# Analysis of Variance (ANOVA)

# Review: Two-Sample T-Test

- Comparison between two groups
  - √ (two groups in a categorical variable like Female/Male or Hispanic/non-Hispanic)
- Observed samples from each population
- Assume underlying normality in each population
- Use rank-based methods when not normal

#### **Notes:**

• Analysis of Variance (ANOVA) is an **extension** of the two-sample t-test.

### Limitations of T-Test

- Single classification variable with only two groups
  - ✓ What if there are more than two groups?
  - ✓ What about the case of more than one categorical variable?

- ANOVA enables testing with more than one categorical variable and more than two groups
  - o Example: With ANOVA, we can study the effect of gender and race on salary at the **same** time.
- ANOVA enables you to compare the **mean** values for more than 3 levels

### When do we use ANOVA model?

- Setting: Continuous response & Categorical (grouping) variables
- Goal: Analyze the difference among groups and study the behaviors of response variable depending on grouping variable
  - (E.g.) we are interested in blood sugar (continuous);
- Variable1: treatment (placebo/ treatment1/ treatment2)
- Variable2: diet (vegetarian/ vegan/ else)
- Variable3: exercise (<1 hr/ between 1 and 3 hrs/ >3 hrs)

#### Want to answer:

- Does type of treatment (or diet or exercise) affect blood sugar?
- If so, which treatment is the most efficient?
- Does diet help to decrease blood sugar?

- **Continuous response** = variable of interest (ex. Salary, Blood Sugar)
- **Categorical Variables** = grouping variables (ex. Race / Gender, Treatment / Diet / Exercise)

### **ANOVA** model

Kind of extension of two-sample t-test

- Compare means of two groups
- T-test can be applied only when **both** groups follow **normal** (parametric test)
- Two types of t-test under equal variance or unequal variance assumption



#### Similar in ANOVA test

- Can compare means from more than three groups
- Assumptions for classic ANOVA (again, parametric test):
  - \*\*Normality for all groups, equal variances, iid sample\*\*
  - More specific statement at slide 11
- Modified test when groups have different variances (welch's ANOVA)

- iid = independent samples
- Follows normal distribution? = **Classic ANOVA** | Doesn't follow normal distribution? = **Welch's ANOVA**

### **Definitions**

- One-way analysis of variance: ANOVA based on a single categorical predictor variable
- Two-way analysis of variance: based on 2 independent categorical predictor variables
- N-way analysis of variance: based on *n* independent categorical variables

- One-way ANOVA = Salary based on Race
- Two-way ANOVA = Salary based on Gender and Race
- N-way ANOVA = Salary based on Gender, Race, and Age

**Interaction (Synergy) Effect Example:** Combination of Caramel and Cheese variables

- **Response**: How much popcorn that gets eaten (g / oz)
- Cheese: No cheese (Level 0) | Cheese (Level 1)
- **Caramel**: No caramel (Level 0) | Caramel (Level 1)

- Main effect: effect of single categorical predictor
- Interaction: the combined effect of combination of categorical predictors -> for example, synergy effect

Cheese

10

20

0

Caramel

20

70

g Eaten

Caramel

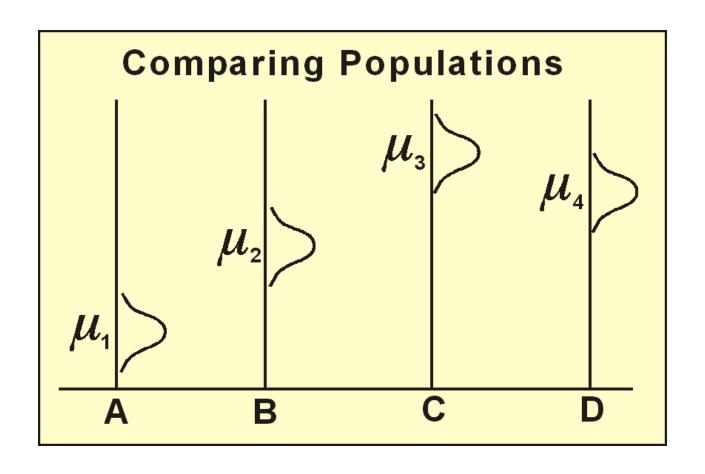
- First-order interaction: an interaction between two categorical predictors
- N-th order interaction: interaction of a categorical predictor with *n* other categorical predictors
  - hard interpretation or potential overfitting issue. In practice, include them only when needed
- Balanced data: data with an equal number of observations in each cell
- Unbalanced data: at least one cell has different number of observations

- Salary Example:
  - O Gender **main effect** or Race **main effect** (they effect salary **separately** depending on the level of Gender or Race)
  - Gender and Race **combined** effect on Salary (interaction effect)

<sup>\*</sup>Effect of one variable depends on the level of the other variable

<sup>\*</sup> Parallel lines = no interaction = one variable does NOT depend Definitions

# ANOVA assumption overview



# **ANOVA Hypotheses**

- Null Hypothesis: There are no mean differences between the groups on response
  - O H0:  $μ_1 = μ_2 = ... = μ_g$ , where g is the number of groups.
    - ➤ E.g., means of salary are same regardless of different education levels
- Alternate Hypothesis:
  - H1: <u>At least ONE of the group</u> means is significantly different from the others in the population

NOTE: But we do not know which group has larger or smaller mean

# **ANOVA Hypotheses**

• For the interactions: (for multi-way ANOVA like 2-way, 3-way ....)

We can also test the null hypothesis for interactions.

Null hypothesis:

H0: There is no interaction between independent variables in the population.

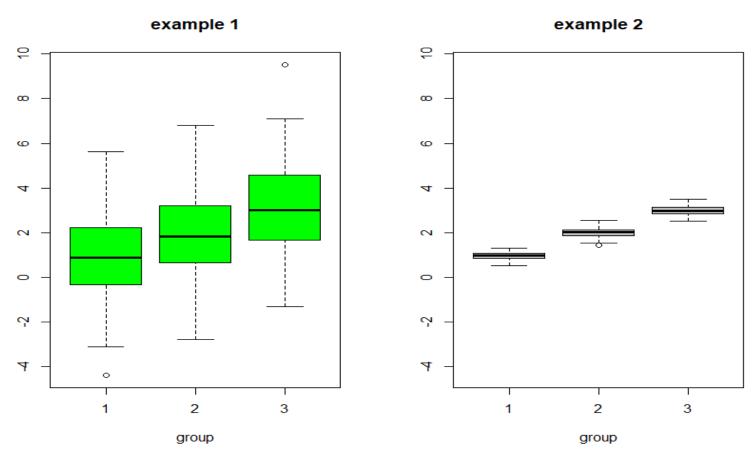
Alternative hypothesis:

H1: There is an interaction between independent variables in the population.

# Assumptions of ANOVA

- 1) The response (dependent) variable is continuous
- Populations from which samples were drawn follow normal distribution
  - i.e., Each group should be normally distributed
  - ✓ Note: ANOVA relatively robust to violations of normality
- 3) Populations from which samples were drawn must have equal variances (Homogeneity of Variance)
  - ✓ Need to perform equal variance test before applying ANOVA
- 4) Observations must be independent of one another

### The F test



- Between group mean variation (differences) are same for both examples
- How about within group variation?

### The F test

- Use F-test when all assumptions are satisfied
- The F test uses the F <u>statistic</u> to determine if there are any significant main effects or interactions
- Formula and Intuition:

F = Between groups variation/ Within group variation

Make a conclusion based on p-values of F-test

### The F test

• If the F-statistic <u>is NOT statistically significant</u>, then you are done and there is no reason to conduct additional analyses. No difference among groups is found.

- If the F-statistic is <u>statistically significant</u>:
  - All you know now is that there is at least one mean that differs from the another.
    - √ To determine which mean(s) differ, you need to conduct post-hoc
      test
    - ✓ Able to get the information which group has significantly larger of smaller mean value

# Example: ToothGrowth

- Response: Tooth length (continuous variable)
- Supplement: VC or OJ
- Dose: 0.5, 1 or 2
  - ✓ Should be coded as a **factor** not as a numeric

# Example: One-way ANOVA

- Install package "car"
- Perform analysis of variance of Toothlength as a function of Dose
  - Check balanced of unbalanced
  - II. Run one-way ANOVA with aov() or possibly, Im()
  - III. Check equal variance assumption levene's test
    - H0: all groups have the same variances vs.
       Ha: at least one group has different variance
    - Could use Welch adjustment if equal variance assumption is not valid
  - IV. Check Normality assumption check diagnostics plot
    - o qq plot and residual plot
  - V. What is conclusion?

# Example: One-way ANOVA

- ANOVA table interpretation
- R-Square value for predictive power of the model
- Significance of **Dose** as a predictive variable
- Conclusion about impact of Dose on tooth growth

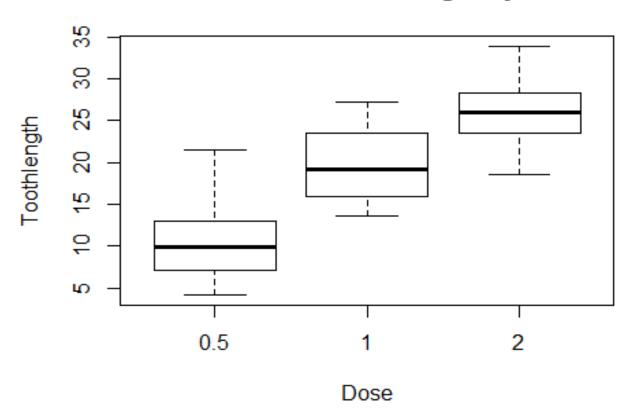
```
tooth$Dose= as.factor(tooth$Dose)
str(tooth)

## 'data.frame': 60 obs. of 3 variables:
## $ Toothlength: num 4.2 11.5 7.3 5.8 6.4 10 11.2 11.2 5.
2 7 ...
## $ Supplement : Factor w/ 2 levels "OJ", "VC": 2 2 2 2 2 2 2 2 2 2 2 ...
## $ Dose : Factor w/ 3 levels "0.5", "1", "2": 1 1 1 1 1 1 1 1 1 1 1 1 1 ...
```

#### Balance or Unbalanced?

boxplot(Toothlength ~ Dose, data=tooth, main="distributio
n of tooth length by dose")

#### distribution of tooth length by dose



```
aov.res= aov(Toothlength~Dose, data=tooth)
  summary(aov.res)
                 Df Sum Sq Mean Sq F value Pr(>F)
  ##
  ## Dose 2 2426
                             1213 67.42 9.53e-16 ***
  ## Residuals 57 1026
                               18
  ## ---
  ## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
Total sum of squares (3452)
(variation of Toothlength)
```

= sum of squares by Dose (2426) (variation of Toothlength explained by Dose)

+ sum of squares by Error (1026)

(variation of Toothlength not be explained by the model)

H0: Dose has no effect on tooth growth  $(\mu_{0.5} = \mu_1 = \mu_2)$ 

Ha: Does has an effect on tooth growth (at least one group in Dose has different mean of tooth length

## [1] 0.7028642

- To calculate R-square, need to run anova with Im()
- Results from Im() and aov() are exactly identical

```
lm.res= lm(Toothlength ~ Dose, data=tooth)
anova(lm.res)
## Analysis of Variance Table
##
## Response: Toothlength
            Df Sum Sq Mean Sq F value Pr(>F)
##
         2 2426.4 1213.2 67.416 9.533e-16 ***
## Dose
## Residuals 57 1025.8 18.0
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
' 1
                         R-square: percentage of variation in a
summary(lm.res)$r.squared
```

R-square: percentage of variation in a response variable that is explained by the model (Dose)

H0: all groups in Dose have the same variance Ha: at least one group has different variance

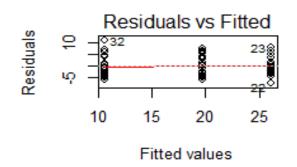
```
leveneTest(aov.res)
## Levene's Test for Homogeneity of Variance (center = median)
## Df F value Pr(>F)
## group 2 0.6457 0.5281
## 57
```

#### Welch's ANOVA – when homogeneity assumption is violated

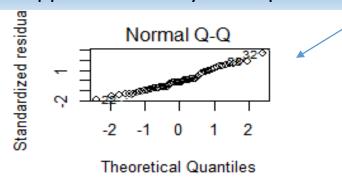
```
oneway.test(Toothlength ~ Dose, data=tooth, var.equal=FALSE)
##
## One-way analysis of means (not assuming equal variances)
##
## data: Toothlength and Dose
## F = 68.401, num df = 2.000, denom df = 37.743, p-value = 2.81
2e-13
```

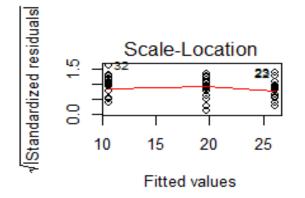
 Normality check – use diagnostics plot instead of rigorous shapiro test by group

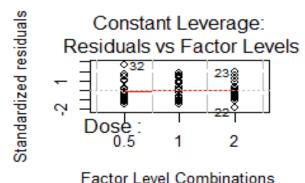
```
par(mfrow=c(2,2))
plot(aov.res)
```



If normal qq plot shows almost straight line, It supports normality assumption.







# Multiple Comparisons

- Pairwise comparison with two-sample t-test
  - If there are 3 groups, there will be total 3 comparisons
  - (group1 vs. group2), (group1 vs. group3) and (group2 vs. group3)
- Making many comparisons at once!!
- Need to account for increased probability of making wrong decision
- Need correction in calculating p-value from t-test
  - Scheffe method, Tukey's Method, etc.
- Should know how to interpret the result. What is null hypothesis and what kind of conclusion can we make?

```
Pairwise t-test with modified p-values:
ScheffeTest(aov.res)
                                 H0: \mu_1 = \mu_{0.5} vs. H1: \mu_1 \neq \mu_{0.5}
##
     Posthoc multiple comparisons of means: Scheffe Test
##
       95% family-wise confidence level
##
##
## $Dose
##
        diff lwr.ci upr.ci
## 1-0.5 9.130 5.758155 12.501845 4.3e-08
## 2-0.5 15.495 12.123155 18.866845 1.2e-15
          6.365 2.993155 9.736845 7.6e-05 ***
## 2-1
##
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Able to get information which pairs are significantly different Final conclusion:

All three different Dose have different effect on tooth length and specifically, Dose 2 > Dose 1 > Dose 0.5

```
TukeyHSD(aov.res)
    Tukey multiple comparisons of means
##
      95% family-wise confidence level
##
##
## Fit: aov(formula = Toothlength ~ Dose, data = tooth)
##
## $Dose
        diff
##
                     lwr
                                     p adj
                               upr
## 1-0.5 9.130 5.901805 12.358195 0.00e+00
## 2-0.5 15.495 12.266805 18.723195 0.00e+00
## 2-1 6.365 3.136805 9.593195 4.25e-05
```

- Different method but we can interpret the output in the same way
- In practice, Scheffe and Tukey are popular

# Example: Two-way ANOVA

- Two main effects (Dose, Supplement) and their interaction
- Interpret significance of model, terms, etc.
- Model validity check (check assumptions)
- Interpretation of Post-hoc test result

```
aov.res2 <- aov(Toothlength ~ Dose * Supplement , data = too th) 
 H0: Supplement has no effect on tooth growth (\mu_{OJ} = \mu_{VC}\,) Ha: Supplement has an effect on tooth growth (at least one group in supplement has different mean of tooth length; \mu_{OJ} \neq \mu_{VC}\,)
```

#### summary(aov.res2)

```
## Dose 2 2426.4 1213.2 92.000 < 2e-16 ***

## Supplement 1 205.4 205.4 15.572 0.000231 ***

## Dose:Supplement 2 108.3 54.2 4.107 0.021860 *

## Residuals 54 712.1 13.2

## ---

## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

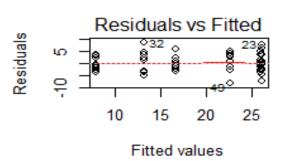
HO: no interaction between type and supplement
```

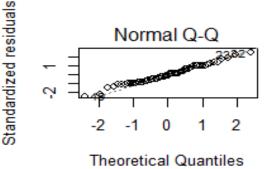
Ha: exist an interaction between type and supplement

```
leveneTest(aov.res2)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
## Df F value Pr(>F)
## group 5 1.7086 0.1484
## 54
```

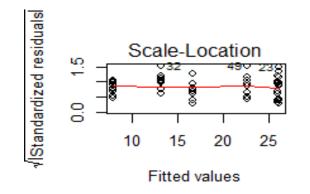
```
par(mfrow=c(2,2))
plot(aov.res2)
```

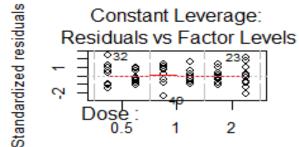




# Model validity check

- Homogeneity of variance?
- Normality?





Factor Level Combinations

```
lm.res2= lm(Toothlength ~ Dose * Supplement, data=tooth)
summary(lm.res2)$r.squared # R-square
## [1] 0.7937246
```

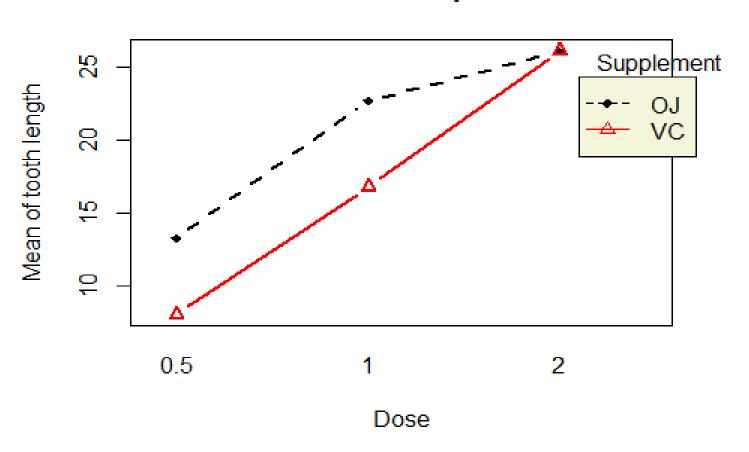
- Compare R-square from one-way ANOVA model (Dose)
- R-square always increases as the model gets bigger (larger number of independent variables)

ScheffeTest(aov.res2)

.... (omitted)

```
##
    Posthoc multiple comparisons of means: Scheffe Test
##
      95% family-wise confidence level
##
                                           Focus on post-hoc
##
                                           analysis for
## $Dose
                                           main effects
##
          diff lwr.ci upr.ci pval
## 1-0.5 9.130 5.16355 13.09645 3.8e-08 *
## 2-0.5 15.495 11.52855 19.46145 3.9e-16 *
                                         * Dose effect:
         6.365 2.39855 10.33145 0.00014
## 2-1
                                           Dose2>Dose1>Dose0.5
##
## $Supplement
##
        diff
                lwr.ci
                           upr.ci
                                    pval
                                           Supplement effect:
## VC-0J -3.7 -6.938593 -0.4614069 0.0153
                                           VC < OJ
##
## $`Dose:Supplement`
##
                  diff
                           lwr.ci
                                       upr.ci
                                                 pval
                  9.47
                                   15.0794079 5.5e-05
## 1:03-0.5:03
                         3.860592
```

#### Interaction plot



### Some notes:

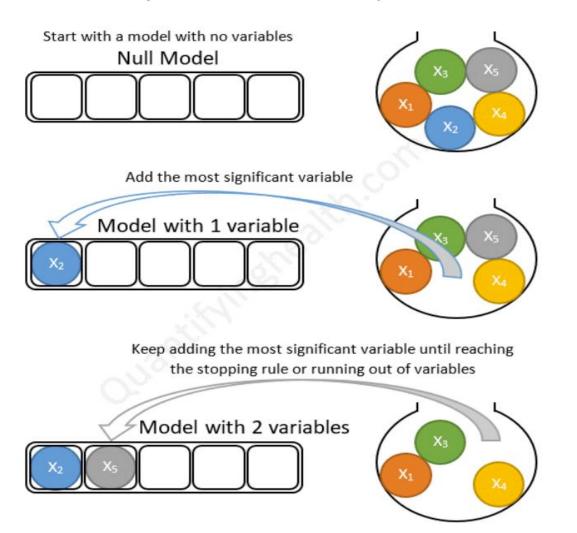
- Significance of model <-> R-square
   (0 or not) (prediction power)
- Model with higher R-square is always better?
  - O What about the model with R-square = 100%?
  - O What is the goal of the analysis?
- In post-hoc analysis, it can happen e.g.,  $\mu_{0.5}=\mu_1$  and  $\mu_{0.5}=\mu_2$  but  $\mu_1=\mu_2$ 
  - Why it happens and how can we make a conclusion?

### **Model Selection**

- For the case of n-way ANOVA, the largest model with all possible interactions has (2<sup>n</sup>-1) terms
- How to choose the best model?
- > Forward selection/ Backward elimination
- ➤ Stepwise selection (Backward + Forward)

### Forward selection

Forward stepwise selection example with 5 variables:



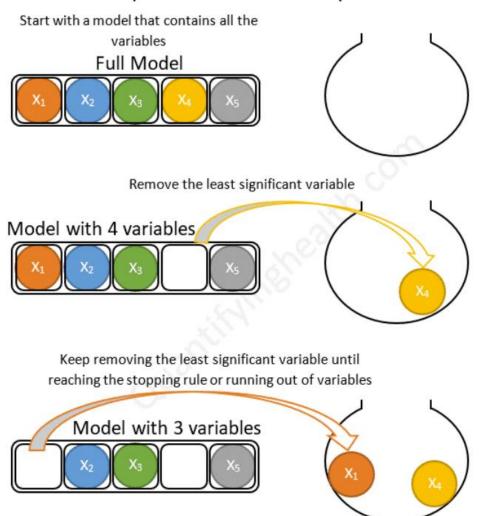
source: https://quantifyinghealth.com/stepwise-selection/

### Forward selection

- 1. Begins with a model that contains no variables (called the Null Model)
- 2. Then starts adding the most significant variables one after the other
- Until a pre-specified stopping rule is reached; Specifically, until there is no more variable which has p-value smaller than significance level (in general 0.05, but not necessarily)
- Once a variable is entered, there is no chance to be out
- Final model may include insignificant variables

### **Backward elimination**

Backward stepwise selection example with 5 variables:



source: https://quantifyinghealth.com/stepwise-selection/

### **Backward elimination**

- 1. Begins with a model that contains all variables under consideration (called the *Full Model*)
- 2. Then starts removing the least significant variables one after the other
- 3. Until a pre-specified stopping rule is reached no more variable with p-value greater than significance level (0.05 but not necessarily)
- Once a variable is eliminated, there is no chance to be in
- All variables in the final model are always significant

### **Model Selection**

- What should we do if interaction term (X1\*X2) is significant but main effect (X1 or X2) is not?
  - In practice, if main effects are not significant, we do not include interaction between them even if it is significant
  - 1. Forward/ backward/ stepwise selection on main effect model first
  - 2. Test interaction among significant main effects
- Use package "MASS" in R
  - Use AIC criteria instead of p-value, but idea is the same
  - AIC will be covered in linear regression

### **Practice**

- Using the grass.csv, let's start with a model that includes Method, Variety, and Group as independent variables and Yield as the response variable.
- Perform model selection
  - Backward elimination manually
  - Forward selection manually
  - Stepwise selection using stepAIC() in package "MASS"
- Find the final model from each approach