

## Topic 4: Orthogonal Contrasts

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ANOVA is a useful and powerful tool to compare several treatment means. In comparing  $t$  treatments, the null hypothesis tested is that the  $t$  true means are all equal ( $H_0: \mu_1 = \mu_2 = \dots = \mu_t$ ). If the F test is "significant," one accepts the alternate hypothesis, which merely states that they are not all equal (i.e. at least one mean is different). Since the test does not tell you *which* mean(s) is/are different, the information provided by this initial F test is quite limited.

Further comparisons to determine which specific treatment means are different can be carried out by further **partitioning the treatment sum of squares (SST)**, thereby providing arguments (i.e. numerators) for additional, more targeted F tests. The orthogonal contrast approach to mean separation is described as *planned F tests*. These tests are planned in the sense that the questions of interest are decided upon *before* looking at the data. In fact, the questions of interest dictate the treatment structure from the very beginning of the experiment. With planned tests, therefore, there is *a priori* knowledge, based either on biological considerations or on the results of preliminary investigations, as to which comparisons are most interesting to make.

Said another way, if the investigator has specific questions to be answered, treatments are chosen (i.e. a treatment structure is designed) to provide information and statistical tests to answer those specific questions. An experienced investigator will select treatments so that SST can be partitioned perfectly to answer as many **independent** (i.e. orthogonal) questions as there are degrees of freedom for treatments in the ANOVA. Consequently, another name of these tests is single degree of freedom tests.

### Definitions of contrast and orthogonality

"Contrast" is mathematical jargon for a linear combination of terms (a polynomial) whose coefficients sum to zero. In ANOVA, "contrast" assumes a more limited definition: A contrast (Q) is a linear combination of two or more factor level means whose coefficients sum to zero:

$$Q = \sum_{i=1}^t c_i \bar{Y}_i, \text{ with the constraint that } \sum_{i=1}^t c_i = 0$$

As an example, consider a comparison of two means, the simplest contrast:

$$\mu_1 = \mu_2$$

This is the same as  $\mu_1 - \mu_2 = 0$ , where  $c_1 = 1$ ,  $c_2 = -1$ , and  $c_1 + c_2 = 0$

The constraint on the sum of coefficients is essential; and, for convenience, the  $c_i$ 's are usually integers. The terms  $\bar{Y}_i$  are usually the treatment means, though technically they can also be the treatment sums.

Now consider a pair of two contrasts:

$$Q_1 = \sum_{i=1}^t c_i \bar{Y}_i \quad \text{and} \quad Q_2 = \sum_{i=1}^t d_i \bar{Y}_i$$

These two contrasts are said to be **orthogonal** to one another if the sum of the products of their corresponding coefficients is zero:

$$\text{Orthogonal if } \sum_{i=1}^t c_i d_i = 0 \quad (\text{or } \sum_{i=1}^t \frac{c_i d_i}{n_i} = 0 \text{ for unbalanced designs})$$

So, orthogonality is a property of a set of two contrasts. A set of more than two contrasts is said to be orthogonal only if each and every pair within the set exhibits pairwise orthogonality, as defined above. To declare a set of four contrasts ( $Q_1 - Q_4$ ) to be orthogonal, therefore, one must show that each of the six possible pairs are orthogonal: ( $Q_1$  and  $Q_2$ ,  $Q_1$  and  $Q_3$ ,  $Q_1$  and  $Q_4$ ,  $Q_2$  and  $Q_3$ ,  $Q_2$  and  $Q_4$ ,  $Q_3$  and  $Q_4$ ).

Why do we care? Remember in our discussion of CRDs that the total SS could be perfectly partitioned into two parts: The treatment SS and the error SS:

$$\text{TSS} = \text{SST} + \text{SSE}$$

This perfect partitioning is possible due to the fact that, mathematically, SST and SSE are orthogonal to one another. We can pull them out and look at each one separately because they are statistically unrelated to one another. This gives us powerful insight into the relative signal (SST) and noise (SSE) in the experiment because, fortunately, SST and SSE are also meaningful quantities from an experimental perspective.

In an analogous way, orthogonal contrasts allow us to partition SST (i.e. decompose the relatively uninformative  $H_0$  of the ANOVA) into a maximum of  $(t - 1)$  meaningful and targeted comparisons involving different combinations of means.

Suppose a set of  $(t - 1)$  contrasts is orthogonal. If the SS for each contrast are added together, their sum will exactly equal SST for the original experiment. This means that an experiment can be partitioned into  $(t - 1)$  separate, independent experiments, one for each contrast.

**Example:** Suppose we are testing three treatments [ $T_1$ ,  $T_2$ , and  $T_3$  (control)], and the treatment means are  $\mu_1$ ,  $\mu_2$ , and  $\mu_3$ . The null hypothesis for the ANOVA is  $H_0: \mu_1 = \mu_2 = \mu_3$ , which uses both treatment degrees of freedom in one test ( $df_t = t - 1 = 2$ ). But since there are two treatment degrees of freedom, there are in principle two *independent comparisons* that can be made. For example, one could in principle test the two hypotheses that  $\mu_1$  and  $\mu_2$  are not significantly different from the control:  $\mu_1 = \mu_3$  and  $\mu_2 = \mu_3$ . As usual, we represent the means  $\mu_i$  with their estimates  $\bar{Y}_i$ .

1.  $\mu_1 = \mu_3$  can be rewritten  $1\mu_1 + 0\mu_2 - 1\mu_3 = 0$   
The coefficients of this contrast are:  $c_1 = 1, c_2 = 0, c_3 = -1$
2.  $\mu_2 = \mu_3$  can be rewritten  $0\mu_1 + 1\mu_2 - 1\mu_3 = 0$   
The coefficients of this contrast are:  $d_1 = 0, d_2 = 1, d_3 = -1$

These linear combinations of means are **contrasts** because:

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

However, these contrasts are **not orthogonal** because:

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

So, not every pair of hypotheses can be tested using this approach. In addition to summing to 0, the  $c_i$  coefficients are almost always taken to be integers, a constraint which severely restricts their possible values. For  $t = 3$ , such a set of values are:

$$c_1 = 1, c_2 = 1, c_3 = -2 \quad \text{and} \quad d_1 = 1, d_2 = -1, d_3 = 0$$

These are **contrasts** since  $(1 + 1 + (-2) = 0$  and  $1 + (-1) + 0 = 0$ ).

These are **orthogonal** because  $(c_1 d_1 + c_2 d_2 + c_3 d_3 = 1 + (-1) + 0 = 0)$ .

Just as not all sets of hypotheses can be asked using orthogonal contrasts, not all sets of orthogonal contrasts correspond to meaningful (or interesting) hypotheses. In this example, the contrasts are, in fact, interesting. The hypotheses they define are:

- 1) The average of the two treatments is equal to the control (i.e. is there a significant average treatment effect?); and
- 2) The two treatments are equal to one another (i.e. is one treatment significantly different from the other?).

Orthogonal contrasts are just simple linear polynomials that meet the constraints discussed above. In terms of general form, then, all contrasts are the same. But the hypotheses they represent can be divided into two general categories, **class (or group) comparisons** and **trend analyses**.

## Class (or group) comparisons

The first category of hypotheses we can pose using orthogonal contrasts is *class (or group) comparisons*. Such contrasts compare specific treatment means or combinations of treatment means, grouped in some meaningful way. To illustrate the use of orthogonal contrasts in class comparisons here, we will use the data given in Table 4.1 below. The analysis of variance for this experiment is given in Table 4.2.

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

Treatment	Replications					Total	Mean
Control	4.23	4.38	4.10	3.99	4.25	20.95	4.19
HCl	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	18.20	3.64

t = 4, r = 5, overall mean = 3.86

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

Source	df	SS	MS	F
Total	19	1.0113		
Treatment	3	0.8738	0.2912	33.87
Error	16	0.1376	0.0086	

Disregarding the data entirely, the *treatment structure* of this experiment suggests that the investigator had several specific questions in mind from the very beginning:

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

These are *planned* questions and so are appropriate candidates for posing via orthogonal contrasts. To do this, we must restate these questions mathematically, as linear combinations of treatments. In the following table (Table 4.3), coefficients are shown that translate these three planned questions into contrasts.

**Table 4.3** Orthogonal coefficients for partitioning the treatment sum of squares of Table 4.2 among three independent tests.

Comparisons	Control	HCl	Propionic	Butyric
Control vs. acid	3	-1	-1	-1
Inorganic vs. organic	0	2	-1	-1
Between organics	0	0	1	-1

The first contrast (first row of coefficients) compares the control group to the average of the three acid-treated groups, as can be seen from the following manipulations:

$$\begin{aligned}
 3\mu_{\text{Cont}} - 1\mu_{\text{HCl}} - 1\mu_{\text{Prop}} - 1\mu_{\text{But}} &= 0 \\
 3\mu_{\text{Cont}} &= 1\mu_{\text{HCl}} + 1\mu_{\text{Prop}} + 1\mu_{\text{But}} \\
 \mu_{\text{Cont}} &= (1/3)*(1\mu_{\text{HCl}} + 1\mu_{\text{Prop}} + 1\mu_{\text{But}}) \\
 \text{Mean of the control group} &= \text{Mean of all acid-treated groups}
 \end{aligned}$$

The  $H_0$  for this first contrast is that there is no average effect of acid treatment on seedling growth. Since this null hypothesis involves only two group means, it costs 1 df.

The second contrast (second row of coefficients) compares the inorganic acid group to the average of the two organic acid groups:

$$\begin{aligned}
 0\mu_{\text{Cont}} + 2\mu_{\text{HCl}} - 1\mu_{\text{Prop}} - 1\mu_{\text{But}} &= 0 \\
 2\mu_{\text{HCl}} &= 1\mu_{\text{Prop}} + 1\mu_{\text{But}} \\
 \mu_{\text{HCl}} &= (1/2)*(1\mu_{\text{Prop}} + 1\mu_{\text{But}}) \\
 \text{Mean of the HCl group} &= \text{Mean of all organic acid-treated groups}
 \end{aligned}$$

The  $H_0$  for this second contrast is that the effect of the inorganic acid treatment on seedling growth is no different from the average effect of organic acid treatment. Since this null hypothesis involves only two group means (different means than before), it also costs 1 df.

Finally, the third contrast (third row of coefficients) compares the two organic acid groups to each other:

$$\begin{aligned}
 0\mu_{\text{Cont}} + 0\mu_{\text{HCl}} + 1\mu_{\text{Prop}} - 1\mu_{\text{But}} &= 0 \\
 1\mu_{\text{Prop}} &= 1\mu_{\text{But}}
 \end{aligned}$$

The  $H_0$  for this third contrast is that the effect of the propionic acid treatment on seedling growth is no different from the effect of butyric acid treatment. Since this null hypothesis involves only two group means (different means than before), it also costs 1 df. At this point, we have spent all our available degrees of freedom ( $df_{\text{trt}} = t - 1 = 4 - 1 = 3$ ).

Because each of these questions are contrasts (each row of coefficients sums to zero) and because the set of three questions is orthogonal (verify this for yourself), these three questions perfectly partition SST into three components, each with 1 df. The SS associated with each of these contrasts serve as the numerators for three separate F tests, one for each comparison. The

critical F values for these single df tests are based on 1 df in the numerator and  $df_{\text{Error}}$  in the denominator. All of this can be seen in the expanded ANOVA table below.

**Table 4.4** Orthogonal partitioning of SST via contrasts.

Source	df	SS	MS	F
<b>Total</b>	19	1.0113		
<b>Treatment</b>	3	0.8738	0.2912	33.87
<b>1. Control vs. acid</b>	1	0.7415	0.7415	86.22
<b>2. Inorg. vs. Org.</b>	1	0.1129	0.1129	13.13
<b>3. Between Org.</b>	1	0.0194	0.0194	2.26
<b>Error</b>	16	0.1376	0.0086	

Notice that  $SST = SS_{\text{Contrast1}} + SS_{\text{Contrast2}} + SS_{\text{Contrast3}}$ . This perfect partitioning of SST among its degrees of freedom is a direct consequence of the orthogonality of the posed contrasts. When comparisons are not orthogonal, the SS for one comparison may contain (or be contained by) part of the SS of another comparison. Therefore, the conclusion from one test may be influenced (or contaminated) by another test and the SS of those individual comparisons will not sum to SST.

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

$$SS(Q) = MS(Q) = \frac{(\sum c_i \bar{Y}_i)^2}{\sum (c_i^2 / r_i)}$$

This expression simplifies to  $\frac{(\sum c_i \bar{Y}_i)^2}{(\sum c_i^2) / r}$  in balanced designs (all r's equal)

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

$$SS_2 (\text{inorg. vs. org.}) = [3.64 + 3.73 - 2(3.87)]^2 / [(6)/5] = 0.11$$

$$SS_3 (\text{between org.}) = [-3.64 + 3.73]^2 / [(2)/5] = 0.02$$

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

From this analysis, we conclude that in this experiment all three acids significantly reduce seedling growth ( $F = 86.22$ ,  $p < 0.01$ ), that the organic acids cause significantly more reduction than the inorganic acid ( $F = 13.13$ ,  $p < 0.01$ ), and that the difference between the organic acids is not significant ( $F = 2.26$ ,  $p > 0.05$ ).

## Construction of coefficients for class comparisons

(Little & Hills p 66)

Contrast coefficients for a class comparison can always be determined by writing the null hypothesis in mathematical form, moving all terms to the same side of the equation, and multiplying by whatever factor is needed to turn the coefficients into integers. This is a general strategy. What follows are more recipe-like, step-by-step operations to arrive at the same results:

1. When the two groups of means being compared each contain 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 extends to the case of more than one treatment per group.
2. When 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 it is the sum of *squares* of the comparison that is used in the 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. The coefficients for an *interaction* comparison are determined by simply multiplying the corresponding coefficients of the two underlying main comparisons (see next example).

**Example:** Fertilizer experiment designed as a CRD with four treatments. The four treatments result from all possible combinations of two levels of both nitrogen ( $N_0 = \text{no N}$ ,  $N_1 = 100 \text{ lbs N/ac}$ ) and phosphorus ( $P_0 = \text{no P}$ ,  $P_1 = 20 \text{ lbs P/ac}$ ).

The questions intended by this treatment structure are:

1. Is there an effect of N on yield?
2. Is there an effect of P on yield?
3. Is there an interaction between N and P on yield? (Other equivalent ways of stating this last question: a) Is the effect of N the same in the presence of P as it is in the absence of P? b) Is the effect of P the same in the presence of N as it is in the absence of N?)

The following table (Table 4.5) presents the contrast coefficients for these planned questions.

**Table 4.5** Contrast coefficients for the three planned questions

	$N_0P_0$	$N_0P_1$	$N_1P_0$	$N_1P_1$
Effect of N	-1	-1	1	1
Effect of P	-1	1	-1	1
<b>Interaction</b> (N $\times$ P)	1	-1	-1	1

The coefficients for the first two comparisons are derived using rule 1. The coefficients for the last comparison (an interaction comparison) are derived by simply multiplying the coefficients of the first two lines.

Note again 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. This set of comparisons is therefore orthogonal. This implies that the conclusion drawn for any one comparison is independent of (not influenced by) the others.

## Trend comparisons

Experiments are often designed to characterize 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.) on some response variable (e.g. yield, disease severity, pest pressure, growth, etc.). In these situations, the experimenter is interested in investigating and describing the dose response relationship. Such an analysis is concerned with overall trends and **not** with pairwise comparisons.

The simplest example involves a single factor at three levels. This is a very common situation in genetic experiments, where the levels are 1) Zero dose of allele A in homozygous BB individuals, 2) One dose of allele A in heterozygous AB individuals, and 3) Two doses of allele A in homozygous AA individuals. With the use of molecular markers, it is now easy to genotype the individuals of a segregating population and classify them into one of these three groups (AA, AB, BB). Of course, these individuals may also be phenotyped for a certain trait of interest. Suppose 40 segregating  $F_2$  individuals are genotyped for a certain marker and the nitrogen content of their seeds are measured. The data for such an experiment are shown in Table 4.6.

**Table 4.6** Genetic example of orthogonal contrasts. Nitrogen content (mg) of seeds of three different genotypes.

Genotype (BB) 0 doses, A allele	Genotype (AB) 1 dose, A allele	Genotype (AA) 2 doses, A allele
12.0	13.5	13.8
12.5	13.8	14.5
12.1	13.0	13.9
11.8	13.2	14.2
12.6	13.0	14.1
	12.8	
	12.9	
	13.4	
	12.7	
	13.6	

Unequal replication is common in genetic experiments due to segregation ratios. In  $F_2$  populations, the expected ratio of homozygous to heterozygous individuals is 1:2:1, which is what we see in the dataset above. Each individual is an independent replication of its respective genotype; so there are five replications of genotype BB, ten replications of genotype AB, and five replications of genotype AA in this experiment. The "treatment" is dosage of the A allele, and the response variable is seed nitrogen content.

With three levels of dosage, the most complicated response the data can reveal is a quadratic relationship between dosage (D) and N content:

$$N = aD^2 + bD + c$$

This quadratic relationship is comprised of two components: A linear component (slope b), and a quadratic component (curvature a). It just so happens that, with 2 treatment degrees of freedom ( $df_{\text{trt}} = t - 1 = 3 - 1 = 2$ ), we can construct orthogonal contrasts to probe each of these components.

To test the hypothesis that  $b = 0$  (i.e. there is zero slope in the overall dosage response relationship), we choose  $H_0: \mu_{BB} = \mu_{AA}$ . If the means of the two extreme dosages are equal,  $b = 0$ . As a contrast, this  $H_0$  takes the form:  $1\mu_{BB} + 0\mu_{AB} - 1\mu_{AA} = 0$ .

To test the hypothesis that  $a = 0$  (i.e. there is zero curvature in the overall dosage response relationship), we choose  $H_0: \mu_{AB} = (1/2)(\mu_{AA} + \mu_{BB})$ . Because the dosage levels are equally spaced (0, 1, 2), a perfectly linear relationship (i.e. zero curvature) would require that the average of the extreme dosage levels  $[(1/2)(\mu_{AA} + \mu_{BB})]$  exactly equal the mean of the heterozygous group ( $\mu_{AB}$ ). As a contrast, this  $H_0$  takes the form:  $1\mu_{BB} - 2\mu_{AB} + 1\mu_{AA} = 0$ .

A quick inspection shows each of these polynomials to be contrasts (i.e. their coefficients sum to zero) as well as orthogonal to each other ( $1*1 + 0*(-2) + (-1)*1 = 0$ ).

Constructing F tests for these contrasts follows the exact same procedure we saw above in the case of class comparisons. So this time, let's use R:

**R script:**

```
#The ANOVA
genedose_mod<-lm(N ~ A_Dose, genedose_dat)
anova(genedose_mod)

#Need to assign contrast coefficients
# Contrast 'Linear'      -1,0,1
# Contrast 'Quadratic'  1,-2,1
contrastmatrix<-cbind(c(-1,0,1),c(1,-2,1))
contrastmatrix

contrasts(genedose_dat$A_Dose)<-contrastmatrix
genedose_dat$A_Dose

#The ANOVA, with partitioned SST
genedose_contrast_mod<-aov(N ~ A_Dose, genedose_dat)
summary(genedose_contrast_mod,
        split = list(A_Dose = list("Linear" = 1, "Quadratic" = 2)))
```

The resultant ANOVA table:

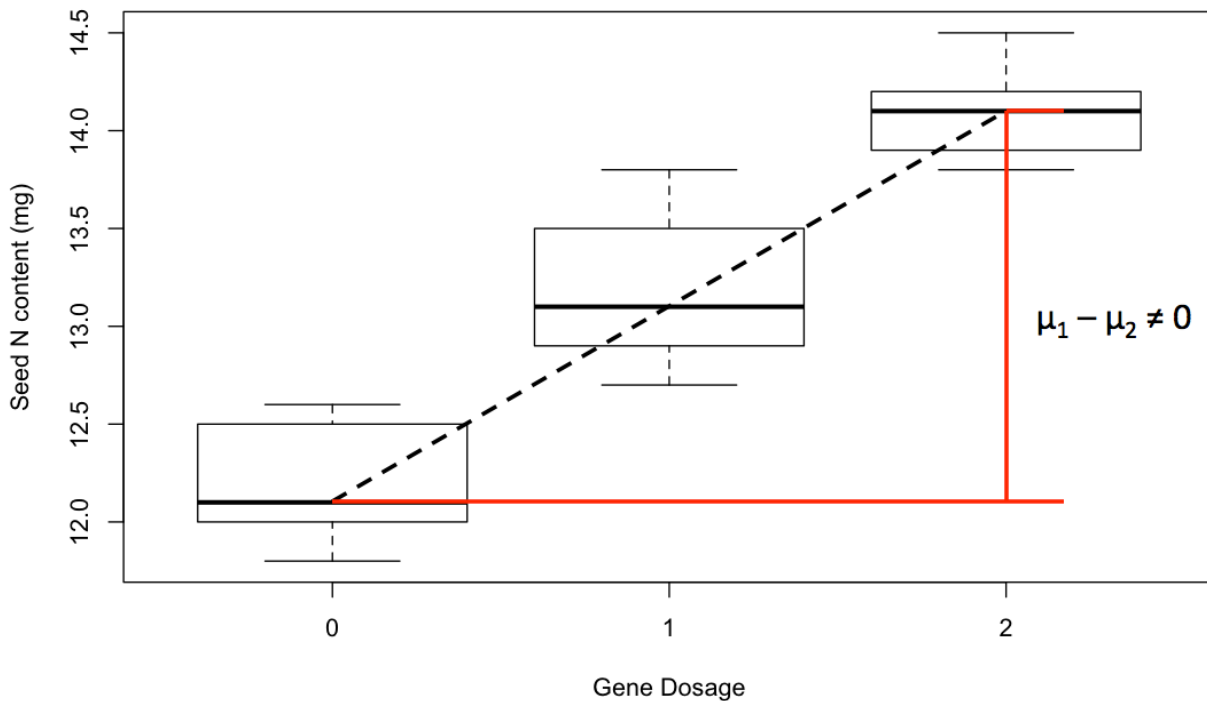
Source	df	SS	MS	F	p
<b>Total</b>	19	11.022			
<b>Model</b>	<b>2</b>	<b>9.033</b>	4.5165	38.60	< 0.0001
<b>Error</b>	17	1.989	0.117		
<b>R-Square:</b>	<b>0.819543</b>				

Contrast	df	SS	MS	F	p
<b>Linear</b>	<b>1</b>	<b>9.025</b>	9.025	77.14	< 0.0001
<b>Quadratic</b>	<b>1</b>	<b>0.008</b>	0.008	0.07	0.7969

The fact that the contrast SS sum perfectly to the SST is a verification of their orthogonality. The significant linear contrast ( $p < 0.0001$ ) leads us to reject its  $H_0$ . There does appear to be a significant, nonzero linear component to the response. The nonsignificant quadratic contrast ( $p = 0.7969$ ), however, leads us not to reject its  $H_0$ . There does not appear to be a significant, nonzero quadratic component to the response. All this can be seen quite easily in the following combined boxplot:

### Effect of gene dosage on N content



We would conclude from all this that the dosage response of nitrogen seed content to the presence of allele A is linear.

Before we move on, notice that when there is no significant quadratic response, 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  $F_{1,17} = 4.45$ ). The reason for this: In the linear contrast,  $MS = SS/1$ , while in the complete Model,  $MS = SS/2$  (i.e. the full SST is divided in half and arbitrarily assigned equally to both effects). When a quantitative factor exhibiting a significant linear dose response is measured at several levels, it is not uncommon for the overall treatment F test to fail to be significant. This is because the overall treatment F test allocates the full SST across many small higher order effects (quadratic, cubic, quartic, quintic, etc.), all of which are truly nonsignificant. This obscures the significance of the true linear effect. In such cases, contrasts significantly increase the power of the test.

Here is a similar dataset, but now the response variable is days to flowering (DTF).

**Table 4.7** Days to flowering (DTF) of seeds of three different genotypes.

Genotype (BB) 0 doses, A allele	Genotype (AB) 1 dose, A allele	Genotype (AA) 2 doses, A allele
58	71	73
51	75	68
57	69	70
59	72	71
60	68	67
	73	
	69	
	70	
	71	
	72	

The R coding is identical in this case.

The resultant ANOVA table:

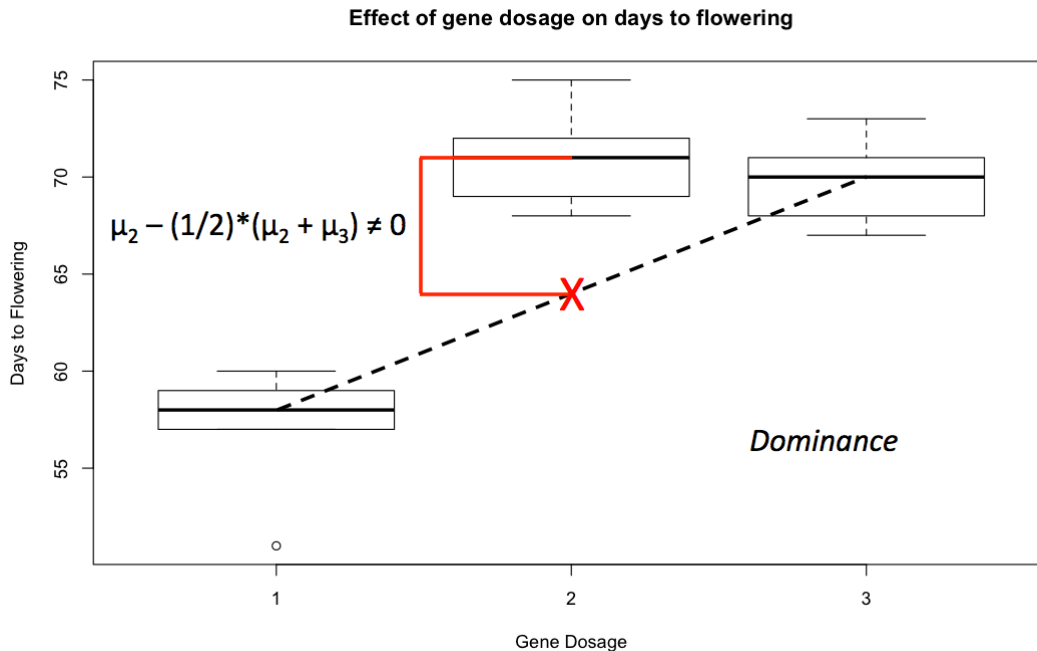
Source	df	SS	MS	F	p
<b>Total</b>	19	811.20			
<b>Model</b>	<b>2</b>	<b>698.40</b>	349.200	52.63	< 0.0001
<b>Error</b>	17	112.80	6.635		
<b>R-Square:</b>	<b>0.860947</b>				

Contrast	df	SS	MS	F	p
<b>Linear</b>	<b>1</b>	<b>409.6</b>	409.6	61.73	< 0.0001
<b>Quadratic</b>	<b>1</b>	<b>288.8</b>	288.8	43.52	< 0.0001

Again, the contrast SS sum perfectly to the SST, a verification of their orthogonality. The significant linear contrast ( $p < 0.0001$ ) leads us to reject its  $H_0$ . There does appear to be a significant, nonzero linear component to the response. And the significant quadratic contrast ( $p < 0.0001$ ), leads us to reject its  $H_0$  as well. There does appear to be a significant, nonzero quadratic component to the response.

All this can be seen quite easily in the following combined boxplot:



We would conclude from all this that the dosage response of nitrogen seed content to the presence of allele A has both a linear and a quadratic component. In genetic terms, there is dominance.

If we were to analyze this last example via a simple linear regression, we would obtain the following results:

Source	df	SS	MS	F	p
Total	19	811.20			
Model	1	409.60	409.6	18.36	0.0004
Error	18	401.60	22.3		

$$401.6 = 112.8 + 288.8$$

The F value is smaller ( $18.36 < 61.73$ ) because the quadratic SS (288.8) is now included in the error sum of squares ( $401.6 = 112.8 + 288.8$ ). The message: An ANOVA with linear and quadratic contrasts is more sensitive to linear effects than a linear regression test. A quadratic regression test, however, will yield identical results to our analysis using contrasts.

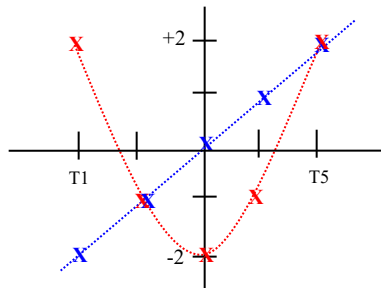
## Coefficients for trend comparisons

The  $c_i$  coefficients used for trend comparisons (linear, quadratic, cubic, quartic, etc.) among *equally spaced* treatments are listed below, taken from Table 15.12 (ST&D 390).

**Table 4.8** Contrast coefficients for trend comparisons for *equally spaced* treatments

Number of Treatments	Response Component	c1	c2	c3	c4	c5	c6
2	Linear	-1	1				
3	Linear	-1	0	1			
	Quadratic	1	-2	1			
4	Linear	-3	-1	1	3		
	Quadratic	1	-1	-1	1		
	Cubic	-1	3	-3	1		
5	Linear	-2	-1	0	1	2	
	Quadratic	2	-1	-2	-1	2	
	Cubic	-1	2	0	-2	1	
	Quartic	1	-4	6	-4	1	
6	Linear	-5	-3	-1	1	3	5
	Quadratic	5	-1	-4	-4	-1	5
	Cubic	-5	7	4	-4	-7	5
	Quartic	1	-3	2	2	-3	1
	Quintic	-1	5	-10	10	-5	1

As argued in the two previous examples, the values of these coefficients ultimately can be traced back to simple geometric arguments. The plot below shows the values of the linear and quadratic coefficients for  $t = 5$ :



To illustrate the procedure for evaluating a trend response when more treatment levels are involved, we will use the data from Table 4.9 below.

**Table 4.9** Partitioning SST using orthogonal polynomials. Yield of Ottawa Mandarin soybeans grown in MN (bushels / acre). [adapted from ST&D 387]

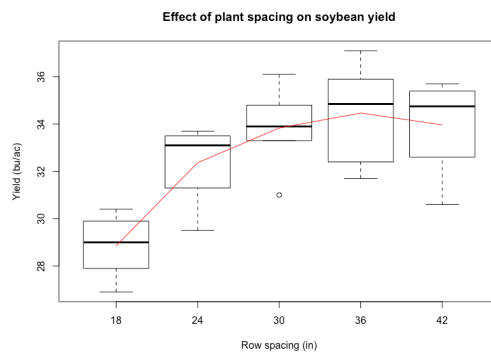
Rep.	Row spacing (in inches)				
	18	24	30	36	42
1	30.4	32.9	31.0	35.6	32.6
2	26.9	29.5	34.5	34.1	35.7
3	29.9	33.5	34.8	32.4	35.1
4	29.4	31.3	33.3	31.7	35.4
5	28.6	33.3	33.3	35.9	34.4
6	27.9	33.7	36.1	37.1	30.6
<b>Means</b>	<b>28.85</b>	<b>32.37</b>	<b>33.83</b>	<b>34.47</b>	<b>33.97</b>

First of all, note that the treatment levels are equally spaced (18, 24, 30, 36, 42 → an equal spacing of 6 between adjacent levels). Trend analysis via contrasts is greatly simplified when treatment levels are equally spaced (either arithmetic or log scales).

The contrast coefficients and the analysis:

	Row spacing					$\sum(c_i \bar{Y}_i)^2$	$\sum(c_i^2)/r$	SS	F
	18	24	30	36	42				
<b>Means</b>	<b>28.85</b>	<b>32.37</b>	<b>33.83</b>	<b>34.47</b>	<b>33.97</b>				
Linear	-2	-1	0	1	2	152.11	1.67	91.27	28.8***
Quadratic	2	-1	-2	-1	2	78.62	2.33	33.69	10.6**
Cubic	-1	2	0	-2	1	0.84	1.67	0.50	0.16 NS
Quartic	1	-4	6	-4	1	2.30	11.67	0.20	0.06 NS

In this trend analysis, we perfectly partitioned SST (125.66) among the four degrees of freedom for treatment, each degree of freedom corresponding to an independent, orthogonal contrast (linear, quadratic, cubic, and quartic components to the overall response). We conclude from this analysis that the relationship between row spacing and yield has significant linear and significant quadratic components. The cubic and quartic components are not significant. This can be seen easily in a plot of the data:



## Unequally spaced treatments

There are equations to calculate coefficient similar to those of Table 4.8 for unequally spaced treatment levels and unequal numbers of replications. The ability to compute such sums of squares using orthogonal contrasts was crucial in the days before computers. But now it is easier to implement a regression approach, which does not require equal spacing among treatment levels. R code for a full regression analysis of the soybean yield data:

An R script for a full regression analysis of the soybean yield data:

```
#Read in the dataset again
spacing_dat<-as.data.frame(spacing_dat)

#Assign Spacing as a numeric regression variable
spacing_dat$Spacing<-as.numeric (spacing_dat$Spacing)
str(spacing_dat, give.attr=F)

#Create a new set of variables
Spacing<-spacing_dat$Spacing
Spacing2<-Spacing^2
Spacing3<-Spacing^3
Spacing4<-Spacing^4

anova(lm(Yield ~ Spacing + Spacing2 + Spacing3 + Spacing4, spacing_dat))
```

In regression, we are not interested in the individual levels of the explanatory variable; we are interested in the nature of overall trend. The output:

	<u>Df</u>	<u>Sum Sq</u>	<u>Mean Sq</u>	<u>F value</u>	<u>Pr(&gt;F)</u>	
Spacing	1	<b>91.267</b>	91.267	28.7623	<b>1.461e-05</b>	***
Spacing2	1	<b>33.693</b>	33.693	10.6183	<b>0.003218</b>	**
Spacing3	1	<b>0.504</b>	0.504	0.1589	0.693568	
Spacing4	1	<b>0.197</b>	0.197	0.0621	0.805187	
Residuals	25	79.328	3.173			

This is the exact result we obtained using contrasts (see code and output below):

```
#The ANOVA
spacing_mod<-lm(Yield ~ Spacing, spacing_dat)
anova(spacing_mod)

#Need to assign contrast coefficients
# Contrast 'Linear'          -2,-1,0,1,2
# Contrast 'Quadratic'      2,-1,-2,-1,2
# Contrast 'Cubic'         -1,2,0,-2,1
# Contrast 'Quartic'       1,-4,6,-4,1
contrastmatrix<-cbind( c(-2,-1,0,1,2),
                       c(2,-1,-2,-1,2),
```

```

c(-1,2,0,-2,1),
c(1,-4,6,-4,1))
contrastmatrix

contrasts(spacing_dat$Spacing)<-contrastmatrix
spacing_dat$Spacing

spacing_contrast_mod<-aov(Yield ~ Spacing, spacing_dat)
summary( spacing_contrast_mod,
        split = list(Spacing = list( "Linear" = 1,
                                     "Quadratic" = 2,
                                     "Cubic" = 3,
                                     "Quartic" = 4)))

```

### The results:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Spacing	4	125.66	31.42	9.900	6.08e-05	***
Spacing: Linear	1	91.27	91.27	28.762	1.46e-05	***
Spacing: Quadratic	1	33.69	33.69	10.618	0.00322	**
Spacing: Cubic	1	0.50	0.50	0.159	0.69357	
Spacing: Quartic	1	0.20	0.20	0.062	0.80519	
Residuals	25	79.33	3.17			

So, as long as the treatment levels are equally spaced, the results are the same for both analyses. The multiple regression analysis can be used with unequally spaced treatments, but the orthogonal contrast analysis, with the provided coefficients, cannot.

### Some remarks on treatment levels for trend analysis

The selection of dose levels for a material depends on the objectives of the experiment. 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 initial assumption of linearity. It is good practice to use one extra level so that 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 model is 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. These variations cause difficulty in analyzing and interpreting combined experiments that are conducted over different years or across different locations. Furthermore, true response models are rarely known. For these reasons, agricultural experiments usually require four to six levels to characterize a dose-response curve.

Final comment about orthogonal contrasts:

Powerful as they are, contrasts are not always appropriate.

**If you have to choose, meaningful hypotheses  
are more desirable than orthogonal ones!**