

Chapter 8: The Analysis of Variance (ANOVA)

Comparisons of Several Population Means

1. ANOVA model
2. ANOVA table and F-test
3. Pairwise comparisons of means (ANOVA setting)
4. Checking ANOVA Assumptions
5. Kruskal-Wallis Test (nonparametric test)
6. Transformations for One Way ANOVA
7. Power for a One-Way Model (Overall F-test)

Examples:

1. Rice Example: One-Way ANOVA
2. Poppies Example: Transformations in the One-Way Model
3. Power in a One-Way

Rice Example: (See “**Rice: One Way ANOVA**” R example)

Goal is to compare effects of 4 acids on the growth of rice seedlings.

$t = 4$ trts = 4 acids: control, acetic, proponic, butyric

$n_T = 20$ dishes (with shoots) randomly assigned to trts ($n = 5$ dishes/acid).

Let y_{ij} = dry wt. after seven days for j^{th} dish of the i^{th} treatment (acid),
 $i=1,\dots,t=4, j=1,\dots,n_i=n=5$

Let: $\bar{y}_1, \bar{y}_2, \bar{y}_3, \bar{y}_4$ be the sample means

and $\mu_1, \mu_2, \mu_3, \mu_4$ be the population means (unknown)

We could use two-sample t-tests to compare the groups, but there are some problems with this idea:

1. In order to test all possible pairs, we would need to run 6 tests. Inconvenient!
2. We get many different estimated pooled variances.
3. Multiple testing problem. We will discuss this in Chapter 9.

1. ANOVA Model

Two equivalent model statements for One-Way ANOVA

1. Means Model (No Intercept) : $y_{ij} = \mu_i + \varepsilon_{ij}$

μ_i = population group/trt means

$$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4$$

H_A : one or more difference exists

2. Effects Model (Default) : $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

μ =reference or intercept, α_i = group/trt effects (NOT Type I error!)

$$H_0 : \alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = 0$$

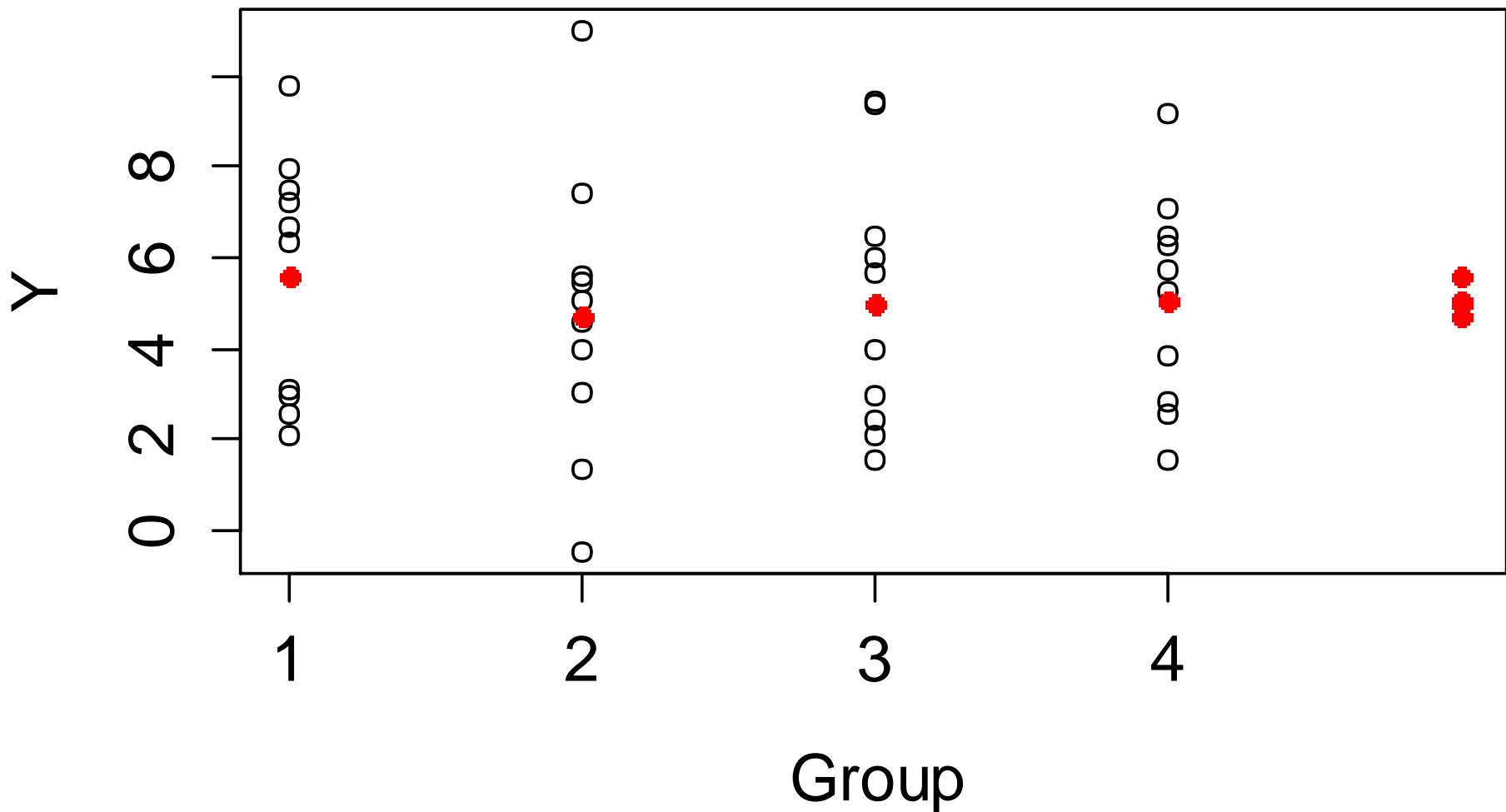
H_A : one or more α_i is not zero

We will use the Analysis of Variance (ANOVA) F-test to test the equality of means. This test has a NON-directional alternative.

The Idea of ANOVA

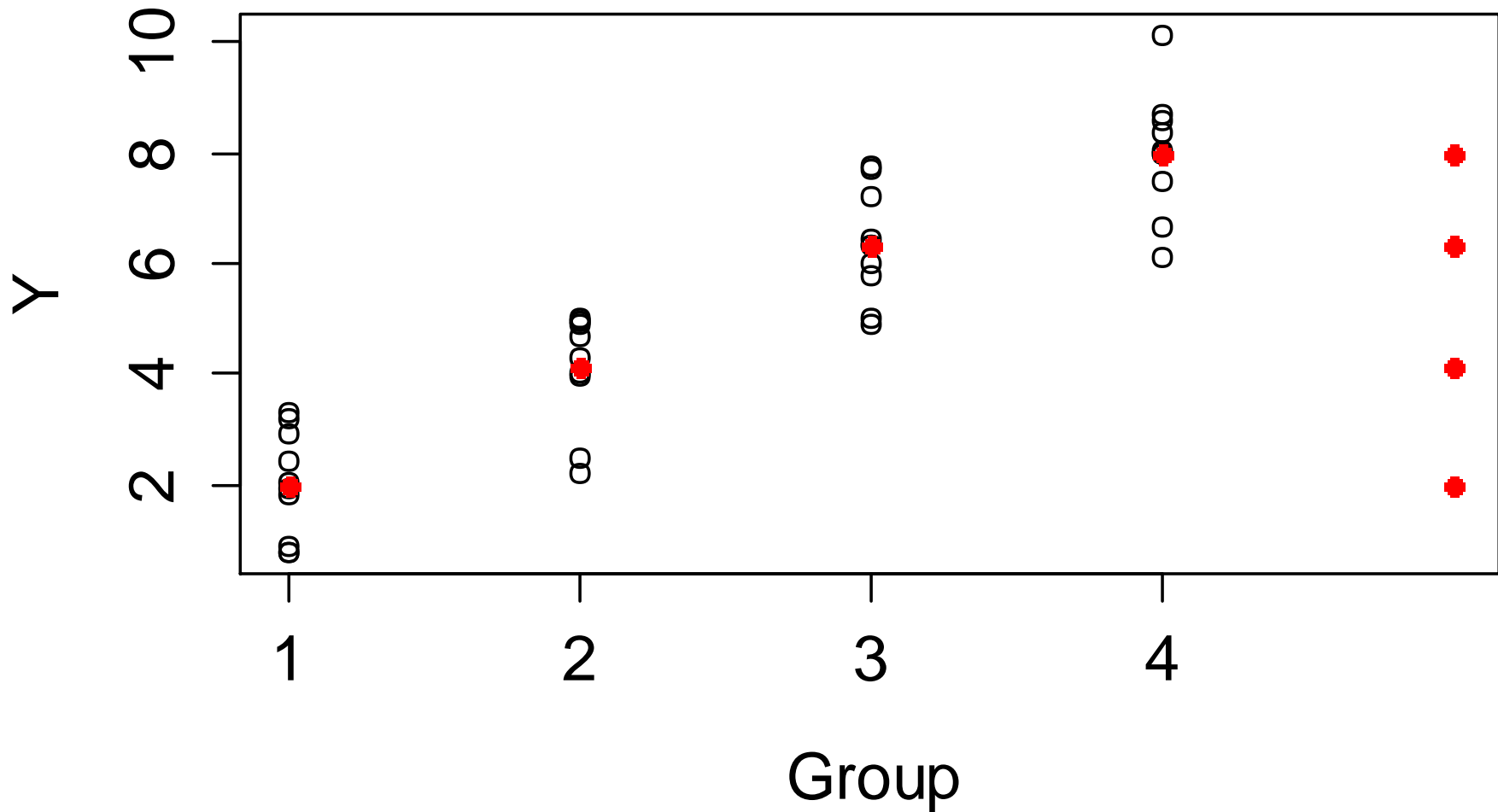
Example of True H_0 ($\mu_1=\mu_2=\mu_3=\mu_4$)

Variation between group means is not large compared to variation within groups.



Example of False H_0

Variation between group means is large compared to variation within groups.



The Idea of ANOVA

If H_0 is true (using either notation), then the model may be written:

$$y_{ij} = \mu + \varepsilon_{ij} \quad (\text{where } \mu \text{ is the common mean})$$

We test H_0 by forming an F-ratio based on two estimates of variance:

$$F = \frac{s_B^2}{s_W^2} = \frac{MSTrt}{MSResid}, \quad \text{where}$$

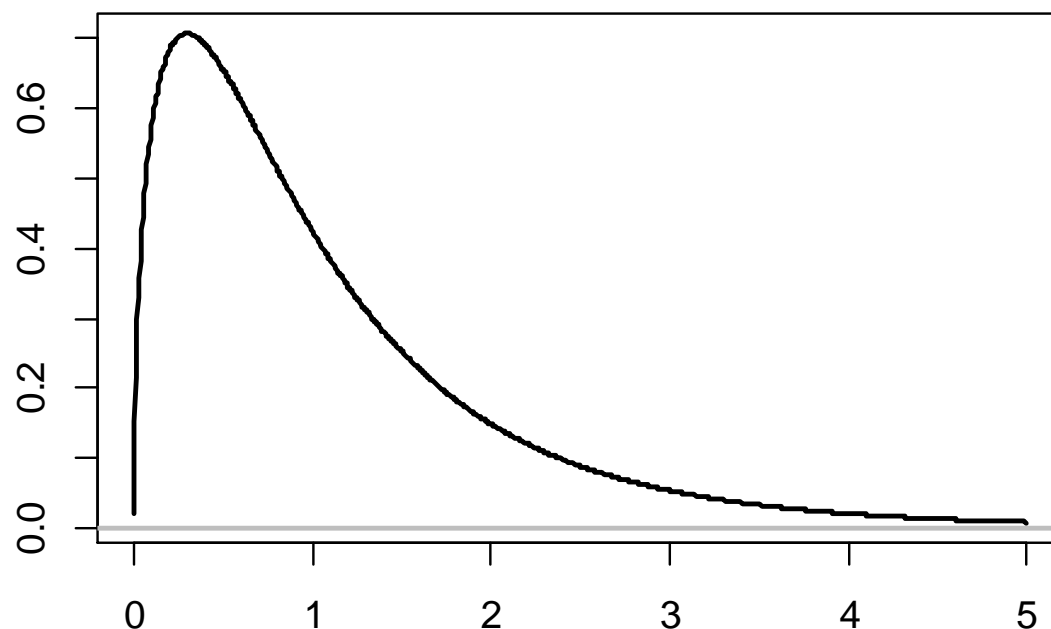
s_W^2 is an estimate of σ^2 formed by pooling sample variances.

s_B^2 is an estimate of σ^2 formed using only sample means.

$$s_W^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \dots + (n_t - 1)s_t^2}{n_T - t} = \frac{SSResid}{n_T - t} = MSResid$$

$$s_B^2 = \frac{\sum_{i=1}^t n_i (\bar{y}_{i.} - \bar{y}_{..})^2}{t - 1} = \frac{SSTrt}{t - 1} = MSTrt$$

- s_W^2 is a pooled estimate of the variance σ^2 that is valid whether H_0 is true or not, because it is based on differences within treatment groups.
- s_B^2 is an estimate of $\sigma^2 + [\sum n_i(\mu_i - \mu)^2]/(t-1)$ where $\mu = (\sum n_i \mu_i)/n_T$. Hence, when H_0 is true, s_B^2 is a valid estimate of σ^2 . If H_0 is false, then s_B^2 will tend to be too big (because it is “contaminated” by mean differences).
- If H_0 is true, the F-statistic will be distributed with the F-distribution with $df_1=t-1$ and $df_2=n_T-t$, where t is # of trts, n_T is the total sample size.



$df_1=3, df_2=16$

$F_{0.05, 3, 16} = 3.24$

2. The ANOVA table and F-test

We can partition the sums of squares:

$$\sum_{ij} (y_{ij} - \bar{y}_{..})^2 = \sum_i n_i (\bar{y}_{i.} - \bar{y}_{..})^2 + \sum_{ij} (y_{ij} - \bar{y}_{i.})^2$$

SSTotal
SS Trt
SS Resid

(corrected for the mean)

<u>Source</u>	<u>SS</u>	<u>df</u>	<u>MS = SS/df</u>	<u>F-test</u>
Trt	$SSTrt$	$t-1$	$s_B^2 = SSTrt/(t-1)$	$F = MSTrt/MSResid$
Resid	$SSResid$	$n_T - t$	$s_W^2 = SSResid/(n_T - t)$	
Total	$SSTotal$	$n_T - 1$		

ANOVA F-test for the Equality of Means

Assumptions: Random sample, independent observations, normally distributed residuals, equality of variances.

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_t$$

H_A : one or more difference exists

Test Statistic:
$$F = \frac{MSTrt}{MS Resid}$$

Reject H_0 if $F > F_{\alpha, ndf, ddf}$

where $df1=ndf=t-1$ (df Trt) and $df2=ddf=n_T-t$ (df Resid)

P-value is calculated as the area to the right of F (test statistic).

NOTE: $MSResid = MSError = MSWithin = s_W^2 = \hat{\sigma}^2$

$$MSTrt = MSBetween$$

Rice Example: One Way ANOVA

- In R, use `lm()` or `aov()`. See “**Rice: One Way ANOVA**” example.
- **NOTE:** Be sure “trt” variable is a factor! Check using `str()`.
- In Rcmdr, choose Statistics -> Means -> One-Way ANOVA.

```
> OneWayFit <- lm(weight ~ trt, data = rice)
> anova(OneWayFit)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	3	1.2199	0.4066	103.5	1.08e-10 ***
Residuals	16	0.0628	0.0039		

Reject H_0 because $p\text{-value} < 0.0001$

Can also compare test statistic to table value and get the same

conclusion: Reject H_0 because calculated $F = 103.5 > F_{0.05, 3, 16} = 3.24$.

Based on the P-value for the F-test we conclude that there is extremely strong evidence indicating differences among the treatment means.

But which means are different?

The linear model **lm()** function can be used to fit a broad class of linear models. This includes one-way ANOVA (this chapter) and simple linear regression (CH11) and many others. Again, be sure **trt** is defined as a factor! This can be done using **str()**.

The analysis of variance **aov()** function is a “wrapper” for the **lm()** function.

From **?aov**:

- The main difference between **lm()** and **aov()** is the way **print**, **summary**, etc handle the fit. **aov()** is expressed in the traditional language of ANOVA rather than that of linear models.
- **aov()** is designed for balanced designs, and the results can be hard to interpret without balance. Beware that missing values in the response will likely lose the balance.

Note: In STAT512, we will use **lm()** and **Anova()** function from the **car** package!

A note about ANOVA vs t-test when there are $t=2$ groups:

An ANOVA with $t=2$ groups is equivalent to running a two-sample t-test assuming equal variances.

The p-values testing $H_0: \mu_1 = \mu_2$ will be identical.

The test statistics are related as follows $F = t^2$.

Note that F-test is non-directional where as the t-test can easily accommodate a one-sided alternative.

3. Pairwise Comparison of Means (in ANOVA setting)

We will discuss this topic more in Chapter 9. Here we just look at one method: the **Least Significant Difference (LSD) method**. These are **unadjusted** comparisons, meaning **not adjusted for multiple testing**.

Assumptions: Random sample, independent observations, normally distributed residuals, equal variances.

$$H_0 : \mu_i = \mu_j \quad 1 \leq i, j \leq t \text{ (any two)}$$

$$H_A : \mu_i \neq \mu_j$$

Test Statistic:

$$t = \frac{\bar{y}_i - \bar{y}_j}{s_W \sqrt{\frac{1}{n_i} + \frac{1}{n_j}}}$$

$$\text{df} = n_T - t = \text{dfResid}$$

Reject H0 if $|t| > t_{\alpha/2}$

NOTE: $s_W = \hat{\sigma} = \sqrt{MS \text{ Resid}}$

Confidence Interval for the difference between means

Assumptions: Random sample, independent observations, normally distributed residuals, equal variances

$$(\bar{y}_i - \bar{y}_j) \pm t_{\alpha/2} s_W \sqrt{\frac{1}{n_i} + \frac{1}{n_j}}$$

If $n_i = n_j = n$ (group sizes are equal) the ME reduces to:

$$ME = LSD = t_{\alpha/2} s_W \sqrt{\frac{2}{n}}$$

df = $n_T - t$ = df Resid

Recall: $s_W = \hat{\sigma} = \sqrt{MS_{Resid}}$

Note that we will Reject H_0 ($\mu_i - \mu_j = 0$), whenever $|\bar{y}_i - \bar{y}_j| > ME$ (because 0 will not be contained in the CI).

So, when group sizes are equal, we will Reject H_0 for any pair (i,j) where $|\bar{y}_i - \bar{y}_j| > LSD = ME$.

Rice Example: Pairwise comparisons

t = 4 treatment groups. All $n_i = n = 5$

df = dfResid = $n_T - t = 20 - 4 = 16$. $t_{\alpha/2} = 2.120$ for $\alpha = 0.05$

Two treatment means are significantly different if:

$$|\bar{y}_{i.} - \bar{y}_{j.}| > t_{\alpha/2} s_W \sqrt{\frac{2}{n}} = (2.120) \sqrt{0.00393} \sqrt{\frac{2}{5}} = 0.0840$$

$$95\% \text{ME} = \text{LSD}_{0.05} = 0.0840$$

The result is sometimes reported in a display.

Order the means first:

Butyric	Propion	Acetic	Control
3.660	3.728	3.768	4.282
<hr/>			<hr/>

Then connect any two means that are not significantly different with a line (or group with the same letter or number).

Rice Example: Example CI

A 95% CI for $\mu_{\text{Control}} - \mu_{\text{Acetic}}$:

$$\bar{y}_i - \bar{y}_j \pm t_{\alpha/2} s_W \sqrt{\frac{2}{n}}$$
$$(4.282 - 3.768) \pm 0.0840$$
$$(0.430, 0.598)$$

Notes on Pairwise Comparison of Means in the One-Way ANOVA setting

1. In R, we will use the `emmeans()` function from the `emmeans` package to get p-values for all pairwise comparisons . See “**Rice One Way ANOVA**” example.
2. For now, we will use the unadjusted pairwise comparisons. But in practice Tukey adjustment is often used to correct for multiple testing (see CH9 notes).
3. Computer programs and publications often put letter or number superscripts next to members of the same LSD group (rather than underlines). In R, we can use the `CLD()` function to do this.
4. If there are unequal sample sizes for groups, then statistical significance is based on magnitude of difference and sample size. For this reason, there is no fixed LSD (=ME) value when sample sizes are unequal. Resulting p-values, CIs, etc are fine. But “lines”/CLD display should be interpreted with caution.

Rice Example: simple summary statistics vs emmeans()

```
> SumStats
```

	trt	n	mean	sd	se
1	acetic	5	3.768	0.06140033	0.02745906
2	butyric	5	3.660	0.06442049	0.02880972
3	control	5	4.282	0.06058052	0.02709243
4	propion	5	3.728	0.06418723	0.02870540

```
> library(emmeans)
> LMFit <- lm(weight ~ trt, data = rice)
> emmeans(LMFit, pairwise ~ trt, adjust = "none")
$emmeans
```

trt	emmean	SE	df	lower.CL	upper.CL
acetic	3.768	0.02802677	16	3.708586	3.827414
butyric	3.660	0.02802677	16	3.600586	3.719414
control	4.282	0.02802677	16	4.222586	4.341414
propion	3.728	0.02802677	16	3.668586	3.787414

“Simple summary” statistics vs emmeans()

For one-way ANOVA the simple means and emmeans will be the same, even if the sample sizes are not balanced across groups.

However, there will be a difference between the “simple” SE and the model based SE:

“Simple” SE for group $i = s_i / \sqrt{n_i}$

Model based SE for group $i = s_W / \sqrt{n_i}$

The difference is that the simple SE allows standard deviation to be estimated separately for each group.

While the model based SE uses a pooled estimate (s_W). This makes sense because the ANOVA model assumes equal variance.

Note that if sample sizes are equal, the model based SE will be the same for all groups. This is true for the Rice example.

Rice Example: Pairwise Comparisons using emmeans()

```
> library(emmeans)
> LMFit <- lm(weight ~ trt, data = rice)
> emout <- emmeans(LMFit, pairwise ~ trt, adjust = "none")
> emout
```

contrast	estimate	SE	df	t.ratio	p.value
acetic - butyric	0.108	0.03963584	16	2.725	0.0150
acetic - control	-0.514	0.03963584	16	-12.968	<.0001
acetic - propion	0.040	0.03963584	16	1.009	0.3279
butyric - control	-0.622	0.03963584	16	-15.693	<.0001
butyric - propion	-0.068	0.03963584	16	-1.716	0.1055
control - propion	0.554	0.03963584	16	13.977	<.0001

```
> CLD(emout$emmeans, adjust = "none")
```

trt	emmean	SE	df	lower.CL	upper.CL	.group
butyric	3.660	0.02802677	16	3.600586	3.719414	1
propion	3.728	0.02802677	16	3.668586	3.787414	12
acetic	3.768	0.02802677	16	3.708586	3.827414	2
control	4.282	0.02802677	16	4.222586	4.341414	3

4. Checking the ANOVA Assumptions

1. Random sample, independent observations
2. Residuals are normally distributed: $\varepsilon_{ij} \sim N(0, \sigma^2)$
QQ plot of residuals
Test of normality for residuals
3. Equality (Homogeneity) of variances: $\text{Var}(\varepsilon_{ij}) = \sigma^2$
Plot of residuals vs fitted (predicted) values
Levene's test

We will be using the **residuals** to check assumptions 2 and 3.

Residuals are calculated as $e_{ij} = y_{ij} - \bar{y}_i$.

Fitted/predicted values $\hat{\mu}_i = \bar{y}_i$ are the sample means.

Note: Both diagnostic plots (and more) can be generated in R by applying the `plot()` function to a `lm` or `aov` object.

Checking for Normality of Residuals:

1. A Q-Q plot of the residuals is a useful graphical tool for checking normality. The Q-Q plot is generally more useful than a histogram of the residuals for this purpose.
2. Tests of normality can be used (ex: Shapiro-Wilks test).

Important Note: When you check the data for normality, check the residuals, not the observations themselves. The errors (i.e., deviations from group means) are assumed to follow a common normal distribution but the observations themselves may come from *different* normal distributions. So, when the means are very different, the combined data will often look non-normal, even when the residuals are close to normal.

Checking for Equality of Variance (Homogeneity of Variance):

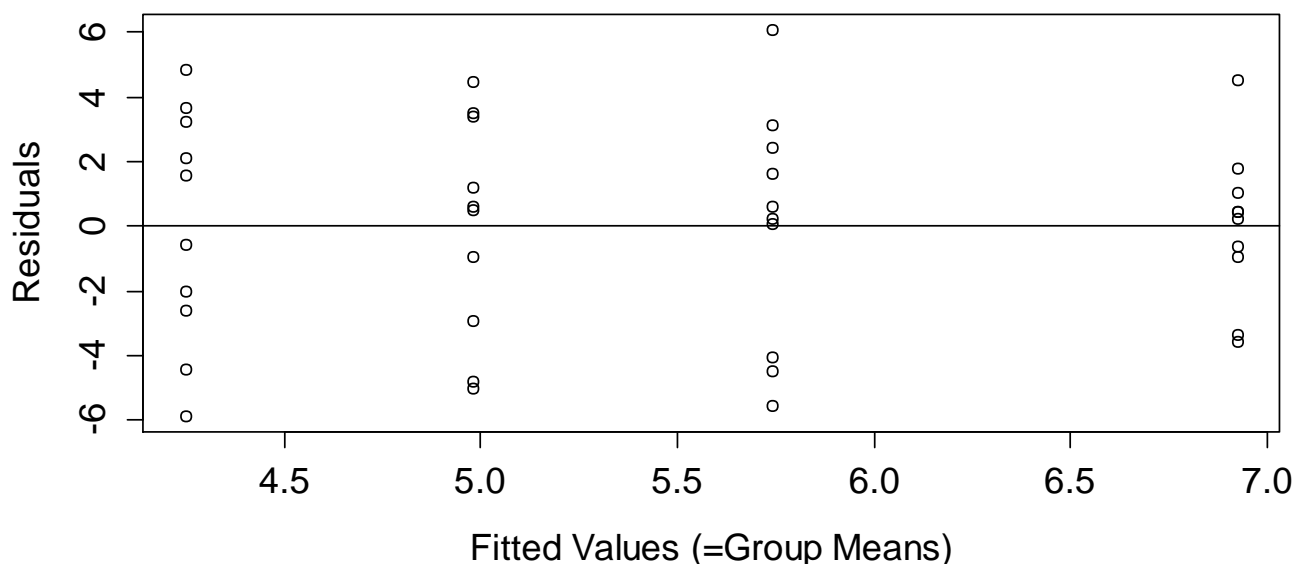
Check plot of residuals versus predicted values (or fitted values). Plots are usually more helpful than the formal tests.

The predicted/fitted value in the i^{th} group is: $\bar{y}_i = \hat{\mu}_i$

The residual for the i,j obs. = $y_{ij} - \bar{y}_i$.

Primarily interested in checking for equal “scatter” (representative of equal variance), but sometimes we can also detect “skew” and/or outliers in the residual diagnostic plot.

Megaphone shape is common when assumption of equal variances is NOT met. See slide 25.



Levene's Test for homogeneity of variances

$$H_0 : \sigma_1^2 = \sigma_2^2 = \dots = \sigma_t^2$$

H_A : one or more is different

Levene's test is a generally satisfactory test (even in the absence of normality) and can be done in R.

In R, use `leveneTest()` from the `car` package.

In Rcmdr, choose Statistics -> Variances -> Levene's test.

See “**One Way ANOVA**” example.

Test is based on ANOVA on the absolute differences from the group means:

$$Z_{ij} = |y_{ij} - \bar{y}_i|$$

The default in R is to use absolute deviations from the median (center=“median”) sometimes called Brown-Forsythe method, this method is considered to be more robust.

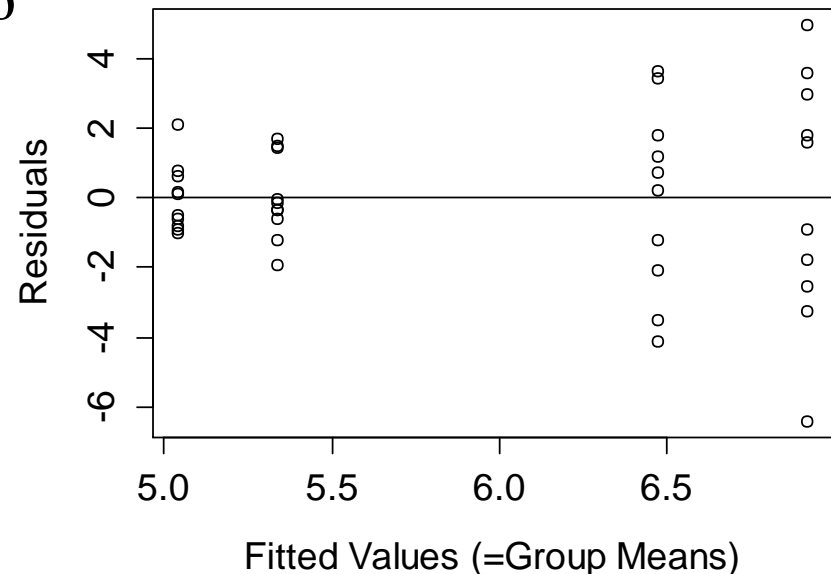
To get the classic Levene's test, use center=“mean”.

Comments about ANOVA assumptions

1. The ANOVA F-test is “robust” to the assumptions of normality and equal variances. This is especially true when group sizes are equal.
2. Pairwise comparisons of means is not accurate if the variances are not close to equal.

Example: $s_w = \sqrt{\text{MSResid}} = 1.66$

Group	n	mean	sd
1	10	5.041818	0.9711052
2	10	5.334920	1.2085462
3	10	6.470208	2.6851316
4	10	6.918262	3.5500434



The “pooled” standard deviation overestimates the standard deviation for groups 1 and 2, and underestimates the standard deviation for groups 3 and 4.

5. The Kruskal-Wallis Test

The Kruskal-Wallis test is a non-parametric alternative to the one-way ANOVA F-test.

When the normality of the errors is in doubt, a rank based test is a reasonable alternative. All observations are sorted by size, ranks assigned (n_T for largest, 1 for smallest). The sum of the ranks for the i^{th} group is T_i .

H_0 : the t groups have identical distributions

H_A : not all of the distribution are the same

$$\text{T.S.: } H = \frac{12}{n_T(n_T + 1)} \left(\sum_i \frac{T_i^2}{n_i} \right) - 3(n_T + 1)$$

R.R : Reject if H exceeds χ_α^2 with $\text{df} = t - 1$

(See O & L for modification to the formula when there are ties.)

Notes on Kruskal-Wallis Test

1. The Kruskal-Wallis test is an extension of the Wilcoxon Rank Sum test for more than two groups.
2. In R, use `kruskal.test()`.
In Rcmdr, choose Statistics -> Nonparametric tests -> Kruskal-Wallis test.
3. It assumes identical shaped distributions (which implies homogeneous variances) with possible location differences, but it is often used even when the variances are not equal, and seems to perform acceptably.
4. Often interpreted as a test for differences in medians.
5. Kruskal-Wallis test is less powerful than the ANOVA F-test if ANOVA assumptions are met.
6. Pairwise comparisons can be done using Dunn's test. Multiple testing adjustments can be applied.

6. Transformations for One Way ANOVA

If ANOVA assumptions are not satisfied on the original scale (Y), then you can consider using a transformed response variable.

Transforming the response makes the model harder to interpret, so we don't want to do it unless it's really necessary.

The literature may have examples of transformations that are common in a particular field. **But the way to decide what transformation is appropriate is by fitting the model with the transformed response and checking the diagnostic plots.**

See “Transformations for One Way ANOVA”

Common transformations include:

- **Square root:** $YT = \sqrt{Y}$ or $YT = (Y)^{0.5}$ (in R)

Example: Count data (calls to switchboard, insects in trap, etc)

- **Power:** $YT = 1/Y$ or $YT = Y^{-2}$ (in R)

- **Log:** $YT = \log(Y)$ or $YT = \log_{10}(Y)$ (in R)

Examples: Chemical concentrations or hormone levels

Important Note: Watch out for $y=0$ values which will be undefined after log transformation! If this is not properly accounted for, then these values will be treated as “missing”. A simple, common solution is to add a small positive constant before log transformation. You might use $y_T = \log(y+1)$ when the y 's are 0 to 20, and use $y_T = \log(y+0.01)$ when the y 's are 0 to 0.25. These are just suggestions!

- The use of transformations like $YT = Y^{(0.75)}$ is rare. Transformations like $YT = Y^{(0.63)}$ are seldom used.

The **Box-Cox** approach is a systematic method for choosing a transformation. This approach should only be used for $y > 0$!

The general form of the Box-Cox transformation is:

$$g(y_i) = (y_i^\lambda - 1)/\lambda$$

where λ is a constant to be determined from the data.

If $\lambda = 1$, then no transformation is needed.

If $\lambda = 2$, then model Y^2 (instead of Y).

If $\lambda = -1$, then model $Y^{-1} = 1/Y$ (instead of Y).

If $\lambda = 0.5$, then model $Y^{0.5} = \text{sqrt}(Y)$ (instead of Y).

If $\lambda = 0$, then model $\log(Y)$ (instead of Y)

We will use the `boxcox()` function from the MASS package to create a Box-Cox plot to choose λ .

Interpretation after transformation

The means of the transformed variables are not the same as the means of the original variables. However, if the means are significantly different based on the analysis in the transformed scale, it is reasonable to conclude that the means in the original scale are also significantly different.

What should a researcher report when it was necessary to do the ANOVA using a transformed scale?

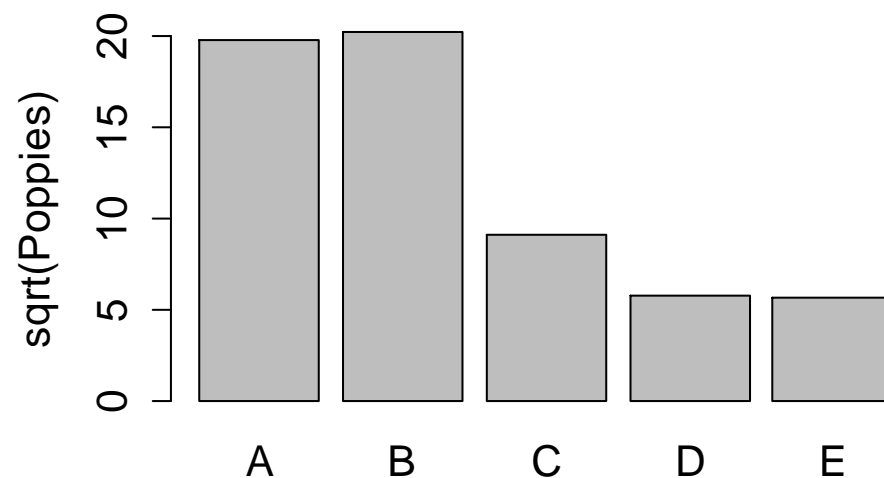
Three options:

1. Present the means on the transformed scale.
2. Present the means on the original scale, but note that analysis was done on transformed data.
3. Back transform to the original scale.

Option 1: Present the means (or graphs) and the comparison of means in the transformed scale. This is a reasonable option when the scale is common in that field of study (e.g. log in chemistry, sqrt in radiological sciences.)

Example: Poppy plants in oats (see R example).

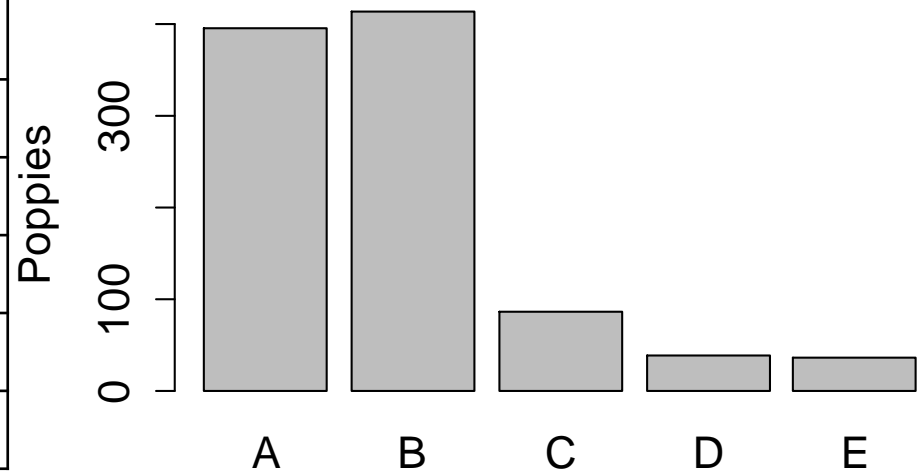
Trt	Orig Scale	Sqrt Scale	Back Transformed
A	394.75	19.83	393.09
B	413.00	20.23	409.07
C	86.75	9.08	82.47
D	37.75	5.75	33.11
E	35.25	5.64	31.89



Option 2: Present the means (or graphs) in the original scale, but with the comparison of means based on the transformed scale. Describe what you have done in your methods section and in a footnote to the table or graph of means.

A LSD value based on the transformed scale cannot be used on means based on the original scale. Also, means that are farther apart will not necessarily be more significantly different than means that are closer together.

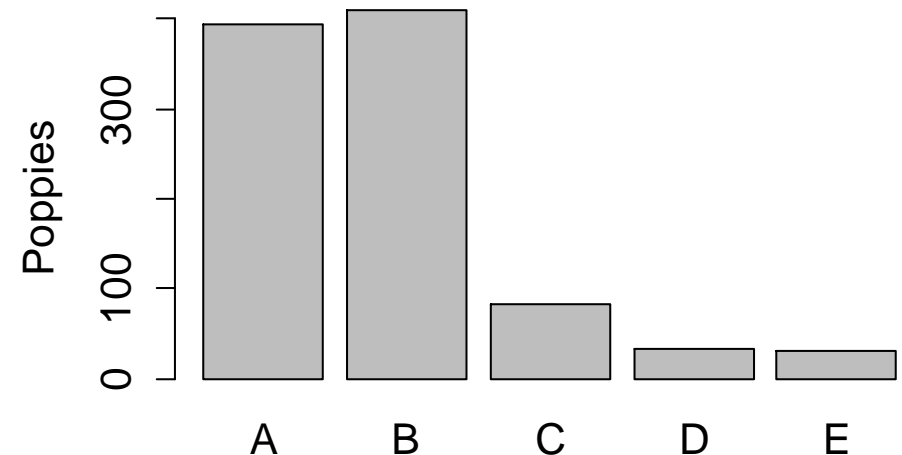
Trt	Orig Scale	Sqrt Scale	Back Transformed
A	394.75	19.83	393.09
B	413.00	20.23	409.07
C	86.75	9.08	82.47
D	37.75	5.75	33.11
E	35.25	5.64	31.89



Option 3: Compute the means using the transformed variable, but “back-transform” the means before presentation. To back-transform a square root transformed variable, square the mean.

Backtransformed means can be substantially lower than the means in the original scale, particularly when the transformation is log.

Trt	Orig Scale	Sqrt Scale	Back Transformed
A	394.75	19.83	393.09
B	413.00	20.23	409.07
C	86.75	9.08	82.47
D	37.75	5.75	33.11
E	35.25	5.64	31.89



Comments about Interpretation after log Transformation:

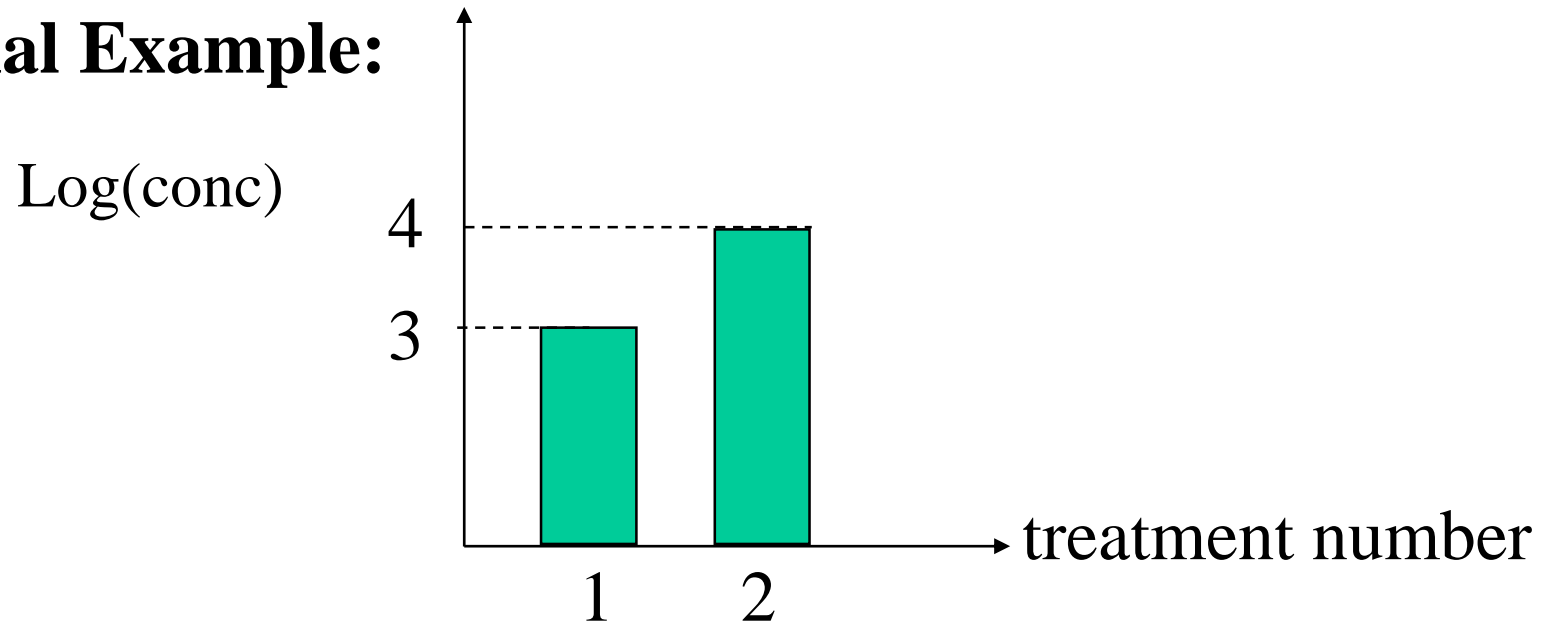
There can be interpretational advantages to doing an analysis in the log scale. Because $\log(x/y) = \log(x) - \log(y)$, differences between means in the log analysis can be interpreted as ratios in the original scale.

In bioinformatics, it is common to use a \log_2 transformation to satisfy ANOVA assumptions, but also because the differences can be interpreted as \log_2 fold change (FC) values.

$$\log_2(2/1) = +1, \log_2(1/2) = -1$$

When using a log transformation, it is common to see people back transform the difference to a “ratio” scale after analysis.

A Lognormal Example:



$$\text{Est. trt diff (log scale)} = \bar{x}_1 - \bar{x}_2 = 3 - 4 = -1$$

$$\text{Ratio of trt means (orig. scale)} = \frac{\text{trt 1 mean}}{\text{trt 2 mean}} = e^{\mu_1 - \mu_2}$$

$$e^{\bar{x}_1 - \bar{x}_2} = e^{3-4} = e^{-1} = 1 / 2.718 = 0.368 \approx 37 \%$$

Trt1 concentration is 37% Trt2 concentration! (e^{LCL}, e^{UCL})

A C.I. for this % is given by:

(where (LCL,UCL) is a C.I. for the difference in the means of the log data)

7. Sample Size and Power in the ANOVA F-test

As with all of the other sample size calculations, we need:

- 1) A conjecture about the within-group standard deviation σ .
- 2) Identification of the true alternative that we want to detect:
conjectures for $\mu_1, \mu_2, \dots, \mu_t$.

Power is a function of the “noncentrality” parameter for the F-distribution, given the alternative:

$$\lambda = \frac{n \sum_{i=1}^t (\mu_i - \bar{\mu})^2}{\sigma^2}$$

Power increases as sample size and the differences among the true means increase, and decreases as the error standard deviation increases.

Power can be computed in R using the **pf** function:

```
fcrit=qf(0.95, dfn, dfd)
power = 1 - pf(fcrit, dfn, dfd, lambda)
```

Power can be computed using `power.anova.test()`

See: “**Power for the One-Way Model**” Example

Power for ANOVA F-test using Lenth

<http://homepage.stat.uiowa.edu/~rlenth/Power/>

* Choose Balanced ANOVA, then Built-in models = “One Way ANOVA”, Study the Power of = “F-tests”. (We will discuss the Differences/Contrasts option in the Ch9 notes.)

