

## Topic 4. Orthogonal contrasts [ST&D p. 183]

The analysis of variance method is a useful and powerful tool to compare several treatment means. In comparing  $k$  treatments, the null hypothesis tested is that the  $k$  true means are all equal ( $H_0: \mu_1 = \mu_2 = \dots = \mu_k$ ). If a significant F test is found, one accepts the alternative hypothesis, which merely states that they are not all equal. Further comparisons to determine which treatments are different can be carried out by further **partitioning of the treatment sum of squares** to provide additional F tests to answer planned questions. The contrast or orthogonal approach to mean separation requires an *a priori* knowledge, either based on biological considerations or on the results of preliminary experimentation. This is why the tests are sometimes called *planned* F tests.

If the investigator has specific questions to be answered the treatments employed are designed to provide information and statistical tests to answer those questions. An experienced investigator will select treatments so that the treatment sum of squares can be partitioned to answer as many **independent** questions as there are degrees of freedom for treatments in the ANOVA. Consequently, another name of these tests is single degree of freedom tests. When the comparisons are independent, they are said to be orthogonal.

### 4. 1. Definition of contrast and orthogonality [ST&D p. 183]

A **contrast** is a linear sum of the form

$$Q = \sum_{i=1}^m c_i \bar{Y}_i; \text{ with } \sum_{i=1}^m c_i = 0$$

It is essential that the sum of the coefficients for each comparison is zero. The terms  $\bar{Y}_i$  are the treatment means (it can also be the treatment sums), and  $m \leq t$ , is the number of treatments. For convenience, the  $c_i$ 's are usually integers. A contrast always has a single degree of freedom.

Suppose  $Q_c = \sum_{i=1}^m c_i \bar{Y}_i$ ; and  $Q_d = \sum_{i=1}^m d_i \bar{Y}_i$  are two contrasts.

Then they are **orthogonal** if the sum of the products of the corresponding coefficients of any two comparisons is zero.

$$\sum_{i=1}^m c_i d_i = 0 \quad (\text{or } \sum_{i=1}^m c_i d_i / n_i = 0 \text{ for unbalanced designs})$$

The idea of orthogonal contrasts is to separate out the null hypotheses into physically meaningful ones involving different combinations of means. Orthogonality is a very important property because of the following: Suppose a set of  $t - 1$  contrasts for  $t$  degrees of freedom is orthogonal. If the treatment sums of squares SST for each contrast are added together, their sum is the treatment SST

for the original experiment. This means that the experiment can be partitioned into  $t - 1$  separate independent experiments, one for each contrast.

**Example**

Suppose we are testing three treatments, T1, T2 and T3 (control). There are then two degrees of freedom for treatments. Let the treatment means be denoted  $\mu_1$ ,  $\mu_2$ , and  $\mu_3$ . The usual null hypothesis is then  $H_0: \mu_1 = \mu_2 = \mu_3$  which uses both degrees of freedom in one test. Since there are two degrees of freedom, there are in principle two independent comparisons that can be made.

For example, one could in principle test the hypotheses that T1 and T2 are not significantly different from the control:  $\mu_1 = \mu_3$  and  $\mu_2 = \mu_3$ . As usual, we represent the mean  $\mu_i$  with the sample mean

For  $\mu_1 = \mu_3$  ( $1\mu_1 + 0\mu_2 - 1\mu_3 = 0$ ) the coefficients are:  $c_1 = 1$ ,  $c_2 = 0$ ,  $c_3 = -1$

For  $\mu_2 = \mu_3$  ( $0\mu_1 + 1\mu_2 - 1\mu_3 = 0$ ) the coefficients are:  $d_1 = 0$ ,  $d_2 = 1$ ,  $d_3 = -1$

These linear combinations of means are **contrast** since

$$\sum_{i=1}^m c_i = 0 \quad (1 + 0 + (-1) = 0); \text{ and } \sum_{i=1}^m d_i = 0 \quad (0 + 1 + (-1) = 0).$$

However these contrasts are not orthogonal because:

$$\sum_{i=1}^m c_i d_i \neq 0 \quad (c_1 d_1 + c_2 d_2 + c_3 d_3 = 0 + 0 + 1 = 1).$$

This shows that not every pair of hypotheses can be tested using this approach. In addition to adding up to 0, the  $c_i$  coefficients are almost always taken to be integers. This severely restricts the possible values. For  $t = 3$  such a set of values are  $c_1 = 1$ ,  $c_2 = 1$ ,  $c_3 = -2$ ; and  $d_1 = 1$ ,  $d_2 = -1$ ,  $d_3 = 0$ .

The hypotheses defined by these orthogonal coefficients are 1) the average of the two treatments is not significantly different from the control, and 2)  $\mu_1$  is not significantly different from  $\mu_2$ .

These are **contrasts** since

$$(1 + 1 + (-2) = 0 \text{ and } 1 + (-1) + 0 = 0)$$

and are **orthogonal** because

$$(c_1 d_1 + c_2 d_2 + c_3 d_3 = 1 + (-1) + 0 = 0).$$

There are two general kinds of linear combinations:

- **Cass comparisons**
- **Trend comparisons.**

## 4.2. Class comparisons

The first application of orthogonal contrasts is in *class comparisons*. This does ANOVA on groups, or *classes* other than the simple treatment groups. The procedure is illustrated by an example on p. 185 of ST&D, which involves the mint data discussed in Topic 3.

To illustrate orthogonal contrasts in *class comparisons* we will use the data given in Table 4.1. The analysis of variance for this experiment is given in Tables 4.2.

**Table 4.1.** Results (mg shoot dry weight) of an experiment (CRD) to determine the effect of seed treatment by acids on the early growth of rice seedlings.

Treatments	Replications					Total	Mean
						$Y_{i.}$	$\bar{Y}_{i.}$
Control	4.23	4.38	4.1	3.99	4.25	20.95	4.19
HC1	3.85	3.78	3.91	3.94	3.86	19.34	3.87
Propionic	3.75	3.65	3.82	3.69	3.73	18.64	3.73
Butyric	3.66	3.67	3.62	3.54	3.71	18.2	3.64
Overall						$Y_{..} = 77.13$	$\bar{Y}_{..} = 3.86$

**Table 4.2.** ANOVA of data in Table 4.1.

Source of Variation	df	Sum of Squares	Mean Squares	F
Total	19	1.0113		
Treatment	3	0.8738	0.2912	33.87
Exp. Error	16	0.1376	0.0086	

A set of questions the investigator may have designed the treatments to answer is,

- 1) Do acid treatments decrease seedling growth?
- 2) Are organic acids different from inorganic acids?
- 3) Is there a difference in the effects of the two organic acids?

To facilitate answering these questions, a table of coefficients is shown (Table 4.3) to define the linear combinations among treatments.

**Table 4.3.** Orthogonal coefficients for partitioning the treatment sum of squares of Table 4.-2 into three independent tests.

		Control	HC1	Propionic	Butyric
	Totals	20.95	19.34	18.64	18.2
Comparisons	Means	4.19	3.87	3.73	3.64
	Control vs. acid	+3	-1	-1	-1
	Inorganic vs. organic	0	-2	+1	+1
	Between organics	0	0	+1	-1

These coefficients can be used to partition the sum of squares of treatments (SST) into three components each with 1 degree of freedom to provide for an  $F$  test for each of the comparisons. The critical  $F$  is based on 1 df for the numerator and df for error in the denominator (single df  $F$  tests).

**Rules for the construction of coefficients for class comparisons** (Little & Hills p 66).

1. In comparing the means of two groups, each containing the same number of treatments assign +1 to the members of one group and -1 to the members of the other. Thus for line 3 in Table 4.3, we are comparing two means, and assign coefficients of 1 (of opposite sign) to each. The same procedure is extended to the case of more than one treatment in each group.
2. In comparing groups containing different numbers of treatments, assign to the first group coefficients equal to the number of treatments in the second group; to the second group, assign coefficients of opposite sign, equal to the number of treatments in the first group. Thus, if among 5 treatments, the first two are to be compared to the last three, the coefficients would be +3, +3, -2, -2, -2. In Table 4.3, where the control mean is compared with the mean of the three acids, we assign a 3 to the control and a 1 to each of the three acids. Opposite signs are then assigned to the two groups. It is immaterial as to which group gets the positive or negative sign since the sum of squares of the comparison will be calculated and used to form an F-test.
3. The coefficients for any comparison should be reduced to the smallest possible integers for each calculation. Thus, +4, +4, -2, -2, -2, -2. should be reduced to +2, +2, -1, -1, -1, -1.
4. At times, a comparison component may be an interaction of two other comparisons. The coefficients for this comparison are determined by multiplying the corresponding coefficients of the two comparisons (Table 4.4)

**Table 4.4.** Fertilizer experiment with 4 treatments, 2 levels of **N** and 2 of **P**.

	$N_0P_0$	$N_0P_1$	$N_1P_0$	$N_1P_1$
Between N	-1	-1	1	1
Between P	-1	1	-1	1
<b>Interaction</b> (N×P)	1	-1	-1	1

The coefficients for the 1<sup>st</sup> two comparisons are derived by rule 1. The interaction coefficients are the result of multiplying the coefficients of the first two lines.

Note that the sum of the coefficients of each comparison is zero and that the sum of the cross products of any two comparisons is also zero. When these two conditions are met, the comparisons are said to be orthogonal. This implies that the conclusion drawn for one comparison is independent of (not influenced by) the others.

The computation of the sum of squares for a single degree of freedom  $F$  test for linear combinations of *treatment means* is

$$SS(Q) = MS(Q) = \frac{(\sum c_i \bar{Y}_i)^2}{(\sum c_i^2)/r} \text{ or } \frac{(\sum c_i \bar{Y}_i)^2}{\sum (c_i^2 / r_i)} \text{ for unbalanced designs}$$

$$SS_1 (\text{control vs. acid}) = [3(4.19) - 3.64 - 3.73 - 3.87]^2 / [(12)/5] = 0.74$$

$$SS_1 (\text{Inorg. vs. org.}) = [3.64 + 3.73 - 2(3.87)]^2 / [(6)/5] = 0.11$$

$$SS_1 (\text{between org.}) = [-3.64 + 3.73]^2 / [(2)/5] = 0.02$$

**Note:** ST&D formulas for contrasts (p.184) are for *treatment totals* and not for *treatment means*. The treatments means formula is required for the unbalanced designs.

**Table 4.5.** Orthogonal partitioning of treatments of Table 4.2 (SS are not identical because of the rounding errors in the calculations of the means).

Source of Variation	df	SS	MS	F
Total	19	1.0113		
Treatment	3	0.8738	0.2912	33.87
Control vs. acid	1	0.7415	0.7415	86.22
Inorg. vs. Org.	1	0.1129	0.1129	13.13
Between Org.	1	0.0194	0.0194	2.26
Error	16	0.1376	0.0086	

From the above analysis we conclude that in this experiment all three acids significantly reduce seedling growth ( $p < 0.01$ ), that organic acids cause more reduction than the inorganic acid ( $p < 0.01$ ) and that the difference between the organic acids is not significant ( $p > 0.05$ ).

Note that the single degrees of freedom sum of squares add up to the sum of squares of treatments. This will always happen when the individual comparisons are orthogonal. The maximum number of orthogonal comparisons equals the degrees of freedom for the treatment sum of squares. When comparisons are not orthogonal, the sum of squares for one comparison may contain (or be contained by) part of the sum of squares of another comparison. Therefore, the conclusion from one test may be influenced by another test and the sum of squares of those individual comparisons will not add up to the sum of squares for treatments.

### 4.3. Trend comparisons

Experiments are often designed to study the effect of increasing levels of a factor, e.g., increments of a fertilizer, planting dates, doses of a chemical, concentrations of a feed additive, etc. In these situations, the experimenter is interested in the dose response relationship. The statistical analysis should evaluate the trend of the response and **not** be concerned with pairwise comparisons.

The simplest example is one with three levels. This is very common in genetic experiments, where the levels are zero dose of allele A in homozygous BB individuals, one dose of allele A in heterozygous AB individuals, and two doses of allele A in homozygous AA individuals. With the use of molecular marker techniques it is now easy to score the genotype of the individuals of a segregating population and measure the phenotype that needs to be analyzed. Suppose 40 F<sub>2</sub> individuals are analyzed for a molecular marker and the Nitrogen content of the seed is measured in each F<sub>2</sub>.

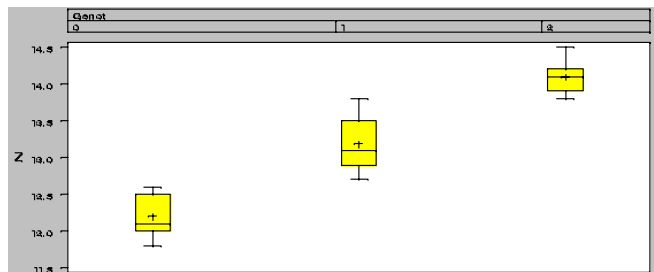
**Table 4.6.** Genetic example of orthogonal contrasts

0 A allele (BB)		1 A Allele (AB)		2 A Alleles (AA)	
Nitrogen	Flowering	Nitrogen	Flowering	Nitrogen	Flowering
12.0	58	13.5	71	13.8	73
12.5	51	13.8	75	14.5	68
12.1	57	13.0	69	13.9	70
11.8	59	13.2	72	14.2	71
12.6	60	13.0	68	14.1	67
		12.8	73		
		12.9	69		
		13.4	70		
		12.7	71		
		13.6	72		

## SAS program

```
proc glm;
  class genot;
  model N Flo= genot;
  contrast 'Lineal'   genot -1  0  1;
  contrast 'Quadratic' genot  1 -2  1;
run; quit;
```

## Dependent Variable: N

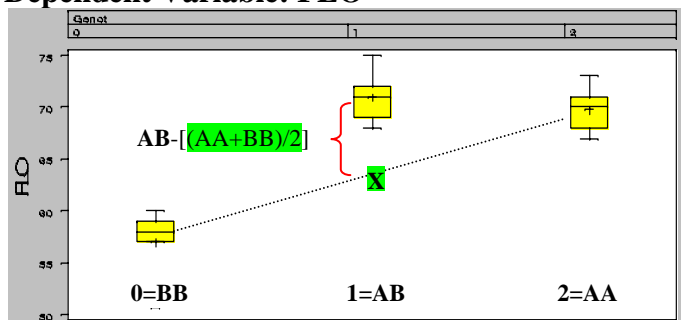


Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	<b>9.033</b>	4.5165	38.60	0.0001
Error	17	1.989	0.1170		
Corrected Total	19	11.022			

R-Square: 0.819543

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
<b>Lineal</b>	1	<b>9.025</b>	9.0250	<b>77.14</b>	0.0001
<b>Quadratic</b>	1	<b>0.008</b>	0.0080	0.07	0.7969

## Dependent Variable: FLO



Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	698.40	349.200	52.63	0.0001
Error	17	<b>112.80</b>	6.635		
Corrected Total	19	811.20			

R-Square: 0.86

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
<b>Lineal</b>	1	409.6	409.6	<b>61.73</b>	0.0001
<b>Quadratic</b>	1	288.8	<b>288.8</b>	<b>43.52</b>	0.0001

The significant quadratic contrast indicates that the response is not lineal. In genetic terms there is dominance. Note that when there is no significant quadratic response (variable N) the F value of the linear response (77.14, critical value  $F_{2, 17}=3.59$ ) is twice as large as the Model F value (38.50, critical value  $F_{1, 17}=4.45$ ). In the lineal contrast  $MS=SS/1$  and in the complete Model both effects are averaged

and  $MS=SS/2$ . When a quantitative factor with a linear effect is measured at several levels, it is not uncommon for the overall treatment F test to fail to be significant since it averages the small higher order effects with that of the linear effect, thereby reducing the power of the overall test.

If the last example is analyzed by lineal regression

Source	DF	SS	MS	F Value	Pr > F
Model	1	409.6	409.6	18.36	0.0004
Error	18	401.6	22.3		
Total	19	811.2			

$$401.6 = 112.8 + 288.8$$

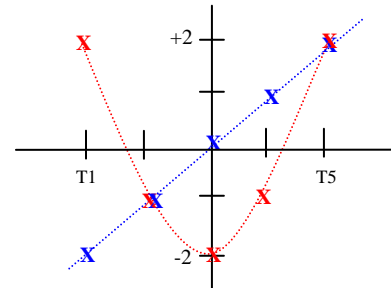
The F value is smaller because the quadratic SS is now included in the error sum of squares (401.6). The ANOVA with the lineal and quadratic contrasts is more sensitive than the linear regression test.

### Coefficients for trend comparisons

The  $c_i$  coefficients used for trend comparisons (linear, quadratic, cubic, quartic, etc) for different number of **equally spaced** treatments are listed below and in Table 15.12 (ST&D p390).

Coefficients for trend comparisons for **equally spaced** treatments

No. of trtmt.	Degree polynom.	T1	T2	T3	T4	T5	T6
2	1	-1	+1				
3	1	-1	0	+1			
3	2	+1	-2	+1			
4	1	-3	-1	+1	+3		
4	2	+1	-1	-1	+1		
4	3	-1	+3	-3	+1		
5	1	-2	-1	0	+1	+2	
5	2	+2	-1	-2	-1	+2	
5	3	-1	+2	0	-2	+1	
5	4	+1	-4	+6	-4	+1	
6	1	-5	-3	-1	+1	+3	+5
6	2	+5	-1	-4	-4	-1	+5
6	3	-5	+7	+4	-4	-7	+5
6	4	+1	-3	+2	+2	-3	+1
6	5	-1	+5	-10	+10	-5	+1



To illustrate the procedure to evaluate a trend response, we will use the data of Table 15.11 (ST&D p387) but using the blocks just as replications in a CRD. The four df for treatment, and the sum of squares for treatments (125.66) will be partitioned using linear, quadratic, cubic, and quartic contrasts. Note that the coefficients provided in Table 15.12 are for equally spaced treatment levels. The

evaluation of a trend analysis is simplified when treatment levels are equally spaced either (arithmetic or log scales).

Note that the linear SS is the same as the SS in a linear regression model. In this example the linear trend accounts for 73% of the variability due to row spacing and the quadratic trend for the remaining 27%. Therefore it is this relationship between row spacing and yield that is important and not pairwise comparisons.

Partition of row spacing SS by use of orthogonal polynomials

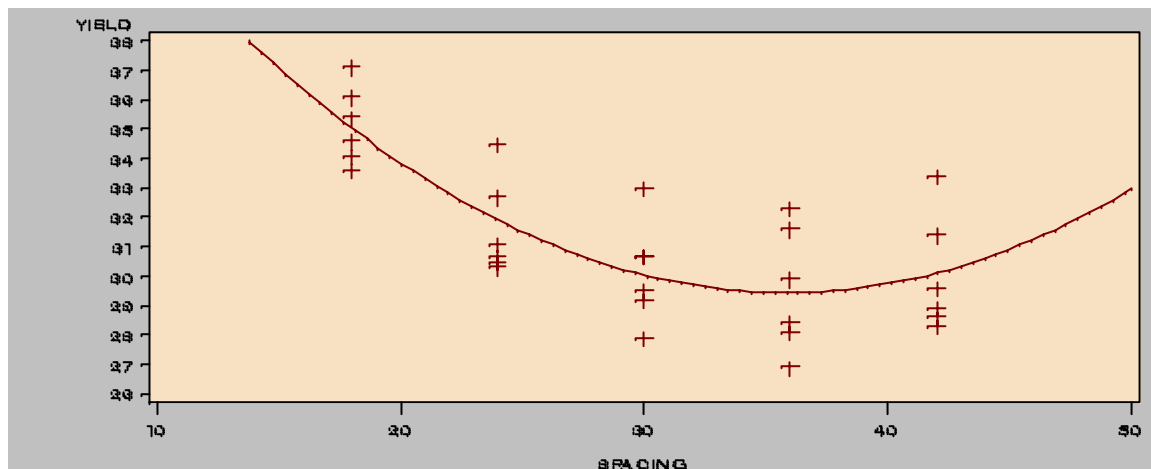
**ST&D Table 15.11 Page 387**

**Yield of Ottawa Mandarin soybeans grown in MN, in bushels per acre.**

Rep.*	Row spacing (in inches)				
	18	24	30	36	42
1	33.6	31.1	33.0	28.4	31.4
2	37.1	34.5	29.5	29.9	28.3
3	34.1	30.5	29.2	31.6	28.9
4	34.6	32.7	30.7	32.3	28.6
5	35.4	30.7	30.7	28.1	29.6
6	36.1	30.3	27.9	26.9	33.4
<b>Means</b>	<b>31.15</b>	<b>31.63</b>	<b>30.17</b>	<b>29.53</b>	<b>30.03</b>

\* Original example with blocks, treated as reps in this example

	Row spacing and Treatment Totals					$(\sum c_i \bar{Y}_i)^2$	$(\sum c_i^2) / r$	SS	F
	18	24	30	36	42				
<b>Effect</b>	<b>31.15</b>	<b>31.63</b>	<b>30.17</b>	<b>29.53</b>	<b>30.03</b>				
Linear	-2	-1	0	+1	+2	152.3	10/6	91.27	28.76 **
Quadratic	+2	-1	-2	-1	+2	78.5	14/6	33.69	10.72 **
Cubic	-1	+2	0	-2	+1	0.8	10/6	0.50	0.16 NS
Quartic	+1	-4	+6	-4	+1	2.4	70/6	0.20	0.16 NS
Total								125.66	



## Unequally spaced treatments

There are equations to calculate coefficient similar to those of Table 15.12 for unequally spaced treatment levels and unequal number of replications. The ability to compute such sum of squares by orthogonal contrast was crucial in the days before computers. With computers it is easier to implement a regression approach, which does not require equal spacing of levels (ST&D p388).

```
data stp387reg;
title 'Multiple regression CRD';
input S yield;
cards;
18 33.6
...;
proc glm;
  model yield= S S*S S*S*S S*S*S*S;
run; quit;
```

(note the absence of a `class` statement)

Dependent Variable: yield

Source	DF	SS	Mean Square	F Value	Pr > F
Model	4	125.7	31.4	9.90	<.0001
Error	25	79.3	3.2		
Corrected Total	29	205.0			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
S	1	91.3	91.3	28.76	<.0001
S*S	1	33.7	33.7	10.62	0.0032
S*S*S	1	0.5	0.5	0.16	0.6936
S*S*S*S	1	0.2	0.2	0.06	0.8052

## Same as

```
data stp387reg;
title 'Contrast CRD';
input S yield;
cards;
18 33.6
...;
proc glm;
  class S;
  model yield=S;
  contrast 'linear' S -2 -1 0 +1 +2;
  contrast 'Quadratic' S +2 -1 -2 -1 +2;
  contrast 'Cubic' S -1 +2 0 -2 +1;
  contrast 'Quartic' S +1 -4 +6 -4 +1;
run; quit;
```

Dependent Variable: yield

Source	DF	SS	Mean Square	F Value	Pr > F
Model	4	125.7	31.4	9.90	<.0001
Error	25	79.3	3.2		
Corrected Total	29	205.0			

Contrasts

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Linear	1	91.3	91.3	28.76	<.0001
Quadratic	1	33.7	33.7	10.62	0.0032
Cubic	1	0.5	0.5	0.16	0.6936
Quartic	1	0.2	0.2	0.06	0.8052

**Same result in both analyses. The multiple regression analysis can be used with unequally spaced treatments, but the Contrast analysis not.**

### **Some remarks on treatment levels for trend analysis**

The selection of dose levels for a material depends on the objectives the experimenter wishes to accomplish. If it is known that a certain response is linear over a given dose range and one is only interested in the rate of change, two doses will suffice, one low and one high. However, with only two doses there is no information available to verify the assumption of linearity. It is good practice to use one extra level so that the deviation from linearity can be estimated and tested. Similarly, if a quadratic response is expected, a minimum of four dose levels are required to test whether or not a quadratic would be appropriate.

The variability in agricultural data is generally greater than for physical and chemical laboratory studies, as the experimental units are subject to less controllable environmental influences. Also, responses vary from year to year and location to location. These variations cause difficulty in analyzing and interpreting combined experiments that are conducted over a few years or for a few locations. Furthermore, true response models are rarely known. For these reasons agricultural experiments require several dose levels, usually four to six levels to characterize a dose-response curve. It is usually desirable to have the doses equally spaced, arranging the levels in an arithmetic or logarithmic series. Experiments with equally spaced doses have the advantages of easy analysis and more power in determining the true response curve.

Final comment about orthogonal contrasts:

Desirable as it is to have independent tests, it is more important to construct sets of contrasts to attain the objectives of the investigation. <b>Practically meaningful contrasts are more desirable than simple orthogonal ones!</b>
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