Statisitcal Thinking in Biology Research

Terry Neeman and Timothee Bonnet

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Acknowledgemnts and warning

Key ideas for today

- Statistics in biology is the study of biological variation

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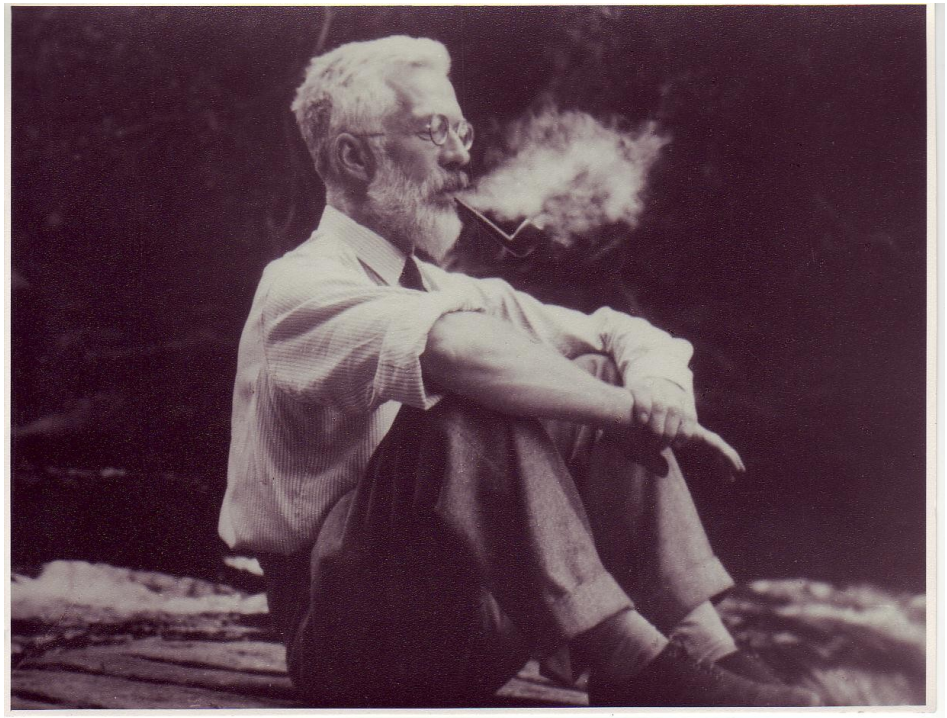
- Statistics in biology is the study of biological variation
- Statistical ideas about biological variation inform the design of experiments
- Statistical ideas about biological variation inform the analysis of experiments
- Statistical thinking is an essential component of scientific thinking

A bit of history of statistical methods

R.A. Fisher: 1890-1962



Statistical Principles for Research Workers (1925)

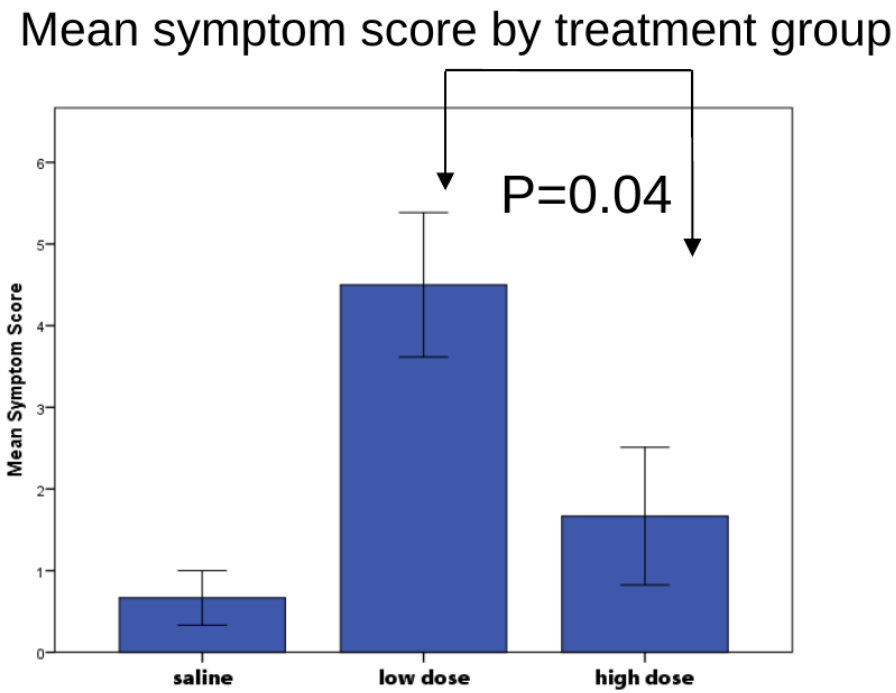
A bit of history of statistical methods

R.A. Fisher: 1890-1962

Statistical Principles for Research Workers (1925)

1. Cautionary tales from the front
2. Introduction to Statistical Modelling

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



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

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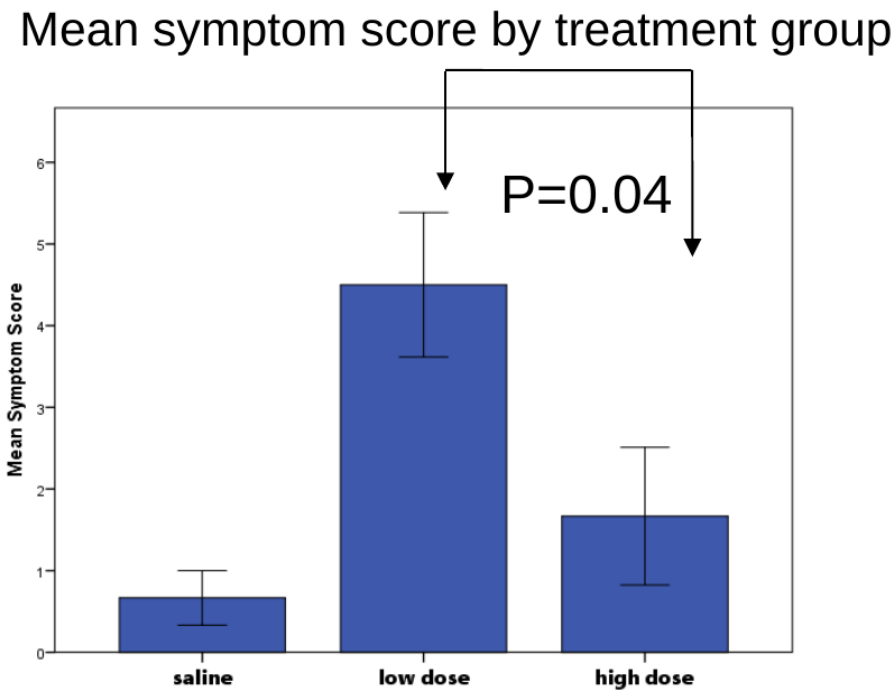

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Do you think the vaccine works? What is strange?

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

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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 1:
saline

Cage 2:
Low Dose

Cage 3:
High Dose

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

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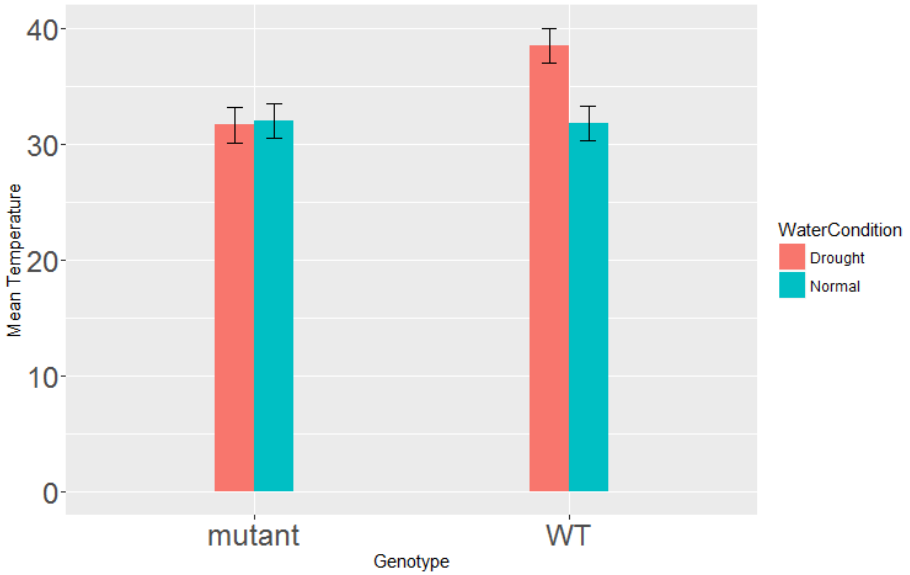

Are temperature mechanisms modified in a genetically modified tomato plant?

- 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

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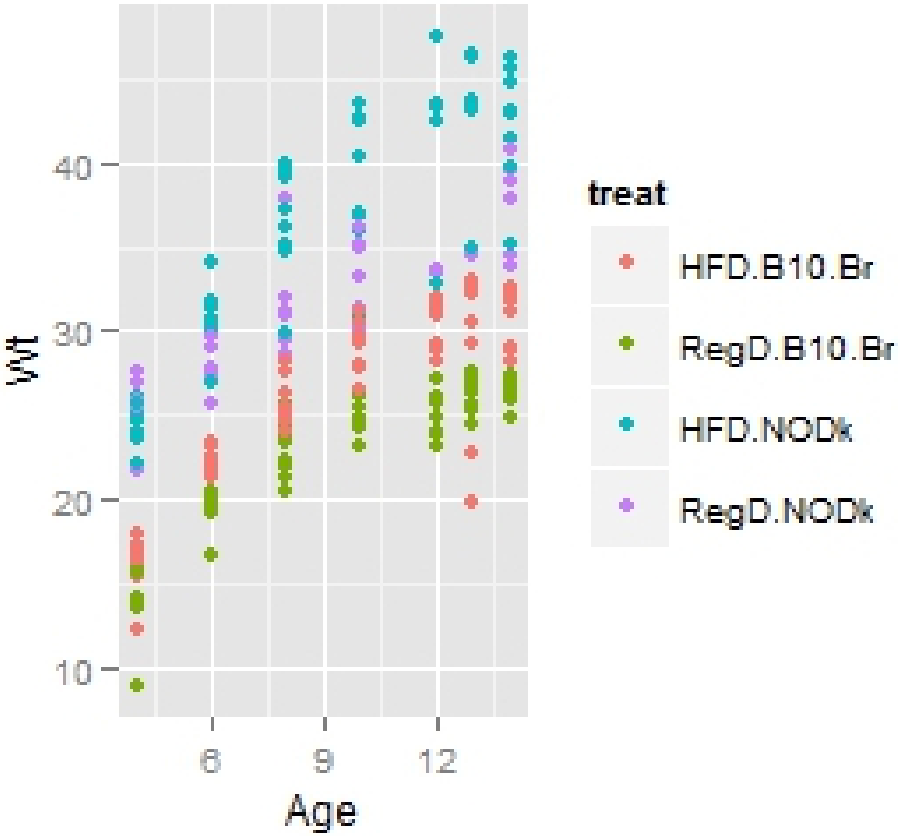
Research question: Are mice susceptible to obesity when exposed to a high fat diet?



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)

Acknowledgements: Ainy Hussain, PhD student 2013

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

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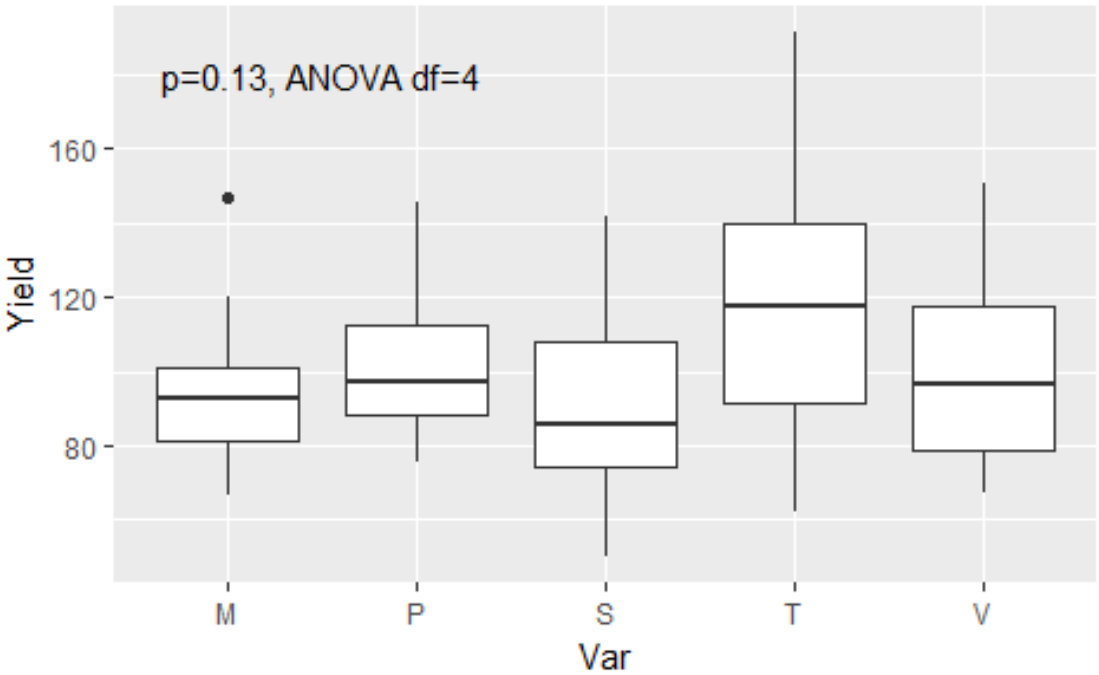

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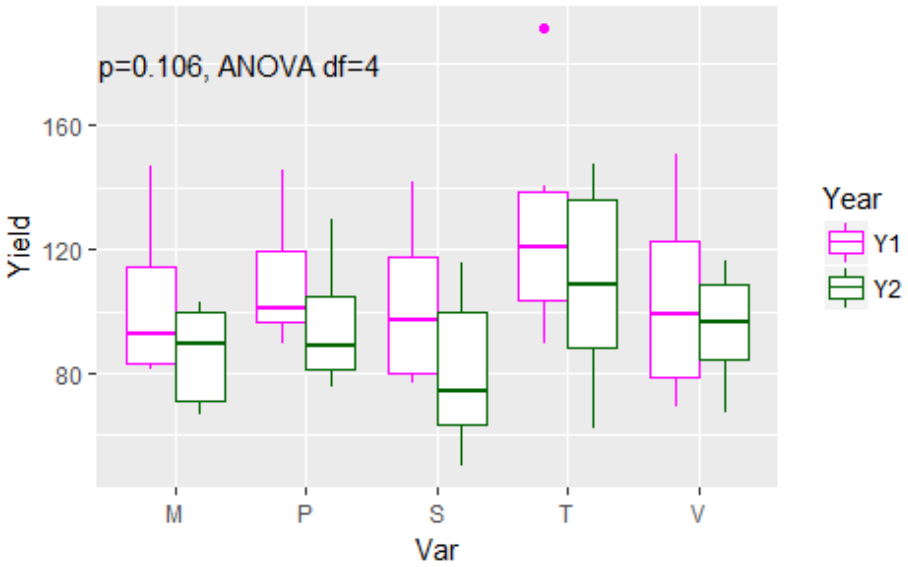

Controlling for other sources of variation:

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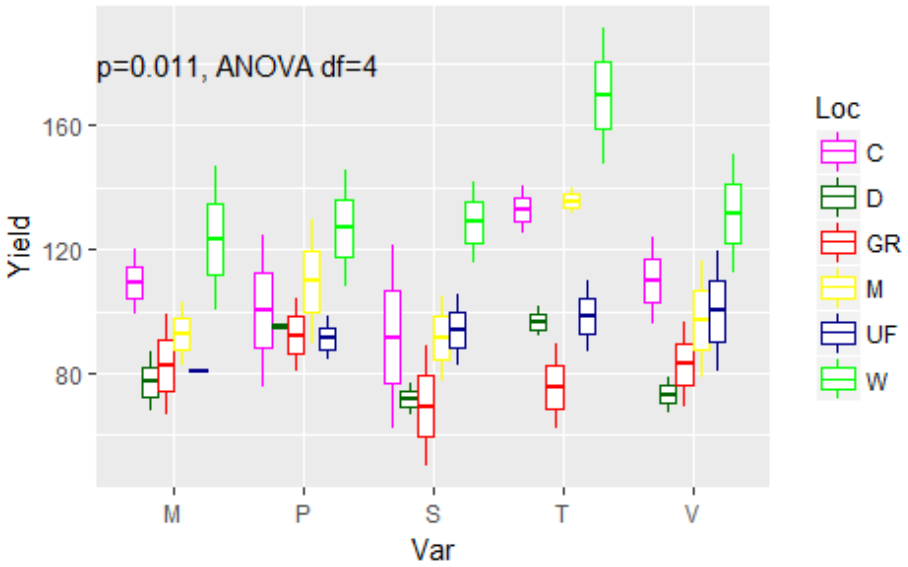

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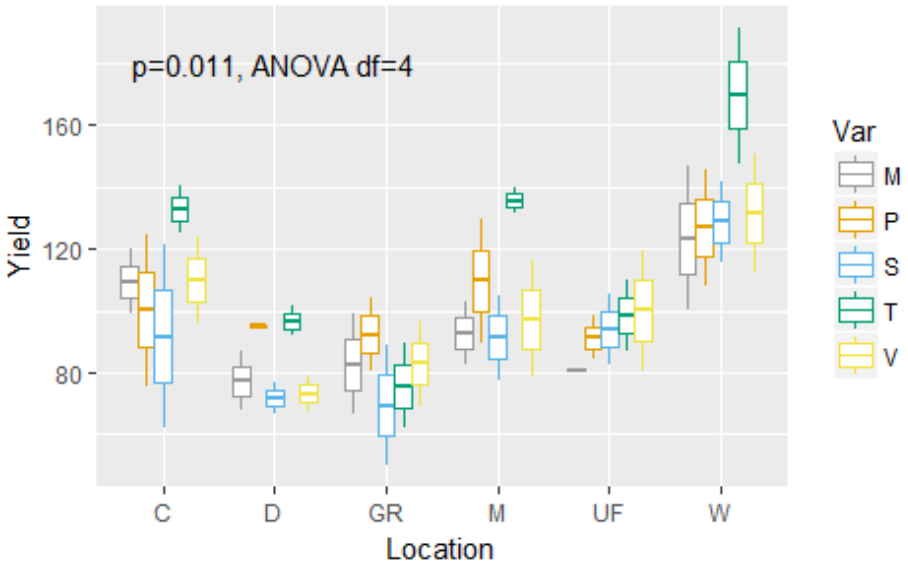

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Research question: How does cold duration impact upon germination in alpine plant A. glacialis?

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?

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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.**

1. Cautionary tales from the front
2. Introduction to Statistical Modelling

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

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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

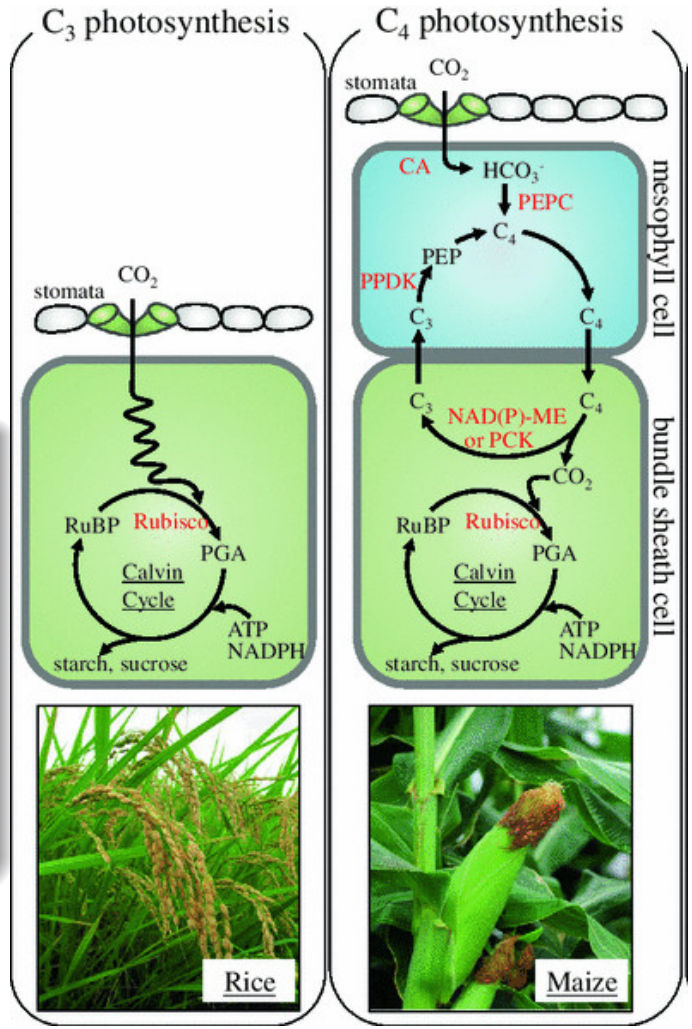
- To compare the mean response of an organism/system to a set of different experimental conditions.
  - Obtain estimate of "Treatment effect"
  - Is this "effect" different in subgroups of interest?
- What are the most important factors influencing the mean response?
- Subsidiary question: how can we 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



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Statistical model

DATA = Mean for C3 + Difference C4-C3 + Noise

$DATA = A + D + \epsilon$

A and D are the model PARAMETERS.

We want to infer whether D is different from 0

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T-test

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- No blocking factor / corrections

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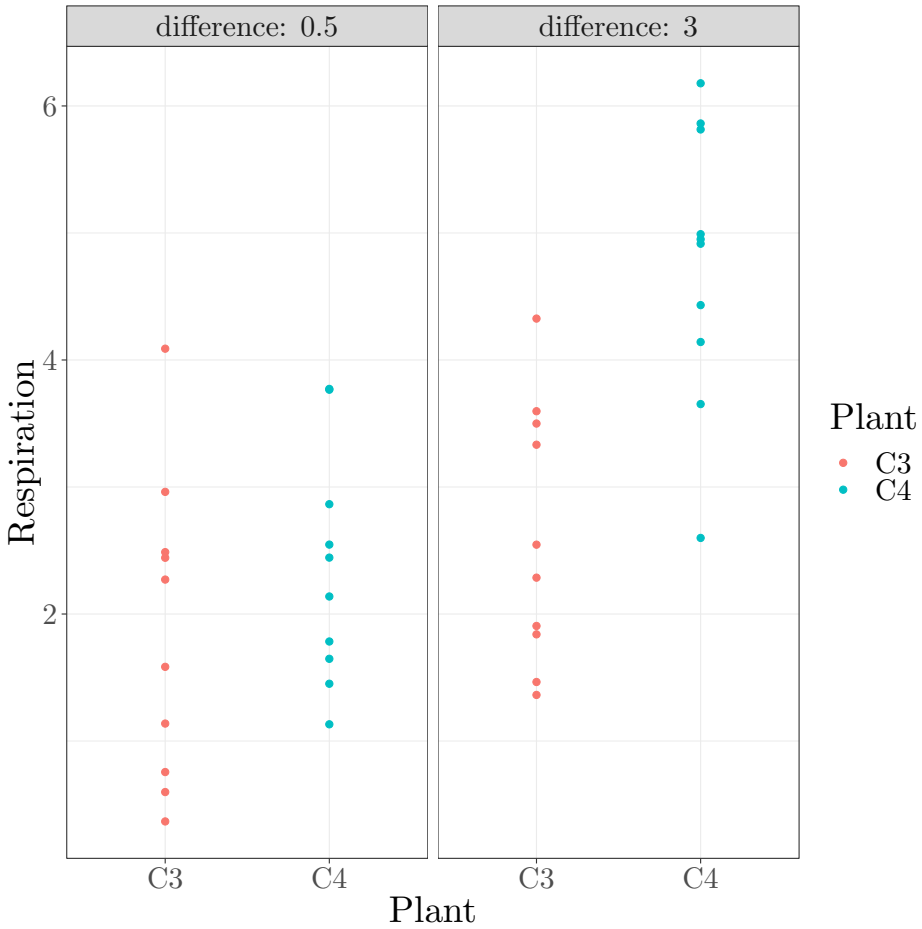
$$t = \frac{D}{\text{Variation of } \epsilon} \times \frac{\text{Sample Size}}{\sqrt{2}}$$

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



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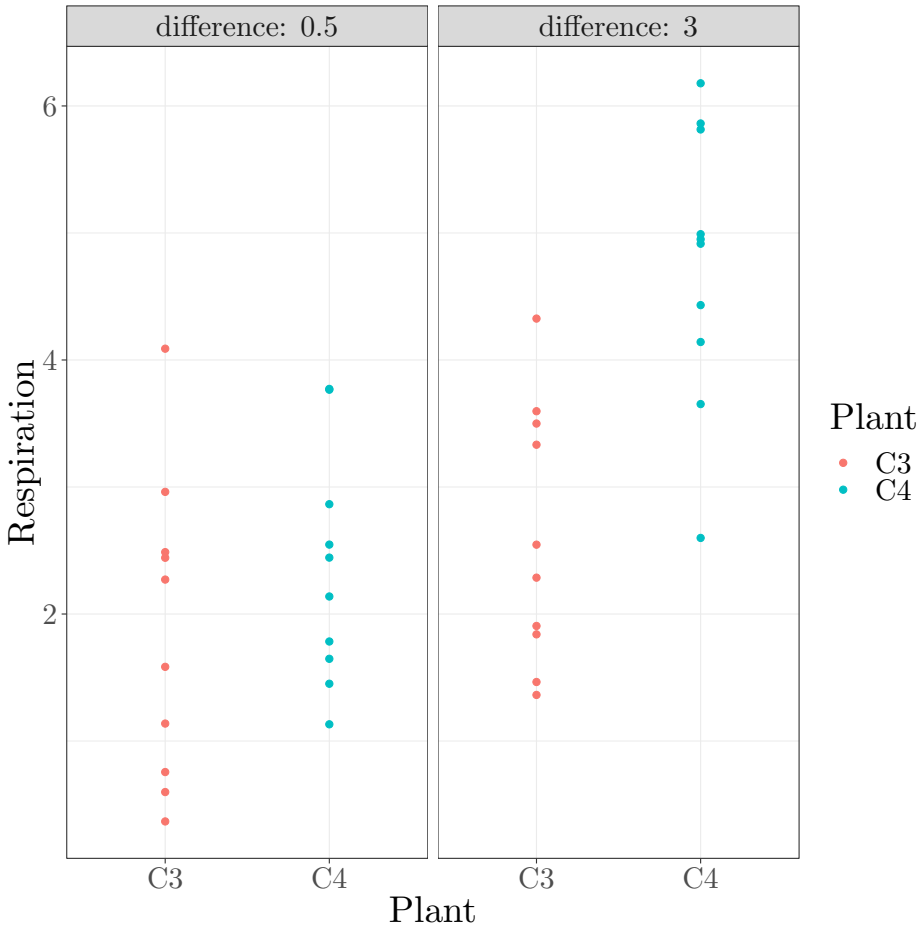

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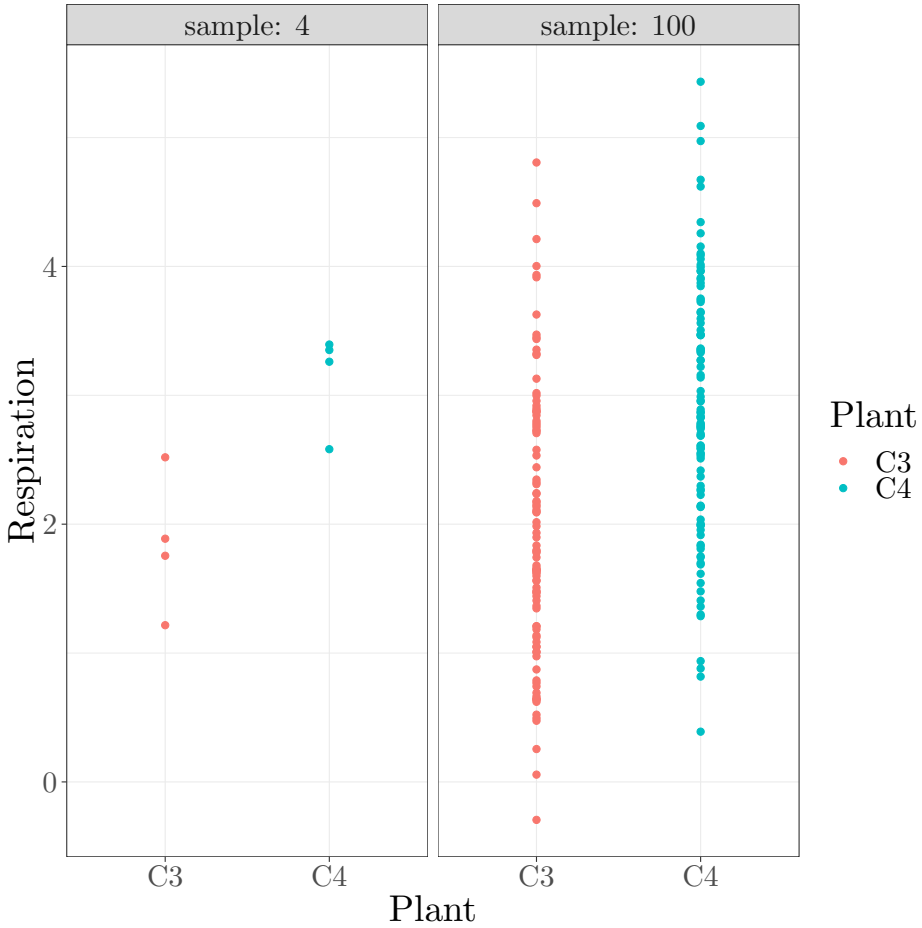

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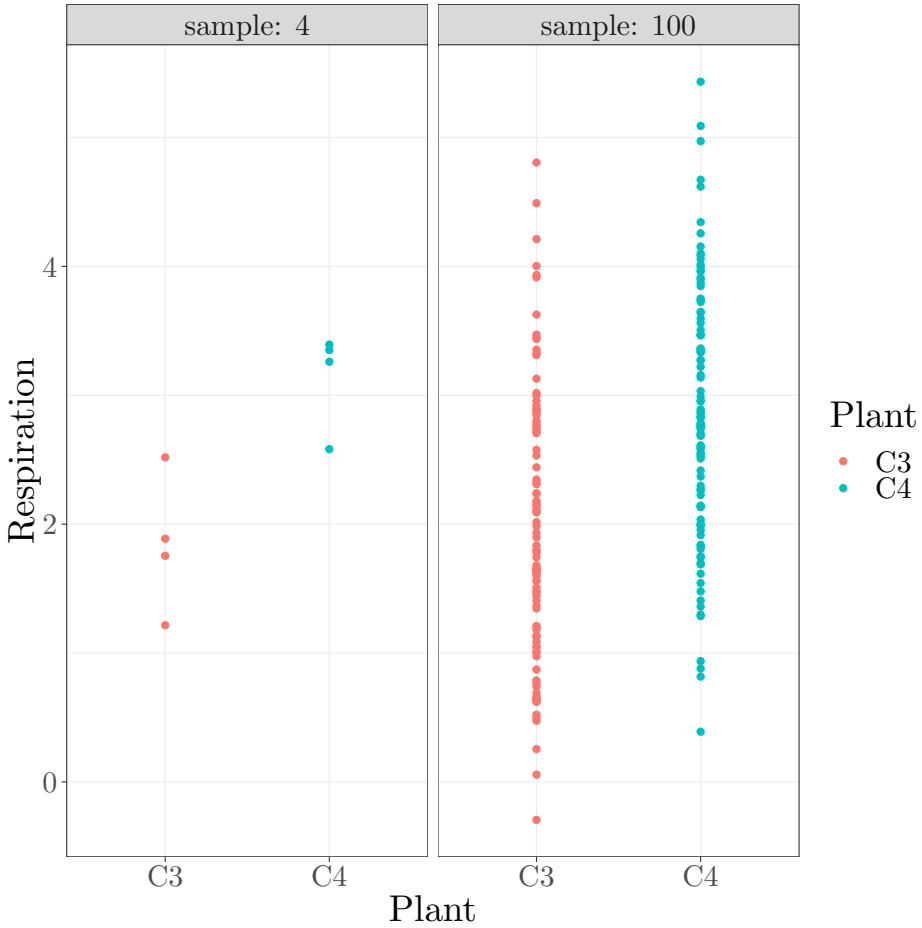

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1. Large true difference between the means
2. Large sample size

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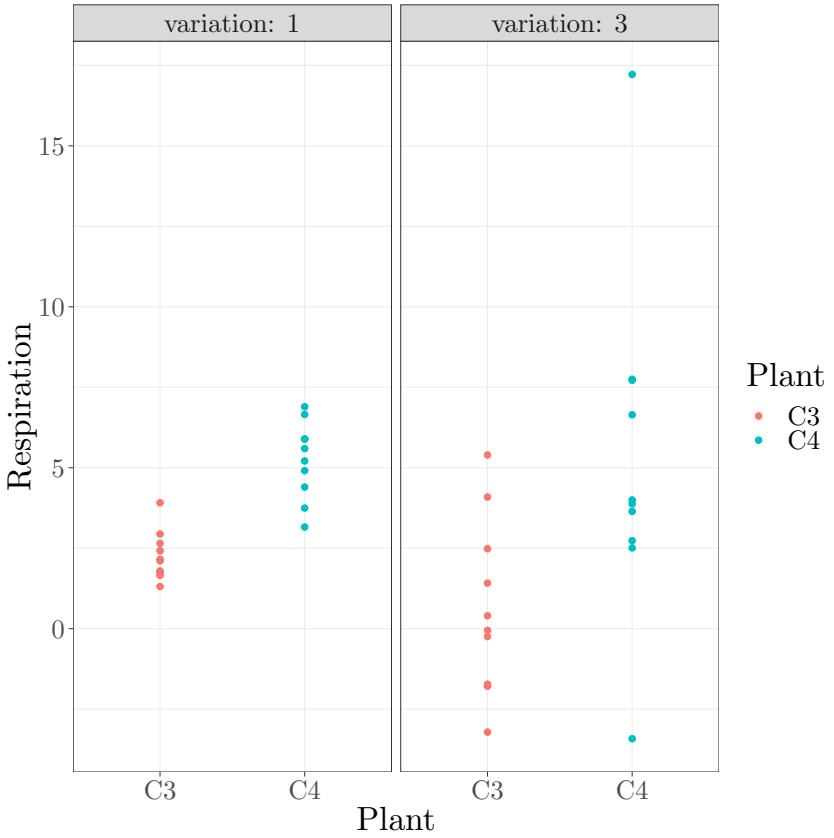

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Is it easier when unexplained variation is 1 or when it is 3?

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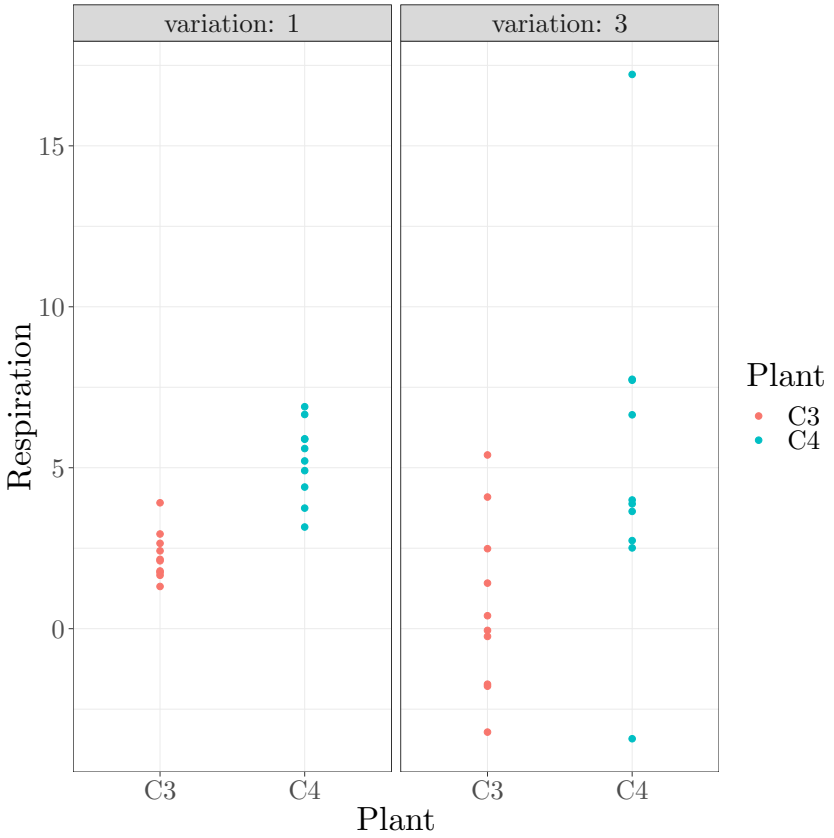

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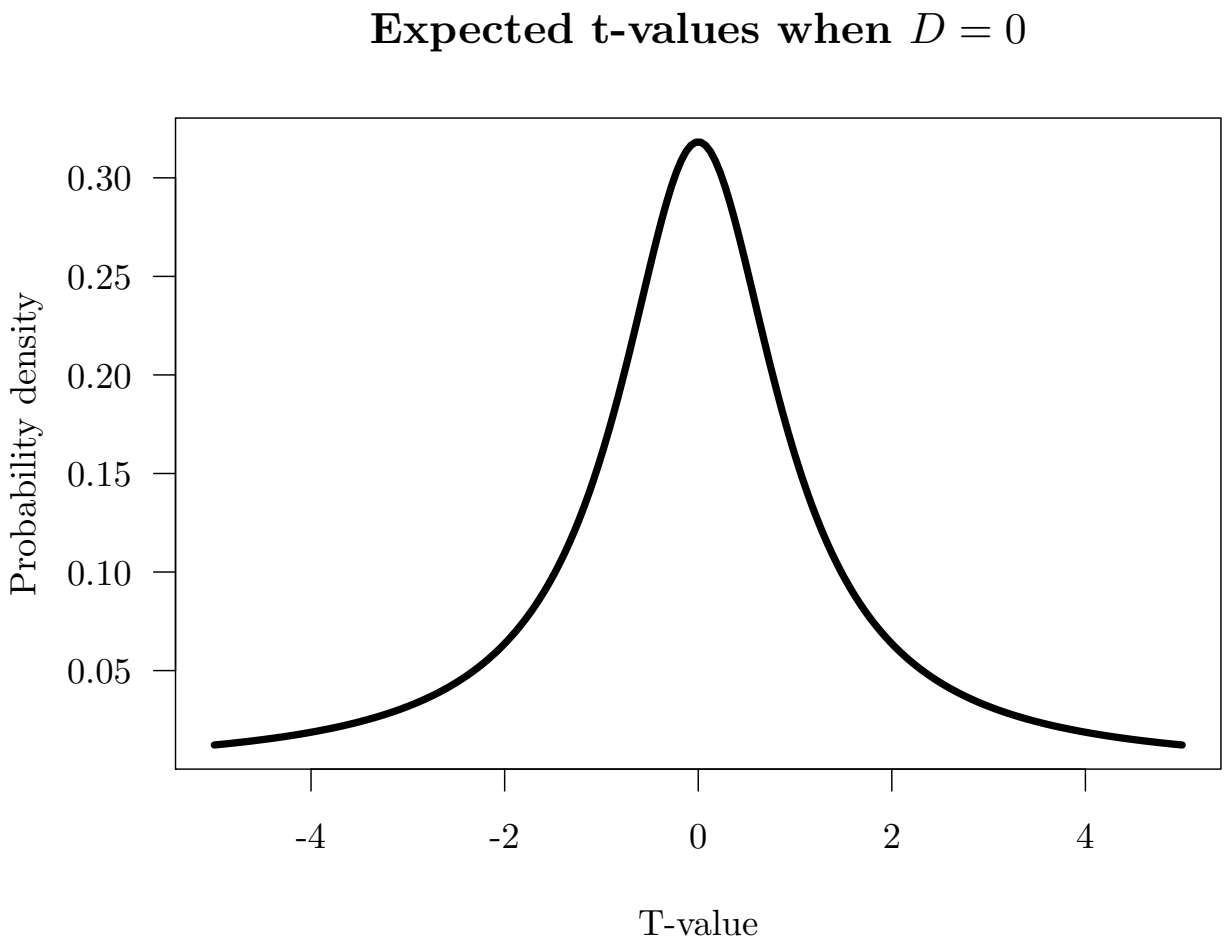
Is it easier when unexplained variation is 1 or when it is 3?

What makes t large:

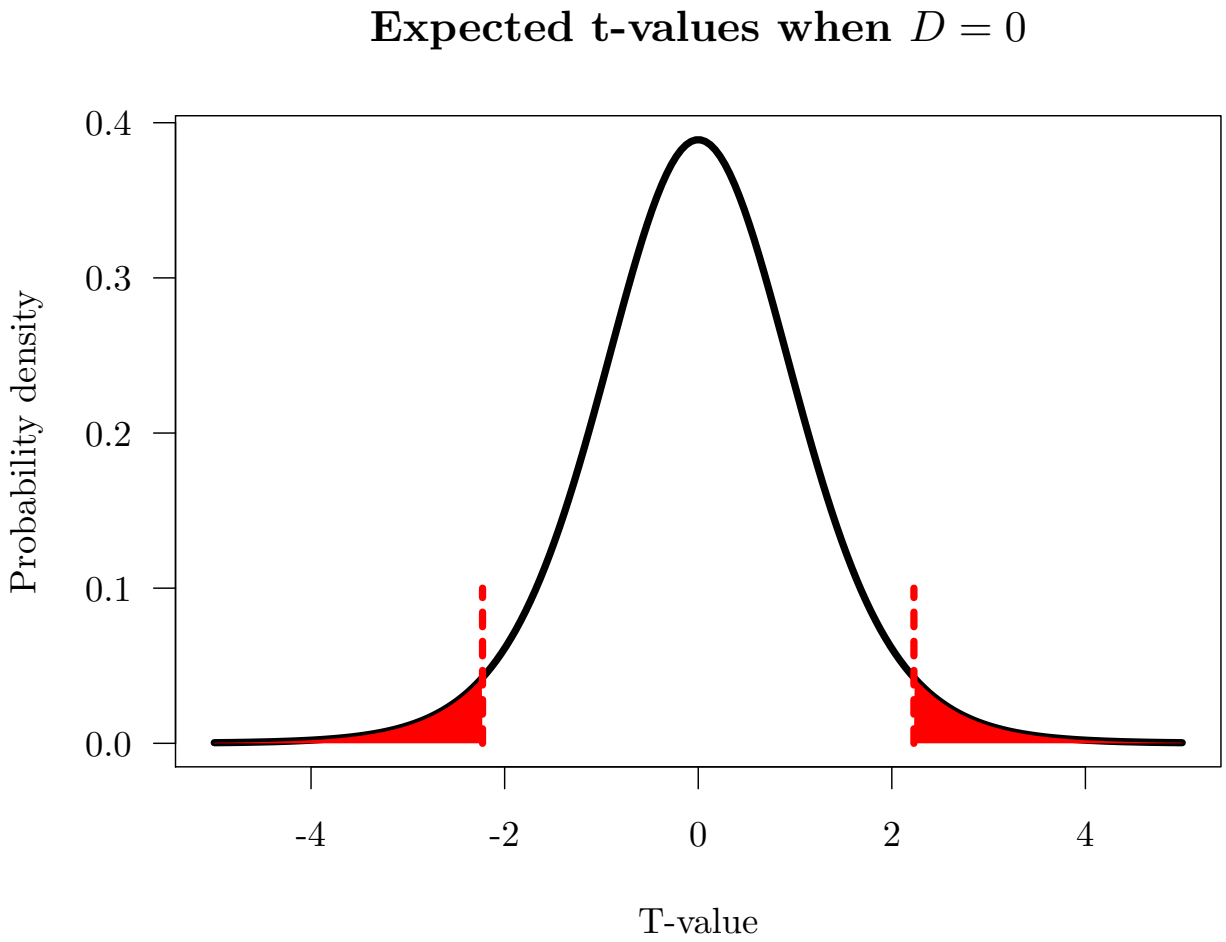
1. Large true difference between the means
2. Large sample size
3. Small unexplained variation

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



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



But really, what is a p-value?

Candy practical

You got 5 Halloween candies out of the bag. Does the bag contain more Halloween than normal candies?

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")
```

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Subset data by Variation (High and Low)

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

Compare C3 and C4 plants in "High Variation" subset

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

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Compare C3 and C4 plants in "High Variation" subset

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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)
```

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

```
             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 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()`

```
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
```

Compare the output from t.test, aov and lm

All is one. . .

All is one...

...but `lm()` rules!

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