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
- 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**

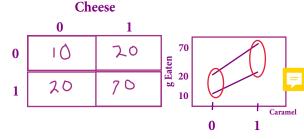
Definitions

- 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



- **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)

* Parallel lines = no interaction = one variable does NOT depend Definitions
level of other variable



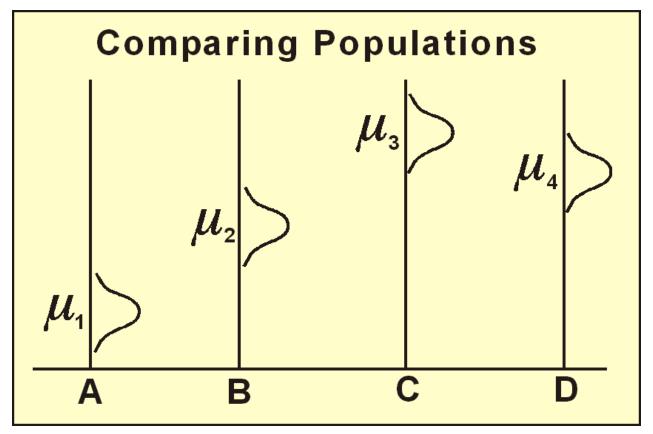
- Main effect: effect of single categorical predictor
- Interaction: the combined effect of combination of categorical predictors -> for example, synergy effect

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)
 - o Gender and Race **combined** effect on Salary (interaction effect)

^{*}Effect of one variable depends on the level of the other variable

ANOVA assumption overview



Four levels - The image represents a comparison of population means (locations) that all follow a normal distribution and have an equal variance.

ANOVA Hypotheses

- Null Hypothesis: There are no mean differences between the groups on response
 - \circ 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: At least ONE of the group means is significantly different from the others in the population
 - ➤ NOTE: But we do not know which group has larger or smaller mean
 - Reference hypothesis example and notes in Notebook app

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.

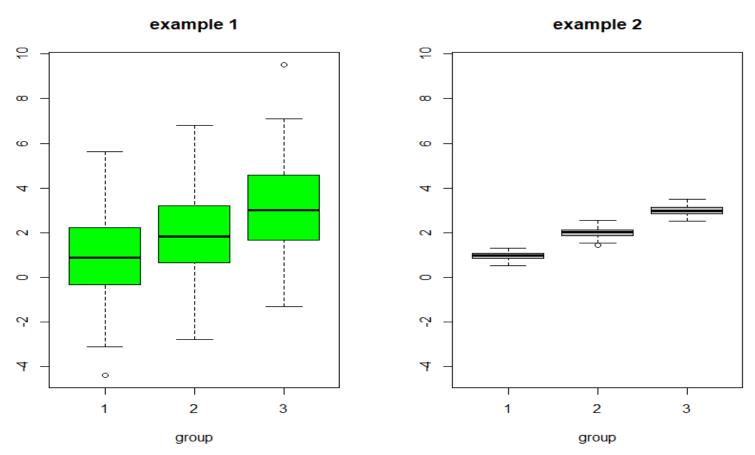
Alternative hypothesis:

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?
 - Reference notes in Notebook app

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
 - Small F Statistic: Do not reject the null, supports the null hypothesis.
 - Large F Statistic: Reject the null, supports the alternative hypothesis

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?

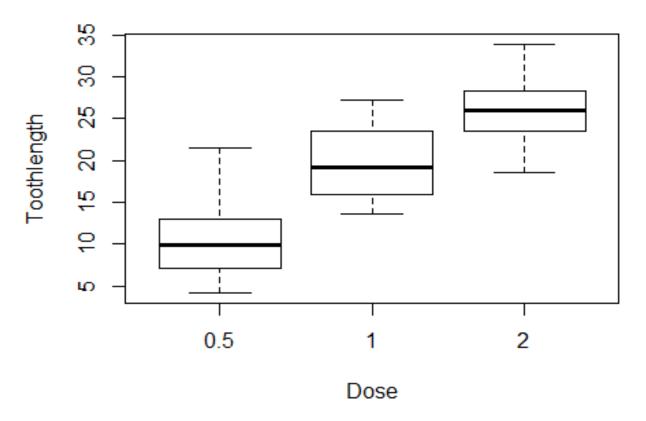
Example: One-way ANOVA

- 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

Balance or Unbalanced?

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

distribution of tooth length by dose



- Null Hypothesis: No Dose Effect
- Alternative Hypothesis: There exists a significant dose effect.
- Highlight = SS Model (Between Group Variation) Name of the Group Variable = Dose The variation of Y that can be explained by Dose aov.res= aov(Toothlength~Dose, data=tooth) summary(aov.res) o p-value calculated based on F statistic Df Sum Sq Mean Sq F value Pr(>F) ## 1213 67.42 **9.53e-16** *** 2426 ## Dose ## Residuals 57 **1026** 18 o 1026 - variation of Y that can't be explained by Dose ## ---0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ## Signif. codes:

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)

O Small p-value means we reject the Null Hypothesis. There exists a significant dose effect.

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

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

summary(lm.res)\$r.squared
[1] 0.7028642

o 70% of variation of Y can be explained by Dose.

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

One-way ANOVA example: o Null Hypothesis: Equal Variance Alternative Hypothesis: No Equal Variance

H0: all groups in Dose have the same variance

Ha: at least one group has different variance

Used for checking equal variance (up to 2 levels)

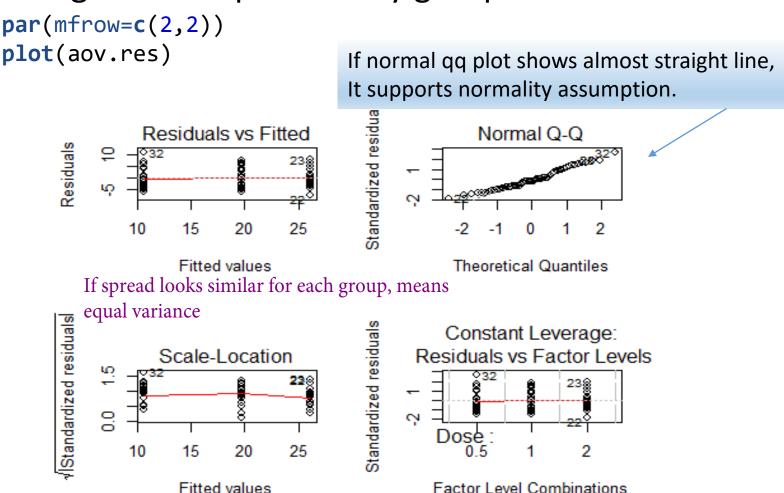
```
leveneTest(aov.res)
## Levene's Test for Homogeneity of Variance (center = median)
          Df F value Pr(>F)
##
## group 2 0.6457 0.5281
##
          57
                         larger p-value: Can't reject the null, therefore, Equal Variance
```

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
```

If the p-value is small, we reject the null and must perform a Welch's ANOVA

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



Multiple Comparisons

Post Hoc Test

- 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
 Because making multiple comparisons increases the probability of making
 the wrong decision, we need to make a correction in calculating p-value
- 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?

specifically, Dose 2 > Dose 1 > Dose 0.5

```
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
##
                                                      '>' means significantly different
##
           Dose 1 mean > Dose 0.5 mean; Dose 2 mean > Dose 0.5 mean; Dose 2 mean > Dose 1 mean
## $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 | *** Ho: \mu2 = \mu0.5 vs. H1: \mu2! = \mu0.5
            6.365 | 2.993155 | 9.736845 | 7.6e-05 | *** Ho: \mu2 = \mu1 vs. H1: \mu2! = \mu1
## 2-1
##
               diff: the estimated difference between the mean values of first group and second group
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
  All dose groups have significantly different mean values of tooth length.
  Able to get information which pairs are significantly different
   Final conclusion:
  All three different Dose have different effect on tooth length and
```

Reference notes from prior slide, same rules apply

```
TukeyHSD(aov.res)
    Tukey multiple comparisons of means
##
      95% family-wise confidence level
##
##
## Fit: aov(formula = Toothlength ~ Dose, data = tooth)
##
## $Dose
         diff
##
                     lwr
                               upr
                                      p adj
## 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_{OI} = \mu_{VC})
```

highlight: Refers to the variation of the tooth length that can be explained by the respective line item.

Ha: Supplement has an effect on tooth growth (at least one group in supplement has different mean of tooth length; $\mu_{OJ} \neq \mu_{VC}$)

Because the p-value is small for each line, we reject the null. Dose and Supplement

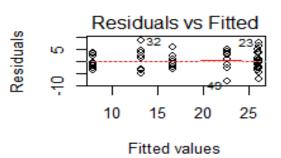
```
summary(aov.res2)
                          both have an effect on Tooth growth and there exists an interaction between dose and
                         supplement.
                     Df Sum Sq Mean Sq F value
##
                      2 2426.4 1213.2 92.000 < 2e-16
## Dose
                         205.4 205.4 15.572 0.000231
## Supplement
## Dose:Supplement
                         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
           H0: no interaction between type and supplement
```

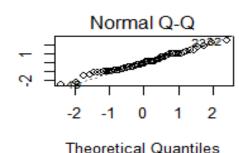
Ha: exist an interaction between type and supplement

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

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

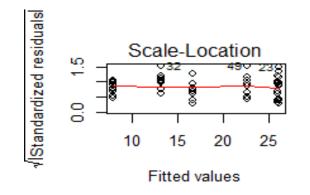
```
par(mfrow=c(2,2))
plot(aov.res2)
```

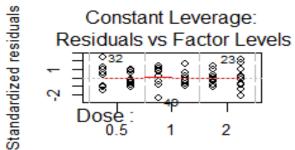




Model validity check

- 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
```

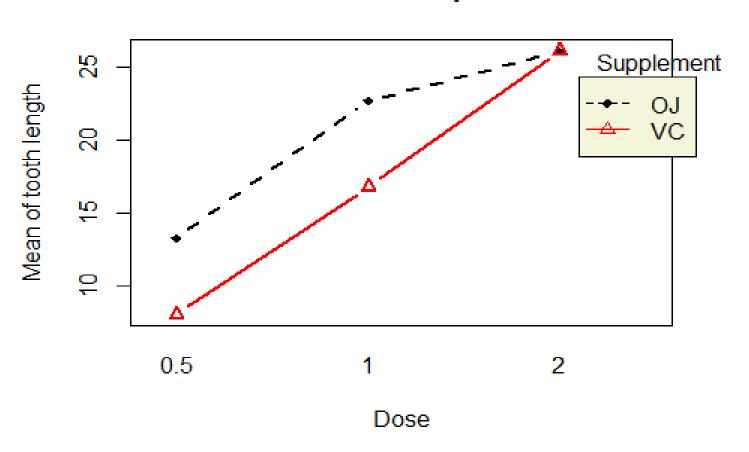
- Compare R-square from one-way ANOVA model (Dose)
- R-square always increases as the model gets bigger (larger number of independent variables)

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 $\mu_1 !=\mu_2$
 - O Why it happens and how can we make a conclusion?

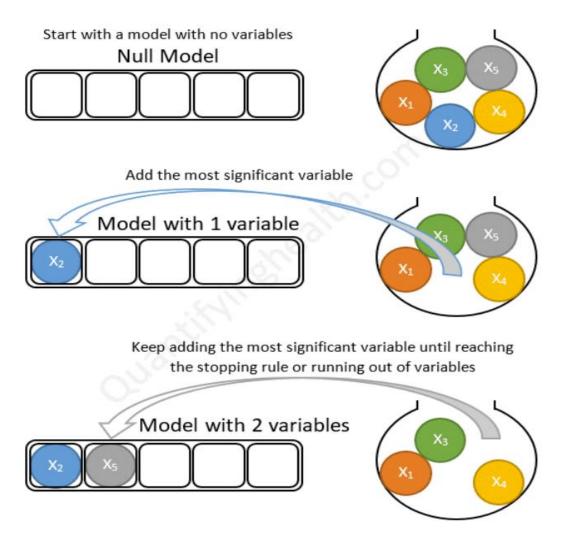
Model Selection

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

Forward selection

Add one by one and then re-run. Exclude the variables that have a p-value above the significance level. Do this until you run out of variables.

Forward stepwise selection example with 5 variables:



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

Forward selection

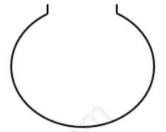
Add one by one and then re-run. Exclude the variables that have a p-value above the significance level.

- 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:

Start with a model that contains all the variables
Full Model

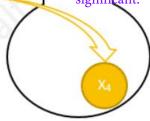


Remove the least significant variable

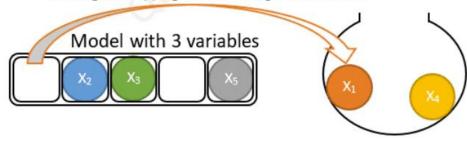
Remove one by one and then re-run. When doing this, the a variable that was previously insignificant may become significant.

Model with 4 variables





Keep removing the least significant variable until reaching the stopping rule or running out of 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