Topic 9: Factorial treatment structures

Introduction

A common objective in research is to investigate the effect of each of a number of variables, or **factors**, on some response variable. In earlier times, factors were studied one at a time, with separate experiments devoted to each one. But RA Fisher pointed out that important advantages are gained by combining the study of several factors in the same experiment. In a **factorial experiment**, the treatment structure consists of *all possible combinations of all levels of all factors under investigation*. Factorial experimentation is highly efficient because each experimental unit provides information about all the factors in the experiment. Factorial experiments also provide a systematic method of investigating the relationships among the effects of different factors (i.e. interactions).

Terminology

The different classes of treatments in an experiment are called **factors** (e.g. Fertilization, Medication, etc.). The different categories *within* each factor are called **levels** (e.g. 0, 20, and 40 lbs N/acre; 0, 1, and 2 doses of an experimental drug, etc.). We will denote different factors by upper case letters (A, B, C, etc.) and different *levels* by lower case letters with subscripts (a_1, a_2, etc.). The mean of experimental units receiving the treatment combination a_ib_i will be denoted "Mean(a_ib_i)".

We will refer to a factorial experiment with two factors and two levels for each factor as a 2x2 factorial experiment. An experiment with 3 levels of Factor A, 4 levels of Factor B, and 2 levels of Factor C will be referred to as a 3x4x2 factorial experiment. Etc.

Example of a 2x2 factorial

An example of a CRD involving two factors: Nitrogen levels (N_0 and N_1) and phosphorous levels (P_0 and P_1), applied to a crop. The response variable is yield (lbs/acre). The data:

Factor		**A = N level**			
	Level	**$a_1 = N_0$**	**$a_2 = N_1$**	**Mean (ab_i)**	**a_2-a_1**
B = P level	**$b_1 = P_0$**	40.9	47.8	44.4	6.9 (*se* A,b_1)
	$b_2 = P_1$	42.4	50.2	46.3	7.8 (*se* A,b_2)
	Mean (a_ib)	41.6	49	45.3	7.4 (*me* A)
	b_2-b_1	1.5 (*se* B,a_1)	2.4 (*se* B,a_2)	1.9 (*me* B)	

The differences a_2 - a_1 (at each leavel of B) and b_2 - b_1 (at each level of A) are called the **simple effects of a and b**, respectively, denoted (*se* A) and (*se* B). The averages of the simple effects are the **main effects of a and b**, respectively, denoted (*me* A) and (*me* B).

One way of using this data is to consider the effect of N on yield at each P level separately. This information could be useful to a grower who is constrained to use one or the other P level. This is called analyzing the *simple effects* (*se*) of N. The simple effects of applying nitrogen are to increase yield by 6.9 lb/acre for P_0 and 7.8 lb/acre for P_1.

It is possible that the effect of N on yield is the same whether or not P is applied. In this case, the two simple effects estimate the same quantity and differ only due to experimental error. One is then justified in averaging the two simple effects to obtain a mean yield response of 7.4 lb/acre. This is called the *main effect* (*me*) of N on yield. If the effect of P is independent of N level, then one could do the same thing for this factor and obtain a main effect of P on yield response of 1.9 lb/acre.

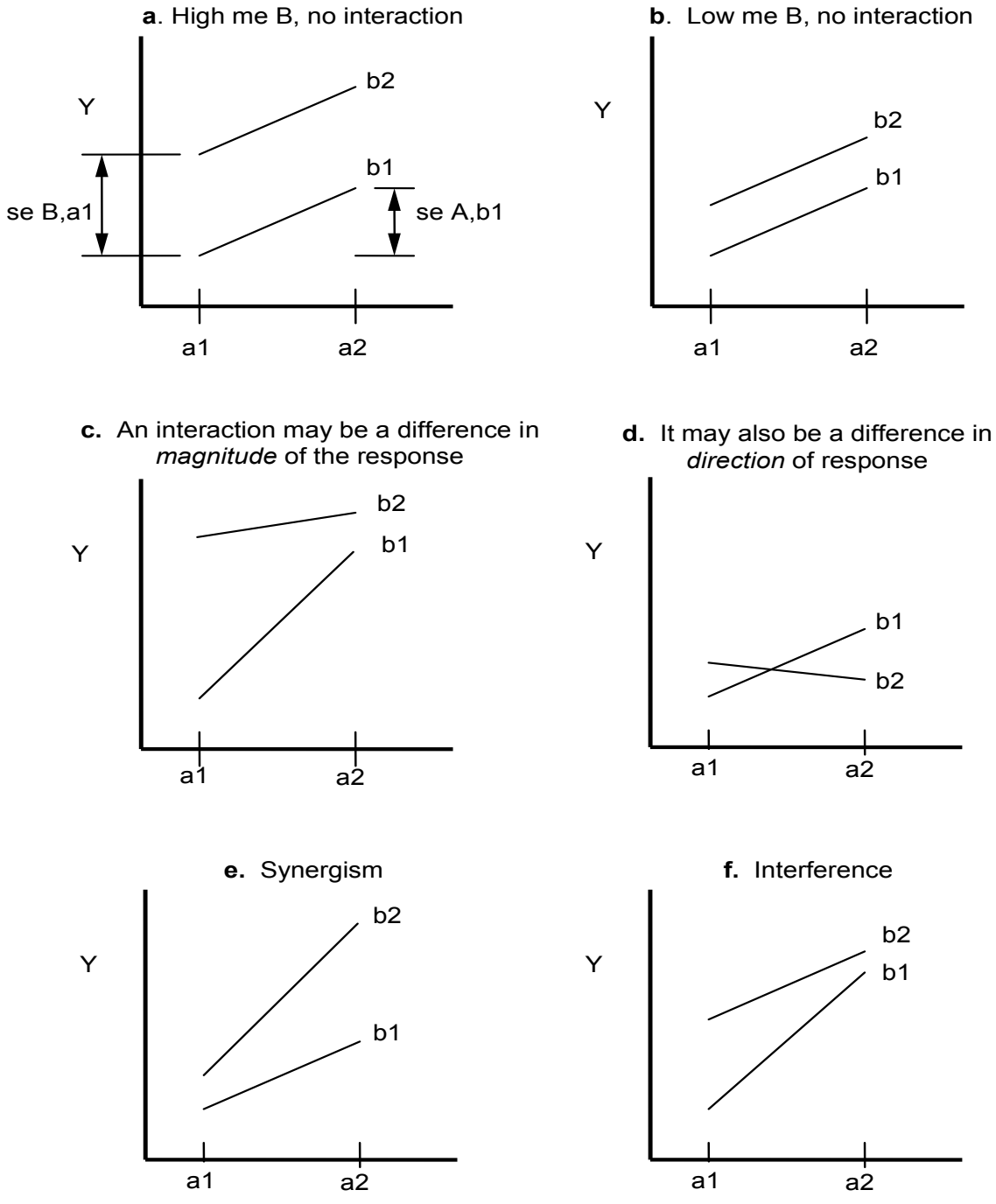
Interaction

If the simple effects of Factor A are the same across all levels of Factor B, the two factors are said to be **independent**. In such cases, it is appropriate to analyze the main effects of each factor. It may, however, be the case that the effects are not independent. For example, one might expect the application of P to permit a higher expression of the yield potential of the N application. In that case, the effect of N in the presence of P would be much larger than the effect of N in the absence of P. When the effect of one factor depends on the level of another factor, the two factors are said to exhibit an **interaction**.

An interaction is a measure of the difference in the effect of one factor at the different levels of another factor. Interaction is a common and fundamental scientific idea.

One of the primary objectives of factorial experiments, other than efficiency, is to study the interactions among factors. The sum of squares of an interaction measures the departure of the group means from the values expected on the basis of purely additive effects. In common biological terminology, a large *positive* deviation of this sort is called **synergism**. When drugs act synergistically, the result of the interaction of the two drugs may be above and beyond the simple addition of the separate effects of each drug. When the combination of levels of two factors *inhibit* each other's effects, we call it **interference**. Both synergism and interference increase the interaction SS.

These differences between the simple effects of two factors, also known as **first-order interactions** or **two-way interactions**, can be visualized in the following *interaction plots*:



In interaction plots, perfect additivity (i.e. no interaction) is indicated by perfectly parallel lines. Significant departures from parallel indicate significant interactions.

Reasons for carrying out factorial experiments

1. *To investigate interactions*: If factors are not independent, single factor experiments provide a disorderly, incomplete, and often quite misleading picture of the system. More than this, most of the interesting questions today concern interactions.

2. *To establish the dependence or independence of factors of interest*: In the initial phases of an investigation, pilot or exploratory factorial experiments can establish which factors are independent and can therefore be justifiably analyzed in separate experiments.

3. *To offer recommendations that must apply over a wide range of conditions*: One can introduce "subsidiary factors" (e.g. soil type) into an experiment to ensure that any recommended results apply across a necessary range of circumstances.

Some disadvantages of factorial experiments

1. The total possible number of treatment level combinations increases rapidly as the number of factors increases. For example, to investigate 7 factors (3 levels each) in a factorial experiment requires, at minimum, 2187 experimental units.

2. Higher order interactions (three-way, four-way, etc.) are very difficult to interpret. So a large number of factors complicates the interpretation of results.

Differences between nested and factorial experiments (Biometry 322-323)

Looking at data table, it is easy to get confused between nested and factorial experiments. Consider a factorial experiment in which leaf discs are grown in 10 different tissue culture media (all possible combinations of 5 different types of sugars and 2 different pH levels). In what way does this differ from a *nested* design in which each sugar solution is prepared twice, so there are two batches of sugar for each treatment? The following tables represent both designs, using asterisks to represent measurements of the response variable (leaf growth).

2x5 factorial experiment

	Sugar Type				
	1	2	3	4	5
pH_1	* *	* *	* *	* *	* *
pH_2	* *	* *	* *	* *	* *

Nested experiment

	Sugar Type				
	1	2	3	4	5
Batch 1	* *	* *	* *	* *	* *
Batch 2	* *	* *	* *	* *	* *

The data tables look very similar, so what's the difference here? The factorial analysis implies that the two pH classes are **common** across the entire study (i.e. pH level 1 is a specific pH level that is the same across all sugar treatments). By analogy, if you were to analyze the nested experiment as a two-way factorial ANOVA, it would imply that Batches are common across the

entire study. But this is not so. Batch 1 for Treatment 1 has no closer relation to Batch 1 for Treatment 2 than it does to Batch 2 for Treatment 2. "Batch" is an ID, and Batches 1 and 2 are simply arbitrary designations for two randomly prepared sugar solutions for each treatment.

Now, if all batches labeled 1 were prepared by the same technician on the same day, while all batches labeled 2 were made by someone else on another day, then "1" and "2" would represent meaningfully common classes across the study. In this case, the experiment could properly be analyzed using a two–way ANOVA with Technicians/Days as blocks (RCBD).

While they are both require two-way ANOVAs, RCBD's differ from true factorial experiments in their *objective*. In this example, we are not interested in the effect of the batches or in the interaction between batches and sugar types. Our main interest is to control for this additional source of variation so that we can better detect the differences among treatments; toward this end, we assume there to be no interactions.

When presented with an experimental description and its accompanying dataset, the critical question to be asked to differentiate **factors** from **experimental units or subsamples** is this: Do the classes in question have a consistent meaning across the experiment, or are they simply ID's? Notice that ID (or dummy) classes can be swapped without affecting the analysis (switching the names of "Batch 1" and "Batch 2" within any given Sugar Type has no consequences) whereas factor classes cannot (switching "pH_1" and "pH_2" within any given Sugar Type will completely muddle the analysis).

The two-way factorial analysis (Model I ANOVA)

The linear model

The linear model for a two-way factorial analysis is

$$Y_{ijk} = \mu + \tau_{Ai} + \tau_{Bj} + (\tau_A\tau_B)_{ij} + \varepsilon_{ijk}$$

Here τ_{Ai} represents the main effect of factor A (i = 1,...,a), τ_{Bj} represents the main effect of factor B, (j = 1,...,b), $(\tau_A\tau_B)_{ij}$ represents the *interaction* of factor A level i with factor B level j, and ε_{ijk} is the error associated with replication k of the factor combination ij (k = 1,..,r). In dot notation:

$$Y_{ijk} = \overline{Y}_{...} + (\overline{Y}_{i..} - \overline{Y}_{...}) + (\overline{Y}_{.j.} - \overline{Y}_{...}) + (\overline{Y}_{ij.} - \overline{Y}_{i..} - \overline{Y}_{.j.} + \overline{Y}_{...}) + (Y_{ijk} - \overline{Y}_{ij.})$$

	main effect factor A	main effect factor B	interaction effect (A*B)	experimental error

The null hypotheses for a two-factor experiment are $\tau_{Ai} = 0$, $\tau_{Bj} = 0$, and $(\tau_A\tau_B)_{ij} = 0$. The F statistics for each of these hypotheses may be interpreted independently due to the orthogonality of their respective sums of squares.

$$TSS = SSA + SSB + SSAB + SSE$$

The ANOVA

In the ANOVA for two-way factorial experiments, the Treatment SS is partitioned into three orthogonal components: a SS for each factor and an interaction SS. This partitioning is valid even when the overall F test among treatments is not significant. Indeed, there are situations where one factor, say B, has no effect on the response variable and hence contributes no more to the SST than one would expect by chance along. In such a circumstance, a significant response to A might well be lost in an overall test of significance. In a factorial experiment, the overall SST is rightly understood to be an intermediate computational quantity rather than an end product (i.e. a numerator for an F test).

In a two factor experiment (a x b), there are a total of ab treatment combinations and therefore (ab – 1) treatment degrees of freedom. The main effect of factor A has (a – 1) df and the main effect of factor B has (b – 1) df. The interaction (AxB) has (a – 1)(b – 1) df. With r replications per treatment combination, there are a total of (rab) experimental units in the study and, therefore, (rab – 1) total degrees of freedom.

General ANOVA table for a two-way CRD factorial experiment:

Source	df	SS	MS	F
Factor A	a - 1	SSA	MSA	MSA/MSE
Factor B	b - 1	SSB	MSB	MSB/MSE
AxB	(a - 1)(b - 1)	SSAB	MSAB	MSAB/MSE
Error	ab(r - 1)	SSE	MSE	
Total	rab - 1	TSS		

The interaction SS is the variation due to the departures of group means from the values expected on the basis of additive combinations of the two factors' main effects. 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 (i.e. one may compare the means of one factor across all levels of the other factor).
>
> Significant interaction: Subsequent analysis (mean comparisons, contrasts, etc.) are performed on the simple effects (i.e. one must compare the means of one factor separately for *each level of the other factor*).

Relationship between factorial experiments and experimental design

Experimental designs are characterized by the method of randomization: how were the treatments assigned to the experimental units? In contrast, factorial experiments are characterized by a certain *treatment structure*, with no requirements on how the treatments are randomly assigned to experimental units. A factorial treatment structure may occur within *any* experimental design.

Example of a 4 x 2 factorial experiment within three different experimental designs:

Since Factor A has 4 levels (1, 2, 3, 4) and Factor B has 2 levels (1, 2), there are eight different treatment combinations: (11, 12, 13, 14, 21, 22, 23, 24).

CRD with 3 replications

24 23 13 23 24 14 13 23 11 24 12 14 22 13 12 21 21 11 22 12 11 22 21 14

RCBD with 3 blocks

13 12 21 23 11 24 14 22	**12 11 24 23 13 22 21 14**	**24 14 22 21 11 13 23 12**

8 x 8 Latin Square

24	**11**	**22**	**12**	**13**	**14**	**23**	**21**
21	**23**	**13**	**14**	**22**	**12**	**11**	**24**
12	**14**	**24**	**11**	**23**	**21**	**22**	**13**
13	**22**	**21**	**24**	**11**	**23**	**14**	**12**
23	**12**	**11**	**13**	**21**	**22**	**24**	**14**
14	**24**	**23**	**22**	**12**	**13**	**21**	**11**
11	**21**	**12**	**23**	**14**	**24**	**13**	**22**
22	**13**	**14**	**21**	**24**	**11**	**12**	**23**

Example of a 2 x 3 factorial experiment within an RCBD with no significant interactions (ST&D 391)

Data: The number of quack-grass shoots per square foot after spraying with maleic hydrazide. Treatments are maleic hydrazide applications rates (**R**: 0, 4, and 8 lbs/acre) and delay in cultivation after spraying (**D**: 3 and 10 days).

D	R	Block 1	Block 2	Block 3	Block 4	Means
3	0	15.7	14.6	16.5	14.7	15.38
	4	9.8	14.6	11.9	12.4	12.18
	8	7.9	10.3	9.7	9.6	9.38
10	0	18.0	17.4	15.1	14.4	16.23
	4	13.6	10.6	11.8	13.3	12.33
	8	8.8	8.2	11.3	11.2	9.88
Means		12.30	12.62	12.72	12.60	12.56

The R Code

```
#The ANOVA
quack_mod<-lm(Number ~ D + R + D*R + Block, quack_dat)  ← WHAT'S MISSING??
anova(quack_mod)
```

```
Note: If there were only 1 replication per D-R combination (i.e. only 1
block) you could not include the D*R interaction in the model.  There would
not be enough error df.
```

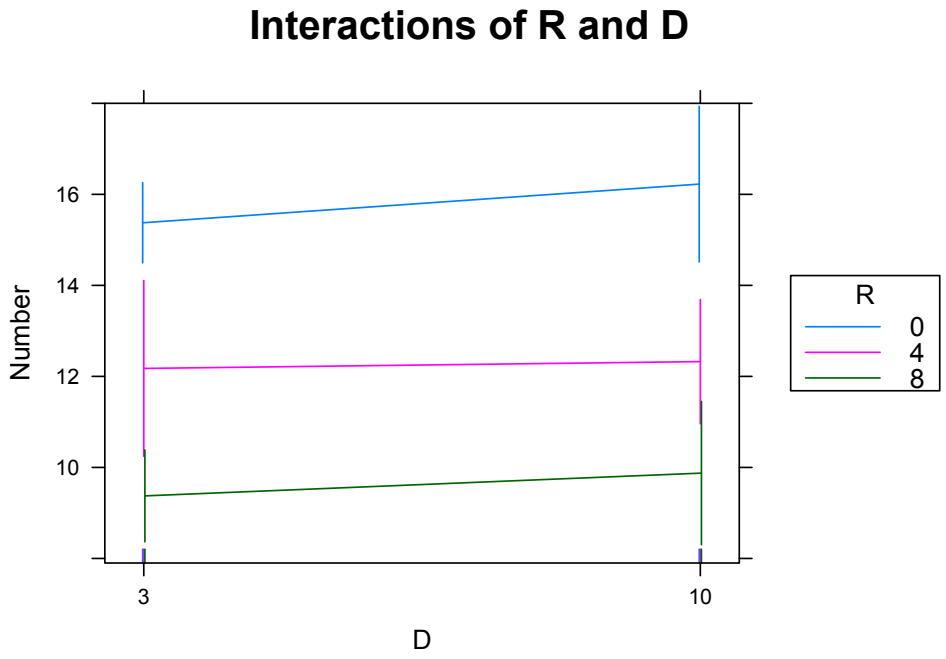
The output

```
Analysis of Variance Table
```

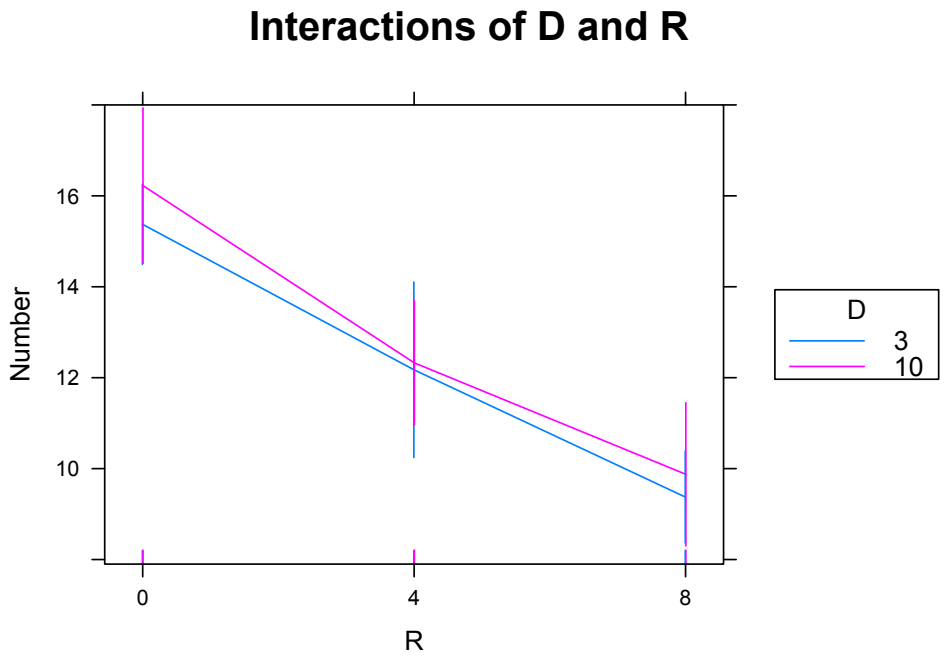
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
D	1	1.500	1.500	0.5713	0.4614	
R	2	153.663	76.832	29.2630	6.643e-06	***
Block	3	0.582	0.194	0.0738	0.9731	
D:R	2	0.490	0.245	0.0933	**0.9114**	**NS**
Residuals	15	39.383	2.626			

Note that the 15 error df =
Block*D (3 df) + Block*R (6 df) + Block*D*R (6 df)

Essentially parallel lines in an interaction plot, as those observed in this case, indicate the absence of an interaction.



The lines of this plot are essentially parallel because the difference between D levels is roughly the same at all R levels. This non-interaction can be seen from the perspective of either factor:



Here, the lines are essentially parallel because the difference between R levels is approximately the same at all levels of D.

If no interaction is present, you proceed by analyzing the *main effects*.

If there is no significant interaction, you are justified in analyzing the effect of R without regard for the level D because the effect of R does not depend on the level of D, and vice versa. Detailed comparisons of the mean effects can be performed using contrasts or an appropriate multiple comparison test. Representative analyses:

```
#Analyzing the main effects
library(agricolae)
HSD.test(quack_mod, "D")
HSD.test(quack_mod, "R")
```

Tukey means separations

```
D,  means
alpha: 0.05 ; Df Error: 15
Critical Value of Studentized Range: 3.014325
Honestly Significant Difference: 1.409971

Means with the same letter are not significantly different.

Groups, Treatments and means
a      10    12.81
a      3     12.31
```

```
R,  means
alpha: 0.05 ; Df Error: 15
Critical Value of Studentized Range: 3.673378
Honestly Significant Difference: 2.104414

Means with the same letter are not significantly different.

Groups, Treatments and means
a      0     15.8
b      4     12.25
c      8     9.625
```

```
#Performing a trend analysis on the factor R
# Contrast 'Linear'     -1,0,1
# Contrast 'Quadratic' 1,-2,1
contrastmatrix<-cbind(c(-1,0,1),c(1,-2,1))
contrasts(quack_dat$R)<-contrastmatrix

quack_Rcontrast_mod<-aov(Number ~ D + R + D*R + Block, quack_dat)
summary(quack_Rcontrast_mod, split = list(R = list("Linear" = 1, "Quadratic"
 = 2)))
```

Contrasts (trend analysis of R)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
D	1	1.50	1.50	0.571	0.461	
R	2	153.66	76.83	29.263	6.64e-06	***
R: Linear	1	152.52	152.52	58.092	1.56e-06	***
R: Quadratic	1	1.14	1.14	0.435	0.520	
Block	3	0.58	0.19	0.074	0.973	
D:R	2	0.49	0.25	0.093	0.911	
D:R: Linear	1	0.12	0.12	0.047	0.832	
D:R: Quadratic	1	0.37	0.37	0.140	0.714	
Residuals	15	39.38	2.63			

9.7.4.2. Partitioning the Interaction Sum of Squares

It is possible to find significant interaction components within an overall non-significant interaction!

In Topic 4, we discussed how it is possible to find a significant 1 df contrast despite an overall non-significant treatment F test. The concept here is similar. When you divide the Interaction SS by the Interaction df to determine the Interaction MS, you are cutting that SS into **equal parts**. But it is possible that one component of the interaction (e.g. D * R Linear) is bigger than another (e.g. D * R quadratic), and that that part *is* significant.

Look again at the contrast output above. The trend analysis using orthogonal contrasts partitioned not only the SS for the factor R but also the SS of the interaction D*R. In this way, R makes partitioning the Interaction SS very easy. This can be done another way as well, by "opening up" the factorial treatment structure, as described below:

To manually partition the D*R interaction (2 df), you first need to create a variable, say "TRT," whose values are the full set of factorial combinations of D and R levels. The values of TRT for this example would be:

D3 R0 = TRT 1	D10 R0 = TRT 4
D3 R4 = TRT 2	D10 R4 = TRT 5
D3 R8 = TRT 3	D10 R8 = TRT 6

Now we are back in familiar territory. We have "opened up" the factorial treatment structure, redefining it as a simple one-way classification. Now we can simply analyze TRT and use contrasts to partition the interaction, as you've seen before.

Modifying the original data table:

D	R	TRT	Block	Number
3	0	1	1	15.7
3	0	1	2	14.6
3	0	1	3	16.5
…	…	…	…	…
10	8	6	2	8.2
10	8	6	3	11.3
10	8	6	4	11.2

Representative code for this approach:

```
#Performing a trend analysis on the factor TRT
# TRT Levels:                 1   2   3   4   5   6
# Contrast 'R Linear'         -1  0   1   -1  0   1
# Contrast 'R Quadratic'      1   -2  1   1   -2  1
# Contrast 'D'                1   1   1   -1  -1  -1
# Contrast 'R Lin * D'        -1  0   1   1   0   -1
# Contrast 'R Quad * D'       1   -2  1   -1  2   -1
contrastmatrix<-cbind(c(-1,0,1,-1,0,1),c(1,-2,1,1,-2,1),
      c(1,1,1,-1,-1,-1),c(-1,0,1,1,0,-1),c(1,-2,1,-1,2,-1))
contrasts(quack_dat$TRT)<-contrastmatrix

quack_Rcontrast_mod<-aov(Number ~ TRT + Block, quack_dat)
summary(quack_Rcontrast_mod, split = list(TRT = list("Lin R" = 1, "Quad R" =
 2, "D" = 3, "Lin R * D" = 4, "Quad R * D" = 5)))
```

The output:

```
                  Df Sum Sq Mean Sq F value   Pr(>F)
TRT                5 155.65   31.13  11.857 8.92e-05 ***
  TRT: Lin R       1 152.52  152.52  58.092 1.56e-06 ***
  TRT: Quad R      1   1.14    1.14   0.435    0.520
  TRT: D           1   1.50    1.50   0.571    0.461
  TRT: Lin R * D   1   0.12    0.12   0.047    0.832
  TRT: Quad R * D  1   0.37    0.37   0.140    0.714
Block              3   0.58    0.19   0.074    0.973
Residuals         15  39.38    2.63
```

Here we have successfully partitioned the Treatment SS into its five single-df components, two of which are interaction components. Compare this output to that of the previous factorial analysis:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
D	1	1.50	1.50	0.571	0.461	
R	2	153.66	76.83	29.263	6.64e-06	***
R: Linear	**1**	**152.52**	152.52	58.092	1.56e-06	***
R: Quadratic	**1**	**1.14**	1.14	0.435	0.520	
Block	3	0.58	0.19	0.074	0.973	
D:R	2	0.49	0.25	0.093	0.911	
D:R: Linear	**1**	**0.12**	0.12	0.047	0.832	
D:R: Quadratic	**1**	**0.37**	0.37	0.140	0.714	
Residuals	15	39.38	2.63			

By "opening up" the factorial treatment structure, we have successfully partitioned the SS of R into its two single-df components and the SS of the D*R interaction into its two single-df components. In this case, no significant interaction components were found "hiding" inside the overall non-significant interaction.

Is it worth partitioning the Interaction SS?

To answer this, divide it by 1 and test for significance.
If that is not significant, it is not worth partitioning the Interaction SS because no significance is found even when *all* the variation is assigned to one component (1 df) of the interaction.