

## One Way ANOVA - II

Orthogonal Contrasts

ANOVA Models

Random vs. Fixed Effects

Testing Assumptions

Normality

Homoscedasticity

Kruskal-Wallis Non-parm ANOVA

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## Orthogonal Contrasts

Previously we discussed procedures to separate means using *a posteriori* tests (performed after the fact). These are known generically as *multiple comparison procedures* (MCPs).

There is another method which is more powerful that utilizes an *a priori* (before the fact) approach. This approach utilizes *1 df comparisons*, but must be specified *prior* to the analysis.

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## Orthogonal Contrasts

- Example 1 -

A forest manager is responsible for the selection and purchase of chainsaws for her field crew. Her primary interest is worker safety. She is provided with data on chainsaw kickback values (degrees of deflection) for A = 4 brands of chainsaws with N = 5 observations each. The obvious null hypothesis is:

$$H_0: \mu_A = \mu_B = \mu_C = \mu_D \quad (\text{ANOVA})$$



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## Orthogonal Contrasts

- Example 1: Data -



Model:	A	B	C	D
	42	28	57	29
	17	50	45	40
	24	44	48	22
	39	32	41	34
	43	61	54	30

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## Orthogonal Contrasts

- Example 1: ANOVA, NCSS -

Analysis of Variance Table

Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Chainsaw	3	1080	360	3.56	0.038233*	0.675356
S	16	1620	101.25			
Total (Adjusted)	19	2700				
Total	20					

\* Term significant at alpha = 0.05




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## Orthogonal Contrasts

- Example 1 -

However, suppose additional information was available. Suppose it was known that chainsaws model A & D were homeowner models and chainsaws B & C were industrial grade. Now additional comparisons can be made:

- 1) Homeowner vs. Industrial  
 $H_{02}: (\mu_A + \mu_D) = (\mu_B + \mu_C)$
- 2) Model A vs. Model D  
 $H_{03}: \mu_A = \mu_D$
- 3) Industrial model B vs. C  
 $H_{04}: \mu_B = \mu_C$




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## Orthogonal Contrasts

In essence, the investigator is able to explore a whole host of relationships that would not otherwise be addressable, and hence the power of the *a priori* approach.

Each null hypothesis is a linear combination of the treatment means. A set of linear combinations of this type is called a set of *orthogonal contrasts*.

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## Orthogonal Contrasts

A set of linear combinations must satisfy two mathematical properties in order to be orthogonal contrasts:

- 1) The sum of the coefficients in each linear contrast must sum to zero, and
- 2) The sum of the products of the corresponding coefficients in any two contrasts must equal zero.

Let's return to the example to see how this is so...

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## Orthogonal Contrasts

-Example 1-

We can re-write each of the 4 null hypotheses into linear combination form. When a treatment is equality or is not being considered it has a coefficient of zero.

When the treatment is part of the comparison, it takes a coefficient value in proportion to the number of comparisons involved.

In other words...

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## Orthogonal Contrasts

- Example 1-

$$H_{01}: (0)\mu_A + (0)\mu_B + (0)\mu_C + (0)\mu_D$$

$$H_{02}: (1/2)\mu_A - (1/2)\mu_B - (1/2)\mu_C + (1/2)\mu_D$$

$$H_{03}: (1)\mu_A + (0)\mu_B + (0)\mu_C - (1)\mu_D$$

$$H_{04}: (0)\mu_A + (1)\mu_B - (1)\mu_C + (0)\mu_D$$

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## Orthogonal Contrasts

- Example 1-

Note how coefficients are derived:

$$H_{02}: (1/2)\mu_A - (1/2)\mu_B - (1/2)\mu_C + (1/2)\mu_D$$

Originated from:

$$H_{02}: (\mu_A + \mu_D) = (\mu_B + \mu_C)$$

$$H_{02}: (\mu_A + \mu_D)/2 - (\mu_B + \mu_C)/2 = 0 \text{ or,}$$

$$H_{02}: +1/2 \mu_A + 1/2 \mu_D - 1/2 \mu_B - 1/2 \mu_C = 0$$

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## Orthogonal Contrasts

- Example 1-

Property #1: Coefficients sum to zero.

$$H_{01}: (0) + (0) + (0) + (0) = 0$$

$$H_{02}: (1/2) - (1/2) - (1/2) + (1/2) = 0$$

$$H_{03}: (1) + (0) + (0) - (1) = 0$$

$$H_{04}: (0) + (1) - (1) + (0) = 0$$

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## Orthogonal Contrasts

- Example 1-

Property #2: Sum of the products of the coefficients in pairwise comparisons = 0.

Contrast 2 vs 3:

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

Contrast 2 vs. 4:

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

Contrast 3 vs. 4:

$$(1)(0) + (0)(1) + (0)(-1) + (-1)(0) = 0$$

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## Orthogonal Contrasts

- Example 1-

A set of contrasts is orthogonal if every pair of contrasts is orthogonal. An experiment with  $A$  treatments can have several sets of mutually orthogonal contrasts, but each set is limited to  $A - 1$  possibilities.

If the experimenter can plan for the use of orthogonal contrasts at the time of experimental design, a much stronger and richer set of hypotheses can be explored.

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```
> chainsaw<-read.csv("C:/TEMPR/Chainsaw.csv")
> chainsaw
  Chainsaw Kickback
1      a           42
2      a           17
3      a           24
4      a           39
5      a           43
6      b           28
7      b           50
8      b           44
9      b           32
10     b           61
11     c           57
12     c           45
13     c           48
14     c           41
15     c           54
16     d           29
17     d           40
18     d           22
19     d           34
20     d           30
```

## Orthogonal Contrasts

- Example 1-



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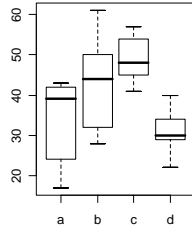
## Orthogonal Contrasts

- Example 1-

```
> summary(chainsaw)
Chainsaw Kickback
a:5      Min.   :17.00
b:5      1st Qu.:29.75
c:5      Median :40.50
d:5      Mean   :39.00
          3rd Qu.:45.75
          Max.   :61.00

> attach(chainsaw)
> boxplot(Kickback ~ Chainsaw)

> tapply(Kickback, Chainsaw, mean)
 a b c d
33 43 49 31
```



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## Orthogonal Contrasts

- Example 1-

```
> anova(lm(Kickback ~ Chainsaw))

Analysis of Variance Table

Response: Kickback
 Df Sum Sq Mean Sq F value Pr(>F)
Chainsaw  3 1080.00  360.00  3.5556 0.03823 *
Residuals 16 1620.00  101.25
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Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05
                 '.' 0.1 ' ' 1
```

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## Orthogonal Contrasts

- Example 1-

```
> contrasts(Chainsaw)<-
+   cbind(c(1,-1,-1,1),c(1,0,
+   0,-1),c(0,1,-1,0))

> contrasts(Chainsaw)
 [,1] [,2] [,3]
a    1    1    0
b   -1    0    1
c   -1    0   -1
d    1   -1    0
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```

> A2<-aov(Kickback~Chainsaw)
> summary.lm(A2)

Call:
aov(formula = Kickback ~ Chainsaw)

Residuals:
    Min       1Q   Median       3Q      Max
-16.00  -8.25   0.00   7.25  18.00

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  39.000     2.250  17.333 8.58e-12 ***
Chainsaw1    -7.000     2.250  -3.111 0.00672 ***
Chainsaw2     1.000     3.182   0.314 0.75738
Chainsaw3    -3.000     3.182  -0.943 0.35980
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 10.06 on 16 degrees of freedom
Multiple R-Squared:  0.4,    Adjusted R-squared: 0.2875
F-statistic: 3.556 on 3 and 16 DF,  p-value: 0.03823

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## Orthogonal Contrasts

- Example 2 -

To demonstrate the outcome, let's look at one more example (briefly).

Suppose 5 insecticides are being evaluated for their efficacy of protecting nursery grown tree seedlings from fungus and insects.

The 5 insecticides all contain the same base compound; then one of two insecticide compounds is added (A or B); in addition, an anti-fungal agent is either added or not.

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## Orthogonal Contrasts

- Example 2 -

Treatment	Additive
I	None (control; base only)
II	Compound A
III	Compound B
IV	Compound A + anti-fungal
V	Compound B + anti-fungal

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## Orthogonal Contrasts

- Example 2 -  
Resulting ANOVA Table

Source	df	SS	MS	F	P
Among Insecticides	4	136.8	34.2	39.8	0.01
Additive vs No Additive	1	2.8	2.8	3.3	NS
Compound A vs Compound B	1	116.6	116.6	135.6	0.001
Compound A vs Compound A + AF	1	14.9	14.9	17.4	0.05
Compound B vs Compound B + AF	1	2.5	2.5	2.9	NS
Within Insecticides	15	13.0	0.86		

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## ANOVA Models

The appeal of the basic ANOVA model is that it can be applied to many different experimental situations.

Invariably, when flexibility is present, confusion seems to proliferate because there are numerous decisions that need to be made by the experimenter.

One of these assumptions has to do with the nature of the effects--are they fixed or random?

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## Fixed Effects Model

The basic examples we have done so far are collectively referred to as "Fixed Effects Models" (FEM) or "Type-I Models".

In this type of linear model, it is assumed that the experimenter has narrowed down the treatment choices to a specific set.

The model is "fixed" because if another researcher were to repeat the experiment, s/he would use the *exact* same treatments again.

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## Random Effects Model

Recognize that there is an entirely different set of models referred to as "Random Effects Models" (REM) or "Type-II Models."

This type of model is less interested in differences among group means of the treatments and more interested in variability among treatment groups.

Here, the treatments are a *random sample* of the treatments being tested.

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## Model Effects

- Examples-

The chainsaw example (4 brands: A,B,C,D) was a FEM because if the experiment were repeated, the same 4 chainsaws would need to be used (although the experiment could have been designed differently to be a REM).

Often in ecology & evolutionary biology, one includes "individuals" or "populations" as a main effect in the ANOVA model. These are REMs because if repeated, another researcher would use different individuals or populations of the same species.

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## Random Effects Model

When the REM is utilized, the investigator is interested in  $\sigma^2_A$  (the variance among all possible treatment groups).

The ANOVA is used to test  $H_0: \sigma^2_A = 0$

If  $H_0$  is rejected, there is evidence of variability among groups. *The inference applies to all individuals or populations of the species, NOT just the particular organisms sampled.*

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## FEM vs REM

Note that the major difference between the FEM and REM has to do with what the  $MS_A$  is estimating:

FEM:  $MS_a$  estimates  $\sigma^2 + N\Sigma\alpha_i^2 / (A-1)$

REM:  $MS_a$  estimates  $\sigma^2 + N \sigma^2_A$

$MS_e$  estimates  $\sigma^2$  for both FEM and REM

Fortunately, the numerical procedures for the calculation of fixed and random effects is the same; just the assumptions differ a bit.

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## FEM vs REM

One of the major differences between FEM and REM has to do with the follow-up procedures.

For the FEM we use standard MCPs, Orthogonal Contrasts, or advanced methods of linear estimation (not discussed).

For REM we are interested in the intraclass correlation, an estimate of the total variance that is due to the differences among the treatments.

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## REM & Intraclass Correlation

- Example -

Suppose a plant geneticist is interested in the heritability of resistance to fungal pathogens.

He selects 30 plants and takes 2 cuttings of each (propagates 2 identical genotypes of each plant). Each of the plants is exposed to a fungal pathogen and after 4 weeks of growth each plant is measured for biomass.

Here, the  $A = 30$  pairs of cuttings are the treatment groups. Each group has  $N = 2$ . This is a REM.

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## REM & Intraclass Correlation

- Example -  
Resulting ANOVA Table

Source	df	SS	MS	F
Among Pairs	29	25921	894	4.43
Within Pairs	30	6050	202	

Since  $F_{0.05,29,30} = 1.847$ , reject  $H_0$ , there is sig. variability among pairs, evidence of genetic heritability.

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## REM & Intraclass Correlation

- Example -

Now, since  $MS_A$  estimates  $\sigma^2 + N\sigma_A^2$  and  $MS_E$  estimates  $\sigma^2$ , the investigator computes the intraclass  $r_i$  as:

$$\hat{\sigma}^2 = MS_E = 202$$

$$\hat{\sigma}_A^2 = \frac{MS_A - MS_E}{N} = \frac{894 - 202}{2}$$

$$r_i = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_A^2 + \hat{\sigma}^2} = \frac{346}{346 + 202} = 0.631$$

In other words, 63.1% of the variance is due to differences among the pairs of cuttings.

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## REM & Intraclass Correlation

Note that by definition,  $r_i$  can only range from 0 to 1.

When  $r_i$  is calculated, the investigator is interested in the percentage of variance due to the treatments.

The specific percentage that is meaningful is dependent upon the experiment. Sometimes the experimenter may want  $r_i$  to be large, other times small.

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## ANOVA Models

Recognize that there are many situations in which the REM should be used. This recognition becomes more important with subsequent more complex models.

We must also be aware of what the MS estimates in order to determine what is a valid F-test.

The value or linear combination of values estimated by the MS is called the Expected Mean Square or EMS.

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## Assumptions of ANOVA

The assumptions for analyzing one-way classification data are simply a straightforward extension of what we have already done when examining two-sample data for the  $t$ -test.

Generally speaking, the ANOVA  $F$ -test is not very sensitive to minor violations of the normality assumption. The test is also moderately insensitive to minor violations of the homogeneous variance assumption, provided the sample sizes are equal and not too small.

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## Assumptions of ANOVA

Just like regression, the assumptions for ANOVA can most easily be tested by examining the residuals.

$$Y_{ij} = \mu + \tau_i + E_{ij} \quad \text{where } i = 1 \dots n_i \quad j = 1 \dots \tau$$

where  $E_{ij}$  is the random error

The residual  $e_{ij}$  of the  $j$ -th observation for the  $i$ -th treatment is

$$e_{ij} = Y_{ij} - \bar{Y}_{i+}$$

$$\text{and } \bar{Y}_{i+} = \mu + \tau_i + \bar{E}_{i+}$$

subtracting...

$$e_{ij} = E_{ij} - \bar{E}_{i+}$$

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## Assumptions of ANOVA

Thus, one can examine the  $e_{ij}$  values using the same procedures as regression.

The best place to start is a histogram, normal probability plot, and tests of normality (same procedures we have already examined) using the residuals.

Equality of variance can be examined graphically with a residuals plot, followed by an explicit test of homogeneity: (1) Hartley's F-max, (2) Bartlett's test, (3) Scheffé-Box test, or (4) Modified Levene test.

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## Tests for Homogeneity of Variance

Hartley's F-max test was examined when discussing the two-sample  $t$ -test. It is simply the max variance divided by the min variance.

Bartlett's test is computationally a bit more prolonged. A nice description and worked example can be found in Zar (1999; Example 10.13, p. 203).

Both Hartley's and Bartlett's are sensitive to departures from normality, so this needs to be determined first. Further, Hartley's test requires equal sample sizes.

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## Bartlett's Tests for Homogeneity of Variance

```
> tapply(Kickback, Chainsaw, var)
  a      b      c      d
138.5 180.0  42.5  44.0
```

```
> bartlett.test(Kickback ~ Chainsaw)
```

```
      Bartlett test of homogeneity of variances
```

```
data: Kickback by Chainsaw
Bartlett's K-squared = 2.9447, df = 3, p-value
= 0.4002
```

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## Tests for Homogeneity of Variance

The Scheffé-Box test is less sensitive to departures from normality and can be used for unequal sample sizes, but requires the data to be acquired in a stratified group fashion.

Perhaps the best overall test (because of its insensitivity to sample size and normality) to examine the homogeneity of variance assumption is the Modified Levene Equal Variance test. Here, all variates are redefined by subtracting the median of each subgroup and running a one-way ANOVA on these redefined variates. If you fail to reject the null hypothesis, conclude that variances are equal. This is the test employed by NCSS.

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## Kruskal-Wallis ANOVA

If the data are non-normally distributed or the variances are heterogeneous, and neither or both can be corrected via transformation or outlier manipulation, then a nonparametric option exists using ranked data.

The Kruskal-Wallis tests statistic H is calculated as:

$$H = \frac{n \left[ \sum_i \left( \bar{r}_i - \frac{N+1}{2} \right)^2 \right]}{N(N+1)/12}$$

H is then compared to a chi-square table to determine significance at a specified alpha and df.

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## Kruskal-Wallis ANOVA

-Example-

Let's return to our original chainsaw example and assume that the data were either non-normally distributed or had heterogeneous variances:

- 1) transform the data in to ranks
- 2) determine the SS among groups for rank data
- 3) determine H using the previous equation
- 4) determine significance via chi-square table

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Model:	A		B		C		D	
	Deg	Rnk	Deg	Rnk	Deg	Rnk	Deg	Rnk
	42	12	28	4	57	19	29	5
	17	1	50	17	45	15	40	10
	24	3	44	14	48	16	22	2
	39	9	32	7	41	11	34	8
	43	13	61	20	54	18	30	6
<b>Sum R</b>	<b>38</b>		<b>62</b>		<b>79</b>		<b>31</b>	
<b>Mean <math>r_i</math></b>	<b>7.6</b>		<b>12.4</b>		<b>15.8</b>		<b>6.2</b>	




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**Kruskal-Wallis ANOVA**  
-Example-

Step 3:  $H = 293.0 / 35.0 = 8.371$

Step 4:  $\chi^2_{0.05,3} = 7.815$

$H_{calc} > \chi^2_{table}$  therefore reject  $H_0$  (same result as with ANOVA)

There is also a post-hoc MCP that can be applied to differentiate among groups...

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**Kruskal-Wallis ANOVA**  
-Example-

```
> kruskal.test(Kickback ~ Chainsaw)

Kruskal-Wallis rank sum test

data: Kickback by Chainsaw
Kruskal-Wallis chi-squared = 8.3714,
df = 3, p-value = 0.03893
```

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## Kruskal-Wallis ANOVA

$Z_{ij}$  is determined and applied just as you would a Fisher's LSD following an ANOVA:

$$Z_{ij} = \frac{\left| \frac{R_i}{n_i} - \frac{R_j}{n_j} \right|}{\sqrt{\frac{N(N+1)}{12} \left( \frac{1}{n_i} + \frac{1}{n_j} \right)}}$$

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*Stay Tuned  
More ANOVA Designs to Follow...*

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