Lecture 13
Topic 9: Factorial treatment structures (Part II)

Increasing precision by partitioning the error sum of squares:

$$F = \frac{s^2_{among}}{s^2_{within}} = \frac{MST}{MSE} = \frac{\frac{SST}{df_{trt}}}{\frac{SSE}{df_e}}$$

Blocking:
SSE (CRD) → SSB + SSE (RCBD)

Increasing precision by partitioning the treatment sum of squares:

$$F = \frac{s^2_{among}}{s^2_{within}} = \frac{MST}{MSE} = \frac{\frac{SST}{df_{trt}}}{\frac{SSE}{df_e}}$$

Contrasts:
SST → (t – 1) orthogonal contrasts

A factorial treatment structure ensures that the TSS can be partitioned into very specific orthogonal components (main effects and interaction effects):

$$\overbrace{\text{SSA} + \text{SSB} + \textit{SS(AB)}}^{\text{SST}}$$

$$\overbrace{\text{SSA} + \text{SSB} + \text{SSC} + \textit{SS(AB)} + \textit{SS(AC)} + \textit{SS(BC)} + \textit{SS(ABC)}}^{\text{SST}}$$

The significance of the interaction F test determines what kind of subsequent analysis is appropriate:

> No significant interaction: Subsequent analysis (mean comparisons, contrasts, etc.) are performed on the *main effects*.
>
> Significant interaction: Subsequent analysis (mean comparisons, contrasts, etc.) are performed on the *simple effects*.

Another example of partitioning the Interaction SS

A 3x3 factorial experiment was conducted to determine the effects of vernalization genes ***Vrn1*** and ***Vrn2*** on flowering time (days to flowering) in wheat. 102 plants from a segregating population (parents A and B) were characterized with molecular markers and the number of alleles of parent A indicated *for each of the two genes* (BB = 0, AB = 1, AA = 2).

Gene			***Vrn2***		
	Genotype		**BB**	**AB**	**AA**
		Dose A	**0**	**1**	**2**
Vrn1	**BB**	**0**	00 = *Type 1*	01 = *Type 2*	02 = *Type 3*
	AB	**1**	10 = *Type 4*	11 = *Type 5*	12 = *Type 6*
	AA	**2**	20 = *Type 7*	21 = *Type 8*	22 = *Type 9*

```
#The ANOVA
vrn_mod<-lm(Days ~ Vrn1 + Vrn2 + Vrn1*Vrn2, vrn_dat)
```

Results (obtained via Anova(), part of the "car" package, using partial SS)

```
             Sum Sq Df   F value     Pr(>F)
(Intercept) 858102  1 7761.1659 < 2.2e-16 ***
Vrn1          4435  2   20.0558 5.734e-08 ***
Vrn2         21310  2   96.3683 < 2.2e-16 ***
Vrn1:Vrn2      808  4    1.8267    0.1303  NS
Residuals    10282 93
```

Is it worth partitioning the Interaction SS?

$$F_{Calc} = \frac{\frac{SS(AB)}{1}}{MSE} = \frac{807.86071}{110.56357} = 7.31$$

$$F_{Crit} = F_{0.05,1,93} \approx 3.96$$

Partitioning the interaction

```
#The same contrasts are used for factors Vrn1 and Vrn2
# Contrast 'Linear'    -1,0,1
# Contrast 'Quadratic' 1,-2,1
contrastmatrix<-cbind(c(-1,0,1),c(1,-2,1))

contrasts(vrn_dat$Vrn1)<-contrastmatrix
contrasts(vrn_dat$Vrn2)<-contrastmatrix

vrn_Rcon_mod<-lm(Days ~ Vrn1 + Vrn2 + Vrn1*Vrn2, vrn_dat)
summary(vrn_Rcon_mod)
```

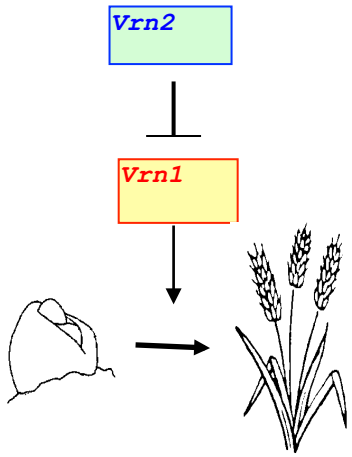
```
             Estimate Std. Error t value Pr(>|t|)
Vrn11        -9.45893    1.87007  -5.058 2.12e-06 ***
Vrn12         2.20747    0.79774   2.767 0.006822 **
Vrn21        22.67063    1.87398  12.098  < 2e-16 ***
Vrn22        -3.06971    0.79467  -3.863 0.000207 ***
Vrn11:Vrn21  -6.30952    2.64122  -2.389 0.018920 *
Vrn12:Vrn21   0.35317    1.08924   0.324 0.746484    NS
Vrn11:Vrn22  -0.06488    1.08250  -0.060 0.952335    NS
Vrn12:Vrn22  -0.59239    0.49082  -1.207 0.230512    NS
```

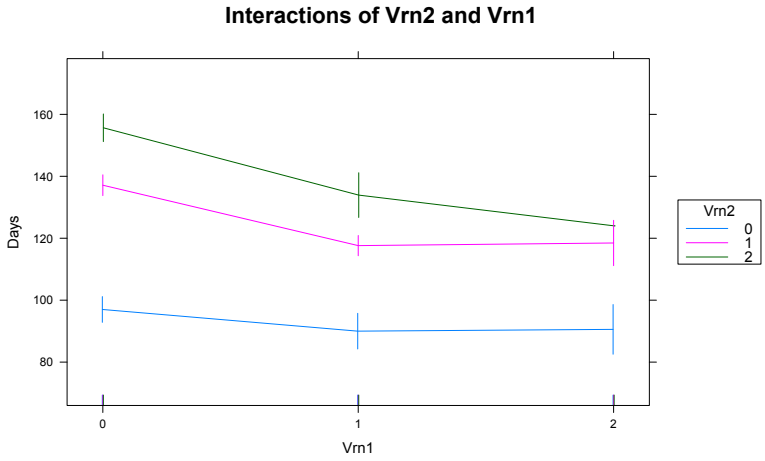
There is a significant *linear by linear* component of the interaction!

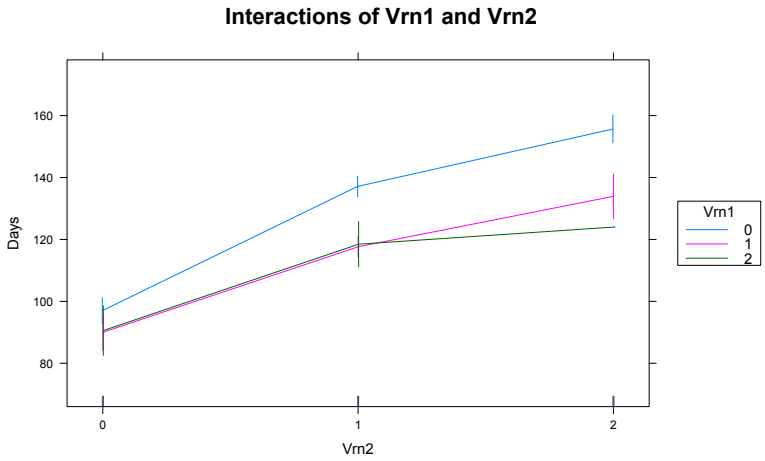
Great. What does that mean?

A linear x linear interaction:

The effect of one factor (full dose vs. no dose) is different depending on the level (full dose vs. no dose) of the other factor.







2x2 CRD with a significant interaction (testing simple effects)

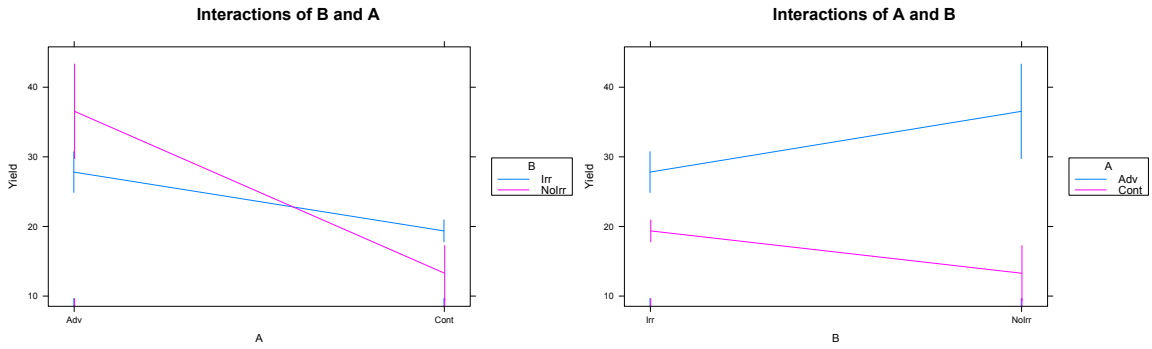
In this CRD with five replications per treatment combination, Factor A is genotype of mungbean (Control vs. Advanced Line) and Factor B is drought stress (Not irrigated vs. Irrigated). The response variable is yield.

Factor B	A			
	Control	Advanced	Means	*se* (A,B_i)
Not Irrigated	19.36	27.81	23.585	8.45
Irrigated	13.28	36.53	24.905	23.25
Means	16.32	32.17	24.245	
se (B,A_i)	6.08	-8.72		

```
#The ANOVA
mung_mod<-lm(Yield ~ A + B + A*B, mung_dat)
anova(mung_mod)
```

Output

```
           Df  Sum Sq Mean Sq F value    Pr(>F)
A           1 1256.75 1256.75 52.9263 1.859e-06 ***
B           1    8.71    8.71  0.3669  0.553198
A:B         1  273.95  273.95 11.5370  0.003686 **
Residuals  16  379.92   23.75
```



If an interaction is present in a fixed-effects model, the next step is to analyze the simple effects.

An easy way of testing simple effects is to subset the data according to the levels of the factors involved and conduct individual ANOVAs within each level:

```
#Analyze the simple effects by subsetting the data...
mung_Irr_dat<-subset(mung_dat, mung_dat$B == "Irr")
mung_NoIrr_dat<-subset(mung_dat, mung_dat$B == "NoIrr")
mung_Cont_dat<-subset(mung_dat, mung_dat$A == "Cont")
mung_Adv_dat<-subset(mung_dat, mung_dat$A == "Adv")

#...and then performing multiple ANOVAs
anova(lm(Yield ~ A, mung_Irr_dat))
anova(lm(Yield ~ A, mung_NoIrr_dat))
anova(lm(Yield ~ B, mung_Cont_dat))
anova(lm(Yield ~ B, mung_Adv_dat))
```

The results (F tests pulled from the four separate ANOVAs):

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Geno (Irr)	1	178.591	178.591	24.802	0.001079	**
Geno (NoIrr)	1	1352.10	1352.10	33.559	0.0004084	***
Irr (Cont)	1	92.477	92.477	7.7607	0.02371	*
Irr (Adv)	1	190.18	190.183	5.3461	0.04952	*

Compare this to the previous F test for the *main effect* of Irrigation:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Geno	1	1256.75	1256.75	52.9263	1.859e-06	***
Irr	1	8.71	8.71	0.3669	**0.553198**	**NS**
Geno:Irr	1	273.95	273.95	11.5370	0.003686	**
Residuals	16	379.92	23.75			

Why not use contrasts to probe simple effects?

These contrasts are not orthogonal (prove it to yourself), so the results you obtain are not identical to those above.

Example of a factorial experiment with subsamples

Suppose in the quack-grass shoots experiment that *two* random areas of 1 square foot each were evaluated in each plot (each R-D combination):

D	R	Block	Plot	Area	Number
3	0	1	1	1	14.7
3	0	2	1	1	13.6
3	0	3	1	1	15.5
…	…	…	…	…	…
10	8	2	1	2	9.2
10	8	3	1	2	12.3
10	8	4	1	2	12.2

The "Plot" and "Area" columns are here just to be very explicit about the fact that there is one experimental unit (Plot) per D:R:Block combination, and there are two subsamples (Areas) per experimental unit. The correct linear model in R, however, does not make use of either of these classification variables:

```
#The ANOVA
quack_mod<-lm(Number ~ D + R + D*R + Block + D:R:Block, quack_dat)
anova(quack_mod)
```

The output:

```
           Df  Sum Sq Mean Sq F value    Pr(>F)
D           1   3.000   3.000  1.5000   0.23256
R           2 307.327 153.663 76.8317 3.693e-11 ***
Block       3   1.163   0.388  0.1939   0.89952
D:R         2   0.980   0.490  0.2450   0.78464
D:R:Block  15  78.767   5.251  2.6256   0.01698 *
Residuals  24  48.000   2.000
```

Using the correct error term (D:R:Block) for testing D, R, Block, and D:R...

```
           Df  Sum Sq Mean Sq F value    Pr(>F)
D           1   3.000   3.000  0.5713   0.46144
R           2 307.327 153.663 29.2636 6.642e-06 ***
Block       3   1.163   0.388  0.0739   0.97309
D:R         2   0.980   0.490  0.0933   0.91143
D:R:Block  15  78.767   5.251  2.6256   0.01698 *
Residuals  24  48.000   2.000
```

The objective of analyzing the experiment using the individual subsample values is simply to understand the sources of variation in the experimental error:

```
#Calculating components of variance
#library(lme4)
quackCV_mod<-lmer(Number ~ D + R + D*R + Block + (1|Block:D:R), quack_dat)
summary(quackCV_mod)
```

The output:

```
Random effects:
 Groups     Name        Variance Std.Dev.
 Block:D:R (Intercept) 1.626    1.275
 Residual              2.000    1.414
Number of obs: 48, groups: Block:D:R, 24
```

If one plot (EU) costs $50 and one subsample costs $5…

$$n_{sub} = \sqrt{\frac{C_{e.u.} * s^2_{sub}}{C_{sub} * s^2_{e.u.}}} = \sqrt{\frac{50 * 2.00}{5 * 1.63}} = 3.5$$

…the optimum allocation of resources would be to take more than 3 subsamples per plot (i.e. 4).

Three-way ANOVA (Model I, fixed-effects model)

Three or more factors may be analyzed simultaneously, each at different levels.

However…

As the number of factors increases, the required number of experimental units becomes very large.

It may not be possible to run all the tests on the same day or to hold all of the material in a single controlled environmental chamber.

There arise a large number of higher-order interactions that are difficult to interpret.

The linear model:

$$\mu_{ijkl} = \mu + \tau_{Ai} + \tau_{Bj} + \tau_{Ck} + (\tau_A\tau_B)_{ij} + (\tau_A\tau_C)_{ik} + (\tau_B\tau_C)_{jk} + (\tau_A\tau_B\tau_C)_{ijk} + \varepsilon_{ijklm}$$

Example

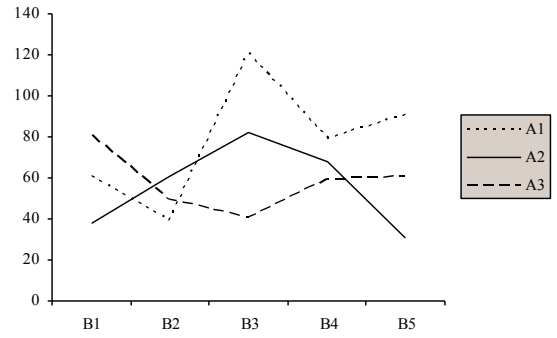
C.J. Monlezun (1979) Two-dimensional plots for interpreting interactions in the three-factor analysis of variance model. *The American Statistician* **33**: 63-69

The following group means from a hypothetical 3x5x2 experiment are used to illustrate an example with **no three-way interaction**.

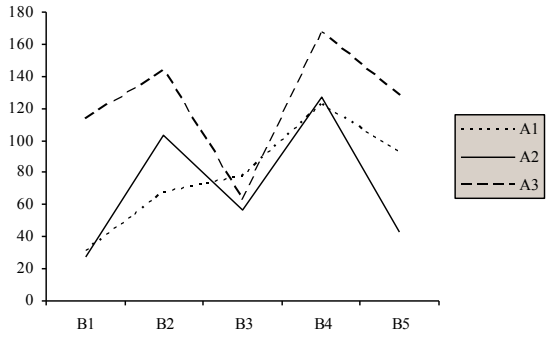
	A1C1	**A2C1**	**A3C1**	**A1C2**	**A2C2**	**A3C2**
B1	61	38	81	31	27	113
B2	39	61	49	68	103	143
B3	121	82	41	78	57	63
B4	79	68	59	122	127	167
B5	91	31	61	92	43	128

There is a two-way interaction between factors A and B at *both* levels of factor C:

A x B at C_1



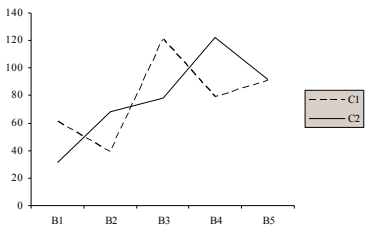
A x B at C_2



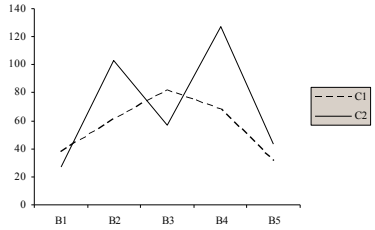
So, the ***first order interaction*** (A:B) has two values: (A:B at C_1) and (A:B at C_2). The interaction term (A:B) is the average of these values.

Similarly, there is a two-way interaction between factors B and C at *each* level of factor A:

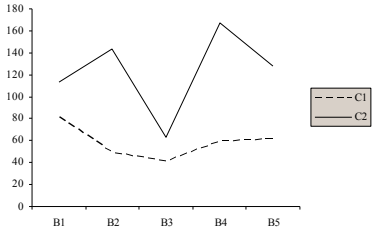
B x C at A_1



B x C at A_2



B x C at A_3

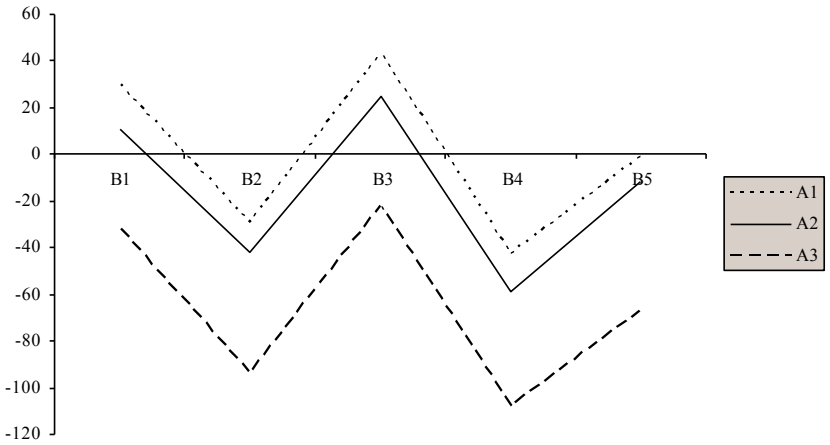


Five interaction plots are needed to visualize the two-way A x C interactions at each level of B.

Visualizing a three-way interaction:

A	B	C	Response	Effect of C (C_1 - C_2)
1	1	1	**61**	**30**
2	1	1	38	11
3	1	1	81	-32
1	1	2	**31**	
2	1	2	27	
3	1	2	113	
1	2	1	39	-29
2	2	1	61	-42
3	2	1	49	-94
1	2	2	68	
2	2	2	103	
3	2	2	143	
1	3	1	121	43
2	3	1	82	25
3	3	1	41	-22
1	3	2	78	
2	3	2	57	
3	3	2	63	
1	4	1	79	-43
2	4	1	68	-59
3	4	1	59	-108
1	4	2	122	
2	4	2	127	
3	4	2	167	
1	5	1	91	-1
2	5	1	31	-12
3	5	1	61	-67
1	5	2	92	
2	5	2	43	
3	5	2	128	

A x B, where Response = Effect of C



Each line represents one level of A.

The *average* of each line represents the average effect of C for each level of A.

While these *averages* differ among lines (i.e. A:C is significant), their *differences* are fairly constant across all levels of B.

The roughly parallel nature of the lines in this interaction plot shows us that the difference in the effects of C at the different levels of A do not vary significantly across the levels of B. [*Translation*: No significant three-way interaction.]

Example: Let A be nitrogen level and B be plow depth. In a simple two-factor experiment:

A significant *two-way interaction* (A:B) means that the *crop's response to nitrogen* varies, depending on plow depth.

Now introduce a third factor C, which could be soil type.

A significant *three-way interaction* (A:B:C) means that *the effect of plow depth on the crop's response to nitrogen* varies, depending on the soil type.