Statisitcal Thinking in Biology Research
Chapter 1

Terry Neeman and Timothée Bonnet

November 27, 2018

Acknowledgemnts and warning

Key ideas for today

- Statistics in biology is the study of biological variation

Key ideas for today

- Statistics in biology is the study of biological variation
- Statistical ideas about biological variation inform the design of experiments

Key ideas for today

- Statistics in biology is the study of biological variation
- Statistical ideas about biological variation inform the design of experiments
- Statistical ideas about biological variation inform the analysis of experiments

Key ideas for today

- Statistics in biology is the study of biological variation
- Statistical ideas about biological variation inform the design of experiments
- Statistical ideas about biological variation inform the analysis of experiments
- Statistical thinking is an essential component of scientific thinking

A bit of history of statistical methods
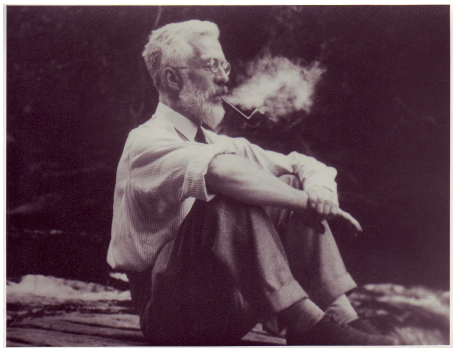
R.A. Fisher: 1890-1962

Statistical Principles for Research Workers (1925)

A bit of history of statistical methods
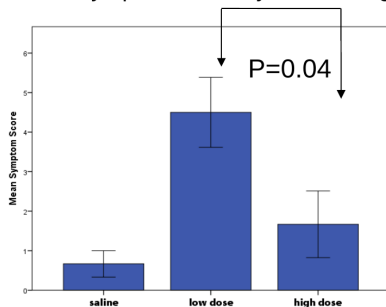
R.A. Fisher: 1890-1962

Statistical Principles for Research Workers (1925)

Message 1: A small p-value is not always evidence of a treatment effect

Mean symptom score by treatment group

Vaccine challenge experiment:

- 6 mice/group (saline/low dose/high dose)
- All mice challenged with Shigella
- Followed for 14 days
- Outcome: Symptom score average Days 2 - 8

One-way ANOVA (post-hoc Bonferroni) p=0.04

Message 1: A small p-value is not always evidence of a treatment effect
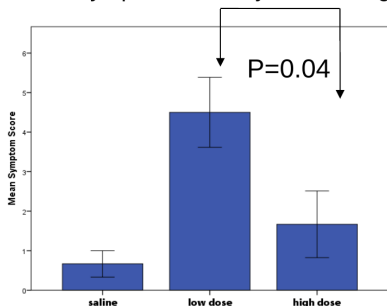
Mean symptom score by treatment group

Vaccine challenge experiment:

- 6 mice/group (saline/low dose/high dose)
- All mice challenged with Shigella
- Followed for 14 days
- Outcome: Symptom score average Days 2 - 8

One-way ANOVA (post-hoc Bonferroni) p=0.04

Do you think the vaccine works? What is strange?

Message 1: A small p-value is not always evidence of a treatment effect

Message 1: A small p-value is not always evidence of a treatment effect

Experimental design

The observed difference in outcome could be the result of:

- Cage effects
- Mouse strain effects

These effects are CONFOUNDED with treatment effect

Cage 2:
Low Dose
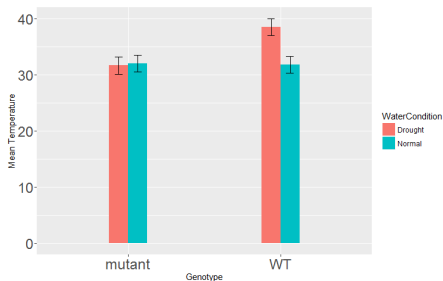
Cage 1:
saline

Cage 3:
High Dose

Message 2: p-values from simple comparisons cannot tell us when differences are "different"

Message 2: p-values from simple comparisons cannot tell us when differences are "different"

Are temperature mechanisms modified in a genetically modified tomato plant?

- Genotypes: WT/mutant
- Water condition: Normal/Drought
- Leaf temperature measured

Comparisons made using t-tests

Evidence of difference + No evidence of difference $\neq$ Evidence that differences are different.

Message 3: Interpreting experimental results needs more than t-tests

Message 3: Interpreting experimental results needs more than t-tests

Research question: Are mice susceptible to obesity when exposed to a high fat diet?
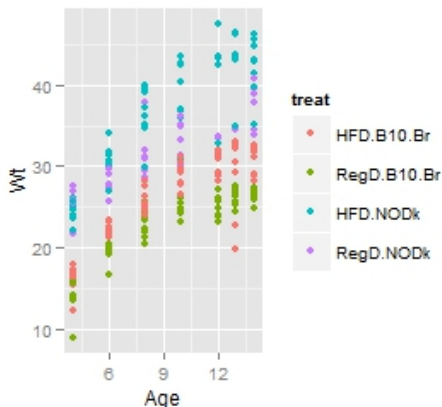
Experimental set-up:

- 37 mice: 16 NODk /21 WT
- Randomised to either regular or high fat diet
- Monitored for 14 weeks
- Outcome measure: Body weight (g)
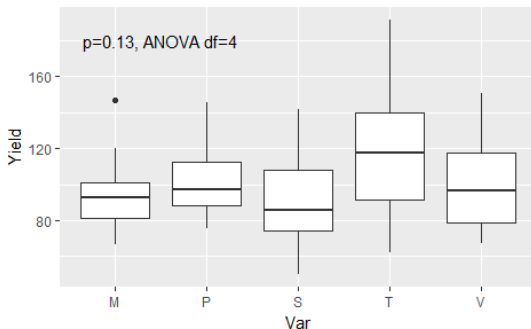- Experimental factors: Diet (2), Strain (2), Time (8)

Message 4: Knowing how to combine information across subgroups can improve inference

Message 4: Knowing how to combine information across subgroups can improve inference

Comparing yield in five barley varieties (1930s)
Experimental factors: 5 varieties of barley, 6 locations, 2 time points.
Outcome measure: yield

Acknowledgements: MASS R-package

Message 4: Knowing how to combine information across subgroups can improve inference

Comparing yield in five barley varieties (1930s)
Experimental factors: 5 varieties of barley, 6 locations, 2 time points.
Outcome measure: yield

Controlling for other sources of variation:

- Controlling for year = comparing yield WITHIN years and combining these

Acknowledgements: MASS R-package

Message 4: Knowing how to combine information across subgroups can improve inference

Comparing yield in five barley varieties (1930s)
Experimental factors: 5 varieties of barley, 6 locations, 2 time points.
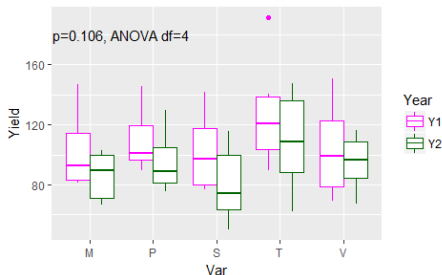Outcome measure: yield

Controlling for other sources of variation:

- Control for year = compare yield WITHIN years and combine these

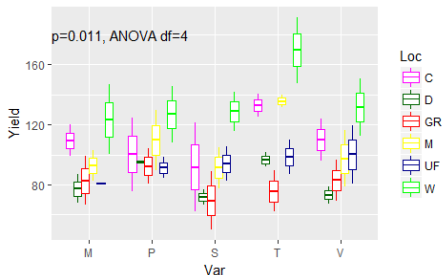- Control for location = compare yield WITHIN locations and combine these

Acknowledgements: MASS R-package

Message 4: Knowing how to combine information across subgroups can improve inference
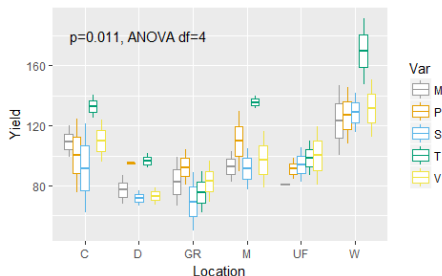

Controlling for other sources of variation:

- Control for year = compare yield WITHIN years and combine these

- Control for location = compare yield WITHIN locations and combine these

Acknowledgements: MASS R-package

Message 5: Knowing what factors contribute to the variation in outcome helps design experiments and analyses

Message 5: Knowing what factors contribute to the variation in outcome helps design experiments and analyses

Research question: How does cold duration impact upon germination in alpine plant A. glacialis?

Experimental set-up:

- Seed collections from alpine region in Australia
- 3 Regions- low/high altitude
- 4 sets of Petri dishes
- 4 cabinet shelves
- Response - % germinated

What other factors are important to consider when comparing cold duration?

Summary

1. A small p-value is not always evidence of a treatment effect. **Good experimental design matters.**

Summary

1. A small p-value is not always evidence of a treatment effect. **Good experimental design matters.**

2. p-values from simple comparisons cannot tell us when differences are "different". **For each question / comparison, a specific test**

Summary

1. A small p-value is not always evidence of a treatment effect. **Good experimental design matters.**

2. p-values from simple comparisons cannot tell us when differences are "different". **For each question / comparison, a specific test**

3. Interpreting experimental results needs more than t-tests. **Need a statistical model of the experiment, matching scientific question.**

Summary

1. A small p-value is not always evidence of a treatment effect. **Good experimental design matters.**

2. p-values from simple comparisons cannot tell us when differences are "different". **For each question / comparison, a specific test**

3. Interpreting experimental results needs more than t-tests. **Need a statistical model of the experiment, matching scientific question.**

4. Combining information across subgroups can improve inference. **A statistical model enables accumulation of evidence across experiments.**

Summary

1. A small p-value is not always evidence of a treatment effect. **Good experimental design matters.**

2. p-values from simple comparisons cannot tell us when differences are "different". **For each question / comparison, a specific test**

3. Interpreting experimental results needs more than t-tests. **Need a statistical model of the experiment, matching scientific question.**

4. Combining information across subgroups can improve inference. **A statistical model enables accumulation of evidence across experiments.**

5. Knowing what factors contribute to the variation in outcome matters. **A statistical model allows one to incorporate effect of other factors in the analysis.**

Introduction to Statistical Modelling

- What is a statistical model?

- Modelling outcomes:
    - a summary of data
    - a prediction model
    - an explanatory model

- Model – may take many different functional forms

- Model – a conceptualization of the experiment

Introduction to Statistical Modelling

- What is a statistical model?

- Modelling outcomes:
    - a summary of data
    - a prediction model
    - an explanatory model

- Model – may take many different functional forms

- Model – a conceptualization of the experiment

ALWAYS BEGIN WITH A RESEARCH QUESTION

Key components of a statistical model of an experiment

- Outcome measure
  - ▶ Response variable
  - ▶ Measure of interest

- Experimental factors
  - ▶ Conditions that can be manipulated
  - ▶ Conditions of interest (e.g. genotype, gender)
  - ▶ Main questions: do the conditions impact upon the outcome measure?

- Blocking factors
  - ▶ Conditions (not of interest) that may impact upon the outcome measure
  - ▶ Sources of variation in the experiment that need to be controlled for
  - ▶ Clustering of experimental units

ALWAYS BEGIN WITH A RESEARCH QUESTION

Key Objectives of a statistical model of an experiment

- To compare the mean response of an organism/system to a set of different experimental conditions.
  - Obtain estimate of "Treatment effect"
  - Is this "effect" different in subgroups of interest?

- What are the most important factors influencing the mean response?

- Subsidiary question: how can we design our experiment in future to more efficiently test our hypotheses?

Example 1: Does dark respiration differ between C3 and C4 plants?
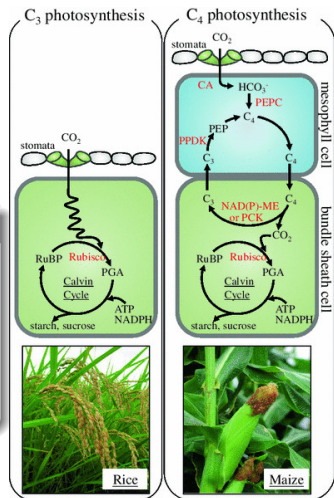
Outcome measure: dark respiration
Experimental factor: Plant type (C4/C3)
Data: 6 plants each of C4, C3

Can calculate

- Observed overall mean
- Observed mean C3 plants
- Observed mean C4 plants
- Variation around each mean

Example 1: Does dark respiration differ between C3 and C4 plants?

Can calculate

- Observed overall mean
- Observed mean C3 plants
- Observed mean C4 plants
- Variation around each mean

Statistical model

Respiration = Mean for C3 + Difference C4-C3 * (is C4?) + Noise

Example 1: Does dark respiration differ between C3 and C4 plants?

Can calculate

- Observed overall mean
- Observed mean C3 plants
- Observed mean C4 plants
- Variation around each mean

Statistical model

Respiration = Mean for C3 + Difference C4-C3 * (is C4?) + Noise

$response = A + D \times predictor + \epsilon$

A and D are the model PARAMETERS.

We want to infer whether D is different from 0

Example 1: Does dark respiration differ between C3 and C4 plants?

$response = A + D \times predictor + \epsilon$

Can we separate the signal D from the noise ϵ ?

Example 1: Does dark respiration differ between C3 and C4 plants?

$response = A + D \times predictor + \epsilon$

Can we separate the signal D from the noise ϵ ?

T-test

- Outcome is a continuous variable
- Experimental factor is one factor with 2 conditions
- No blocking factor / corrections

Example 1: Does dark respiration differ between C3 and C4 plants?
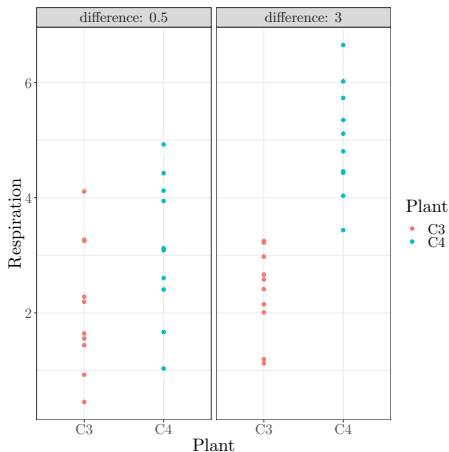
$response = A + D \times predictor + \epsilon$

Can we separate the signal D from the noise ϵ ?

T-test

- Outcome is a continuous variable
- Experimental factor is one factor with 2 conditions
- No blocking factor / corrections

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

When can we know whether $D \neq 0$?

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

Is it easier when the true difference is 0.5 or when it is 3 ?

When can we know whether $D \neq 0$?

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

Is it easier when the true difference is 0.5 or when it is 3 ?

1. Large true difference between the means

When can we know whether $D \neq 0$?

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

Is it easier when sample size is 4 or when it is 100?

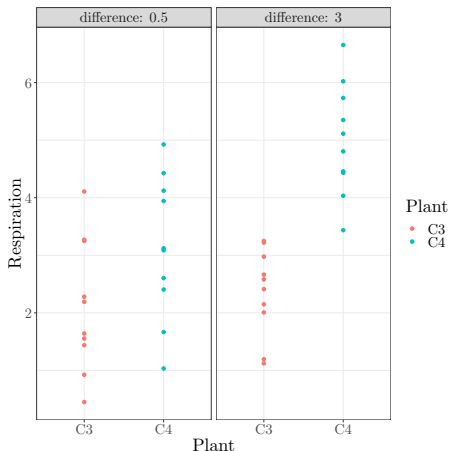# When can we know whether $D \neq 0$?

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

Is it easier when sample size is 4 or when it is 100?

1. Large true difference between the means

2. Large sample size
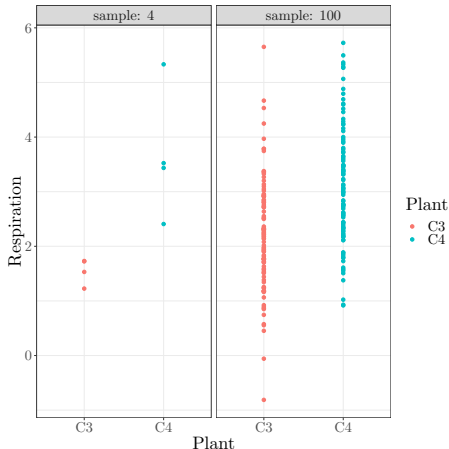
When can we know whether $D \neq 0$?

$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

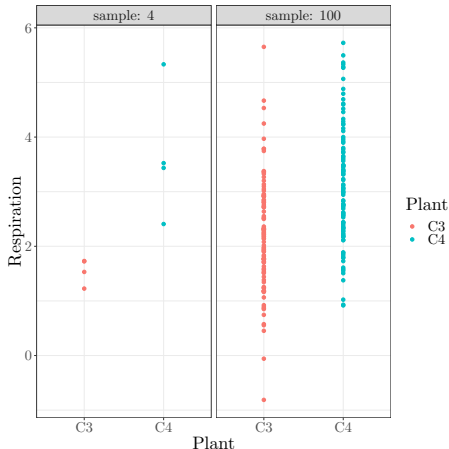Is it easier when unexplained variation is 1 or when it is 3?

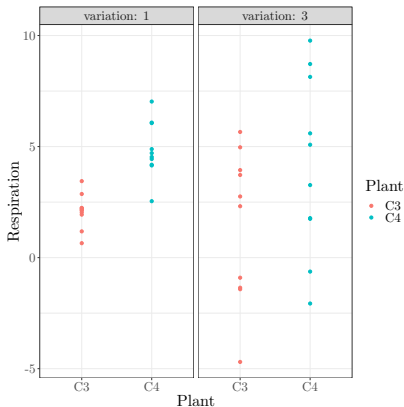# When can we know whether $D \neq 0$?
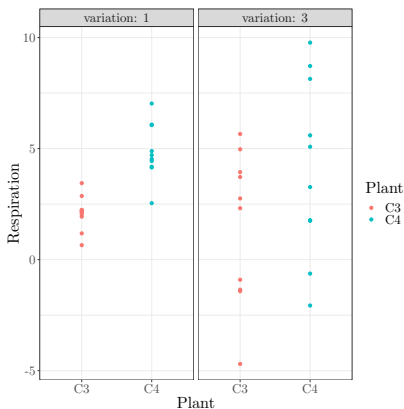
$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

Is it easier when unexplained variation is 1 or when it is 3?

What makes t large:

1. Large true difference between the means

2. Large sample size

3. Small unexplained variation

When can we know whether $D \neq 0$?

Expected t-values when $D = 0$
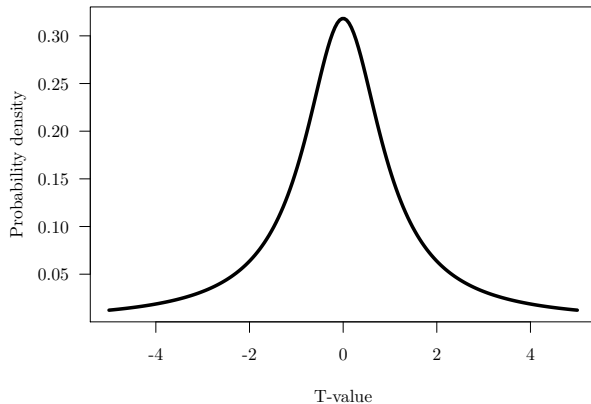
When can we know whether $D \neq 0$?

p-value: probability (area under curve) of getting a value as extreme as what you observed, when D=0

Expected t-values when $D = 0$
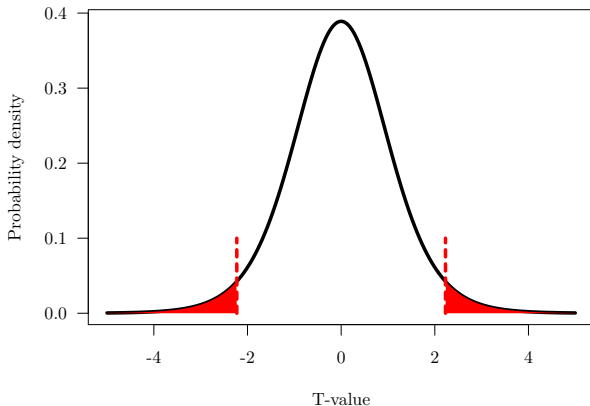
But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one. . . looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.
- Are there more Halloween than Fruit candies in that pack?

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.
- Are there more Halloween than Fruit candies in that pack?
- You decide to use statistics to find out

How to?

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.
- Are there more Halloween than Fruit candies in that pack?
- You decide to use statistics to find out

How to?

- Draw 5 candies out of the pack

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.
- Are there more Halloween than Fruit candies in that pack?
- You decide to use statistics to find out

How to?

- Draw 5 candies out of the pack
- Write down how many Halloween candies

But really, what is a p-value?

Candy practical

- You got a pack of 20 candies with a mix of Halloween and Fruit candies
- You pick one, it's a Halloween one... looks quite disgusting. You put in back
- You pick a second one. Again a disgusting Halloween candy! You put it back
- And so on, until 5 candies. You wonder if you have been cheated.
- Are there more Halloween than Fruit candies in that pack?
- You decide to use statistics to find out

How to?

- Draw 5 candies out of the pack
- Write down how many Halloween candies
- How often is it 5?

But really, what is a p-value?

- Draw 5 candies out of the pack
- Write down how many Halloween candies
- How often is it 5?

But really, what is a p-value?

- Draw 5 candies out of the pack
- Write down how many Halloween candies
- How often is it 5?
- Estimate the p-value for the test "candies have same frequency"

But really, what is a p-value?

- Draw 5 candies out of the pack

- Write down how many Halloween candies

- How often is it 5?

- Estimate the p-value for the test "candies have same frequency"

- Redo the experiment in R, using random sampling (rbinom)

But really, what is a p-value?

- Draw 5 candies out of the pack

- Write down how many Halloween candies

- How often is it 5?

- Estimate the p-value for the test "candies have same frequency"

- Redo the experiment in R, using random sampling (rbinom)

- What is the correct null-distribution?

Back to C3/C4 plants. Analyse real data in R

1. Set working directory (setwd('' / '')) or create a R-project
2. Load and check data

```
resp <- read.csv("d_respiration.csv")
str(resp)
View(resp)
```

3. Visualize data

```
library(ggplot2)
ggplot(resp,aes(Plant_type,rrarea,colour=Plant_type))+
    geom_point()+facet_wrap(~Variation)
```

Fit a t-test in R: `t.test()`

Subset data by Variation (High and Low)

```
resp_H <- subset(resp,Variation == "High")
resp_L <- subset(resp,Variation == "Low")
```

Fit a t-test in R: `t.test()`

Subset data by Variation (High and Low)

```
resp_H <- subset(resp,Variation == "High")
resp_L <- subset(resp,Variation == "Low")
```

Compare C3 and C4 plants in "High Variation" subset

```
t.test(rrarea~Plant_type, data=resp_H, var.equal=TRUE)
```

Fit a t-test in R: `t.test()`

Subset data by Variation (High and Low)

```
resp_H <- subset(resp,Variation == "High")
resp_L <- subset(resp,Variation == "Low")
```

Compare C3 and C4 plants in "High Variation" subset

```
t.test(rrarea~Plant_type, data=resp_H, var.equal=TRUE)
```

```
Two Sample t-test
data:  rrarea by Plant_type
t = -0.93776, df = 10, p-value = 0.3705
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.7619349  0.7181446
sample estimates:
mean in group C3 mean in group C4
        2.720021         3.241916
```

Fit a t-test in R: `t.test()`

Subset data by Variation (High and Low)

```
resp_H <- subset(resp,Variation == "High")
resp_L <- subset(resp,Variation == "Low")
```

Compare C3 and C4 plants in "High Variation" subset

```
t.test(rrarea~Plant_type, data=resp_H, var.equal=TRUE)
```

```
Two Sample t-test
data:  rrarea by Plant_type
t = -0.93776, df = 10, p-value = 0.3705
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.7619349  0.7181446
sample estimates:
mean in group C3 mean in group C4
        2.720021         3.241916
```

Fit a t-test in R: `t.test()`

Compare C3 and C4 plants in "Low Variation" subset

```
t.test(rrarea~Plant_type, data=resp_L, var.equal=TRUE)
```

Fit an anova in R: aov()

```
aov1 <- aov(rrarea~Plant_type, data=resp_H)
summary(aov1)
```

Fit an anova in R: aov()

```
aov1 <- aov(rrarea~Plant_type, data=resp_H)
summary(aov1)
```

```
           Df Sum Sq Mean Sq F value Pr(>F)
Plant_type  1  0.817  0.8171   0.879   0.37
Residuals  10  9.292  0.9292
```

Fit an anova in R: `aov()`

```
aov1 <- aov(rrarea~Plant_type, data=resp_H)
summary(aov1)
```

```
            Df Sum Sq Mean Sq F value Pr(>F)
Plant_type   1  0.817  0.8171   0.879   0.37
Residuals   10  9.292  0.9292
```

$response = A + D \times predictor + \epsilon$

Fit a linear model in R: `lm()`

```
lm1<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lm1)
```

Fit a linear model in R: `lm()`

```
lm1<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lm1)
```

```
lm(formula = rrarea ~ Plant_type, data = resp_H)

Residuals:
    Min      1Q  Median      3Q     Max
-1.7380 -0.4201 -0.1437  0.6706  1.6754

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.7200     0.3935   6.912 4.13e-05 ***
Plant_typeC4  0.5219     0.5565   0.938     0.37
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9639 on 10 degrees of freedom
Multiple R-squared:  0.08083,	Adjusted R-squared:  -0.01109
F-statistic: 0.8794 on 1 and 10 DF,  p-value: 0.3705
```

Fit a linear model in R: `lm()`

```
lm1<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lm1)
```

```
lm(formula = rrarea ~ Plant_type, data = resp_H)

Residuals:
    Min      1Q  Median      3Q     Max
-1.7380 -0.4201 -0.1437  0.6706  1.6754

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.7200     0.3935   6.912 4.13e-05 ***
Plant_typeC4  0.5219     0.5565   0.938     0.37
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9639 on 10 degrees of freedom
Multiple R-squared:  0.08083,	Adjusted R-squared:  -0.01109
F-statistic: 0.8794 on 1 and 10 DF,  p-value: 0.3705
```

Fit a linear model in R: `lm()`

```
library(emmeans)
emmeans(lm1, ~Plant_type)
```

```
 Plant_type   emmean        SE df lower.CL upper.CL
 C3         2.720021 0.3935305 10 1.843180 3.596861
 C4         3.241916 0.3935305 10 2.365076 4.118757

Confidence level used: 0.95
```

Fit a linear model in R: `lm()`

```
library(emmeans)
emmeans(lm1, ~Plant_type)
```

```
 Plant_type    emmean        SE df lower.CL upper.CL
 C3          2.720021 0.3935305 10 1.843180 3.596861
 C4          3.241916 0.3935305 10 2.365076 4.118757

Confidence level used: 0.95
```
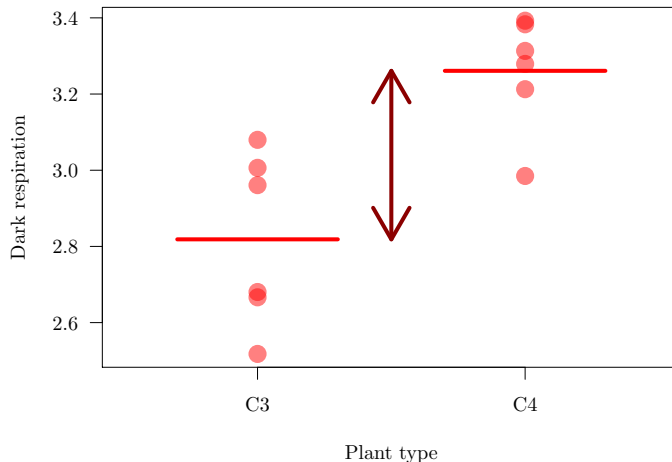
response = $A + D \times$ *predictor* $+ \epsilon$

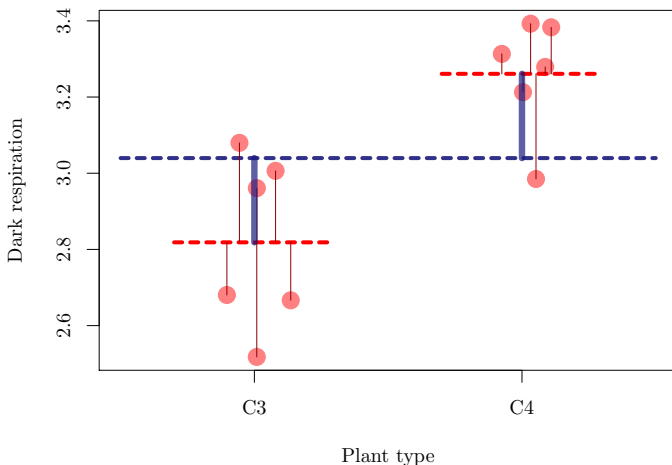# Compare the output from t.test, aov and lm

Three equivalent ways to look at data

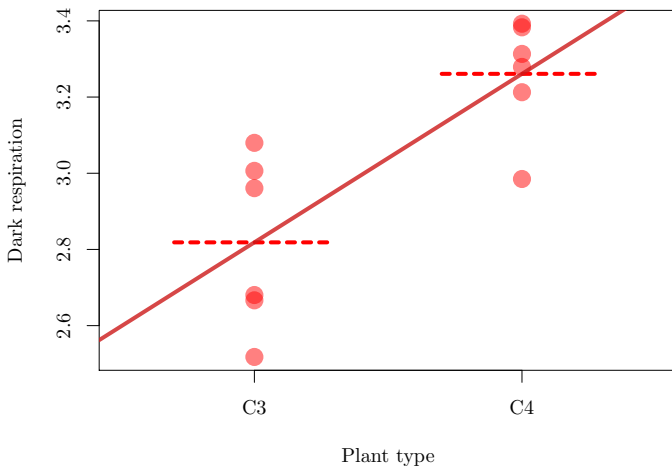T-test, focus on difference between two means

Three equivalent ways to look at data

ANOVA, focus on variation within VS. between

Three equivalent ways to look at data

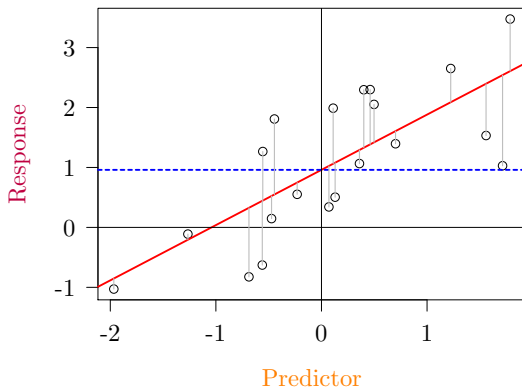Linear regression, focus on rate of change

All is one. . .

All is one...

...but lm() rules (IMH)

- t-test, ANOVA, regression and others can be mathematically equivalent
- In R, lm() and related functions can do them all...
- ...and much more!

Focus on linear models

Response = **Intercept** + **Slope** × **Predictor** + **Error**

A simple linear model

Response = **Intercept** + **Slope** × **Predictor** + Error

```
lm(response ~ 1 + predictor1 + predictor2, data=data)
```

equivalent to

```
lm(response ~ predictor1 + predictor2, data=data)
```

equivalent to

```
lm(response ~ predictor2 + predictor1, data=data)
```

- Intercept can be explicit or implicit
- Can remove intercept with ...~ 0 + ...
- Error is implicit
- Feed the option data= to keep code short, reliable and flexible
- Order of predictors do not matter

1. Scientific question

General approach

1. Scientific question
↓
2. Model and Statistical question

General approach

1. Scientific question
↓
2. Model and Statistical question ⟷ 3. Data collection

General approach

1. Scientific question
↓
2. Model and Statistical question ⟷ 3. Data collection
↓
4. Estimation
4.b Uncertainty and statistical significance

1. Scientific question
↓
2. Model and Statistical question ⟷ 3. Data collection
↓
4. Estimation
4.b Uncertainty and statistical significance

↓

5. Check assumptions, visualize results
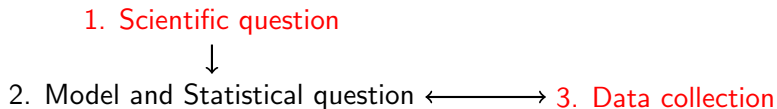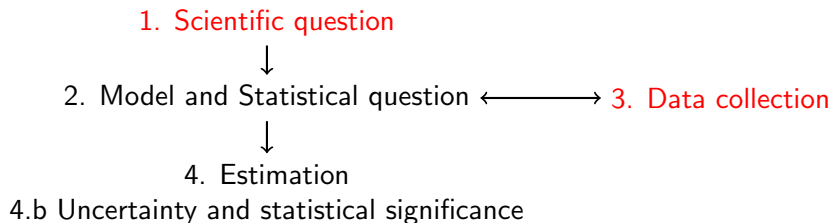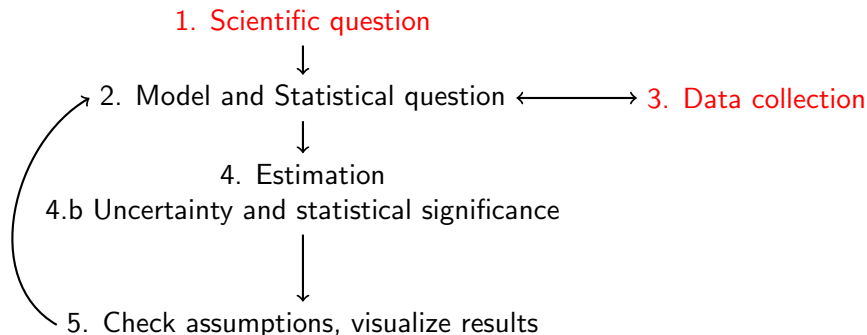
General approach
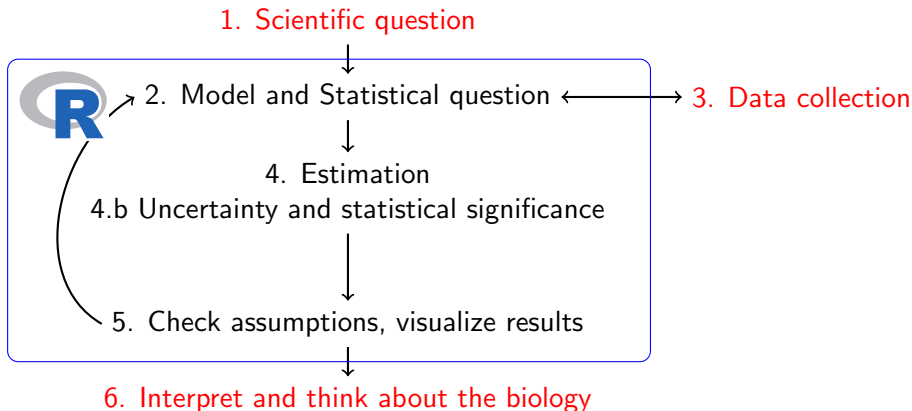
Back to C3/C4

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lmL)
```

Back to C3/C4

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lmL)
```

```
...
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.81857    0.07856  35.878 6.72e-12 ***
Plant_typeC4   0.44235    0.11110   3.982  0.00259 ** ---
...
```

Estimation:

$response = A + D \times predictor + \epsilon$

$A = ?, D = ?$

Back to C3/C4

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lmL)
```

```
...
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.81857    0.07856  35.878 6.72e-12 ***
Plant_typeC4   0.44235    0.11110   3.982  0.00259 ** ---
...
```

Estimation:

$response = A + D \times predictor + \epsilon$

$A = 2.81857$, $D = 0.44235$

Back to C3/C4

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lmL)
```

```
...
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.81857    0.07856  35.878 6.72e-12 ***
Plant_typeC4  0.44235    0.11110   3.982  0.00259 ** ---
...
```

Estimation:
response = A + D × predictor + ϵ
$A = 2.81857$, $D = 0.44235$
Uncertainty:
For D SE= 0.11110 ; p-value=0.00259

Back to C3/C4

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
summary(lmL)
```

```
...
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.81857    0.07856  35.878 6.72e-12 ***
Plant_typeC4  0.44235    0.11110   3.982  0.00259 ** ---
...
```

Estimation:
response $= A + D \times$ *predictor* $+ \epsilon$
$A = 2.81857$, $D = 0.44235$
Uncertainty:
For D SE$= 0.11110$; p-value$=0.00259$

What do we do next?

Check assumptions, visualize results

Check assumptions, visualize results

Linear model basic assumptions

- Predictor not perfectly correlated
  Risk: Model won't run, unstable convergence, or huge SE

Check assumptions, visualize results

Linear model basic assumptions

- Predictor not perfectly correlated
  Risk: Model won't run, unstable convergence, or huge SE

- Little error in predictors
  Risk: bias estimates (underestimate with Gaussian error)

Check assumptions, visualize results

Linear model basic assumptions

- Predictor not perfectly correlated
  Risk: Model won't run, unstable convergence, or huge SE

- Little error in predictors
  Risk: bias estimates (underestimate with Gaussian error)

- Gaussian error distribution
  Risk: Poor predictions

Check assumptions, visualize results

Linear model basic assumptions

- Predictor not perfectly correlated
  Risk: Model won't run, unstable convergence, or huge SE

- Little error in predictors
  Risk: bias estimates (underestimate with Gaussian error)

- Gaussian error distribution
  Risk: Poor predictions

- Homoscedasticity (constant error variance)
  Risk: Over-optimistic uncertainty, unreliable predictions

Check assumptions, visualize results

Linear model basic assumptions

- Predictor not perfectly correlated
  Risk: Model won't run, unstable convergence, or huge SE

- Little error in predictors
  Risk: bias estimates (underestimate with Gaussian error)

- Gaussian error distribution
  Risk: Poor predictions

- Homoscedasticity (constant error variance)
  Risk: Over-optimistic uncertainty, unreliable predictions

- Independence of error
  Risk: Bias and over-optimistic uncertainty

Check assumptions, visualize results

Assessing model assumptions in R:

```
lmL<-lm(rrarea ~ Plant_type, data = resp_L)
plot(lmL)
summary(lmL)
```

Check assumptions, visualize results

Visualize and report results

```
lm1.results<-summary(emmeans(lm1,~Plant_type))

ggplot(lm1.results,aes(Plant_type,emmean, fill=Plant_type))+
  geom_bar(stat="identity", width=.4)+
  geom_errorbar(aes(ymin =lm1.results$lower.CL,
  ymax = lm1.results$upper.CL), width=.2)+
  ylim(0,4)+
  geom_point(data=resp_L, aes(x=Plant_type, y=rrarea), color="red")+
  labs(y = "Dark Respiration (units)")+
  geom_text(aes(x=1.5, y=3.5, label="p=0.002"))
```

Check assumptions, visualize results
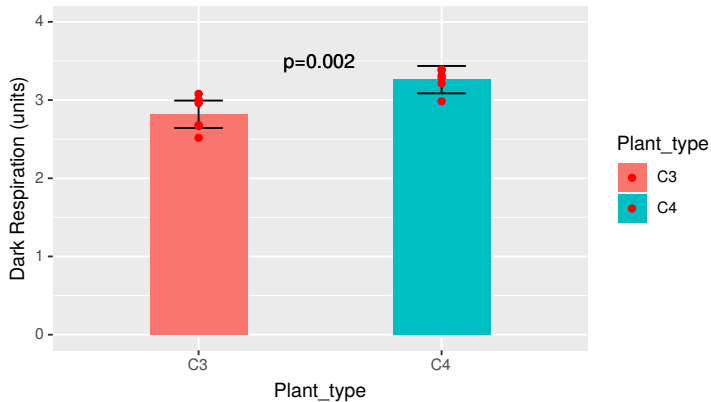
Another example

Compare wheat yields between 3 varieties

- Outcome measure: Tonnes/hectare
- Experimental factor: Variety (new/newPLUS/standard)
- Data: 6 plots/ variety

How many parameters in this model?

Results from Wheat Yield Experiment with 3 Varieties
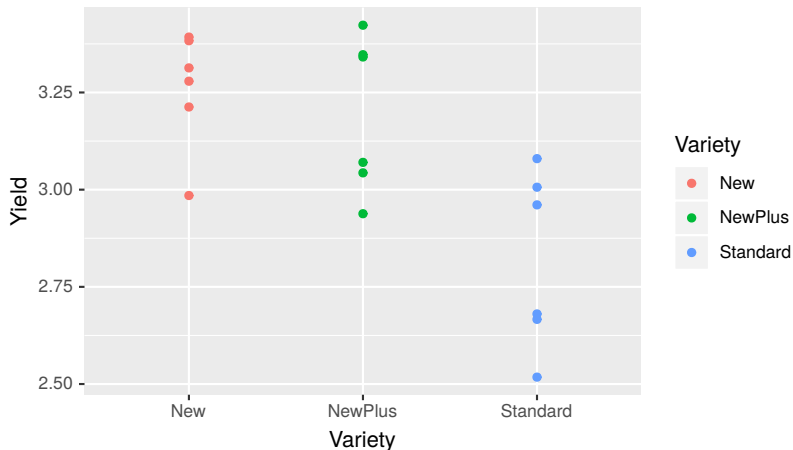
Analyse these data in R

```
wheat2<-read.csv("wheat yield PLUS.csv")
str(wheat2) #check data types for each variable
View(wheat2) #View data
ggplot(wheat2, aes(Variety, Yield, colour=Variety)) +
            geom_point()
```

Sample analysis in R: 1-way ANOVA

```
aov1=aov(Yield~Variety, data = wheat2)
summary(aov1)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	0.6820	0.3410	8.951	0.00276 **
Residuals	15	0.5714	0.0381		

ANOVA

- Compares means between TWO or MORE GROUPS
- Relies on F-statistic $= \frac{\text{Between-groups variance}}{\text{Within groups variance}} = \frac{\text{Explained}}{\text{Unexplained}}$
- One test for significance of all groups

Sample analysis in R: 1-way ANOVA

```
aov1=aov(Yield~Variety, data = wheat2)
summary(aov1)
```

```
            Df  Sum Sq   Mean Sq  F value  Pr(>F)
Variety      2  0.6820    0.3410    8.951  0.00276 **
Residuals   15  0.5714    0.0381
```

ANOVA

- Compares means between TWO or MORE GROUPS
- Relies on F-statistic $= \frac{\text{Between-groups variance}}{\text{Within groups variance}} = \frac{\text{Explained}}{\text{Unexplained}}$
- One test for significance of all groups

But which groups are different???

Using emmeans to extract group estimates

```
emmeans(aov1, ~Variety)
```

```
Variety    emmean   SE   df   lower.CL   upper.CL
 Standard   2.81    0.079  15   2.64       2.98
 New        3.26    0.079  15   3.09       3.43
 NewPlus    3.19    0.079  15   3.02       3.36

Confidence level used: 0.95
```

Using emmeans to compare groups

```
emmeans(aov1, pairwise~Variety)
```

```
$contrasts
 contrast               estimate     SE   df   t.ratio  p.value
 Standard - New           -0.44    0.112  15   -3.925   0.0036
 Standard { NewPlus       -0.37    0.112  15   -3.330   0.0120
 New - NewPlus             0.06    0.112  15    0.595   0.8248

P value adjustment: tukey method for comparing a family of 3 estimates
```

Equivalent with `lm()`

```
lm2<-lm(Yield ~ Variety, data = wheat2)
anova(lm2)
summary(lm2)
```

```
Analysis of Variance Table
 Response: Yield
          Df   Sum Sq  Mean Sq    F value   Pr(>F)
Variety    2  0.68203  0.34101    8.9513    0.002764 **
Residuals 15 0.57145  0.03810
```

```
Coefficients:
               Estimate  Std. Error   t value    Pr(>|t|)
(Intercept)     2.81857   0.07968     35.372     7.26e-16 ***
VarietyNew      0.44235   0.11269      3.925     0.00135 **
VarietyNewPlus  0.37529   0.11269      3.330     0.00457 **
```

Equivalent with `lm()`

```
emmeans(lm2, pairwise~Variety)
```

```
$emmeans
 Variety    emmean      SE      df    lower.CL  upper.CL
 Standard   2.81      0.0796    15     2.64      2.98
 New        3.26      0.0796    15     3.09      3.43
 NewPlus    3.19      0.0796    15     3.02      3.36

Confidence level used: 0.95

$contrasts
 contrast              estimate      SE      df    t.ratio  p.value
 Standard - New          -0.44     0.112    15    -3.925   0.0036
 Standard - NewPlus      -0.37     0.112    15    -3.330   0.0120
 New - NewPlus           0.067     0.112    15     0.595   0.8248

P value adjustment: tukey method for comparing a family of 3 estimates
```
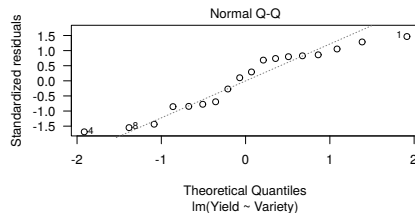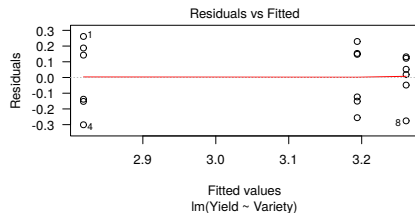
Assessing model assumptions for `lm()`

```
plot(lm2)
```

Summary of results with post hoc comparisons: