# ANOVA (Analysis of Variance)

### One way ANOVA

#### **ANOVA: Introduction**

- Many studies involve comparisons between more than two groups of subjects.
- If the outcome is numerical, ANOVA can be used to compare the means between groups.
- ANOVA is an abbreviation for the full name of the method: ANalysis Of Variance - Invented by R.A. Fisher in the 1920's

#### **ANOVA: Introduction**

- > The response variable is the variable you're comparing
- The factor variable is the categorical variable being used to define the groups
- we will assume k samples (groups)
- The one-way is because each value is classified in exactly one way
- Examples include comparisons by gender, race, political party, color, etc.

#### **ANOVA Assumptions**

- The observations are from a random sample and they are independent from each other
- The observations are normally distributed within each group
- ANOVA is still appropriate if this assumption is not met when the sample size in each group is at least 30.
- It is not required to have equal sample sizes in all groups.
- > The variances are approximately equal between groups
- If the ratio of the largest SD / smallest SD < 2, this assumption is considered to be met.

#### Why ANOVA instead of multiple t-tests

If you are comparing means between more than two groups, why not just do several two sample t-tests to compare the mean from one group with the mean from each of the other groups?

- Before ANOVA, this was the only option available to compare means between more than two groups.
- > The problem with the multiple t-tests approach is that as the number of groups increases, the number of two sample t-tests also increases.
- As the number of tests increases the probability of making a Type I error also increases.

## ANOVA: a single test for multiple comparisons

- The advantage of using ANOVA over multiple t-tests is that ANOVA will identify if any two of the group means are significantly different with a single test.
- If the significance level is set at 0.05, the probability of a Type I error for ANOVA = 0.05 regardless of the number of groups being compared.
- If the ANOVA F-test is significant, further comparisons can be done to determine which groups have significantly different means.

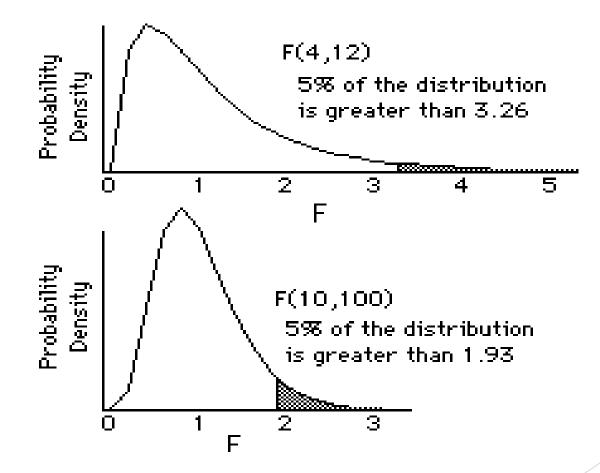
#### **ANOVA Hypotheses**

The Null hypothesis for ANOVA is that the means for all groups are equal:

$$H_{_{0}}: \mu_{_{1}} = \mu_{_{2}} = \mu_{_{3}} = \cdots = \mu_{_{k}}$$

- > The Alternative hypothesis for ANOVA is that at least two of the means are not equal.
- > The test statistic for ANOVA is the ANOVA F-statistic.

#### F table

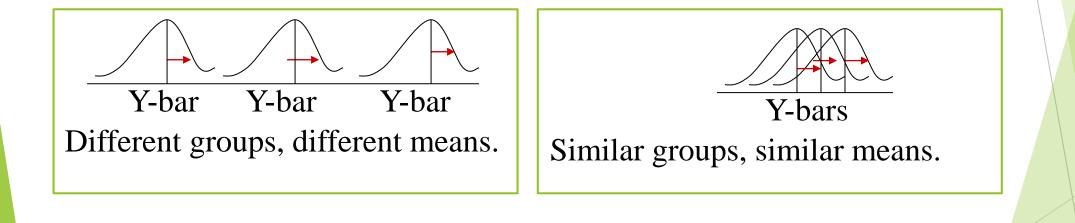


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#### Analysis of Variance

- ANOVA is used to compare means between three or more groups, so why is it called Analysis of VARIANCE?
- The ANOVA F-test is a comparison of the average variability between groups to the average variability within groups.
- The variability within each group is a measure of the spread of the data within each of the groups.
- The variability between groups is a measure of the spread of the group means around the overall mean for all groups combined.
- F = average variability between groups average variability within groups

#### Analysis of Variance



#### **ANOVA: F-statistic**

- > If variability between groups is large relative to the variability within groups, the F-statistic will be large.
- > If variability between groups is similar or smaller than variability within groups, the F-statistic will be small.
- > If the F-statistic is large enough, the null hypothesis that all means are equal is rejected.

#### Total variation in two parts

- The difference of each observation from the overall mean can be divided into two parts:
- The difference between the observation and the group mean
- The difference between the group mean and the overall mean
- > ANOVA makes use of this partitioned variability.

#### SST = SSW + SSB

- This partitioned relationship is also true for the squared differences:
- The variability between each observation and the overall (or grand) mean is measured by the 'sum of squares total' (SST)
- The variability within groups is measured by the 'sum of squares within' (SSW).  $\sum_{i=1}^{k} (n_i 1) s_i^2$ .
- The variability between groups is measured by the 'sum of squares between' (SSB).  $\sum_{i=1}^{k} n_i (\bar{x}_i \bar{x})^2$ ,

#### Mean Square Within and Mean Square Between

- The mean squares are measures of the average variability and are calculated from the sum of squares divided by the degrees of freedom.
- > MSW = SSW/(N-j)
- MSW has N-j degrees of freedom where N= total number of observations and j= # groups
- ➤ MSB = SSB / (j-1)
- MSB = j-1 degrees of freedom where j= number of groups
- F-statistic = MSB / MSW

One-Way	/ ANOVA Su	immary Tab	le	
Source of	Degrees	Sum of	Mean	F
Variation	of Freedom	Squares	Square (Variance)	
Treatment	p - 1	SST	MST = SST/(p - 1)	MST MSE
Error	n - p	SSE	MSE = SSE/(n - p)	
Total	n - 1	SS(Total) = SST+SSE		

#### ANOVA example:

mobility The hypothetical data below represent mobility scores (higher score indicates improved mobility) for 3 groups of patients: Control group did not receive any therapy Treatment group 1 received physical therapy, Treatment group 2 received counseling and physical therapy. Assume that the mobility scores are normally distributed.

Control	Treat #1	Treat #2
35	38	47
38	43	53
42	45	42
34	52	45
28	40	46
39	46	37

#### ANOVA

- > State the Hypotheses
- Null Hypothesis:  $\mu$  control =  $\mu$  trt1 =  $\mu$  trt2
- Alternative Hypothesis: at least two of the means ( $\mu$  control ,  $\mu$  trt1 ,  $\mu$  trt2 ) are not equal
- ANOVA is always a two-sided test
- ANOVA will identify if at least two means are significantly different but will not identify which two (or more) means are different.
- > Set the significance level  $\alpha$  = 0.05.

# Check for Equality of Variance between groups

- Calculate the SD for each group
- Control: 4.86
- Trt 1: 4.94
- Trt 2: 5.33
- Calculate the ratio:
- largest SD / smallest SD = 5.33 / 4.86 = 1.1
- Since the ratio < 2, assume equality of variance between groups.

#### Calculate Overall Mean and Group Means

- > Overall mean = average of all 18 observations = 41.7
- Group means = average of the observations in each group
- Control mean = 36
- Treatment 1 mean = 44
- Treatment 2 mean = 45

### Calculate the Within Sum of Squares: SSW

- Square the difference between each observation and it's group mean and sum the 18 terms.
- > The SSW for the control group (mean = 36)
- (35 36)2 + (38 36)2 + (42 36)2 + (34 36)2 + (28 36)2 + (39 36)2 = 118
- > The SSW for the treatment 1 group (mean = 44)
- (38 44)2 + (43 44)2 + (45 44)2 + (52 44)2 + (40 44)2 + (46 44)2 = 122
- > The SSW for the treatment 2 group (mean = 45)
- (47 45)2 + (53 45)2 + (42 45)2 + (45 45)2 + (46 45)2 + (37 45)2 = 142
- SSW = sum of SSW for each group = 118 + 122 + 142 = 382

### Calculate the Between Sum of Squares: SSB

- The overall mean = 41.7
- > The three group means are:
- Control: mean = 36
- Treatment 1: mean = 44
- Treatment 2: mean = 45
- For each group square the difference between the group mean and the overall mean and multiply by the group sample size, then sum these 3 terms for the SSB:
- SSB =  $6^{*}(36 41.7)2 + 6^{*}(44 41.7)2 + 6^{*}(45 41.7)2 = 292$

#### Calculate MSB and MSW

- The Mean Square Between (MSB) is the average variability between groups
- MSB = SSB /( j-1) SSB = 292, j = 3
- MSB = 292 / (3-1) = 146
- The Mean Square Within (MSW) is the average variability within groups:
- MSW = SSW / (N-j)
- SSW = 382, N = 18, j= 3
- MSW = 382 / (18 3) = 25.47

#### Calculate the ANOVA F-statistic

- The ANOVA F-statistic = MSB/MSW
- > The ANOVA F-statistic will be large when there is more variability between the groups than within the groups.
- If the variability between groups and within groups is approximately equal the ANOVA F-statistic will be small (close to 1.0)
- > The Null hypothesis of equal means between groups is rejected if the F-statistic is large enough.
- > ANOVA F-statistic for example = 146/25.47 = 5.73

#### Sampling Distribution of ANOVA Fstatistic

- The sampling distribution of the ANOVA F-statistic is the Fdistribution
- non-negative since all F-statistics are positive.
- indexed by two degrees of freedom
- Numerator df = number of groups minus 1 (j-1)
- Denominator df = total sample size minus number of groups (N-j)
- The shape of the F-distribution varies depending on the two degrees of freedom

#### Find the p-value of the ANOVA F-statistic

- The p-value of the ANOVA F-statistic is the right tail area greater than the F-statistic under the F-distribution with (num. df, den. df)
- > The F-statistic for the example data = 5.73
- > The df for the F-distribution are (3-1) = 2 for the numerator and (18-3) = 15 for the denominator.
- the p-value = 0.014

#### Decision about the Hypotheses

- Since the p-value of 0.014 < the significance level of 0.05, the null hypothesis of equality between all three group means is rejected.
- > We can conclude that that AT LEAST two of the means are significantly different.
- How many of the means are significantly different? Which of the means are different?
- > Post-hoc tests are done to answer these questions.

#### ANOVA: post-hoc comparisons

- A significant ANOVA F-test is evidence that not all means are equal but it does not identify which means are significantly different.
- Methods used to find group differences after the ANOVA null hypothesis has been rejected are called post-hoc tests.
- Post-hoc is Latin for 'after-this'
- Post-hoc comparisons should only be done when the ANOVA F-test is significant.

#### Adjustments for Multiple Comparisons

- When multiple comparisons are being done it is customary to adjust the significance level of each individual comparison so that the overall experiment significance level remains at 0.05
- For an ANOVA with 3 groups, there are 3 combinations of t-tests.
- A conservative adjustment (Bonferroni adjustment) is to divide 0.05/3 so that alpha for each test = 0.017. Each comparison will be significant if the p-value < 0.017</p>

#### Post-hoc comparison: Control and Treatment 1

- Results of the two-sample t-test to compare mean mobility scores between the control group and treatment 1 group:
- Control mean score = 36
- Treatment 1 mean score = 44
- P-value for two-sample t-test = 0.0179
- This is not significant at the adjusted α-level of 0.017 but would be considered marginally significant since it is close to 0.017.
- Conclusion: After adjusting for multiple comparisons, the control group mean mobility score is marginally significantly less than the mean mobility score for the group that received physical therapy (p = 0.0179).

### Post-hoc comparison: Control and Treatment 2

- > A two-sample t-test is done to compare mean mobility scores between the control group and treatment 2 group:
- Control mean score = 36
- Treatment 2 mean score = 45
- P-value for two-sample t-test = 0.012
- > This is a significant difference at the adjusted  $\alpha$ -level of 0.017
- Conclusion: After adjusting for multiple comparisons, the control group mean mobility score is significantly less than the mean mobility score for the group that received physical therapy and counseling (p = 0.012).

### Post-hoc comparison: Treatment 1 and Treatment 2

- > A two-sample t-test is done to compare mean mobility scores between the two treatment groups.
- Treatment 1 mean score = 44
- Treatment 2 mean score = 45
- P-value for two-sample t-test = 0.743
- There is not a significant difference between the two treatment groups (at either the adjusted or un-adjusted level).
- Conclusion: There is no significant difference in mean mobility score between the two treatment groups (p = 0.743).

#### **ANOVA** summary

ANOVA was done to evaluate differences in mean mobility score between three groups: a control group, a group that received physical therapy only and a group that received physical therapy and counseling. The significant ANOVA F- test result indicated that at least two of the mean mobility scores were significantly different. Post-hoc t-tests with adjusted  $\alpha$ -level = 0.017 (Bonferroni adjustment for multiple comparisons) were done. Results of the post-hoc comparisons indicated a significant difference between the control group and the treatment group with both physical therapy and counseling (p = 0.012), a marginally significant difference between the control group and the treatment group with physical therapy only (p = 0.0179) and no significant difference between the two treatment groups.

#### Multiple Comparison Tests

- > Bonferroni procedure
- > Duncan Multiple range test
- > Dunnett's multiple comparison test
- Newman-Keuls test
- Scheffe's test
- > Tukey's test
- Holm t-test

#### **Other ANOVA Procedures**

- > One-way ANOVA is Analysis of Variance for one factor
- More than one factor can be used for a two, three or four-way ANOVA
- > A continuous variable can be added to the model
- this is Analysis of Covariance (ANCOVA)
- Repeated Measures ANOVA can handle replicated measurements on the same observation unit (subject)

### Two way ANOVA

#### Factorial Design

- > Experimental Units (Subjects) Are Assigned Randomly to Treatments
  - Subjects are Assumed Homogeneous
- > Two or More **Factors** or Independent Variables
  - Each Has 2 or More Treatments (Levels)
- > Analyzed by Two-Way ANOVA

#### The Two-way ANOVA

- We need to test for the *independent* and *combined* effects of multiple variables on performance. We do this with an ANOVA that asks:
  - (i) how different from each other are the means for levels of Variable A?
  - (ii) how different from each other are the means for levels of Variable B?
  - (iii) how different from each other are the means for the treatment *combinations* produced by A and B together?

#### The Two-way ANOVA

The first two of those questions are questions about main effects of the respective independent variables.

The third question is about the *interaction* effect, the effect of the two variables considered simultaneously.

#### The Two-way ANOVA

#### > Main effect

• A main effect is the effect on performance of one treatment variable considered in isolation (ignoring other variables in the study)

#### Interaction

• an interaction effect occurs when the effect of one variable is different across levels of one or more other variables

#### Two-way ANOVA - hypothesis test for A

 $H_0$ : No difference among means for levels of A  $H_A$ : At least two A means differ significantly

Test statistic: 
$$F = MS_A$$
  
 $MS_E$ 

**Rej. region:** 
$$F_{obt} < F_{(2, 12, .05)} = 3.89$$

Decision: Reject  $H_0$  - variable A has an effect.

Two Way ANOVA

#### Two-way ANOVA - hypothesis test for B

 $H_0$ : No difference among means for levels of B  $H_A$ : At least two B means differ significantly

Test statistic: 
$$F = MS_B$$
  
 $MS_E$ 

**Rej. region:** 
$$F_{obt} < F_{(1, 12, .05)} = 4.75$$

Decision: Reject  $H_0$  - variable B has an effect.

Two Way ANOVA

#### Two-way ANOVA - hypothesis for AB

 $H_0$ : A & B do not interact to affect mean response  $H_A$ : A & B do interact to affect mean response

Test statistic: 
$$F = MS_{AB}$$
  
 $MS_{E}$ 

Rej. region: 
$$F_{obt} < F_{(2, 12, .05)} = 3.89$$

Decision: Reject H<sub>0</sub> - A & B do interact...

Two Way ANOVA

### Two-Way ANOVA without replication Summary Table

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	
A (Row)	a - 1	SS(A)	MS(A)	MS(A) MSE	
B (Column)	b - 1	SS(B)	MS(B)	MS(B) MSE	
Error	(a-1)(b-1)	SSE	MSE		
Total	n - 1	SS(Total)			

#### Two-Way ANOVA with replication Summary Table

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
A (Row)	a - 1	SS(A)	MS(A)	MS(A) MSE
B (Column)	b - 1	SS(B)	MS(B)	MS(B) MSE
AB (Interaction)	(a-1)(b-1)	SS(AB)	MS(AB)	MS(AB) MSE
Error	n - ab	SSE	MSE	
Total	n - 1	SS(Total)		