# Odds and ends

Observational vs. experimental

*key is whether researcher assigns treatment or not*

Goals experiment

1) Eliminating bias
2) Reduce sampling error

# Reduce bias

- Controls
- Random assignment to treatments
- Blinding

# Reducing sampling error

$$t = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{s_p^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

*"Signal"*

*"Noise"*

*Can be achieved with smaller s or larger n.*

# Reducing sampling error

- Replication
- Balance (equal sample size)
- Blocking
- Extreme treatments

$$SE_{\bar{Y}_1 - \bar{Y}_2} = \sqrt{s_p^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}.$$

# Comparing the means of more than two groups

# Analysis of variance (ANOVA)

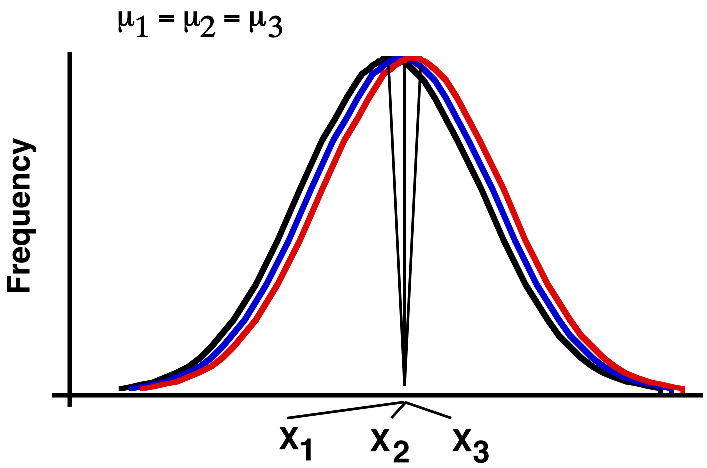
- Like a *t*-test, but can compare more than two groups
- Asks whether any of two or more means is different from any other.
- In other words, is the variance among groups greater than zero?

# Null hypothesis for simple ANOVA

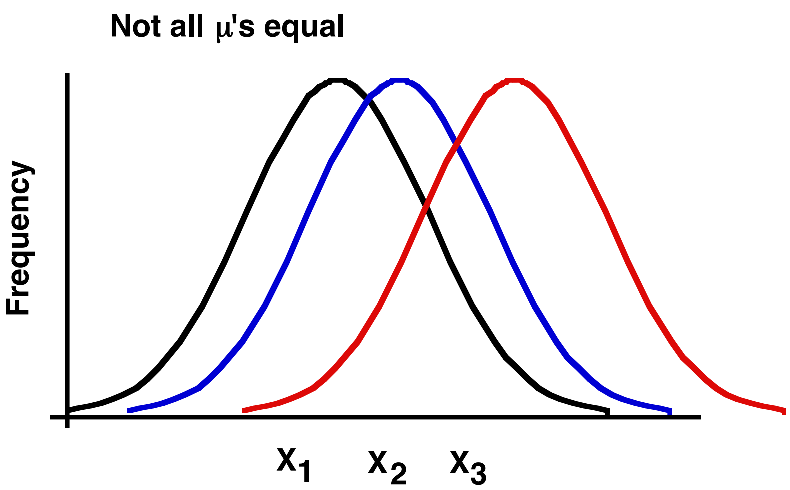
- $H_0$ : Variance among groups = 0

OR

- $H_0$ : $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \ldots \mu_k$

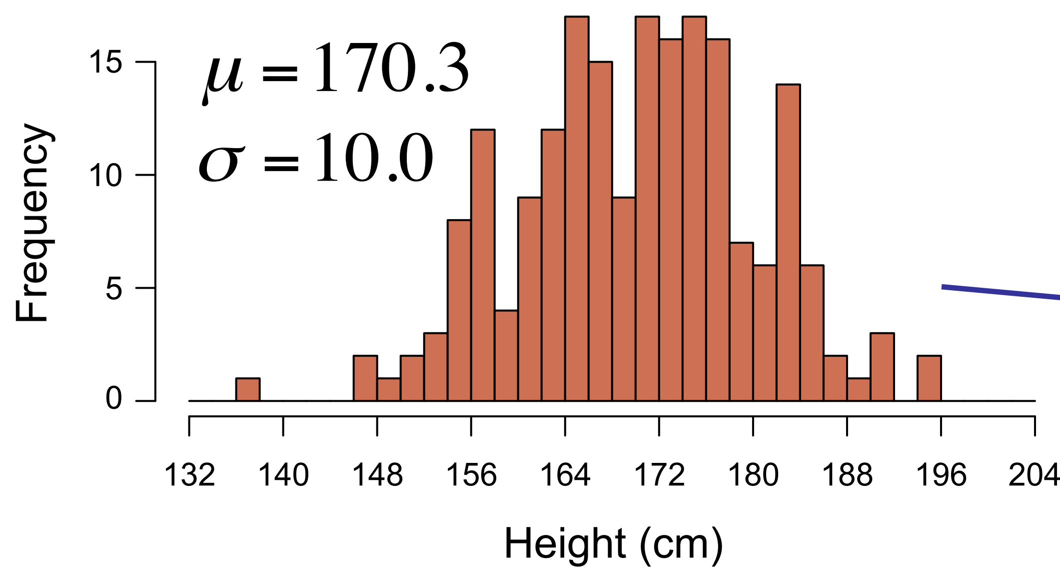



$H_0$: all populations have equal means




$H_A$: at least one population mean is different.

**Heights of BIOL300 students (*N* = 202)**
(2014 Spring; self-reported, outlier removed)

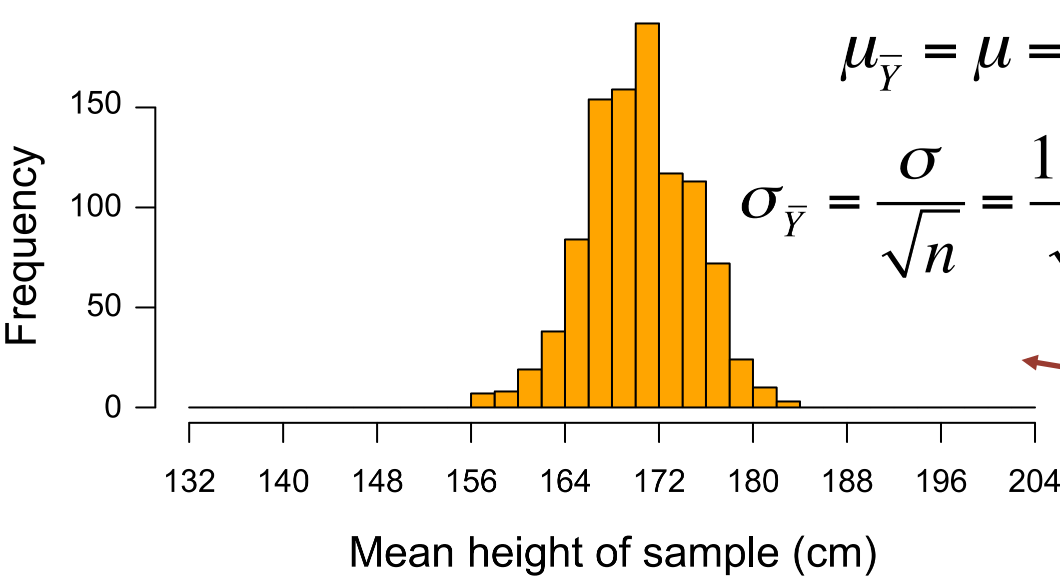



$$\mu = 170.3$$
$$\sigma = 10.0$$

Sampling distribution of Y

*If we draw multiple samples from the same population, we are also drawing sample means from a distribution.*

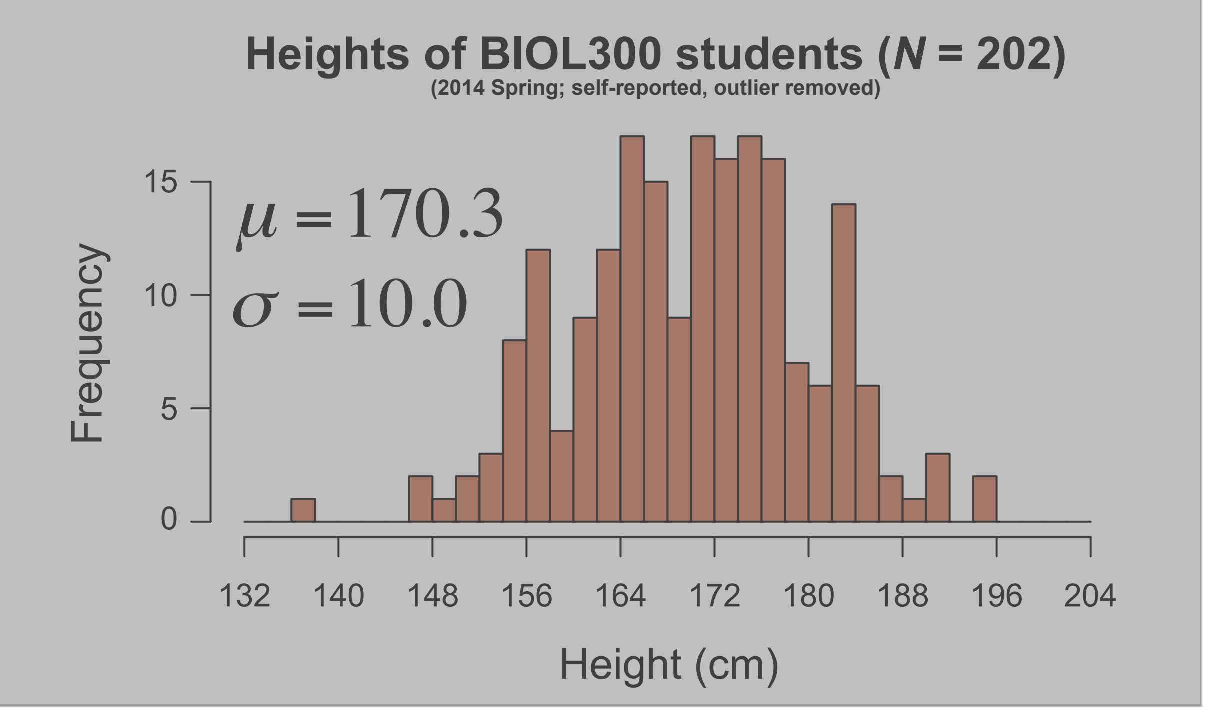
**Mean heights of samples of size 5**
(1000 samples)




$$\mu_{\bar{Y}} = \mu = 170.3$$

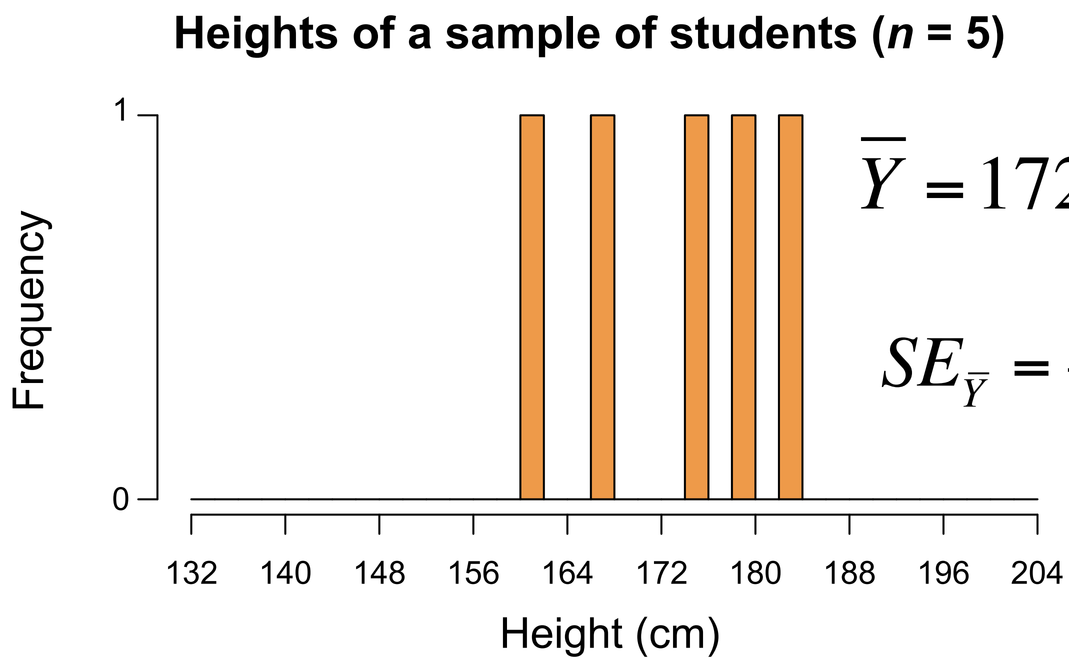
$$\sigma_{\bar{Y}} = \frac{\sigma}{\sqrt{n}} = \frac{10.0}{\sqrt{5}} = 4.5$$

Sampling distribution of $\bar{Y}$




*In most cases, we don't know the real population distribution.*

*We only have a sample.*




$$\bar{Y} = 172.8 \qquad s = 9.2$$

$$SE_{\bar{Y}} = \frac{s}{\sqrt{n}} = \frac{9.2}{\sqrt{5}} = 4.1$$

*We use this as an estimate of* $\sigma_{\bar{Y}}$

Under the null hypothesis, the sample mean of each group should vary because of sampling error.

The standard deviation of sample means, when the true mean is constant, is the standard error:

$$\sigma_{\bar{x}} = \frac{\sigma_x}{\sqrt{n}}$$

Note that we used standard errors in t-tests, e.g.: $t = \dfrac{\bar{Y}_1 - \bar{Y}_2}{SE_{\bar{Y}_1 - \bar{Y}_2}}$

Squaring the standard error, the variance among sample means due to sampling error should be:

$$\sigma_{\bar{x}}^{2} = \frac{\sigma_{x}^{2}}{n}$$

***In ANOVA, we work with variances rather than standard deviations.***

Alternatively, if the null is FALSE:

$$\sigma_{\bar{x}}^2 = \frac{\sigma_x^2}{n} + Variance\left[\mu_i\right]$$

If the null hypothesis is <u>not</u> true, the variance among sample means should be equal to the variance due to sampling error *plus* the real variance among population means.

With ANOVA, we test whether the variance among true group means is greater than zero.

$$Variance\left[\mu_i\right] > 0\,?$$

We do this by asking whether the variance among sample means is greater than expected under the null hypothesis:

$$\sigma_{\bar{x}}^2 > \frac{\sigma_x^2}{n}\,?$$

*But, our data is affected by sampling error, such that we only have estimates of the above.*

$$\sigma_{\bar{x}}^{2} > \frac{\sigma_{x}^{2}}{n}?$$

$$n\sigma_{\bar{x}}^{2} > \sigma_{x}^{2}?$$

| Population parameters | | Estimates from sample |
|---|---|---|
| $n\sigma_{\bar{x}}^{2}$ | is estimated by the "Mean Squares Group" | $MS_{group}$ |
| *'n times variance among groups'* | | |
| $\sigma_{x}^{2}$ | is the variance within groups, estimated by the "Mean Squares Error" | $MS_{error}$ |
| *'variance within groups'* | | |

# Mean squares group

Abbreviation: $MS_{group}$

Variance in mean due to sampling error

Estimates this parameter: $$n\left(\frac{\sigma_x^2}{n}+Variance\left[\mu_i\right]\right)$$

Variance in mean due to differences in means

Formula: $$MS_{groups}=\frac{SS_{groups}}{df_{groups}}$$

# Mean squares group

*i* = *group*
*j* = *indiv.*

$$SS_{group} = \sum n_i \left( \bar{X}_i - \bar{X} \right)^2$$

$\bar{X}_i$ is the mean of group $i$, and

$\bar{X} = \sum_i \sum_j X_{ij} / N$ is the overall mean.

$df_{groups} = k\text{-}1$ (where k is the number of groups)

$$MS_{groups} = \frac{SS_{groups}}{df_{groups}}$$

# Mean squares error

Abbreviation: $MS_{error}$

Estimates this parameter: $\sigma_x^2$

Formula: $MS_{error} = \dfrac{SS_{error}}{df_{error}}$

# Mean squares error

Error sum of squares =

$$SS_{error} = \sum df_i s_i^2 = \sum s_i^2 (n_i - 1)$$

Error degrees of freedom =

$$df_{error} = \sum df_i = \sum \left(n_i - 1\right) = N - k$$

$$MS_{error} = \frac{SS_{error}}{df_{error}} = \frac{\sum s_i^2 (n_i - 1)}{N - k}$$

$MS_{error}$ is like the pooled variance in a 2-sample *t*-test:

$$s_p^2 = \frac{df_1 s_1^2 + df_2 s_2^2}{df_1 + df_2}$$

# Test statistic: *F*

If $H_0$ is true, then $$n\,\sigma_{\bar{x}}^2 = \sigma_x^2$$

In other words: $$F = \frac{n\,\sigma_{\bar{x}}^2}{\sigma_x^2} = 1$$

**But, the above refer to population parameters. We must estimate F from samples with: $MS_{group} / MS_{error}$**

# *F* if null hypothesis is false:

We test whether the *F* ratio is greater than one, as it would be if $H_0$ is false:

$$F = \frac{n\left(\frac{\sigma_x^2}{n} + Variance\left[\mu_i\right]\right)}{\sigma_x^2} > 1$$

***But we must take into account sampling error. Often, F calculated from data will be greater than one even when the null is true. Hence we must compare F to a null distribution.***

# ANOVA table

| **Source** | **SS** | **df** | **MS** | **F** | ***P*** |
|---|---|---|---|---|---|
| Group | | | | | |
| Error | | | | | |
| Total | | | | | |

An ANOVA table is a convenient way to keep track of the important calculations.

Scientific papers often report ANOVA results with ANOVA tables.

# Example: Body temperature of squirrels in low, medium and hot environments

Wooden & Walsberg (2004) **Body temperature and locomotor capacity in a heterothermic rodent.** *Journal of Experimental Biology* 207:41-46.

# Squirrel body temperature data (° C)

Cold: 30.4, 31.0, 31.2, 31.0, 31.5, 30.4, 30.6, 31.1, 31.3, 31.9, 31.4, 31.6, 31.5, 31.4, 30.3, 30.5, 30.3, 30.0, 30.8, 31.0

Warm: 36.3, 37.5, 36.9, 37.2, 37.5, 37.7, 37.5, 37.7, 38.0, 38.0, 37.6, 37.4, 37.9, 37.2, 36.3, 36.2, 36.4, 36.7, 36.8, 37.0, 37.7

Hot: 40.7, 40.6, 40.9, 41.1, 41.5, 40.8, 40.5, 41.0, 41.3, 41.5, 41.3, 41.2, 40.7, 40.3, 40.2, 41.3, 40.7, 41.6, 41.5, 40.5

# Hypotheses

$H_0$: Mean body temperature is the same for all three groups of squirrels.

$H_A$: At least one of the three is different from the others.

# Summary data

| Group | $\bar{x}$ | $s$ | $n$ |
|---|---|---|---|
| Cold | 31.0 | 0.551 | 20 |
| Warm | 37.2 | 0.582 | 21 |
| Hot | 41.0 | 0.430 | 20 |

Total sample size: $N = \sum n = 20 + 21 = 20 = 61$

# Error mean square for squirrels

$$SS_{error} = \sum df_i s_i^2$$

$$= 19(0.551)^2 + 20(0.582)^2 + 19(0.430)^2$$

$$= 16.1$$

$$df_{error} = 19 + 20 + 19 = 58$$

$$MS_{error} = \frac{16.1}{58} = 0.277$$

# Squirrel mean squares group:

$$\bar{X} = \frac{20(31.0) + 21(37.2) + 20(41.0)}{20 + 21 + 20} = 36.4$$

$$SS_{group} = \sum n_i \left(\bar{X}_i - \bar{X}\right)^2$$

$$SS_{group} = 20(31.0\text{-}36.4)^2 + 21(37.2\text{-}36.4)^2 + 20(41.0\text{-}36.4)^2$$

$$= 1015.7$$

# Squirrel mean squares group:

$df_{group} = k - 1 = 3\text{-}1 = 2$

$$MS_{groups} = \frac{SS_{groups}}{df_{groups}} = \frac{1015.7}{2} = 507.9$$

# The test statistic for ANOVA is *F*

$$F = \frac{MS_{group}}{MS_{error}} = \frac{507.9}{0.277} = 1834.7$$

$MS_{group}$ is always in the numerator;
$MS_{error}$ is always in the denominator.

# Compare to $F_{\alpha(1),\text{df\_group},\text{df\_error}}$

$$F_{0.05(1),2,58} = 3.15.$$

Since 1835 > 3.15, we know $P < 0.05$ and we can reject the null hypothesis.

The variance in sample group means is bigger than expected given the variance within sample groups.

Therefore, at least one of the groups has a population mean different from another group.

# ANOVA table – squirrel data

| **Source** | **SS** | **df** | **MS** | **F** | ***P*** |
|---|---|---|---|---|---|
| Group | 1015.7 | 2 | 507.9 | 1834.7 | <0.0001 |
| Error | 16.1 | 58 | 0.277 | | |
| Total | 1031.8 | 60 | | | |

# Variability explained: $R^2$

- $R^2$ is the fraction of variation that is explained by group differences:

$$R^2 = \frac{SS_{groups}}{SS_{total}}$$

- For Squirrel study:

$R^2 = 1015.7 / 1031.8 = 0.98$

# Assumptions of ANOVA

- Random samples.

- Normal distributions for each population.
  (Small deviations are OK)

- Equal variances for all populations. (*Homoscedasticity*)
  (But OK if balanced samples, and variance difference less than factor of 10)

# Kruskal-Wallis test

- A non-parametric test similar to a single factor ANOVA
- Uses the ranks of the data points
- Conceptually similar to Mann-Whitney U test.

# Multiple-factor ANOVA
## (an advanced topic)

- A *factor* is a categorical variable

- ANOVAs can be generalized to look at more than one categorical variable at a time

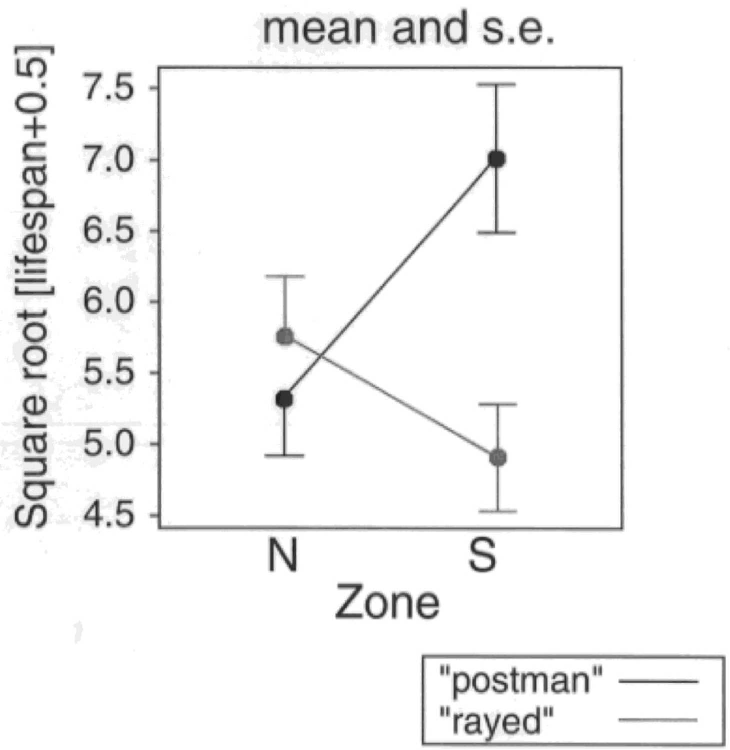
- Not only can we ask whether each categorical variable affects a numerical variable, but also do they interact in affecting the numerical variable.

# 2-factor ANOVA: Example

"rayed" *Heliconius erato* "postman"

This experiment uses two "morphs”:
the rayed morph from the "north,
and the postman morph from the "south."

# Testing multiple hypotheses




$H_0$: Mean lifespans are the same in both geographical zones.

$H_0$: Mean lifespans are the same for both morphs.

$H_0$: There is no interaction between geographical zone and morph.

# *Heliconius* ANOVA table

| Source of variation | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Zone | 9.1 | 1 | 9.1 | 0.96 | 0.327 |
| Morph | 34.6 | 1 | 34.6 | 3.68 | 0.056 |
| Zone*Morph | 80.5 | 1 | 80.5 | 8.59 | 0.004 |
| Error | 1837.9 | 196 | 9.38 | | |

# Fixed vs. random effects

1. *Fixed effects*: With fixed effects, the treatments are chosen by the experimenter. They are not a random subset of all possible treatments.
   (e.g., specific drug treatments, specific diets, season...)

2. *Random effects*: With random effects, the groups are a random sample from all possible groups.
   (e.g., family, location, ...)
   Useful for analysis of ***repeatability*** of measurements.

For single-factor ANOVAs, there is no difference in the statistics for fixed or random effects.

# ANOVA's v. *t*-tests

An ANOVA with *2 groups* is mathematically equivalent to a two-tailed 2-sample *t*-test.

# Multiple comparisons

Probability of a Type I error in *N* tests =
$1-(1-\alpha)^N$

For 20 tests, the probability of at least one Type I error is ~65%.

# "Bonferroni correction" for multiple comparisons

Uses a smaller $\alpha$ value:

$$\alpha' = \frac{\alpha}{\text{number of tests}}$$

# Which groups are different?

After finding evidence for differences among means with ANOVA, sometimes we want to know:

*Which groups are different from which others?*

**Planned comparisons**: similar to two-sample
t-test, but using all samples to estimate SE.

**Unplanned comparisons**: Need to adjust for inflated
Type 1 error rate.
One method: the Tukey-Kramer test

# The Tukey-Kramer test

Done after finding variation among groups with single-factor ANOVA.

Compares all group means to all other group means

# The wood-wide web

Trees (and other plants) are often connected by roots via mycorrhizae, which allow the exchange of resources.

# Test for carbon transfer between birch and Douglas fir; Comparing effects of shading on fir

Net amount of carbon transferred from birch to fir

| Shade treatment | Sample mean | Sample standard deviation | Sample size |
|---|---|---|---|
| Deep shade | 18.33 | 6.98 | 5 |
| Partial shade | 8.29 | 4.76 | 5 |
| No shade | 5.21 | 3.00 | 5 |

Simard et al. (1997) **Net transfer of carbon between ectomycorrhizal tree species in the field.** *Nature* 388:579-582.

# ANOVA results

| Source of variation | SS | df | MS | F | *P* |
|---|---|---|---|---|---|
| Groups (treatments) | 470.704 | 2 | 235.352 | 8.784 | 0.004 |
| Error | 321.512 | 12 | 26.792 | | |
| Total | 792.216 | 14 | | | |

# Order the group means

| No shade | Partial shade | Deep shade |
| --- | --- | --- |
| $\bar{Y}_3$ | $\bar{Y}_2$ | $\bar{Y}_1$ |
| 5.21 | 8.29 | 18.33 |

# Null hypotheses for Tukey-Kramer

$$H_0: \ \mu_1 = \mu_2$$

$$H_0: \ \mu_1 = \mu_3$$

$$H_0: \ \mu_2 = \mu_3$$

# Why not use a series of two-sample *t*-tests?

Multiple comparisons would cause the *t*-tests to reject too many true null hypotheses.

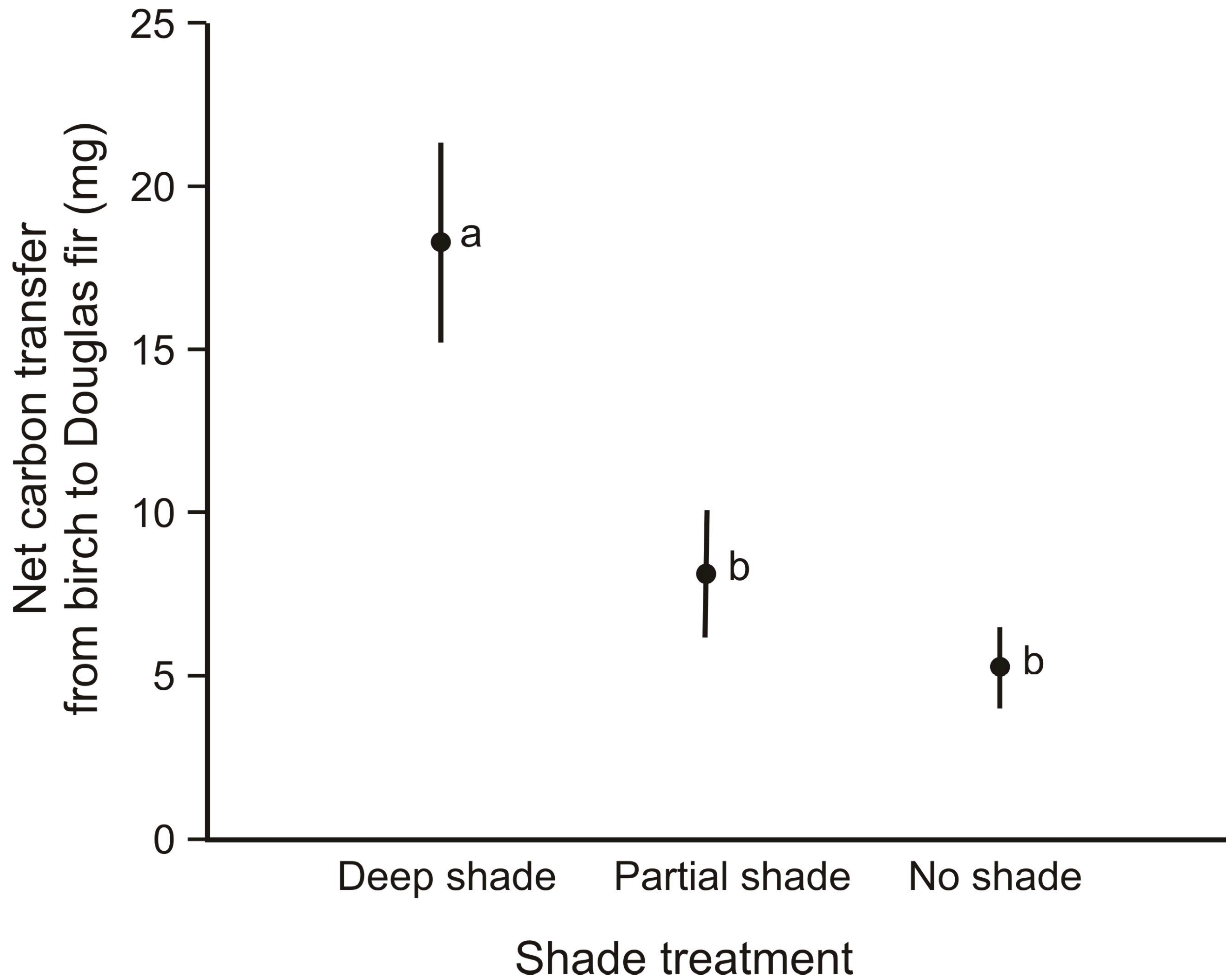
Tukey-Kramer adjusts for the number of tests.

Tukey-Kramer also uses information about the variance within groups from all the data, so it has more power than a *t*-test with a Bonferroni correction.

# Tukey-Kramer Results

| **Group *i*** | **Group *j*** | $\bar{Y}_i - \bar{Y}_j$ | **SE** | **Test statistic** $q$ | **Critical value** $q_{0.05,3,12}$ | **Conclusion** |
|---|---|---|---|---|---|---|
| Deep | No | 13.12 | 3.273693 | 4.008 | 2.67 | Reject $H_0$ |
| Deep | Partial | 10.04 | 3.273693 | 3.067 | 2.67 | Reject $H_0$ |
| Partial | No | 3.08 | 3.273693 | 0.941 | 2.67 | Do not reject $H_0$ |

$$SE = \sqrt{MS_{error}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$



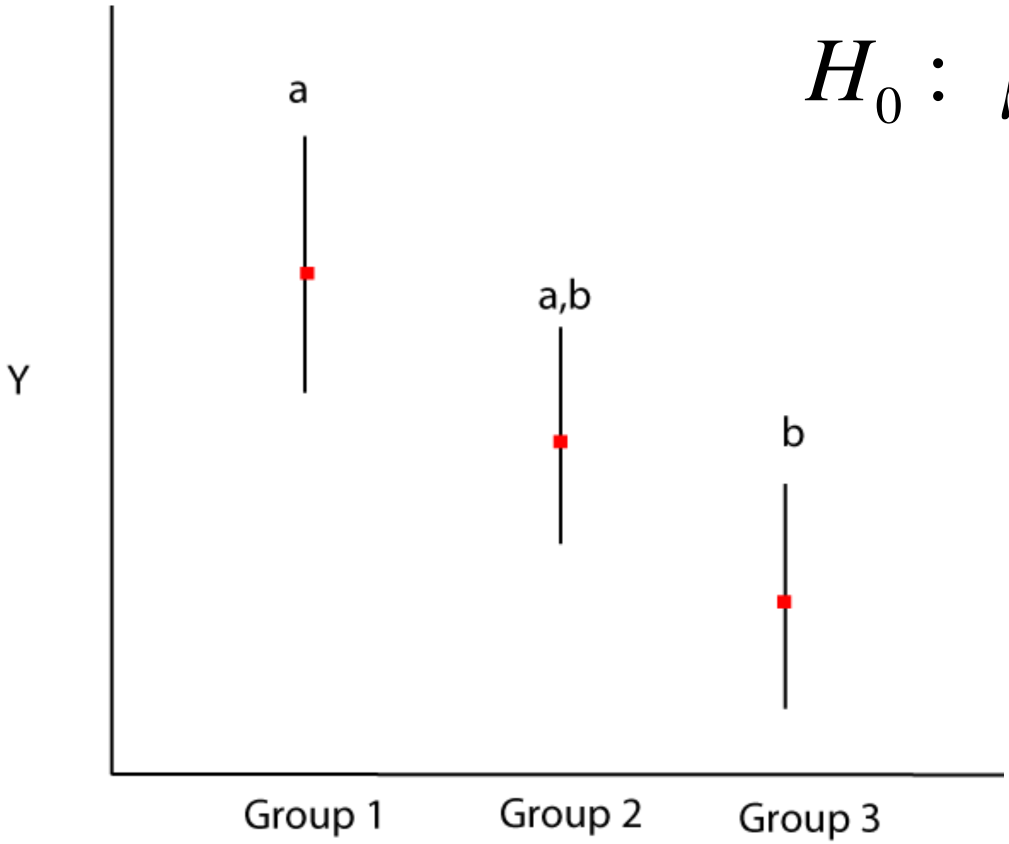

Groups which cannot be distinguished share the same letter.

Another (imaginary) example:

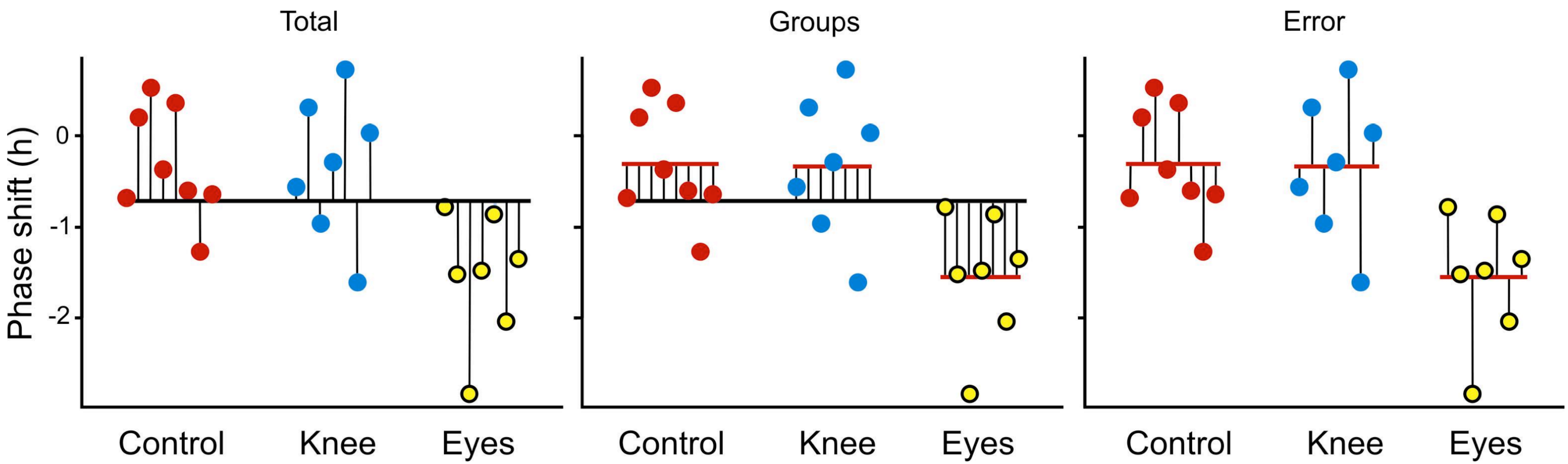
$$H_0: \mu_1 = \mu_2$$ Cannot reject

$$H_0: \mu_1 = \mu_3$$ Reject

$$H_0: \mu_2 = \mu_3$$ Cannot reject



With the Tukey-Kramer method, the probability of making at least one Type 1 error throughout the course of testing all pairs of means is no greater than the significance level $\alpha$.




$$SS_{total} = SS_{groups} + SS_{error}$$

# Announcement

- lab report due today
- new assignment on Friday
- the sun is shining

# Comparing the means of more than two groups

Chapter 15

# Analysis of variance (ANOVA)

- Like a *t*-test, but can compare more than two groups
- Asks whether any of two or more means is different from any other.
- In other words, is the variance among groups greater than zero?

# Null hypothesis for simple ANOVA

- $H_0$ : Variance among groups = 0

OR

- $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \ldots \mu_k$

Under $H_0$, the sample mean of each group should vary because of sampling error.

standard deviation of sample means

=

standard error (when the true mean is constant)

$$\sigma_{\bar{x}} = \frac{\sigma_x}{\sqrt{n}}$$

Squaring the standard error, the variance among sample means due to sampling error should be:

$$\sigma_{\bar{x}}^2 = \frac{\sigma_x^2}{n}$$

# Under $H_0$ FALSE:

var(sample mean) = var(sample) + var(real means)

*expected due to sampling error*

*present of means of groups are different*
*($H_0$ is **false**)*

# Under $H_0$ FALSE:

$$\sigma_{\bar{x}}^{2} = \frac{\sigma_{x}^{2}}{n} + Variance\left[\mu_{i}\right]$$

variance among sample means =
variance due to sampling error
+
real variance among population means

With ANOVA, we test whether the variance among true group means is greater than zero.

$$Variance\left[\mu_i\right] > 0\,?$$

With ANOVA, we test whether the variance among true group means is greater than zero.

$$Variance\left[\mu_i\right] > 0 ?$$

We do this by asking whether the variance among sample means is greater than expected under the null hypothesis:

$$\sigma_{\bar{x}}^2 > \frac{\sigma_x^2}{n} ?$$

Above is based on population parameters

but…..

we only have *estimates* through our samples.

*Variance among groups* $\sigma^2_{\bar{x}} > \dfrac{\sigma^2_x}{n}$ ? $\dfrac{\textit{Variance within groups}}{n}$

$$n\,\sigma^2_{\bar{x}} > \sigma^2_x\ ?$$

**Population parameters** $\longrightarrow$ **Estimates from sample**

$$\sigma_{\bar{x}}^2 > \frac{\sigma_x^2}{n} ?$$

$$n\sigma_{\bar{x}}^2 > \sigma_x^2 ?$$

**Population parameters** **Estimates from sample**

$n\sigma_{\bar{x}}^2$ is estimated by the "Mean Squares Group" $MS_{group}$

*'n times variance among groups'*

$\sigma_x^2$ is the variance within groups, estimated by the "Mean Squares Error" $MS_{error}$

*'variance within groups'*

*How to calculate the $MS_{group}$ and $MS_{error}$ from sample*

# Mean squares group

Abbreviation: $MS_{group}$

Variance in mean due to sampling error

Estimates this parameter: $$n\left(\frac{\sigma_x^2}{n} + Variance\left[\mu_i\right]\right)$$

Variance in mean due to differences in means

Formula: $$MS_{groups} = \frac{SS_{groups}}{df_{groups}}$$

# Mean squares group

*i* = group
*j* = indiv.

$$SS_{group} = \sum n_i \left(\overline{X}_i - \overline{X}\right)^2$$

$\bar{X}_i$ *is the mean of group i, and*

$$\bar{X} = \sum_{i=1}^{k} \sum_{j=1}^{n_i} \frac{X_{ij}}{N}$$

$df_{groups} = k - 1$(where k is the number of groups)

$$MS_{groups} = \frac{SS_{groups}}{df_{groups}}$$

# Mean squares error

Abbreviation: $MS_{error}$

Estimates this parameter: $\sigma_x^2$

Formula: $MS_{error} = \dfrac{SS_{error}}{df_{error}}$

# Mean squares error

Error sum of squares =

$$SS_{error} = \sum df_i s_i^2 = \sum s_i^2 (n_i - 1)$$

Error degrees of freedom =

$$df_{error} = \sum df_i = \sum \left(n_i - 1\right) = N - k$$

$$MS_{error} = \frac{SS_{error}}{df_{error}} = \frac{\sum s_i^2 (n_i - 1)}{N - k}$$

# Test statistic: *F*

If $H_0$ is true, then $$n\,\sigma_{\bar{x}}^2 = \sigma_x^2$$

In other words: $$F = \frac{n\,\sigma_{\bar{x}}^2}{\sigma_x^2} = 1$$

But, refers to population parameters.
We must estimate F from samples with:
$MS_{\text{group}} / MS_{\text{error}}$

# *F* if null hypothesis is false:

We test whether the *F* ratio is greater than one, as it would be if $H_0$ is false:

$$F = \frac{n\left(\frac{\sigma_x^2}{n} + Variance\left[\mu_i\right]\right)}{\sigma_x^2} > 1$$

# Sampling error

- Take into account sampling error

- Often, F calculated from data will be greater than one even when the null is true

- Hence we must compare F to a null distribution.

# Using sample to get F

$$F = \frac{MS_{groups}}{MS_{errors}}$$

# ANOVA table

| Source | SS | df | MS | F | *P* |
|---|---|---|---|---|---|
| Group | | | | | |
| Error | | | | | |
| Total | | | | | |

An ANOVA table is a convenient way to keep track of the important calculations.

Scientific papers often report ANOVA results with ANOVA tables.

# Example: Body temperature of squirrels in low, medium and hot environments

Wooden & Walsberg (2004). *Journal of Experimental Biology* 207:41-46.

# Hypotheses

$H_0$: Mean body temperature is the same for all three groups of squirrels.

$H_A$: At least one of the three is different from the others.

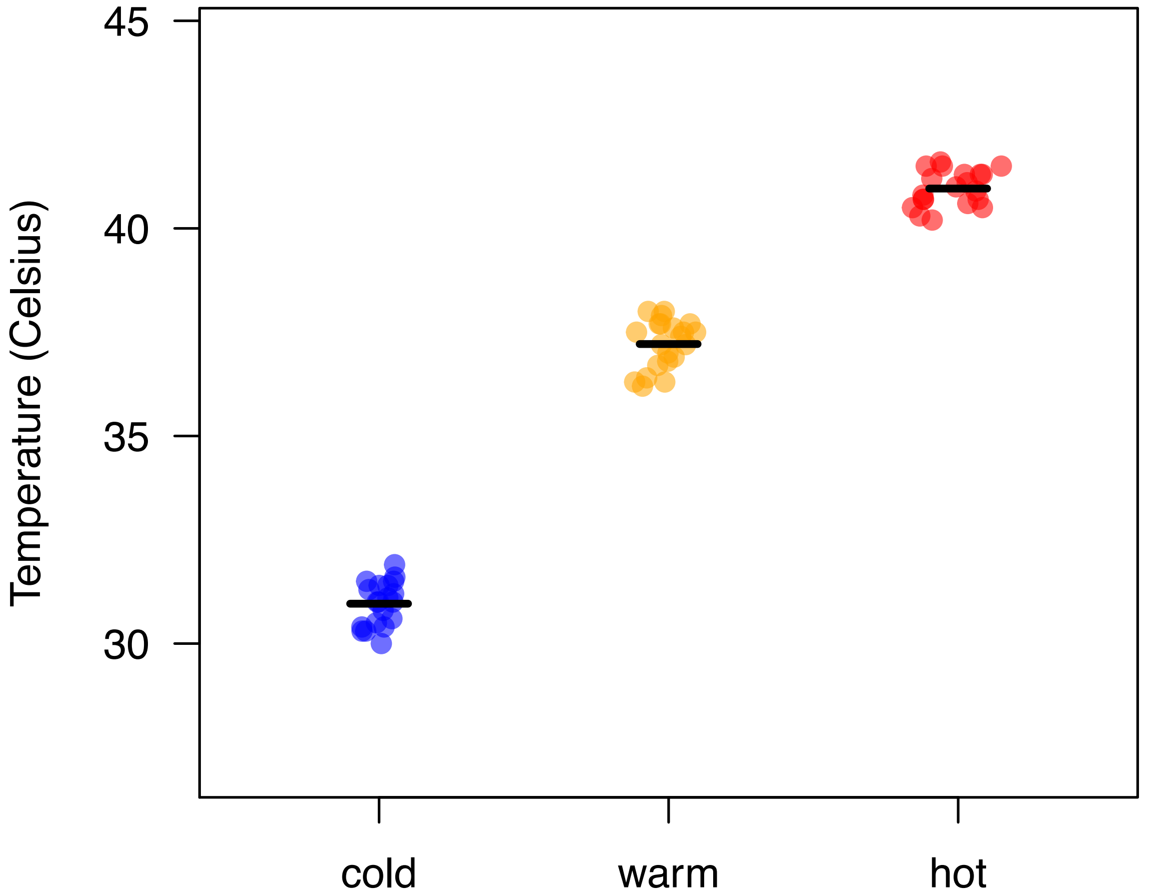
# Squirrel body temperature data (° C)

Cold: 30.4, 31.0, 31.2, 31.0, 31.5, 30.4, 30.6, 31.1,
31.3, 31.9, 31.4, 31.6, 31.5, 31.4, 30.3, 30.5,
30.3, 30.0, 30.8, 31.0

Warm: 36.3, 37.5, 36.9, 37.2, 37.5, 37.7, 37.5, 37.7,
38.0, 38.0, 37.6, 37.4, 37.9, 37.2, 36.3, 36.2,
36.4, 36.7, 36.8, 37.0, 37.7

Hot: 40.7, 40.6, 40.9, 41.1, 41.5, 40.8, 40.5, 41.0,
41.3, 41.5, 41.3, 41.2, 40.7, 40.3, 40.2, 41.3,
40.7, 41.6, 41.5, 40.5

# Plot the data



# Summary data

| Group | $\bar{x}$ | ***s*** | ***n*** |
|---|---|---|---|
| Cold | 31.0 | 0.551 | 20 |
| Warm | 37.2 | 0.582 | 21 |
| Hot | 41.0 | 0.430 | 20 |

Total sample size: $N = \sum n = 20 + 21 = 20 = 61$

# Next steps

Calculate:

- $MS_{error}$
- $MS_{group}$
- F value

# $MS_{error}$ for squirrels

$$SS_{error} = \sum df_i s_i^2$$

$$= 19(0.551)^2 + 20(0.582)^2 + 19(0.430)^2$$

$$= 16.1$$

$$df_{error} = 19 + 20 + 19 = 58$$

$$MS_{error} = \frac{16.1}{58} = 0.277$$

# $MS_{group}$ squares group:

$$\overline{X} = \frac{20(31.0) + 21(37.2) + 20(41.0)}{20 + 21 + 20} = 36.4$$

$$SS_{group} = \sum n_i \left(\overline{X}_i - \overline{X}\right)^2$$

$$SS_{group} = 20(31.0\text{-}36.4)^2 + 21(37.2\text{-}36.4)^2 + 20(41.0\text{-}36.4)^2$$

$$= 1015.7$$

# $MS_{group}$ squares group:

$df_{group} = k - 1 = 3\text{-}1\text{=}2$

$$MS_{groups} = \frac{SS_{groups}}{df_{groups}} = \frac{1015.7}{2} = 507.9$$

# $F$ statistic

$$F = \frac{MS_{group}}{MS_{error}} = \frac{507.9}{0.277} = 1834.7$$

$MS_{\text{group}}$ is always in the <u>numerator</u>;
$MS_{\text{error}}$ is always in the <u>denominator</u>.

# Compare to $F_{\alpha(1),df\_group,df\_error}$

$$F_{0.05(1),2,58} = 3.15.$$

Since 1835 > 3.15, we know $P < 0.05$ and we can reject the null hypothesis.

The variance in sample group means is bigger than expected given the variance within sample groups.

Therefore, at least one of the groups has a population mean different from another group.

# ANOVA table – squirrel data

| Source | SS | df | MS | F | *P* |
|---|---|---|---|---|---|
| Group | 1015.7 | 2 | 507.9 | 1834.7 | <0.0001 |
| Error | 16.1 | 58 | 0.277 | | |
| Total | 1031.8 | 60 | | | |

# Variability explained: $R^2$

- $R^2$ is the fraction of variation that is explained by group differences:

$$R^2 = \frac{SS_{groups}}{SS_{total}}$$

- For Squirrel study:

$R^2 = 1015.7 / 1031.8 = 0.98$

# Assumptions of ANOVA

- Random samples.
- Normal distributions for each population.
  (Small deviations are OK)
- Equal variances for all populations. (*Homoscedasticity*)
  (But OK if balanced samples, and variance difference less than factor of 10)

# Kruskal-Wallis test

- A non-parametric test similar to a single factor ANOVA
- Uses the ranks of the data points
- Conceptually similar to Mann-Whitney U test.

# What’s up next

- Multi factor anova
- Testing multiple hypotheses
- Correct for multiple testing
- Tukey-Kramer test

# Multiple-factor ANOVA
## (an advanced topic)

- A *factor* is a categorical variable

- ANOVAs can be generalized to look at more than one categorical variable at a time

- Not only can we ask whether each categorical variable affects a numerical variable, but also do they interact in affecting the numerical variable.

# 2-factor ANOVA: Example

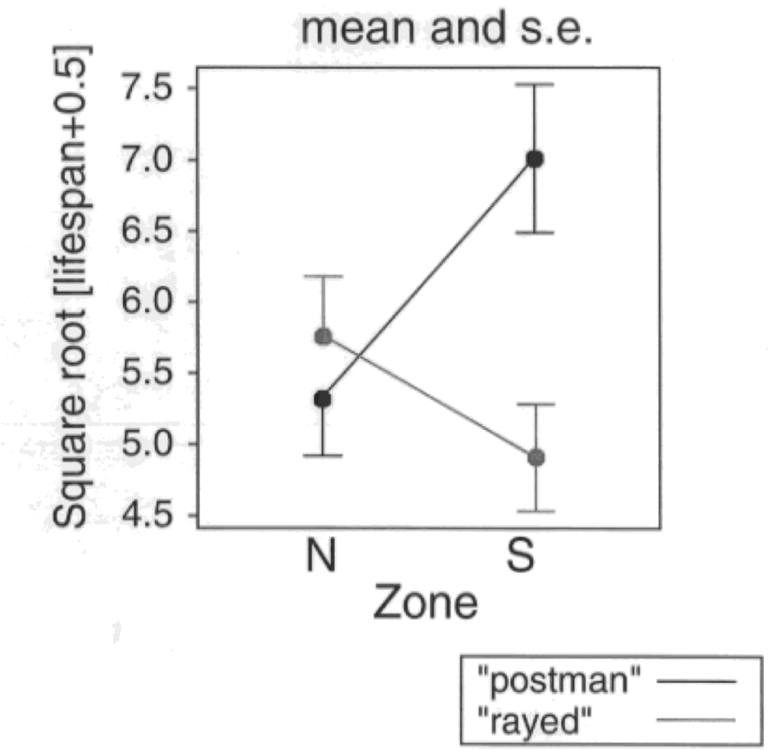
"rayed"

*Heliconius erato*

"postman"

This experiment uses two "morphs”:
the rayed morph from the "north,
and the postman morph from the "south."

# Testing multiple hypotheses




$H_0$: Mean lifespans are the same in both geographical zones.

$H_0$: Mean lifespans are the same for both morphs.

$H_0$: There is no interaction between geographical zone and morph.

# *Heliconius* ANOVA table

| Source of variation | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Zone | 9.1 | 1 | 9.1 | 0.96 | 0.327 |
| Morph | 34.6 | 1 | 34.6 | 3.68 | 0.056 |
| Zone*Morph | 80.5 | 1 | 80.5 | 8.59 | 0.004 |
| Error | 1837.9 | 196 | 9.38 | | |

# Fixed vs. random effects

1. *Fixed effects*: With fixed effects, the treatments are chosen by the experimenter. They are not a random subset of all possible treatments.
   - (e.g., specific drug treatments, specific diets, season...)

2. *Random effects*: With random effects, the groups are a random sample from all possible groups.
   - (e.g., family, location, ...)
   - Useful for analysis of ***repeatability*** of measurements.

For single-factor ANOVAs, there is no difference in the statistics for fixed or random effects.

# ANOVA's v. *t*-tests

An ANOVA with *2 groups* is mathematically equivalent to a two-tailed 2-sample *t*-test.

# Multiple comparisons

Probability of a Type I error in *N* tests = $1-(1-\alpha)^N$

For 20 tests, the probability of at least one Type I error is ~65%.

# "Bonferroni correction" for multiple comparisons

Uses a smaller $\alpha$ value:

$$\alpha' = \frac{\alpha}{\text{number of tests}}$$

# Which groups are different?

After finding evidence for differences among means with ANOVA, sometimes we want to know:

*Which groups are different from which others?*

**Planned comparisons**: similar to two-sample t-test, but using all samples to estimate SE.

**Unplanned comparisons**: Need to adjust for inflated Type 1 error rate.

One method: the Tukey-Kramer test

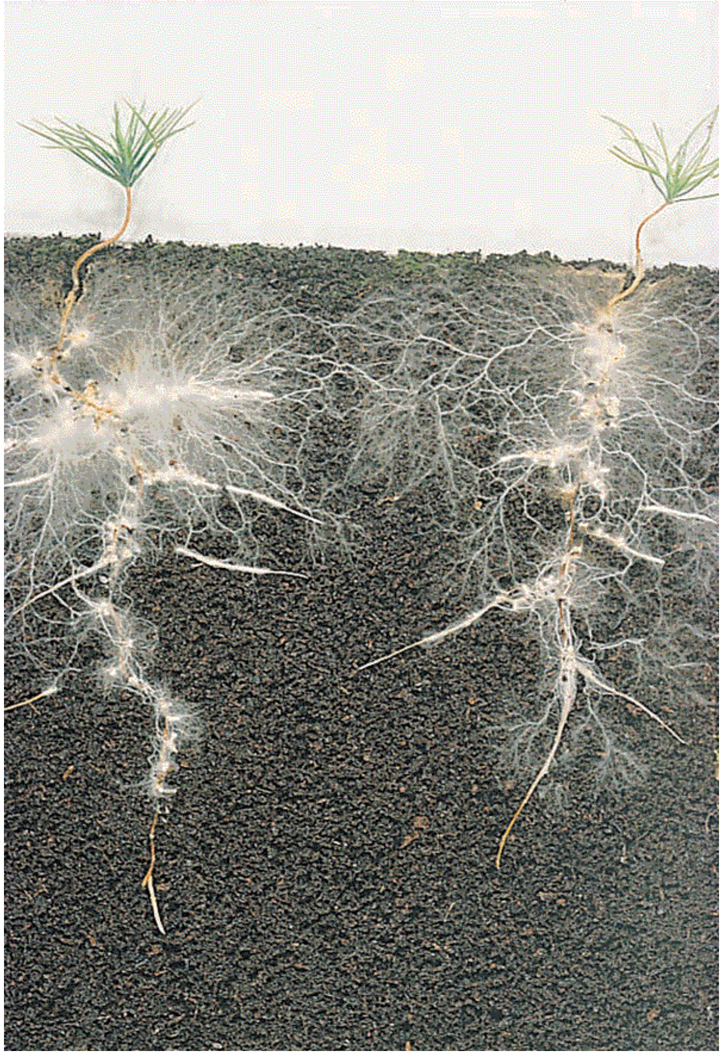
# The Tukey-Kramer test

Done after finding variation among groups with single-factor ANOVA.

Compares all group means to all other group means

# The wood-wide web

Trees (and other plants) are often connected by roots via mycorrhizae, which allow the exchange of resources.



# Test for carbon transfer between birch and Douglas fir;
# Comparing effects of shading on fir

Net amount of carbon transferred from birch to fir

| Shade treatment | Sample mean | Sample standard deviation | Sample size |
|---|---|---|---|
| Deep shade | 18.33 | 6.98 | 5 |
| Partial shade | 8.29 | 4.76 | 5 |
| No shade | 5.21 | 3.00 | 5 |

Simard et al. (1997) **Net transfer of carbon between ectomycorrhizal tree species in the field.** *Nature* 388:579-582.

# ANOVA results

| Source of variation | SS | df | MS | F | *P* |
|---|---|---|---|---|---|
| Groups (treatments) | 470.704 | 2 | 235.352 | 8.784 | 0.004 |
| Error | 321.512 | 12 | 26.792 | | |
| Total | 792.216 | 14 | | | |

# Order the group means

| No shade | Partial shade | Deep shade |
|---|---|---|
| $\bar{Y}_3$ | $\bar{Y}_2$ | $\bar{Y}_1$ |
| 5.21 | 8.29 | 18.33 |

# Null hypotheses for Tukey-Kramer

$$H_0: \ \mu_1 = \mu_2$$

$$H_0: \ \mu_1 = \mu_3$$

$$H_0: \ \mu_2 = \mu_3$$

# Why not use a series of two-sample *t*-tests?

Multiple comparisons would cause the *t*-tests to reject too many true null hypotheses.

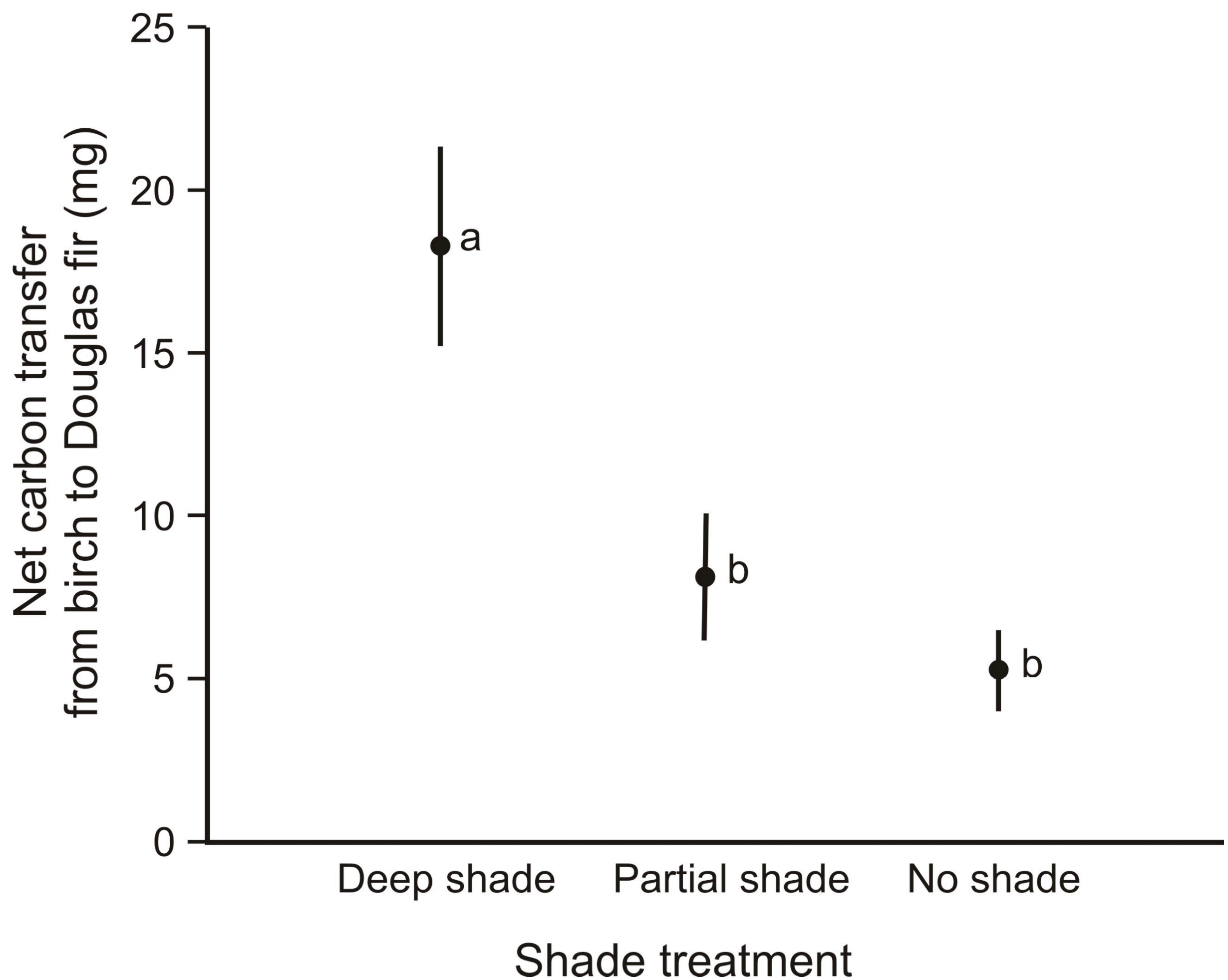
Tukey-Kramer adjusts for the number of tests.

Tukey-Kramer also uses information about the variance within groups from all the data, so it has more power than a *t*-test with a Bonferroni correction.

# Tukey-Kramer Results

| Group $i$ | Group $j$ | $\bar{Y}_i - \bar{Y}_j$ | SE | Test statistic $q$ | Critical value $q_{0.05,3,12}$ | Conclusion |
|---|---|---|---|---|---|---|
| Deep | No | 13.12 | 3.273693 | 4.008 | 2.67 | Reject $H_0$ |
| Deep | Partial | 10.04 | 3.273693 | 3.067 | 2.67 | Reject $H_0$ |
| Partial | No | 3.08 | 3.273693 | 0.941 | 2.67 | Do not reject $H_0$ |

$$SE = \sqrt{MS_{error}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$




Groups which cannot be distinguished share the same letter.

Another (imaginary) example:

$$H_0: \mu_1 = \mu_2$$ Cannot reject

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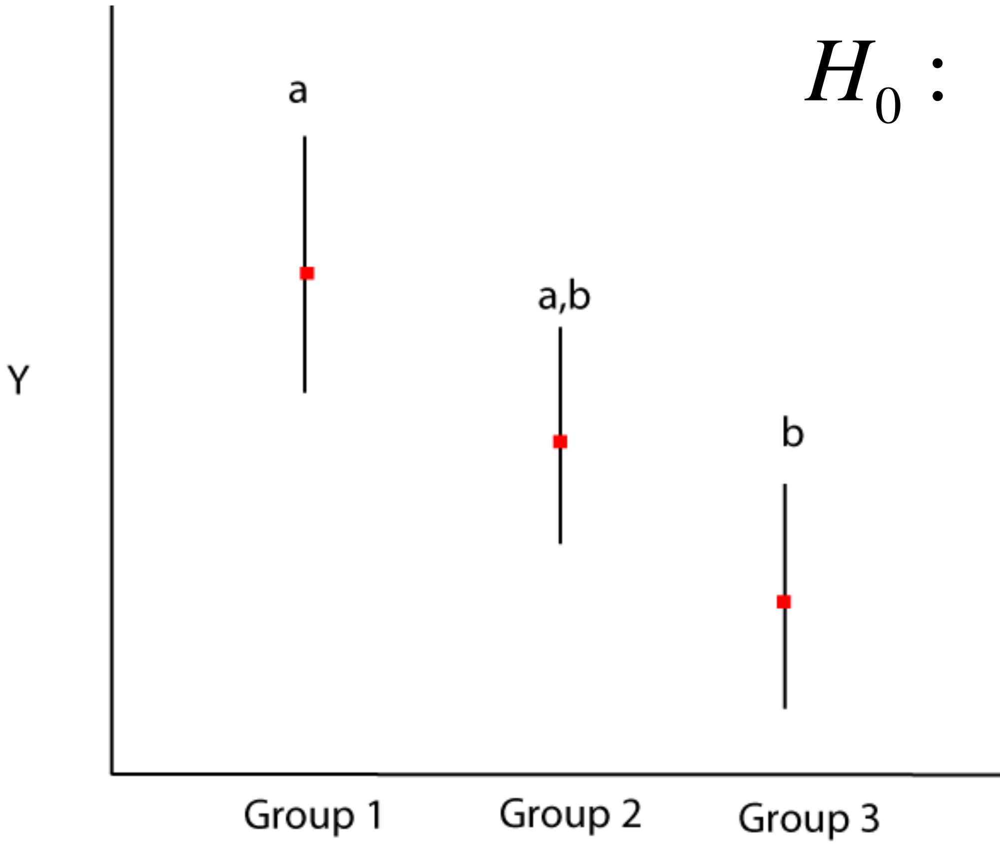
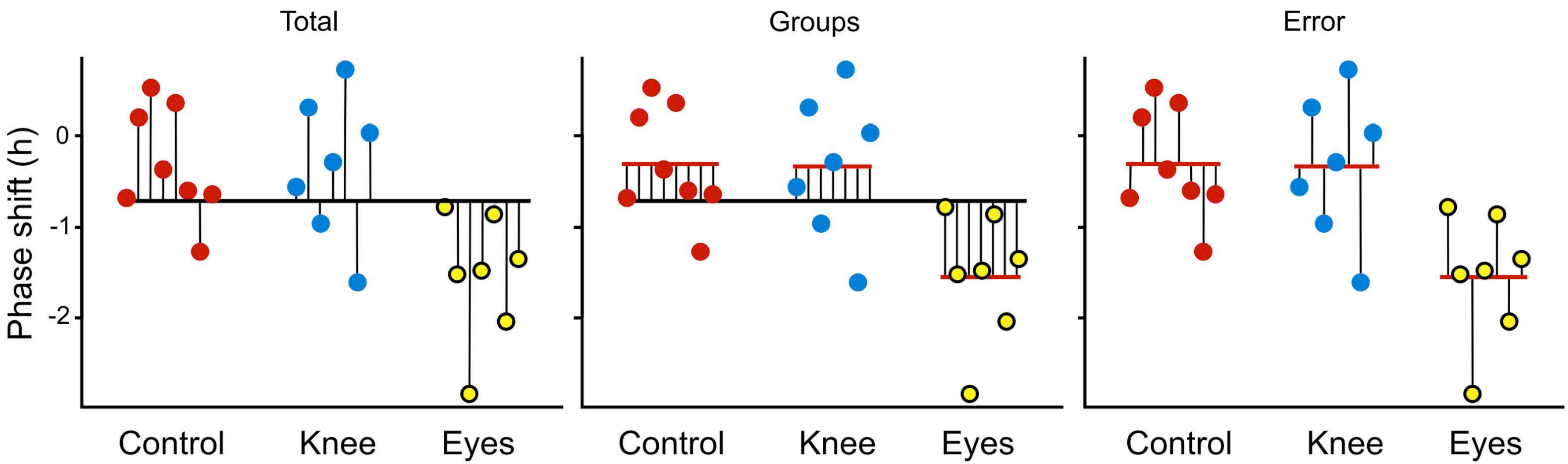


With the Tukey-Kramer method, the probability of making at least one Type 1 error throughout the course of testing all pairs of means is no greater than the significance level $\alpha$.




$$SS_{total} = SS_{groups} + SS_{error}$$

# Announcement

- new assignment available after class on Connect
- Next week your TA will discuss during lab the lab report analyses
- Labs & Easter:

  -Lab 9: March 21-24 for Mon-Thur sections and April 1st for the Friday section.

  -Lab 10: everyone April 4-8

- the sun is still shining

# Comparing the means of more than two groups

Chapter 15

# Analysis of variance (ANOVA)

- Asks whether any of two or more means is different from any other.

- In other words, is the variance among groups greater than zero?

# Null hypothesis for simple ANOVA

- $H_0$ : Variance among groups = 0

OR

- $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4 = \ldots \mu_k$

## Under $H_0$ :

var(sample mean) = var(sample)

*expected due to sampling error*

Squaring the standard error, the variance among sample means due to sampling error should be:

$$\sigma_{\bar{x}}^2 = \frac{\sigma_x^2}{n}$$

# Under $H_0$ FALSE:

var(sample mean) = var(sample) + var(real means)

*expected due to sampling error*

*present of means of groups are different*
*($H_0$ is **false**)*

## Under $H_0$ FALSE:

$$\sigma_{\bar{x}}^2 = \frac{\sigma_x^2}{n} + Variance\left[\mu_i\right]$$

variance among sample means =
variance due to sampling error
+
real variance among population means

With ANOVA, we test whether the variance among true group means is greater than zero.

$$Variance\left[\mu_i\right] > 0\,?$$

With ANOVA, we test whether the variance among true group means is greater than zero.

$$Variance\left[\mu_i\right] > 0 \,?$$

We do this by asking whether the variance among sample means is greater than expected under the null hypothesis:

$$\sigma_{\bar{x}}^2 > \frac{\sigma_x^2}{n} \,?$$

$$\sigma_{\bar{x}}^{2} > \frac{\sigma_{x}^{2}}{n} ?$$

$$n\,\sigma_{\bar{x}}^{2} > \sigma_{x}^{2} ?$$

**Population parameters** | **Estimates from sample**

$n\,\sigma_{\bar{x}}^{2}$ is estimated by the "Mean Squares Group" $MS_{group}$

*'n times variance among groups'*

$\sigma_{x}^{2}$ is the variance within groups, estimated by the "Mean Squares Error" $MS_{error}$

*'variance within groups'*

# Test statistic: *F*

If $H_0$ is true, then $$n\,\sigma_{\bar{x}}^2 = \sigma_x^2$$

In other words: $$F = \frac{n\,\sigma_{\bar{x}}^2}{\sigma_x^2} = 1$$

# *F* if null hypothesis is false:

We test whether the *F* ratio is greater than one, as it would be if $H_0$ is false:

$$F = \frac{n\left(\dfrac{\sigma_x^2}{n} + Variance\left[\mu_i\right]\right)}{\sigma_x^2} > 1$$

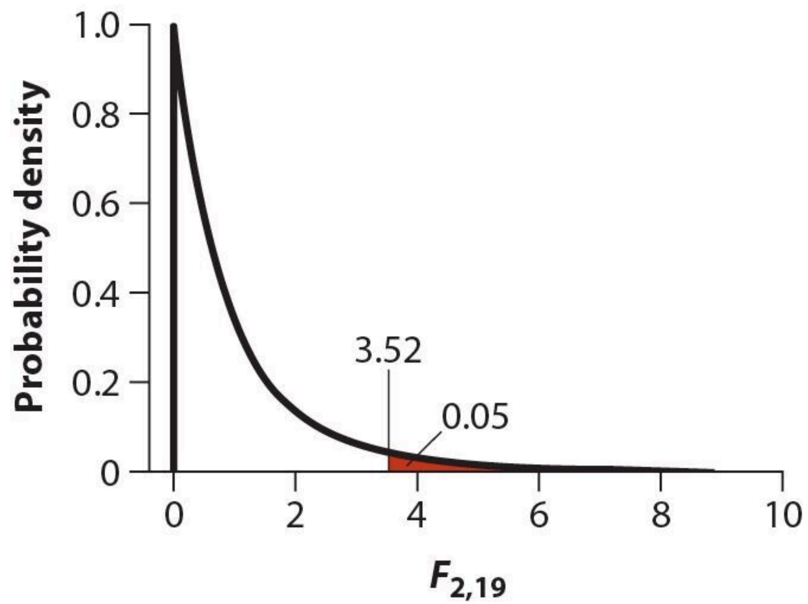
# Using sample to get F

$$F = \frac{MS_{groups}}{MS_{errors}}$$

# Compare to $F_{\alpha(1),\text{df\_group},\text{df\_error}}$

$$F_{0.05(1),2,58} = 3.15.$$

*Means we are looking at the right hand tail of the F distribution*



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# Today

- Multi factor anova
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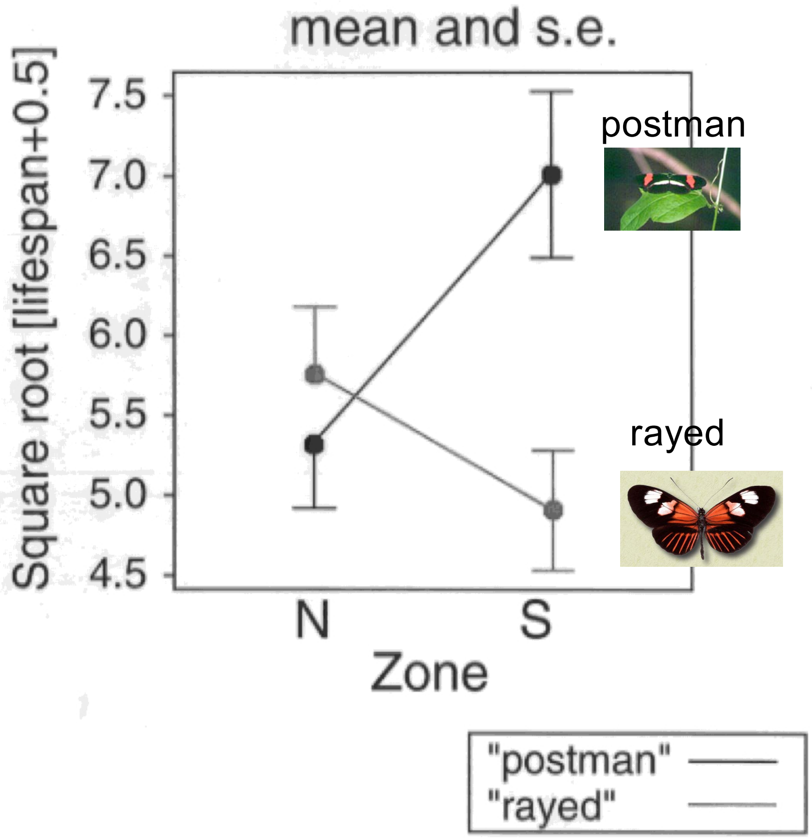


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Probability of a Type I error in *N* tests =

$$1-(1-\alpha)^N$$

*Probability you don't make a Type I error N times in a row.*

# Multiple comparisons

Probability of a Type I error in *N* tests =
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For 20 tests, the
probability of at least one
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Uses a smaller $\alpha$ value:

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# Which groups are different?

After finding evidence for differences among means with ANOVA, sometimes we want to know:

*Which groups are different from which others?*

**Planned comparisons**: similar to two-sample t-test, but using all samples to estimate SE.

*pooled sample variance ($MS_{error}$) based on all k groups*

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# Tukey-Kramer Results

| **Group *i*** | **Group *j*** | $\bar{Y}_i - \bar{Y}_j$ | **SE** | **Test statistic** $q$ | **Critical value** $q_{0.05,3,12}$ | **Conclusion** |
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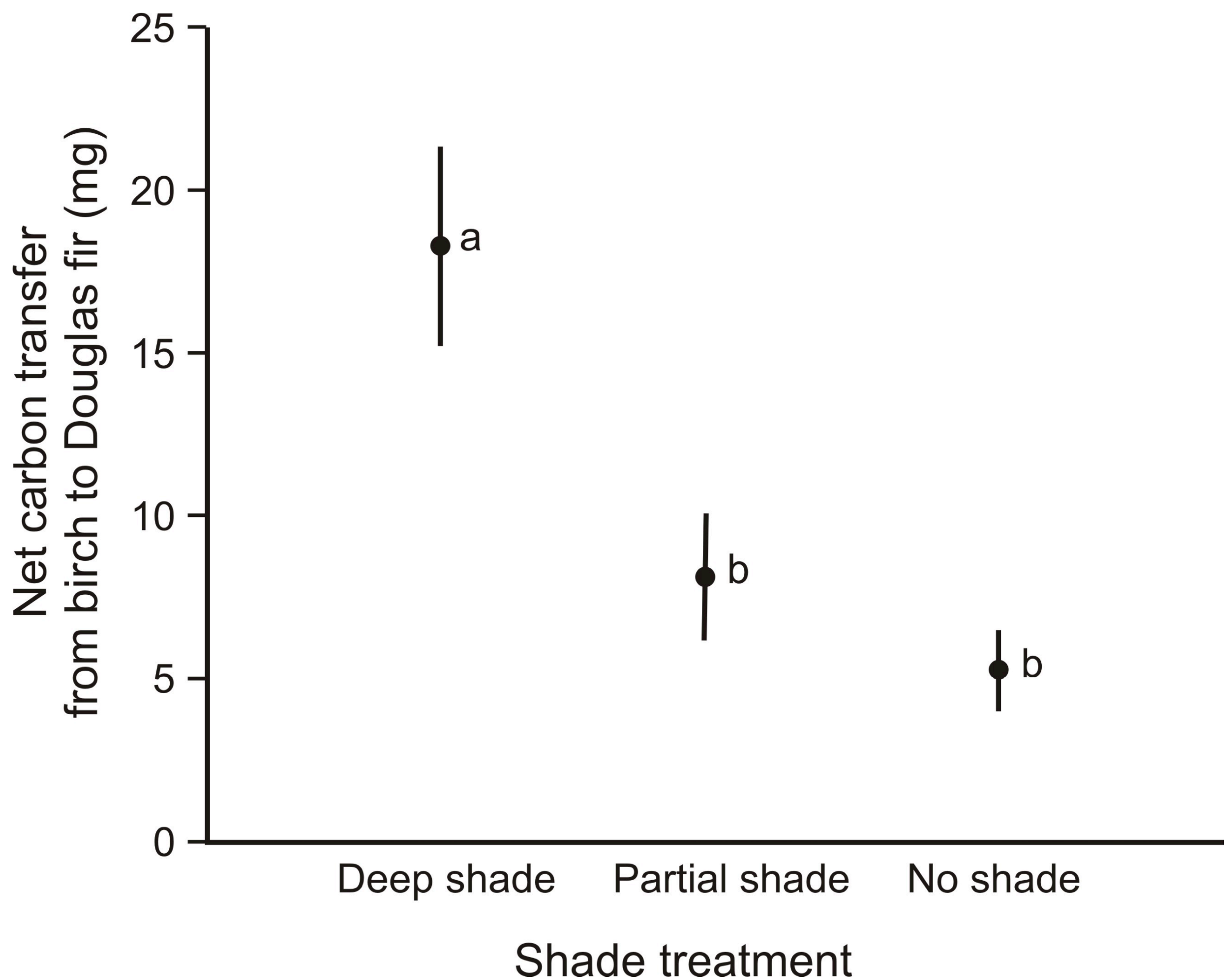


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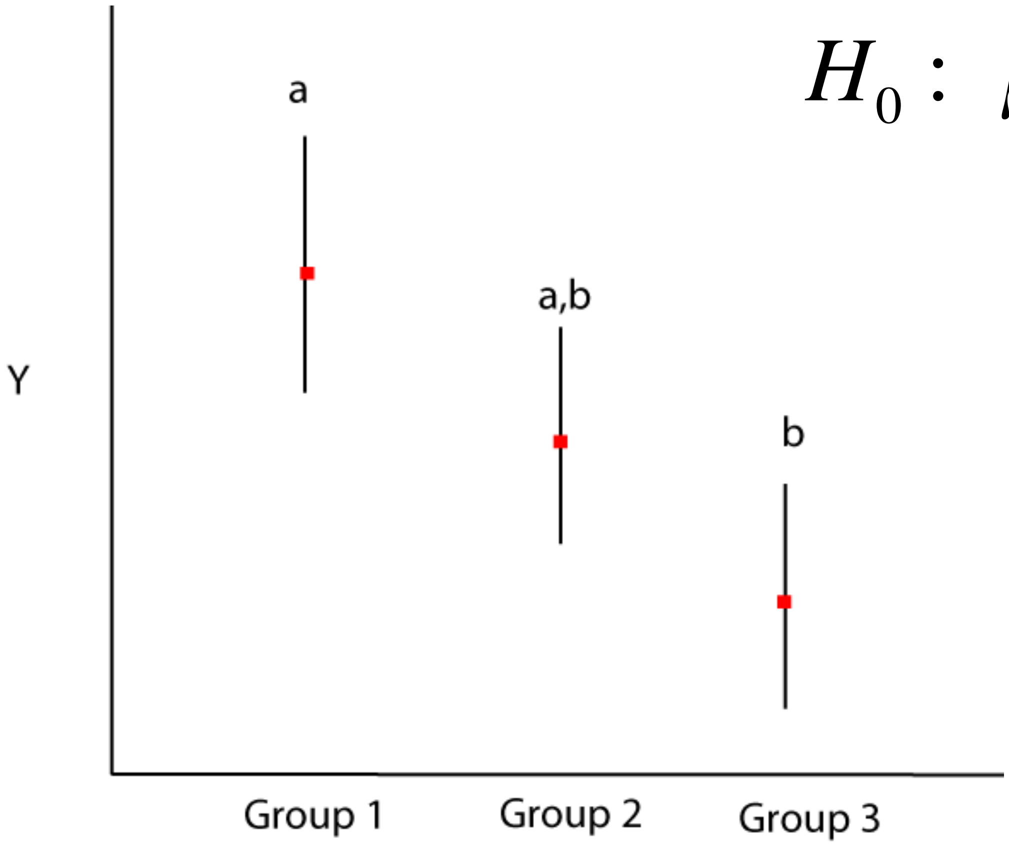

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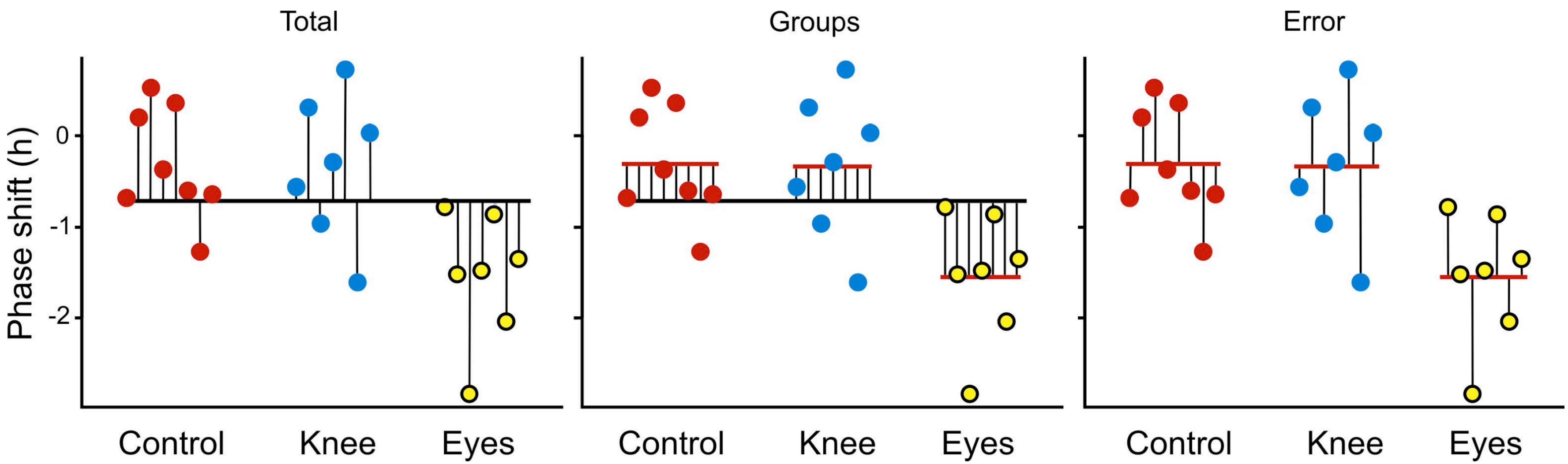


$$SS_{total} = SS_{groups} + SS_{error}$$