Topic 9. Factorial Experiments [ST&D Chapter 15]

9. 1. 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. Later, R. A. 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 observation provides information about all the factors in the experiment. Factorial experimentation also provides a systematic method of investigating the relationships among the effects of different factors (i.e. interactions).

9. 2. 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 (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.

9. 3. Example of a 2x2 factorial

Below is 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).

Factor			A = N level		
	Level	**a_1** = N_0	**a_2** = N_1	Mean (a_{bi})	a_2-a_1
	b_1 = P_0	40.9	47.8	44.4	6.9 (*se* A,b_1)
B = P level	**b_2** = P_1	42.4	50.2	46.3	7.8 (*se* A,b_2)
	Mean (a_ib)	41.6	49.0	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 and b_2 - b_1 are called the **simple effects**, denoted (*se* A) and (*se* B). The differences between the means are the **main effects,** 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 lbs/acre for P_0 and 7.8 lbs/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 looking at the difference between the two means to obtain a main yield response of 7.4 lbs/acres. This is called the *main effect* (*me*) of N on yield. If the effect of P is the same at any N level then one could do the same thing for this factor to get a main effect of 1.9 lb/a.

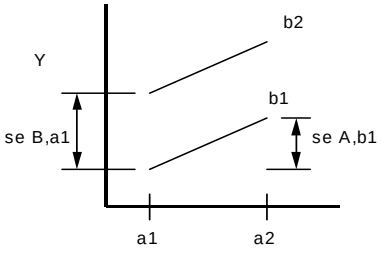
9. 4. Interaction

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.**

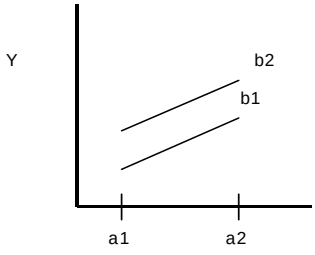
An interaction is a measure of the difference in the effect of one factor at the different levels of another factor. Interaction is a common and fundamental scientific idea.

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 *inhibits* each other's effects, we call it **interference**. Both synergism and interference increase the interaction SS.

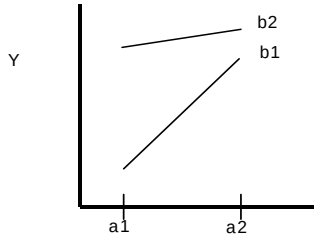
These differences between the simple effects of two factors, also known as **first-order interactions or two-way interactions,** can be visualized in the following *interaction plots*.



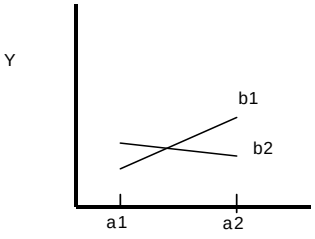
High me B, no interaction



Low me B, no interaction



Interaction may be a difference in magnitude of response



Interaction may be a difference in direction of response

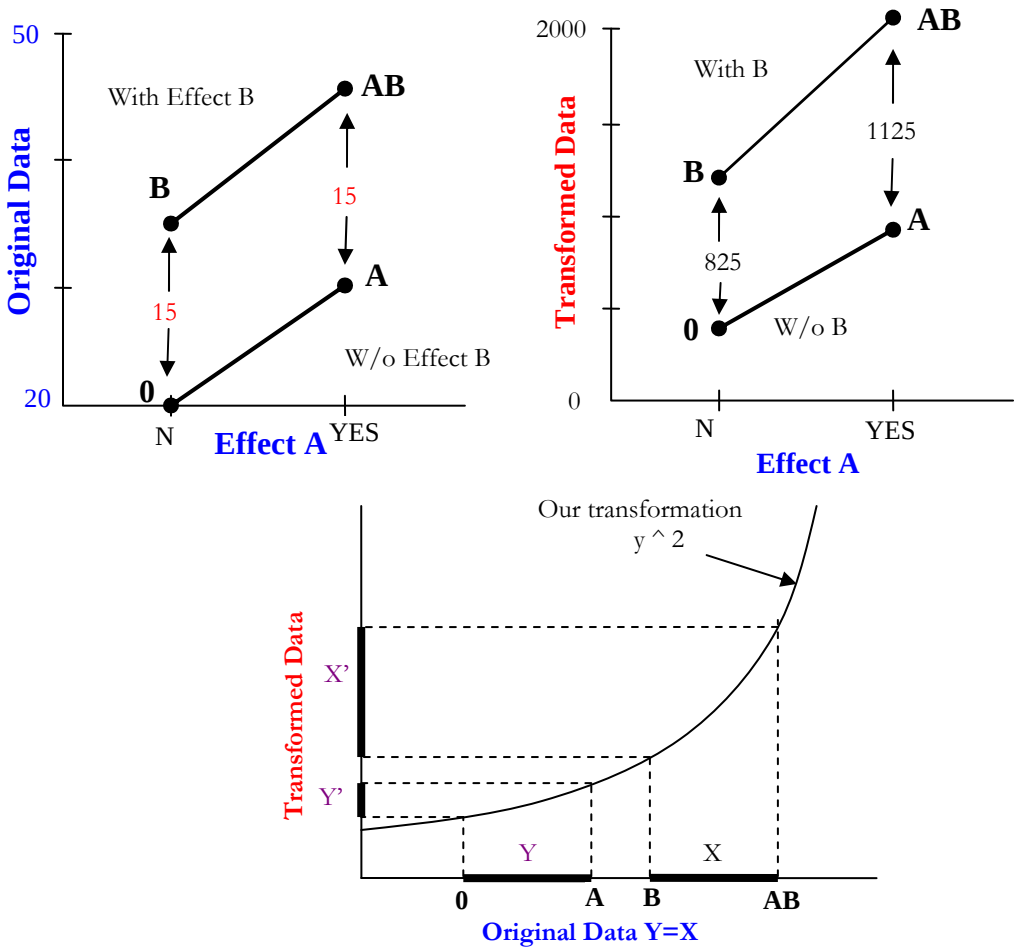
Pitfalls of Interpreting Interactions in Transformed Data

	0	A	B	AB
Y	20	30	35	45
Y^2	400	900	1225	2025

A: increases 10; B increases 15; A and B increases 25.
Perfectly additive and therefore parallel lines (left figure)

After transformation according to Y^2 (bottom figure)
A: adds 500 in the absence of B but 800 in its presence!
Non-additive effect and not parallel lines (right figure)

In interaction plots, perfect additivity (i.e. no interaction) is indicated by perfectly parallel lines



Transformation of perfectly additive data may result in non additive results (significant interaction).

9. 5. 1. 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 more fully 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.

9. 5. 2. 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 greatly complicates the interpretation of results.

9. 6. Differences between nested and factorial experiments (Biometry pages 322-323)

People are often confused between nested and factorial experiments. Consider a factorial experiment in which growth of leaf discs was measured in tissue culture with five different types of sugars at two 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.

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 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).

9. 7. The two-way factorial analysis (for fixed-effects model or Model I)

9. 7. 1. The linear model for two-way factorial experiments

The linear model for a two-way factorial analysis is

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Here α_i represents the main effect of factor A i, i = 1,...,a, β_j represents the main effect of factor B, j = 1,...,b, $(\alpha\beta)_{ij}$ 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 i; main effect factor j; interaction effect; experimental error

The null hypotheses for a tw- factor experiment are $\alpha_i = 0$, $\beta_j = 0$, and $(\alpha\beta)_{ij} = 0$. The F statistic s for each of these hypotheses may be interpreted independently due to the orthogonality of their respective sum of squares (they are equivalent to orthogonal contrasts). The sum of squares equation becomes:

T SS = SSA + SSB + SSAB + SSE.

9. 7. 2. ANOVA for a two-way factorial design (for fixed-effects model or Model I)

In the ANOVA for two-way factorial experiments, the Treatments SS is partitioned into three orthogonal components: a SS for each factor and an interaction. 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 n effect on A and hence contributes no more to the SST than one would expect by chance; a significant response to A might well be lost in an overall test of significance. In a factorial experiment the overall SST is more often just an intermediate computational quantity rather than an end product.

In a two-way factorial (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*).

9. 7. 3. Relationship between factorial experiments and experimental design

While an experimental design is concerned with the assignment of treatments to experimental units, a factorial experiment is concerned with the structure of treatments. The factorial structure may be placed into any experimental design.

Example of a 4 x 2 Factorial experiment replicated in different designs

- Factor A at 4 levels (1, 2, 3, 4)

- Factor B at 2 levels (1, 2)
- Eight different combinations of both factors: 11 12 13 14 21 22 23 24

CRD with 3 replicates of the factorial experiment

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

9. 7. 4. 1. Example of a 2 x 3 factorial organized in a Randomized Complete Block Design with no significant interactions (ST&D Table 15.3 p 391)

Square root of the number of quack-grass shoots per square foot after spraying with maleic hydrazide. Treatments are maleic hydrazide applications rates (**R**) of 0, 4, and 8 lb/acre, and days delay in cultivation after spray (**D,** 3 or 10 days)

D	R	Block 1	Block 2	Block 3	Block 4	Total
3	0	15.7	14.6	16.5	14.7	61.5
	4	9.8	14.6	11.9	12.4	48.7
	8	7.9	10.3	9.7	9.6	37.5
10	0	18.0	17.4	15.1	14.4	64.9
	4	13.6	10.6	11.8	13.3	49.3
	8	8.8	8.2	11.3	11.2	39.5
Totals		73.8	75.7	76.3	75.6	301.4

SAS Program

```
data STDp391;
input D R block number @@;
cards;
 3 0 1 15.7            3 4 1  9.8            3 8 1  7.9
 3 0 2 14.6            3 4 2 14.6            3 8 2 10.3
 3 0 3 16.5            3 4 3 11.9            3 8 3  9.7
 3 0 4 14.7            3 4 4 12.4            3 8 4  9.6
10 0 1 18.0           10 4 1 13.6           10 8 1  8.8
10 0 2 17.4           10 4 2 10.6           10 8 2  8.2
10 0 3 15.1           10 4 3 11.8           10 8 3 11.3
10 0 4 14.4           10 4 4 13.3           10 8 4 11.2
proc GLM;
  class D R block;
  model number= block D R D*R;
  means D|R / lsd;
  contrast 'R lineal'     R -1  0  1;
  contrast 'R quadratic'  R  1 -2  1;
run; quit
If you have 1 rep only (1 block) you can not include the D*R in the model
```

Dependent Variable: NUMBER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	156.235000	19.529375	7.44	0.0005
Error	15	39.383333	2.625556		
Corrected Total	23	195.618333			
BLOCK	3	0.581667	0.193889	0.07	0.9731
D	1	1.500000	1.500000	0.57	0.4614
R	2	153.663333	76.831667	29.26	0.0001
D*R	2	0.490000	0.245000	0.09	0.9114

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

T tests (LSD) for variable: NUMBER

T Grouping	Mean	N	D
A	12.8083	12	10
A	12.3083	12	3

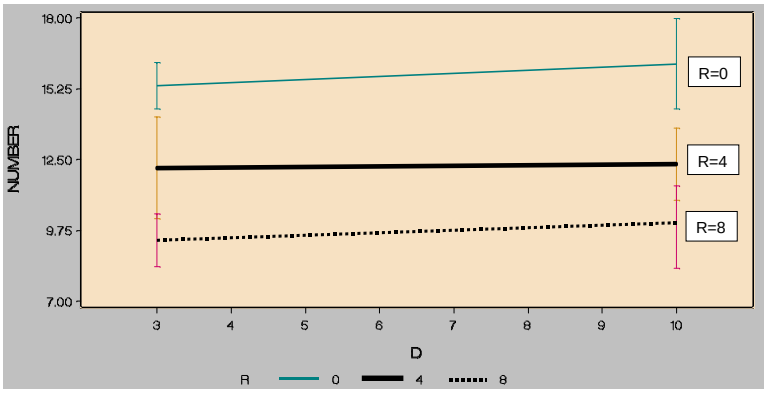
T Grouping	Mean	N	R
A	15.8000	8	0
B	12.2500	8	4
C	9.6250	8	8

D	R	N	Mean	SD
3	0	4	15.3750000	0.89953692
3	4	4	12.1750000	1.97040605
3	8	4	9.3750000	1.03077641
10	0	4	16.2250000	1.74427635
10	4	4	12.3250000	1.39373599
10	8	4	9.8750000	1.60701587

Dependent Variable: NUMBER

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
R lineal	1	152.522500	152.522500	58.09	0.0001
R quadratic	1	1.140833	1.140833	0.43	0.5198

The figure below was produced using the Analyst application. Within the Factorial ANOVA window there is an option to produce plots of the dependent means for the two-way effects. Parallel lines, as those observed in this graphic indicate absence of interaction. The differences among "R" doses are the same for the different "D" levels. As a consequence of these constant differences the lines are parallel.



If no interactions are present the next step is the analysis of the main effects.

Multiple comparisons can be performed using the means of the main effects using CONTRAST or the multiple comparison tests described on Topic 5. This strategy is represented in the program by lines:

```
means D|R / lsd;
contrast 'R lineal'      R -1  0  1;
contrast  'R quadratic'  R  1 -2  1;
```

9. 7. 4. 2. Partitioning of the SS for the interaction in independent parts

It is possible that significant interaction components are hidden in a non-significant interaction!

This is the similar concept as a significant contrast within a non significant ANOVA we discussed in section 4. When you divide your SS interaction by the df you are cutting that SS in **equal parts**. However, it is possible that a part of the interaction is bigger than the other (example a D by R lineal > D by R quadratic), and that that part is significant.

We will learn now how to partition an interaction to test this possibility. If you want **multiple comparisons of the D*R combinations**, you can create a variable, say TRT, whose values are the combinations of values of D and R. The values of TRT for the previous example would be

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

D10 R4= TRT 5
D10 R8= TRT 6.

Then analyze TRT means as if TRT were a one-way classification of the data and use contrast to partition the interaction. The contrasts in blue are the two interaction contrasts (A good discussion is available in "SAS System for Linear Models, 3rd Ed. P 94-104).

```
proc glm order=data;
  class TRT block;
  model number= block TRT;
   contrast  'D'                              TRT  1  1  1 -1 -1 -1;
   contrast  'R lineal'                       TRT -1  0  1 -1  0  1;
   contrast  'R quadratic'                    TRT  1 -2  1  1 -2  1;
   contrast  'Interaction lineal R * D'    TRT -1  0  1  1  0 -1;
   contrast  'Interaction Quadratic R * D' TRT  1  -2 1 -1  2 -1;

run;quit;
```

Factorial analysis opened as an RCBD: TRT with 6 levels

Model number = TRT block;

Class Level Information

Class	Levels	Values
TRT	6	1 2 3 4 5 6
block	4	1 2 3 4

Dependent Variable: number

Source	DF	SS	MS	F Value	Pr > F
Model	8	156.235	19.529	7.44	0.0005
Error	15	39.383	2.626		
Corr. Total	23	195.618			

Source	DF	SS	MS	F Value	Pr > F
block	3	0.582	0.194	0.07	0.9731
TRT	**5**	155.653	31.131	11.86	<.0001

Contrast	DF	Contrast SS	MS	F Value	Pr > F
D	**1**	1.500	1.500	0.57	0.4614
R lineal	**1**	152.522	152.522	58.09	<.0001
R quadratic	**1**	1.141	1.141	0.43	0.5198
Int R L*D	**1**	**0.123**	0.122	0.05	0.8319
Int R Q*D	**1**	**0.367**	0.367	0.14	0.7135

Previous analysis as a Factorial

Model number= D R D*R block;

Class Level Information

Class	Levels	Values
D	2	1 2
R	3	1 2 3
block	4	1 2 3 4

Source	DF	SS	MS	F Value	Pr > F
BLOCK	3	0.582	0.194	0.07	0.9731
D	1	1.500	1.500	0.57	0.4614
R	2	153.663	76.832	29.26	0.0001
D*R	**2**	**0.490**	0.245	0.09	0.9114

Contrast	DF	Contrast SS	MS	F Value	Pr > F
R lineal	1	152.522	152.522	58.09	0.0001
R quadratic	1	1.141	1.141	0.43	0.5198

To decide if it is worth to partition the Interaction SS, divide it by 1 and test the significance. If this is not significant, it is not worth to partition the Interaction SS because even if all the variation is assigned to one component of the interaction, it will not be significant

9.7.4.3 Another example of a partition of Interaction SS

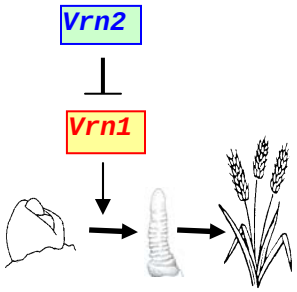
Partition of interaction example: effect of ***Vrn1*** and ***Vrn2*** genes on flowering.

Each plant from a segregating population from a cross between parents A and B (N=102) was characterized with molecular markers and the number of alleles of parent A indicated (BB= 0, AB=1, AA=2).

The auxiliary variable "**type**" represents each combination of ***Vrn1*** and ***Vrn2*** classes.

```
data interpart;
input type Vrn1 Vrn2 days;
cards;
1  0 0 89       1  0 0 97       1  0 0 101      1  0 0 100
1  0 0 98       2  0 1 133      2  0 1 144      2  0 1 148
2  0 1 148      2  0 1 138      2  0 1 130      2  0 1 133
2  0 1 128      2  0 1 130      2  0 1 137      2  0 1 141
2  0 1 134      2  0 1 133      2  0 1 138      2  0 1 131
2  0 1 148      3  0 2 163      3  0 2 153      3  0 2 161
3  0 2 153      3  0 2 156      3  0 2 148      4  1 0 109
4  1 0  83      4  1 0  87      4  1 0 103      4  1 0 110
4  1 0  81      4  1 0  99      4  1 0  98      4  1 0  83
4  1 0  78      4  1 0  92      4  1 0  92      4  1 0  91
4  1 0  85      4  1 0  83      4  1 0  66      5  1 1 122
5  1 1 121      5  1 1 121      5  1 1 122      5  1 1 125
5  1 1 118      5  1 1 123      5  1 1 124      5  1 1 125
5  1 1 108      5  1 1 112      5  1 1 126      5  1 1 118
5  1 1  98      5  1 1 116      5  1 1 106      5  1 1 117
5  1 1 110      5  1 1 113      5  1 1 129      5  1 1 116
6  1 2 140      6  1 2 125      6  1 2 178      6  1 2 136
6  1 2 132      6  1 2 133      6  1 2 135      6  1 2 134
6  1 2 125      6  1 2 125      6  1 2 128      6  1 2 121
6  1 2 128      6  1 2 135      7  2 0  91      7  2 0 103
7  2 0  81      7  2 0  99      7  2 0  88      7  2 0  99
7  2 0  73      8  2 1 137      8  2 1 118      8  2 1 120
8  2 1 153      8  2 1  86      8  2 1 114      8  2 1 126
8  2 1 120      8  2 1 120      8  2 1 118      8  2 1 119
8  2 1 106      8  2 1 112      8  2 1 111      8  2 1 117
9  2 2 124      9  2 2 124
;
proc glm order=data;
 class vrn1 vrn2;
 model days= vrn1|vrn2;
 contrast 'Lineal Vrn1'        vrn1 -1 0 1;
 contrast 'Quadratic Vrn1'     vrn1 1 -2 1;

proc glm order=data;
```

1	2	3	4	5	6	7	8	9	Type
0	0	0	1	1	1	2	2	2	***Vrn1***
0	1	2	0	1	2	0	1	2	***Vrn2***



```
 class type;
 model days= type;
 contrast 'Lineal   Vrn1' Type -1 -1 -1  0  0  0  1  1  1;
 contrast 'Quadrat  Vrn1' Type  1  1  1 -2 -2 -2  1  1  1;
 contrast 'Lineal   Vrn2' Type -1  0  1 -1  0  1 -1  0  1;
 contrast 'Quadrat  Vrn2' Type  1 -2  1  1 -2  1  1 -2  1;
 contrast 'Int l by l'    Type  1  0 -1  0  0  0 -1  0  1;
 contrast 'Int l by q'    Type -1  2 -1  0  0  0  1 -2  1;
 contrast 'Int q by l'    Type -1  0  1  2  0 -2 -1  0  1;
 contrast 'Int q by q'    Type  1 -2  1 -2  4 -2  1 -2  1;
run; quit;
```

3x3 Factorial

Class	Levels	Values
Vrn1	3	0 1 2
Vrn2	3	0 1 2

Source	DF	SS	MS	F Value	Pr > F
Model	8	38006	4751	42.97	<.0001
Error	93	10282	111		
Corrected Total	101	48288			

Source	DF	Type III SS	MS	F Value	Pr > F
Vrn1	2	4435	2217	20.06	<.0001
Vrn2	2	21310	10655	96.37	<.0001
Vrn1*Vrn2	4	808	202	1.83	**0.1303 NS**

Contrast		DF	SS	MS	F Value	Pr > F
Lineal	Vrn1	1	2829	2829	25.58	<.0001
Quadrat	Vrn1	1	847	847	7.66	0.0068

Partition of interaction using one way ANOVA and contrasts

Class	Levels	Values
type	9	1 2 3 4 5 6 7 8 9

Source	DF	SS	MS	F Value	Pr > F
Type	8	38006	4751	42.97	<.0001
Error	93	10282	111		
Corrected Total	101	48288			

Contrast		DF	SS	MS	F Value	Pr > F
Lineal	Vrn1	1	2829	2829	25.58	<.0001
Quadrat	Vrn1	1	847	847	7.66	0.0068
Lineal	Vrn2	1	16181	16181	146.35	<.0001
Quadrat	Vrn2	1	1650	1650	14.92	0.0002
Int l by l		1	631	631	5.71	**0.0189**
Int l by q		1	0	0	0.00	0.9523
Int q by l		1	12	12	0.11	0.7465
Int q by q		1	161	161	1.46	0.2305

Note that even though the interaction in the 3x3 factorial is not significant, **the lineal by lineal interaction is significant**.

Note also that the Lineal and Quadratic contrast for the main ***Vrn1*** are identical in both analyses.

9.7.4.4. Example of a nested factor within a factorial design

Assume that in the quack-grass shoots experiment (9.7.4.1), two random samples of 1 square foot were taken in each plot (each R – D combination). The values for the two subsamples were created to give an average identical to the value in the previous exercise. The correct design includes subsamples, nested within the interaction R*D*Block.

```
data STDp391;
input D R Block plot number @@;
cards;
   3 0 1 1 14.7  3 4 1 1  8.8  3 8 1 1  6.9    3 0 1 1 16.7  3 4 1 1 10.8  3 8 1 1  8.9
   3 0 2 1 13.6  3 4 2 1 13.6  3 8 2 1  9.3    3 0 2 1 15.6  3 4 2 1 15.6  3 8 2 1 11.3
   3 0 3 1 15.5  3 4 3 1 10.9  3 8 3 1  8.7    3 0 3 1 17.5  3 4 3 1 12.9  3 8 3 1 10.7
   3 0 4 1 13.7  3 4 4 1 11.4  3 8 4 1  8.6    3 0 4 1 15.7  3 4 4 1 13.4  3 8 4 1 10.6
  10 0 1 1 17.0 10 4 1 1 12.6 10 8 1 1  7.8   10 0 1 1 19.0 10 4 1 1 14.6 10 8 1 1  9.8
  10 0 2 1 16.4 10 4 2 1  9.6 10 8 2 1  7.2   10 0 2 1 18.4 10 4 2 1 11.6 10 8 2 1  9.2
  10 0 3 1 14.1 10 4 3 1 10.8 10 8 3 1 10.3   10 0 3 1 16.1 10 4 3 1 12.8 10 8 3 1 12.3
  10 0 4 1 13.4 10 4 4 1 12.3 10 8 4 1 10.2   10 0 4 1 15.4 10 4 4 1 14.3 10 8 4 1 12.2
      ;
      proc GLM;
        class D R Block plot;
        model number= Block D R D*R plot(D*R*Block);
        random plot(D*R*Block);
        test h= D    e= plot(D*R*Block);
        test h= R    e= plot(D*R*Block);
        test h= D*R  e= plot(D*R*Block);

      proc varcomp Method= Type1;
        class D R Block plot;
        model number= Block D R D*R plot(D*R*Block);
      run; quit;
```

Source	DF	SS	MS	F	Pr > F
Model	23	391.2	17.0	8.51	<.0001
Error	24	48.0	2.0		
Corrected Total	47	439.2			

Source	DF	SS	MS	F	Pr > F
Block	3	1.16	0.39	0.19	0.90
D	1	3.00	3.00	1.50	~~0.23~~
R	2	307.33	153.66	76.83	~~<.0001~~
D*R	2	0.98	0.49	0.24	~~0.7846~~
plot(D*R*Block)	15	78.77	5.25	2.63	0.0170

Tests of Hypotheses Using MS for plot(D*R*Block) as Error Term

Source	DF	SS	MS	F Value	Pr > F
D	1	3.00	3.00	0.57	**0.4614**
R	2	307.33	153.66	29.26	**<.0001**
D*R	2	0.98	0.49	0.09	**0.9114**

Variance Component	Estimate	%
Var(Block)	-0.40528	0
Var(D)	0.10458	1
Var(R)	9.57333	72
Var(D*R)	-0.59514	0
Var(plot(D*R*Block))	1.62556	12
Var(Error)	2.00000	15

Plot= $50 Subsample= $5
Optimum allocation
SQRT[(50*2)/(5*1.62]= 3.5
Use 3-4 subsamples

$$Ns = \sqrt{\frac{C_{e.u.} * s^2_{SUB}}{C_{SUB} * s^2_{e.u.}}}$$

Note that the first PROC GLM produce wrong results because SAS uses automatically the last error. Once you specify the correct error (`plot(D*R*Block)`) for each hypothesis (h= D, or h=D, or H=D*R) SAS will divide by the correct error term. The real replication is the block and not the two subsamples. If you are confused by this analysis, use the average of the subsamples and you will get a correct result (remember similar exercise in Homework 3. problem 5).

The output indicates the relative contribution of each component to the variance. In this case the mayor component is the significant R factor and within the error term the variance between subsamples is similar to the variance between the replications.

The objective of introducing a nested factor is to understand the sources of variance in the error term. This information can be used later to optimize the distribution of resources between the number of samples and subsamples, as indicated above.

9. 7. 5 Two-way factorial in a CRD with one replication per cell

When only one observation per treatment combination is available, there is no source of variation to estimate the experimental error. However, the interaction effect can be used as error term if it is possible to assume that there are no significant interactions between the factors. Tukey's additivity test can be used to test the presence of some of these interactions.

The interaction is not specified in the model, and the interaction variation is used as an estimate of the experimental error. In the following table only the first block from the previous example is used as an example of a CRD.

```
proc glm;
 class D R;
 model Y= D R;
```

Dependent Variable: NUMBER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	81.5	27.2	25.87	0.0375
Error	2	2.1	1.1		
Corrected Total	5	83.6			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
D	1	8.2	8.2	7.77	0.4349
R	2	73.3	36.7	34.86	0.0279

Note that the error SS is estimated by the SS interaction. If the interaction is non significant, SS_{error} and $SS_{interaction}$ estimate the same error and the conclusions are valid

9. 7. 6 Example with significant interaction (fixed-effects model, ST&D, p. 358)

The interpretation of factorial experiments is often complicated when the interactions are large. This is especially true if the effects change direction, as they do in this example. Factor A in this experiment is time of bleeding of a lamb, and Factor B is treatment vs. no treatment with estrogen. Here are the treatment totals of the 5 replications

Factor		A= time		
	Level	(a1)= A.M.	(a2)= P.M.	Total
B= estrogen	(b1)= control	Mean of 5 obs.: 66.39	Mean of 5 obs.: 182.67	249.06
	(b2)= treated	Mean of 5 obs.: 96.80	Mean of 5 obs.: 139.06	235.86
	Total	163.19	321.73	484.92

SAS analysis

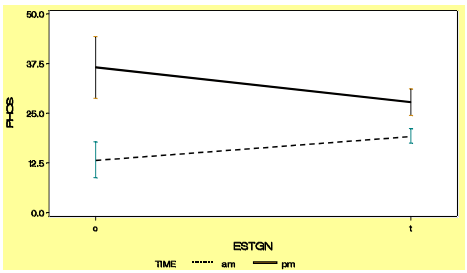
```
data fact1;
input id time $ estgn $ phos @@;
cards;
1 am c  8.53  2 am t 17.53  3 pm c 39.14  4 pm t 32.00
1 am c 20.53  2 am t 21.07  3 pm c 26.20  4 pm t 23.80
1 am c 12.53  2 am t 20.80  3 pm c 31.33  4 pm t 28.87
1 am c 14.00  2 am t 17.33  3 pm c 45.80  4 pm t 25.06
1 am c 10.80  2 am t 20.07  3 pm c 40.20  4 pm t 29.33
;
proc glm;
  class time estgn;
  model phos=time|estgn;
proc glm;
  class id;
  model phos= id;
  contrast 'Between time within control' id  1  0 -1  0;
  contrast 'Between time within treated' id  0  1  0 -1;
  contrast 'Between estrogen levels, am' id  1 -1  0  0;
  contrast 'Between estrogen levels, pm' id  0  0  1 -1;

run; quit;
```

OUTPUT

First PROC GLM

Dependent Variable: PHOS

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIME	1	1256.74658	1256.74658	52.93	0.0001
ESTGN	1	8.71200	8.71200	0.37	**0.5532**
TIME*ESTGN	1	273.94802	273.94802	11.54	**0.0037**
ERROR	16	379.92000	23.75000		



The interaction is significant, which means that the simple effects are heterogeneous. Non-parallel lines, as those observed in this graphic indicate interaction.

If interactions are present in a fixed-effects model the next step is the analysis of the simple effects.

One general way of testing the simple effects is using the **by** statement (always use **proc sort** before, to sort by the variable used in the by statement).

```
proc sort;
  by time;
proc glm;
  class estgn;
  model phos= estgn;
  means estgn / Hovtest= Levene;
   by time;

proc sort;
  by estgn;
proc glm;
  class time;
  model phos=time;
  means time / Hovtest= Levene;
   by estgn;
run; quit;
```

You need to test the assumptions for each one way ANOVA

One Way Anovas	DF	Contrast SS	Mean Square	F Value	Pr > F
Between time within Control	1	1352.10384	1352.10384	0.0004	
Between time within treated	1	178.59076	178.59076	0.0011	
Between estrogen level am	1	92.47681	92.47681	0.0237	
Between egstrogen level pm	1	190.18321	190.18321	0.0495	

An alternative way when there are clear preplanned hypotheses is to use an ID variable and solve the simple effects by contrasts (ST&D page 362). These contrasts are not orthogonal. The results are not identical since they use different MSE. We will generally use the first approach.

Second PROC GLM (with id as a class variable)

Source	DF	SS	MS	F Value	Pr > F
ID	3	1539.40660	513.13553	21.61	0.0001
Error	16	379.92328	23.74520		
Corrected Total	19	1919.32988			

CONTRASTS

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Between time within Control	1	1352.10384	1352.10384	56.94	0.0001
Between time within treated	1	178.59076	178.59076	7.52	0.0145
Between estrogen level am	1	92.47681	92.47681	3.89	0.0660
Between estrogen level pm	1	190.18321	190.18321	8.01	0.0121

9. 8. Three way ANOVA (fixed-effects model)

There is no reason to restrict the factorial design to a consideration of only two factors. Three or more factors may be analyzed simultaneously each at different levels. However, as the number of factors increases, even without replication within a subgroup, the experimental units necessary becomes very large. It is frequently impossible or prohibitive in cost to carry out such an experiment. A 4x4x4 factorial requires 64 experimental units to represent each combination of factors. Moreover, if only 64 e.u. are used, there will be no replication to estimate the basic experiment error and some interactions would have to be used as an estimate of experimental error (on the assumption that no added interaction effect is present).

There are also logistic difficulties with such large experiments. It may not be possible to run all the tests in one day or to hold all of the material in a single controlled environmental chamber. Thus treatments may be confounded with undesired effects if different treatments are applied under not quite the same experimental conditions.

The third problem that accompanies a factorial ANOVA with several main effects is the large number of possible interactions. A two-way ANOVA has only one interaction, A X B. A three-factor factorial has three **first-order interactions**, A X B, A X C, and B X C.; and a **second-order interaction**, A X B X C.

The fixed model is assumed to be: $\mu_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk}$

A four-factor factorial has 6 first-order interactions, four second-order interactions, and one **third-order interaction** (A X B X C X D). The numbers of interactions go up rapidly as the numbers of factors increase. The testing of their significance, and more importantly, their interpretation becomes exceedingly complex.

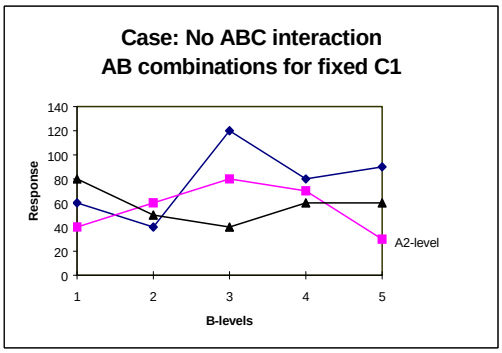
9. 8. 1. Example of a three-way factorial ANOVA

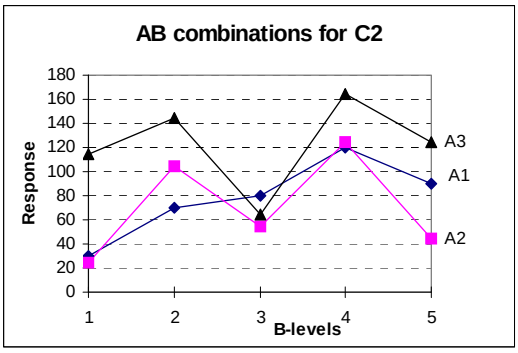
(Taken from: C.J. Monlezun.1979. Two-dimensional plots for interpreting interactions in the three-factor analysis of variance model. The American Statistician 33:63-69.)

The following hypothetical population means for a 3x5x2 experiment are used to illustrate an example with **no three-way interactions**. A graphic technique to show the three way interactions is discussed.

	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

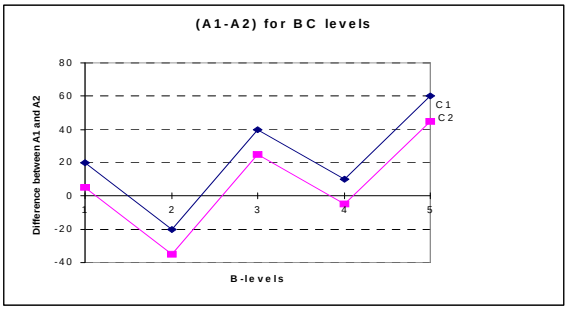
The lines of mean plots for fixed C1 (left, figure next page) and C2 (right) levels are not parallel indicating a two-way interaction between A and B in both levels of C. The ***first order interaction*** (AxB) now has two values: (AxB, c_1) and (AxB, c_2). The interaction term (AxB) is the average of these.

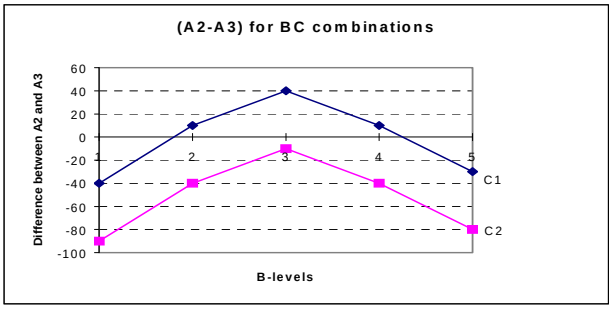




If, however, **the differences between different levels of A** are taken over levels of say, B, for the two different C levels, the plot of these differences reveals no interaction between BC. The lack of BC interaction with the differences between levels of A indicates that no ABC interaction is present in these means, i.e. $(\alpha\beta\gamma)_{ijk} = 0$. A graphical check of whether $(\alpha\beta\gamma)_{ijk} = 0$ is satisfied in the general situation requires a-1 different graphs.

Phrasing these results in words, we can say that factors A and B interact in the same way for all levels of factor C.





The interpretation of a three-factor interaction is that, for example, the effect of factor A depends on the precise combination of factors B and C. For example if A is nitrogen level (0 or 3 cwt/a) and B is plow depth (7 or 11 in.). In a two-factor experiment, a significant AxB interaction indicates that the crop has a different response to N depending on plow depth. Now introduce the third factor C, which is soil type (loam or sand). Then a nonzero (AxBxC) would mean that the amount of difference in their response to N as a function of plow depth depends on the soil type.