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**
  - 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

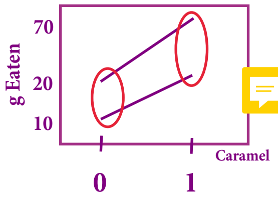
Interaction (Synergy) Effect Example: Combination of Caramel and Cheese variables

- **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)

*Effect of one variable depends on the level of the other variable

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

Caramel \ Cheese	0	1
0	10	20
1	20	70

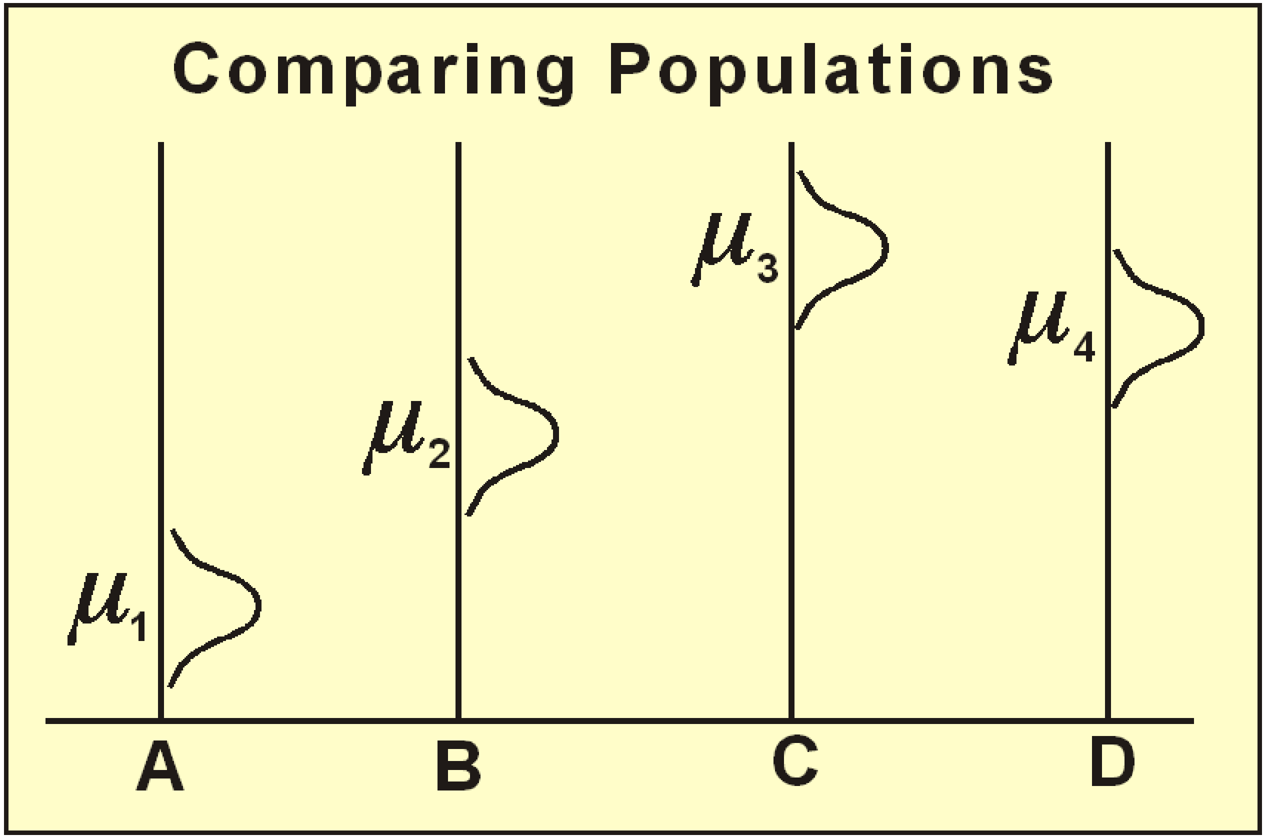


Definitions

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

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
  - **H0: $\mu_1 = \mu_2 = \ldots = \mu_g$,**

    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.

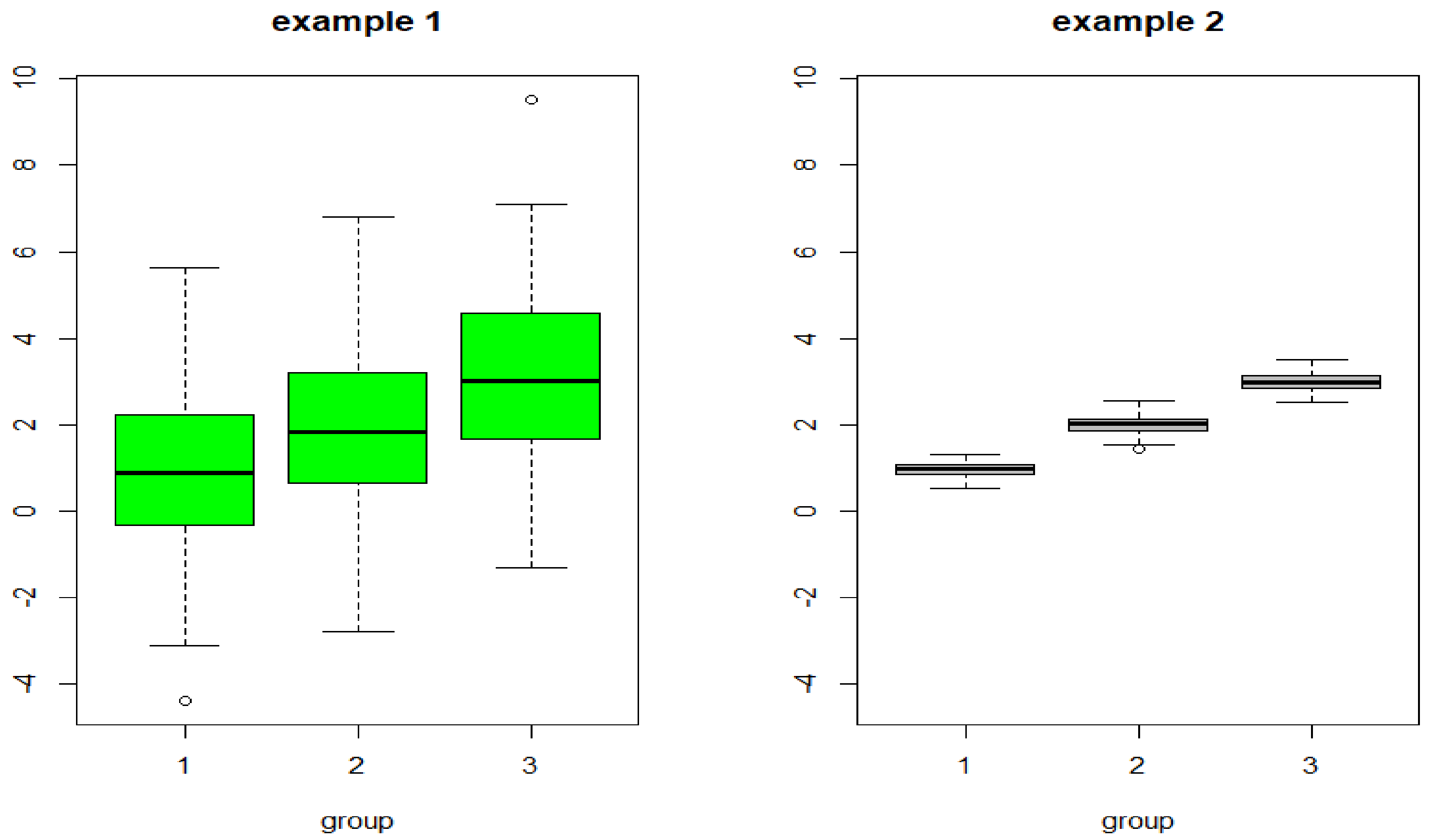
- Alternative hypothesis:
  H1: There is an interaction between independent variables in the population.

Assumptions of ANOVA

1) The response (dependent) variable is **continuous**
2) 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**

○ Reference assumptions notes in Notebook app

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 *statistic* 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 is NOT statistically significant, then you are done and there is no reason to conduct additional analyses. No difference among groups is found.

- If the F-statistic is statistically significant:
  - 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**

I. Check balanced of unbalanced

II. Run one-way ANOVA with **aov()** or possibly, **lm()**

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

- 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

One-way ANOVA example:

```
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 ...
##  $ Dose       : Factor w/ 3 levels "0.5","1","2": 1 1 1 1
1 1 1 1 1 1 ...
```

- Change Dose to factor so that we can run the ANOVA test

- Balance or Unbalanced?

```
table(tooth$Dose); table(tooth$Supplement)
```

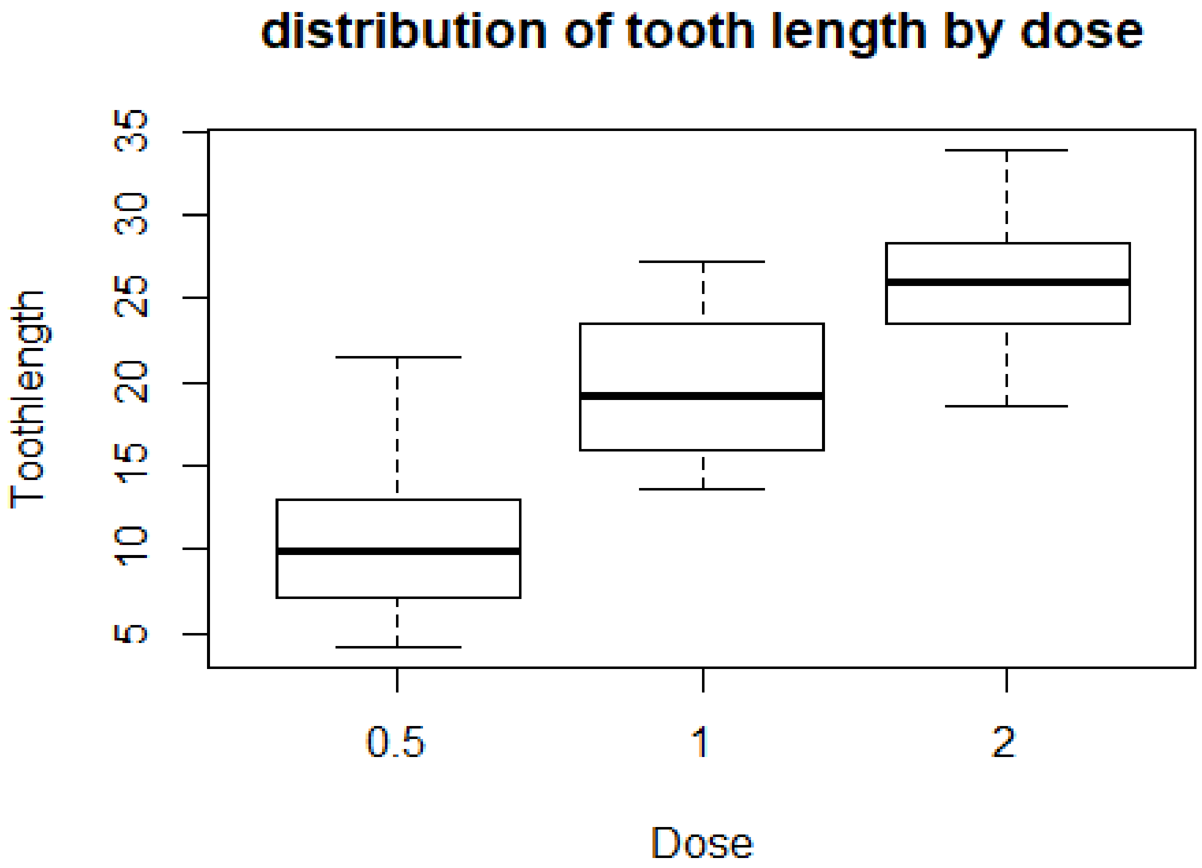
```
##
## 0.5   1   2
##  20  20  20

##
## OJ VC
## 30 30
```

- 20 observation per group and 30 observations per group
    - This indicates Balance

One-way ANOVA example:

- Compare the means of each group

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



One-way ANOVA example:

- Null Hypothesis: No Dose Effect
- Alternative Hypothesis: There exists a significant dose effect.

- Name of the Group Variable = Dose

- Highlight = SS Model (Between Group Variation)
  - The variation of Y that can be explained by Dose

```
aov.res= aov(Toothlength~Dose, data=tooth)

summary(aov.res)
```

- p-value calculated based on F statistic

```
##              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 '
' 1
```

- 1026 - variation of Y that can't be explained by Dose

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

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

One-way ANOVA example:

- To calculate R-square, need to run anova with **lm()**
- Results from **lm()** 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)
## Dose       2 2426.4  1213.2  67.416 9.533e-16 ***
## Residuals 57 1025.8    18.0
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
' 1
```

```
summary(lm.res)$r.squared
```

```
## [1] 0.7028642
```

- 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:

- 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
```

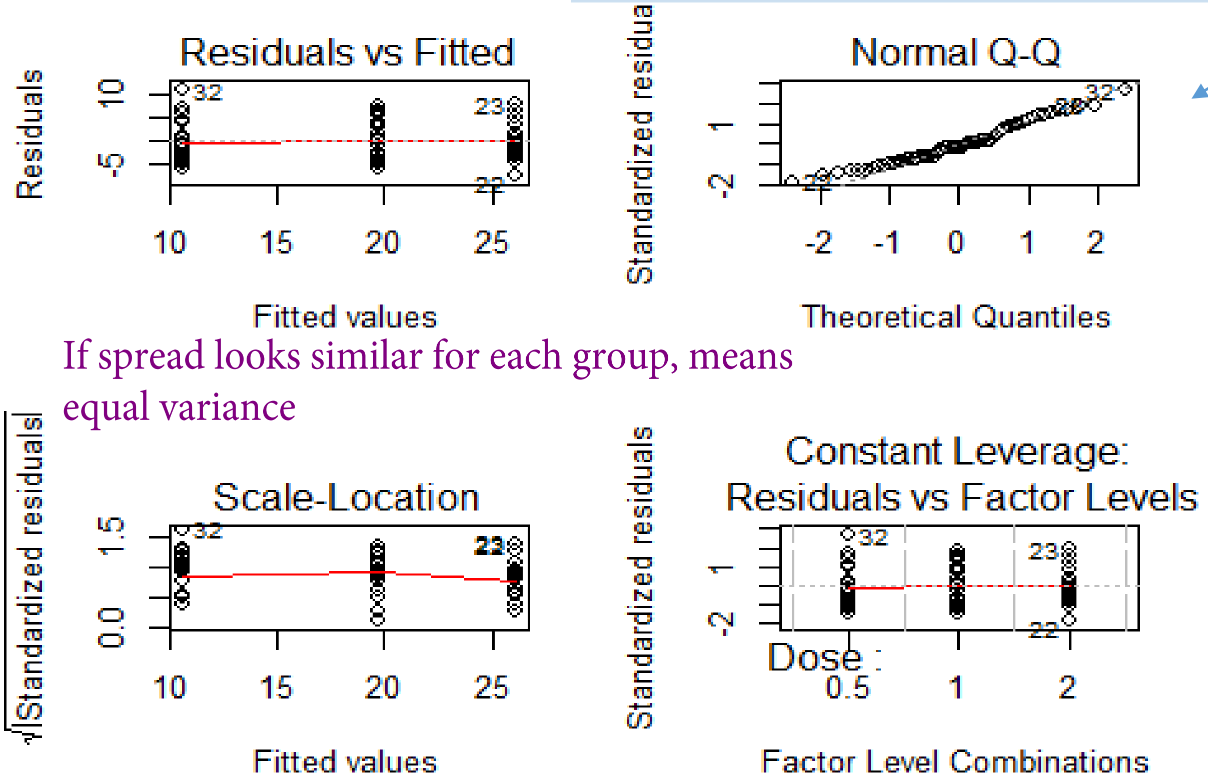
- If the p-value is small, we reject the null and must perform a Welch's ANOVA

One-way ANOVA example:

- 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.



If spread looks similar for each group, means equal variance

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?**

One-way ANOVA example:

Hypothesis is for the first comparison (Dose 1 and 0.5)

Pairwise t-test with modified p-values:

H0: $\mu_1 = \mu_{0.5}$ vs. H1: $\mu_1 \neq \mu_{0.5}$

```
ScheffeTest(aov.res)

##
##   Posthoc multiple comparisons of means: Scheffe Test
##     95% family-wise confidence level
##
## $Dose
##          diff    lwr.ci    upr.ci    pval
## 1-0.5   9.130  5.758155 12.501845 4.3e-08 ***
## 2-0.5  15.495 12.123155 18.866845 1.2e-15 ***
## 2-1     6.365  2.993155  9.736845 7.6e-05 ***
##
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

'>' means significantly different

Dose 1 mean > Dose 0.5 mean ; Dose 2 mean > Dose 0.5 mean ; Dose 2 mean > Dose 1 mean

Ho: μ2 = μ0.5 vs. H1: μ2 != μ0.5

Ho: μ2 = μ1 vs. H1: μ2 != μ1

diff: the estimated difference between the mean values of first group and second group

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 specifically, Dose 2 > Dose 1 > Dose 0.5

One-way ANOVA example:

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

Two-way ANOVA example:

```
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; $\mu_{OJ} \neq \mu_{VC}$)

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

Because the p-value is small for each line, we reject the null. Dose and Supplement both have an effect on Tooth growth and there exists an interaction between dose and supplement.

```
summary(aov.res2)
```

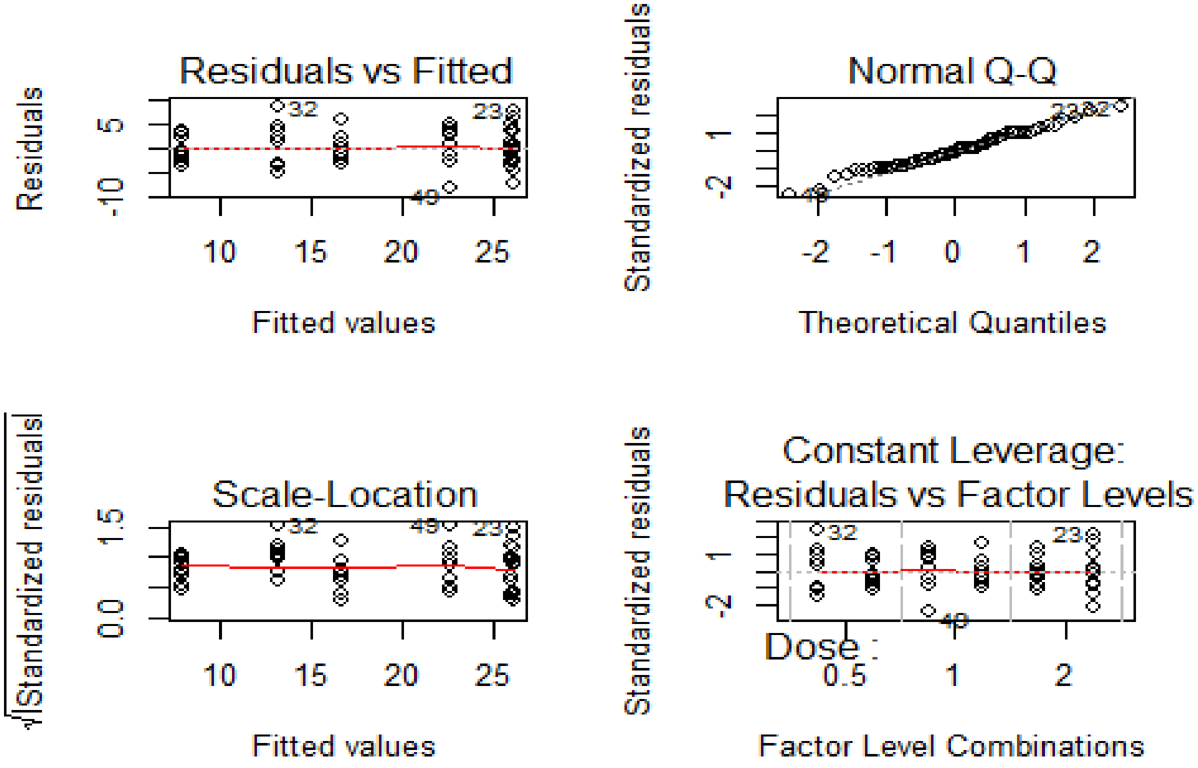
```
##                 Df Sum Sq Mean Sq F value   Pr(>F)
## Dose             2 2426.4  1213.2  92.000  < 2e-16 ***
## Supplement       1  205.4   205.4  15.572 0.000231 ***
## Dose:Supplement  2  108.3    54.2   4.107 0.021860 *
## Residuals       54  712.1    13.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1
' ' 1
```

H0: no interaction between type and supplement

Ha: exist an interaction between type and supplement

Two-way ANOVA example:

```
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?

Two-way ANOVA example:

```
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)

Two-way ANOVA example:

```
ScheffeTest(aov.res2)

##
##   Posthoc multiple comparisons of means: Scheffe Test
##     95% family-wise confidence level
##
## $Dose
##          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 *
## 2-1    6.365  2.39855 10.33145 0.00014 *
##
## $Supplement
##        diff    lwr.ci     upr.ci   pval
## VC-OJ -3.7 -6.938593 -0.4614069 0.0153 *
##
## $`Dose:Supplement`
##                   diff    lwr.ci     upr.ci    pval
## 1:OJ-0.5:OJ       9.47   3.860592  15.0794079 5.5e-05 ***
```

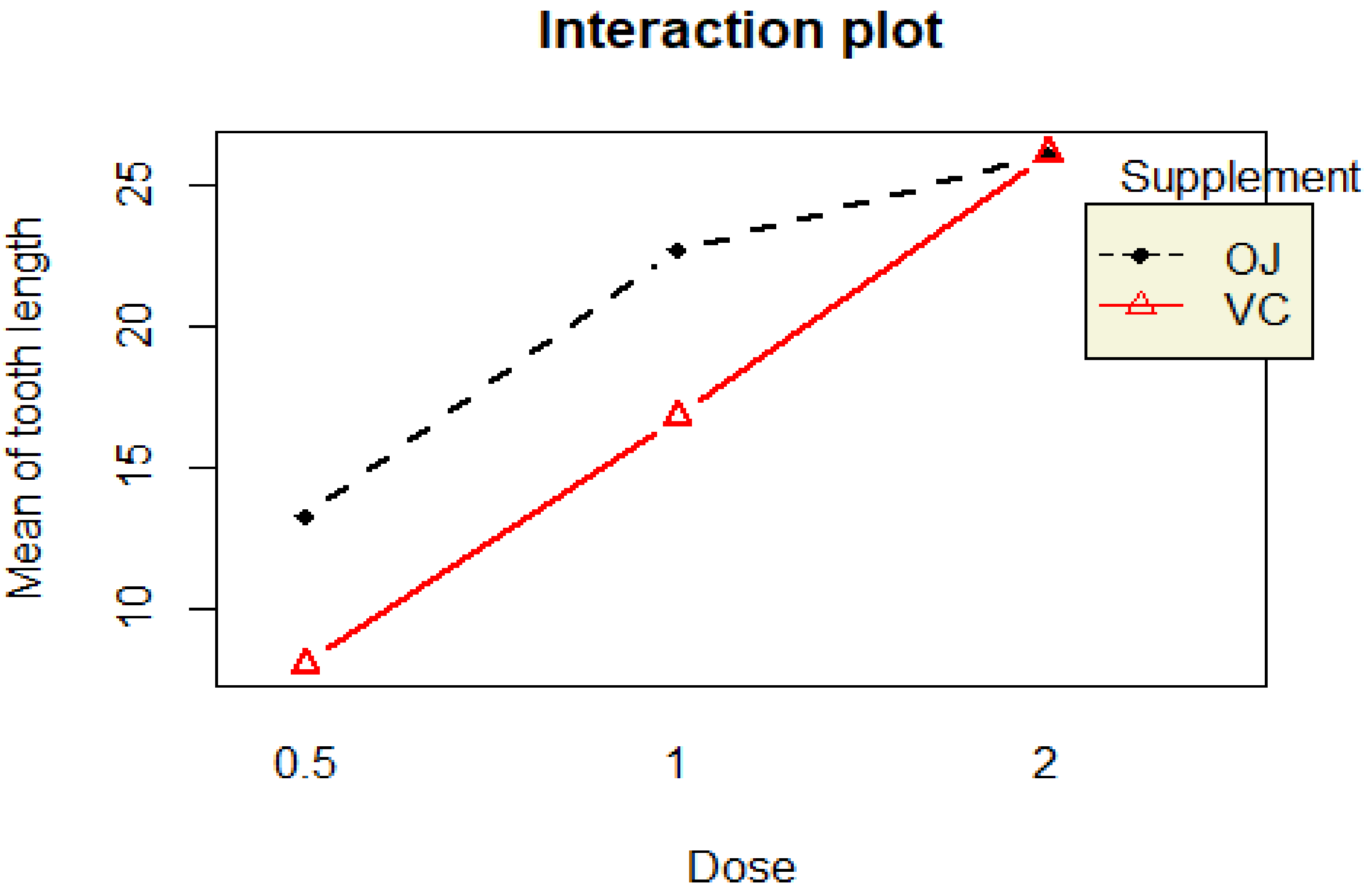
..... (omitted)

Focus on post-hoc analysis for main effects

Dose effect:
Dose2>Dose1>Dose0.5

Supplement effect:
VC < OJ

Two-way ANOVA example:



Some notes:

- Significance of model <-> R-square
  (0 or not) (prediction power)

- Model with higher R-square is always better?
  - What about the model with R-square = 100%?
  - 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 $\mu_1 \mathrel{!=} \mu_2$

  - Why it happens and how can we make a conclusion?

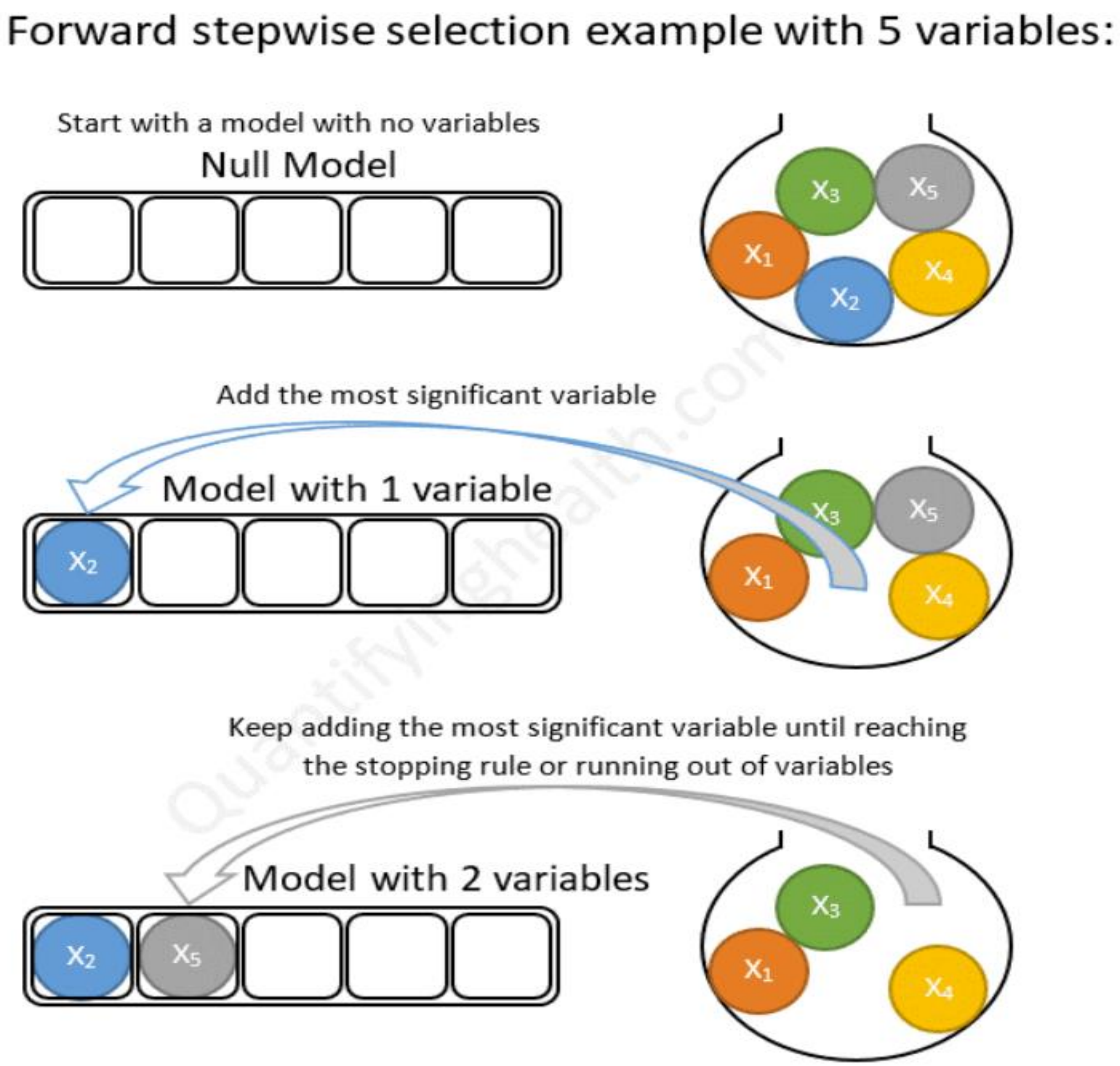
Model Selection

- For the case of n-way ANOVA, the largest model with all possible interactions has (2^n-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:
Start with a model with no variables
Null Model
X_3
X_5
X_1
X_4
X_2
Add the most significant variable
Model with 1 variable
X_2
X_3
X_5
X_1
X_4
Keep adding the most significant variable until reaching the stopping rule or running out of variables
Model with 2 variables
X_2
X_5
X_3
X_1
X_4

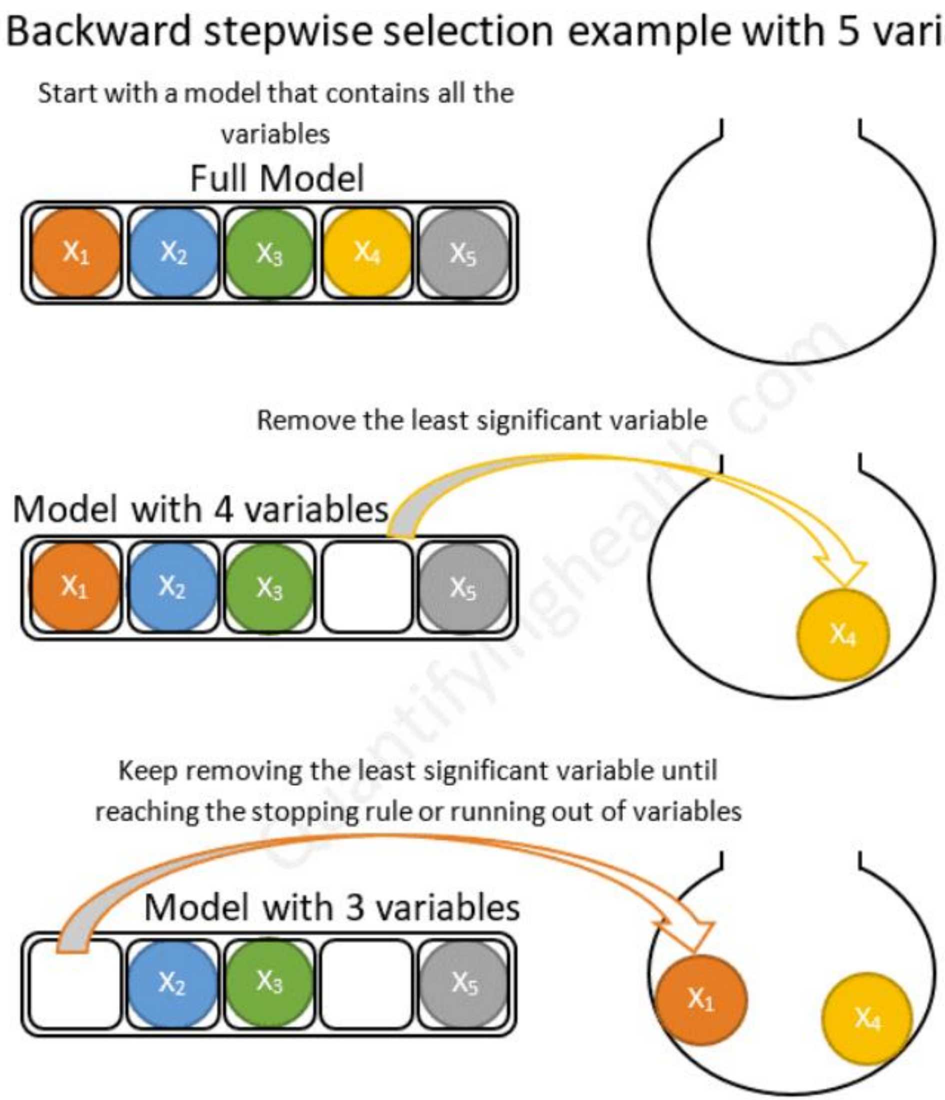
source: https://quantifyinghealth.com/stepwise-selection/

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
3. 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

Model with 4 variables

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

Model with 3 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