# Unit 6
# Introduction to Analysis of Variance

*"Always graph results of an analysis of variance"*
*- Gerald van Belle.*

**Analysis of variance is just a special case of normal theory regression.** The response variable is continuous and assumed distributed normal, (same as what we learned in Unit 5 – Regression. In an analysis of variance, however, *all* the predictor variables are categorical variables called *factors.* The possible values of the categorical predictors are called *levels*.

| | |
|---|---|
| One-way analysis of variance: | 1 categorical predictor with multiple levels |
| Two-sample t-test: | One-way analysis of variance with 2 levels |
| Two-way analysis of variance: | 2 categorical predictors (factors), regardless of the # levels |
| Three-way analysis of variance: | 3 categorical predictors/factors . *And so on.* |

**So why the fuss?** If an analysis of variance model is just multiple predictor linear regression model with discrete predictors only and no continuous predictors, then why do we need to distinguish this setting as a special case? There's a reason. The framework of analysis of variance works wonderfully for the analysis of many experimental designs.

In a **factorial design**, there are observations at every combination of levels of the factors (e.g., for sex at birth at 2 levels and political affiliation at 3 levels for a total of 2 x 3 = 6 groups, the data include observations for each combination of sex at birth and political affiliation). Factorial designs are good for exploring *interactions (effect modification)* between factors. An interaction between factor I and factor II is said to exist when the response to factor II depends on the level of factor I and vice versa.

In a **nested or hierarchical design**, such as a two-level nested design, the analysis is of units (e.g., patients) that are clustered by level of factor I (e.g., hospital) which are in turn clustered by level of factor II (e.g., city). Nested designs are good for controlling for *confounding*.

A special type of nested design is the **longitudinal or repeated measurements design**. Repeated measurements are clustered within subjects and the repeated measurements are made over a meaningful dimension such as time (e.g., growth over time in children) or space. The analysis of repeated measurements data is introduced elsewhere.

Unit 6 is an introduction to analysis of variance.

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# Learning Objectives

When you have finished this unit, you should be able to:

- **Explain how** *analysis of variance is a special case of normal theory linear regression***.**
- **Perform and interpret a** *one way analysis of variance***.**
- **Explain what is meant by a** *multi-way* **analysis of variance.**
- **Explain what is meant by a** *factorial design* **analysis of variance.**
- **Explain the meaning of** *interaction* **of two factors.**
- **Explain what is meant by a** *nested design* **analysis of variance.**
- **Perform and interpret a** *two-way factorial analysis of variance***, including an** *assessment of interaction***.**

## Introduction to Annoying Notation!
## Your Roadmap for Keeping Track

- With apologies, we move from Y to X:
  **Whereas previously, outcomes are represented using the notation Y**
  **In analysis of variance, often, outcomes are represented using the notation X**

- Keeping track of subscripts:
  **Subscripts are needed to identify group and individual**

  **In the ONE WAY anova ($X_{ij}$)**
  - **"i" tells you the group, the level of the ONE categorical predictor (also called factor); (e.g., i=1 for "male sex at birth")**
  - **"j" tells you the individual within that group ( e.g., j=3 indexes "Robert")**

  **In the TWO WAY anova ($X_{ijk}$)**
  - **"i" tells you the level of the FIRST categorical predictor (also called Factor I)**
  - **"j" tells you the level of the SECOND categorical predictor (also called Factor II)**
  - **"k" tells you the individual within the two way classification of groups**

- Subscripts that are DOTS are telling you that you are looking at a total
  **For example:**

  $X_{ij.}$ = **sum of all the observations solely for the group defined by factor I at level=i, factor II at level=j; hence, no "k" subscript**

  $X_{i..}$ = **sum of all the observations for the group defined by factor I at level=i, So this is the sum taken over all levels of factor II (hence no "k" and no "j" subscript)**

  $X_{...}$ = **sum of all the observations (hence no "k" and no "j" and no "i" subscripts)**

- There are a lot of variances floating around and these now have subscripts too. Mostly. Your key:

  $S^2$ = **Sample variance of the entire pool all the observations (with no regard to group)**

  $S_i^2$ = **Sample variance of ONLY the observations in the group defined by the categorical predictor at level = "i"**

- **And those pesky bars on top**: BARS ON TOP are telling you that you are looking at an **average**

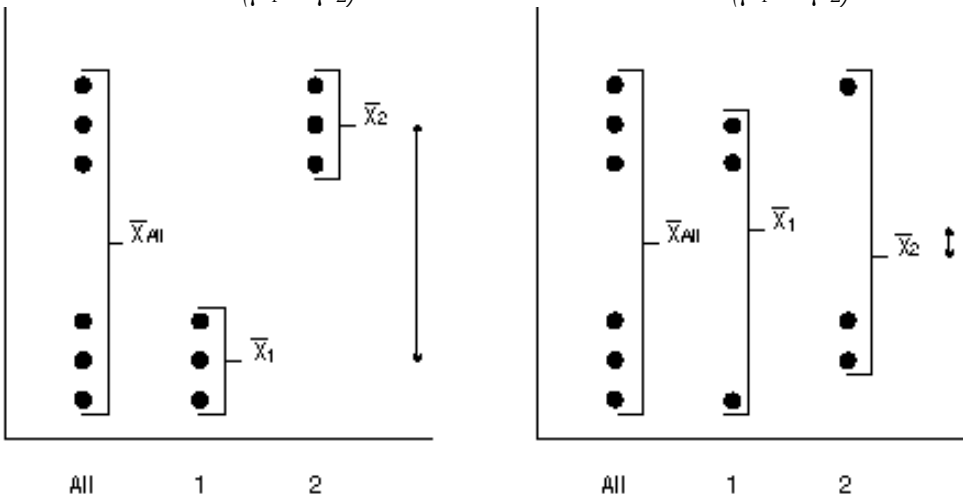
## 1. The Logic of Analysis of Variance

### Analysis of Variance might seem like a misnomer, but it's not...

- Analysis of variance, like normal theory regression introduced in Unit 5, is the modeling and analysis of means. We ask: is the variability among the group level means statistically significantly greater than the variability of individuals within a group? Think "signal-to-noise".

- Consider the following scenarios.

Scenario 1 ($\mu_1 \neq \mu_2$) Scenario 2 ($\mu_1 = \mu_2$)




| **Scenario 1**<br>**means are *different***<br>$\mu_1 \neq \mu_2$<br>variability of means > variability within groups | **Scenario 2**<br>**means are the *same***<br>$\mu_1 = \mu_2$<br>variability of means ~ variability within groups |
|---|---|
| $S_1^2$ and $S_2^2$ each summarize "noise" controlling for location. | $S_1^2$ and $S_2^2$ each summarize "noise" controlling for location. |
| The size of $\lvert\bar{X}_1 - \bar{X}_2\rvert$ is larger than "noise" | $\lvert\bar{X}_1 - \bar{X}_2\rvert$ is within the neighborhood of "noise". |
| $S^2$ is <u>larger</u> than $S_1^2$ and $S_2^2$ because it is made larger by the extra variability among individuals due to change in location. | $S^2$ is <u>similar</u> in size to $S_1^2$ and $S_2^2$ because it does not have to accommodate an extra source of variability because of location differences between the two groups. |

When the sample size in each group is the same (and equal to n in each group), it's easier to see how analysis of variance is an analysis of the *variability of the means*. **When the samples sizes are not equal, the algebra is not so tidy.**

## Example - Stress and Heart Rate (…. Or all things friends and pets! …)

Source: Gerstman BB. Basic Biostatistics: Statistics for Public Health Practice, pp 259-262. The data used by Gerstman are from Allen et al (1991) Presence of human friends and pet dogs as moderators of autonomic responses to stress in women. *J. Personality and Social Psychology* 61(4); 582-589.

Does the companionship of a pet provide psychological relief to its owners when they are experiencing stress? In an experiment to address this question, consenting participants were randomized to one of three conditions: 1- Pet Present, 2-Friend Present, or 3-Neither friend nor pet present. Each participant was then exposed to a stressor (it happened to be mental arithmetic). The response variable is X = heart rate.

Selected Summary Statistics, by Group:

| | Group 1<br>Pet Present | Group 2<br>Friend Present | Group 3<br>Neither Pet nor Friend |
|---|---|---|---|
| **n** | $n_1 = 15$ | $n_2 = 15$ | $n_3 = 15$ |
| $\bar{X}$ | $\bar{X}_1 = 73.48$ | $\bar{X}_2 = 91.33$ | $\bar{X}_3 = 82.52$ |
| **S** | $S_1 = 9.97$ | $S_2 = 8.34$ | $S_3 = 9.24$ |
| $S^2$ | $S_1^2 = 99.40$ | $S_2^2 = 69.57$ | $S_3^2 = 85.41$ |
| $(n-1)S^2$<br>$= \sum(X-\bar{X})^2$ | $(n_1-1)S_1^2 = \sum_{j=1}^{n_1}(X_{1j}-\bar{X}_1)^2$<br>$= 1{,}391.57$ | $(n_2-1)S_2^2 = \sum_{j=1}^{n_2}(X_{2j}-\bar{X}_2)^2$<br>$= 974.05$ | $(n_3-1)S_3^2 = \sum_{j=1}^{n_3}(X_{3j}-\bar{X}_3)^2$<br>$= 1{,}195.70$ |

### Analysis of Variance Question:

Do these data provided statistically significant evidence that the means of the stress scores (group 1 v 2 v 3) differ by group? (ie – that $\mu_1$, $\mu_2$, and $\mu_3$ are not all equal to each other)?

## The Reasoning in an Analysis of Variance

*Proof by Contradiction*
*Signal-to-Noise*

The intuition of analysis of variance modeling is most easily appreciated in a one way fixed effects analysis of variance where the sample size in each group is the same.

1. *As we always do in statistical hypothesis testing .. We begin by provisionally entertaining as true the null hypothesis. Here, null hypothesis $H_O$: the means are equal.*

   - We assume the null hypothesis is true, then apply this model to the observed data. We then look to see if its application has led to an unlikely result. If so, this warrants rejection of the null hypothesis of equality of $\mu_1$, $\mu_2$, and $\mu_3$. And we conclude that we have statistically significant evidence that at least one mean is different from the others.

2. *Because analysis of variance is a special case of normal theory regression, the assumptions are the same. We need these assumptions for computing p-values and constructing confidence intervals.*

   Assumptions for a One Way Fixed Effects Analysis of Variance:
   - $X_{11} \cdots X_{1n1}$ are distributed Normal ( $\mu_1$ , $\sigma^2$ )
   - $X_{21} \cdots X_{2n2}$ are distributed Normal ( $\mu_2$ , $\sigma^2$ )
   - $X_{31} \cdots X_{3n3}$ are distributed Normal ( $\mu_3$ , $\sigma^2$ )
   - The variances are all equal to $\sigma^2$.
   - The observations are all independent.

3. *Specify $H_O$ and $H_A$.*

   $H_O$: $\mu_1 = \mu_2 = \mu_3$
   $H_A$: not

4. *"Reason" an appropriate test statistic* ***(Signal-to-Noise).***

**NOISE:**

**"Within Group Variability = Noise"** **(Answers: What is the variability of individuals about their own group means?)**

- In each of groups 1, 2, and 3 we obtain the separate, group-specific, $S_1^2$, $S_2^2$, and $S_3^2$
- *Under the assumption of a common* $\sigma^2$, each of $S_1^2$, $S_2^2$, and $S_3^2$ is an estimate of the same common $\sigma^2$.
- So we combine the 3 separate estimates of the common variance $\sigma^2$ into a single (better) guess of the common variance $\sigma^2$. To do this, we compute a weighted average. The weights are the degrees of freedom of each of $S_1^2$, $S_2^2$, and $S_3^2$

*Noise* is the *within group variability* and estimates the variability among individuals, controlling for location.

$$\text{Estimate of } \sigma^2 = \hat{\sigma}^2_{within} = \frac{\sum_{i=1}^{3}(n_i - 1)S_i^2}{\sum_{i=1}^{3}(n_i - 1)}$$

Nice. The expected value of this estimate is the assumed common error variance

$$E\left[\frac{\sum_{i=1}^{3}(n_i - 1)S_i^2}{\sum_{i=1}^{3}(n_i - 1)}\right] = E\left[\hat{\sigma}^2_{within}\right] = \sigma^2$$

**SIGNAL:**

**"Between Group Variability = Signal"** **(Answers: What is the variability among the group means themselves?)**

An appealing (because it is so intuitive) strategy would be to calculate the sample variance of the group specific means $\bar{X}_1, \bar{X}_2$ and $\bar{X}_3$. What we actually do is a slight modification of this intuition. The modification takes into account group specific sample sizes. Specifically:

- We construct a *special* little data set with sample of size = 3, defined:

$$X_1^* = \sqrt{n_1}\bar{X}_1 = \sqrt{15}(73.4831) = 284.59882$$
$$X_2^* = \sqrt{n_2}\bar{X}_2 = \sqrt{15}(91.3251) = 353.70059$$
$$X_3^* = \sqrt{n_3}\bar{X}_3 = \sqrt{15}(82.5241) = 319.61446$$

The sample mean of these "special data set" means is $\bar{X}^* = 319.3046$

$\sigma^2_{BETWEEN}$

**The** expected value **of the sample variance of our "starry" group means, $X_1^*$, $X_2^*$, and $X_3^*$ is** $\sigma^2_{BETWEEN}$**:**

$$E\left[\frac{\sum_{i=1}^{3}\left(X_i^* - \bar{X}^*\right)^2}{(3-1)}\right] = \sigma^2_{between}$$

*The expected value of the variability of the "starry" group means will be smaller or larger depending on how different the true means are, that is, depending on whether the null is true or not.*

**Null true:** When the null hypothesis $H_O$ is true (the means are equal), *and only when $H_O$ is true*

$$E\left[\frac{\sum_{i=1}^{3}\left(X_i^* - \bar{X}^*\right)^2}{(3-1)}\right] = \sigma^2_{between} = \sigma^2$$

**Alternative true:** Otherwise (when the alternative hypothesis $H_A$ is true), the sample variance of $X_1^*$, $X_2^*$, and $X_3^*$ is an estimate of a quantity ($\sigma^2_{between}$) that is larger than $\sigma^2$.

$$E\left[\frac{\sum_{i=1}^{3}\left(X_i^* - \bar{X}^*\right)^2}{(3-1)}\right] = \sigma^2_{between} = \sigma^2 + \Delta \quad \text{where}$$

$\Delta$ is a measure of the "differentness" of the means, positive!

$$\Delta = function\ (\mu_1, \mu_2, \mu_3) > 0$$

Thus, the "signal" is related to the group specific means and, in particular, how different they are:

$$\Delta = function(\mu_1, \mu_2, \mu_3) > 0$$

*In case you are interested*: In a one-way analysis of # groups = K and equal sample size = n in each group:

$$\Delta = \frac{n\sum_{i=1}^{K}(\mu_i - \bar{\mu})^2}{K-1}$$

## SIGNAL – to - NOISE:

Just as we have learned for statistical hypothesis tests in 1 and 2 sample inference, our test statistic is a "signal-to-Noise" comparison that compares the *between* group means variability to the *within* groups variability. Here, it is defined as follows

$$\frac{\text{Noise + Signal}}{\text{Noise}} = \frac{\text{Variability among a function of the group means}}{\text{Variability of individuals within groups}}$$

$$= \frac{\text{Var}(\bar{X}_1^*, \bar{X}_2^*, \bar{X}_3^*)}{\text{" weighted (using df) sum of } S_1^2, S_2^2, \text{ and } S_3^2}$$

$$= \left[\frac{\hat{\sigma}^2_{between}}{\hat{\sigma}^2_{within}}\right]$$

$$= \frac{\left[\sum_{i=1}^{3}\left(\bar{X}_i^* - \bar{\bar{X}}^*\right)^2 / (3-1)\right]}{\left[\left\{\sum_{i=1}^{3}(n_i-1)S_i^2\right\} \Big/ \left\{\sum_{i=1}^{3}(n_i-1)\right\}\right]}$$

### 5. *Perform the calculations.*

Using the values in the table on page 6, we have

$$\hat{\sigma}^2_{within} = \frac{\sum_{i=1}^{3}(n_i-1)S_i^2}{\sum_{i=1}^{3}(n_i-1)} = \frac{(1391.57+974.05+1195.70)}{(14+14+14)}$$

$$= 84.79$$

Using the values of our starry means $\bar{X}_i^*$ on page 8, we also have

$$\hat{\sigma}^2_{between} = \frac{\sum_{i=1}^{3}\left(\bar{X}_i^* - \bar{\bar{X}}^*\right)^2}{(3-1)} = 1{,}193.836$$

**Overall F Test.** We use the F distribution to compare the variability of the means to the variability of individuals about their own means.

- Luckily, these two quantities are independent. When the null hypothesis $H_O$ is true:
- Overall $F = \dfrac{\left[\hat{\sigma}^2_{between}/\sigma^2\right]}{\left[\hat{\sigma}^2_{within}/\sigma^2\right]}$ is distributed F with

Numerator degrees of freedom = $(k - 1) = (3-1) = 2$
Denominator degrees of freedom = $\sum (n_i - 1) = (3)(15-1) = 42$

| | Expected Value of | | | | |
|---|---|---|---|---|---|
| | $\hat{\sigma}^2_{within}$ | $\hat{\sigma}^2_{between}$ | $\hat{\sigma}^2_{between}/\hat{\sigma}^2_{within}$ | $F$ | p-value |
| $H_O$ true (means equal) | $\sigma^2$ | $\sigma^2$ | $1$ | $1$ | Large |
| $H_A$ true (means NOT equal) | $\sigma^2$ | $\sigma^2 + \Delta$ | $>1$ | $>1$ | Small |
| | | | | | |

For our data, F = 1193.836 / 84.79 = 14.08
The accompanying p-value is Probability[ $F_{df=2,42} \geq 14.08$ ] = .00002.

***6. "Evaluate" findings and report.***

The assumption of the null hypothesis of equal means has led to an *extremely unlikely* result! The null hypothesis chances were approximately, 2 chances in 100,000 of obtaining 3 means of groups that are as different from each other as are 73.48, 91.33, and 82.52. The null hypothesis is rejected.

***7. Interpret in the context of biological relevance.***

This analysis provides statistically significant evidence of group differences in heart rate, depending on companionship by pet or by friend. But we do not know which, or if both, provides the benefit!! Okay then! I think we should all have lots of friends and lots of pets!

## 2. Introduction to Analysis of Variance Modeling

***Preliminary note*** - See again page 4. In the pages that follow, I am using the notation X to refer to the *outcome variable* in analysis of variance. Previously, in unit 5, I used the notation Y (Sorry for the annoyance!)

Analysis of variance models, like regression models, have an identifiable basic structure.

**Structure of Analysis of Variance Model**
**Observed = mean + random error**

$$X = \mu + \varepsilon$$

Observed — Expected is modeled — Random error

- $\mu$ - This is the (model) expected value of X which we write as $\mu = E[X]$ = linear model(stuff)
- $\varepsilon$ - This is the idea of "random error", "error in measurement", "noise"
- Subscripts –Subscripts keep track of group membership and persons within groups.
  *For example*: $X_{ij}$ = Observed value for $j^{th}$ person in the $i^{th}$ group.

A special feature of analysis of variance models is their use of subscripts.

**Example – One way fixed effects analysis of variance.**

**Structure of One-Way Fixed Effects Analysis of Variance Model**
**Observed = mean + random error**

Subscripts:
"i" identifies the level of the one predictor variable (factor).
"j" identifies the individual within the group defined by level.

$$X_{ij} = \mu_i + \varepsilon_{ij}$$

| Observed data value for "$j^{th}$ person in $i^{th}$ group/level | Expected value is the mean of $i^{th}$ group/level | Random error for "$j^{th}$" observation of the $i^{th}$ level group mean. |
|---|---|---|

## Definition
## The One Way Fixed Effects Anova Model

In a one way fixed effects analysis of variance (anova) model, $E(X_{ij}) = \mu_i$ *is completely general.* The factor level group means can be anything. We are not modeling them as lying on a line or lying on any sort of functional form for that matter (e.g., polynomial). Recall. The notation E( ) is referring to the modelled expected value.

**Example – Heart rate and stress, *continued* -**
In this analysis, the null hypothesis was that the means are all the same. The alternative hypothesis said simply "the means are not all the same"

$H_O$: $\mu_1 = \mu_2 = \mu_3$
$H_A$: At least one is different .

- More generally, suppose the number of groups = K, instead of 3 in the heart rate example.

- KEY to subscripts for $X_{ij}$ in a one way fixed effects anova.

  **Subscript "i"** (identifies group)
  The first subscript will be "i" and will index the groups i=1 , …, K.

  **Subscript "j"** (identifies individual within the group)
  The second subscript will be "j" and will index the jth individual in the ith group j = 1 , … , $n_i$.

  $n_{i.}$ = sample size in the ith group

Let

$\mu_i$ = mean for persons in the subpopulation that is the $i^{th}$ group
$\mu$ = overall mean, over the entire population, (that is over all subpopulations)

**Deviation from means model**. This is a nifty *re-write* that rewrites $\mu_i$ as a new expression that is equal to itself. This is done by adding and subtracting $\mu$ to $\mu_i$.

$$\mu_i = \mu + (\mu_i - \mu)$$

mean for group "i" — overall mean — deviation from mean specific to $i^{th}$ group

Same nifty trick to obtain a rewrite of the observed $X_{ij}$. Notice (below) that we are adding and subtracting two things this time:

$$X_{ij} = \bar{X} + \left(\bar{X}_i - \bar{X}\right) + \left(X_{ij} - \bar{X}_i\right)$$

One more nifty maneuver lets us express the variability of individuals about the overall mean as the sum of two contributions. This is useful for analysis purposes.

$$\left(X_{ij} - \bar{X}\right) = \left(\bar{X}_i - \bar{X}\right) + \left(X_{ij} - \bar{X}_i\right)$$

Each source (between or within) contributes its own share to the total variability via the following (wonderful) result.

$$\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}\right)^2 = \sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(\bar{X}_i - \bar{X}\right)^2 + \sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}_i\right)^2$$

total variability — variability of means "Between groups" — variability of individuals "Within groups"

We keep track of all this in an analysis of variance table.

| Source | df[a] | Sum of Squares | Mean Square | Variance Ratio = F |
|---|---|---|---|---|
| Between groups (due model) | ( K-1 ) | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(\bar{X}_i - \bar{X}\right)^2$ | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(\bar{X}_i - \bar{X}\right)^2 \Big/ (K-1)$ = $\hat{\sigma}^2_{between}$ | $F = \dfrac{\hat{\sigma}^2_{between}}{\hat{\sigma}^2_{within}}$ |
| Within Groups (due error/residual) | $\sum_{i=1}^{K}(n_i - 1)$ | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}_i\right)^2$ | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}_i\right)^2 \Big/ \sum_{i=1}^{K}(n_i - 1)$ = $\hat{\sigma}^2_{within}$ | |
| Total | N – 1 | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}\right)^2$ | | |

[a] degrees of freedom

Note: Just to be clear:

N = grand total sample size, taken over all groups = $\sum_{i=1}^{K} n_i$

## Example – continued from page 5: Stress and Heart Rate

*(Source: Gerstman BB. Basic Biostatistics: Statistics for Public Health Practice, pp 259-262).*
The data used by Gerstman are from Allen et al (1991) Presence of human friends and pet dogs as moderators of autonomic responses to stress in women. *J. Personality and Social Psychology* 61(4); 582-589.

Data

| Treatment | | |
|---|---|---|
| **1 = Pet Present** | **2 = Friend Present** | **3 = Neither Pet, Nor Friend** |
| 69.17 | 99.69 | 84.74 |
| 68.86 | 91.35 | 87.23 |
| 70.17 | 83.40 | 84.88 |
| 64.17 | 100.88 | 80.37 |
| 58.69 | 102.15 | 91.75 |
| 79.66 | 89.82 | 87.45 |
| 69.23 | 80.28 | 87.78 |
| 75.98 | 98.20 | 73.28 |
| 86.45 | 101.06 | 84.52 |
| 97.54 | 76.91 | 77.80 |
| 85.00 | 97.05 | 70.88 |
| 69.54 | 88.02 | 90.02 |
| 70.08 | 81.60 | 99.05 |
| 72.26 | 86.98 | 75.48 |
| 65.45 | 92.49 | 62.65 |

Analysis of Variance Table

| Source | df[a] | Sum of Squares | Mean Square | Variance Ratio = F |
|---|---|---|---|---|
| Between groups | 2 | 2387.69 | $\hat{\sigma}^2_{between} = 1193.84$ | $F = \frac{\hat{\sigma}^2_{between}}{\hat{\sigma}^2_{within}} = 14.08$ |
| Within Groups | 42 | 3561.30 | $\hat{\sigma}^2_{within} = 84.79$ | |
| Total | 44 | 5948.99 | | |

[a] degrees of freedom

## R Illustration

**Descriptives by group.**

```
library(FSA)                                    #    Summarize( ) in package {FSA} provides a nice layout for anova
load(file="pets.Rdata")


pets$group <- as.factor(pets$group)                                                  # KEY: YVAR ~ GROUPVAR (must be factor)
FSA::Summarize(hrt_rate~group,data=pets,digits=2,na.rm=TRUE)
##                    group  n  mean   sd   min    Q1 median    Q3    max
## 1            Pet Present 15 73.48 9.97 58.69 69.02  70.08 77.82  97.54
## 2         Friend Present 15 91.33 8.34 76.91 85.19  91.35 98.95 102.20
## 3 Neither Friend nor Pet 15 82.52 9.24 62.65 76.64  84.74 87.62  99.05
```

**One-Way ANOVA**

```
aov1 <- aov(hrt_rate ~ group, data=pets)                                              # KEY:  aov(YVAR ~ GROUPVAR, data= )
anova(aov1)
## Analysis of Variance Table
##
## Response: hrt_rate
##           Df Sum Sq Mean Sq F value     Pr(>F)
## group      2 2387.7 1193.84  14.079 0.00002092 ***
## Residuals 42 3561.3   84.79
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

*MATCH!* Hand calculations and R results match. Phew.

## 3. The One Way Fixed Effects Analysis of Variance

*Not sure what "fixed" means?*

Stay tuned. Fixed versus Random Effects are Introduced in Section 5b

**The two independent samples t-test is a One Way Fixed Effects Anova with # groups = K = 2.** The stress and heart rate example (see again, page 6) is a one way fixed effects analysis of variance with K=3. A two sample t-test is also one way analysis of variance; K=2. *If you square the value of your t-test, the result is the value of the anova F-test.*

| **2 independent groups t-test**<br>The T-Test "Lense" | **2 independent groups t-test**<br>The One-Way Anova "Lense" |
|---|---|
| Is the "signal" $(\bar{X}_1 - \bar{X}_2)$ large relative to "noise" where "noise" = SE$(\bar{X}_1 - \bar{X}_2)$? | Is the variability of $(\bar{X}_1, \bar{X}_2)$ large relative to "noise" where "noise" = weighted average of $S_1^2, S_2^2$ |
| $t = \dfrac{(\bar{X}_1 - \bar{X}_2)}{SE(\bar{X}_1 - \bar{X}_2)}$ | $F = \dfrac{\text{function of variability of data } \bar{X}_1, \bar{X}_2}{\text{function of variability of "noise" } S_1^2, S_2^2}$ |
| = measure of distance of $(\bar{X}_1 - \bar{X}_2)$ from 0, in units of SE. | = measure of variability among $\bar{X}_1, \bar{X}_2$ ("signal") compared to to variability of individuals within groups ("noise") |

Assumptions are those of Normal Theory Linear Regression

1. *Normality*. The observed outcomes are random draws from conditional Normal probability distributions.

$$\text{Group 1: } X_{11} \ldots X_{1n_1} \text{ are a simple random sample from a Normal}(\mu_1, \sigma^2)$$
$$\text{Group 2: } X_{21} \ldots X_{2n_2} \text{ are a simple random sample from a Normal}(\mu_2, \sigma^2)$$
$$\textbf{Etc.}$$
$$\text{Group K: } X_{K1} \ldots X_{1n_K} \text{ are a simple random sample from a Normal}(\mu_K, \sigma^2)$$

2. *Constant variance*. The K separate variance parameters are equal

**3.** *Independence* The observations are independent

**4.** *Null and Alternative Hypotheses*

$H_O : \mu_1 = \ldots = \mu_K$

$H_A$ : At least one is different

## One Way Fixed Effects Analysis of Variance
## Model of E[ $X_{ij}$ ] = $\mu_i$

*Deviation from Means Coding*

Recall -
"i" is the first subscript. It tells you the level/group of the one predictor variable (factor).
"j" is the second subscript. It tells you the individual within the group.

Model for the mean in the ith group -

(1) $E[X_{ij}] = \mu_i$

$= [\mu + \tau_i]$ where

(2) $\sum_{i=1}^{K} \tau_i = 0$

Introduction to the $\tau_i$: The "different-ness" of each mean is captured in the $\tau_1, \ldots, \tau_K$. Notice the following:

Group1: $\mu_1 = \mu + [\mu_1 - \mu] = \mu + \tau_1$ says that $[\mu_1 - \mu] = \tau_1$

…

Group K: $\mu_k = \mu + [\mu_K - \mu] = \mu + \tau_K$ says that $[\mu_K - \mu] = \tau_K$

*pssst* ....**This is deviation of means coding**

- By definition, $\sum_{i=1}^{K} \tau_i = 0$
- If the means are NOT EQUAL, then *at least one* $\tau_i = [\mu_i - \mu] \neq 0$

## One Way Analysis of Variance
## Fixed Effects Model

Setting:

K levels/groups of the predictor/factor are indexed i= 1, 2, …. K
Group specific sample sizes: $n_1, n_2, \ldots, n_K$
$X_{ij}$ = Observation for the jth individual in the ith group

The one way analysis of variance fixed effects model of $X_{ij}$ is defined as follows:

$$X_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where

$\mu$ = grand mean

$\tau_i = [\mu_i - \mu]$

$$\sum_{i=1}^{K} \tau_i = 0$$

and $\varepsilon_{ij}$ is random error distributed Normal$(0,\sigma^2)$

Estimation

| Parameter | Estimate using Sample Data |
|---|---|
| $\mu$ = overall or "grand" mean | $\bar{X}_{..}$ |
| $\mu_i$ = mean for group defined by level "i" of predictor | $\bar{X}_{i.}$ |
| $\tau_i = [\mu_i - \mu]$ measures "differentness" of ith group mean relative to overall or "grand" mean | $[\bar{X}_{i.} - \bar{X}]$ |
| $\sigma^2$ = the assumed common within group variance | $\hat{\sigma}^2_{within} = \dfrac{\sum_{i=1}^{K}(n_i - 1)S_i^2}{\sum_{i=1}^{K}(n_i - 1)}$ |

| | Expected Value of | | | | |
|---|---|---|---|---|---|
| | $\hat{\sigma}^2_{within}$ | $\hat{\sigma}^2_{between}$ | $\hat{\sigma}^2_{between}/\hat{\sigma}^2_{within}$ | $F$ | p-value |
| $H_O$ true (means equal) | $\sigma^2$ | $\sigma^2$ | 1 | 1 | Large |
| $H_A$ true (means NOT equal) | $\sigma^2$ | $\sigma^2 + \Delta$ | $>1$ | $>1$ | Small |

***Where …***

$$\Delta = \frac{n\sum_{i=1}^{K}(\mu_i - \bar{\mu})^2}{K-1}$$

**where n= common sample size in each group.**

## Example

Consenting physical therapy patients were randomized into 3 treatments. Thus, the single predictor is the factor predictor treatment and it has K=3 levels. The sample size was n=4 in each group. At follow-up, each participant was given a test to measure treatment effectiveness. The following scores were obtained.

| Treatment | | |
|---|---|---|
| **1** | **2** | **3** |
| 75 | 25 | 100 |
| 80 | 75 | 80 |
| 75 | 25 | 100 |
| 50 | 75 | 40 |
| $\bar{X}_1=70$ | $\bar{X}_2=50$ | $\bar{X}_3=80$ |
| $X_1^*=\sqrt{n}\left[\bar{X}_1\right]=(2)(70)=140$ | $X_2^*=\sqrt{n}\left[\bar{X}_2\right]=(2)(50)=100$ | $X_3^*=\sqrt{n}\left[\bar{X}_3\right]=(2)(80)=160$ |

$$\bar{X}^* = \sum_{i=1}^{3} X_i^* \Big/ 3 = 133.33$$

$H_O$: $\mu_1 = \mu_2 = \mu_3$
$H_A$: not

**Step 1: Test the assumption of equality of variances.**

*Stay tuned.* Tests of the assumption of equality of variances are discussed later (see, Section 4a). These are of limited usefulness for two reasons:

(1) Tests of equality of variance tend to be sensitive to the assumption of normality.

(2) Analysis of variance methodology is pretty robust to violations of the assumption of a common variance.

**Step 2: Estimate the varibility of individuals within group ("noise"). This will be a weighted average of the k separate sample variances.**

$$\hat{\sigma}^2_{within} = \frac{\sum_{i=1}^{K=3}(n_i-1)S_i^2}{\sum_{i=1}^{3}(n_i-1)} = \frac{5450.00}{9} = 605.56$$

**Step 3:** **Estimate the variability of the means between groups ("noise + signal"). This will be a sample variance calculation for our starry means data set comprised of**

$$X_1^* = \sqrt{n}\bar{X}_1, \quad X_2^* = \sqrt{n}\bar{X}_2, \quad X_3^* = \sqrt{n}\bar{X}_3\text{'s}$$

**Forgot what's meant by "noise", "signal to noise" and "noise + signal"? Take a look back at page 10.**

$$\hat{\sigma}^2_{between} = \left[ \frac{\sum_{i=1}^{3} \left( X_i^* - \bar{X}^* \right)^2}{(3-1)} \right] = \frac{1866.67}{2} = 933.33$$

**Step 4:** **Construct an analysis of variance table that shows the partitioingof the total variability into its two components, "between (model)" and "between (residual)". Perform an overall F-test that compares the fitted model to the "intercept-only" model. The null hypothesis says that the means are equal.**

| Source | df[a] | Sum of Squares | Mean Square | Overall F = Variance Ratio |
|---|---|---|---|---|
| Between groups (due model) | **( K-1 ) = 2** | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(\bar{X}_i - \bar{X}\right)^2$ **= 1866.67** | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(\bar{X}_i - \bar{X}\right)^2 \Big/ (K-1)$ **= $\hat{\sigma}^2_{between}$ = 933.33** | $F = \frac{\hat{\sigma}^2_{between}}{\hat{\sigma}^2_{within}} = 1.54$ |
| Within Groups (due error/residual) | $\sum_{i=1}^{K}(n_i - 1) = 9$ | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}_i\right)^2$ **= 5450.00** | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}_i\right)^2 \Big/ \sum_{i=1}^{K}(n_i - 1)$ **= $\hat{\sigma}^2_{within}$ = 605.56** | |
| Total | **(N – 1)**[b] | $\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(X_{ij} - \bar{X}\right)^2$ **= 7316.67** | | |

[a] degrees of freedom

[b] N = grand total sample size, taken over all groups = $\sum_{i=1}^{K} n_i$

**p-value = Pr [$F_{DF=2,9} \geq 1.54$ ] = .27**

Conclusion. The null hypothesis is *not* rejected. These data do not provide statistically significant evidence of differences in group means, that effectiveness of treatment is different, depending on the type of treatment received ("1" versus "2" versus "3").

## R

```
Input data directly
pt_table=read.table(text="
txgroup yscore
1.00    75.00
1.00    80.00
1.00    75.00
1.00    50.00
2.00    25.00
2.00    75.00
2.00    25.00
2.00    75.00
3.00    100.00
3.00    80.00
3.00    100.00
3.00    40.00",header=TRUE)
ptdata <- as.data.frame.matrix(pt_table)
ptdata$txgroup <- as.factor(ptdata$txgroup)                    # Predictor variable must be of type factor

Anova.

aov2 <- aov(yscore ~ txgroup, data=ptdata)                     # MODEL:  YVAR ~ GROUPVAR, data=DATAFRAME
anova(aov2)
## Analysis of Variance Table
##
## Response: yscore
##           Df Sum Sq Mean Sq F value Pr(>F)
## txgroup    2 1866.7  933.33  1.5413 0.2657
## Residuals  9 5450.0  605.56
```

Interpretation: This matches the hand calcluation. The null hypothesis is *not* rejected. Conclude these data do not provide statistically significant evidence that effectiveness of treatment is different, depending on the type of treatment received ("1" versus "2" versus "3").

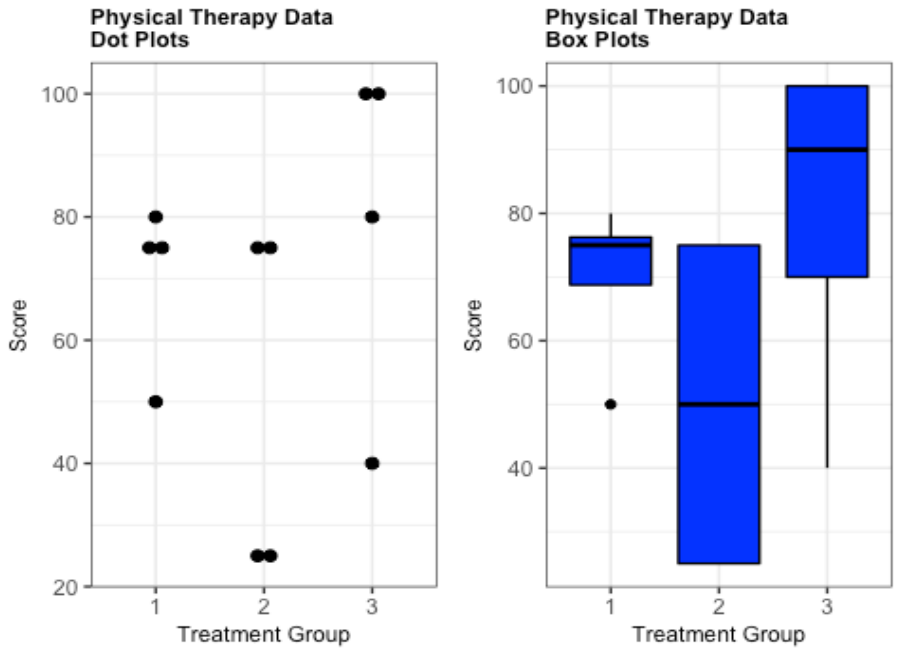
## Step 5: Don't forget to look at your data!

### R Illustration

```
library(FSA)

Numerical Descriptives

# Descriptives - Overall
FSA::Summarize(yscore ~ 1,data=ptdata,digits=2, na.rm=TRUE)              # Tip: (yvar ~ 1) for descriptives overall

##      n   mean     sd    min     Q1 median     Q3    max
##  12.00  66.67  25.79  25.00  47.50  75.00  80.00 100.00

# Descriptives - by Treatment Group                                  # KEY: (yvar ~ groupvar) for descriptives, by group
FSA::Summarize(yscore ~ txgroup,data=ptdata,digits=2, na.rm=TRUE)


##   txgroup n mean    sd min    Q1 median     Q3 max
## 1       1 4   70 13.54  50 68.75     75  76.25  80
## 2       2 4   50 28.87  25 25.00     50  75.00  75
## 3       3 4   80 28.28  40 70.00     90 100.00 100
```

Graphical Descriptives

```
library(ggplot2)
library(gridExtra)



# panel 1 = side-by-side dot plot (Preferred for small to moderate sample sizes)
p1 <- ggplot(data=ptdata,aes(x=factor(txgroup),y=yscore))  +
         geom_dotplot(dotsize=0.75,binaxis = "y",
               stackdir = "center",binpositions="all")     +
         xlab("Treatment Group")                            +
          ylab("Score")                                     +
         ggtitle("Physical Therapy Data \nDot Plots")      +
         theme_bw()                                        +
         theme(axis.text = element_text(size = 9),
               axis.title = element_text(size = 9),
               plot.title = element_text(size = 9, face = "bold"))

# panel 2 = side-by-side box plot (better for large sample sizes)
p2 <- ggplot(data=ptdata,aes(x=factor(txgroup),y=yscore))  +
        geom_boxplot(color="black",fill="blue")             +
        xlab("Treatment Group") + ylab("Score")             +
        ggtitle("Physical Therapy Data \nBox Plots")       +
        theme_bw() +
        theme(axis.text = element_text(size = 9),
              axis.title = element_text(size = 9),
              plot.title = element_text(size = 9, face = "bold"))

# Create arrange the two panels (side-by-side) in one graph using grid.arrange( ) in {gridExtra}
gridExtra::grid.arrange(p1, p2, ncol=2)
```



## 4. Checking Assumptions of the One Way Analysis of Variance

### a. Tests for Homogeneity of Variance

**Violation of the assumption of homogeneity of variances is sometimes, *but not always*, a problem.**

- Recall that the denominator of the overall F statistic in a one way analysis of variance is the "within group mean square." It is a weighted average of the separate within group variance estimates $S^2$ and, as such, is an estimate of the assumed common variance.
    - When the within group variance parameters $\sigma_1^2, \sigma_2^2, \ldots, \sigma_K^2$ are at least reasonably similar, then the within group mean square is a good summary of the within group variability.
- The overall F test for equality of means in an analysis of variance is reasonably robust to moderate violations of the assumption of homogeneity of variance.
- However, pairwise t-tests and hypothesis tests of contrasts are not robust to violations of homogeneity of variance, as when the $\sigma_1^2, \sigma_2^2, \ldots, \sigma_K^2$ are very unequal.

**Tests of homogeneity of variance are appropriate for the one-way analysis of variance only.**
There are a variety of tests available.

- **F Test for Equality of Two Variances –** This was introduced in BIOSTATS 540 Unit 10, Section 2d *Hypothesis Testing, p 24* ( https://people.umass.edu/biep540w/pdf/10.%20TWO%20Sample%20Inference%202019.pdf
- **Bartlett's test** - This test has high statistical power when the assumption of normality is met. However, it is very sensitive to the assumption of normality.
- **Levene's test** – This test has the advantage of being much less sensitive to violations of normality. Its disadvantage is that it has less power than Bartlett's test.
- **Brown-Forsythe test**, also called **Levene (med) test** – Similar to Levene's Test.

## Bartlett's Test

- $H_O$: $\sigma_1^2 = \sigma_2^2 = \ldots = \sigma_K^2$
  $H_A$: At least one $\sigma_i^2$ is unequal to the others
- Obtain the K separate group-specific sample variances $S_1^2,\ldots,S_K^2$
- Obtain $\hat{\sigma}^2_{within} = \dfrac{\sum_{i=1}^{K}(n_i-1)S_i^2}{\sum_{i=1}^{K}(n_i-1)}$ the estimate of the (null hypothesis) common $\sigma^2$
- Compute $B = [\ln(\hat{\sigma}^2_{within})]\left(\sum_{i=1}^{K}(n_i\text{-}1)\right) - \sum_{i=1}^{K}(n_i\text{-}1)\ln\left(S_i^2\right)$ *note – Some texts use B as the test statisticst.*
- Compute $C = 1 + \dfrac{1}{3(K\text{-}1)}\left\{\sum_{i=1}^{K}\dfrac{1}{(n_i\text{-}1)} - \dfrac{1}{\sum_{i=1}^{K}(n_i\text{-}1)}\right\}$ *-note- C is a correction factor*
- Compute Bartlett Test Statistic = $\dfrac{B}{C}$ *note – the distribution of B/C is better approximated by chi square.*
- When the null hypothesis is true, Bartlett Test Statistic is distributed Chi square (df=K-1)
  - Reject null for large values of Bartlett Test

## Levene's Test ("Dispersion variable Analysis of Variance")

The idea of Levene's test and its modification is to create a new random variable, a *dispersion random variable* which we will represent as d and that is a measure of how the variances are different. Levene's test (and its modifications) is a one way analysis of variance on a dispersion random variable d.

- $H_O$: $\sigma_1^2 = \sigma_2^2 = \ldots = \sigma_K^2$
  $H_A$: At least one $\sigma_i^2$ is unequal to the others
- Compute $d_{ij} = |X_{ij} - \bar{X}_i|$
  .
- Perform a one way analysis of variance of the $d_{ij}$
- When the null hypothesis is true, the Levene Test One Way Analysis of Variance is distributed F (numerator df = K-1, denominator df = N-K)
  - Reject null for large values of Levene Test One Way Anova F

## Brown and Forsythe Modification of Levene's Test ("Dispersion variable Analysis of Variance")

The Brown and Forsythe modification of Levene's test utilizes as its dispersion random variable d the absolute deviation from the group median.

- $H_O: \sigma_1^2 = \sigma_2^2 = \ldots = \sigma_K^2$

  $H_A:$ At least one $\sigma_i^2$ is unequal to the others

- Compute $\tilde{d}_{ij} = | X_{ij} - \text{median}(X_{i1}, X_{i2}, \ldots, X_{in_i}) |$

  .

- Perform a one way analysis of variance of the $\tilde{d}_{ij}$

- When the null hypothesis is true, the Brown and Forsythe One Way Analysis of Variance is distributed F (numerator df = K-1, denominator df = N-K)

  - Reject null for large values of Brown and Forsythe Test One Way Anova **F**

R Illustration

```
library(car)                                                    # leveneTest( ) in package {car}

# Bartlett Test
bartlett.test(yscore~txgroup, data=ptdata)
##
##  Bartlett test of homogeneity of variances
##
## data:  yscore by txgroup
## Bartlett's K-squared = 1.5601, df = 2, p-value = 0.4584

Interpretation:  Do NOT reject the null (p-value = .46).  Assumption of the null has NOT led to a contradiction.
Conclude there is NO statistically significant evidence that the variances are unequal.

# Brown and Forsythe Test in R is called a "Modified Levene Test"

car::leveneTest(yscore~txgroup,data=ptdata)
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  2     1.8   0.22
##        9

Interpretation:  Same.  Do NOT reject the null (p-value = .22).  Assumption of the null has NOT led to a
contradiction.  Conclude there is NO statistically significant evidence that the variances are unequal.
```

## Statistics in Practice: Guidelines for Assessing Homogeneity of Variance

- Look at the variances (or standard deviations) for each group first!

  - Compare the numeric values of the variances (or standard deviations)
    If the ratio of the standard deviations is less than 3 (or so), it's okay
    not to worry about homogeneity of variances

  - Construct a side-by-side box plot of the data and have a look at the sizes of the boxes.

- Second, assess the reasonableness of the normality assumption. This is important to the validity of the tests of homogeneity of variances.

- Levene's test of equality of variances is least affected by non-normality; it's a good choice.

- Bartlett's test should be used with caution, given its sensitivity to violations of normality.

## b. Graphical Assessments and Tests of Normality

Graphical assessments and tests of normality were introduced previously. See again BIOSTATS 640 Unit 5, Regression & Correlation, page 47.

Analysis of Variance methods are reasonably robust to violation of the assumption of normality in analysis of variance.

- The assumption of normality in a one way analysis of variance is that within each of the K groups the individual $X_{ij}$ for $j = 1, 2, \ldots n_i$ are assumed to be a simple random sample from a Normal( $\mu_i$ , $\sigma^2$ ),

- The analysis of variance is actually relying on normality of the sampling distribution of the means $\bar{X}_i$ for $i = 1, 2, ..., K$. This is great because we can appeal to the central limit theorem.

- Thus, provided that the sample sizes in each group are reasonable (say 20-30 or more), and provided the underlying distributions are not too too different from normality, then the analysis of variance is reasonably robust to violation of the assumption of normality.

**As with normal theory regression, assessments of normality are of two types in analysis of variance.**

- Preliminary is to calculate the residuals:
  - For $X_{ij}$ = observation for "j"th person in group=i
  - Residual $r_{ij}$ = ( $X_{ij} - \bar{X}_{i.}$ ) difference between observed and mean for group

- **1. Graphical Assessments of the distribution of the residuals:**
  - Dot plots with overlay normal
  - Quantile-Quantile plots using referent = normal

- **2. Numerical Asessments:**
  - Calculation of skewness and kurtosis statistics
  - Shapiro Wilk test of normality
  - Kolmogorov Smirnov/Lillefors tests of normality
  - Anderson Darling/Cramer von Mises tests of normality

## Statistics in Practice: Guidelines for the Handling of Violations of Normality

- **Small sample size setting (within group sample size n < 20, approximately):**
  - **Replace the normal theory one-way analysis of variance with a Kruskal Wallis** nonparametric one way analysis of variance. You can find a detailed description in Unit 3, Nonparametrics

- **Large sample size setting:**
  If you *really must*, consider a normalizing data transformation. Possible transformations include the following:

  (1) Logarithmic Transformation: $X^* = \ln(X+1)$ helps positive skewness

  (2) Square Root Transformation: $X^* = \sqrt{X + 0.5}$ helps heteroscedasticity

  (3) Arcsine Transformation: $p^* = \text{arcsine}\sqrt{p}$ for outcome 0 to 100 percentage

  (4) If your data are actual proportions of the type X/n and you have X and n consider Anscombe Arcsine Transformation:

$$p^* = \text{arcsine}\sqrt{\frac{X+\frac{3}{8}}{n+\frac{3}{4}}}$$

## 5. Introduction to More Complicated Designs

**What if we have more than one categorical predictor, each with multiple levels?** So far we have considered just one categorical predictor with possibly multiple levels: the one-way analysis of variance design.

- E.g., predictor was TREATMENT with 3 levels ("Pet", "Friend", "Neither pet nor friend")

When the number of predictors is itself several, depending on the research question, analysis of variance methods can become more complicated for a variety of reasons, including but not limited to the following-

(1) The model may have more terms, including interactions and/or adjustment for confounding Fitting and interpretation become more challenging.

(2) One or more of the terms in the model might be measured with error instead of being fixed – Estimates of variance, their interpretation and confidence interval construction are more involved.

(3) The partitioning of total variability might not be as straightforward as what we have seen – Understanding and working with analysis of variance tables and, especially, knowing which F test to use, can be hard.

### a. Balanced versus Unbalanced

**Balanced versus unbalanced refers to sample size**. When the within group sample sizes are unequal, the partitioning of the total variability looks a bit complicated, but the intuition is the same as we saw for the one way fixed effects analysis of variance with equal sample sizes per group.

**BALANCED**

The sample size in each cell is the same.

Equality of sample size makes the analysis easier.

Specifically, the partitioning of SSQ is straightforward

A 2 way balanced anova with n=1 is called the randomized block design

**UNBALANCED**

The sample sizes in the cells are different.

The partitioning of SSQ is no longer straightforward.

Here, a regression approach (reference cell coding – this comes later) is sometimes easier to follow.

## b. Fixed versus Random Effects

***Hack!*** **"Fixed" versus "random" is more complicated than you might think.**

- **The distinction has to do with** *how the inferences will be used*
- **The formal analysis of variance is largely** *unchanged.*

There exist a number of definitions of "fixed" versus "random" effects. Among them are the following.

- **Fixed effects** are levels of effects chosen by the investigator, whereas
  **Random effects** are selected at random from a larger population

- **Fixed effects** are either (1) levels chosen by the investigator or (2) *all* the levels possible
  **Random effects** are a random sample from some universe of all possible levels.

- **Fixed effects** are effects that are interesting in themselves, whereas
  Effects are investigated as **random** if the underlying population is of interest.

### Examples

- **Examples of Fixed Effects** – (1) Treatment; (2) Sex at birth

- **Examples of Random Effects** – (1) litter of animals (this is an example of a random block); (2) interviewer in a data collection setting where there might be multiple interviewers or raters.

### Illustration of "Fixed" versus "Random" Thinking

**In "fixed" versus "random", the null hypotheses are slightly different.** Consider a one way anova analysis which explores variations in SAT scores, depending on the university affiliation of the students.

- Outcome is $X_{ij}$ = SAT score for "j"th individual at University "i"

- Factor is University with

  i = 1 if University is Massachusetts
  2 if University is Wisconsin
  3 if University is Alaska

- Subscript "j" indexes student within the University

- **FIXED effects perspective**

  **Interest pertains only to the 3 Universities** (MA, WI, and AK). → Null hypothesis is

  $H_0$: $\mu_{Massachusetts} = \mu_{Wisconsin} = \mu_{Alaska}$

- **RANDOM effects perspective**

  **Massachusetts, Wisconsin, and Alaska are a random sample** from the population of universities in the US. → Null hypothesis is

  $H_0$: Mean SAT scores are equal at all American Universities

## c. Factorial versus Nested

**The distinction "factorial" versus "nested" is an important distinction pertaining to the discovery of interaction or effect modification (factorial) versus control for confounding (nested).**

To discover effect modification → Factorial Design
To control for (eliminate) confounding → Nested Design

Consider the context of a two way analysis of variance that explores Factor A at "a" levels and Factor B at "b" levels

### FACTORIAL

All combinations of factor A and factor B are investigated.

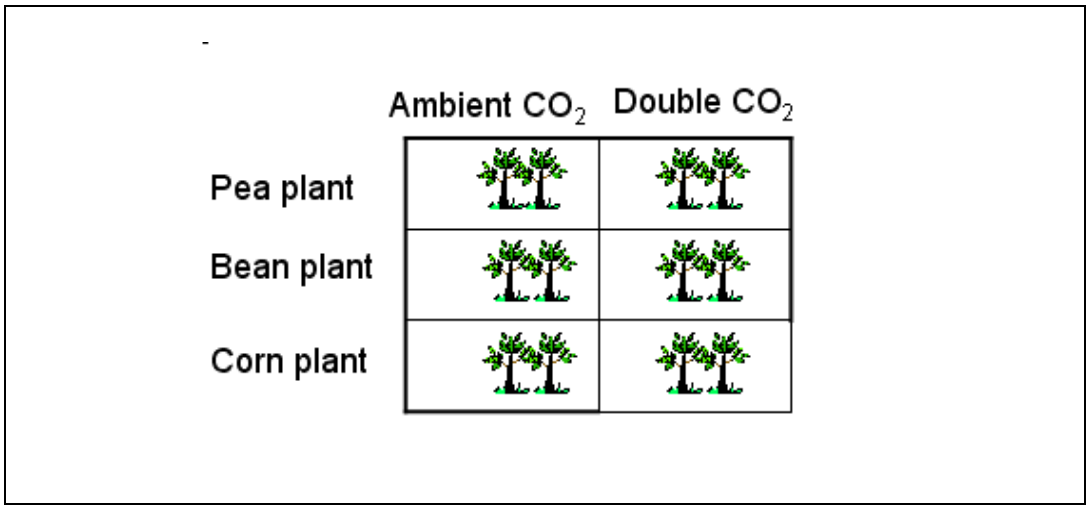
Factorial design permits investigation of A x B interaction

Thus, good for exploration of effect modification, synergism, etc.

Frequently used in public health, observational epidemiology

**Example of Factorial Design.**

**Factor A = Plant at 3 levels (rows) and Factor B = $CO_2$ at 2 levels (columns)**




Note: All (3)(2) = 6 combinations of plant x $CO_2$ are investigated

**NESTED**

The levels of the second factor are nested in the first.

*Confounding (by Factor A) of the Factor B- Outcome relationship* is controlled through use of stratification on Factor A

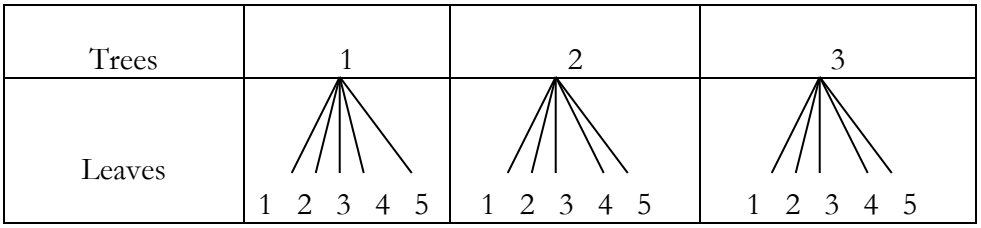
Familiar examples are *hierarchical, split plot, repeated measures, mixed models.*

Nested designs are frequently used in biology, psychology, and complex survey methodologies.

Factor A, the stratifying variable, is sometimes called the "primary sampling unit."

**Example of Nested Design.**

**Factor A = Trees at 3 levels and Factor B = Leaf at 5 levels, nested within its trees of belonging!**



## 6. Some Other Analysis of Variance Designs

***What design should you use?***
It depends! Specifically, the answer depends on (1) the research question; and (2) knowledge of underlying biology and, specifically, knowledge of external influences that might be effect modifying or confounding or both; and (3) availability of sample size.

Briefly, three other analysis of variance designs are introduced here

a. The Randomized Complete Block Design
b. Two Way Fixed Effects Analysis of Variance – Equal cell numbers
c. The Two Way Hierarchical or Nested Design

### a. The Randomized Complete Block Design

**Example -**
An investigator wishes to compare 3 treatments for HIV disease. However, it is suspected that response to treatment might be confounded by baseline cd4 count. The investigator seeks to control (better yet, eliminate) confounding. To accomplish this, consenting subjects are grouped into 8 "homogeneous" blocks according to cd4 count. Within each block, baseline cd4 counts are assumed to be similar.

Within each block, there are 3 subjects, *one per treatment*. Assignment of subject to treatment within each block is *randomized*.

Data Layout –

| **Block is Stratum of cd4 count, i=** | **Treatment, j=** | | |
|---|---|---|---|
| **1** | Drug 3 | Drug 1 | Drug 2 |
| **2** | Drug 2 | Drug 1 | Drug 3 |
| **3** | … | … | … |
| **4** | … | … | … |
| **5** | … | … | … |
| **6** | … | … | … |
| **7** | … | … | … |
| **8** | Drug 1 | Drug 3 | Drug 2 |

While there are two factors ..

- The row factor is called a blocking factor; its influence on outcome is *not* of interest. But we do want to control for its possible confounding effect of baseline CD4 count

- Only the column factor is of interest – Treatment (drug) at 3 levels.

**A characteristic of a randomized complete block design is that the sample size is ONE in each *Treatment* × *Block* combination.**

## Randomized Complete Block Design Model

Setting:

I indexes level of Factor 1 which are <u>blocks</u> indexed i= 1, 2, …. I
J indexes level of Factor 2 which are <u>treatments</u> indexed j=1, 2, …., J
Sample size is 1 in each block x treatment combination
$X_{ij}$ = Observation for the one individual in the $i^{th}$ block who received the $j^{th}$ group/treatment

The <u>randomized complete block design model</u> of $X_{ij}$ is defined as follows:

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where -

$\mu$ = grand mean

$$\alpha_i = [\mu_{i.} - \mu] \text{ and } \sum_{i=1}^{I} \alpha_i = 0$$

$$\beta_j = [\mu_{.j} - \mu] \text{ and } \sum_{j=1}^{J} \beta_j = 0$$

and - $\varepsilon_{ij}$ is random error distributed Normal$(0,\sigma^2)$

## Randomized Complete Block Design Analysis of Variance

"i" indexes level of Factor 1, block, i=1 …. I
"j" indexes level of Factor 2, treatment, j = 1 …. J

$\mu_{ij}$ = Mean [ Outcome ] for drug "j" in block "i"

i = 1 .. I In this example, I = 8 because there are 8 blocks
j = 1, 2, …, J In this example, J = 3 because there are 3 treatments

$E[ X_{ij} ] = \mu_{ij} = \mu + \alpha_i + \beta_j \rightarrow$

$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$ where

$$(1) \quad \alpha_i = [\,\mu_{i.} - \mu\,] \text{ and } \sum_{i=1}^{I} \alpha_i = 0$$

$$(2) \quad \beta_j = [\,\mu_{.j} - \mu\,] \text{ and } \sum_{j=1}^{J} \beta_j = 0$$

$$X_{ij} = \bar{X}_{..} + [\,\bar{X}_{i.} - \bar{X}_{..}\,] + [\,\bar{X}_{.j} - \bar{X}_{..}\,] + [\,X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}\,] \quad \textit{algebraic identity}$$

- $\varepsilon_{ij}$ is assumed distributed Normal(0, $\sigma^2$)

- Because n=1 in each cell, block x treatment interactions, if they exist, cannot be estimated. (Bummer – this means we cannot assess affect modification)

## Total SSQ and its Partitioning

$$X_{ij} = \bar{X}_{..} + [\,\bar{X}_{i.} - \bar{X}_{..}\,] + [\,\bar{X}_{.j} - \bar{X}_{..}\,] + [\,X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}\,] \rightarrow$$

$$[\,X_{ij} - \bar{X}_{..}\,] = [\,\bar{X}_{i.} - \bar{X}_{..}\,] + [\,\bar{X}_{.j} - \bar{X}_{..}\,] + [\,X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}\,].$$

Squaring both sides and summing over all observations yields (because the cross product terms sum to zero!)

$$\sum_{i=1}^{I}\sum_{j=1}^{J}[\,X_{ij} - \bar{X}_{..}\,]^2$$

$$= \sum_{i=1}^{I}\sum_{j=1}^{J}[\,\bar{X}_{i.} - \bar{X}_{..}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}[\,\bar{X}_{.j} - \bar{X}_{..}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}[\,X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}\,]^2$$

$$= J\sum_{i=1}^{I}[\,\bar{X}_{i.} - \bar{X}_{..}\,]^2 + I\sum_{j=1}^{J}[\,\bar{X}_{.j} - \bar{X}_{..}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}[\,X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}\,]^2$$

**Analysis of Variance Table**

| Source | df[a] | Sum of Squares | E (Mean Square) | F |
|---|---|---|---|---|
| **Due block** | ( I-1 ) | $J\sum_{i=1}^{I}\left(\bar{X}_{i.}-\bar{X}..\right)^2$ | $\sigma^2 + J\left[\frac{\sum_{i=1}^{I}\alpha_i^2}{(I-1)}\right]$ | $F=\frac{MSQ_{block}}{MSQ_{residual}}$ df = (I-1), (I-1)(J-1) |
| **Due treatment** | (J-1) | $I\sum_{j=1}^{J}\left(\bar{X}_{.j}-\bar{X}_{..}\right)^2$ | $\sigma^2 + I\left[\frac{\sum_{j=1}^{J}\beta_j^2}{(J-1)}\right]$ | $F=\frac{MSQ_{treatment}}{MSQ_{residual}}$ df = (J-1), (I-1)(J-1) |
| **Residual** | (I-1)(J-1) | $\sum_{i=1}^{I}\sum_{j=1}^{J}\left(X_{ij}-\bar{X}_{i.}-\bar{X}_{.j}+\bar{X}_{..}\right)^2$ | $\sigma^2$ | |
| **Total** | IJ – 1 | $\sum_{i=1}^{I}\sum_{j=1}^{J}\left(X_{ij}-\bar{X}_{..}\right)^2$ | | |
| | | | | |

[a] degrees of freedom

## b. The Two Way Fixed Effects Analysis of Variance

**Example -**

Fish growth is thought to be influenced by light or by temperature or by both and possibly by both in combination. To explore this, an investigator considered all possible combinations of light at 2 levels and water temperature at 3 levels. Thus the total number of combinations of light and temperature is 2 x 3 = 6. This is an example of a *two way factorial analysis of variance.*

Outcome X = fish growth at six weeks

- Factor 1 is Light at 2 levels (low and high)
- Factor 2 is Water Temperature at 3 levels (cold, lukewarm, warm)
- **$X_{ijk}$** = growth at six weeks for the **$k^{th}$** fish at **light level=i** and **water temperature=j**

Following are the data

| Light | Water Temp | X = Fish Growth |
|---|---|---|
| 1=low | 1=cold | 4.55 |
| 1=low | 1=cold | 4.24 |
| 1=low | 2=lukewarm | 4.89 |
| 1=low | 2=lukewarm | 4.88 |
| 1=low | 3=warm | 5.01 |
| 1=low | 3=warm | 5.11 |
| 2=high | 1=cold | 5.55 |
| 2=high | 1=cold | 4.08 |
| 2=high | 2=lukewarm | 6.09 |
| 2=high | 2=lukewarm | 5.01 |
| 2=high | 3=warm | 7.01 |
| 2=high | 3=warm | 6.92 |
| | | |

There are 3 analysis questions and, thus, three null hypotheses of interest:

(1) Ho: No effect due to light,
e.g. mean fish length is the same over the two levels of light

(2) Ho: No effect due to temperature
e.g. mean fish length is the same over the three levels of temperature

(3) Ho: Not a differential effect
of one treatment over the levels of the other (e.g. no interaction)

Note – As we will see in the next page, the order in which we test these hypotheses matters!

## Two Way Factorial Analysis of Variance
## Fixed Effects Model

Setting:

I levels of Factor #1 and are indexed i= 1, 2, …. I
J levels of Factor #2 and are indexed indexed j=1, 2, …., J
The sample size for the group defined by Factor 1 at level "i" and Factor 2 at level "j" is $n_{ij}$
"k" indexes the kth observation in the "ij"$^{th}$ group
$X_{ijk}$ = Observation for the k$^{th}$ individual in the jth block of the ith group/treatment

The two way factorial analysis of variance fixed effects model of $X_{ijk}$ is defined as follows:

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

where

$\mu$ = grand mean

$$\alpha_i = [\mu_{i.} - \mu] \text{ and } \sum_{i=1}^{I} \alpha_i = 0$$

$$\beta_j = [\mu_{.j} - \mu] \text{ and } \sum_{j=1}^{J} \beta_j = 0$$

$$(\alpha\beta)_{ij} = \mu_{ij} - [\mu + \alpha_i + \beta_j] = \mu_{ij} - \mu - \alpha_i - \beta_j = \mu_{ij} - \mu - [\mu_{i.} - \mu] - [\mu_{.j} - \mu]$$

$$\sum_{i=1}^{I} (\alpha\beta)_{ij} = 0 \text{ and } \sum_{j=1}^{j} (\alpha\beta)_{ij} = 0$$

and $\varepsilon_{ij}$ is random error distributed Normal$(0,\sigma^2)$

## Two Way Factorial Fixed Effects Analysis of Variance

Let i index light, i = 1, 2.
j index water temperature, j = 1, 2, 3.
k index individual in the $(i, j)^{th}$ group, k = $1, \cdots, n_{ij}$.

$\mu_{ij}$ = Expected mean growth at 6 weeks for fish raised under conditions of light = "i" and water temperature = "j".

$$\mu_{ij} = \mu + [\mu_{i\cdot} - \mu] + [\mu_{\cdot j} - \mu] + [\mu_{ij} - \mu - (\mu_{i\cdot} - \mu) - (\mu_{\cdot j} - \mu)]$$

$\mu$ = Overall population mean

$\alpha_i = [\mu_{i\cdot} - \mu]$ is the light effect. It is estimated by $\left[\bar{X}_{i\cdot\cdot} - \bar{X}_{\cdots}\right]$

$\beta_j = [\mu_{\cdot j} - \mu]$ is the water temperature effect. It is estimated by $\left[\bar{X}_{\cdot j\cdot} - \bar{X}_{\cdots}\right]$

$(\alpha\beta)_{ij} = [\mu_{ij} - \mu - (\mu_{i\cdot} - \mu) - (\mu_{\cdot j} - \mu)]$ is the extra, joint, effect of the $i^{th}$ light level and $j^{th}$ water temperature. It is estimated by

$$\left[\bar{X}_{ij\cdot} - \bar{X}_{\cdots} - \left(\bar{X}_{i\cdot\cdot} - \bar{X}_{\cdots}\right) - \left(\bar{X}_{\cdot j\cdot} - \bar{X}_{\cdots}\right)\right]$$

Thus, an individual response $X_{ij}$ is modeled

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk}$$

$$= \mu + [\mu_{i\cdot} - \mu] + [\mu_{\cdot j} - \mu] + [\mu_{ij} - \mu - (\mu_{i\cdot} - \mu) - (\mu_{\cdot j} - \mu)] + \varepsilon_{ijk}$$

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

Assumptions

- $\sum_{i=1}^{I} \alpha_i = 0$ $\quad\quad$ $\sum_{j=1}^{J} \beta_j = 0$
- $\sum_{i=1}^{I} (\alpha\beta)_{ij} = 0$ $\quad\quad$ $\sum_{j=1}^{J} (\alpha\beta)_{ij} = 0$

## Analysis of Variance

| Source | df[a] | Sum of Squares | Mean Square | F |
|---|---|---|---|---|
| **Due <u>light</u>** | ( I-1 ) | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(\bar{X}_{i..}-\bar{X}_{...}\right)^2$ | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(\bar{X}_{i..}-\bar{X}_{...}\right)^2 \Big/ (I-1)$ $= \hat{\sigma}^2_{light}$ | $F=\frac{\hat{\sigma}^2_{light}}{\hat{\sigma}^2}$ |
| **Due <u>temperature</u>** | (J-1) | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(\bar{X}_{.j.}-\bar{X}_{...}\right)^2$ | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(\bar{X}_{.j.}-\bar{X}_{...}\right)^2 \Big/ (J-1)$ $= \hat{\sigma}^2_{temp}$ | $F=\frac{\hat{\sigma}^2_{temp}}{\hat{\sigma}^2}$ |
| **Due <u>interaction</u>** | (I-1)(J-1) | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(\bar{X}_{ij.}-\bar{X}_{i..}-\bar{X}_{.j.}+\bar{X}_{...}\right)^2$ | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}} \frac{\left(\bar{X}_{ij.}-\bar{X}_{i..}-\bar{X}_{.j.}+\bar{X}_{...}\right)^2}{(I-1)(J-1)}$ $= \hat{\sigma}^2_{light*temp}$ | $F=\frac{\hat{\sigma}^2_{light*temp}}{\hat{\sigma}^2}$ |
| **Within Groups** **(due error)** | $\sum_{i=1}^{I}\sum_{j=1}^{J}\left(n_{ij}-1\right)$ | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_i}\left(X_{ijk}-\bar{X}_{ij.}\right)^2$ | $\frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(X_{ijk}-\bar{X}_{ij.}\right)^2}{\sum_{i=1}^{I}\sum_{j=1}^{J}\left(n_{ij}-1\right)}$ $= \hat{\sigma}^2$ | |
| **Total** | N – 1 | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n_{ij}}\left(X_{ijk}-\bar{X}_{...}\right)^2$ | | |
| | | | | |

[a] degrees of freedom

**A meaningful analysis might proceed in this order.**

**Step 1. Test for no interaction**

- If there is interaction, this means that the effect of light on growth depends on the water temperature and vice versa.

- Accordingly, the meaning that can be given to an analysis of effects of light (Factor I) or an analysis of the effects of water temperature (Factor II) depend on an understanding of interaction

- The correct F statistic tests this interaction: $F=\frac{\hat{\sigma}^2_{\text{light*temperature}}}{\hat{\sigma}^2}$

- Numerator df = (I-1)(J-1).

- Denominator df = $\sum_{i=1}^{I}\sum_{j=1}^{J}\left(n_{ij}-1\right)$

| If interaction is **NOT** significant | If interaction is **SIGNIFICANT** |
|---|---|
| Step 2.<br>Test for main effect of Factor 1<br><br>Use $F=\frac{\hat{\sigma}^2_{\text{factor I}}}{\hat{\sigma}^2}$<br><br>Numerator df = (I-1).<br><br>Denominator df = $\sum_{i=1}^{I}\sum_{j=1}^{J}\left(n_{ij}-1\right)$<br><br>Repeat for Factor 2. | Step 2.<br>STOP. Report report the biological variability you just discovered.<br><br>**NOTE:** It is still possible to assess main effects but take care to understand its meaning in this situation!<br><br>In particular, because the interaction is significant, we have inferred at this point that the response to one level of a factor (eg. Factor II = water temperature) depends on the level of another factor (eg. Factor I = level of light)<br><br>So then, if the analyst decides to collapse the data to a one-way analysis of variance, then the meaning of the analysis of a main effect is that it yields an estimate of the *average* main effect, taken over the levels of the other factor. FYI -This is typically not of interest. |

Example - Continued

| Source | df[a] | Sum Squares | Mean Square | F |
|---|---|---|---|---|
| **Due light** | ( 2-1 ) = 1 | 2.98 | 2.98 = $\hat{\sigma}^2_{\text{light}}$ | $F = \frac{\hat{\sigma}^2_{light}}{\hat{\sigma}^2_{error}} = 10.39$ P =.018 |
| **Due water temp** | (3-1) = 2 | 3.984 | 1.992 = $\hat{\sigma}^2_{\text{temp}}$ | $F = \frac{\hat{\sigma}^2_{temp}}{\hat{\sigma}^2_{error}} = 6.95$ p=.027 |
| **Due interaction** | (I-1)(J-1) = 2 | 1.268 | 0.634 = $\hat{\sigma}^2_{\text{light*temp}}$ | $F = \frac{\hat{\sigma}^2_{light*temp}}{\hat{\sigma}^2_{error}} = 2.21$ p=.191 |
| **Error** | $\sum_{i=1}^{I}\sum_{j=1}^{J}(n_{ij}-1) =$ 6 | 1.721 | 0.2868 = $\hat{\sigma}^2_{\text{error}}$ | |
| **Total** | N – 1 = 11 | 9.953 | | |

[a] **degrees of freedom**

Interpretation:

**Proceed in the right order:**

- STEP #1: Assess for evidence of interaction (biology worth discovering)
  In the "Due Interaction" row, find F-statistic = 2.21 on df =2,6. The p-value is .19, suggesting that there is NO statistically significant evidence of interaction. Not surprising, given the small sample size!

- STEP 2: With no interaction, you can now go on to assess output for strength of main effects.

  * Main Effect of TEMP: F=6.95 on df=2,6. p-value = .03.
    Conclude statistically significant

  * Main Effect of LIGHT: F=10.39 on df=1,6. p-value = .02.
    Conclude statistically significant.

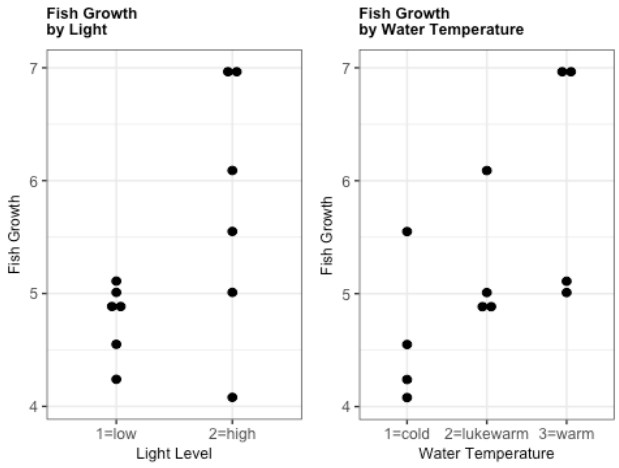
## R

**Load data. Descriptives by group.**

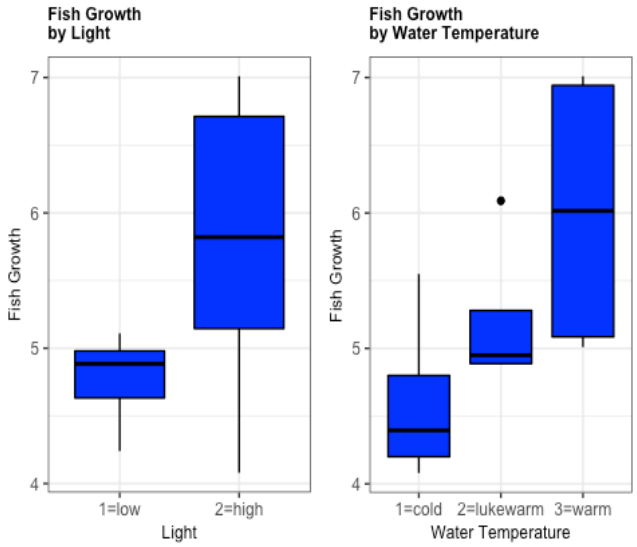
```
library(FSA)                                  # Here, you see the advantage of using Summarize( ) in package {FSA}
load(file="fishgrowth.Rdata")
FSA::Summarize(growth ~ light + temp,data=fishgrowth,digits=2,na.rm=TRUE)

##    light       temp n mean   sd  min   Q1 median   Q3  max
## 1  1=low     1=cold 2 4.39 0.22 4.24 4.32   4.39 4.47 4.55
## 2 2=high     1=cold 2 4.82 1.04 4.08 4.45   4.82 5.18 5.55
## 3  1=low 2=lukewarm 2 4.88 0.01 4.88 4.88   4.88 4.89 4.89
## 4 2=high 2=lukewarm 2 5.55 0.76 5.01 5.28   5.55 5.82 6.09
## 5  1=low     3=warm 2 5.06 0.07 5.01 5.04   5.06 5.08 5.11
## 6 2=high     3=warm 2 6.97 0.06 6.92 6.94   6.96 6.99 7.01
```

**Graphs: 1) side-by-side dotplot 2) side-by-side boxplot**

```
library(ggplot2)
library(gridExtra)

# panel 1a = side-by-side dot over light
p1a <- ggplot(data=fishgrowth,aes(x=factor(light),y=growth))  +
          geom_dotplot(dotsize=0.75,binaxis = "y", stackdir = "center",binpositions="all") +
          xlab("Light Level") + ylab("Fish Growth") +
          ggtitle("Fish Growth \nby Light") +
          theme_bw() +
          theme(axis.text = element_text(size = 9),
                axis.title = element_text(size = 9),
                plot.title = element_text(size = 9, face = "bold"))

# panel 1b = side-by-side dot over temp
p1b <- ggplot(data=fishgrowth,aes(x=factor(temp),y=growth)) +
          geom_dotplot(dotsize=0.75,binaxis = "y", stackdir = "center",binpositions="all") +
          xlab("Water Temperature") + ylab("Fish Growth") +
          ggtitle("Fish Growth \nby Water Temperature") +
          theme_bw() +
          theme(axis.text = element_text(size = 9),
               axis.title = element_text(size = 9),
               plot.title = element_text(size = 9, face = "bold"))

gridExtra::grid.arrange(p1a, p1b, ncol=2)
```



```
# panel 2a = side-by-side box plot over light
p2a <- ggplot(data=fishgrowth,aes(x=factor(light),y=growth)) +
         geom_boxplot(color="black",fill="blue") +
         xlab("Light") + ylab("Fish Growth") +
         ggtitle("Fish Growth \nby Light") +
         theme_bw() +
         theme(axis.text = element_text(size = 9),
               axis.title = element_text(size = 9),
               plot.title = element_text(size = 9, face = "bold"))

# panel 2b = side-by-side box plot over temp
p2b <- ggplot(data=fishgrowth,aes(x=factor(temp),y=growth)) +
         geom_boxplot(color="black",fill="blue") +
         xlab("Water Temperature") + ylab("Fish Growth") +
         ggtitle("Fish Growth \nby Water Temperature") +
         theme_bw() +
         theme(axis.text = element_text(size = 9),
               axis.title = element_text(size = 9),
               plot.title = element_text(size = 9, face = "bold"))

gridExtra::grid.arrange(p2a, p2b, ncol=2)
```




**Two-Way Anova. Fit of Model.**

```
library(car)
# Fit of anova model using R command aov( ).  Assign fit to object that I name aov3.
aov3 <- aov(growth ~ temp + light + temp:light, data=fishgrowth)
anova(aov3)
## Analysis of Variance Table
##
## Response: growth
##            Df Sum Sq Mean Sq F value  Pr(>F)
## temp        2 3.9843 1.99216  6.9462 0.02744 *
## light       1 2.9800 2.98003 10.3906 0.01806 *
## temp:light  2 1.2676 0.63381  2.2099 0.19093
## Residuals   6 1.7208 0.28680
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

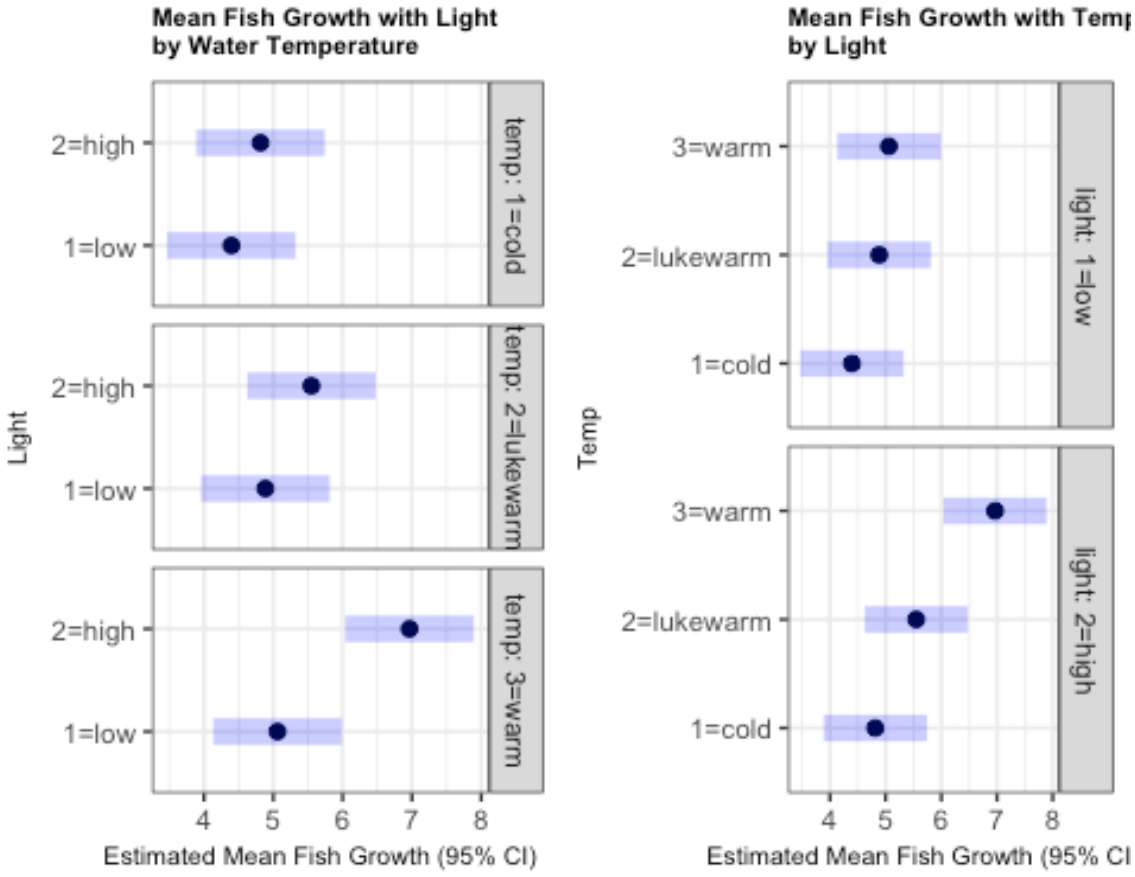
**Two-Way Anova. Tests of Homogeneity of Variance**

```
# tests of homogeneity of variance
# R Modified Levene Test is Brown and Forsythe Test
library(car)
car::leveneTest(growth ~ temp*light, data=fishgrowth)
## Warning in anova.lm(lm(resp ~ group)): ANOVA F-tests on an essentially
## perfect fit are unreliable

## Levene's Test for Homogeneity of Variance (center = median)
##       Df                          F value                Pr(>F)
## group  5 57841111952702865723844244537344 < 0.00000000000000022 ***   Wow. Should have rounded. But forging on..
##        6
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

**Post Fit Estimation of Means and Nifty Plot**

```
library(emmeans)
library(ggplot2)
library(gridExtra)
emm1 = emmeans::emmeans(aov3, specs = "light", by="temp")
emm1
## temp = 1=cold:
##  light  emmean    SE df lower.CL upper.CL
##  1=low    4.39 0.379  6     3.47     5.32
##  2=high   4.82 0.379  6     3.89     5.74
##
## temp = 2=lukewarm:
##  light  emmean    SE df lower.CL upper.CL
##  1=low    4.88 0.379  6     3.96     5.81
##  2=high   5.55 0.379  6     4.62     6.48
##
## temp = 3=warm:
##  light  emmean    SE df lower.CL upper.CL
##  1=low    5.06 0.379  6     4.13     5.99
##  2=high   6.96 0.379  6     6.04     7.89
##
## Confidence level used: 0.95

# Estimated means wrt TEMP separately by LIGHT
emm2 = emmeans::emmeans(aov3, specs = "temp", by="light")
emm2
## light = 1=low:
##  temp       emmean    SE df lower.CL upper.CL
##  1=cold       4.39 0.379  6     3.47     5.32
##  2=lukewarm   4.88 0.379  6     3.96     5.81
##  3=warm       5.06 0.379  6     4.13     5.99
##
## light = 2=high:
##  temp       emmean    SE df lower.CL upper.CL
##  1=cold       4.82 0.379  6     3.89     5.74
##  2=lukewarm   5.55 0.379  6     4.62     6.48
##  3=warm       6.96 0.379  6     6.04     7.89
##
## Confidence level used: 0.95

# Plot - Estimated means (95% CI) wrt LIGHT separately by TEMP
p3a  <- plot(emm1) + theme_bw() +
        labs(x = "Estimated Mean Fish Growth (95% CI)", y = "Light") +
        ggtitle("Estimated Mean Fish Growth with Light \nby Water Temperature") +
        theme(axis.title = element_text(size = 8),
                plot.title = element_text(size = 8, face = "bold"))
```

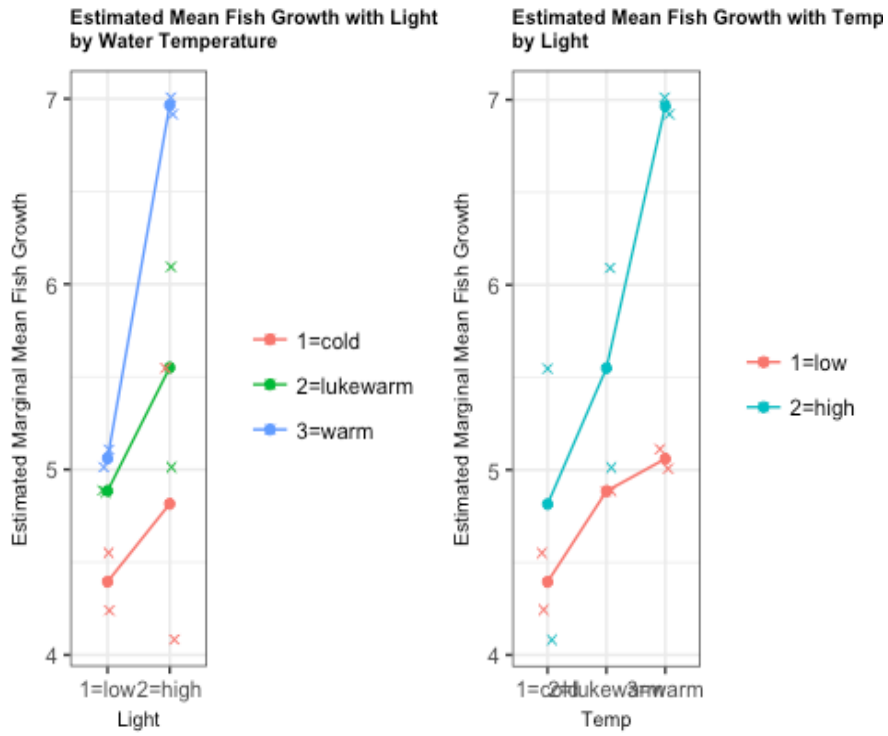
```
# Plot Estimated means (95% CI) wrt TEMP separately by LIGHT
p3b <- plot(emm2) + theme_bw() +
       labs(x = "Estimated Mean Fish Growth (95% CI)", y = "Temp") +
       ggtitle("Estimated Mean Fish Growth with Temp \nby Light") +
       theme(axis.title = element_text(size = 8),
             plot.title = element_text(size = 8, face = "bold"))

gridExtra::grid.arrange(p3a, p3b, ncol=2)
```




**Post Fit Interaction Plots**

```
library(emmeans)
library(ggplot2)
library(gridExtra)
# Interaction Plot Y=growth  X=LIGHT:  separately by TEMP
# emmip(FITOBJECT, STRATIFYVAR ~ XVAR)
p4a <- emmip(aov3, temp ~ light) +
       geom_jitter(aes(x = light, y = growth, colour = temp),
                   data = fishgrowth, pch = 4, width = 0.1) +
       labs(y = "Estimated Marginal Mean Fish Growth", x="Light", colour = "") +
       ggtitle("Estimated Mean Fish Growth with Light \nby Water Temperature") +
       theme_bw() +
       theme(axis.title = element_text(size = 8),
             plot.title = element_text(size = 8, face = "bold"))
```

```
# Interaction Plot Y=growth  X=TEMP:  separately by LIGHT
# emmip(FITOBJECT, STRATIFYVAR ~ XVAR)
p4b <- emmip(aov3, light ~ temp) +
       geom_jitter(aes(x = temp, y = growth, colour = light),
              data = fishgrowth, pch = 4, width = 0.1) +
       labs(y = "Estimated Marginal Mean Fish Growth", x="Temp", colour = "") +
       ggtitle("Estimated Mean Fish Growth with Temp \nby Light") +
       theme_bw() +
       theme(axis.title = element_text(size = 8),
                 plot.title = element_text(size = 8, face = "bold"))

gridExtra::grid.arrange(p4a, p4b, ncol=2)
```




**Summary - The analysis of variance table tells us the following.**

1. Fail to reject the hypothesis of no interaction. Thus tests for main effects using the MSE are meaningful.

2. The statistical test of the null hypothesis of no main effect of temperature is borderline significant.

3. The statistical test of the null hypothesis of no maing effect of light is also borderline significant.

4. Bottom line? NOT ENOUGH SAMPLE SIZE TO DO MUCH HERE…

## c. The Two Way Hierarchical (Nested) Design

**Example -**
An investigator wishes compare nitrogen levels in leaves by treatment at 3 levels. Each treatment is applied to 6 leaves of 4 trees. Thus, the total number of observations is (3 treatments)(4 trees)(6 leaves) = 72

In an example such as this, sampling is done in multiple stages. Here – (1) In stage 1, a random sample of trees is selected (thus "tree" is the primary sampling unit) and (2) in stage 2, within each tree, a random sample of leaves is selected for measurement.

Outcome X = Nitrogen concentration in the leaf
Group variable is tree
Primary sampling unit is leaf (and leaf is nested within tree)

Following are the data

Tree, j (nested within spray)

| | (1)j=1 | (1)j=2 | (1)j=3 | (1)j=4 |
|---|---|---|---|---|
| i=1 Spray 1 | **4.50** | **5.78** | **13.32** | **11.59** |
| | **7.04** | **7.69** | **15.05** | **8.96** |
| | **4.98** | **12.68** | **12.67** | **10.95** |
| | **5.48** | **5.89** | **12.42** | **9.87** |
| | **6.54** | **4.07** | **10.03** | **10.48** |
| | **7.20** | **4.08** | **13.50** | **12.79** |

Tree (nested within spray) – a different set of 4 trees!

| | (2)j=1 | (2)j=2 | (2)j=3 | (2)j=4 |
|---|---|---|---|---|
| i=2 Spray 2 | **15.32** | **14.53** | **10.89** | **15.12** |
| | **14.97** | **14.51** | **10.27** | **13.79** |
| | **14.81** | **12.61** | **12.21** | **15.32** |
| | **14.26** | **16.13** | **12.77** | **11.95** |
| | **15.88** | **13.65** | **10.45** | **12.56** |
| | **16.01** | **14.78** | **11.44** | **15.31** |

Tree (nested within spray) - a different set of 4 trees!

| | (3)j=1 | (3)j=2 | (3)j=3 | (3)j=4 |
|---|---|---|---|---|
| i=3 Spray 3 | **7.18** | **6.70** | **5.94** | **4.08** |
| | **7.98** | **8.28** | **5.78** | **5.46** |
| | **5.51** | **6.99** | **7.59** | **5.40** |
| | **7.48** | **6.40** | **7.21** | **6.85** |
| | **7.55** | **4.96** | **6.12** | **7.74** |
| | **5.64** | **7.03** | **7.13** | **6.81** |

**In this two-way hierarchical (nested) design,**

| | |
|---|---|
| I = # treatments. | In this example, I=3 |
| J = # primary sampling units | In this example, J=4 |
| n = # secondary sampling units, nested | In this example, n=6 |

- Only one factor is of interest – Treatment (spray) at 3 levels.
- The effect of tree is random and is *not* of interest.
- Similarly, the effect of leaf is random and is *not* of interest.

## The Two Way Hierarchical (Nested) Design Model

Setting:

I groups or treatments indexed i= 1, 2, …. I
J primary sampling units nested within each treatments and indexed j=1, 2, …., J
Sample size is n in each treatment x block combination; these are *secondary sampling* units
k indexes the secondary sampling units and are indexed k = 1, 2, …., n
$X_{ijk}$ = Observation for the $k^{th}$ secondary sampling unit of the jth primary sampling unit in the ith group/treatment

The two way hierarchical (nested) design model of $X_{ijk}$ is defined as follows:

$$X_{ijk} = \mu + \alpha_i + b_{(i)j} + \varepsilon_{(ij)k}$$

where

$\mu$ = grand mean

$$\alpha_i = [\mu_{i.} - \mu] \text{ and } \sum_{i=1}^{I} \alpha_i = 0$$

**and**

$b_{(i)j}$ is random and distributed Normal$(0,\sigma_b^2)$

$\varepsilon_{ij}$ is random error distributed Normal$(0,\sigma^2)$

$b_{(i)j}$ and $\varepsilon_{(ij)k}$ are mutually independent

## Two Way Hierarchical (Nested) Analysis of Variance

The nested model, *for the reason of having random effects*, looks a little different from a fixed effects model.

$\mu_i$ = Mean [ Outcome ] for spray "i"

$= \mu + \alpha_i$

$X_{ijk} = \mu + \alpha_i + b_{(i)j} + \varepsilon_{(ij)k}$ where

- The parenthesis notation "(i)j" tells us that tree "j" is nested in spray "i"
- The parenthesis notation "(ij)k" tells us that leaf "k" is nested in the "jth" tree receiving spray = "i"

$$\alpha_i = [\mu_{i.} - \mu] \text{ and } \sum_{i=1}^{I} \alpha_i = 0$$

$$X_{ijk} = \bar{X}_{..} + [\bar{X}_{i..} - \bar{X}_{...}] + [\bar{X}_{ij.} - \bar{X}_{i..}] + [X_{ijk} - \bar{X}_{ij.}] \quad \textit{algebraic identity}$$

### Assumptions

The $b_{(i)j}$ are independent and distributed Normal(0, $\sigma_b^2$)

The $\varepsilon_{(ij)k}$ are independent and distributed Normal(0, $\sigma_e^2$)

The $b_{(i)j}$ and $\varepsilon_{(ij)k}$ are mutually independent

### Total SSQ and its Partitioning

$$X_{ijk} = \bar{X}_{..} + [\bar{X}_{i..} - \bar{X}_{...}] + [\bar{X}_{ij.} - \bar{X}_{i..}] + [X_{ijk} - \bar{X}_{ij.}] \rightarrow$$

$$[X_{ijk} - \bar{X}_{..}] = [\bar{X}_{i..} - \bar{X}_{...}] + [\bar{X}_{ij.} - \bar{X}_{i..}] + [X_{ijk} - \bar{X}_{ij.}].$$

**Squaring both sides and summing over all observations yields** (because the cross product terms sum to zero!)

$$\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}[\,X_{ijk} - \bar{X}_{...}\,]^2$$

$$= \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}[\,\bar{X}_{i..} - \bar{X}_{...}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}[\,\bar{X}_{ij.} - \bar{X}_{i..}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}[\,X_{ijk} - \bar{X}_{ij.}\,]^2$$

$$= Jn\sum_{i=1}^{I}[\,\bar{X}_{i..} - \bar{X}_{...}\,]^2 + n\sum_{i=1}^{I}\sum_{j=1}^{J}[\,\bar{X}_{ij.} - \bar{X}_{i..}\,]^2 + \sum_{i=1}^{I}\sum_{j=1}^{I}\sum_{k=1}^{n}[\,X_{ijk} - \bar{X}_{ij.}\,]^2$$

## Analysis of Variance Table

| Source | df[a] | Sum of Squares | E (Mean Square) | F |
|---|---|---|---|---|
| Due <u>treatment</u> | ( I-1 ) | $Jn\sum_{i=1}^{I}\left(\bar{X}_{i..}-\bar{X}_{...}\right)^2$ | $\sigma_e^2 + n\sigma_b^2 + Jn\left[\frac{\sum_{i=1}^{I}\alpha_i^2}{(I-1)}\right]$ | $F=\frac{MSQ_{treatment}}{MSQ_{within\ treatment\ among\ samples}}$ <br> df = (I-1), I(J-1) |
| *Within treatment* Among <u>samples</u> | I (J-1) | $n\sum_{i=1}^{I}\sum_{j=1}^{J}\left(\bar{X}_{ij.}-\bar{X}_{i..}\right)^2$ | $\sigma_e^2 + n\sigma_b^2$ | $F=\frac{MSQ_{within\ treatment\ among\ samples}}{MSQ_{residual}}$ <br> df = (J-1), IJ(n-1) |
| Residual | IJ(n-1) | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}\left(\bar{X}_{ijk} - \bar{X}_{ij.}\right)^2$ | $\sigma_e^2$ | |
| Total | IJn – 1 | $\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{n}\left(X_{ijk}-\bar{X}_{...}\right)^2$ | | |
| | | | | |

[a] degrees of freedom

*Note:* The correct F test for treatment has in the denominator the mean square for within treatment among samples. This can be appreciated as the correct definition by looking at the expected mean squares.