Classes of statistical designs

Dependent Variable	Independent Variable	
	Continuous	Categorical
Continuous	Regression	t-tests and **ANOVA**
Categorical	Logistic Regression	Tabular

1

COMPARING THE MEANS OF THREE OR MORE SAMPLES or GROUPS (often called *treatments* in experiments)

THE ANALYSIS OF VARIANCE (ANOVA):

One of the most important and used tools in statistics

2

THE ANALYSIS OF VARIANCE (ANOVA) for comparing multiple sample means (groups or treatments)

The problem about "The knees who say night"

By Whitlock and Schluter (2009)

OR

"Bright light behind the knees is just bright light behind the knees"

http://www.genomenewsnetwork.org/articles/08_02/bright_knees.shtml

Resetting the human Circadian rhythm

Extraocular Circadian Phototransduction in Humans

Scott S. Campbell* and Patricia J. Murphy

Physiological and behavioral rhythms are governed by an endogenous circadian clock. The response of the human circadian clock to extraocular light exposure was monitored by measurement of body temperature and melatonin concentrations throughout the circadian cycle before and after light pulses presented to the popliteal region (behind the knee). A systematic relation was found between the timing of the light pulse and the magnitude and direction of phase shifts, resulting in the generation of a phase response curve. These findings challenge the belief that mammals are incapable of extraretinal circadian phototransduction and have implications for the development of more effective treatments for sleep and circadian rhythm disorders.

SCIENCE • VOL. 279 • 16 JANUARY 1998

Data challenged as subjects were exposed to light while knees being illuminated

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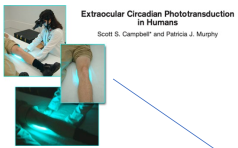
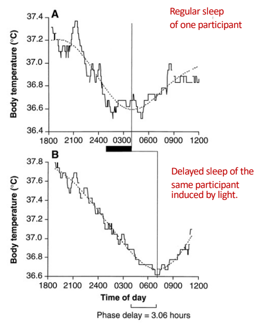
Our core body temperature fluctuates around 37°C, but fluctuates by about 1°C or so throughout the night.

The drop in temperature starts about two hours before you go to sleep, coinciding with the release of the sleep hormone melatonin.





Example of a delay in circadian phase in response to a 3-hour bright light presentation to the popliteal region. Light was presented on one occasion between 0100 and 0400 on night 2 in the laboratory (black bar) while the participant (a 29-year-old male) remained awake and seated in a dimly lit room (ambient illumination <20 lux).

The circadian phase was determined by fitting a complex cosine curve (dotted line

The resulting phase delay was 3.06 hours

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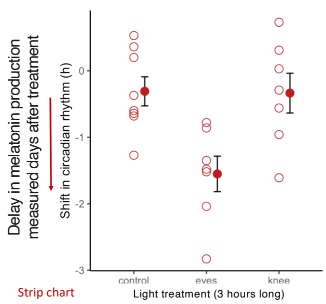
THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

PHYSIOLOGY SCIENCE VOL 297 26 JULY 2002

Absence of Circadian Phase Resetting in Response to Bright Light Behind the Knees

Kenneth P. Wright Jr.* and Charles A. Czeisler



New study challenged the original study (Wright & Czeiler 2002): subjects were exposed to light to knees only.

22 people randomly assigned to one of the three light treatments.

Do these samples originate from statistical populations with the same mean, or do the observed differences in means among samples arise solely from sampling variation within a single population?

Whitlock & Schluter, *The Analysis of Biological Data*, 3e © 2020 W. H. Freeman and Company

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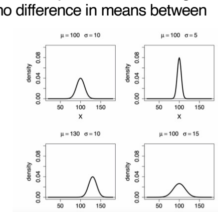
Do these samples originate from statistical populations with the same mean, or do the observed differences in means among samples arise solely from sampling variation within a single population?

Keep in mind that samples may originate from statistical populations that share the same mean but are not necessarily from the same statistical population.

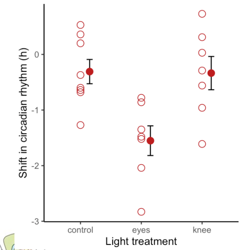
Why is this important? Statistical populations can have identical means but differ in their variances (and other aspects). This indicates that samples could be drawn from distinct populations, yet their means may not differ. This aligns with the null hypothesis (H_0), which assumes no difference in means between groups.

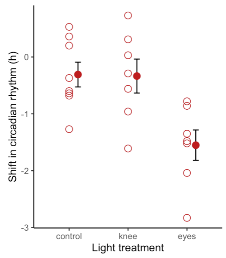


6

THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

Which order of treatments work best?



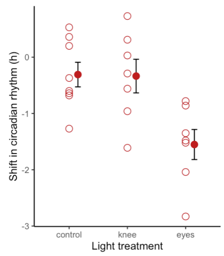


7

THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

H_0: The samples originate from statistical populations that share the same mean., i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.



8

THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

H_0: The samples originate from statistical populations that share the same mean., i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.

Which is to say:

H_0: Differences in group means are solely due to sampling variation from statistical populations that share the same mean.

H_A: Differences in group means are not solely due to sampling variation, indicating the populations may differ in their means.

Remember: ***Sampling error*** is due to sampling variation, i.e., samples that come from the statistical populations sharing the same mean.

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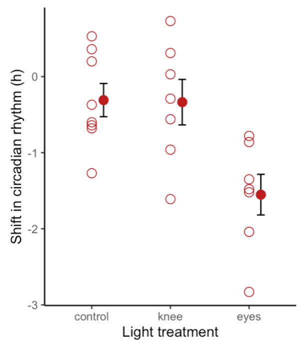
THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

An ANOVA always involves one ***continuous*** response variable (e.g., shift in circadian rhythm) and **one** *categorical* predictor variable.

The categorical variable (predictor) is divided into groups which are often referred as treatments or factor levels.



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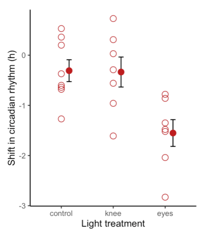
THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

H_0: The samples originate from statistical populations that share the same mean., i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.



As we are studying one single factor (light), we will use a ***one-way ANOVA***.

If two factors were involved (say light and time of experimentation) that would be a two-way ANOVA (not covered in BIOL322).

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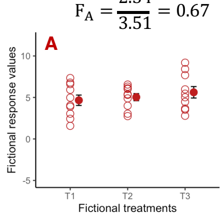
We require a test statistic that can detect variations in means across multiple groups.
The F-statistic achieves this by evaluating the ratio of two variance components.

$$F = \frac{\text{variance among group means (due to “treatment”)}}{\text{variance within groups}}$$

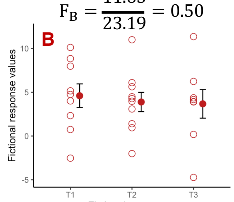
(referred to as error or residual variation, it represents the variation not explained by the differences in means among groups)

Means among groups don't vary much in both data **A** and **B**, but residual variation (within groups) is smaller in **A** than **B**.

$$F_A = \frac{2.34}{3.51} = 0.67$$



$$F_B = \frac{11.63}{23.19} = 0.50$$



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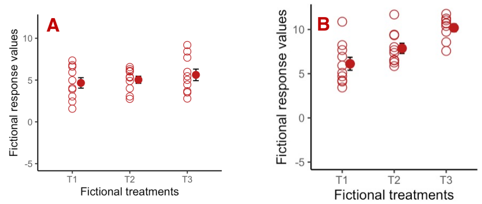
We require a test statistic that can detect variations in means across multiple groups.

The F-statistic achieves this by evaluating the ratio of two variance components.

Means among groups don't vary in **A** but vary in **B**;
residuals variation is similar in **A** than **B.**

$$F_A = \frac{2.34}{3.51} = 0.67 \qquad F_B = \frac{47.41}{3.64} = 13.03$$

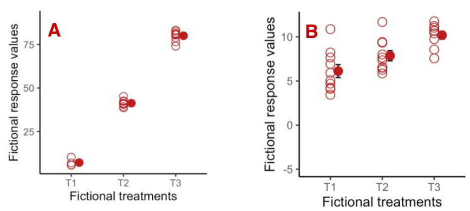


13

We require a test statistic that can detect variations in means across multiple groups:
the F-statistic achieves this by evaluating the ratio of two variance components.

Mean differences among groups are much larger in **A** than **B**;
residuals variation is similar in **A** than **B.** Notice the differences in their Y-scales (the mean differences among groups is huge in **A**).

$$F_A = \frac{14078.0}{5.71} = 2456.90 \qquad F_B = \frac{47.41}{3.64} = 13.03$$

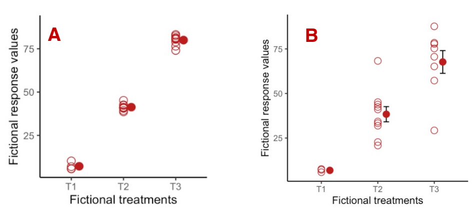


14

HETEROSCEDASTICITY reduces the F-ratio ability to differentiate among differences in means among groups

Means among groups are somewhat similar in **A** than **B**;
A is homoscedastic **B** heteroscedastic

$$F_A = \frac{14078.0}{5.71} = 2456.90 \qquad F_B = \frac{12275.0}{217.9} = 56.34$$



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We require a test statistic that can detect variations in means across multiple groups: the F-statistic achieves this by evaluating the ratio of two variance components.

Let's talk ANOVA "jargon"

$$F = \frac{\text{variance among group means (due to "treatment")}}{\text{variance within groups (caller error or residual variation not explained by the mean within groups)}}$$

You can interpret ANOVA without knowing how it works, but you are less likely to use ANOVA inappropriately if you have some idea of how it works (*Motulsky*)

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We need a test statistic that is sensitive to mean variation across multiple groups (or treatments): The F statistic does that by considering the ratio of two variances (variance components):

Let's talk ANOVA "jargon"

$$F = \frac{\text{variance among group means (due to "treatment")}}{\text{variance within groups (caller error or residual variation not explained by the mean within groups)}}$$

$$F = \frac{\text{Group Mean Square}}{\text{Error Mean Square}} = \frac{MS_{groups}}{MS_{error}}$$

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The F statistic measures the variance among groups but accounting for the variance within groups

The F statistic in the ANOVA context is so important that is more than worth knowing how it works!

$$F = \frac{s_b^2}{s_w^2} = \frac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}$$

- s_b^2: Group Mean Square, MS_{groups} (b=between or among)
- s_w^2: MS_{errors} (w=within groups), Error Mean Square
- $\bar{X}_i$: Mean of each group
- $\bar{\bar{X}}$: Total mean!
- $g-1$: Degrees of freedom of MS_{groups}

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The F-statistic evaluates the variance among group means while accounting for the variance within groups.

The F-statistic plays a crucial role in the ANOVA context, making it well worth understanding how it works!

$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i-1)s_i^2}{\sum_{i=1}^{g}(n_i-1)}}$$

$$\sum_{i=1}^{g}(n_i-1) \rightarrow = (N-g)$$

- Group Mean Square MS_{groups} (b=between or among)
- Error Mean Square MS_{errors} (w=within groups)
- Mean of each group
- Total mean!
- Degrees of freedom of MS_{groups}
- Variance of each group
- Big "N"; sum of all sample sizes across groups
- Number of groups
- Degrees of freedom of MS_{groups}
- Sample size of each group

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A small example: worth doing it "by hand"!

Let's assume two groups for simplicity!

group 1

1 2 3 4 5

$\bar{X}_1 = 3.0$

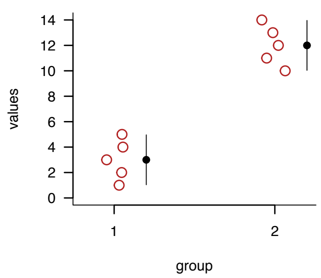
$s_1^2 = 2.5$

group 2

10 11 12 13 14

$\bar{X}_2 = 12.0$

$s_2^2 = 2.5$



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g_1: 1 2 3 4 5

g_2: 10 11 12 13 14

$\bar{X}_1 = 3.0$ $\bar{X}_2 = 12.0$

$s_1^2 = 2.5$ $s_2^2 = 2.5$

$\bar{\bar{X}}$ = (1+2+3+4+5+10+11+12+13+14)/10 = **7.5**

MS_{groups}= variance among group means (due to "treatment")

= (5x(**3.0** - 7.5)^2 + 5x(**12.0** - 7.5)^2)/(2-1) =

202.5/(2-1) = **202.5**

df(MS_{groups}) = g - 1

$$F = \frac{202.5}{MS_{error}} = ???$$

$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i-1)s_i^2}{\sum_{i=1}^{g}(n_i-1)}} \qquad \sum_{i=1}^{g}(n_i-1) \rightarrow = (N-g)$$

- Mean of each group
- Total mean!
- MS_{groups}
- Variance of each group
- Big "N"; sum of all sample sizes across groups

21

g_1: 1 2 3 4 5

g_2: 10 11 12 13 14

Mean of each group

Total mean!

$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i - 1)s_i^2}{\sum_{i=1}^{g}(n_i - 1)}}$$

Variance of each group

Big "N"; sum of all sample sizes across groups

$\sum_{i=1}^{g}(n_i - 1)$ → = (N-g)

MS_{error}

$\bar{X}_1 = 3.0 \quad \bar{X}_2 = 12.0$

$s_1^2 = 2.5 \quad s_2^2 = 2.5$

MS_{error} = variance within groups (residuals)

$MSE_1 = (1-3.0)^2+(2-3.0)^2+(3-3.0)^2+(4-3.0)^2+(5-3.0)^2 =$ **10**

$MSE_2 = (10-12.0)^2+(11-12.0)^2+(12-12.0)^2+(13-12.0)^2+(14-12.0)^2 =$ **10**

$MS_{error} = (MSE_1 + MSE_2)/(N-g) = (10+10)/(10-2) = 20/8 =$ **2.5**

$df(MS_{error}) = N-g = 10 - 2 = 8$

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$\bar{\bar{X}} = (1+2+3+4+5+10+11+12+13+14)/10 =$ **7.5**

$MS_{groups} =$

$= (5\times(\mathbf{3.0} - 7.5)^2 + 5\times(\mathbf{12.0} - 7.5)^2)/(2-1) =$

$202.5/(2-1) =$ **202.5**

$df(MS_{groups}) = g - 1 = 2-1$

$$F = \frac{\mathbf{202.5}}{\mathbf{2.5}} = 81$$

MS_{error} = variance within groups (residuals)

$MSE_1 = (1-3.0)^2+(2-3.0)^2+(3-3.0)^2+(4-3.0)^2+(5-3.0)^2 =$ **10**

$MSE_2 = (10-12.0)^2+(11-12.0)^2+(12-12.0)^2+(13-12.0)^2+(14-12.0)^2 =$ **10**

$MS_{error} = (MSE_1 + MSE_2)/(N-g) = (10+10)/(10-2) = 20/8 =$ **2.5**

$df(MS_{error}) = N-g = 10 - 2 = 8$

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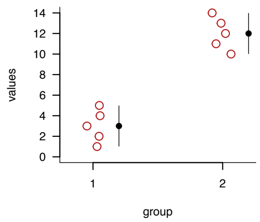
Let's take a power break – 1 minute

24

one-way ANOVA in R - step 1: organizing data in a csv file

E	F
values	group
1	1
2	1
3	1
4	1
5	1
10	2
11	2
12	2
13	2
14	2



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ANOVA in R

Function to run **An** **A**nalysis of **V**ariance (aov)

Vector of observations (1,2,3,4,5,10,11,12,13,14)

Factor identifying group of the observation (1,1,1,1,1,2,2,2,2,2)

```
> aov(data.points~groups)
Call:
   aov(formula = data.points ~ groups)

Terms:
                groups Residuals
Sum of Squares   202.5      20.0
Deg. of Freedom      1         8

Residual standard error: 1.581139
Estimated effects may be unbalanced
```

$$F = \frac{202.5}{2.5} = 81$$

26

NOTE: ANOVA for two groups is equivalent to the two-sample mean t-test

```
> aov(data.points~groups)
Call:
   aov(formula = data.points ~ groups)

Terms:
                groups Residuals
Sum of Squares   202.5      20.0
Deg. of Freedom      1         8

Residual standard error: 1.581139
Estimated effects may be unbalanced
```

$$F = \frac{202.5}{2.5} = 81$$

$$t = \sqrt{F} = \sqrt{\frac{202.5}{2.5}} = 9$$

```
t.test(data.points~groups,var.equal = TRUE)

    Two Sample t-test

data:  data.points by groups
t = -9, df = 8, p-value = 1.853e-05
```

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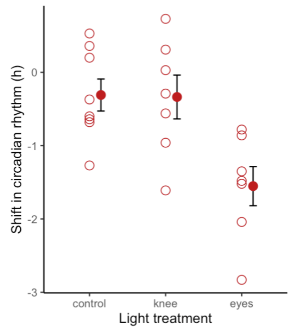
LET's go back to the "The knees who say night"

data in a csv file

treatment	shift
control	0.53
control	0.36
control	0.2
control	-0.37
control	-0.6
control	-0.64
control	-0.68
control	-1.27
knee	0.73
knee	0.31
knee	0.03
knee	-0.29
knee	-0.56
knee	-0.96
knee	-1.61
eyes	-0.78
eyes	-0.86
eyes	-1.35
eyes	-1.48
eyes	-1.52
eyes	-2.04
eyes	-2.83



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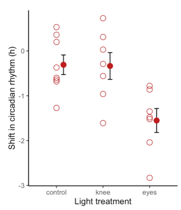
"The knees who say night"



Statistical Conclusion?

H$_0$: The samples originate from statistical populations that share the same mean, i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H$_A$: At least two samples originate from distinct statistical populations with differing means.

```
summary(aov(shift ~ treatment, data=circadian))
            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
---
```

29

"The knees who say night"

```
summary(aov(shift ~ treatment, data=circadian))
            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
```

ANOVA Table – reporting quality

Source of variation	Sum of squares	df	Mean square	F	P
Between	7.224	2	3.612	7.289	0.00447
Within	9.415	19	0.496		

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Remembering the role of degrees of freedom

Source of variation	Sum of squares	df	Mean square	F	P
Between	7.224	2	3.612	7.289	0.00447
Within	9.415	19	0.496		

Keep in mind that calculating the sum of squares involves subtracting from the means, which would introduce bias if not adjusted. To address this, the sum of squares is divided by the degrees of freedom, resulting in mean square deviations.

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"The knees who say night"

ANOVA Table

Source of variation	Sum of squares	df	Mean square	F	P
Between	7.224	2	3.612	7.289	0.00447
Within	9.415	19	0.496		

H_0: The samples originate from statistical populations that share the same mean, i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.

Reject H_0

How does the ANOVA statistical significance test work?

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How to think about the F distribution

The statistical "machinery":

1) Assume that H_0 is true (i.e., the samples originate from statistical populations that share the same mean and variance).

Why assume equal variances? There are infinite ways in which statistical populations can share the same mean but differ in variance. And here statistical populations are also assumed normally distributed (all for convenience of calculus). So, in here, as they share the same mean, variance and are normally distributed, they are in fact the same statistical population.

2) Sample from the statistical population the appropriate number of groups (samples) respecting the sample size of each group.

3) Repeat step 2 a large (or infinite) number of times and each time calculate the F statistic.

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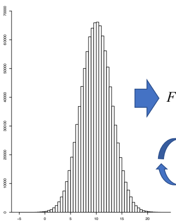
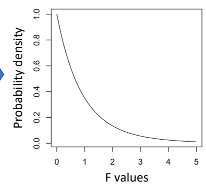
The F (sampling) distribution assuming that H_0 is true

H_0: Differences in group means are solely due to sampling variation from statistical populations that share the same mean.

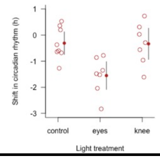


$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i - 1)s_i^2}{\sum_{i=1}^{g}(n_i - 1)}}$$

(8,7,7) observations



Sample from the same (normally distributed) population (i.e., ***H_0 is true***), respecting the original number of groups and their sample sizes.



Control: 8 observations
Eyes: 7 observations
Knee: 7 observations

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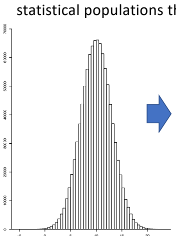
The F (sampling) distribution assuming that H_0 is true

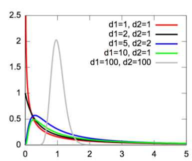
H_0: Differences in group means are solely due to sampling variation from statistical populations that share the same mean.



$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i - 1)s_i^2}{\sum_{i=1}^{g}(n_i - 1)}}$$

Varying the number of groups and the number of observations per group results in different shapes for the F-distribution.

Sample from the same (normally distributed) population (i.e., ***H_0 is true***), respecting the original number of groups and their sample sizes.



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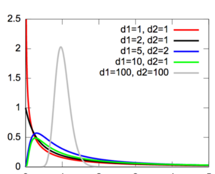
The F distribution assuming that H_0 is true (i.e., the sampling distribution of the test statistic F when H_0 is true).



Mean of each group — Total mean!

$$F = \frac{s_b^2}{s_w^2} = \frac{\dfrac{\sum_{i=1}^{g} n_i(\bar{X}_i - \bar{\bar{X}})^2}{g-1}}{\dfrac{\sum_{i=1}^{g}(n_i - 1)s_i^2}{\sum_{i=1}^{g}(n_i - 1)}}$$

Numerator → df_1

Variance of each group

Big "N"; sum of all sample sizes across groups

$\sum_{i=1}^{g}(n_i - 1)$ = (N-g) → df_2

The numerator degrees of freedom depends on the number of groups (g-1) and the denominator degrees of freedom depends on the total number of observations (N-g)

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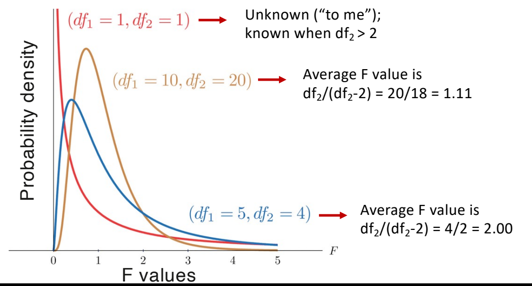
The F (sampling) distribution assuming that H_0 is true

Remember: The average of the t-distribution under $\mathbf{H_0}$ is always zero;

However, the average of the F-distribution expected under $\mathbf{H_0}$ depends on its degrees of freedom.



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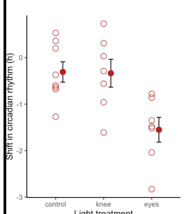
The expected mean value for the F distribution under the null hypothesis as a way to also express the null and alternative hypothesis

H_0: The samples originate from statistical populations that share the same mean, i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.



Which is to say:

H_0: $F = df_2/(df_2-2)$
H_A: $F \neq df_2/(df_2-2)$

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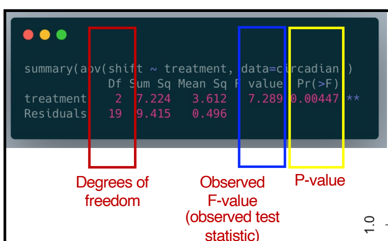
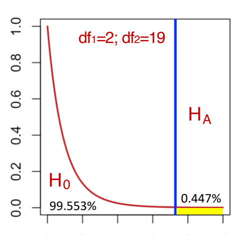


ANOVA is a one-sided (one-tail) statistical test by design; even though the null hypothesis was set as a two-tailed test.

H_0: The samples originate from statistical populations that share the same mean, i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.

The p-value is estimated as the number of F-values in the null distribution equal or greater than the observed F-value (i.e., one tailed-test).



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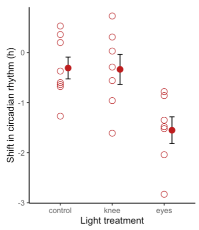
THE ANALYSIS OF VARIANCE (ANOVA)
for comparing multiple sample means (groups or treatments)

H_0: The samples originate from statistical populations that share the same mean,
i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.



Research conclusion: light treatment influences shifts in circadian rhythm.

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ANOVA assumptions

Assumptions for ANOVA are similar to those of the independent two-sample t-test:

Each observation is a random sample from its respective population (which may or may not be the same population).

The variable of interest (e.g., shift in circadian rhythm) is normally distributed within each treatment population (further discussion in a later lecture).

Variances are equal across all populations from which the treatments were sampled. Unequal variances can alter F-values, making them unreliable for measuring differences among means (more on this in a subsequent lecture).

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"The knees who say night"

H_0: The samples originate from statistical populations that share the same mean,
i.e., $\mu_{control} = \mu_{knee} = \mu_{eyes}$.

H_A: At least two samples originate from distinct statistical populations with differing means.

Conclusion?
Significant, but how?

How do we know which group means differ from one another?

Why not simply not contrast all pairs of means using a two-sample mean t-test?
Control vs. knee; control vs. eyes; knee vs. eyes?

More later in the course!

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