

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

9. 1. Introduction

A common problem in research is investigating the effect of each of a number of variables, or **factors**, on some response 'Y'. In earlier times factors were studied one at a time, with separate experiments devoted to each factor. Later, R. A. Fisher pointed out that important advantages are gained by combining the study of several factors in the same **factorial experiment**. In the factorial approach, the investigator compares all treatments that can be formed by combining the **levels** of the different factors. Factorial experimentation is highly efficient, because every observation supplies information about all the factors included in the experiment. Also factorial experimentation is a systematic method of investigating the relationships between the effects of different factors.

9. 2. Terminology

The individual treatments are called **factors**. The treatment levels within a factor are called **levels**. Different factors will be denoted by upper case letters and different levels by lower case letters with subscripts. The mean of observations receiving the combination ab will be denoted (ab) .

A 2×2 factor experiment with two factors and two levels for each factor is denoted as a 2^2 factorial experiment. An experiment with f factors at t levels is denoted as a f^t factorial experiment. If the number of levels in each treatments is different then the notation is $t_A \times t_B$. For example, if factor A has 3 levels and factor B has 5 then it is a 3×5 factorial experiment.

9. 3. Example of a 2x2 factorial

An example of an experiment involving two factors is the application of two nitrogen levels, N_0 and N_1 , and two phosphorous levels, P_0 and P_1 to a crop, with yield (lb/a) as the measured variable. The results are shown here:

| Factor | | A = N level | | | |
|-------------|-----------------------|-------------------------|-------------------------|---------------------|-------------------------|
| | Level | a1 = N_0 | a2 = N_1 | Mean (a_{bi}) | $a_2 - a_1$ |
| B = P level | b1 = P_0 | 40.9 | 47.8 | 44.4 | 6.9 (<i>se A, b1</i>) |
| | b2 = P_1 | 42.4 | 50.2 | 46.3 | 7.8 (<i>se A, b2</i>) |
| | Mean ($a_{i\cdot}$) | 41.6 | 49.0 | 45.3 | 7.4 (<i>me A</i>) |
| | $b_2 - b_1$ | 1.5 (<i>se B, a1</i>) | 2.4 (<i>se B, a2</i>) | 1.9 (<i>me B</i>) | |

The differences $a_2 - a_1$ and $b_2 - b_1$ are the **simple effects**, denoted (*se A*) and (*se B*). The averages of the simple effects are the **main effects**, denoted (*me A*) and (*me B*) or simply (A) and (B).

One way of looking at the data is to separately consider the effect of N on yield for each P level. This information would be useful to a farmer who was constrained to always use the same P level. This is called analyzing the *simple effects* (*se*) of N. They are to increase yield by 6.9 lb/a for P_0 and 7.8 lb/a for P_1 .

It may happen that the effect of N on yield is the same whether P is applied or not. In this case the two simple effects are estimates of the same quantity and differ only by experimental error. One can average the two responses to get 7.4 lb/a; 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.

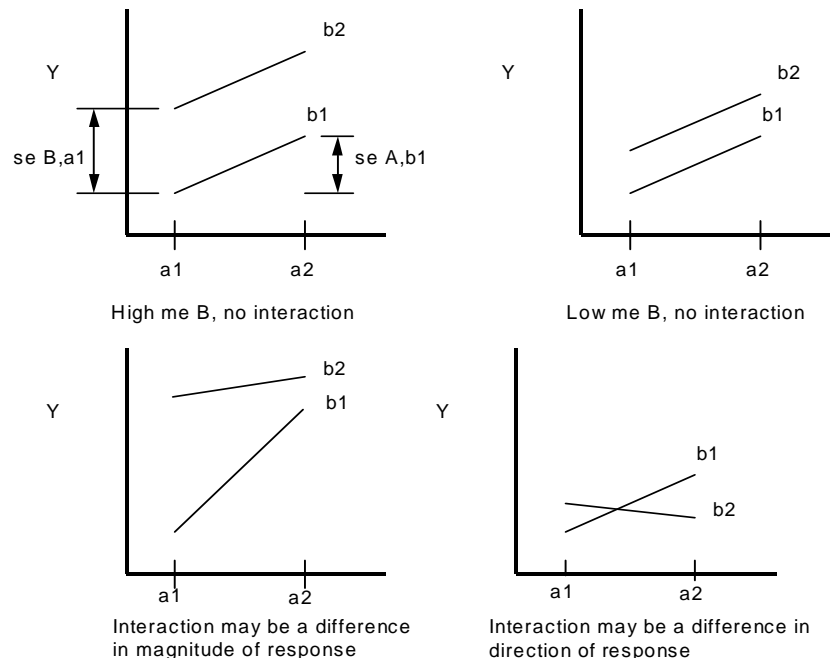
9. 4. Interaction

If the simple effects of Factor A are the same for all levels of Factor B the two factors are said to be **independent**. It may, however be the case that the effects are not independent. One might expect the greater P to permit a higher expression of the yield potential of the N application. In this case the factors have an **interaction**.

Interaction measures the failure of one effect to be the same for each level of other factors. Interaction is a common and a fundamental scientific idea.

One of the main objectives of factorial experiments is to study the interactions between factors. The sum of squares of interaction measures the departure of the group means from the values expected on the basis of additive combinations of the row and column means. 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 separate effects of each drug. When the combination of levels of two factors *inhibit* each other's effect, we call it **interference**. Synergism and interference both tend to magnify the interaction SS.

These differences between simple effects of two factors or **first-order interactions (AxB)** can be visualized in the following graphics.



Pitfalls of Interpreting Interactions in Transformed Data

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

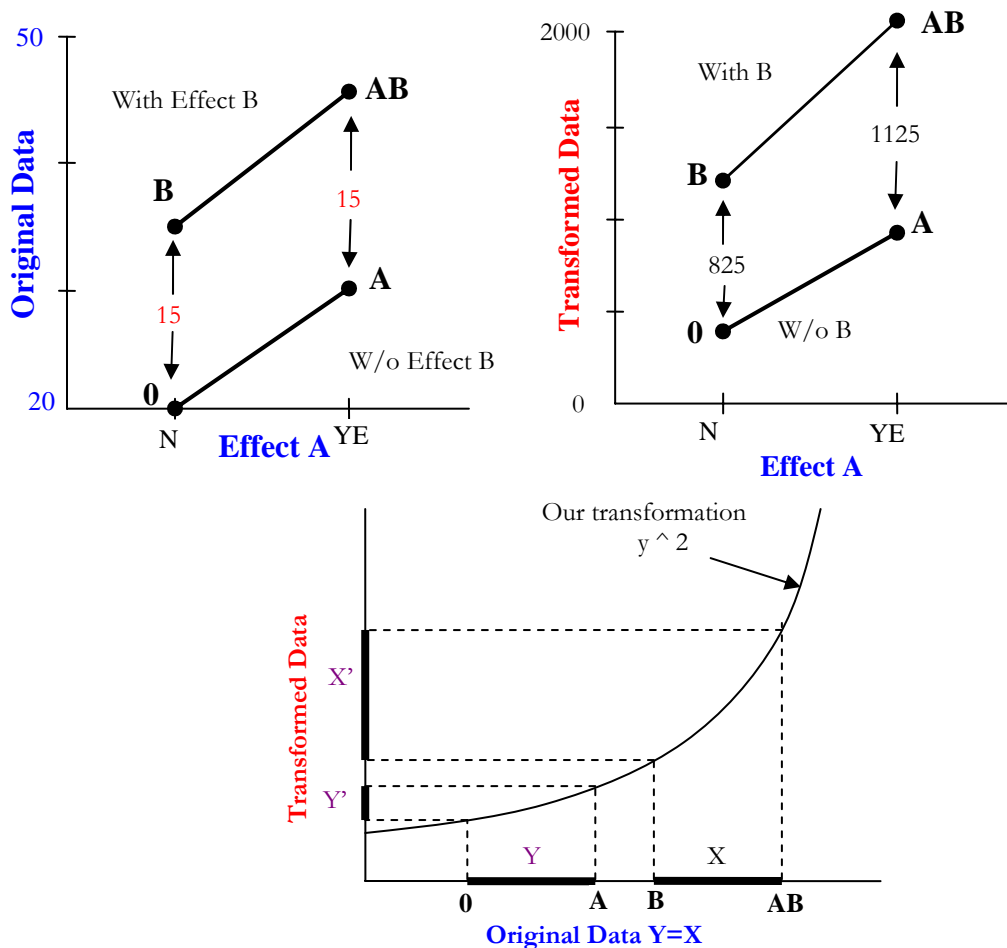
A: increases 10; B increases 15; A and B increases 25.

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)



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

9. 5. 1. Reasons for doing factorial experiments

- *To investigate the interactions of factors:* If the factors are not independent, single factor experiments provide a disorderly and incomplete picture.
- *In exploratory work for quick determination of effects of factors:* In the initial phases of an investigation, factorial experiments can establish which factors are independent and can therefore be more fully analyzed in separate experiments.
- *In experiments designed to lead to recommendations that must apply over a wide range of conditions:* One can introduce "subsidiary factors", for example soil type, so that any recommendations resulting from the experiments can be applied over a broader range of circumstances.

9. 5. 2. Disadvantages of factorial design

- *Large number of combinations required to study several factors at several levels and need a large sized experiment:* 7 factors at 3 levels requires 2187 combinations.
- *Large number of factors complicate the interpretation of high order interactions*

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

Beginners are often confused between nested and factorial ANOVAs. 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 made up for each treatment? The following tables represent both designs using asterisks to represent the measured variable.

| Two way factorial ANOVA | | | | | | Nested ANOVA | | | | | | | | | | |
|-------------------------|---|---|---|---|---|--------------|---|---|---|---|---|---|---|---|---|---------------|
| | 1 | 2 | 3 | 4 | 5 | 1 | | 2 | | 3 | | 4 | | 5 | | Sugar batches |
| <i>pH level 1</i> | * | * | * | * | * | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| <i>pH level 2</i> | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |
| | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | |

Why can we rearrange the first table as a nested design and the second as a factorial? The reason is that the first factorial analysis implies that the two pH classes are **common** to the entire study—that is, that pH level 1 is the same for all sugar treatments. Conversely, if we tried to arrange the data from the nested analysis as a two-way factorial ANOVA, we would imply that batches 1 and 2 had the same meaning for all the sugar concentrations. This is not so. Batch 1 for treatment 1 has no closer relation to batch 1 in treatment 2 than it does to batch 2 in that treatment. Batches 1 and 2 are simply arbitrary

designations for the two randomly prepared sugar solutions that represent each treatment. By contrast, if all batches labeled 1 were prepared in a single recipient the same day, while all batches labeled 2 were made on the following day, the “1” and “2” would represent common information for the study that should properly arrange as two-way ANOVA (RCBD).

This RCBD differs from a true factorial design in the 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 separate this additional source of variation and we assume no interactions.

The critical question to be asked to differentiate factorial and nested designs is always:

Does the arrangement of the data into a two-way table correctly imply a correspondence across the classes?

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-factor analysis is

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

Here α_i represents the main effect of factor A i , $i = 1, \dots, a$, β_j represents the main effect of factor B, $j = 1, \dots, b$, $(\alpha\beta)_{ij}$ represents the interaction of factor A level i with factor B level j ., and ϵ_{ijk} is the error associated with rep k of factor level ij , $k = 1, \dots, r$.

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

The data represent the model as

| | | | |
|-------------|-------------|-------------|--------------|
| main effect | main effect | interaction | experimental |
| factor i | factor j | effect | error |

The null hypotheses for a 2 factor experiment are $\alpha_i = 0$, $\beta_j = 0$, and $(\alpha\beta)_{ij} = 0$. Each F statistic may be interpreted independently. The sum of squares equation becomes $SS = SSA + SSB + SSAB + SSE$.

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

In ANOVA for factorial experiments the SS of *treatments* is partitioned into components for each factor and each interaction. This partition is valid even when the overall F test of no differences among treatments is not significant. There are situations where one factor, say B, does not have any effect on A and hence contributes no more to the SST than can be attributed to 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 used as part of a computational procedure than to supply a numerator for an F test.

In a two factor $a \times b$ experiment there are a total of ab treatment combinations and therefore $ab - 1$ degrees of freedom for treatments. The main effect of factor A has a -1

df and the main effect of factor B has $b - 1$ df. By subtraction the interaction (AxB) has $ab - a - b + 1$ df. A little algebra shows that this is equal to $(a-1)(b-1)$ df. In a 3 factor $a \times b \times c$ experiment, the (AxBxC) interaction has $(a-1)(b-1)(c-1)$ df. In a 2×2 experiment there are 1 df for each main effect and 1 for the interaction.

The ANOVA table for the CRD factorial design is

| 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 | $rab - 1$ | SS | | |

The sum of squares of interaction measures the departure of the group means from the values expected on the basis of additive combinations of the row and column means. When the F test for interaction is not significant the subsequent analysis of this table is different from the analysis if interactions are significant

- No significant interaction: multiple comparisons can be performed on the main effect means.
- Significant interaction: go back to the means and analyze simple effects. 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 4×2 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 | | | | | |
| 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

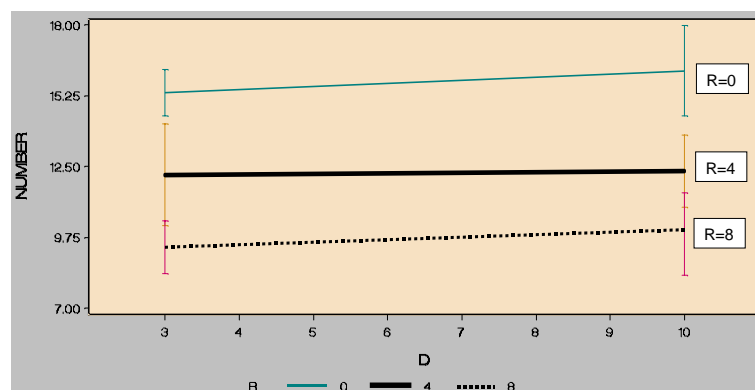
| T Grouping | Mean | N | D |
|------------|---------|----|----|
| A | 12.8083 | 12 | 10 |
| A | 12.3083 | 12 | 3 |

| 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).

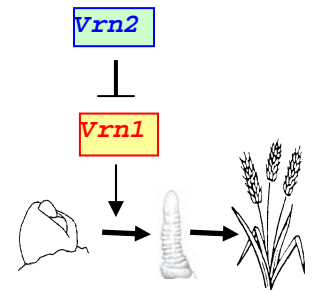
The auxiliary variable “**type**” represents each combination of *Vrn1* and *Vrn2* classes.

```

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;
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 1 by 1 | 1 | 631 | 631 | 5.71 | 0.0189 |
| Int 1 by q | 1 | 0 | 0 | 0.00 | 0.9523 |
| Int q by 1 | 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 | Plot= \$50 Subsample= \$5 |
| Var(D) | 0.10458 | 1 | Optimum allocation |
| Var(R) | 9.57333 | 72 | SQRT[(50*1.62)/(5*2)]= 2.84 |
| Var(D*R) | -0.59514 | 0 | Use 3 subsamples |
| Var(plot(D*R*Block)) | 1.62556 | 12 | $N_S = \sqrt{\frac{C_{e.u.} * s_{SUB}^2}{C_{SUB} * s_{e.u.}^2}}$ |
| Var(Error) | 2.00000 | 15 | |

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 $SS_{\text{interaction}}$ 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 | | | Total |
|-------------|---------------|-----------------------|------------------------|--------|
| | Level | (a1)= A.M. | (a2)= P.M. | |
| 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;

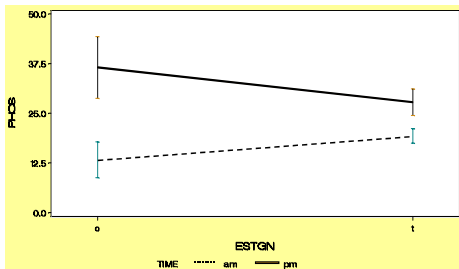
```

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 estrogen 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: $\mu_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk}$

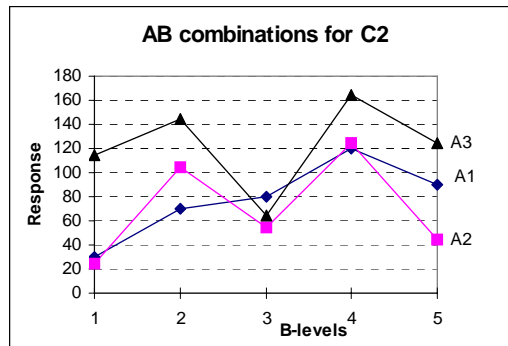
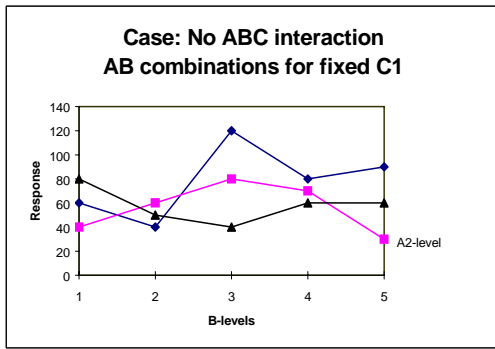
A four-factor factorial has 6 first-order interactions, four second-order interactions, and one **third-order interaction** (A X B X C X D). The numbers of interactions go up rapidly as the numbers of factors increase. The testing of their significance, and more importantly, their interpretation becomes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

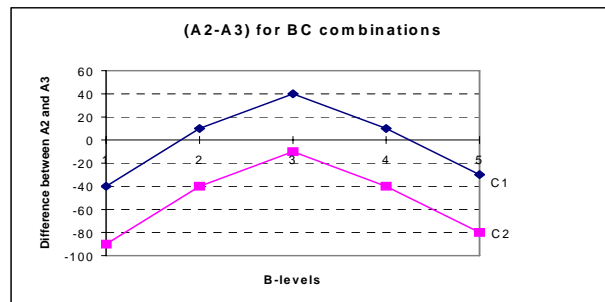
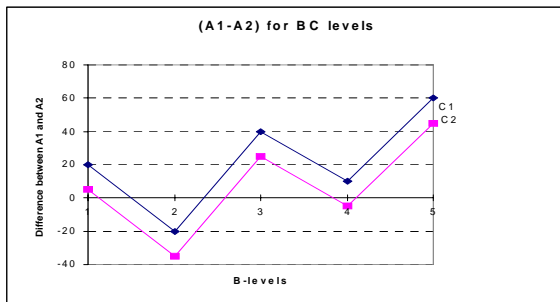
| | A1C1 | A2C1 | A3C1 | A1C2 | A2C2 | A3C2 |
|----|------|------|------|------|------|------|
| B1 | 61 | 38 | 81 | 31 | 27 | 113 |
| B2 | 39 | 61 | 49 | 68 | 103 | 143 |
| B3 | 121 | 82 | 41 | 78 | 57 | 63 |
| B4 | 79 | 68 | 59 | 122 | 127 | 167 |
| B5 | 91 | 31 | 61 | 92 | 43 | 128 |

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, c1) and (AxB, c2). 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.