Lecture 17
Topic 12: The split-plot design and its relatives (Part II - Repeated Measures)

Researchers often make **multiple measurements on the same experimental unit**.

Replications $\neq$ Subsamples

The objectives of **subsampling**:

1. To better estimate the true value of an experimental unit
2. To estimate the components of variance

There is no interest in the effect of <u>time</u> between subsamples on the response variable.

Repeated measures analysis

Researchers often make **multiple measurements on the same experimental unit *because* they are interested in this effect of time**.

Plant height ***over weeks***
Yield of perennial crops ***over seasons***
Animal growth ***over months***
Population dynamics ***over years***

The objectives of **repeated measures**:

1. To characterize the effect of <u>time</u> on the response variable.
2. To characterize the <u>interaction of other factors with time</u> on the response variable.

The **split-plot** principle can be applied to experiments where successive observations are made on the same experimental unit over time (i.e. repeated measurements).

Some examples:

Repeated harvesting of a perennial crop like alfalfa in a fertilizer study
Repeated picking of fruit from the same trees in an irrigation study
Repeated weighing of individuals in a nutrition study
Repeated bird counts in a restoration ecology study

The experimental units to which the treatment levels are assigned are the "Main plots" (A)

Main Plot treatment levels:	Fertilizer levels, irrigation levels, diets, restoration strategies
Experimental units:	One plot, one tree, one person, one site

The several measurements over time are the "Subplots" (B)

Subplot "treatment" levels: Time point 1, Time point 2, …, Time point b

Repeated measures: A subplot consists of data taken from *the entire main plot*

Normal split-plot: A subplot consists of data taken from *a portion of the main plot*

The pattern of dependency among the repeated observations
is used to adjust the degree of freedoms in the ANOVA
to give approximate tests.

Repeated measures ANOVA

ANOVA table of a repeated measurement analysis

Source	df	SS	MS	Conservative df
Among Experimental Units				
Treatment (A)	a-1	SSA	SSA/(a-1)	
Rep:Trt (Error A)	a(n-1)	SS(MPE)	SS(MPE)/a(n-1)	
***Within* Experimental Units**				
Time (B)	b-1	SSB	SSB /(b-1)	**1**
Treatment:Time (A:B)	(b-1)(a-1)	SSAB	SSAB/(b-1)(a-1)	**a-1**
MSE (Error B)	a(b-1)(n-1)	SS(SPE)	SS(SPE)/a(b-1)(n-1)	**a(n-1)**

Split-plot model: Subplot observations are equally correlated.
[Note ANOVA assumes *errors* to be independent, not observations.]

Repeated measures: Subplot observations are not necessarily equally correlated.
[Measures close in time are generally more highly correlated than measures far apart in time.]

To compensate for this assumption of uniform correlation across repeated measurements,
a **conservative approach** is recommended by many statisticians

Example

An experiment to study the differences in quality of four alfalfa cultivars. Five replications of these four varieties were organized according to a CRD, and four cuttings were made of each replication over time:

4 levels of main plot A: **Cultivars** 1 – 4
4 levels of subplot B: **Cut times** 1 – 4 (9/10/16, 5/18/17, 6/25/17, 9/16/17)

Begin with a standard split-plot analysis.

```
rep_meas_mod<-aov(quality ~  A_var +
                             Error(A_var:rep) +
                             B_cut + A_var:B_cut,
                             rep_meas_dat)
summary(rep_meas_mod)
```

The output

```
Error: A_var:Rep
            Df  Sum Sq  Mean Sq  F value  Pr(>F)
A_var        3   2.841   0.9469    7.395  0.00251 **
Residuals   16   2.049   0.1280

Error: Within
              Df  Sum Sq  Mean Sq  F value  Pr(>F)
B_cut          3   37.45   12.483  130.463  <2e-16 ***
A_var:B_cut    9    0.55    0.061    0.636   0.761
Residuals     48    4.59    0.096
```

Impose conservative degrees of freedom

```
Error: Within
              Df  Sum Sq  Mean Sq  F value   Pr(>F)
B_cut          1   37.45    37.45  130.463  4.2e-09 ***
A_var:B_cut    3    0.55    0.183    0.636    0.592
Residuals     16    4.59    0.287
```

Analysis of the main effect of Cultivar (main plot A):

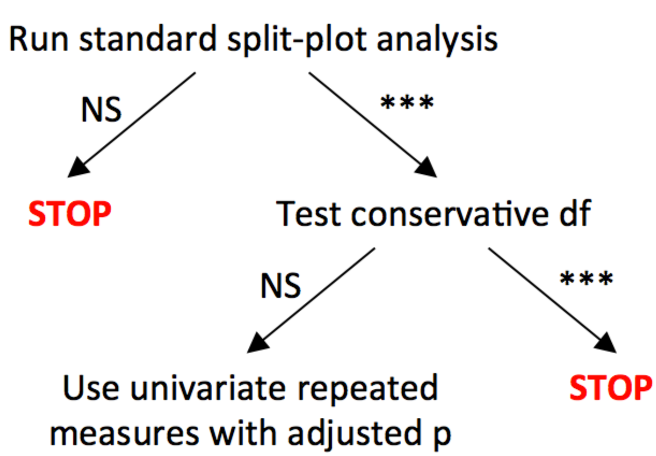
```
MP_comparison<-LSD.test(rep_meas_dat$quality, rep_meas_dat$A_var,
      DFerror = 16, MSerror = 0.1280)
MP_comparison
```

```
       Mean       CV  MSerror       LSD
   2.993789 11.95044    0.128 0.2398399   <-- using MS(A:rep)
                                              as the error

  trt     means  M
1   2 3.269848   a
2   1 3.044449  ab
3   4 2.900750  bc
4   3 2.760109   c
```

Analysis of the main effect of Time (subplot B):

```
SP_comparison<-LSD.test(rep_meas_dat$quality, rep_meas_dat$B_cut,
      DFerror = 16, MSerror = 0.287)
SP_comparison
```

```
MSerror       LSD
  0.287 0.3591347

Tukey groups
 B_cut      means  Grouping
     2   4.099587     a
     3   2.985440      b
     1   2.598901       c
     4   2.291229       c
```



Univariate repeated measures analysis using the ezANOVA() function

If the unadjusted and conservative df approaches produce different conclusions, a more precise test can be obtained using a univariate repeated measures analysis, available in a user-friendly form as part of R's "ez" package.

```
#library(ez)

ezANOVA(
  data = rep_meas_dat,          ← the same data set
  dv = quality,                 ← the column containing the response variable
  wid = rep,                    ← the column containing the e.u. identifier
  within = B_cut,               ← the column containing the repeated measure identifier
  between = A_var,              ← the column containing the main plot identifier
  return_aov = TRUE
)
```

The output:

```
$ANOVA
       Effect DFn DFd           F            p p<.05        ges
2       A_var   3  16   7.3953319 2.511139e-03     * 0.29959303
3       B_cut   3  48 130.4625662 4.432360e-23     * 0.84936627
4 A_var:B_cut   9  48   0.6360617 7.605683e-01       0.07618871

$`Mauchly's Test for Sphericity`
       Effect         W            p p<.05
3       B_cut 0.1127544 5.992606e-06     *
4 A_var:B_cut 0.1127544 5.992606e-06     *

$`Sphericity Corrections`
       Effect       GGe        p[GG] p[GG]<.05       HFe        p[HF] p[HF]<.05
3       B_cut 0.4984411 1.391816e-12         * 0.5382286 2.031184e-13         *
4 A_var:B_cut 0.4984411 6.585475e-01           0.5382286 6.694957e-01
```

Greenhouse-Geisser Epsilon = 0.4984
Huynh-Feldt Epsilon = 0.5382

The output:

```
$ANOVA
        Effect DFn DFd           F            p p<.05        ges
2        A_var   3  16   7.3953319 2.511139e-03     * 0.29959303
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4 A_var:B_cut 0.4984411 6.585475e-01           0.5382286 6.694957e-01
```

```
Greenhouse-Geisser Epsilon = 0.4984
    Huynh-Feldt Epsilon = 0.5382
```

Compare to the split-plot output (full df):

```
Error: A_var:Rep
            Df Sum Sq Mean Sq  F value   Pr(>F)
A_var        3  2.841  0.9469    7.395 0.00251 **
B_cut         3  37.45  12.483 130.463  <2e-16 ***
A_var:B_cut   9   0.55   0.061   0.636   0.761
Residuals    48   4.59   0.096
```

And the conservative df output:

```
Error: Within
              Df Sum Sq Mean Sq F value   Pr(>F)
B_cut          1  37.45   37.45 130.463 4.2e-09 ***
A_var:B_cut    3   0.55   0.183   0.636   0.592
Residuals     16   4.59   0.287
```

This analysis produces the same F values as the previous split-plot analysis but **corrected** p-values for TIME (previous B_cut) and TIME:VAR (previous B_cut*A_var)

Which to use, Split-Plot or REPEATED?

If responses are significant even when the conservative df are used, those conclusions are sound.

In some cases, a repeated measures analysis can solve some problems of correlation; but if many observations are missing, the split-plot approach might offer the only way of analyzing a repeated-measures design.

Are there any other options?

Yes! In some (many) cases, a collection of repeated measures can be distilled into a single response variable that captures the information of interest. By collapsing a suite of repeated measures into a single descriptive number, one can proceed with analyzing the data in more familiar (and robust) ways. Examples:

1. Areas (e.g. AUDPC = area under disease progress curve)
2. Differences (e.g. change between two time points)
3. Trend parameters (e.g. slopes, maxima, minima, decay constants)