Analysis of variance (ANOVA designs)

BIOL322 Lecture 17

Isabella Richmond & Dr. Pedro Peres-Neto

11/10/22

Learning Goals

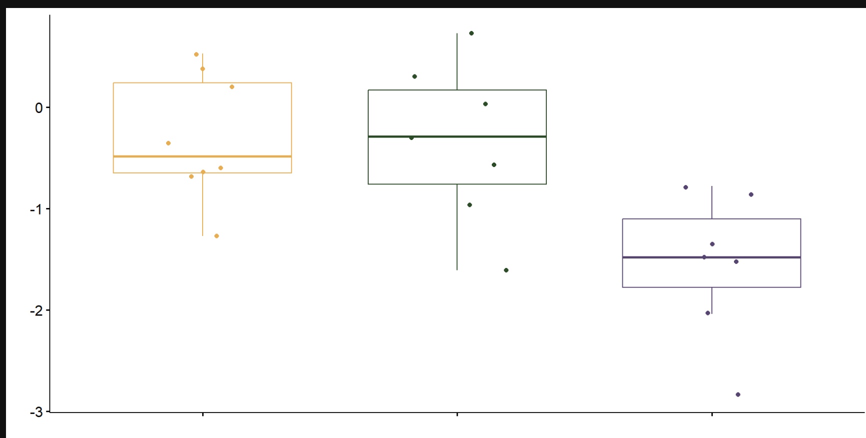
- *Understand* what an ANOVA is
- *Understand* when/how to use an ANOVA
- *Understand* what a F-statistic is and where it comes from
- *Differentiate* between statistical & research conclusions

When do I use an ANOVA?

- You have groups!
- We are explaining *continuous* dependent variables using **categorical** independent variables
  - i.e., if you want to know if there are differences between more than two groups

What is an ANOVA?

- One-way = **one** categorical explanatory variable
- Statistical test used to compare variation across means of 2+ groups



History of ANOVAs

- Invented by Sir R.A. Fisher ("father of modern statistics")
- Used to prove differences between groups to support eugenics (among other things)

Wikipedia

Example - knees are the new eyeballs??

Background: photoreceptor cells influence our circadian rhythm based on how much light they receive & when

- Mammals thought to only have these cells in our eyes
- Did experiment to test if these cells are present behind the knees (Campbell & Murphy, 1998)
- 33 trials of 2 groups: control or experimental (knee)
- Found evidence light exposure to knees can change circadian rhythm! But...

Example - knees are the new eyeballs??

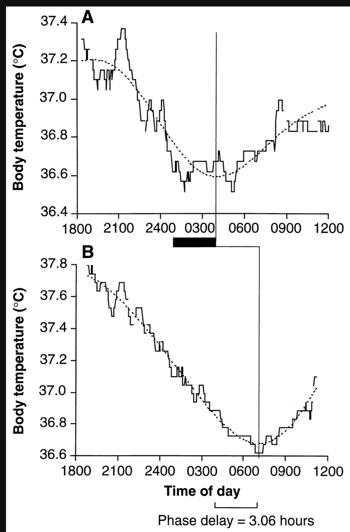


Figure 1. Phase Delay

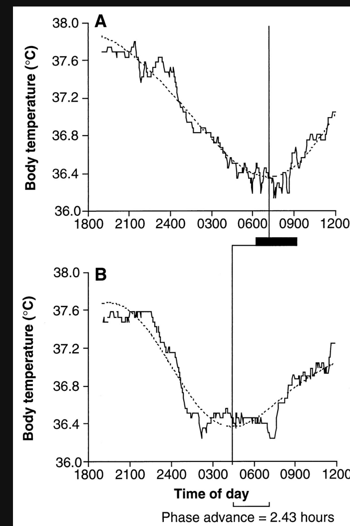
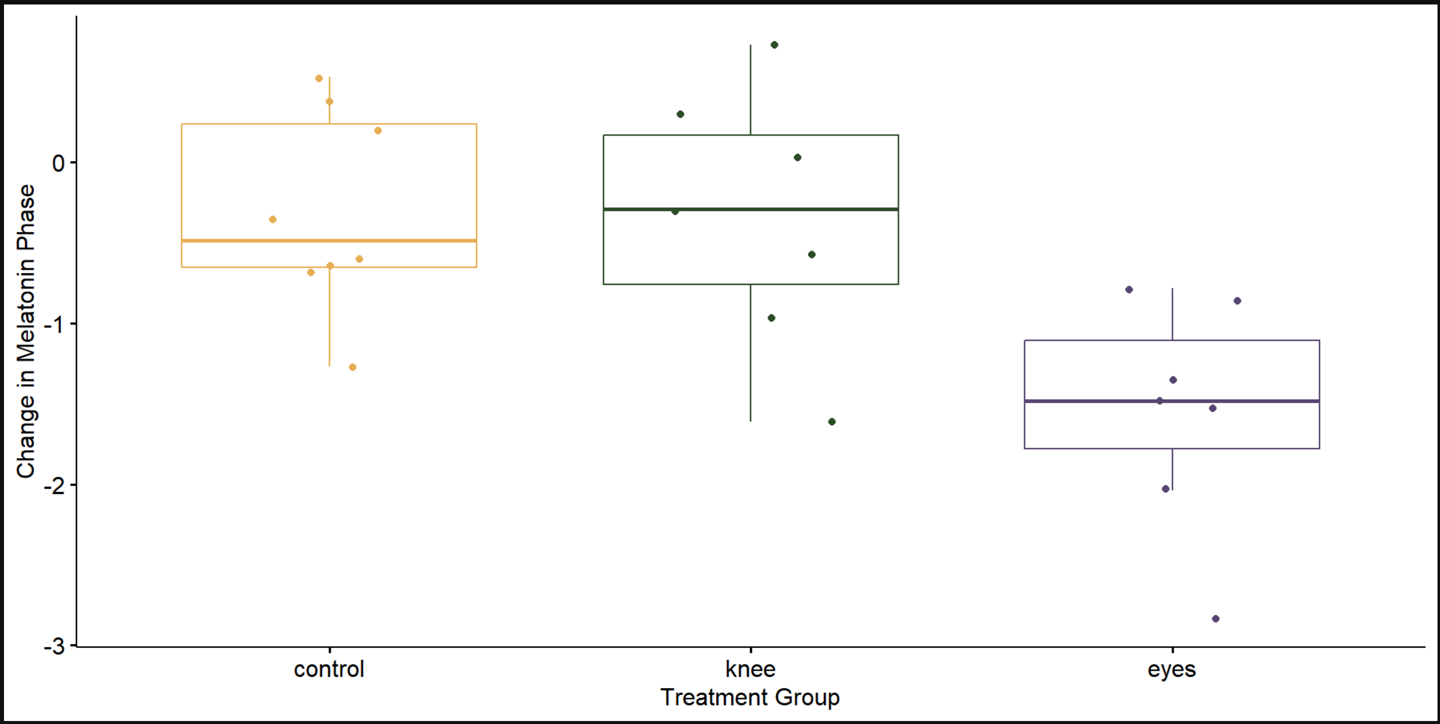


Figure 2. Phase Advance

Example - knees are not the new eyeballs!

- One huge problem in the study highlighted in a rebuttal paper (Wright Jr. & Czeisler 2002)
- Initial study did not cover patients eyes during the experiment!
- New study with 3 groups: control, light exposure with blindfold (knee), light exposure without blindfold (eyes)
- No effect of light exposure to knees

Example - knees are not the new eyeballs!



BREAK

- 2 minute brain break!

Statistical Hypothesis Testing

Null hypothesis & alternative hypothesis framework:

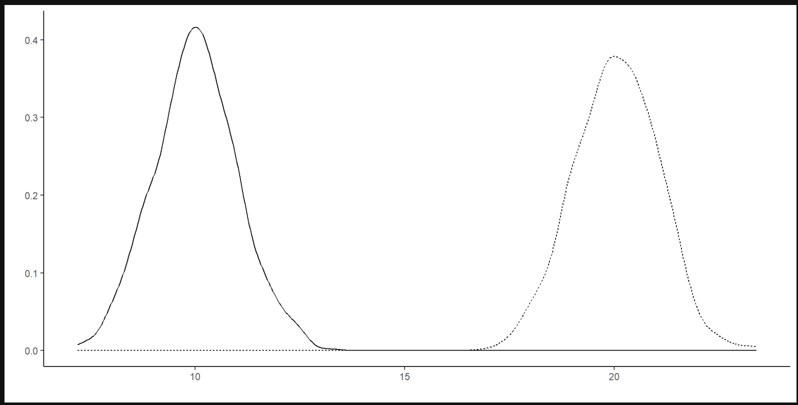
- H_0 = there is no difference in the means across groups
- H_A = at least two groups differ in their means
- we either:
  a. reject the null hypothesis
  b. fail to reject the null hypothesis (**we do not accept H_0**)
- Statistical significance != biological significance

One-Way ANOVAs

- One-way ANOVAs test if there is a statistically significant difference between the means of 2+ groups using the F-statistic
- The more different the groups are, the more likely we are to **reject the null hypothesis** and determine them as significantly different

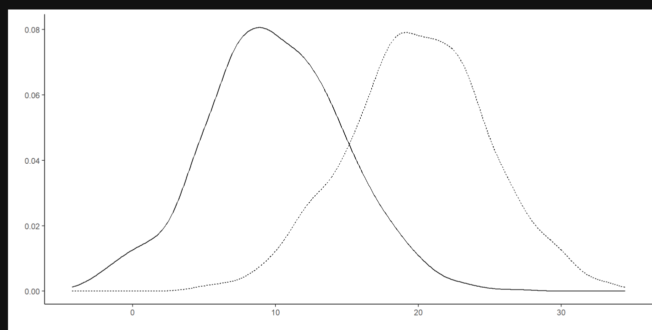
The Sensitivity of the F-Statistic

- The F-statistic penalizes for variance **within groups**
  - as variance **within groups** increases, F-statistic decreases even when the **between group** variance is the same



The Sensitivity of the F-Statistic

- These groups have a lower F-statistic (and therefore are less likely to be statistically significant), even though they have the same means (**between group variance**) as the last slide
  - they just have more **within group** variance



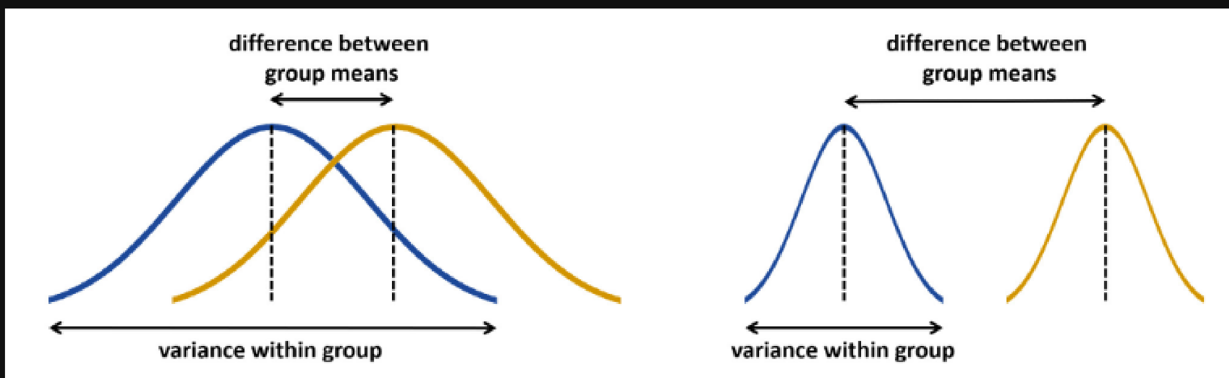
Analysis of Variance (AMONG MEANS)

- ANOVAs are only variance among **means**, not variance in general

$$F = \frac{s^2\ between}{s^2\ within} = \frac{\frac{\sum_{i=1}^{g}(X\,i - X)^2}{(g-1)}}{\frac{\sum_{i=1}^{g}(X - X\,i\,)^2}{(n-g)}}$$

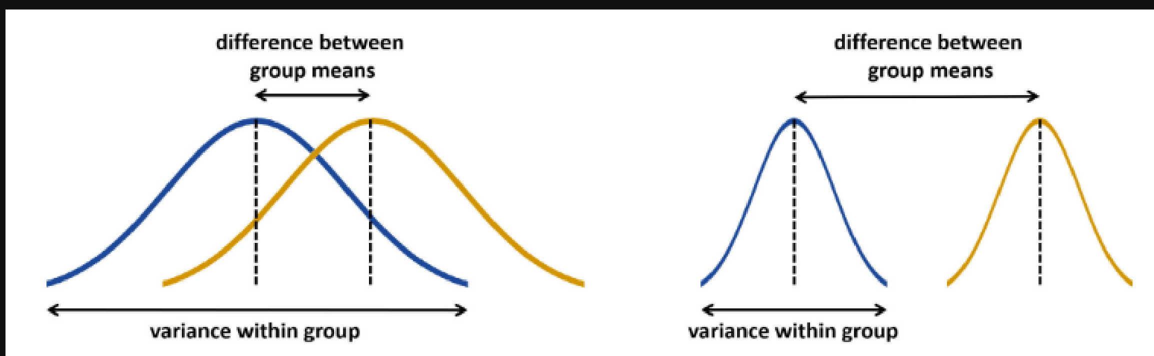
F-Statistic

- Numerator: between group variation **from the global mean**
- Need to divide by number of groups (g) because as group number increases, sum of variation will increase



F-Statistic

- Denominator: within group variation **from the global mean**
- As within group variation increases, F-statistic gets smaller
  - penalizes for within group variation



F-Statistic

- Penalization for within group variation is important because it makes **sampling error** very important

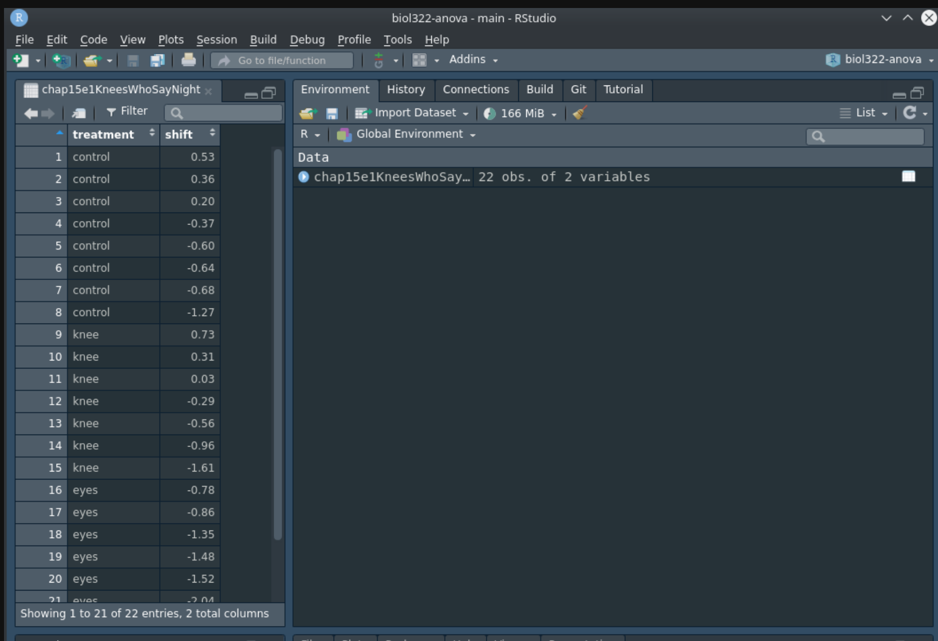
$$F = \frac{s^2_{between}}{s^2_{within}} = \frac{\frac{\sum_{i=1}^{g}(\bar{X}_i - \bar{\bar{X}})^2}{(g-1)}}{\frac{\sum_{i=1}^{g}(X - \bar{X}_i)^2}{(n-g)}}$$

BREAK

- 2 min brain break

Data in R

```r
# read in data
df <- read.csv("/biol322/data/chap15e1KneesWhoSayNight.csv")
View(df)
```



ANOVAs in R

```
# read in data
df <- read.csv("/biol322/data/chap15e1KneesWhoSayNight.csv")
View(df)

# ensure that your group variable is coded as a factor
df$treatment <- as.factor(df$treatment)

# use function `aov` to perform an anova
anova <- aov(shift ~ treatment, data = df)

# look at summary table
summary(anova)
```

Comparison of Two Groups

- $t^2 = F$ when you are comparing two groups

```r
# read in data
df <- read.csv("/biol322/data/chap15e1KneesWhoSayNight.csv")
View(df)

# ensure that your group variable is coded as a factor
df$treatment <- as.factor(df$treatment)

# subset to 2 groups - control & knees
df_s <- df[df$treatment == "control" | df$treatment == "knee", ]

# use function `aov` to perform an anova
anova <- aov(shift ~ treatment, data = df_s)

# use function `t.test` to perform a two sample t-test
ttest <- t.test(shift ~ treatment, data = df_s)
```

$t^2 = F$

ANOVA:

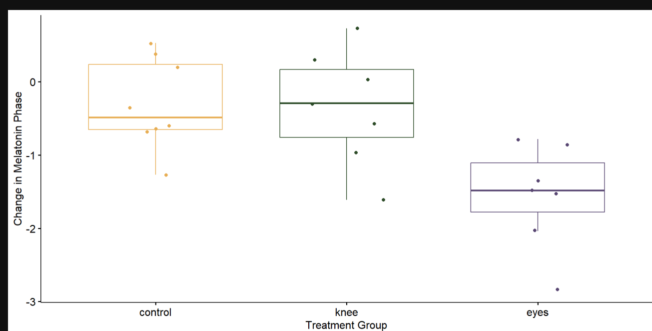
```
            Df Sum Sq Mean Sq F value
Pr(>F)
treatment    1  0.003  0.0027   0.005
0.942
Residuals   13  6.422  0.4940
```

t-test:

```
    Welch Two Sample t-test

data:  shift by treatment
t = 0.072846, df = 11.343, p-value =
0.9432
alternative hypothesis: true
difference in means between group
control and group knee is not equal
to 0
95 percent confidence interval:
 -0.7847452  0.8386737
sample estimates:
mean in group control    mean in
group knee
            0.3087500
```

Statistical Conclusion

- We reject H_0, the groups are significantly different from one another (statistically)
- ANOVAs only tell us if there is a difference, this is the entire statistical conclusion
- We **do not accept H_0**, we only fail to reject



Reporting ANOVAs

- When reporting ANOVAs we always use a clean and organized table to report the:

  a. sum of squares
  b. degrees of freedom
  c. mean square
  d. F statistic
  e. p-value

Reporting ANOVAs

Table 1. ANOVA summary table presenting results from aov, testing if mean changes in circadian rhythm are statistically different across treatment groups.

	df	Sum of Squares	Mean Squares	F	p-value
treatment	2	7.2	3.6	7.3	0.0045
Residuals	19	9.4	0.50	NA	NA

BREAK

- two minute brain break!

ANOVA R Output

```r
# use function `aov` to perform an anova
anova <- aov(shift ~ treatment, data = df)

# look at raw output
print(anova)

# look at summary table
summary(anova)
```

Output:

```
Call:
   aov(formula = shift ~ treatment, data = d)

Terms:
                treatment Residuals
Sum of Squares   7.224492  9.415345
Deg. of Freedom         2        19

Residual standard error: 0.7039492
Estimated effects may be unbalanced
```

ANOVA R Output

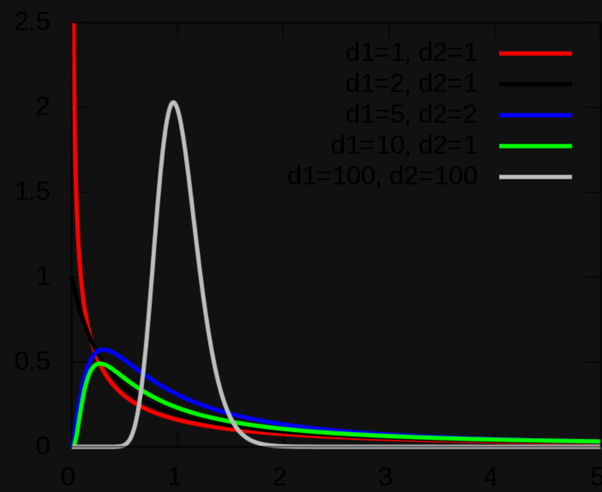
```r
# use function `aov` to perform an anova
anova <- aov(shift ~ treatment, data = df)

# look at raw output
print(anova)

# look at summary table
summary(anova)
```

Output:

```
            Df Sum Sq Mean Sq F value  Pr(>F)   
treatment    2  7.224   3.612   7.289 0.00447 **
Residuals   19  9.415   0.496                   
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ANOVAs are one-tailed tests

- We are only interested in when F is large
- Large F = large variation among group means



Research Conclusion

- Statistical conclusion = H_0 is rejected, groups are statistically different
- **Research conclusion** = there is a difference in the changes of circadian rhythm between control, knee, and eyes groups
  - Previously reported study may be wrong
  - We could do some post-hoc tests to determine where the differences are

ANOVA Assumptions

- Randomly sampled
- Independent observations & groups
- Standard deviation of each group is approximately the same
- Each group has a large n (n > 20 is guide) or is ~ normal
  - The more skewed the data, the higher the n required

Questions?

- Thank you!!
- Extra videos on course bookdown