 0.322 0.354 0.141 0.369 0.400 0.508
```

```
summary(trefoil)
```

```
##       Rep      Sample      Pop1             Pop2             Pop3
##  1      : 6   1:8     Min.   :0.0000   Min.   :0.0610   Min.   :0.1150
##  2      : 6   2:8     1st Qu.:0.1610   1st Qu.:0.2928   1st Qu.:0.3227
##  3      : 6   3:8     Median :0.2640   Median :0.4660   Median :0.4130
##  4      : 6   4:8     Mean   :0.2794   Mean   :0.4904   Mean   :0.4522
##  5      : 6   5:8     3rd Qu.:0.3370   3rd Qu.:0.7013   3rd Qu.:0.5773
##  6      : 6   6:8     Max.   :0.7610   Max.   :1.1820   Max.   :0.8420
##  (Other):12
##       Pop4             Pop5             Pop6             Pop7
##  Min.   :0.1410   Min.   :0.0880   Min.   :0.0430   Min.   :0.1270
##  1st Qu.:0.3548   1st Qu.:0.3673   1st Qu.:0.3615   1st Qu.:0.5573
##  Median :0.5405   Median :0.5135   Median :0.4825   Median :0.6620
##  Mean   :0.6061   Mean   :0.5734   Mean   :0.5234   Mean   :0.6974
##  3rd Qu.:0.7977   3rd Qu.:0.7208   3rd Qu.:0.6520   3rd Qu.:0.8635
##  Max.   :1.5130   Max.   :1.4000   Max.   :1.1150   Max.   :1.3230
##
```

2.1 Wide vs. long format

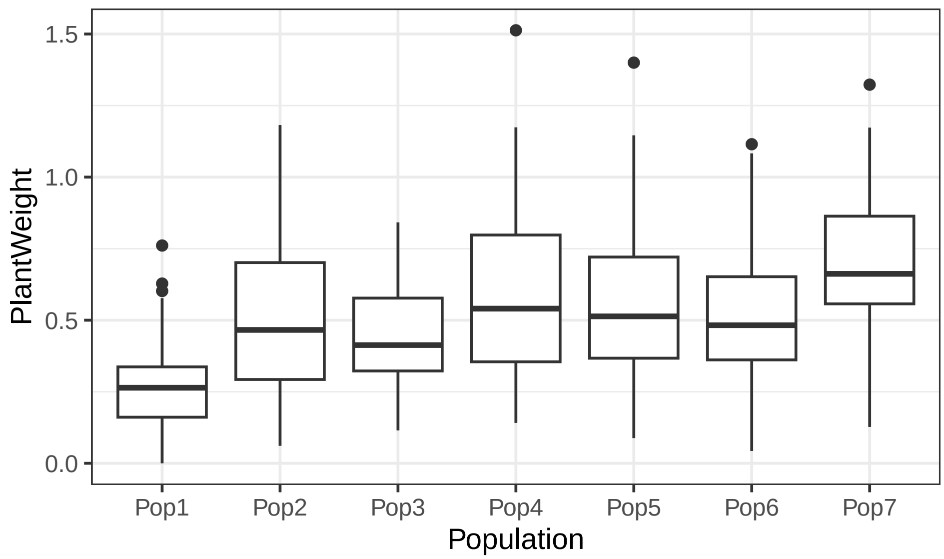
The data above are in "wide" format: there are several observations on a single line (one each for populations 1-7). R requires "long" format for fitting linear models, and the `tidyverse` has a nice function (`pivot_longer`) that enables us to reformat the data. The below code is creating a new data frame by taking all columns that "`starts_with`" `Pop` and creating new columns called `Population` and `PlantWeight`, into which the column header text (e.g., `Pop1`) and associated value (e.g., `0.060`) are input. For example, the first and second row of the new data will have `Population` values equal to `Pop1` and `Pop2` and `PlantWeight` values of `0.060` and `0.238`. For this to work, the column headers for whichever variable you are shifting from wide to long format need to start with a unique string of letters (`Pop` in this case).

```
trefoil_long <- trefoil %>%
   pivot_longer(
   cols = starts_with("Pop"),
   names_to = "Population",
```

```r
  values_to = "PlantWeight")

head(trefoil_long)
```

```
## # A tibble: 6 x 4
##   Rep   Sample Population PlantWeight
##   <fct> <fct>  <chr>            <dbl>
## 1 1     1      Pop1             0.06
## 2 1     1      Pop2             0.238
## 3 1     1      Pop3             0.296
## 4 1     1      Pop4             0.246
## 5 1     1      Pop5             0.318
## 6 1     1      Pop6             0.55
```



2.2 aov()

```r
fit_aov_RCB <- aov(PlantWeight ~ Rep + Population, data=trefoil_long)
summary(fit_aov_RCB)
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)    
## Rep           7  4.432  0.6331   12.54 3.16e-14 ***
## Population    6  5.044  0.8407   16.66  < 2e-16 ***
## Residuals   322 16.251  0.0505                     
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3 lm()

```r
fit_lm_RCB <- lm(PlantWeight ~ Rep + Population, data=trefoil_long)
Anova(fit_lm_RCB, type="III")
```

```
## Anova Table (Type III tests)
## 
## Response: PlantWeight
##              Sum Sq  Df F value    Pr(>F)    
```

```
## (Intercept)  0.1504   1  2.9808   0.08522 .
## Rep          4.4315   7 12.5436 3.155e-14 ***
## Population   5.0441   6 16.6572 < 2.2e-16 ***
## Residuals   16.2514 322
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.4 mixed-effects model

```r
fit_lmer_RCB <- lmer(PlantWeight ~ Population + (1|Rep), data=trefoil_long)
anova(fit_lmer_RCB, type=3)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##            Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Population 5.0441 0.84069     6   322  16.657 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.5 pairwise comparisons

```r
emmeans(fit_lmer_RCB, pairwise~"Population")
```

```
## $emmeans
##  Population emmean     SE   df lower.CL upper.CL
##  Pop1        0.279 0.0528 15.2    0.167    0.392
##  Pop2        0.490 0.0528 15.2    0.378    0.603
##  Pop3        0.452 0.0528 15.2    0.340    0.565
##  Pop4        0.606 0.0528 15.2    0.494    0.718
##  Pop5        0.573 0.0528 15.2    0.461    0.686
##  Pop6        0.523 0.0528 15.2    0.411    0.636
##  Pop7        0.697 0.0528 15.2    0.585    0.810
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast    estimate     SE  df t.ratio p.value
##  Pop1 - Pop2  -0.2111 0.0459 322  -4.603  0.0001
##  Pop1 - Pop3  -0.1728 0.0459 322  -3.768  0.0037
##  Pop1 - Pop4  -0.3267 0.0459 322  -7.124  <.0001
##  Pop1 - Pop5  -0.2940 0.0459 322  -6.412  <.0001
##  Pop1 - Pop6  -0.2440 0.0459 322  -5.320  <.0001
##  Pop1 - Pop7  -0.4181 0.0459 322  -9.117  <.0001
##  Pop2 - Pop3   0.0383 0.0459 322   0.835  0.9812
##  Pop2 - Pop4  -0.1156 0.0459 322  -2.521  0.1551
##  Pop2 - Pop5  -0.0830 0.0459 322  -1.809  0.5426
##  Pop2 - Pop6  -0.0329 0.0459 322  -0.718  0.9915
##  Pop2 - Pop7  -0.2070 0.0459 322  -4.514  0.0002
##  Pop3 - Pop4  -0.1539 0.0459 322  -3.356  0.0153
##  Pop3 - Pop5  -0.1212 0.0459 322  -2.644  0.1165
##  Pop3 - Pop6  -0.0712 0.0459 322  -1.552  0.7127
##  Pop3 - Pop7  -0.2453 0.0459 322  -5.349  <.0001
##  Pop4 - Pop5   0.0326 0.0459 322   0.712  0.9918
##  Pop4 - Pop6   0.0827 0.0459 322   1.804  0.5466
```

```
##  Pop4 - Pop7  -0.0914 0.0459 322  -1.993  0.4214
##  Pop5 - Pop6   0.0501 0.0459 322   1.092  0.9303
##  Pop5 - Pop7  -0.1240 0.0459 322  -2.704  0.1004
##  Pop6 - Pop7  -0.1741 0.0459 322  -3.796  0.0033
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 7 estimates
```

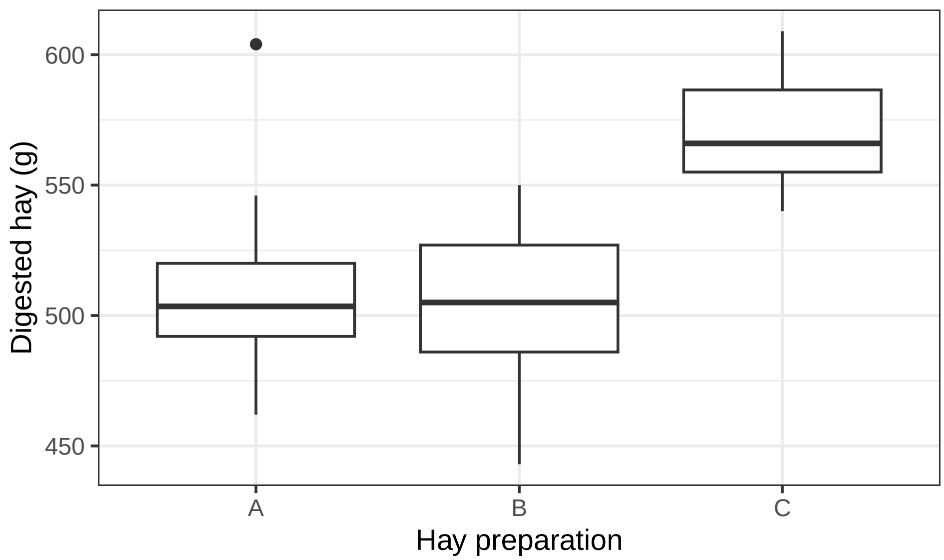
3 Latin squares

An animal scientist was conducting a feeding trial to determine the dry matter digestibility of three different preparations of hay. The scientist had only 18 sheep (experimental units) available for use in the trial, so it was decided to conduct the experiment as a series of six 3×3 Latin squares, run concurrently, with each sheep (columns = sheep) receiving each of three treatments over three consecutive feeding periods (rows = times).

```
sheep <- read.table("sheep.csv", header=T, sep=",",
                    colClasses = c("factor","factor","factor",
                                   "factor","numeric"))

summary(sheep)
```

```
##   square     sheep    letter time       digest
##   1:9    1       : 3   A:18   1:18   Min.   :443.0
##   2:9    10      : 3   B:18   2:18   1st Qu.:495.8
##   3:9    11      : 3   C:18   3:18   Median :525.0
##   4:9    12      : 3                 Mean   :528.0
##   5:9    13      : 3                 3rd Qu.:555.0
##   6:9    14      : 3                 Max.   :609.0
##          (Other):36
```



3.1 aov()

```
fit_aov_LS <- aov(digest ~ square + square/sheep + square/time + letter,
                  data=sheep)
summary(fit_aov_LS)
```

```
##               Df Sum Sq Mean Sq F value   Pr(>F)
## square         5   6718    1344   1.383    0.269
## letter         2  50877   25438  26.180 1.52e-06 ***
## square:sheep  12   7412     618   0.636    0.790
## square:time   12   8852     738   0.759    0.683
## Residuals     22  21377     972
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.2 lm()

```r
fit_lm_LS <- lm(digest ~ square + square/sheep + square/time + letter,
                data=sheep)
anova(fit_lm_LS)
```

```
## Analysis of Variance Table
##
## Response: digest
##              Df Sum Sq Mean Sq F value   Pr(>F)
## square        5   6718  1343.5  1.3827   0.2690
## letter        2  50877 25438.4 26.1796 1.52e-06 ***
## square:sheep 12   7412   617.7  0.6357   0.7901
## square:time  12   8852   737.7  0.7592   0.6832
## Residuals    22  21377   971.7
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.3 mixed-effects model

Apparently, the variation due to sheep and time is small enough that it collapses to 0 in the variance components (see top of summary() output).

```r
fit_lmer_LS <- lmer(digest ~ letter + (1|square/sheep) + (1|square:time), data=sheep)
```

```
## boundary (singular) fit: see help('isSingular')
```

```r
anova(fit_lmer_LS, type=3)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##        Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## letter  50877   25438     2    46  31.087 2.875e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(fit_lmer_LS)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: digest ~ letter + (1 | square/sheep) + (1 | square:time)
##    Data: sheep
##
## REML criterion at convergence: 498
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.9434 -0.5187 -0.1436  0.6654  3.0413
##
## Random effects:
##  Groups       Name        Variance Std.Dev.
##  square:time  (Intercept)   0.00    0.000
##  sheep:square (Intercept)   0.00    0.000
##  square       (Intercept)  58.36    7.639
##  Residual                 818.29   28.606
## Number of obs: 54, groups:  square:time, 18; sheep:square, 18; square, 6
##
## Fixed effects:
```

```
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)  508.778      7.429  21.184  68.487  < 2e-16 ***
## letterB       -4.944      9.535  46.000  -0.519    0.607
## letterC       62.500      9.535  46.000   6.555 4.25e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##         (Intr) lettrB
## letterB -0.642
## letterC -0.642  0.500
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

3.4 pairwise comparisons

```r
emmeans(fit_lmer_LS, pairwise~"letter")
```

```
## $emmeans
##  letter emmean   SE   df lower.CL upper.CL
##  A         509 7.43 18.4      493      524
##  B         504 7.43 18.4      488      519
##  C         571 7.43 18.4      556      587
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate   SE df t.ratio p.value
##  A - B        4.94 9.54 22   0.519  0.8632
##  A - C      -62.50 9.54 22  -6.555  <.0001
##  B - C      -67.44 9.54 22  -7.073  <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

4 R Activity

You will want to ensure that you use Type III sums of squares when conducting ANOVAs (i.e., use marginal and not sequential fits). You will need the following packages to complete this problem set:

```
library(tidyverse)
library(car)
library(lme4)
library(lmerTest)
library(emmeans)
```

A turfgrass management study was initiated in to determine if tillage plan and herbicide applications to control quackgrass (a noxious weed) influenced seed production in a single, commonly grown variety of perennial ryegrass. The experiment was set up as a 3 x 2 factorial with 3 replications (=blocks) in a randomized complete block design (ignore interactions for this problem set, but note that factorial designs are often implemented to evaluate the interactive effect of two treatments on a single response variable). The data are in the "`EPP_seed.txt`" data file.

- Factor 1: Tillage, 3 kinds
  - Spring = spring tillage/spring seeding
  - Fall = fall tillage/fall seeding
  - None = no-tillage/fall seeding

- Factor 2: Herbicide, 2 levels
  - None = no herbicide treatment
  - Applied = herbicide treatment (to control quackgrass)

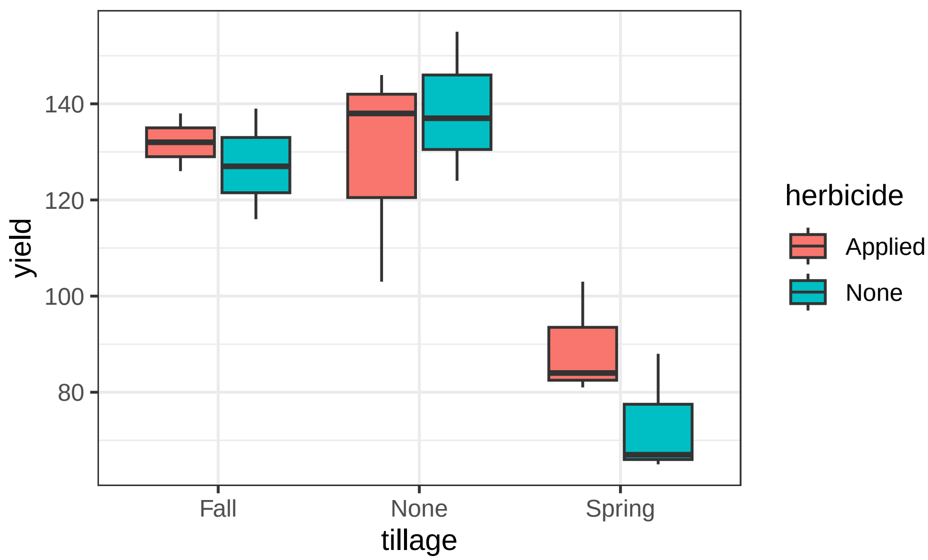
1. Load in the data. Note that `rep` is the column name for blocks.

```r
seed_df <- read.table('EPP_seed.txt', header=T, sep="\t",
                  colClasses = c("factor","factor","factor",NA))
summary(seed_df)
```

```
##  rep     tillage    herbicide      yield
##  1:6   Fall  :6   Applied:9   Min.   : 65.00
##  2:6   None  :6   None   :9   1st Qu.: 91.75
##  3:6   Spring:6               Median :125.00
##                               Mean   :114.94
##                               3rd Qu.:137.75
##                               Max.   :155.00
```

2. Graph the data using a boxplot. In the plot, group the data by tillage treatment on the x-axis and then color each box by herbicide treatment.

```r
ggplot(seed_df, aes(x=tillage, y=yield, fill=herbicide)) +
 geom_boxplot() + theme_bw()
```



3. Conduct an analysis of variance (ANOVA) using the `lm()` and `Anova()` (from the `car` package) commands (i.e., assess if `tillage` and `herbicide` explain variation in `yield`)

```
lm_seed_1 <- lm(yield ~ rep + tillage + herbicide, data=seed_df)
Anova(lm_seed_1, type="III")
```

```
## Anova Table (Type III tests)
##
## Response: yield
##             Sum Sq Df  F value    Pr(>F)
## (Intercept)  50613  1 309.9481 6.148e-10 ***
## rep           1013  2   3.1031   0.08199 .
## tillage      10219  2  31.2911 1.735e-05 ***
## herbicide       60  1   0.3705   0.55408
## Residuals     1960 12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

4. Select a variable you would like to explore further using pairwise comparisons. Explain your reasoning, and then conduct the comparisons.

```
emmeans(lm_seed_1, pairwise~tillage)
```

```
## $emmeans
##  tillage emmean   SE df lower.CL upper.CL
##  Fall     129.7 5.22 12      118    141.0
##  None     133.8 5.22 12      122    145.2
##  Spring    81.3 5.22 12       70     92.7
##
## Results are averaged over the levels of: rep, herbicide
## Confidence level used: 0.95
##
## $contrasts
##  contrast      estimate   SE df t.ratio p.value
##  Fall - None      -4.17 7.38 12  -0.565  0.8410
##  Fall - Spring    48.33 7.38 12   6.551  0.0001
##  None - Spring    52.50 7.38 12   7.116  <.0001
```

```
##
## Results are averaged over the levels of: rep, herbicide
## P value adjustment: tukey method for comparing a family of 3 estimates
```

5. Write 3-4 sentences summarizing your findings using "biologically meaningful" terms.

   Answer: Yield of the perennial rye grass varied significantly between tillage treatments ($F_{1,12} = 31.29, p < 0.0001$) but herbicide had no effect ($F_{1,12} = 0.37, p = 0.55$), indicating that quackgrass is best managed by altering tillage. A pairwise comparison between tillage options indicated that tilling/seeding in spring sigificantly reduced yield by approx. 48g and 53g compared with tilling/seeding in fall ($t_{12} = 6.55, p = 0.0001$) and no tilling/fall seeding ($t_{12} = 7.12, p < 0.0001$). Treatments using fall seeding did not differ ($t_{12} = -0.57, p = 0.84$). Taken together, tillage and seeding should be completed in fall.