ANOVA

ANalysis Of VAriance

Introduction to ANOVA

10/21/2022 (Week 9)

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Outline

ANOVA

One-way ANOVA

Connection with Regression

Two-way ANOVA

Regression or ANOVA/t-tests?

- Regression emphasizes overall weight of an independent variable predictively
    - Prediction
    - Test

- ANOVA/t-tests emphasize "statistical significance" after experiment
    - Test mean differences of a continuous variable between two groups: Two sample t-test
    - Test differences of a continuous variable among multiple groups: **An**alysis **of Va**riance (ANOVA)

Categorical variables

Study how a variable would change according to different factors/levels of a categorical variables (e.g., group variable)
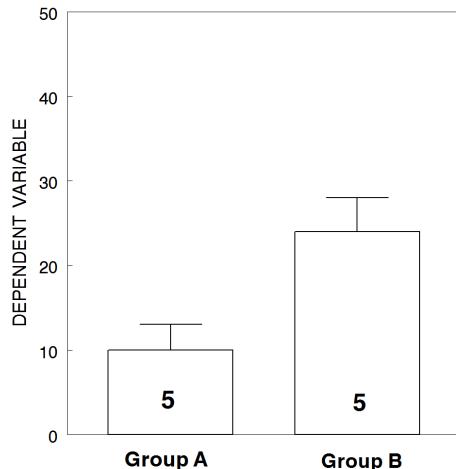
Especially when the categorical variable has factors/levels >2

Factor data type in R

For example, sex variable in the *abalones* dataset contains values for M, F, and I
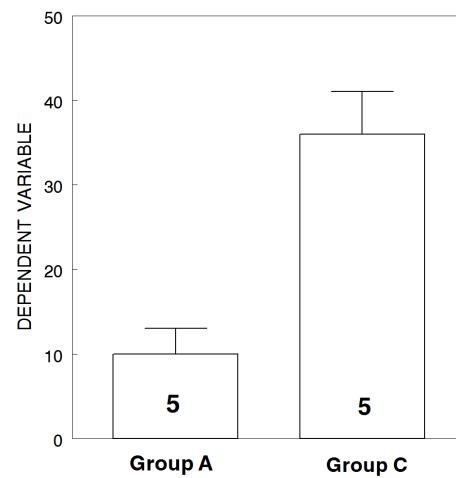
Let A, B & C be 3 levels of one factor: do any differ from the others?

If we do multiple pair-wise Two-sample **t-test**

$H_O: \mu_A = \mu_B$

$\alpha=5\%$

$H_O: \mu_A = \mu_C$

$\alpha=5\%$
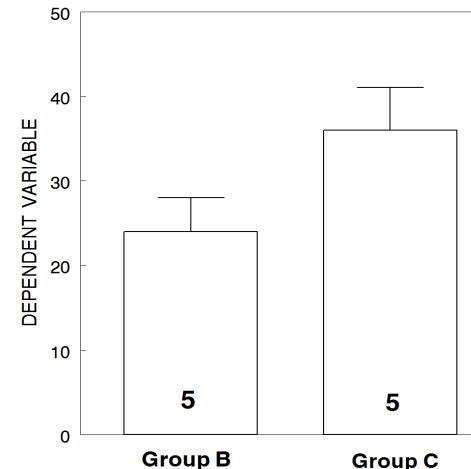
$H_O: \mu_B = \mu_C$

$\alpha=5\%$

Why Multiple Testing Matters

- In general, if we perform m hypothesis tests, what is the probability of at least 1 false positive?

P(Making an error) = α

P(Not making an error) = 1 - α

P(Not making an error in m tests) = $(1 - \alpha)^m$
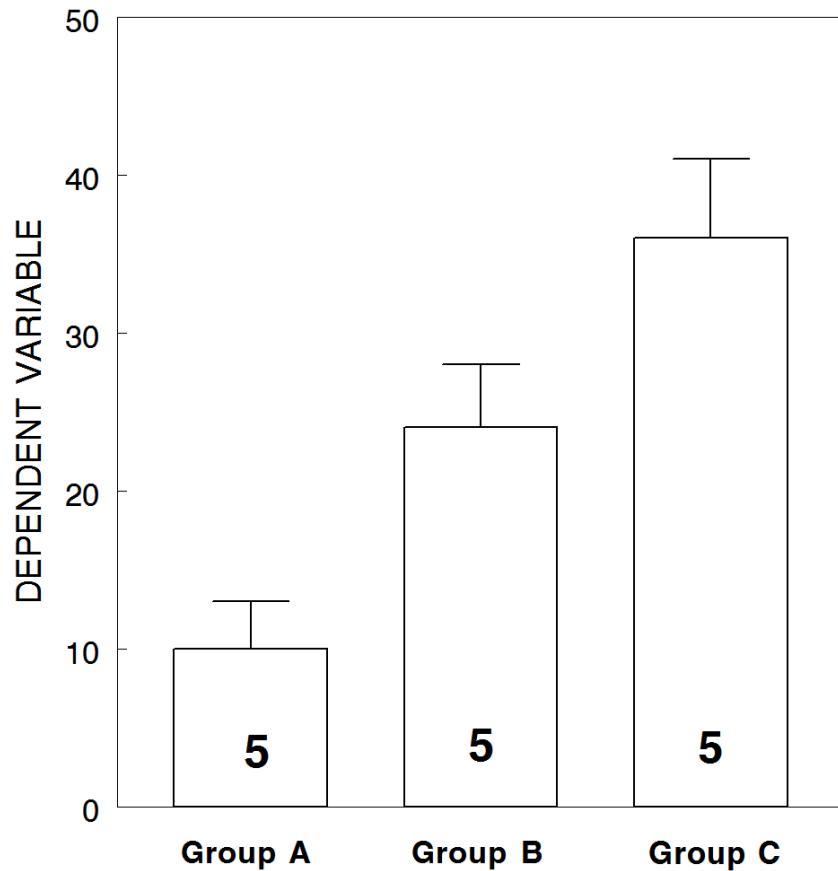
P(Making at least 1 error in m tests) = $1 - (1 - \alpha)^m$

3 Hypothesis tests
Family-wise type1 error: 14.2% = $1 - (1 - 0.05)^3$
(Week 12 Lecture about Multiple Testing)

One-way ANOVA
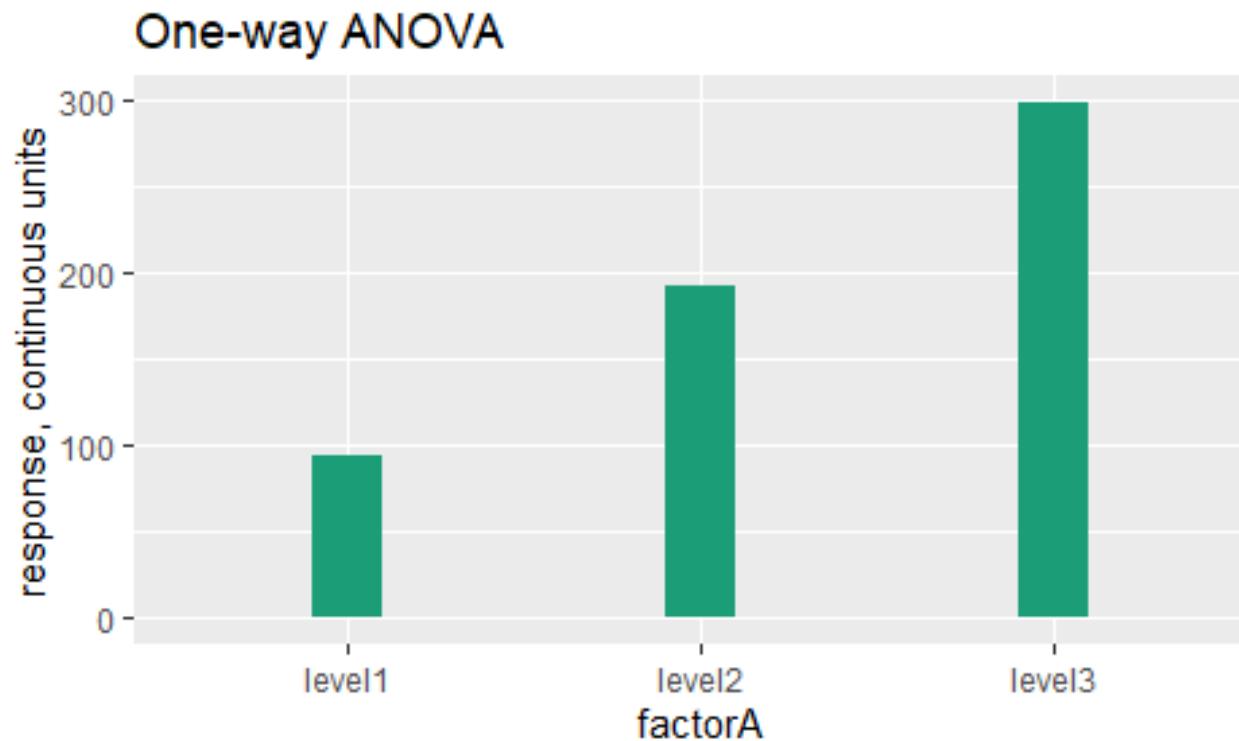
$$H_0: \mu_A = \mu_B = \mu_C$$

$$H_a: \text{At least one of the means is different}$$
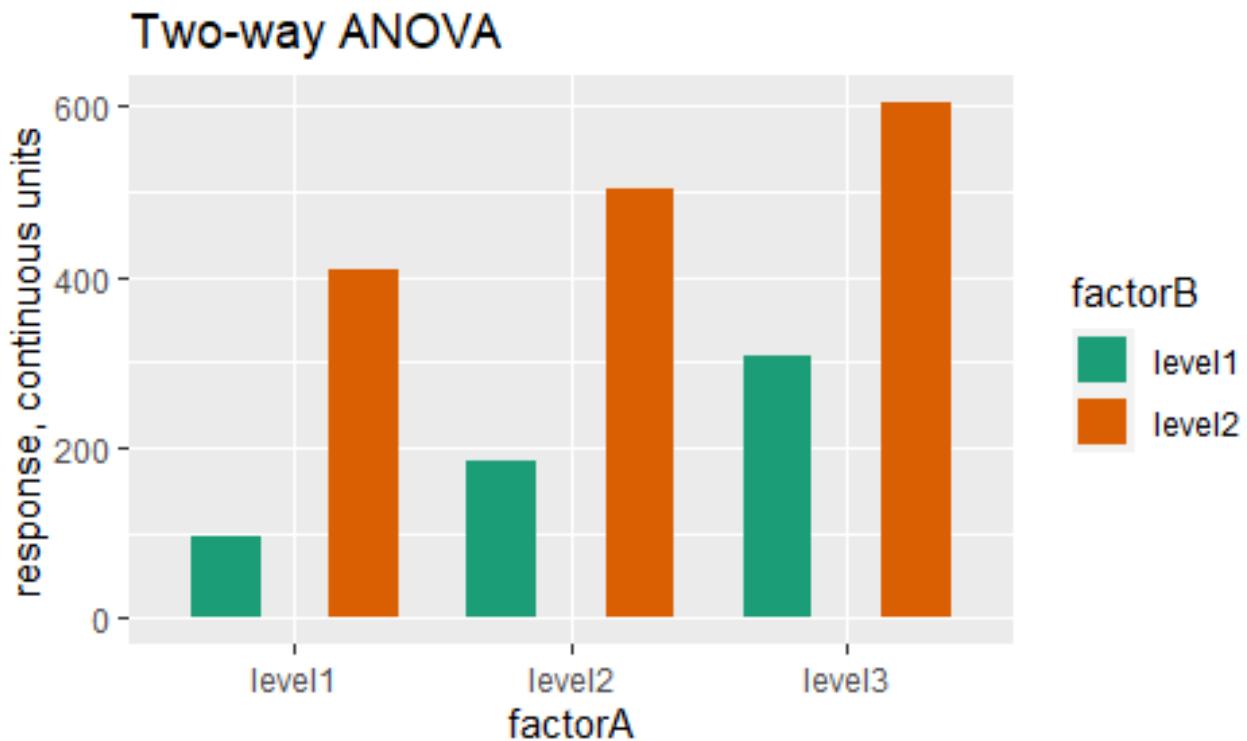
1 Hypothesis test
Family-wise type1 error : 5%

1 question for one factor

## One-way ANOVA
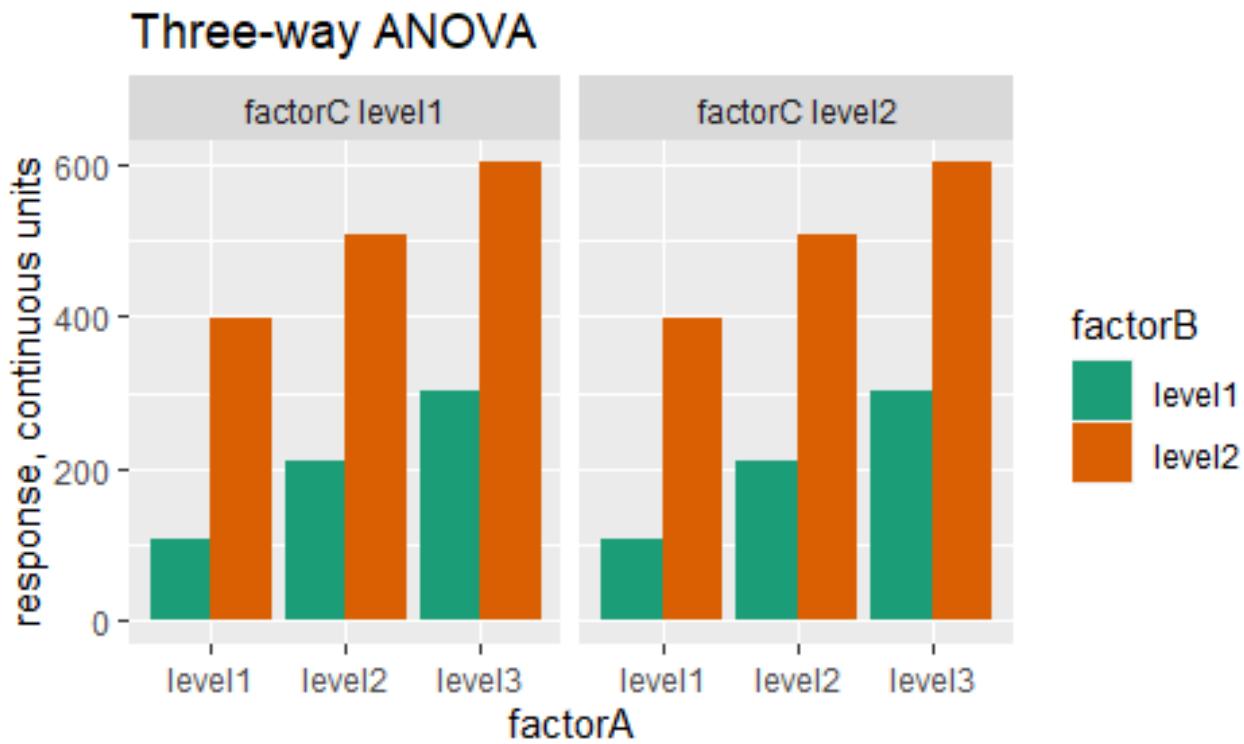
- Is factorA associated with a response?

3 questions for two factors

- Is factorA associated with a response?

- Is factorB associated with a response?

- Is the interaction between factorA and factorB associated with a response?

7 questions for three factors

Three-way ANOVA

- Is factorA associated with a response?
- Is factorB associated with a response?
- Is factorC associated with a response?

- Is the interaction between factorA and factorB associated with a response?
- Is the interaction between factorA and factorC associated with a response?
- Is the interaction between factorB and factorC associated with a response?

- Is the interaction among factorA, factor B, and factorC associated with a response?

One-way ANOVA
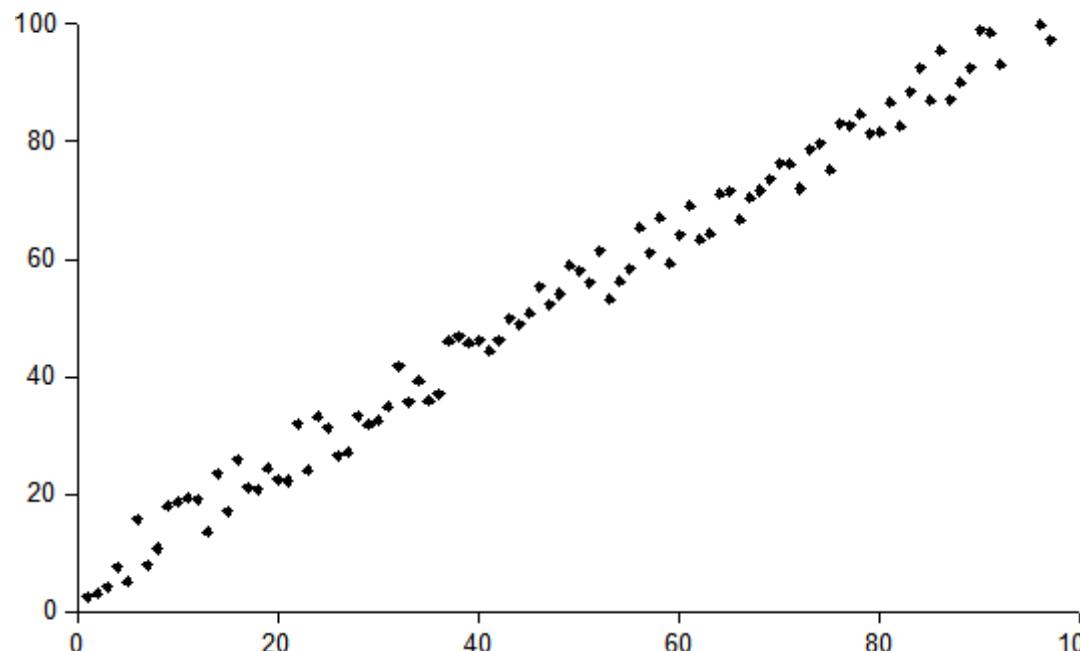
One-way ANOVA
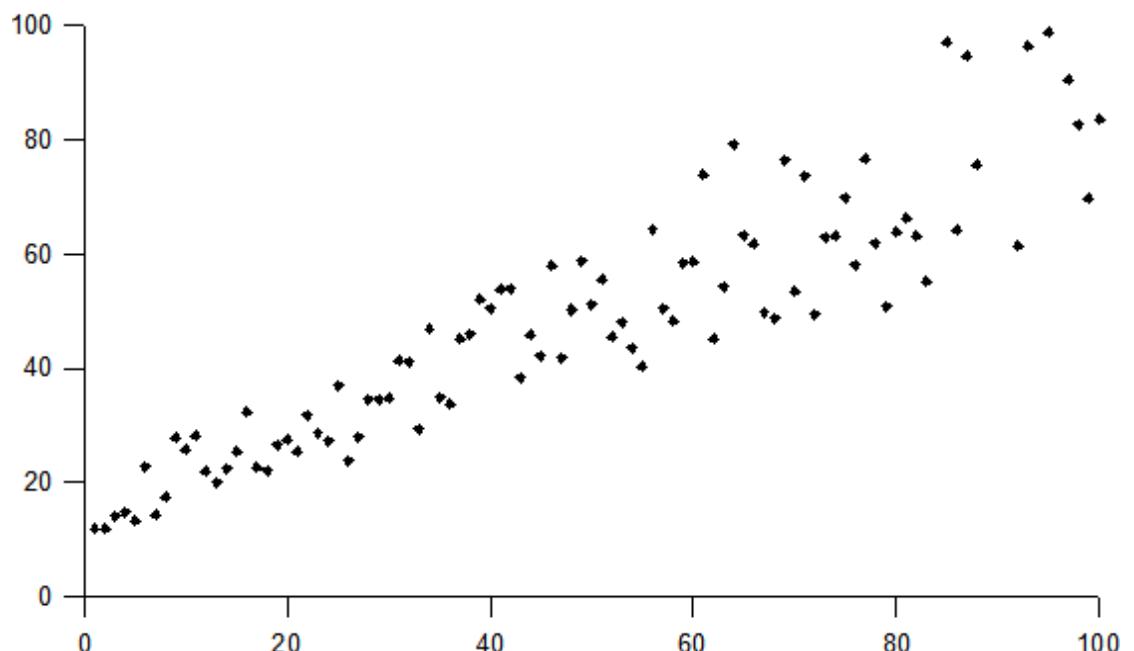
- **Hypothesis**
  - $H_0: \mu_1 = \mu_2 = \cdots = \mu_k$
  - H_a: At least one of the means is different

- **Assumptions**
  - Sample independence
  - Normality of the continuous variable per group
  - Homogeneity of variances (aka, Homoscedasticity): assuming the residuals all have the same variance

Homoscedasticity vs. Heteroscedasticity

Rational of ANOVA

- Partition total data variation into two sources
  - Between levels/groups (model, regression model)
  - Within levels/groups (residuals, error)

- If $H_0 : \mu_1 = \mu_2 = \cdots = \mu_k$ is true, the standardized variances (**between group vs. within group**) are equal to one another

- F test statistic

Grand mean and total deviation

Measurements, Y

$$\bar{Y}_{Grand}$$

$$\bar{Y}_{grand} = \frac{\sum y_{ij}}{N}$$

$$deviate = y_{ij} - \bar{Y}_{grand}$$

Level A   Level B   Level C

$$\sum (y_{ij} - \bar{Y}_{grand})^2 = \text{Sum of Squares Total} = SST$$

$$s^2 = variance = MS_{total} = \frac{SST}{df_{total}}$$

$df_{total} = N-1$

$$s = \sqrt{\frac{SST}{df_{total}}}$$

Partitioning Total Variation

- Variation is simply average Squared Deviations from the Mean

$$\boldsymbol{SST} = SST_{group} + SSE_{residual}$$

$$\sum_{j=1}^{K}\sum_{i=1}^{n_j}\left(\boldsymbol{y_{ij}} - \bar{Y}\right)^2 = \sum_{j=1}^{K} n_j\left(\bar{y}_j - \bar{Y}\right)^2 + \sum_{j=1}^{K}\sum_{i=1}^{n_j}\left(y_{ij} - \bar{y}_j\right)^2$$
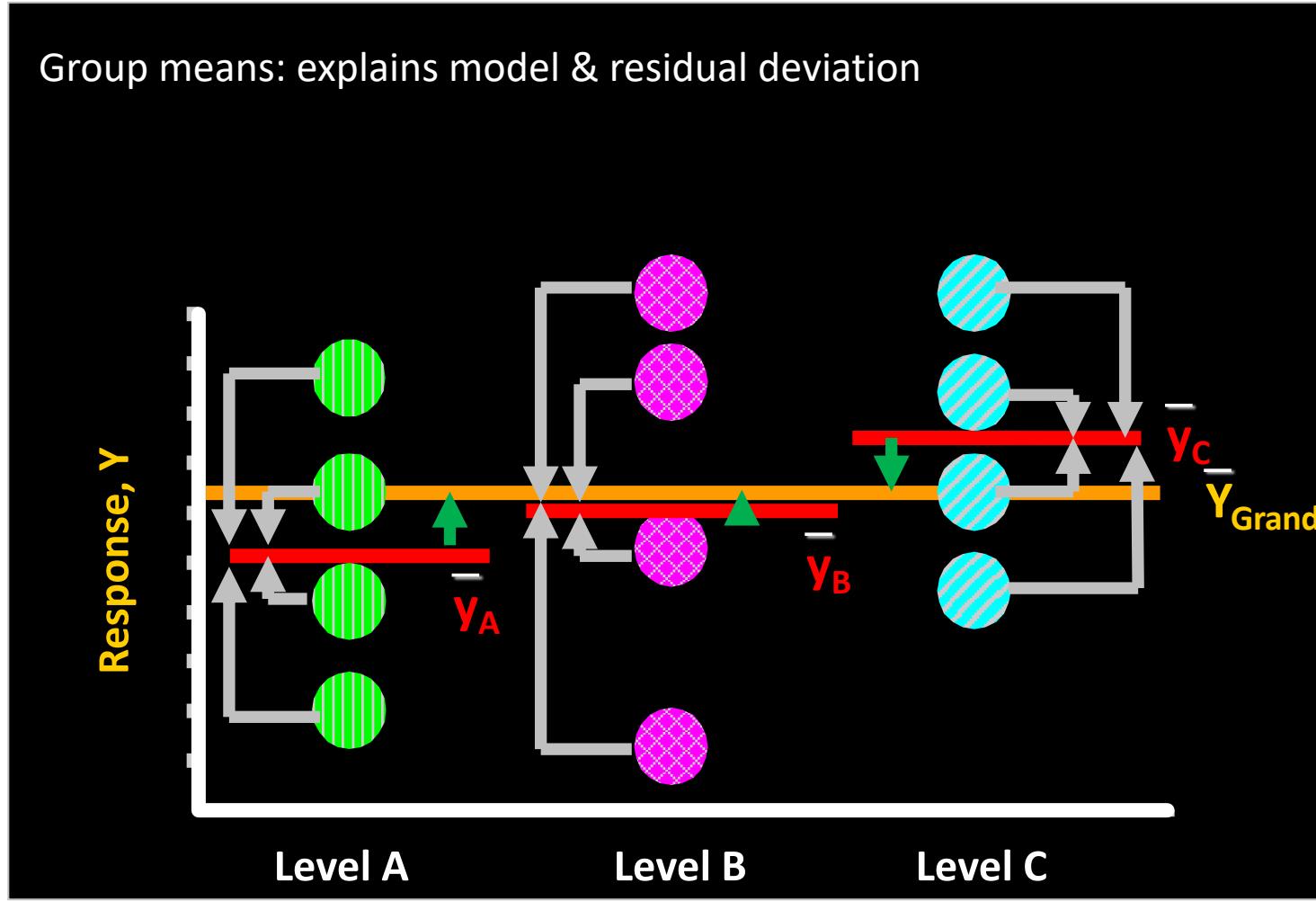
Sum of squared deviations from the grand mean across all N observations

Sum of squared deviations for each group mean from the grand mean

Sum of squared deviations for all observations from each group mean across all K groups

$$N = n_1 + n_2 + \cdots + n_K$$

Group means: explains model & residual deviation

Response, Y

y_A

y_B

y_C

Y_{Grand}

Level A Level B Level C

$$\bar{Y}_{grand} = \frac{\sum y_{ij}}{N}$$

$$group\ means\ \bar{y}_j = \frac{\sum y_i}{n_j}$$

↑ group $deviate = \bar{y}_j - \bar{Y}_{grand}$

↑ $residual\ deviate = y_{ij} - \bar{y}_j$

$\sum n(\uparrow)^2 = Sum\ of\ Squares\ group$

$\sum (\uparrow)^2 = Error\ Sum\ of\ Squares\ residual$

$$MS_{group} = \frac{SST_{group}}{df_{group}}$$

$$MSE_{residual} = \frac{SSE_{residual}}{df_{residual}}$$

$$F_{K-1,\ N-K} \sim \frac{\sum(\uparrow)^2 / df_g = MS_{group}}{\sum(\uparrow)^2 / df_r = MSE_{residual}}$$

$df_g = K-1, \quad df_r = N-K$

16
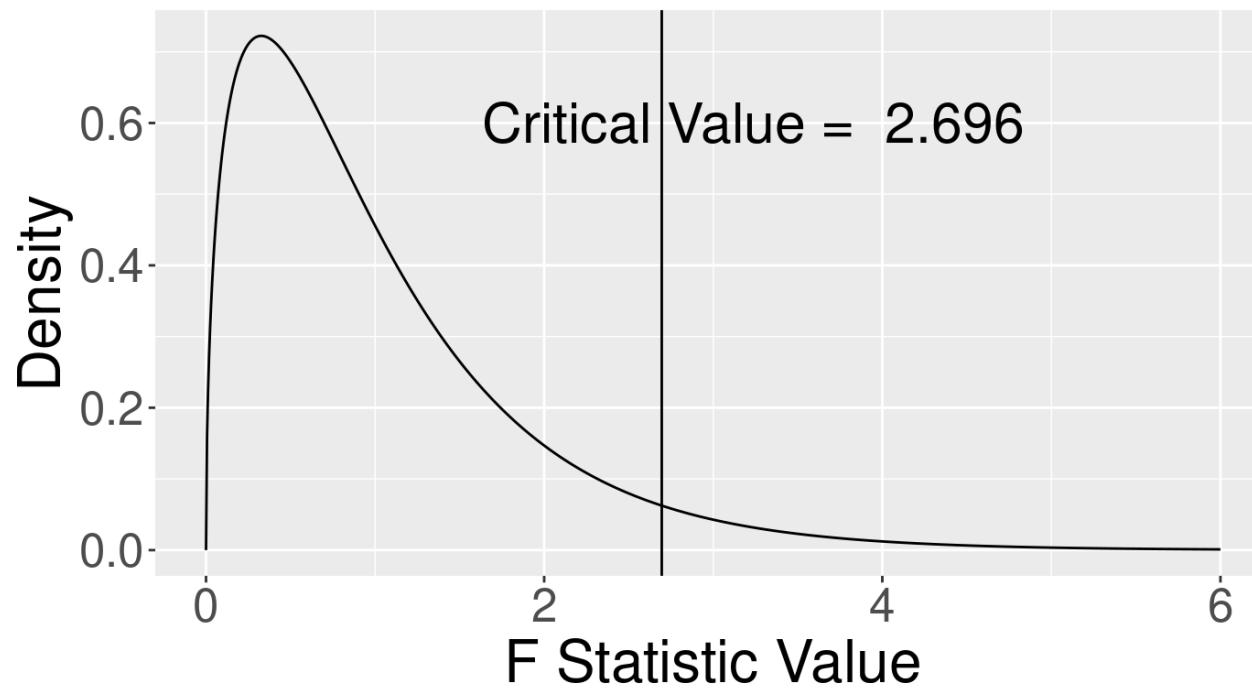
ANOVA F-test
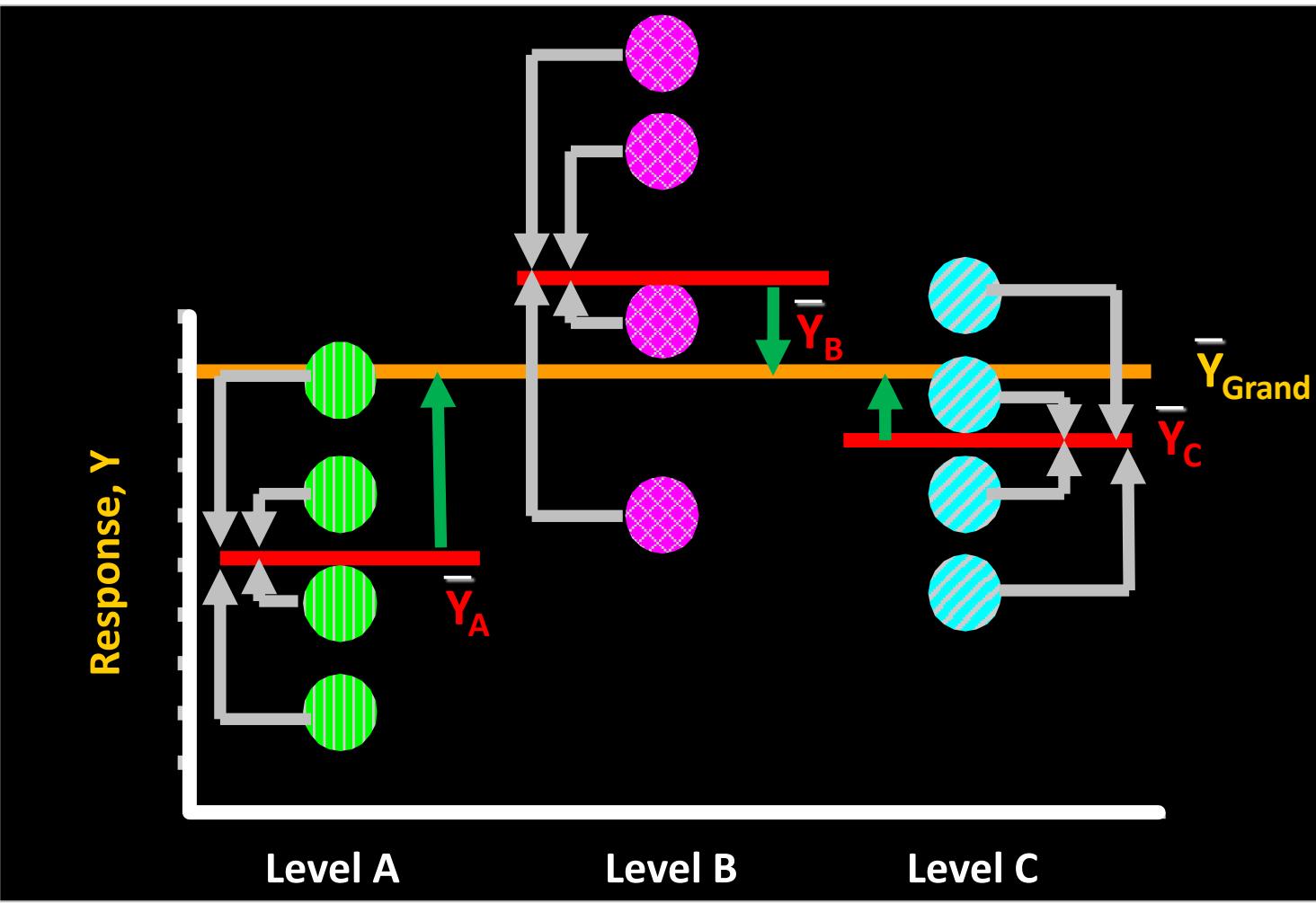
$$F_{df1,df2} = \frac{model \ or \ group \ variance}{residual \ variance} = \frac{MS_{dfg,\,effect}}{MSE_{dfr}}$$

Follows an F-distribution under the NULL hypothesis.

F-distribution df1 = 3, df2 = 100

Critical Value = 2.696

- Null distributions of F(3, 100)

- One-sided critical values of F(3, 100)
  qf(0.95, df1 = 3, df2 = 100)
  2.696

- If our test F is as or more extreme than the critical value, we reject the null hypothesis.

When the factor is associated with the response:

$$F_{K,\ (N-(K+1))} \sim \frac{\sum(\uparrow)^2 / df_g = MS_{group}}{\sum(\uparrow)^2 / df_r = MSE_{residual}}$$

is expected to be greater than the corresponding critical value.

Statistical Analysis of an ANOVA design is usually a two-step process

- Step 1: F Test of the **omnibus** null

$$H_o : \sigma^2_{model} \leq \sigma^2_{residual}$$

- Step 2: Multiple *post hoc* comparisons of group means

$$H_o : \mu_A \leq \mu_B \leq \mu_C ...., \leq \mu_k$$

ANOVA Table

Source of Variation	df	Sum of Squares	MS	F
Group	k-1	SST_G	$\dfrac{SST_G}{k-1}$	$\dfrac{SST_G}{k-1} \Big/ \dfrac{SST_E}{N-k}$
Error	N-k	SST_E	$\dfrac{SST_E}{N-k}$	
Total	N-1	SST		

$SST_G = SST_{group}$

$SST_E = SSE_{residual}$

$\eta^2 = \dfrac{SST_G}{SST_{Total}}$, "ges" generalized eta square in results by ezANOVA()

Equivalent to regression $R^2 = \dfrac{SSR}{SST_{Total}} = 1 - \dfrac{SSE_{residual}}{SST_{Total}}$

Example dataset: a quantitative trait X was measured, and a single SNP was genotyped

Our Data:

AA:	**82, 83, 97**	$\bar{x}_{1.} = (82 + 83 + 97)/3 = 87.3$
AG:	**83, 78, 68**	$\bar{x}_{2.} = (83 + 78 + 68)/3 = 76.3$
GG:	**38, 59, 55**	$\bar{x}_{3.} = (38 + 59 + 55)/3 = 50.6$

- Let X_{ij} denote the data from the ith level and jth observation

- Overall, or **grand mean**, is:

$$\bar{x}_{..} = \sum_{i=1}^{K}\sum_{j=1}^{J}\frac{x_{ij}}{N}$$

$$\bar{x}_{..} = \frac{82 + 83 + 97 + 83 + 78 + 68 + 38 + 59 + 55}{9} = 71.4$$

(X is the continuous response variable Y in the previous slide)

Partitioning Total Variation

- $SST_G = SST_{group}$

- $SST_E = SSE_{residual}$

$$SST \quad = \quad SST_G \quad + \quad SST_E$$

$$\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2 \qquad \sum_{i=1}^{K}n_i \cdot (\bar{x}_{i.}-\bar{x}_{..})^2 \qquad \sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2$$

$(82-71.4)^2+(83-71.4)^2+(97-71.4)^2+$
$(83-71.4)^2+(78-71.4)^2+(68-71.4)^2+$
$(38-71.4)^2+(59-71.4)^2+(55-71.4)^2=$

$3 \cdot (87.3-71.4)^2+$
$3 \cdot (76.3-71.4)^2+$
$3 \cdot (50.6-71.4)^2=$

$(82-87.3)^2+(83-87.3)^2+(97-87.3)^2+$
$(83-76.3)^2+(78-76.3)^2+(68-76.3)^2+$
$(38-50.6)^2+(59-50.6)^2+(55-50.6)^2=$

2630.2 **2124.2** **506**

$$SST \quad = \quad SST_G \quad + \quad SST_E$$

$$\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2 \qquad \sum_{i=1}^{K}n_i \bullet (\bar{x}_{i.}-\bar{x}_{..})^2 \qquad \sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2$$

Partitioning Total Variation

- $SST_G = SST_{group}$

- $SST_E = SSE_{residual}$

Calculating Mean Squares

- To make the sum of squares comparable, we divide each one by their associated degrees of freedom

    - $SST_G = k - 1 \ (3 - 1 = 2)$

    - $SST_E = N - k \ (9 - 3 = 6)$

    - $SST_T = N - 1 \ (9 - 1 = 8)$

$MST_G = MS_{group}$

$MST_E = MSE_{residual}$

- $MST_G = 2124.2 / 2 = 1062.1$

- $MST_E = 506 / 6 = 84.3$
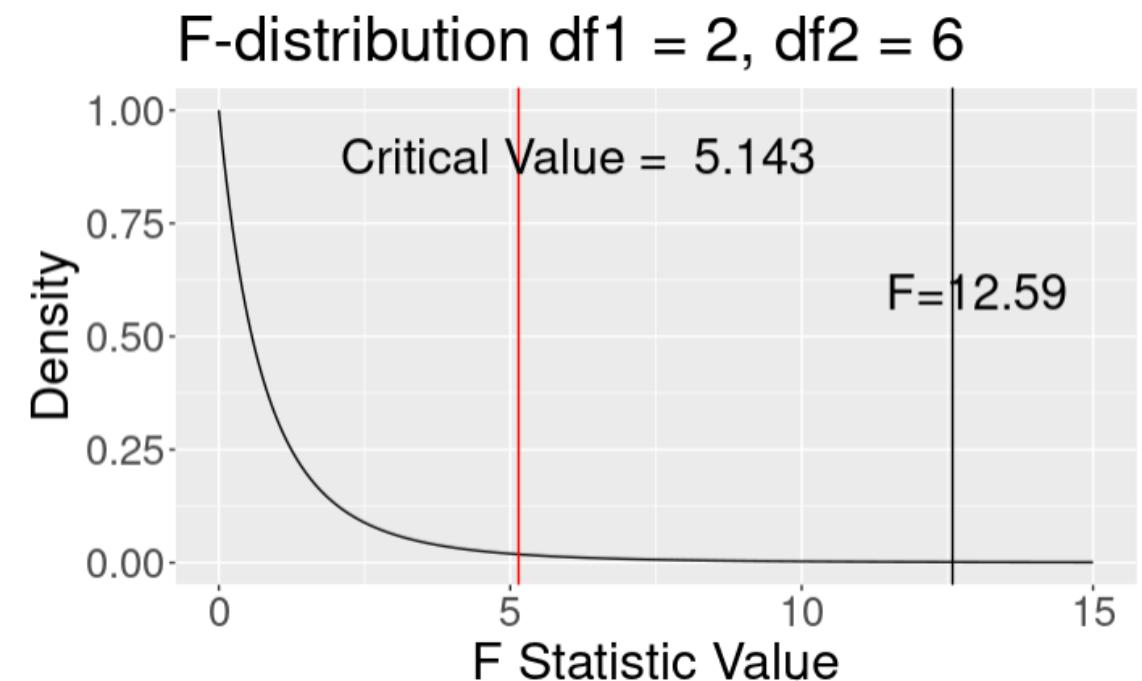
Almost There… Calculating F Statistic

- The test statistic is the ratio of group and error mean squares

$$F = \frac{MST_G}{MST_E} = \frac{1062.2}{84.3} = 12.59$$

- If H_0 is true MST_G and MST_E are equal

- Critical value for rejection region is $F_{\alpha,\, k-1,\, N-k}$

- If we define $\alpha = 0.05$, then $F_{0.05,\, 2,\, 6} = 5.14$

F-distribution df1 = 2, df2 = 6

Critical Value = 5.143

F=12.59

Density

F Statistic Value

How to do ANOVA analysis in R?

- Base R function : **aov()**

- R function: **ezANOVA()** from R library "ez"

```
> aov_2 <- aov(X ~ SNP, data = example_dt1)
> summary(aov_2)
            Df Sum Sq Mean Sq F value  Pr(>F)
SNP          2   2124  1062.1   12.59 0.00712 **
Residuals    6    506    84.3
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

One-way ANOVA by **ezANOVA()** with Completely Randomized Samples

```
> aov_1 <- ezANOVA(data = example_dt1, dv = X, wid = SampleID,
+                       between = SNP, detailed = TRUE, return_aov = TRUE)
> print(aov_1)
$ANOVA
  Effect DFn DFd       SSn SSd       F          p p<.05       ges
1    SNP   2   6 2124.222 506 12.5942 0.007119905     * 0.8076208


$`Levene's Test for Homogeneity of Variance`
  DFn DFd SSn SSd          F         p p<.05
1   2   6   8 330 0.07272727 0.9306614


$aov
Call:
    aov(formula = formula(aov_formula), data = data)

Terms:
                        SNP Residuals
Sum of Squares    2124.222   506.000
Deg. of Freedom          2         6

Residual standard error: 9.183318
Estimated effects may be unbalanced
```

Connection with Linear Regression

Predicted and Residual Values

- **Predicted**, or fitted, values are values of y predicted by the least-squares regression line obtained by plugging in $x_1, x_2, \ldots, x_n$ into the estimated regression line
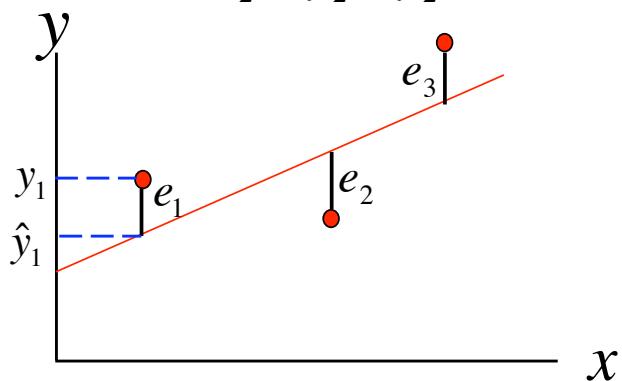
$$\hat{y}_1 = \hat{\beta}_0 - \hat{\beta}_1 x_1$$

$$\hat{y}_2 = \hat{\beta}_0 - \hat{\beta}_1 x_2$$

- **Residuals** are the deviations of observed and predicted values

$$e_1 = y_1 - \hat{y}_1$$

$$e_2 = y_2 - \hat{y}_2$$

Residuals Are Useful!

- They allow us to calculate the error sum of squares (SSE):

$$SSE = \sum_{i=1}^{n}(e_i)^2 = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

- Which in turn allows us to estimate σ^2:

$$\hat{\sigma}^2 = \frac{SSE}{n-2}$$

n is Sample Size

- As well as an important statistic referred to as the coefficient of determination:

$$r^2 = 1 - \frac{SSE}{SST}$$

$$SST = \sum_{i=1}^{n}(y_i - \bar{y})^2$$

Aka. Regression R^2

Multivariate Linear Regression

- Linear regression model to two or more independent/predictor variables

- $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k + \epsilon$

Hypothesis Testing: Model Utility Test (or Omnibus Test)

- The first thing we want to know after fitting a model is whether any of the independent variables (X's) are significantly related to the dependent variable (Y):

$$H_0 \; : \; \beta_1 = \beta_2 = ... = \beta_k = 0$$

$$H_A \; : \; \text{At least one } \beta_1 \neq 0$$

$$f = \frac{R^2}{(1 - R^2)} \bullet \frac{k}{n - (k+1)}$$

n is Sample Size

$$\text{Rejection Region} : \; F_{\alpha, k, n-(k+1)}$$

Equivalent ANOVA Formulation of Omnibus Test

- We can also frame this in our now familiar ANOVA framework

  - partition total variation into two components: **SSE** (unexplained variation) and **SSR** (variation explained by linear model)

Source of Variation	df	Sum of Squares	MS	F
Regression	k	$SSR = \sum (\hat{y}_i - \bar{y})^2$	$\dfrac{SSR}{k}$	$\dfrac{MS_R}{MS_E}$
Error	n-2	$SSE = \sum (y_i - \hat{y}_i)^2$	$\dfrac{SSE}{n-2}$	
Total	n-1	$SST = \sum (y_i - \bar{y})^2$		

$$\text{Rejection Region}: F_{\alpha,k,n-(k+1)}$$

SSR is equivalent to SST_{group}

n is Sample Size

F Test For Subsets of Independent Variables

- A powerful tool in multiple regression analyses is the ability to compare two models

- For instance say we want to compare:

$$\text{Full Model}: y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \varepsilon$$

$$\text{Reduced Model}: y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

- Again, another example of ANOVA:

SSE_R = error sum of squares for reduced model with l predictors

SSE_F = error sum of squares for full model with k predictors

$$f = \frac{(SSE_R - SSE_F)/(k-l)}{SSE_F/([n-(k+1)]}$$

n is Sample Size

Example of Model Comparison

- We have a quantitative trait and want to test the effects at two markers, M1 and M2.

  Full Model: Trait = Mean + M1 + M2 + (M1*M2) + error

  Reduced Model: Trait = Mean + M1 + M2 + error

$$f = \frac{(SSE_R - SSE_F)/(3-2)}{SSE_F/([100-(3+1)]} = \frac{(SSE_R - SSE_F)}{SSE_F/96}$$

$$\text{Rejection Region}: F_{a,\,1,\,96}$$

Model 1. Rings/Age ~ factor(sex) + length + diameter + height + wholeWeight + shuckedWeight + visceraWeight + shellWeight + diameter * height

vs.

Model 2. Rings/Age ~ factor(sex) + length + diameter + height + wholeWeight + shuckedWeight + visceraWeight + shellWeight
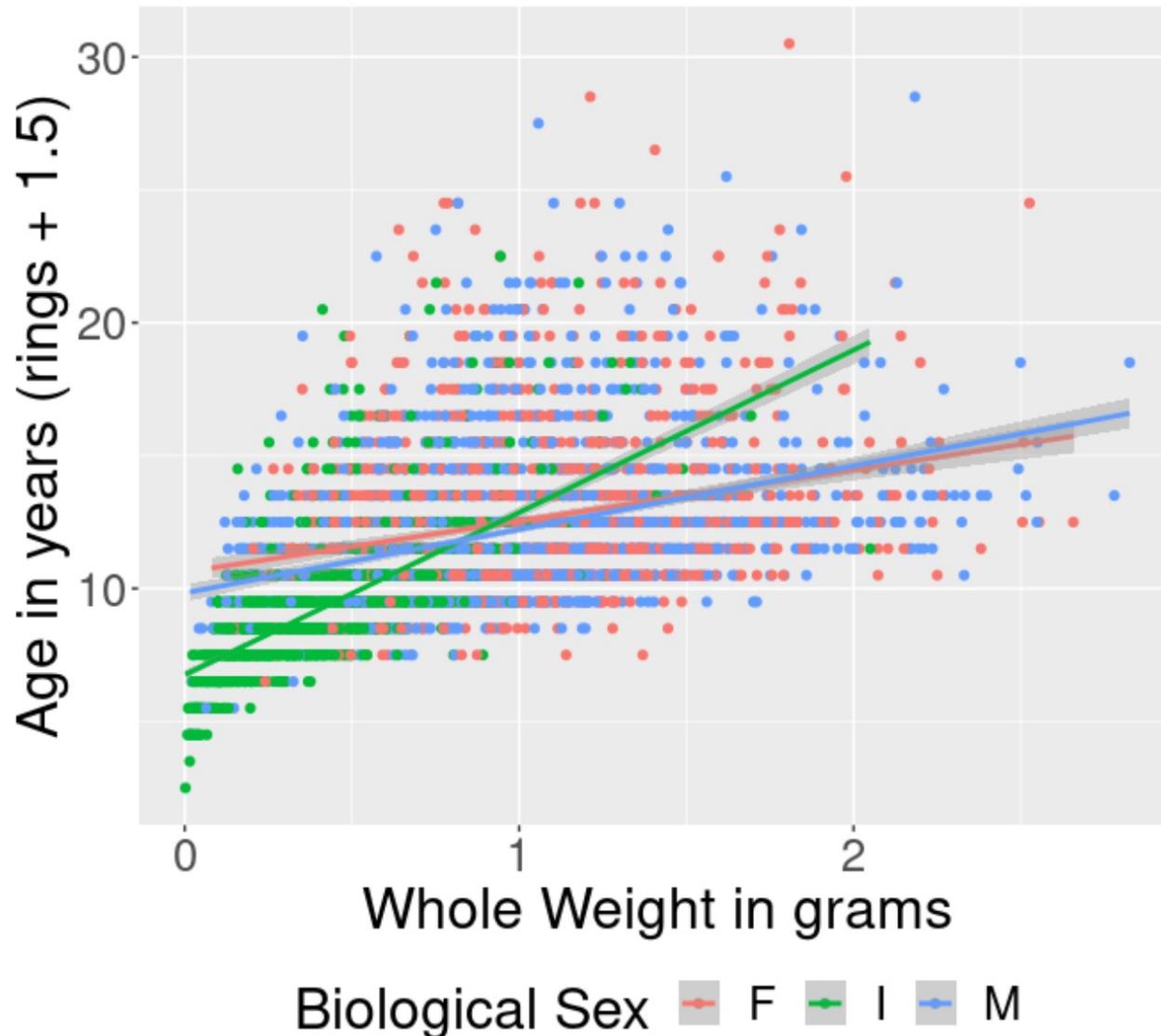
Abalones Dataset

Name	Data Type	Measurement Unit	Description
Sex	nominal	–	M, F, and I (infant)
Length	continuous	mm	Longest shell measurement
Diameter	continuous	mm	perpendicular to length
Height	continuous	mm	with meat in shell
Whole weight	continuous	grams	whole abalone
Shucked weight	continuous	grams	weight of meat
Viscera weight	continuous	grams	gut weight (after bleeding)
Shell weight	continuous	grams	after being dried
Rings	integer	–	+1.5 gives the age in years

Relationship between Abalone age/rings and Whole Weight

Age of Abalones by Whole Weight

Best fit lines shown by sex

Age in years (rings + 1.5)

Whole Weight in grams

Biological Sex   F   I   M

Fit the full model: Model 1

```
> fit1_full <- lm(age ~ factor(sex) + length + diameter + height + wholeWeight + shuckedWeight
+ visceraWeight + shellWeight + diameter * height, data = abalone)
> summary(fit1_full)

Call:
lm(formula = age ~ factor(sex) + length + diameter + height +
    wholeWeight + shuckedWeight + visceraWeight + shellWeight +
    diameter * height, data = abalone)

Residuals:
     Min       1Q   Median       3Q      Max
-12.5374  -1.3104  -0.3387   0.8896  14.3819

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)
(Intercept)         3.09937    0.38314   8.089 7.80e-16 ***
factor(sex)I       -0.72354    0.10201  -7.093 1.54e-12 ***
factor(sex)M        0.04222    0.08256   0.511 0.609110
length             -6.93065    1.92719  -3.596 0.000327 ***
diameter           22.61123    2.54307   8.891  < 2e-16 ***
height             48.84643    4.44716  10.984  < 2e-16 ***
wholeWeight         9.75707    0.72347  13.487  < 2e-16 ***
shuckedWeight     -18.92136    0.81497 -23.217  < 2e-16 ***
visceraWeight      -8.79936    1.29604  -6.789 1.28e-11 ***
shellWeight        11.02196    1.14157   9.655  < 2e-16 ***
diameter:height  -102.44668   11.24096  -9.114  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.173 on 4166 degrees of freedom
Multiple R-squared:  0.5469,    Adjusted R-squared:  0.5458
F-statistic: 502.9 on 10 and 4166 DF,  p-value: < 2.2e-16
```

Fit the subset model: Model 2

```
> fit2 <- lm(age ~ factor(sex) + length + diameter + height + wholeWeight
+ shuckedWeight + visceraWeight + shellWeight, data = abalone)
> summary(fit2)

Call:
lm(formula = age ~ factor(sex) + length + diameter + height +
    wholeWeight + shuckedWeight + visceraWeight + shellWeight,
    data = abalone)

Residuals:
    Min      1Q   Median      3Q     Max
-10.4800  -1.3053  -0.3428   0.8600  13.9426

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      5.39464    0.29157  18.502  < 2e-16 ***
factor(sex)I    -0.82488    0.10240  -8.056 1.02e-15 ***
factor(sex)M     0.05772    0.08335   0.692    0.489
length          -0.45834    1.80912  -0.253    0.800
diameter        11.07510    2.22728   4.972 6.88e-07 ***
height          10.76154    1.53620   7.005 2.86e-12 ***
wholeWeight      8.97544    0.72540  12.373  < 2e-16 ***
shuckedWeight  -19.78687    0.81735 -24.209  < 2e-16 ***
visceraWeight  -10.58183    1.29375  -8.179 3.76e-16 ***
shellWeight      8.74181    1.12473   7.772 9.64e-15 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.194 on 4167 degrees of freedom
Multiple R-squared:  0.5379,    Adjusted R-squared:  0.5369
F-statistic: 538.9 on 9 and 4167 DF,  p-value: < 2.2e-16
```

Model comparison by anova()

```
> anova(fit1_full, fit2)
Analysis of Variance Table

Model 1: age ~ factor(sex) + length + diameter + height + wholeWeight +
    shuckedWeight + visceraWeight + shellWeight + diameter *
    height
Model 2: age ~ factor(sex) + length + diameter + height + wholeWeight +
    shuckedWeight + visceraWeight + shellWeight
  Res.Df   RSS Df Sum of Sq      F      Pr(>F)
1   4166 19669
2   4167 20061 -1   -392.14 83.059 < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- Conclusion: stay with the full model

In-Class Exercise 1 :
## One-way ANOVA

Two-way ANOVA

Two-way ANOVA
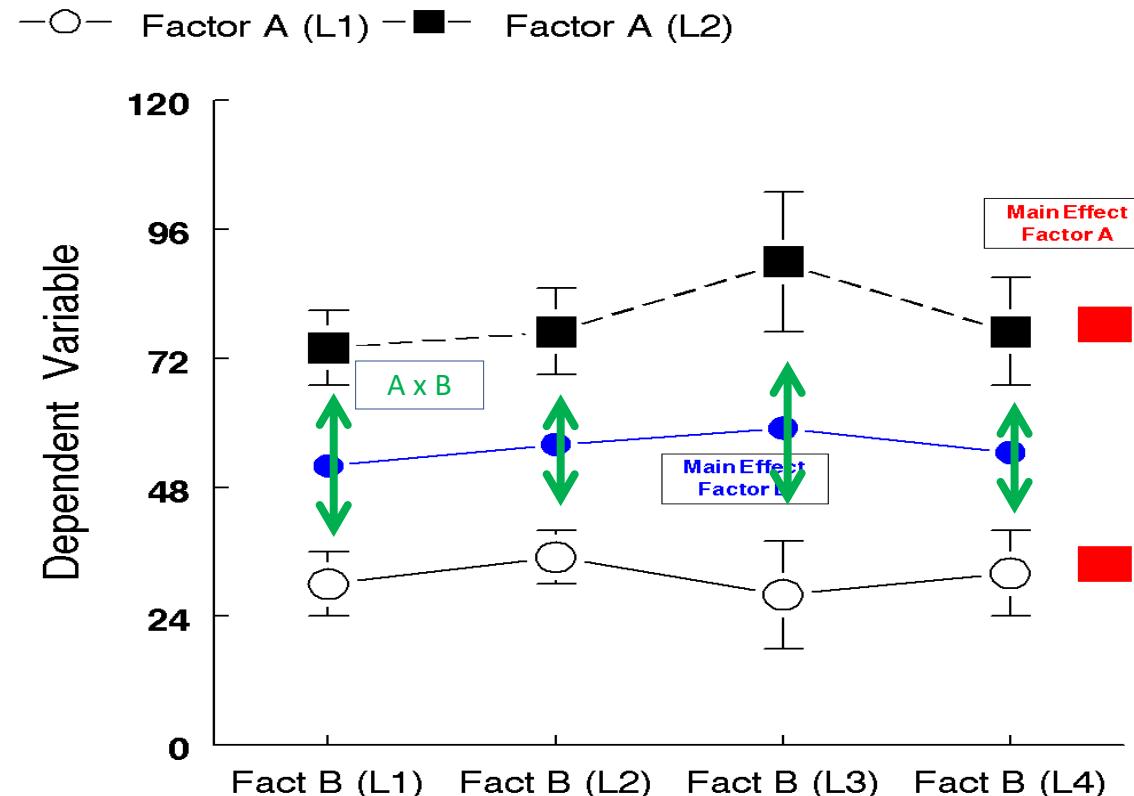
- Participating the total variation with respect to two-way factors/groups:

  - $SST = \boldsymbol{SST_{model}} + SSE_{residual}$

  - $\boldsymbol{SST_{model}} = SST_{factorA} + SST_{factorB} + SST_{AxB\ interaction}$

Two-way ANOVA's Have Three Models
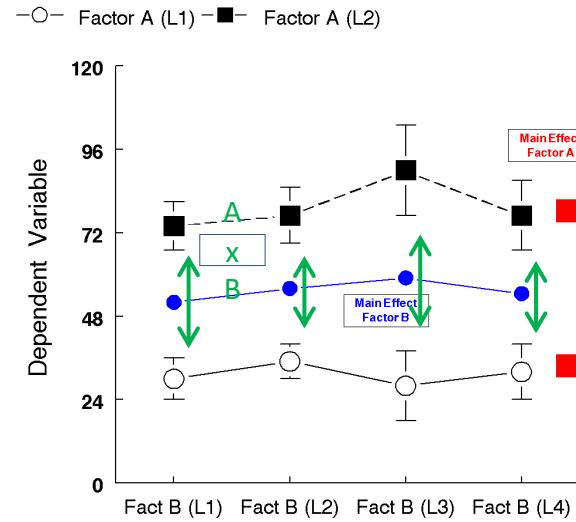
- Main Effect of Factor B (blue symbols, means)
- Main Effect of Factor A (red symbols, means)
- A x B Interaction (green lines, means of differences)

Two-way ANOVA

THREE Null Hypotheses

● Hypothesis for Factor A = *Main Effect of Factor A*
● Hypothesis for Factor B = *Main Effect of Factor B*
● Hypothesis for Interaction between Factor A and Factor B =
   *Factor A x Factor B Interact*
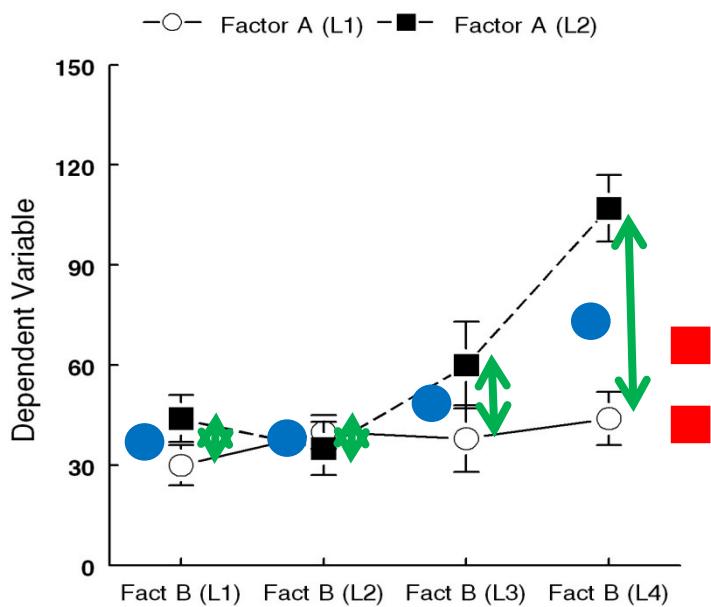
Two-way ANOVA Table with independent/random samples

Source	Sum of Squares	df	Mean Square	F-Ratio
Main Effect-A	SST_A	df_A	MS_A	$MS_A / MSE_{residual}$
Main Effect-B	SST_B	df_B	MS_B	$MS_B / MSE_{residual}$
A X B Interact	SST_{AXB}	df_{AXB}	MS_{AXB}	$MS_{AXB} / MSE_{residual}$
Residual (Error)	$SSE_{residual}$	$df_{residual}$	$MSE_{residual}$	
Total	SST_{Total}	df_{Total}	MS_{Total}	

Two-way ANOVA: 3 omnibus null hypotheses

- Main effect Factor A: $H_0: \sigma_A^2 \leq \sigma_r^2$

- Main effect Factor B: $H_0: \sigma_B^2 \leq \sigma_r^2$

- Interaction AxB: $H_0: \sigma_{A \times B}^2 \leq \sigma_r^2$

**Main effects usually detected when interaction occurs**

Main effect of A?

yes
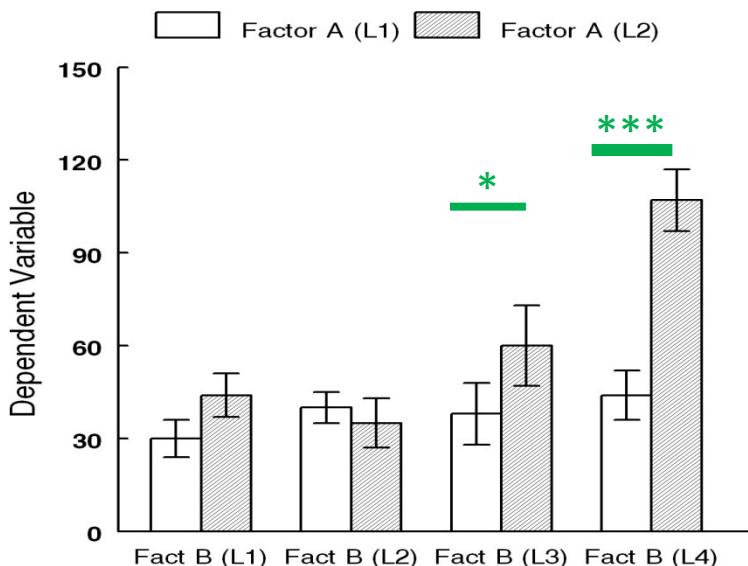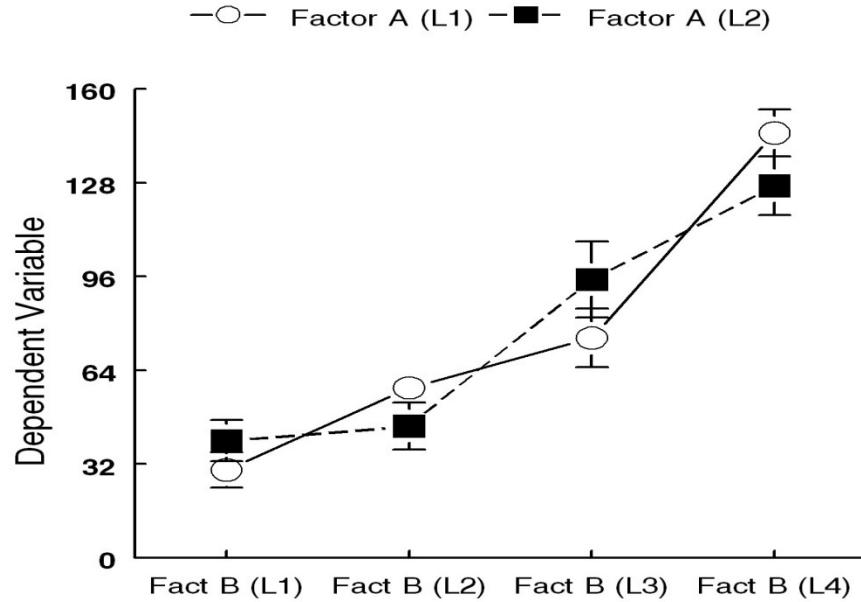
Main effect of B?

yes
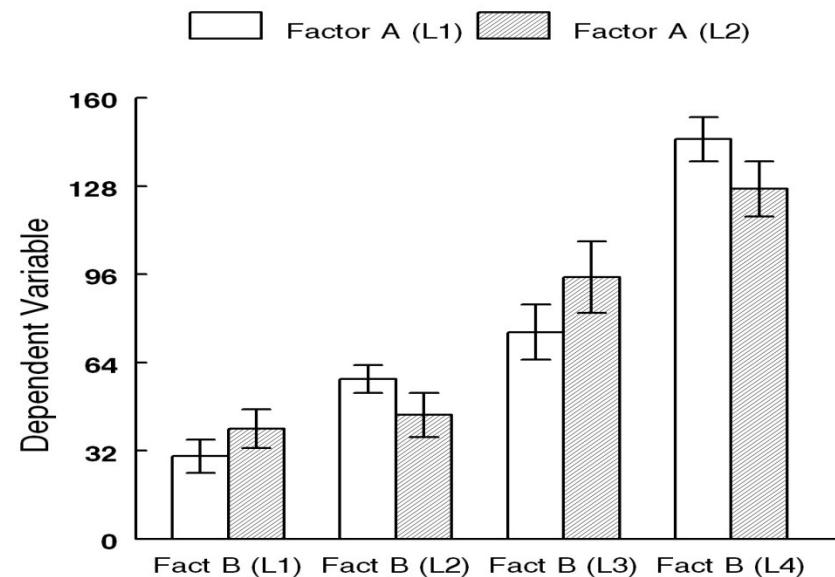
AXB Interaction?

yes

Main effect of A?

none
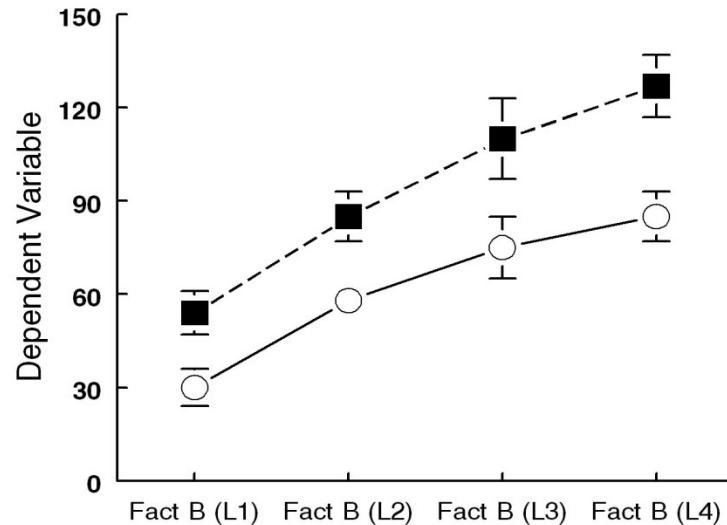
Main effect of B?

yes

AXB Interaction?

none

Main effect of A?

yes

Main effect of B?

yes
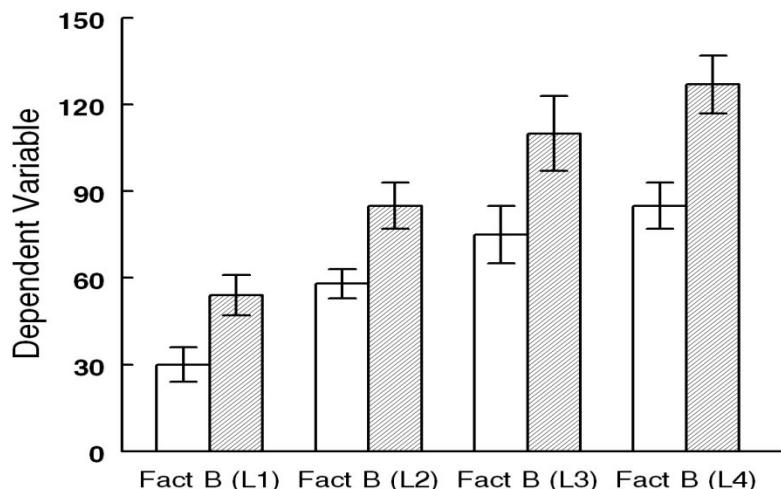
AXB Interaction?

none

## ANOVA: Completely Randomized vs. Related Measures
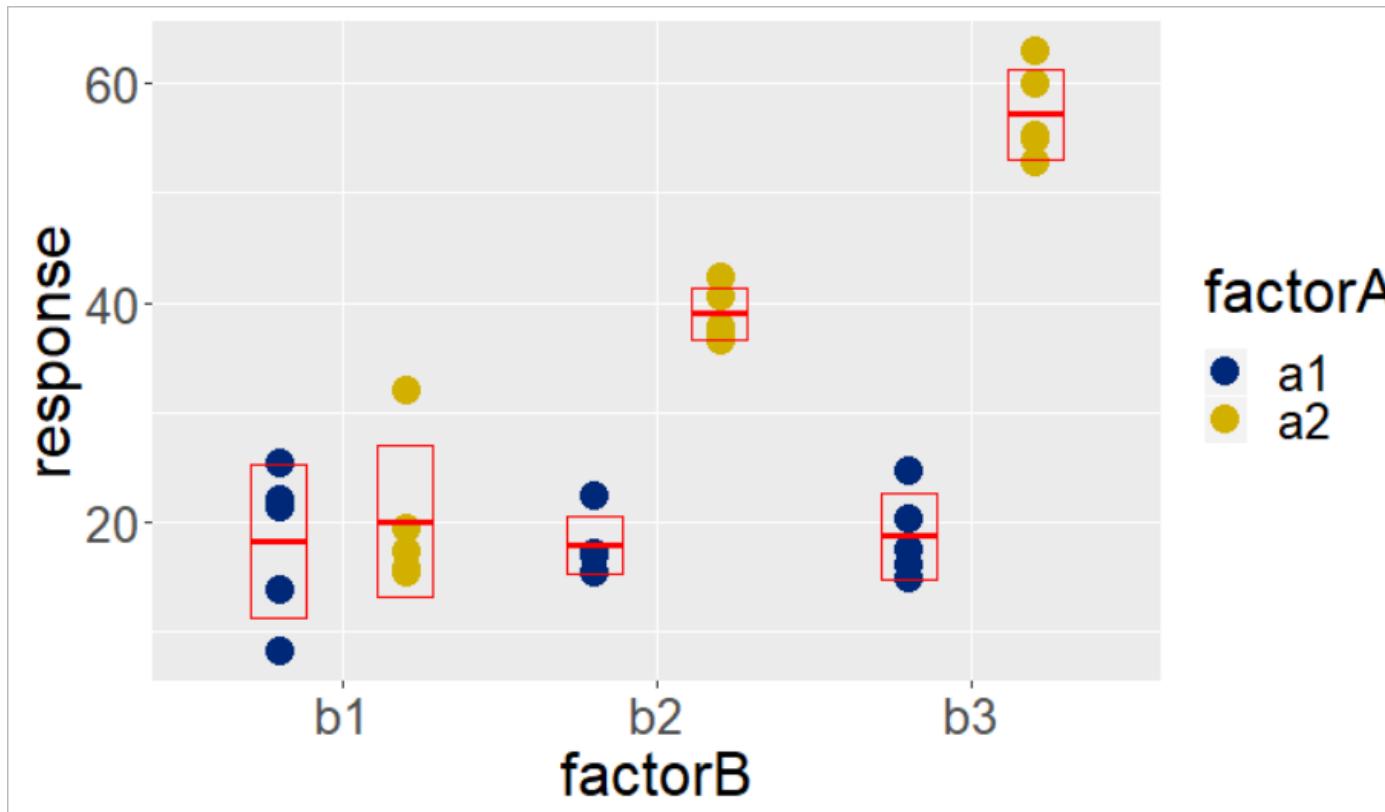
Example related measures:

- before-after
- identical twins
- isogenic littermates
- split tissue
- cell culture
- one cell
- one extract

Question: Are any measurements intrinsically-related?

Yes? The are from the same replicate, use related measures for that factor

Two-way ANOVA Completely Randomized

N=30
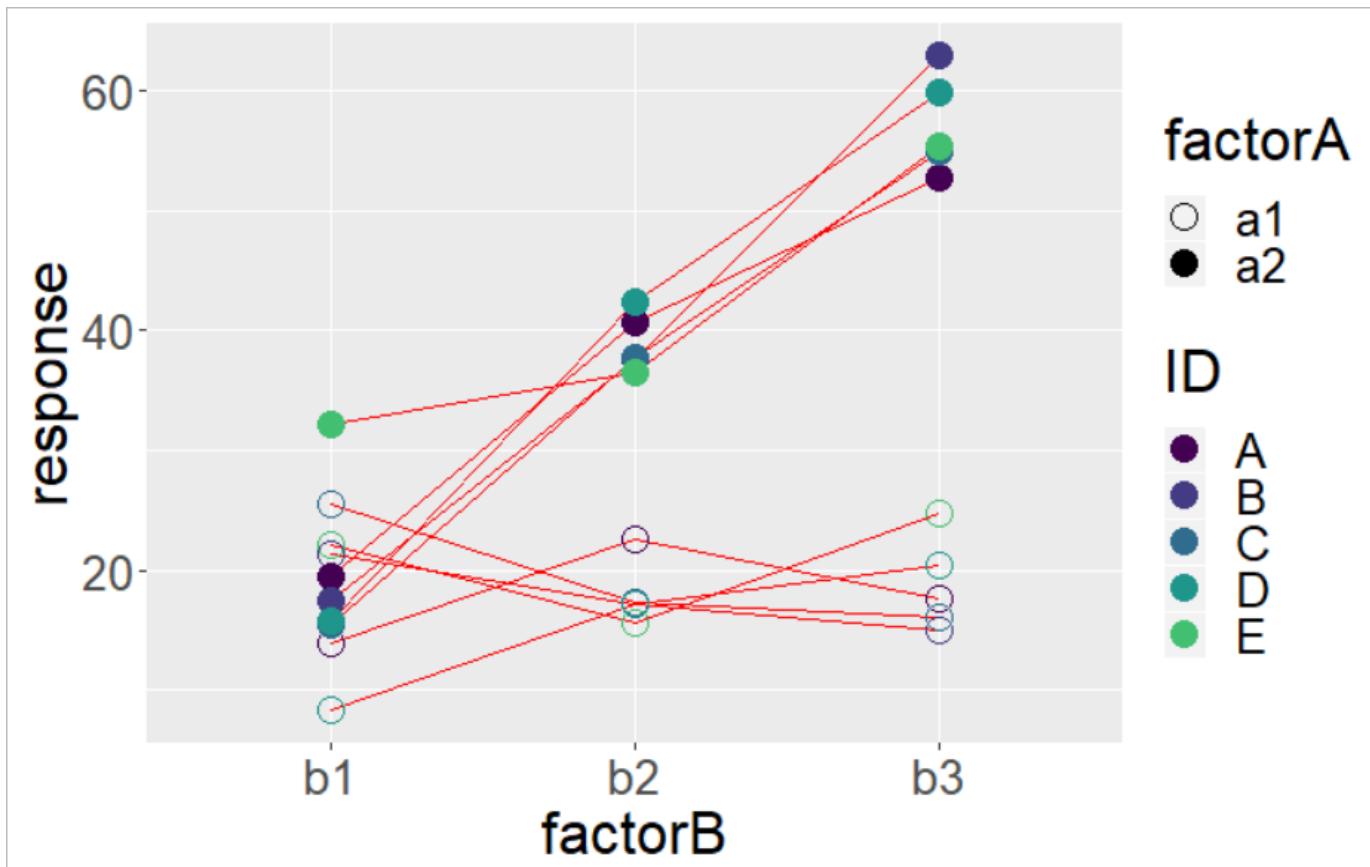
Groups are independent

Two-way ANOVA: Completely Randomized on factorA and factorB

```
out <- ezANOVA(
 …
between= c(factorB,
        factorA),
…
)
```

```
out$ANOVA
Effect            DFn DFd SSn SSd F        p             p<.05 ges
1 factorA           1  24 3123 566 132.3 2.988e-11 *            0.846
2 factorB           2  24 1770 566  37.5 4.117e-08 *            0.757
3 factorA:factorB   2  24 1673 566  35.4 6.871e-08 *            0.747
```
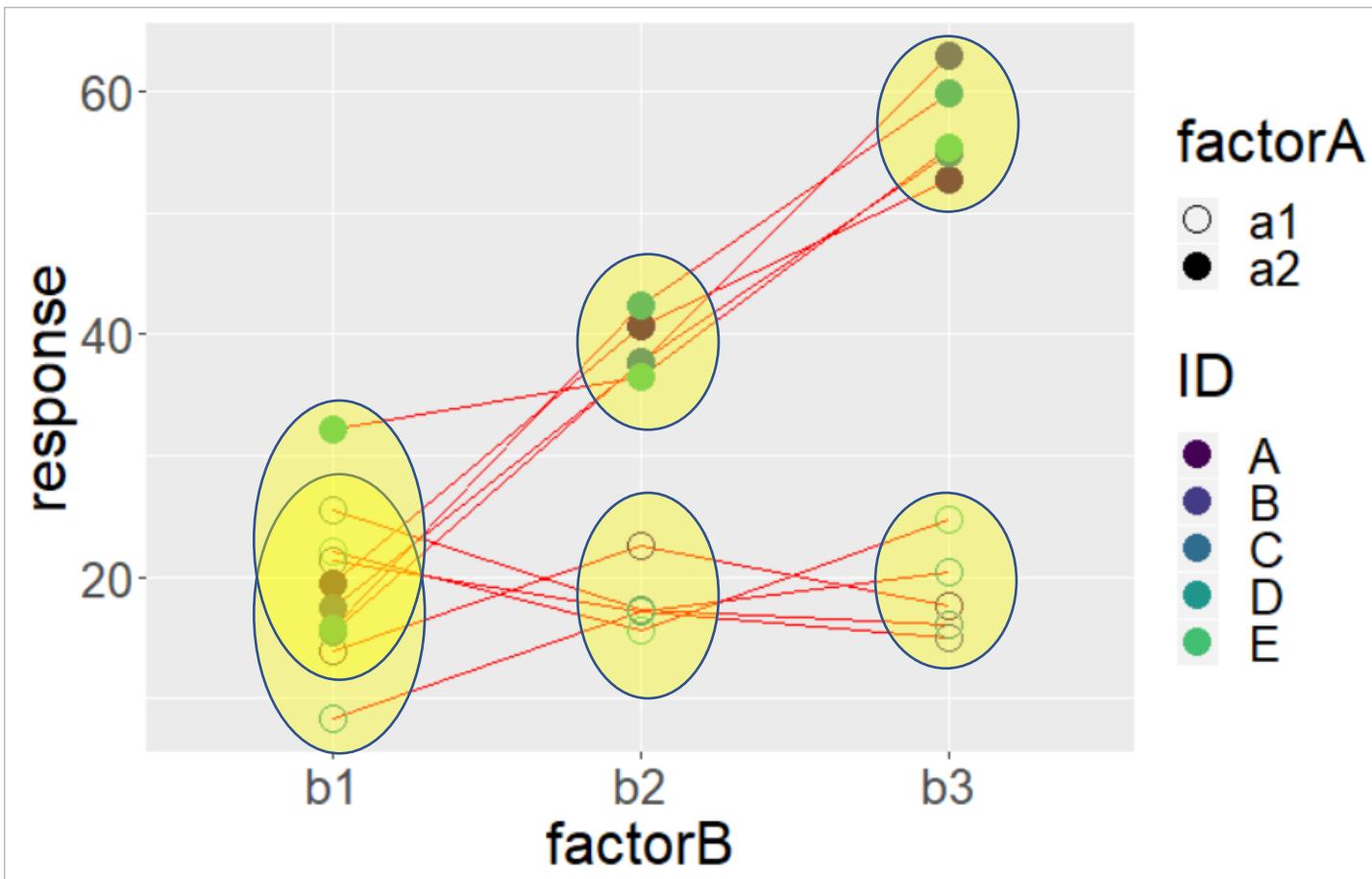
Two-way ANOVA Related Measures

N=5

Group means are irrelevant and deceptive; group comparisons are NOT independent

Group means are not independent! We can't unpair them!!


Every replicate (color) IS independent

Two-way ANOVA : Repeated Measures on factorA and factorB
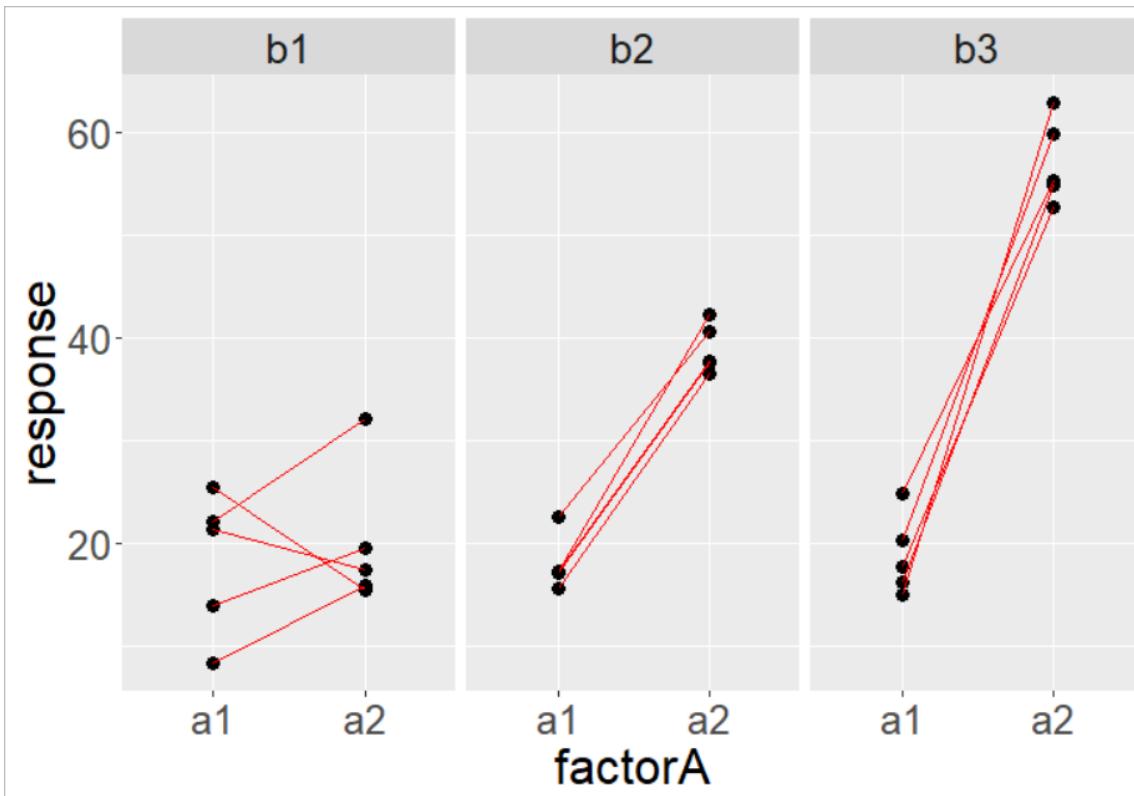
```
out <- ezANOVA(
  …
within = c(factorA,
           factorB),
…
)
```

```
out$ANOVA
Effect             DFn DFd SSn    SSd F       p         p<.05 ges
1 (Intercept)      1   4   24398   53 1816.3  1.811e-06 *     0.977
2 factorA          1   4    3123   46  268.2  8.137e-05 *     0.846
3 factorB          2   8    1770  275   25.6  3.304e-04 *     0.757
4 factorA:factorB  2   8    1673  190   35.1  1.088e-04 *     0.747
```

Two-way ANOVA mixed

Related Measures on factor A
Completely Randomized on factor B

N=15

A groups are
related measures

B groups are
independent

Two-way ANOVA mixed

Related Measures on factor A
Completely Randomized on factor B

out <- ezANOVA(

...

between= factorB,
within = factorA

...

)
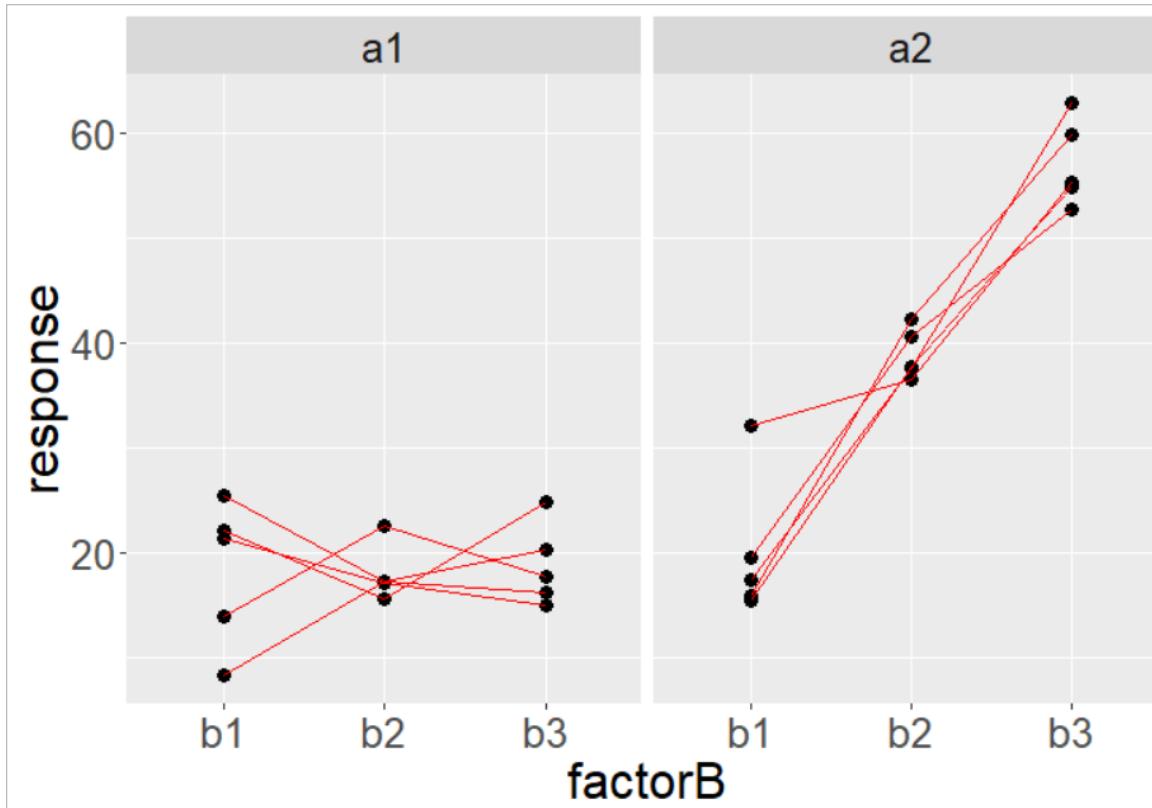
```
out$ANOVA
  Effect              DFn DFd SSn    SSd  F   p           p<.05 ges
1 (Intercept)        1   12  24398  329  887 1.275e-12 * 0.977
2 factorB            2   12  1770   329  32  1.495e-05 * 0.757
3 factorA            1   12  3123   236  158 2.859e-08 * 0.846
4 factorB:factorA    2   12  1673   236  42  3.642e-06 * 0.747
```

Two-way ANOVA mixed

Completely Randomized on factor A
Related Measures on factor B

N=10

A groups are
independent

B groups are
related measures

Two-way ANOVA mixed
Completely Randomized on factor A
Related Measures on factor B

```
out <- ezANOVA(

    ...
    between= factorA,
    within = factorB,

    ...
    )
```
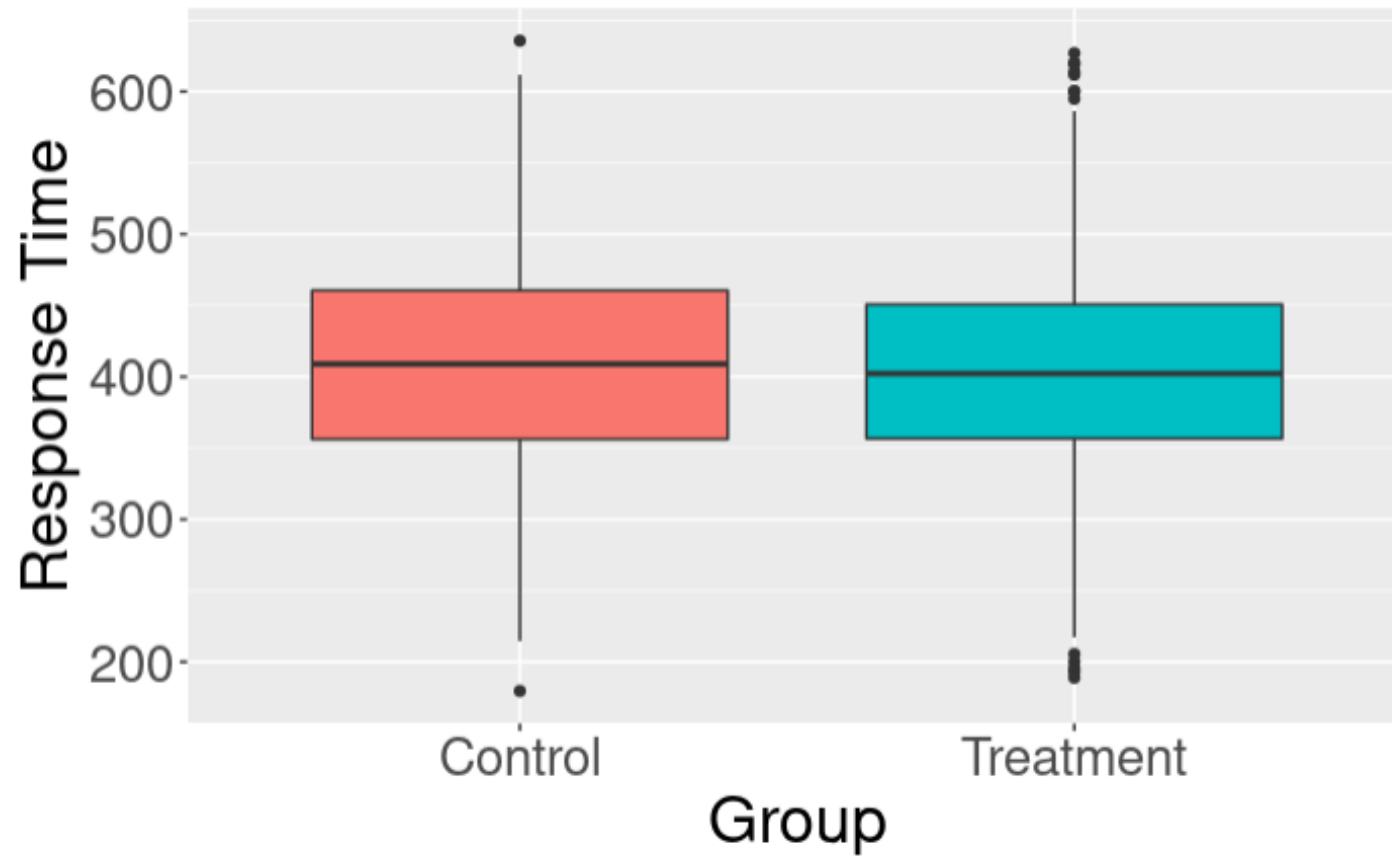
```
out$ANOVA
Effect              DFn DFd SSn    SSd F    p         p<.05 ges
1 (Intercept)       1   8   24398  100 1945 7.700e-11 *     0.977
2 factorA           1   8    3123  100  249 2.595e-07 *     0.846
3 factorB           2  16    1770  466   30 3.564e-06 *     0.757
4 factorA:factorB   2  16    1673  466   28 5.092e-06 *     0.747
```

A data frame with 5760 observations on the following 10 variables.

subnum a factor with levels 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

group a factor with levels Control Treatment

block a numeric vector

trial a numeric vector

cue a factor with levels None Center Double Spatial

flank a factor with levels Neutral Congruent Incongruent

location a factor with levels down up

direction a factor with levels left right

rt a numeric vector

error a numeric vector

Mixed two-way ANOVA

- Response: response time (rt)
- Completely Randomized on group
- Related Measures on cue and flank

```
#Run an ANOVA on the mean correct RT data.
rt_anova = ezANOVA(data = ANT_correct,
                   dv = rt,
                   wid = subnum,
                   within = .(cue,flank),
                   between = group
)
#Show the ANOVA and assumption tests.
print(rt_anova)
```

between: "levels of this factor vary between replicates"
within: "levels of this factor vary within replicates"

Mixed two-way ANOVA results by ezANOVA()

```
$ANOVA
            Effect DFn DFd           F            p p<.05         ges
2            group   1  18   18.430592 4.377562e-04     * 0.07633358
3              cue   3  54  516.605213 1.005518e-39     * 0.89662286
5            flank   2  36 1350.598810 1.386546e-34     * 0.92710583
4        group:cue   3  54    2.553236 6.497492e-02       0.04110445
6      group:flank   2  36    8.768499 7.900829e-04     * 0.07627434
7        cue:flank   6 108    5.193357 9.938494e-05     * 0.11436699
8  group:cue:flank   6 108    6.377225 9.012515e-06     * 0.13686958

$`Mauchly's Test for Sphericity`
            Effect         W         p p<.05
3              cue 0.7828347 0.5366835
4        group:cue 0.7828347 0.5366835
5            flank 0.8812738 0.3415406
6      group:flank 0.8812738 0.3415406
7        cue:flank 0.1737053 0.1254796
8  group:cue:flank 0.1737053 0.1254796

$`Sphericity Corrections`
            Effect       GGe        p[GG] p[GG]<.05       HFe        p[HF] p[HF]<.05
3              cue 0.8652559 1.115029e-34         * 1.0239520 1.005518e-39         *
4        group:cue 0.8652559 7.472046e-02           1.0239520 6.497492e-02
5            flank 0.8938738 3.763312e-31         * 0.9858964 3.964046e-34         *
6      group:flank 0.8938738 1.297752e-03         * 0.9858964 8.438369e-04         *
7        cue:flank 0.6022111 1.546166e-03         * 0.7721473 4.745714e-04         *
8  group:cue:flank 0.6022111 3.424499e-04         * 0.7721473 7.170939e-05         *
```
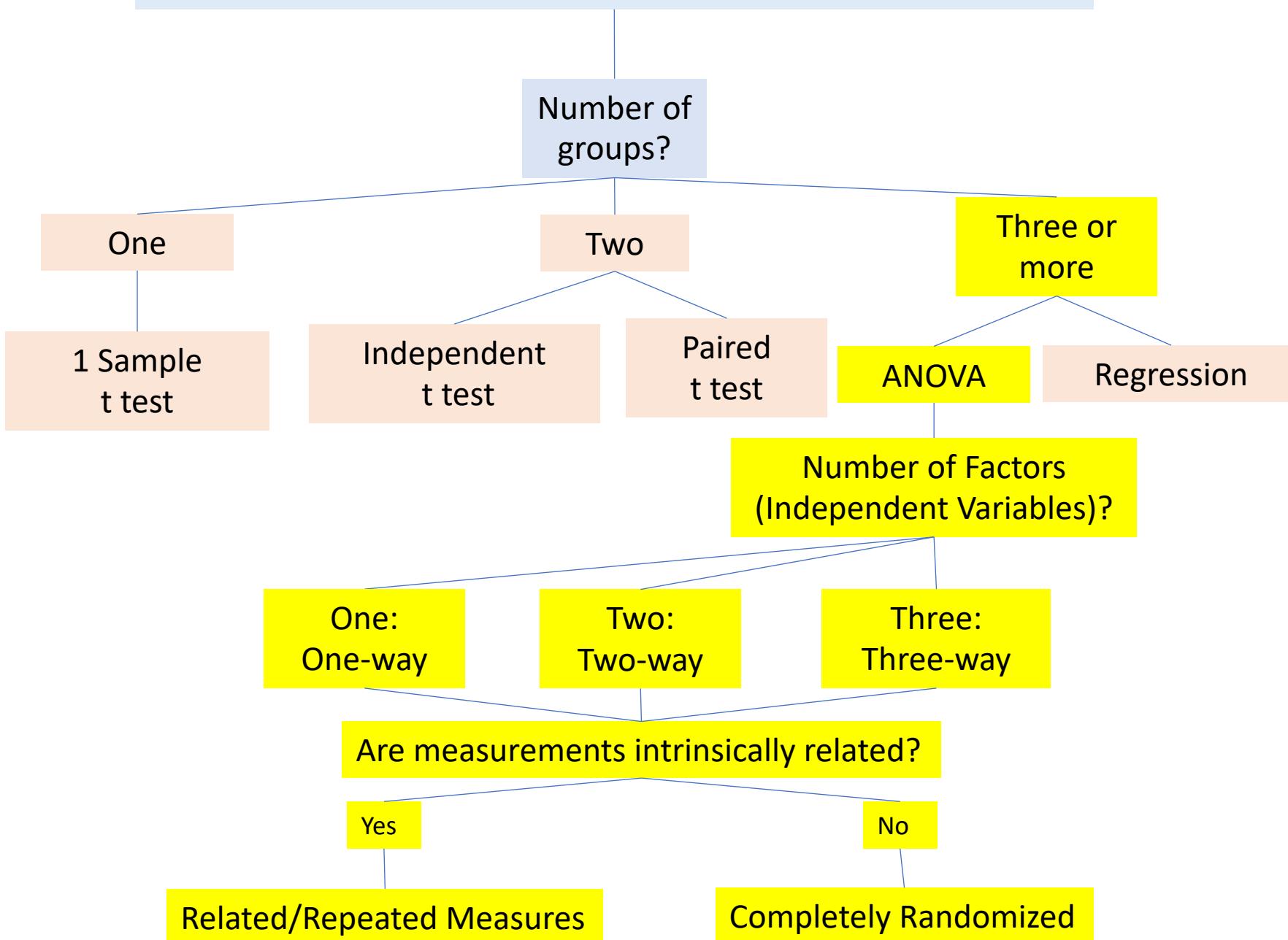
Output variables by ezANOVA():

DFn	Degrees of Freedom in the numerator (a.k.a. DFeffect).
DFd	Degrees of Freedom in the denominator (a.k.a. DFerror).
SSn	Sum of Squares in the numerator (a.k.a. SSeffect).
SSd	Sum of Squares in the denominator (a.k.a. SSerror).
F	F-value.
p	p-value (probability of the data given the null hypothesis).
p<.05	Highlights p-values less than the traditional alpha level of .05.
ges	Generalized Eta-Squared measure of effect size (see in references below: Bakeman, 2005).
GGe	Greenhouse-Geisser epsilon.
p[GGe]	p-value after correction using Greenhouse-Geisser epsilon.
p[GGe]<.05	Highlights p-values (after correction using Greenhouse-Geisser epsilon) less than the traditional alpha level of

HFe	Huynh-Feldt epsilon.
p[HFe]	p-value after correction using Huynh-Feldt epsilon.
p[HFe]<.05	Highlights p-values (after correction using Huynh-Feldt epsilon) less than the traditional alpha level of .05.
W	Mauchly's W statistic

Common mistakes with ANOVA

- Overdesigned. Testing too many factors & levels simultaneously.

- Treating technical replicates as independent

- Not controlling Family-wise Error Rate (FWER) in posthoc tests

- Running Completely Randomized analysis on Related Measures designs
  - Running posthoc range tests when RM

- Never doing *a priori* power / sample size analysis (Week 11 Lecture)

Best Practices

1

Step1: Make a tidy data frame

- One variable per column. One column for the unique subject ID
- Missing data? Exclude or impute (by sample mean)

2

Step2: Plot the data

- Response vs. Factors

3

Step3: Use ez::ezANOVA() or aov()

- Is factor Completely Randomized (between) or Related Measures (within)?

When Data Assumptions Cannot be Satisfied?

Permutation test (Week 13 Lecture)

In-Class Exercise 2 :
Two-way ANOVA