## Lecture 6
## **Topic 4:** Orthogonal contrasts

**ANOVA:** $H_0$: $\mu_1 = \mu_2 = ... = \mu_t$
$H_1$: The mean of at least one treatment group is different

To test this hypothesis, a basic ANOVA allocates the variation among treatment means (SST) equally across the (t – 1) treatment degrees of freedom and asks, "Is this average portion of SST significant?"

$$F = \frac{s^2_{among}}{s^2_{within}} = \frac{ns^2_{\bar{Y}}}{s^2} = \frac{MST}{MSE} = \frac{\frac{SST}{df_{trt}}}{\frac{SSE}{df_{error}}}$$

But what if the variation among treatment means is *not* distributed equally? Isn't there a better way to "spend" our treatment degrees of freedom?

$$TSS = SST + SSE$$
An orthogonal partition of the total SS

$$SST_{df=t-1} = \sum_{i=1}^{t-1} SSContrast_{i(df=1)}$$
An orthogonal partition of the treatment SS

Comparisons to determine which specific treatment means are different can be carried out by **partitioning the treatment sum of squares (SST)**.

The orthogonal contrasts approach to mean separation is described as *planned, single degree of freedom F tests*.

In effect, an experiment can be partitioned into (t – 1) separate, independent experiments, one for each contrast.

**Definitions of contrast and orthogonality**

A contrast (Q) is a linear combination of terms (a polynomial) whose coefficients sum to zero:

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

**Example:** $\mu_1 = \mu_2$

The $c_i$'s are usually integers, and each contrast has one degree of freedom.

Now consider a pair of two contrasts:

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

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

$$\text{Orthogonal if } \sum_{i=1}^{t} c_i d_i = 0$$

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.

The combined SS of a set of (t – 1) orthogonal contrasts will equal SST.

**Example:** Three treatments ($T_1$, $T_2$, and $T_3$ (control)), with means $\mu_1$, $\mu_2$, and $\mu_3$.

With two treatment df, there are in principle two *independent comparisons* we can make.

We *could* test the two hypotheses that $\mu_1$ and $\mu_2$ are not significantly different from the control:

1. $H_0$: $\mu_1 = \mu_3 \rightarrow 1\mu_1 + 0\mu_2 - 1\mu_3 = 0$
   $c_1 = 1, c_2 = 0, c_3 = -1$

2. $H_0$: $\mu_2 = \mu_3 \rightarrow 0\mu_1 + 1\mu_2 - 1\mu_3 = 0$
   $d_1 = 0, d_2 = 1, d_3 = -1$

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

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

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

$$\sum_{i=1}^{t} c_i d_i \neq 0 \ (c_1d_1 + c_2d_2 + c_3d_3 = 1*0 + 0*1 + (-1)*(-1) = 1)$$

| Not every set of hypotheses can be tested using this approach. |
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**1.** Is there a significant average treatment effect?

$$H_0: \ \frac{\mu_1 + \mu_2}{2} = \mu_3 \rightarrow \mu_1 + \mu_2 - 2\mu_3 = 0$$

**2.** Is there a difference between the two treatment effects?

$$H_0: \ \mu_1 = \mu_2 \rightarrow \mu_1 - \mu_2 = 0$$

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

$$c_1d_1 + c_2d_2 + c_3d_3 = 1 + (-1) + 0 = 0$$

| Not all sets of orthogonal contrasts correspond to meaningful (or interesting) hypotheses. |
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## Class comparisons

**Example:** Results of an experiment (CRD) to determine the effect of different acid seed treatments on the early growth of rice seedlings (mg dry weight).

| Treatment | Replications | | | | | Mean |
|---|---|---|---|---|---|---|
| **Control** | 4.23 | 4.38 | 4.10 | 3.99 | 4.25 | 4.19 |
| **HCl** | 3.85 | 3.78 | 3.91 | 3.94 | 3.86 | 3.87 |
| **Propionic** | 3.75 | 3.65 | 3.82 | 3.69 | 3.73 | 3.73 |
| **Butyric** | 3.66 | 3.67 | 3.62 | 3.54 | 3.71 | 3.64 |

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

**Results of the ANOVA**

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

**The planned questions:**

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 the two different organic acids?

**Orthogonal coefficients for partitioning SST among three independent tests:**

| Comparisons | Control | HC1 | Propionic | Butyric |
|---|---|---|---|---|
| **Control vs. acid** | 3 | -1 | -1 | -1 |
| **Inorganic vs. organic** | 0 | 2 | -1 | -1 |
| **Between organics** | 0 | 0 | 1 | -1 |

**Computation of Contrast SS**

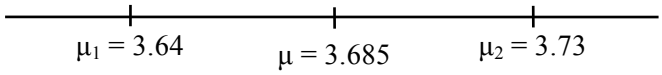
The SS for a single degree of freedom *F* test for linear combinations of ***treatment means*** is

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

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

$SS_1$ (control vs. acid) $= [3(4.19) - 3.64 - 3.73 - 3.87]^2 / [(12)/5] = 0.74$
$SS_2$ (inorg. vs. org.) $= [3.64 + 3.73 - 2(3.87)]^2 / [(6)/5] = 0.11$
$SS_3$ (between org.) $= [-3.64 + 3.73]^2 / [(2)/5] = 0.02$

You obtain the same results via a more intuitive sums of squares approach:




$$SS_3 = r\sum_{i=1}^{2}(\bar{Y}_i - \bar{Y})^2 = 5\left[(3.64 - 3.685)^2 + (3.73 - 3.685)^2\right] = 0.02$$

**Orthogonal partitioning of SST via contrasts:**

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

**We conclude:**

1. All three acids reduce seedling growth ($F = 86.22$, $p < 0.0001$)
2. Organic acids cause more reduction than the inorganic acid ($F = 13.13$, $p = 0.0023$)
3. There is no difference between the organic acids ($F = 2.26$, $p = 0.1529$)

**With a set of (t – 1) orthogonal contrasts:**

1. Contrasts SS add up to the SST
2. The SS for one comparison contains no part of the SS of another comparison
3. The conclusions are independent from each other

**Construction of coefficients for class comparisons**

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.

2. When comparing groups containing different numbers of treatments, assign:

   *First group coefficients* = number of treatments in the second group
   *Second group coefficients* = number of treatments in the first group, with opposite sign

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.

**Example**: A fertilizer experiment designed as a CRD with four treatments. The four treatments result from all possible combinations of two levels of both nitrogen ($N_0$ = no N, $N_1$ = 100 kg N/ha) and phosphorus ($P_0$ = no P, $P_1$ = 20 kg P/ha).

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?

**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 |
| **Interaction** (NxP) | 1 | -1 | -1 | 1 |

If comparisons are orthogonal, 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 on some response variable (i.e. the dose response relationship).

| Such analyses are concerned with overall trends and **not** with pairwise comparisons. |
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**Example:** An experiment is conducted to determine the effect of a certain allele on the nitrogen content of seeds (in mg). The experiment involves a single factor (Allele A) at three levels:

1. Zero doses of allele A (homozygous BB individuals)
2. One dose of allele A (heterozygous AB individuals)
3. Two doses of allele A (homozygous AA individuals)

Nitrogen content (mg) of seeds of three different genotypes.

| Genotype (BB)<br>0 doses, A allele | Genotype (AB)<br>1 dose, A allele | Genotype (AA)<br>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 | |

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

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

**R script:**

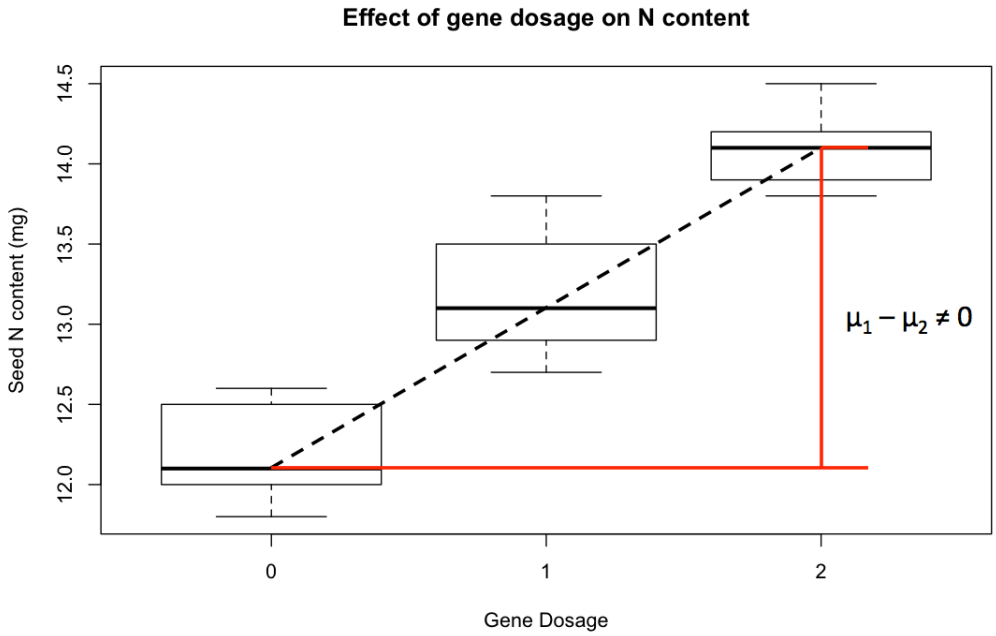
```
#The ANOVA
genedose_mod<-lm(N ~ A_Dose, genedose_dat)
anova(genedose_mod)

#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

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 tables:

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Dose | 2 | 9.033 | 4.5165 | 38.603 | 4.777e-07 | *** |
| Residuals | 17 | 1.989 | 0.1170 | | | |

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Dose: Linear | 1 | 9.025 | 9.025 | 77.137 | 1.00e-07 | *** |
| Dose: Quadratic | 1 | 0.008 | 0.008 | 0.068 | 0.797 | |

**MS Model** = The *average* of the two effects.



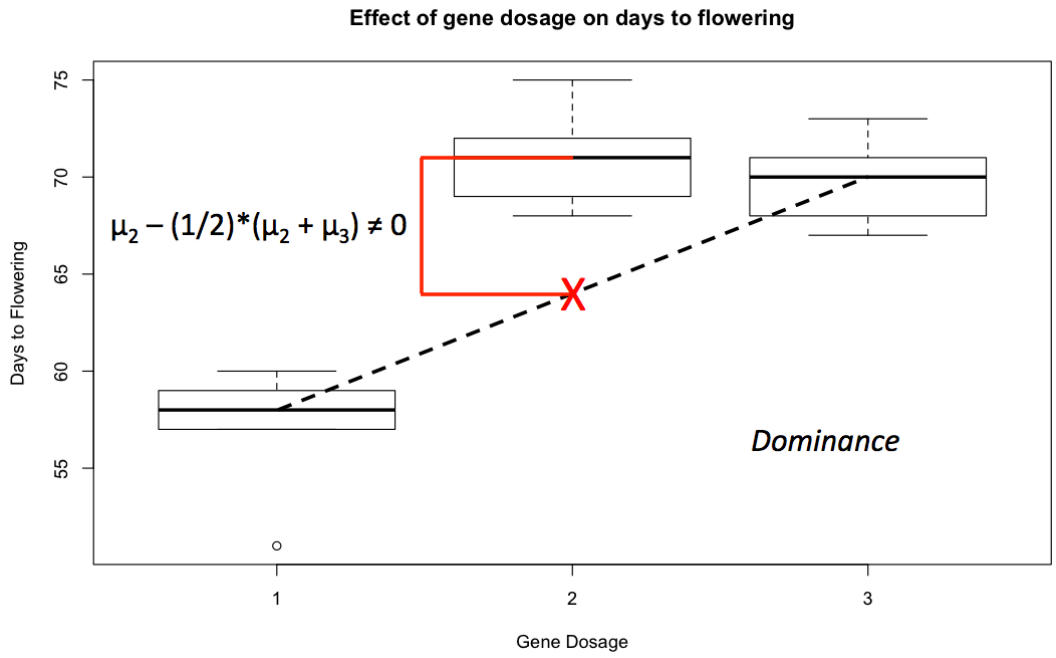
A similar dataset, but now the response variable is days to flowering (DTF).

| Genotype (BB)<br>0 doses, A allele | Genotype (AB)<br>1 dose, A allele | Genotype (AA)<br>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 resultant ANOVA table:

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Dose | 2 | 698.4 | 349.20 | 52.628 | 5.212e-08 | *** |
| Residuals | 17 | 112.8 | 6.64 | | | |

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Dose: Linear | 1 | 409.6 | 409.6 | 61.73 | 4.66e-07 | *** |
| Dose: Quadratic | 1 | 288.8 | 288.8 | 43.52 | 4.53e-06 | *** |



**Coefficients for trend comparisons**

The $c_i$ coefficients used for trend comparisons (linear, quadratic, cubic, quartic, etc.) among ***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 |

**Example:** 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 | 33.6 | 31.1 | 33 | 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 |
| **Means** | **31.15** | **31.63** | **30.17** | **29.53** | **30.03** |

The contrast coefficients and the analysis, by hand:

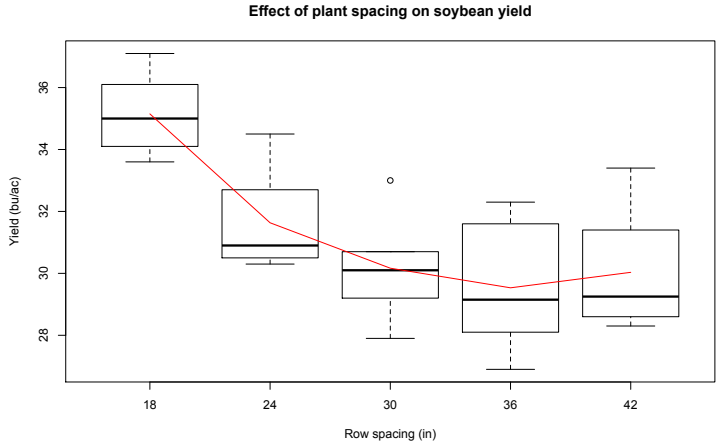
| | Row spacing | | | | | $\sum(c_i\bar{Y}_{i.})^2$ | $\sum(c_i^2)/r$ | SS | F |
|---|---|---|---|---|---|---|---|---|---|
| | 18 | 24 | 30 | 36 | 42 | | | | |
| **Means** | **35.15** | **31.63** | **30.17** | **29.53** | **30.03** | | | | |
| Linear | -2 | -1 | 0 | 1 | 2 | 152.11 | 1.67 | 91.27 | 28.8*** |
| 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 |

Using R:

```
#The ANOVA
anova(lm(Yield ~ Spacing, spacing.dat))

#Assigning contrast coefficients
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))

contrasts(spacing.dat$Spacing)<-contrastmatrix

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
```



**Unequally spaced treatments**

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)

Spacing<-spacing_dat$Spacing
Spacing2<-Spacing^2
Spacing3<-Spacing^3
Spacing4<-Spacing^4

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

**The results:**

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

Same results with both analyses. For unequally spaced treatment levels, regression analysis can be used but orthogonal contrasts with the given coefficients cannot.

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!**