Lesson 17: Comparing Means with ANOVA

TB sections 5.5

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2024-~~10-02~~ 12-2

Learning Objectives

1. Revisit data visualization for a numeric outcome and categorical variable (from Lesson 8).
2. Understand the different measures of variability within an Analysis of Variance (ANOVA) table.
3. Understand the F-statistic and F-distribution that is used to measure the ratio of between group and within group variability.
4. Determine if groups of means are different from one another using a hypothesis test and F-distribution.

Where are we?

Data
- Collecting data
- Categorical vs. Numeric
- Summary statistics
- Data visualization

Probability
- Probability rules
- Independence, conditional
- Random variables and probability distributions
- Linear combinations
- Binomial, Normal, and Poisson

Sampling Variability, and Statistical Inference
- Sampling distributions
- Central Limit Theorem
- Confidence Intervals
- Hypothesis tests

Inference for continuous data/outcomes
- One sample t-test
- 3+ independent samples
- Simple linear regression / correlation
- 2 sample tests: paired and independent
- Power and sample size
- Non-parametric tests

Inference for categorical data/outcomes
- One proportion test
- Fisher's exact test
- Non-parametric tests
- Chi-squared test
- 2 proportion test
- Power and sample size

R
- Basics
- Reproducibility
- Quarto
- Packages
- Data visualization
- Data wrangling
- R Projects
- …

A little while ago…

- We looked at inference for a **single mean** $\hat{\mu}$
- We looked at inference for a difference in **means from two independent samples** $\hat{\mu}_1 - \hat{\mu}_2$

- If there are two groups, we could see if they had different means by testing if the difference between the means were the same (null) or different (alternative)

- What happens when we want to compare two **or more** groups' means?
  - Can no longer rely on the difference in means
  - Need a new method to make inference (ANOVA or Linear Regression!)

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From Lesson 8: Data visualization

- Study investigating whether ACTN3 genotype at a particular location (residue 577) is associated with change in muscle function

- **Categorical variable:** genotypes (CC, TT, CT)

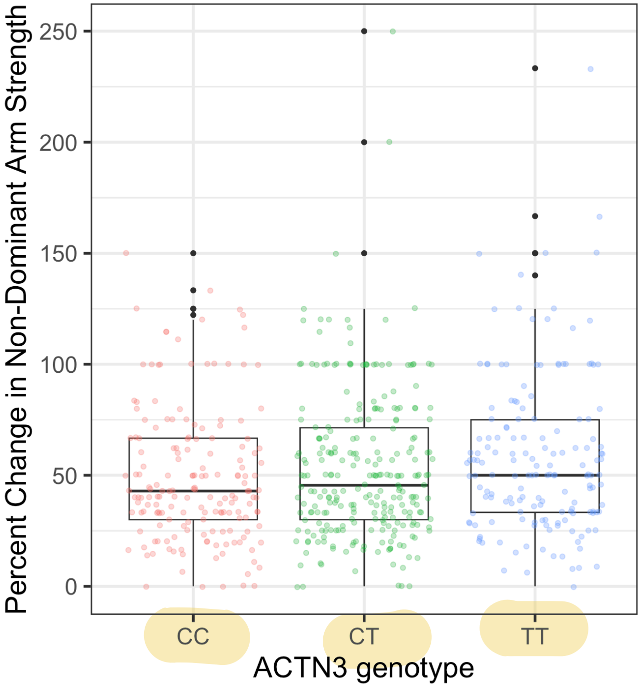
- **Numeric variable:** Muscle function, measured as percent change in non-dominant arm strength

- We can start the investigation by plotting the relationship

From Lesson 8: Side-by-side boxplots with data points

- We can look at the boxplot of percent change for each genotype **with points shown so we can see the distribution of observations better**

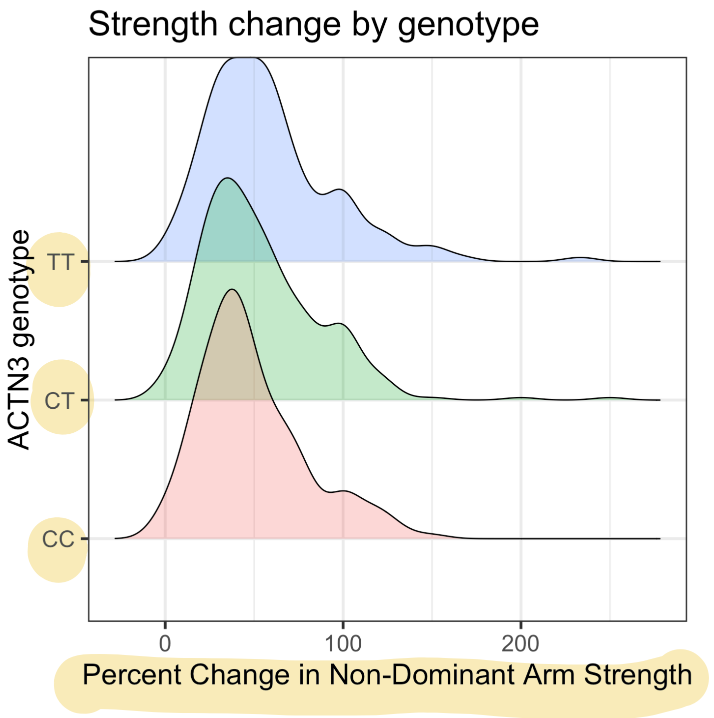
```
ggplot(data = famuss,
       aes(x = actn3.r577x,
           y = ndrm.ch)) +
  geom_boxplot() +
  labs(x = "ACTN3 genotype",
       y = "Percent Change in Non−Dominan
  geom_jitter(aes(color = actn3.r577x),
    alpha = 0.3,
    show.legend = FALSE,
    position = position_jitter(
      height = 0.4))
```



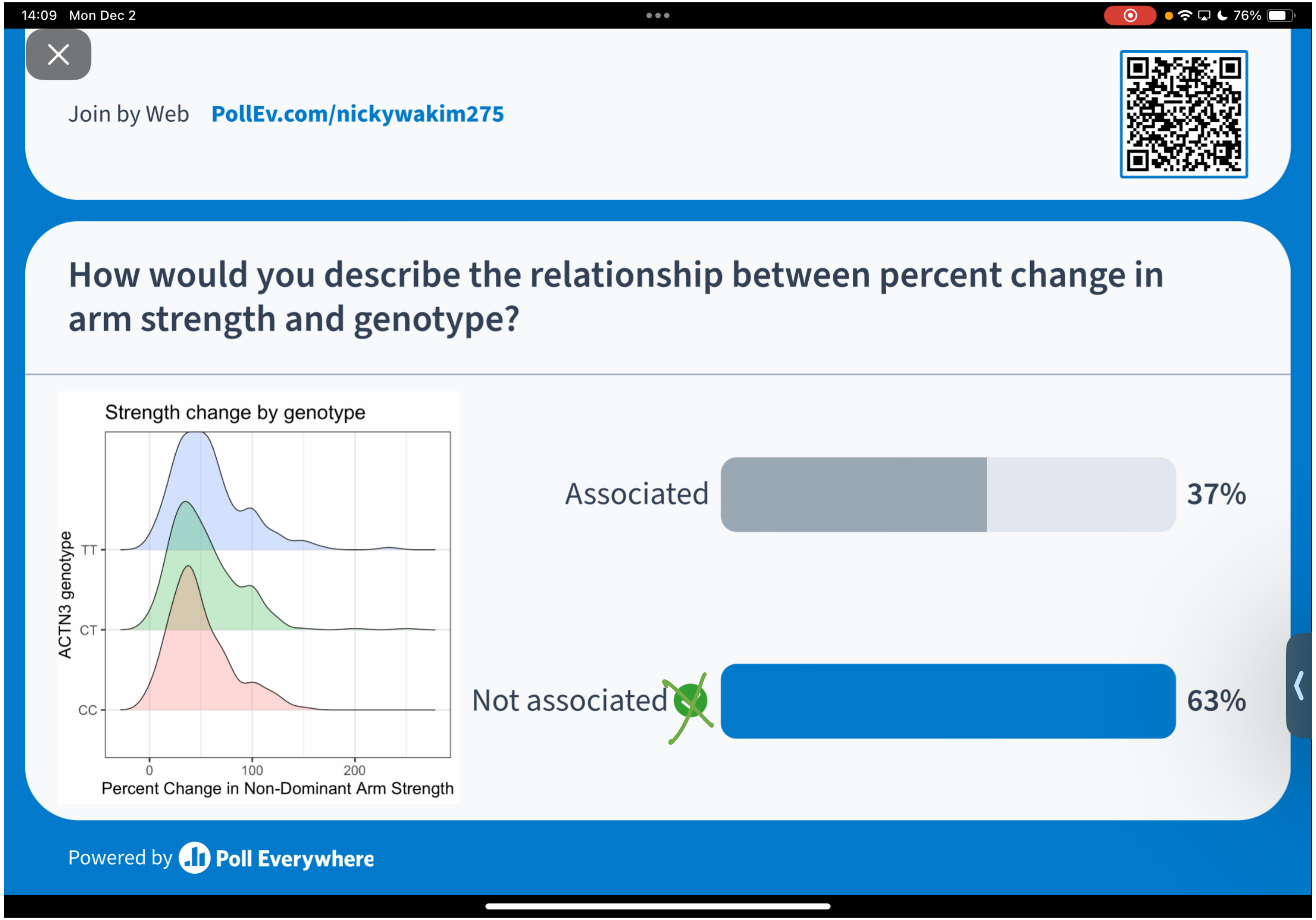
From Lesson 8: Ridgeline plot

- Overlapped densities were easy enough to see with 3 genotypes
- If you have **many categories**, a ridgeline plot might make it easier to see

```
library(ggridges)
ggplot(data = famuss,
       aes(y = actn3.r577x,
           x = ndrm.ch,
           fill = actn3.r577x)) +
  geom_density_ridges(alpha = 0.3,
          show.legend = FALSE) +
  labs(x = "Percent Change in Non-Dominant
       y = "ACTN3 genotype",
       title = "Strength change by genotyp
```



Poll Everywhere Question 1



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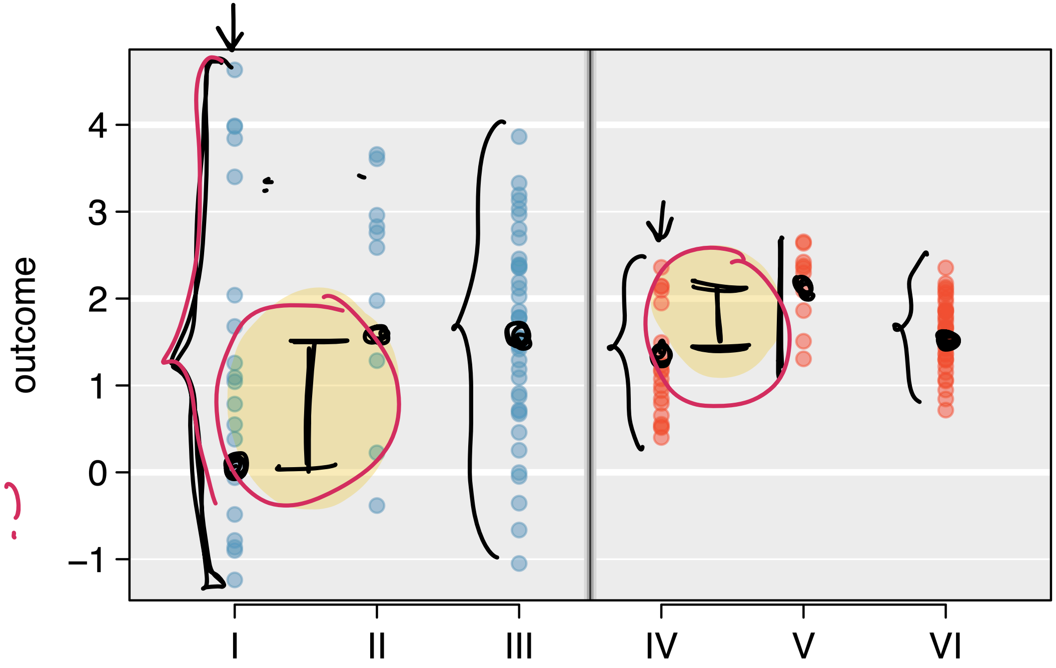
Comparing means

Whether or not two means are significantly different depends on:

- How far apart the **means** are
- How much **variability** there is within each group

Questions:

- How to measure variability **between** groups? how far apart are mean?
- How to measure variability **within** groups?
- How to compare the two measures of variability?
- How to determine significance?



Generic ANOVA table

The "mean square" is the sum of squares divided by the degrees of freedom

Source	df	Sum of Squares	Mean Square	F-Statistic
Groups	k-1	SSG	**MSG** = SSG/(k-1)	$\frac{MSG}{MSE}$
Error	N-k	SSE	**MSE** = SSE/(N-k)	
Total	N-1	SST		

Sum of Squares ↑ variability

Mean Square ↑ average variability

variability b/w grps → Groups

variability w/in grps → Error

The ***F-statistic*** is a ratio of

the average variability ***between*** groups

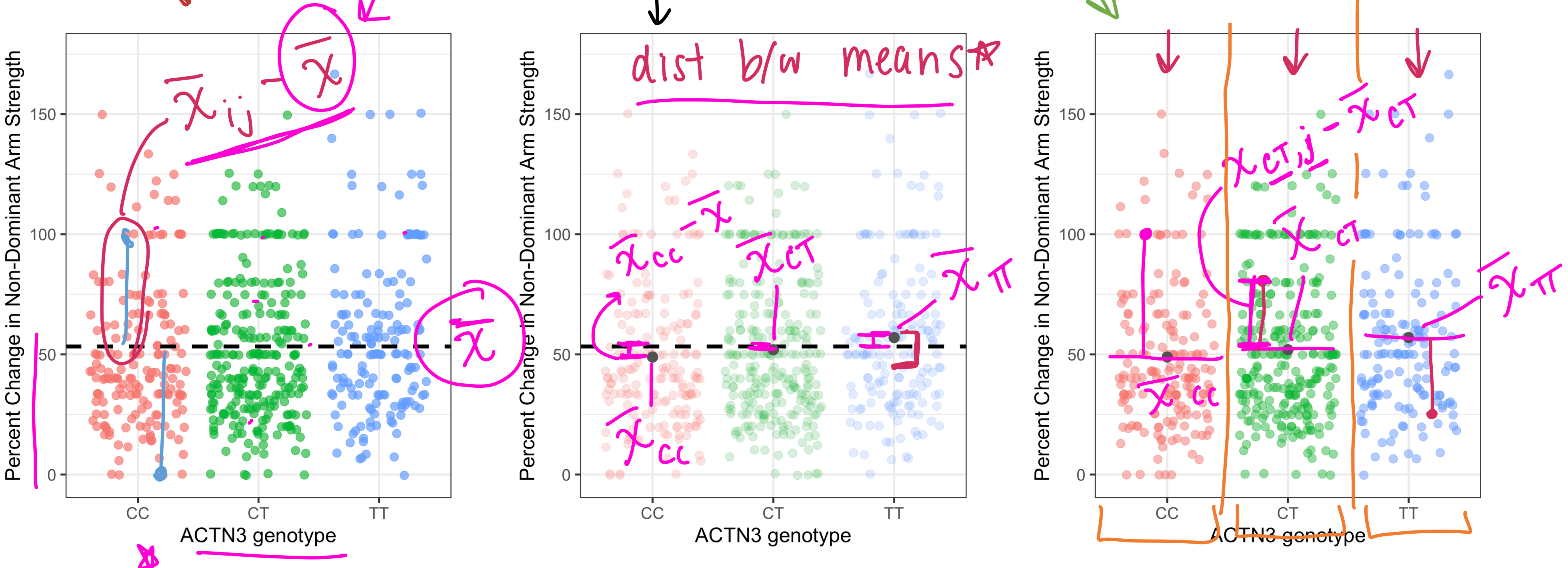
to the average variability ***within*** groups

ANOVA: Analysis of Variance

how far means are from each other

ANOVA compares the variability between groups to the variability within groups

Total variability = **Variability between groups** + **Variability within groups**



ANOVA: Analysis of Variance

Analysis of Variance (ANOVA) compares the variability between groups to the variability within groups

Total variability	=	Variability between groups	+	Variability within groups

$$\sum_{i=1}^{k}\sum_{j=1}^{n_i}(x_{ij}-\bar{x})^2 = \sum_{i=1}^{k} n_i(\bar{x}_i-\bar{x})^2 + \sum_{i=1}^{k}\sum_{j=1}^{n_i}(x_{ij}-\bar{x}_i)^2$$

SST (Total sum of squares)	=	**SSG** (sum of squares due to groups)	+	**SSE** (Error sum of squares)

within grp i, diff b/w the individual's measurement & grp i's mean

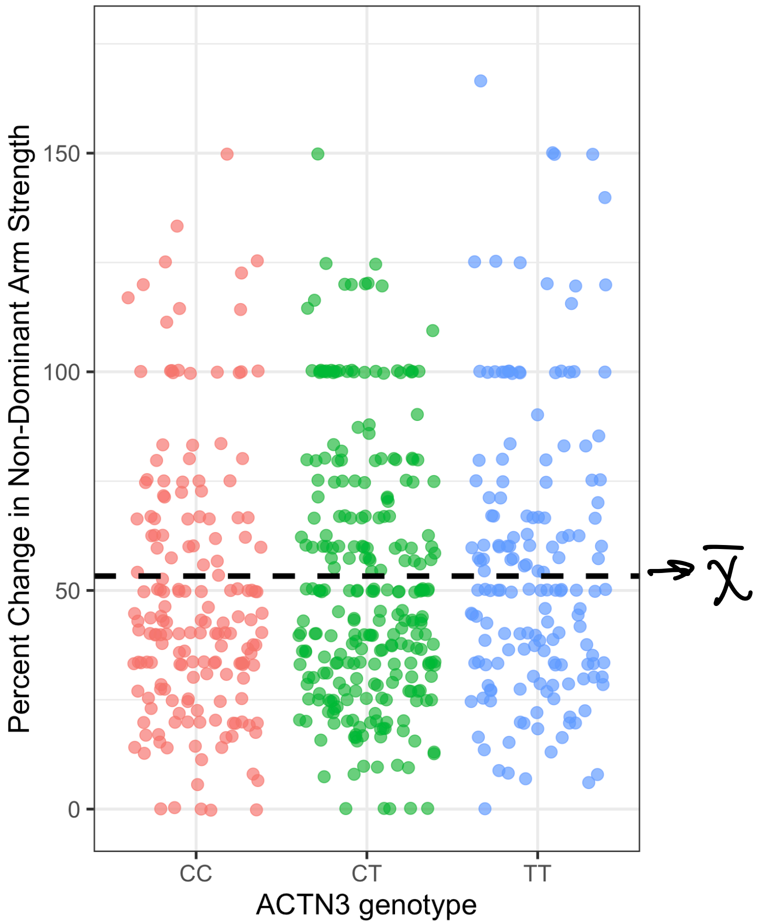
$\bar{x}$ = grand sample mean (mean for all regardless of grp)

Notation

- k groups
- n_i observations in each of the k groups
- Total sample size is $N = \sum_{i=1}^{k} n_i$
- $\bar{x}_i$ = mean of observations in group *i*
- $\bar{x}$ = mean of *all* observations
- s_i = sd of observations in group *i*
- s = sd of *all* observations

Observation	***i* = 1**	***i* = 2**	***i* = 3**	. . .	***i* = *k***	**overall**
j = 1	x_{11}	x_{21}	x_{31}	. . .	x_{k1}	
j = 2	x_{12}	x_{22}	x_{32}	. . .	x_{k2}	
j = 3	x_{13}	x_{23}	x_{33}	. . .	x_{k3}	
j = 4	x_{14}	x_{24}	x_{34}	. . .	x_{k4}	
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\ddots$	$\vdots$	
j = n_i	x_{1n_1}	x_{2n_2}	x_{3n_3}	. . .	x_{kn_k}	
Means	$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_3$	. . .	$\bar{x}_k$	$\bar{x}$
Variance	s_1^2	s_2^2	s_3^2	. . .	s_k^2	s^2

Total Sums of Squares (SST)

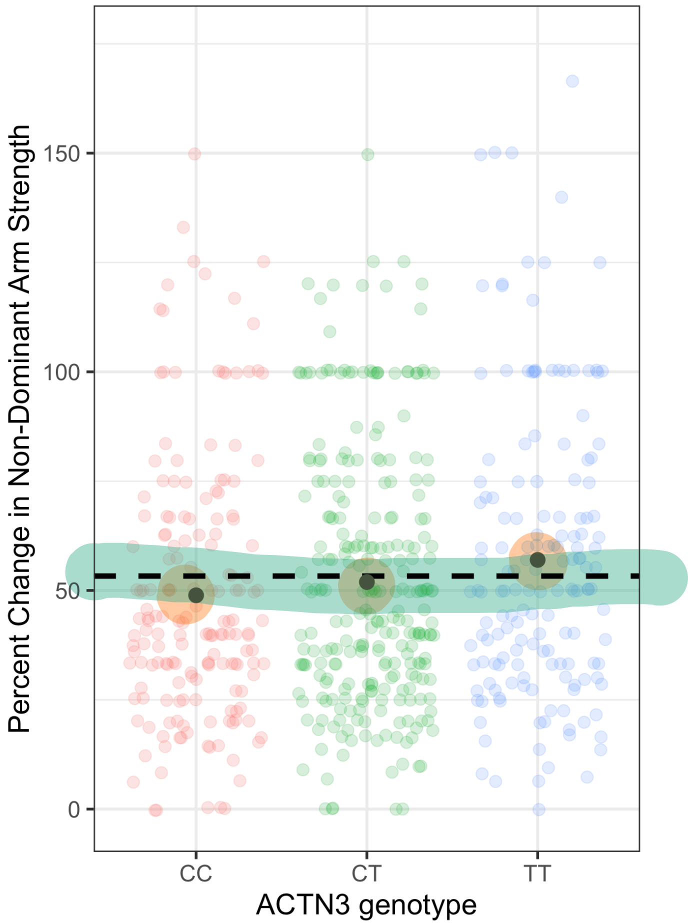


Total Sums of Squares:

$$SST = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (x_{ij} - \bar{x})^2 = (N-1)s^2$$

- where
  - $N = \sum_{i=1}^{k} n_i$ is the total sample size and
  - s^2 is the grand standard deviation of all the observations
- This is the sum of the squared differences between each observed x_{ij} value and the *grand mean*, $\bar{x}$.
- That is, it is the total deviation of the x_{ij}'s from the grand mean.

Sums of Squares due to Groups (SSG)

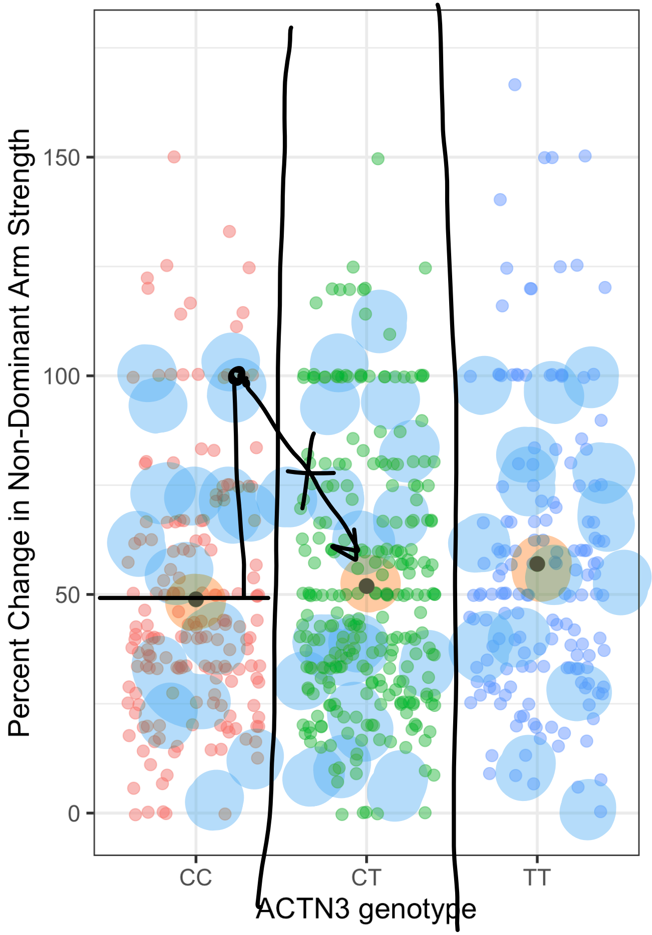


Sums of Squares due to Groups:

$$SSG = \sum_{i=1}^{k} n_i(\bar{x}_i - \bar{x})^2$$

- This is the sum of the squared differences between each *group* mean, $\bar{x}_i$, and the *grand mean*, $\bar{x}$.
- That is, it is the deviation of the group means from the grand mean.
- Also called the Model SS, or SS_{model}.

Sums of Squares Error (SSE)



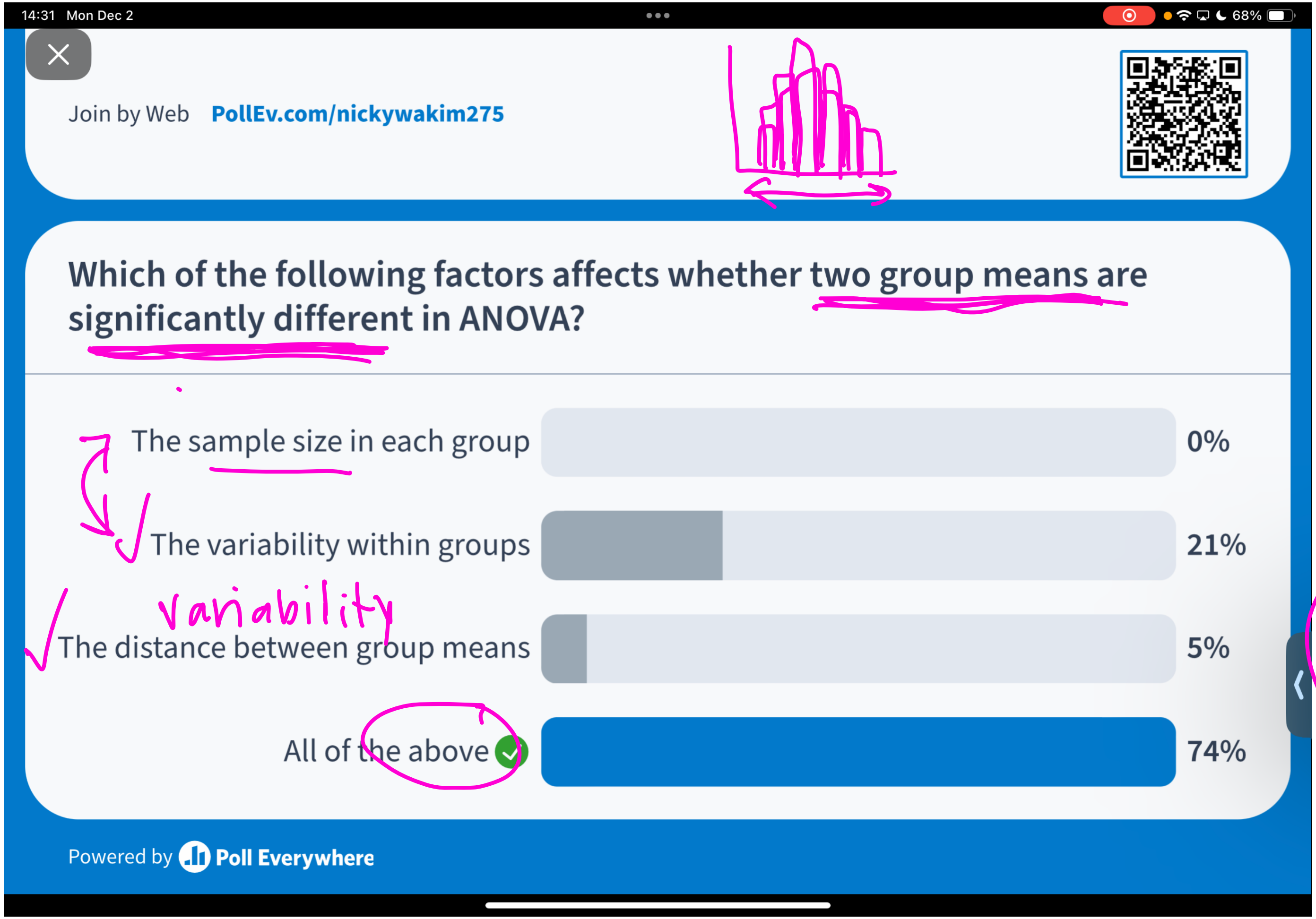
Sums of Squares Error:

$$SSE = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2 = \sum_{i=1}^{k} (n_i - 1)s_i^2$$

where s_i is the standard deviation of the i^{th} group

- This is the sum of the squared differences between each observed x_{ij} value and its group mean $\bar{x}_i$.
- That is, it is the deviation of the x_{ij}'s from the predicted ndrm.ch by group.
- Also called the residual sums of squares, or $SS_{residual}$.

Poll Everywhere Question 2



variability b/w grps — higher

vs.

variability w/in grps — lower

ANOVA table to hypothesis test?

- Okay, so how do we use all these types of variability to run a test?
- How do we determine, statistically, if the groups have different means or not?

↑ F ↑ SSG
↑ var b/w grps

↓ F ↑ SSE
↑ var w/in grps

The "mean square" is the sum of squares divided by the degrees of freedom

Source	df	Sum of Squares	Mean Square	F-Statistic
Groups	k-1	SSG	**MSG** = SSG/(k-1)	$\frac{MSG}{MSE}$
Error	N-k	SSE	**MSE** = SSE/(N-k)	
Total	N-1	SST		

The ***F-statistic*** is a ratio of

the average variability ***between*** groups

to the average variability ***within*** groups

(SST ↑) variability

(Mean Square ↑) average variability — way we standardize the variability

b/w: more variab. here = more evidence that grp means are diff

w/i: more var here = more evidence that grp means are NOT diff

- Answer: We use the F-statistic in a hypothesis test!

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Thinking about the F-statistic

If the groups are actually different, then which of these is more accurate?

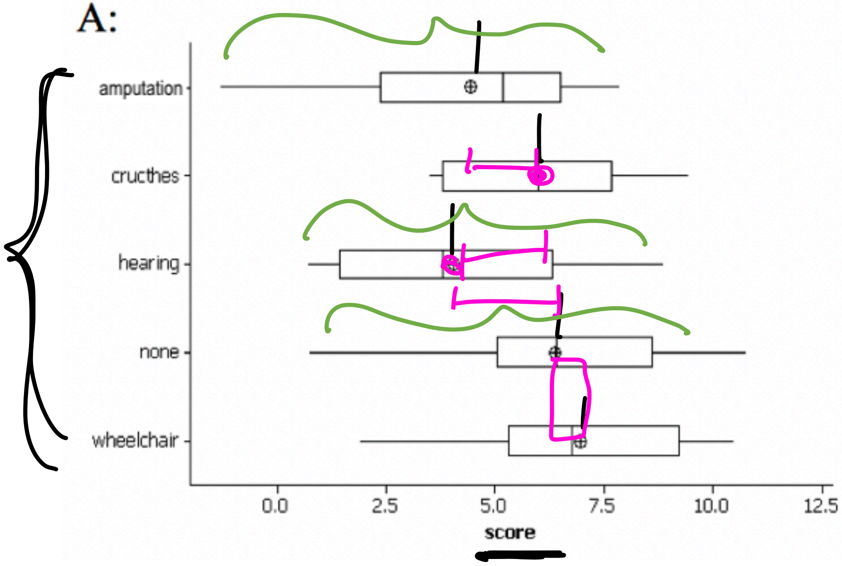
1. **The variability between groups should be higher than the variability within groups**
2. The variability within groups should be higher than the variability between groups

If there really is a difference between the groups, we would expect the F-statistic to be which of these:

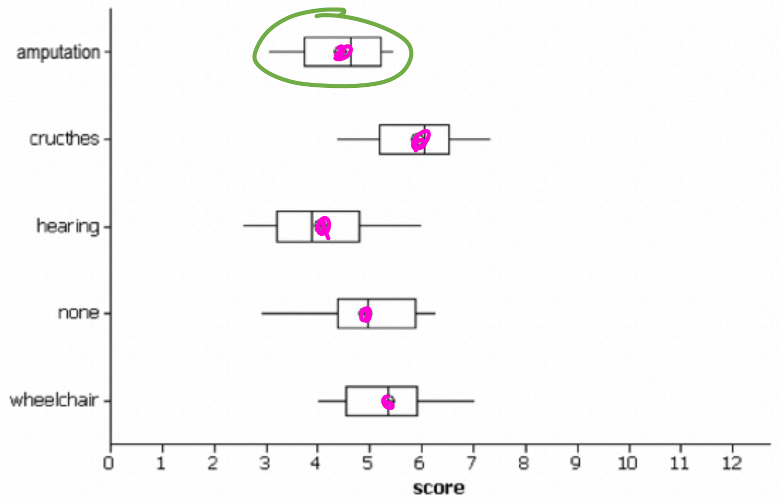
1. **Higher than we would observe by random chance** (aka no assoc)
2. Lower than we would observe by random chance

F statistic higher in B

A:



B:



The F-statistic

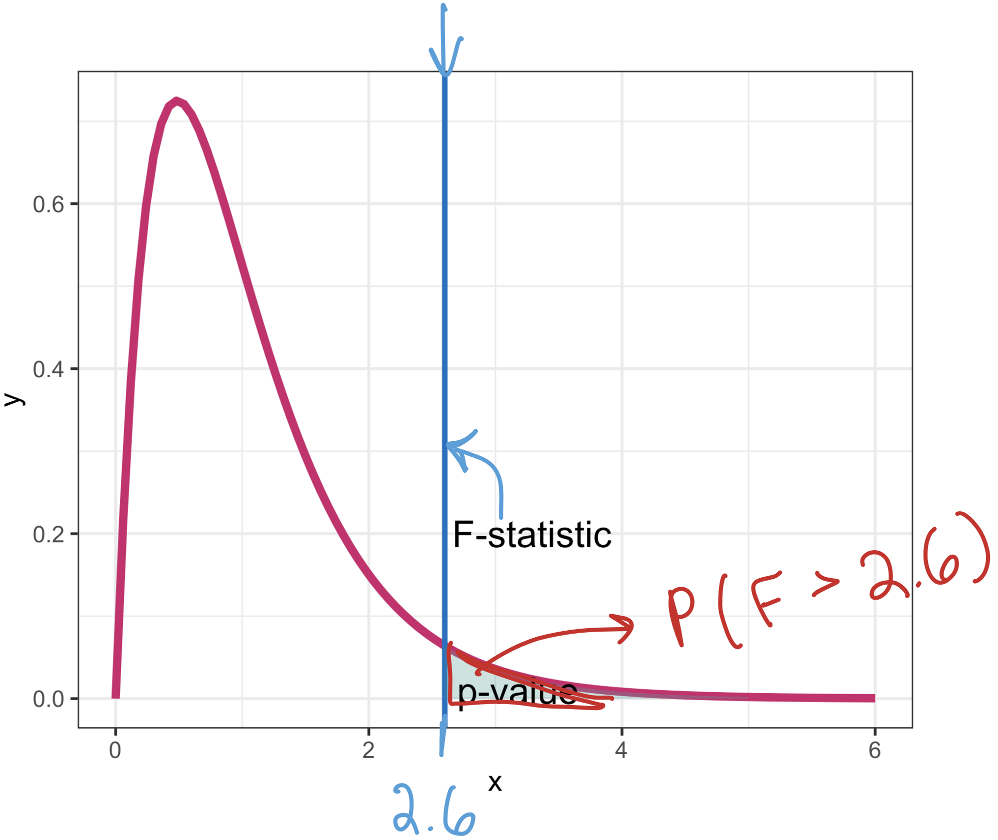
- F-statistic represents the standardized ratio of variability between groups to the variability within the groups

$$F_{stat} = \frac{MSG}{MSE}$$

- F is larger when the variability between groups is larger than variability within groups

The F-distribution

- The F-distribution is skewed right
- The F-distribution has **two different degrees of freedom**:
  - one for the numerator of the ratio (k – 1) and
  - one for the denominator (N – k)
- p-value
  - $P(F > F_{stat})$
  - is always the upper tail
  - (the area as extreme or more extreme)



Poll Everywhere Question 3

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Reference: Steps in a Hypothesis Test

1. Check the **assumptions**
2. Set the **level of significance** α
3. Specify the **null** (H_0) and **alternative** (H_A) **hypotheses**
    1. In symbols
    2. In words
    3. ~~Alternative: one- or two-sided?~~
4. Calculate the **test statistic**.
5. Calculate the **p-value** based on the observed test statistic and its sampling distribution
6. Write a **conclusion** to the hypothesis test
    1. Do we reject or fail to reject H_0?
    2. Write a conclusion in the context of the problem

Step 1: Check assumptions

The sampling distribution is an **F-distribution**, if…

- Sample sizes in each group group are large (each $n \geq 30$) ✓
  - OR the data are relatively normally distributed in each group
- Variability is "similar" in all group groups:
  - Is the within group variability about the same for each group?
  - As a rough rule of thumb, this condition is *violated if the standard deviation of one group is more than double the standard deviation of another group*

Step 1: Check assumptions

- Use R to check both assumptions in our example

```
genotype_groups <- famuss %>%
  group_by(actn3.r577x) %>%
  summarise(count = n(),
            SD = sd(ndrm.ch))
genotype_groups
```

```
# A tibble: 3 × 3
  actn3.r577x count    SD
  <fct>       <int> <dbl>
1 CC            173  30.0
2 CT            261  33.2
3 TT            161  35.7
```

- Counts in each group are greater than 30!

```
max(genotype_groups$SD) / min(genotype_groups$SD)
```

```
[1] 1.191455
```

- Variability in one group vs. another is no more than 1.2 times!

Step 3: Specify Hypotheses

General hypotheses

To test for a difference in means across *k* groups:

$$H_0 : \mu_1 = \mu_2 = \ldots = \mu_k$$
$$\text{vs. } H_A : \text{At least one pair } \mu_i \neq \mu_j \text{ for } i \neq j$$

$\mu_1 = \mu_2 = \mu_3$ but $\mu_3 \neq \mu_4$

Hypotheses test for example

$$H_0 : \mu_{CC} = \mu_{CT} = \mu_{TT}$$
$$\text{vs. } H_A : \text{At least one pair } \mu_i \neq \mu_j \text{ for } i \neq j$$

also H_A:
- $\mu_{CC} \neq \mu_{CT}$ OR
- $\mu_{CT} \neq \mu_{TT}$ OR
- $\mu_{CC} \neq \mu_{TT}$

Step 4-5: Find the test statistic and p-value

- Our test statistic is an F-statistic
  - F-statistic: measurement of the ratio of variability between groups to variability within groups

- Our F-statistic follows an F-distribution
  - Which is why we cannot use something like the Z-distribution nor T-distribution

- So we'll need to find the F-statistic and its corresponding p-value using an F-distribution

Step 4-5: Find the test statistic and p-value

- There are several options to run an ANOVA model (aka calculate F-statistic and p-value)
- Two most common are `lm` and `aov`
  - `lm` = linear model; will be using frequently in BSTA 512

```r
lm(ndrm.ch ~ actn3.r577x,
      data = famuss) %>% anova()
```

```
Analysis of Variance Table

Response: ndrm.ch
             Df Sum Sq Mean Sq F value  Pr(>F)  
actn3.r577x   2   7043  3521.6  3.2308 0.04022 
*
Residuals   592 645293  1090.0                  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*'
0.05 '.' 0.1 ' ' 1
```

```r
aov(ndrm.ch ~ actn3.r577x,
      data = famuss) %>% summary()
```

```
             Df Sum Sq Mean Sq F value Pr(>F)  
actn3.r577x   2   7043    3522   3.231 0.0402 *
Residuals   592 645293    1090                 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*'
0.05 '.' 0.1 ' ' 1
```

Step 6: Conclusion

$$H_0 : \mu_{CC} = \mu_{CT} = \mu_{TT}$$
$$\text{vs. } H_A : \text{At least one pair} \mu_i \neq \mu_j \text{ for } i \neq j$$

- Recall the p-value = 0.0402
- Use α = 0.05
- Do we reject or fail to reject H_0?

Conclusion statement:

- There is sufficient evidence that at least one of the genotype groups has a change in arm strength statistically different from the other groups. (p-value =0.0402)

Final note

- Recall, visually the three looked pretty close
- This is the case that I would also do some work to report the means and standard deviations of each genotype's percent change in non-dominant arm strength.

```
famuss %>%
  group_by(actn3.r577x) %>%
  summarise(count = n(),
            mean = mean(ndrm.ch),
            SD = sd(ndrm.ch))
```

```
# A tibble: 3 × 4
  actn3.r577x count  mean    SD
  <fct>       <int> <dbl> <dbl>
1 CC            173  48.9  30.0
2 CT            261  53.2  33.2
3 TT            161  58.1  35.7
```

Revised conclusion statement:

- For people with CC genotype then mean percent change in arm non-dominant arm strength was 48.9% (SD = 30%). For CT, mean percent change was 53.2% (SD = 33.2%). For TT, mean percent change was 58.1% (SD = 35.7%). There is sufficient evidence that at least one of the genotype groups has a change in arm strength statistically different from the other groups. (p-value =0.0402)