

Introduction to statistical thinking

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April 4th, 2017

Why statistics?

DATA: BY THE NUMBERS



www.phdcomics.com

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Why statistics?

Statistics is at the core of modern research

What is statistics?

Why statistics?

Statistics is at the core of modern research

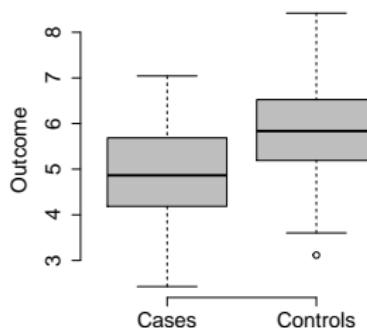
What is statistics?



→ everything from experimental design to figure preparation!

What can we do with statistics?

Comparisons between two groups



For example,

- ▶ Case/control studies
- ▶ Differential expression studies

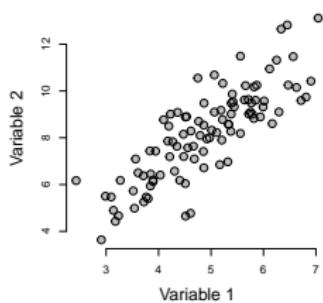
Is this difference *statistically significant*?

What can we do with statistics?

Finding associations between variables

For example,

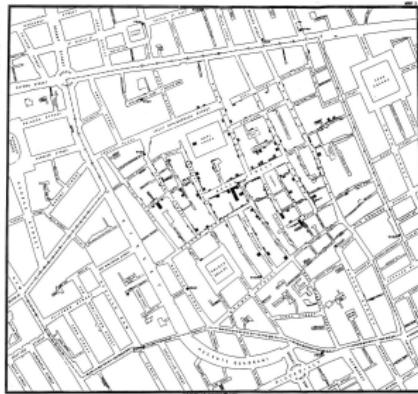
- ▶ Dose level vs survival time
- ▶ Association studies (e.g. GWAS)
- ▶ Co-expression networks



Is there an association between X and Y ?

Which associations are *statistically significant*?

What can we do with statistics?



Public domain image

During the Soho cholera outbreak in 1854, John Snow used statistics to find an **association** between the quality of the water source and cholera cases

John Snow is considered one of the fathers of modern epidemiology

Outline for the course

- ▶ **Introduction to statistics**
 - ▶ Types of data
 - ▶ Descriptive statistics
 - ▶ Practical: descriptive statistics in Rmarkdown
 - ▶ Statistical inference
- ▶ **Hypothesis testing (in R)**
- ▶ **Regression analysis (in R)**
- ▶ **Multiple testing (in R)**

Introduction to statistics

Types of data

Prior to any analysis, it is important to know what type of data is available

Types of data

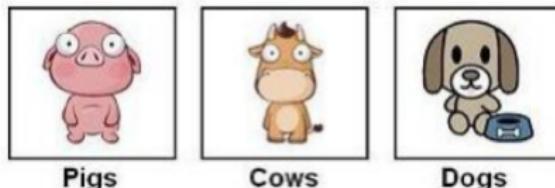
Prior to any analysis, it is important to know what type of data is available

Data can be classified into different **types of variables**:

- ▶ Categorical (nominal)
- ▶ Categorical (ordinal)
- ▶ Discrete
- ▶ Continuous

Types of data: categorical (nominal)

To describe categories



Source: <http://www.restore.ac.uk>

- ▶ No logical order
- ▶ Mutually exclusive fixed categories

Examples: gender, yes/no, cancer type, eye colour, ethnicity, etc.

Types of data: categorical (ordinal)

To describe categories



- ▶ There is a logical order
- ▶ Mutually exclusive fixed categories
- ▶ There is no quantification of the distance between categories

Examples: stress level (1 = low, ..., 7 = high), pain level (low/medium/high), education level (primary, secondary, ...), etc.

Types of data: discrete

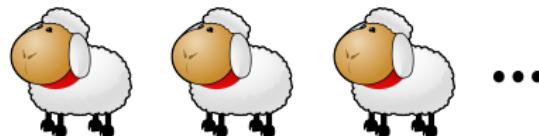


Image modified from Free Stock Photos

- ▶ Fixed (countable) possible set of values
- ▶ Distance between categories can be quantified
- ▶ ... basically, anything counted

Examples: number of tumours, hospital admissions, etc.

Sometimes treated as continuous if range is large!

Types of data: continuous



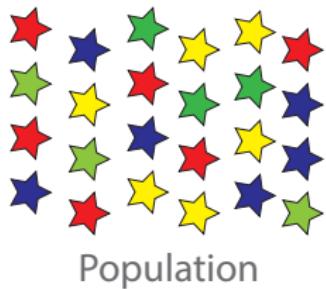
Requirements:

- ▶ Infinite number of possible values
- ▶ Given any two values, one fits between
- ▶ May have finite or infinite range

Examples: height, weight, blood pressure, temperature etc.

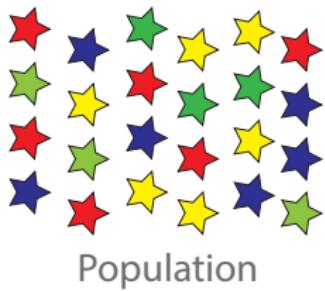
Population versus sample

Census records information from every subject within a population



Population versus sample

Census records information from every subject within a population



Scientific studies typically rely on a subset of a population



Assumption: the sample is a good representative of the population!

Descriptive statistics versus statistical inference

Descriptive statistics

summarizes the information
available in the data

6 x  +
6 x  +
6 x  +
6 x  +

Descriptive statistics versus statistical inference

Descriptive statistics

summarizes the information available in the data

Statistical inference

extrapolates sample information to population level

6 x  +
6 x  +
6 x  +
6 x  +

$$P(\text{Red Star}) = 1/4$$

$$P(\text{Green Star}) = 1/4$$

$$P(\text{Blue Star}) = 1/4$$

$$P(\text{Yellow Star}) = 1/4$$

Assumption: the sample is a good representative of the population!

Descriptive analyses

Descriptive analyses

There are two basic forms of data summary

- ▶ Numerical: frequency tables, summary measures, etc
- ▶ Graphical: histograms, scatter-plots, boxplots, etc

A good summary depends on the data type!

Descriptive analyses: categorical variables

Numerical summary

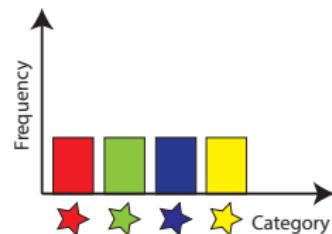
Category	Frequency	Cumulative frequency	Relative frequency	Cum. rel. frequency
★	6	6	0.25	0.25
★	6	12	0.25	0.50
★	6	18	0.25	0.75
★	6	24	0.25	1.00

Descriptive analyses: categorical variables

Numerical summary

Category	Frequency	Cumulative frequency	Relative frequency	Cum. rel. frequency
★	6	6	0.25	0.25
★	6	12	0.25	0.50
★	6	18	0.25	0.75
★	6	24	0.25	1.00

Graphical summary



Descriptive analysis: discrete/continuous variables

Frequency tables are not useful
unless we *categorize* the data

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Frequency tables are not useful
unless we *categorize* the data

Example:

Birth weight	Rel. freq
Very low (<1.5 kg.)	0.05
Low (1.5-2.5 kg.)	0.10
Normal (≥ 2.5 kg.)	0.85

Descriptive analysis: discrete/continuous variables

Frequency tables are not useful unless we *categorize* the data

Instead, we typically prefer *summary measures*

Example:

Birth weight	Rel. freq
Very low (<1.5 kg.)	0.05
Low (1.5-2.5 kg.)	0.10
Normal (≥ 2.5 kg.)	0.85

- ▶ Mean
- ▶ Median
- ▶ Mode
- ▶ Variance
- ▶ Quantiles

Which one?

Descriptive analysis: discrete/continuous variables

Firstly, we can use measures to describe the **location** of the data

Descriptive analysis: discrete/continuous variables

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Mean (\bar{x})

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_n}{n}$$

Median ($x_{50\%}$)

50% of observations lie below $x_{50\%}$

Descriptive analysis: discrete/continuous variables

Firstly, we can use measures to describe the **location** of the data

When the data is normal ...

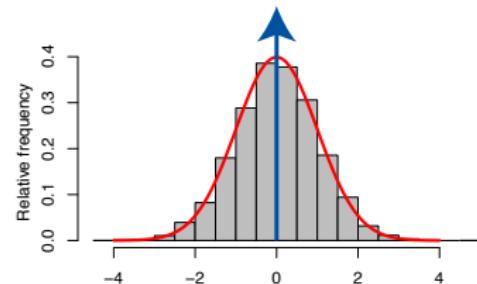
Mean (\bar{x})

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_n}{n}$$

Median ($x_{50\%}$)

50% of observations lie below $x_{50\%}$

Mean = Median



... but that is not always true

Descriptive analysis: example

Suppose we record the number of Facebook friends for 7 colleagues:

311, 345, 270, 310, 243, 5300, 11

Mean (\bar{x})

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_7}{7} = 970$$

Median ($x_{50\%}$)

$$11, 243, 270, \textcolor{red}{310}, 311, 345, 5300 \Rightarrow x_{50\%} = 310$$

Which one provides a better description for the location of the data?

Descriptive analysis: example

Now suppose the data is slightly different:

311, 345, 270, 310, 243, **530**, 11

Mean (\bar{x})

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_7}{7} = 289$$

Median ($x_{50\%}$)

$$11, 243, 270, \textcolor{red}{310}, 311, 345, 530 \Rightarrow x_{50\%} = 310$$

What happened?

Descriptive analysis: discrete/continuous variables

It is also important to summarize the **spread** of the data

Standard deviation ($\text{sd}(x)$)

$$\text{sd}(x) = \sqrt{\frac{(x_1 - \bar{x})^2 + \cdots + (x_n - \bar{x})^2}{n}}$$

Interquartile range ($\text{IQR}(x)$)

$$\text{IQR}(x) = x_{75\%} - x_{25\%}$$

Descriptive analysis: example

In the first example, we have

Standard deviation ($\text{sd}(x)$)

$$\text{sd}(x) = \sqrt{\frac{(x_1 - \bar{x})^2 + \cdots + (x_7 - \bar{x})^2}{7}} = 1912.57$$

Interquartile range ($\text{IQR}(x)$)

$$11, \textcolor{red}{243}, 270, \textcolor{red}{310}, 311, \textcolor{red}{345}, 5300 \Rightarrow \text{IQR}(x) = 345 - 243$$

Descriptive analysis: example

In the second example, we have

Standard deviation ($\text{sd}(x)$)

$$\text{sd}(x) = \sqrt{\frac{(x_1 - \bar{x})^2 + \dots + (x_7 - \bar{x})^2}{7}} = 153.79$$

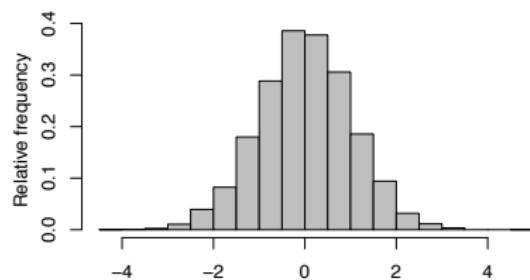
Interquartile range ($\text{IQR}(x)$)

$$11, \textcolor{red}{243}, 270, \textcolor{red}{310}, 311, \textcolor{red}{345}, 530 \Rightarrow \text{IQR}(x) = 345 - 243$$

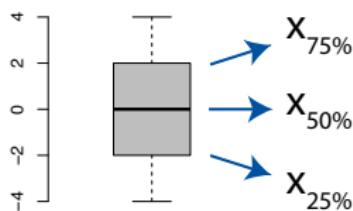
Descriptive analysis: discrete/continuous variables

We can also summarize the distribution of the data **graphically**

Histogram



Boxplot



Before we continue: the normal distribution

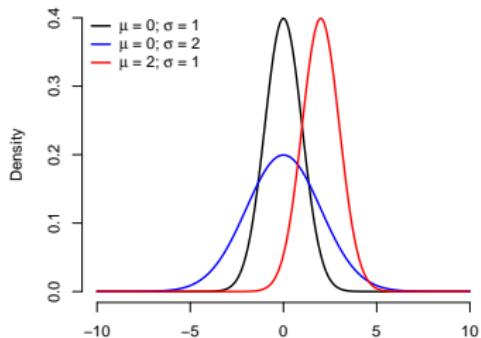
The normal (or Gaussian) distribution is very popular!

Indexed by two parameters:

- ▶ $\mu \rightarrow$ location (mean)
- ▶ σ (or σ^2) \rightarrow spread (standard deviation)

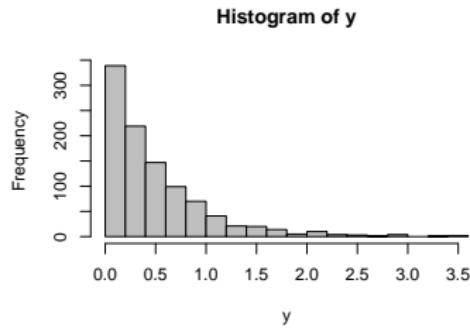
Rule of thumb:

- ▶ $\sim 95\%$ of the data lies between $(\mu - 2\sigma, \mu + 2\sigma)$
- ▶ $\sim 99\%$ of the data lies between $(\mu - 3\sigma, \mu + 3\sigma)$

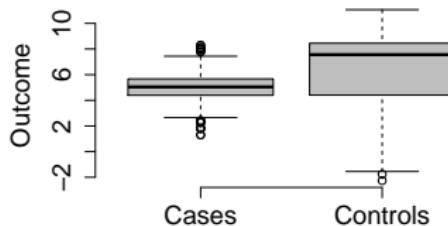
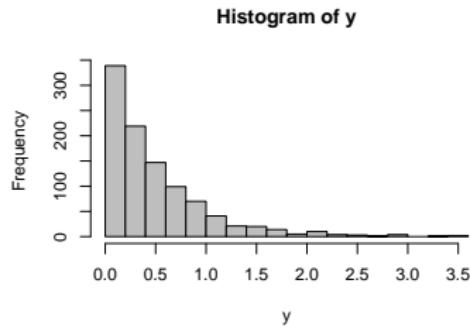


The distribution is symmetric around μ !

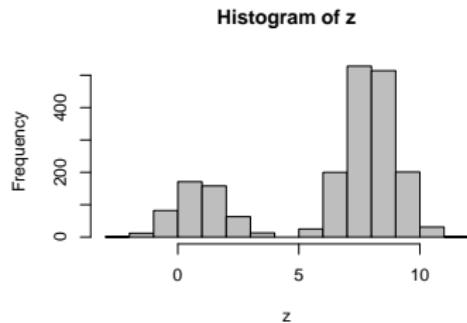
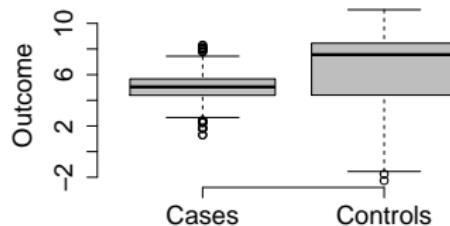
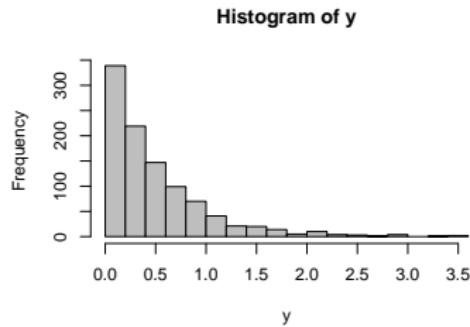
Descriptive analysis: is the data normally distributed?



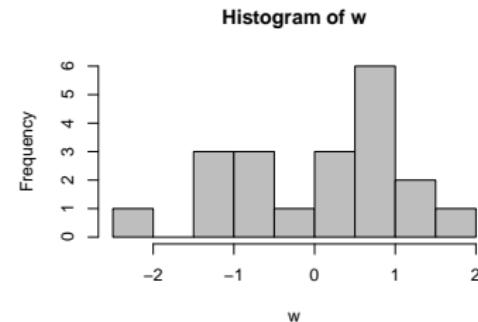
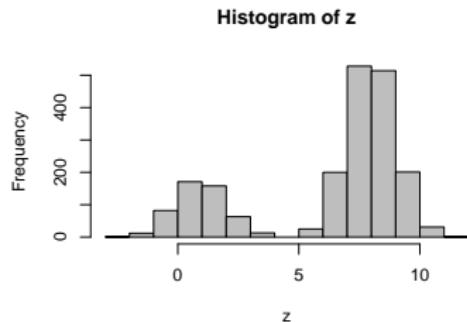
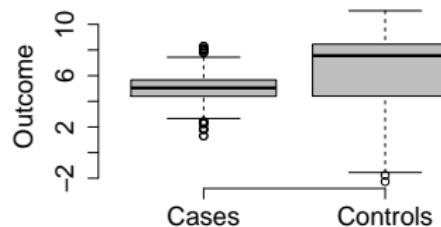
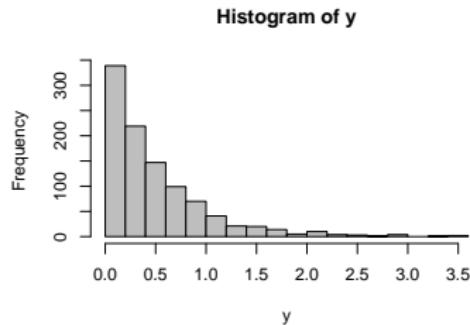
Descriptive analysis: is the data normally distributed?



Descriptive analysis: is the data normally distributed?



Descriptive analysis: is the data normally distributed?

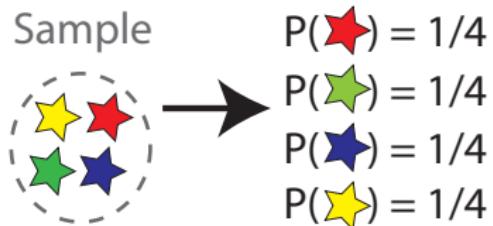


Statistical inference

Statistical inference: estimation

Statistical inference

extrapolates sample information
to population level

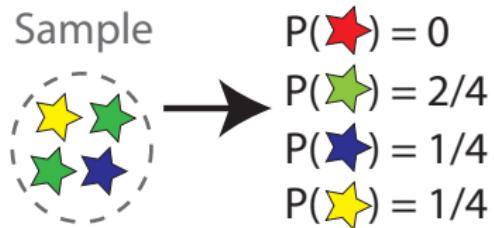


In this example, we want to **estimate** the proportion of red, green, blue and yellow stars in the population based on the observed data

Statistical inference: estimation

Statistical inference

extrapolates sample information
to population level

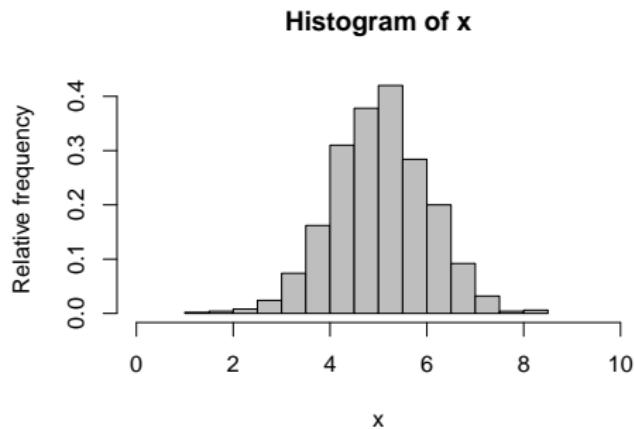


Inference needs to account for uncertainty due to sampling

Note: in this sample we didn't observe red stars!

Statistical inference: estimation

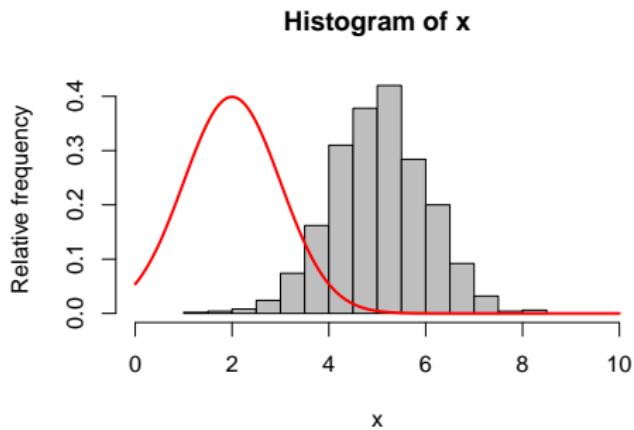
To model the data (e.g. expression of a gene across samples) as being normally distributed with mean μ and standard deviation 1.



The aim here is to estimate the value of μ !

Statistical inference: estimation

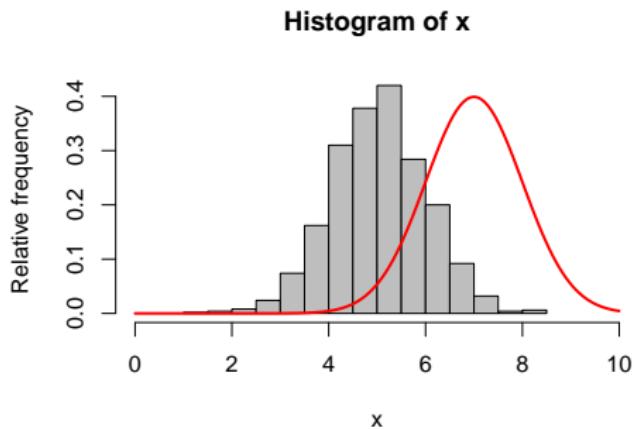
To model the data (e.g. expression of a gene across samples) as being normally distributed with mean μ and standard deviation 1.



$$\mu = 2?$$

Statistical inference: estimation

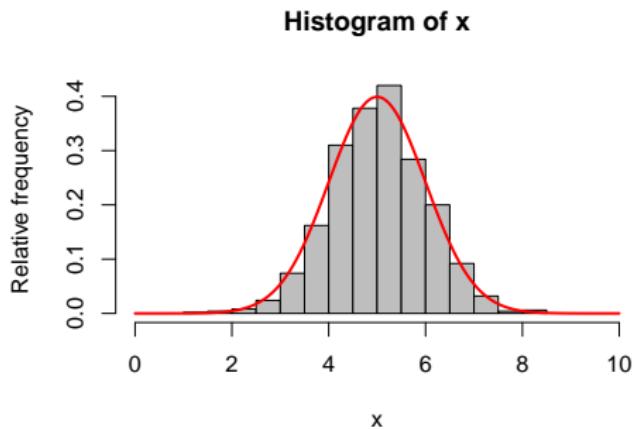
To model the data (e.g. expression of a gene across samples) as being normally distributed with mean μ and standard deviation 1.



$$\mu = 7?$$

Statistical inference: estimation

To model the data (e.g. expression of a gene across samples) as being normally distributed with mean μ and standard deviation 1.



$$\mu = 5?$$

Statistical inference: estimation

We can estimate μ using the observed data through

$$\bar{x} = \frac{x_1 + \dots + x_n}{n}$$

- ▶ How does \bar{x} compare to μ for different sample sizes n ?
- ▶ If the data is normally distributed, with standard deviation σ : how does \bar{x} compare to μ for different values of σ ?

Statistical inference: estimation (shiny app)

This app demonstrates the difference between the true mean μ and the sample mean \bar{x} .

The dataset contains weights of cattle in a feedlot. Cows are randomly sampled from the population by clicking on “Fresh cows”. We can control:

- ▶ The number of cows
- ▶ The true mean μ
- ▶ The true standard deviation σ

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What happens when we change these values?

Statistical inference: estimation

We can estimate μ using the observed data through

$$\bar{x} = \frac{x_1 + \dots + x_n}{n}$$

Estimates of μ that are based on a **finite sample** are not exact:

we need to provide a measure of uncertainty

Statistical inference: estimation

We can estimate μ using the observed data through

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Estimates of μ that are based on a **finite sample** are not exact:

we need to provide a measure of uncertainty

In this case, this can be quantified through the **standard error**:

$$\text{S.E.} = \sigma / \sqrt{n}$$

Statistical inference: estimation

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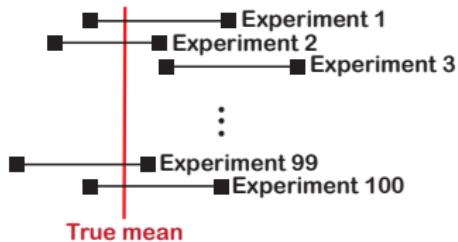
This is consistent with our previous observations

Important: standard error \neq standard deviation

Statistical inference: confidence intervals

A confidence interval (CI) is a *random* interval

In repeated experiments ...
95% of the time CI covers the *true* mean

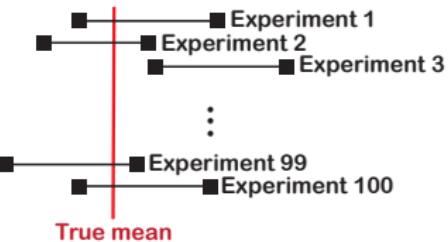


Statistical inference: confidence intervals

A confidence interval (CI) is a *random* interval

In repeated experiments ...

95% of the time CI covers the *true* mean



For the mean, the 95% CI is given by

$$(\bar{x} - 1.96 \text{ S.E.}, \bar{x} + 1.96 \text{ S.E.})$$

Statistical inference: estimation (shiny app)

This app demonstrates the difference between the true mean μ and the sample mean \bar{x} .

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The dataset contains weights of cattle in a feedlot. Cows are randomly sampled from the population by clicking on “Fresh cows”. We can control:

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- ▶ The true mean μ
- ▶ The true standard deviation σ

How does the CI behave when we change these values?

Statistical inference: Central Limit Theorem (CLT)

This formula for the CI assumes the data is **normally distributed**.

What can we do if this is not true?

Statistical inference: Central Limit Theorem (CLT)

This formula for the CI assumes the data is **normally distributed**.

What can we do if this is not true?

If the data is not normally distributed, the **CLT** guarantees this result is still valid, provided the sample size is large:

CLT: if we average across a large number of samples, \bar{x} is normally distributed

Statistical inference: CLT (shiny app)

This app illustrates the CLT.

https://gallery.shinyapps.io/CLT_mean/

What happens when the sample size and the number of samples varies if

1. we simulate data from a Normal distribution?

Statistical inference: CLT (shiny app)

This app illustrates the CLT.

https://gallery.shinyapps.io/CLT_mean/

What happens when the sample size and the number of samples varies if

1. we simulate data from a Normal distribution?
2. we simulate data from a Uniform distribution?

Statistical inference: hypothesis testing

Often, the aim of the analysis is not only **estimation**.

For example,

- ▶ *Is the treatment effective?*
- ▶ *Is a gene differentially expressed?*
- ▶ *Is there an association between genotype and phenotype?*



These questions can be translated as **hypothesis testing** problems

Comic taken from Significance Magazine, December 2008.

Statistical inference: hypothesis testing



Am I pregnant?

Disclaimer: this image was
downloaded from the internet and
does not reflect the life of the
instructors!

Statistical inference: hypothesis testing

Basic setup



Am I pregnant?

Disclaimer: this image was downloaded from the internet and does not reflect the life of the instructors!

Formulate the ‘null’ and alternative hypothesis

Calculate a “test statistic” from the data

Desicion rule

Statistical inference: hypothesis testing

Basic setup (example)



Am I pregnant?

Disclaimer: this image was downloaded from the internet and does not reflect the life of the instructors!

Formulate the ‘null’ and alternative hypothesis

e.g. H_0 : Not pregnant vs H_1 : Pregnant

Calculate a “test statistic” from the data

e.g. summary measure based on hormonal levels

Decision rule

e.g. is the data more extreme than what is expected by chance (for a non-pregnant woman)?

Statistical inference: hypothesis testing

No test is exact:

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis	Correct True positive	Wrong False positive
Do not reject null hypothesis	Wrong False negative	Correct True negative

Statistical inference: hypothesis testing

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Error rates depend on e.g. sample size ... what else?

Statistical inference: hypothesis testing

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Error rates depend on e.g. sample size ... what else?

Beware of multiple testing correction issues!

Statistical inference: hypothesis testing

Suppose that 100 women take the test

- ▶ 70 of them were truly pregnant
- ▶ the test was positive for 75 women
- ▶ the result was wrong for 10 non-pregnant women



Am I pregnant?

Complete the table:

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis		
Do not reject null hypothesis		

Statistical inference: hypothesis testing

Suppose that 100 women take the test

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Am I pregnant?

Complete the table:

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis		10/30
Do not reject null hypothesis		

Statistical inference: hypothesis testing

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Am I pregnant?

Complete the table:

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis	65/70	10/30
Do not reject null hypothesis	5/70	20/30

Statistical inference: hypothesis testing

Typically, two types of error are of main interest:

- ▶ Type I error

$$\alpha = p(\text{Reject } H_0 \text{ when } H_0 \text{ is true})$$

- ▶ Type II error

$$\beta = p(\text{Do not reject } H_0 \text{ when } H_0 \text{ is false})$$

	Null hypothesis does not hold	Null hypothesis holds
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Statistical inference: hypothesis testing

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	Null hypothesis does not hold	Null hypothesis holds
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Most hypothesis tests are designed to control type I error!

Statistical inference: hypothesis testing

In terms of this table

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis	65/70	10/30
Do not reject null hypothesis	5/70	20/30

- ▶ What is the **significance level** of the test?
- ▶ What is the **power** of the test?

Statistical inference: hypothesis testing

In terms of this table

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis	65/70	10/30
Do not reject null hypothesis	5/70	20/30

- ▶ What is the **significance level** of the test?
 - ▶ Type I error (α), i.e. $10/30 = 0.33$
- ▶ What is the **power** of the test?

Statistical inference: hypothesis testing

In terms of this table

	Null hypothesis does not hold	Null hypothesis holds
Reject null hypothesis	65/70	10/30
Do not reject null hypothesis	5/70	20/30

- ▶ What is the **significance level** of the test?
 - ▶ Type I error (α), i.e. $10/30 = 0.33$
- ▶ What is the **power** of the test?
 - ▶ $1 - \text{type II error } (1 - \beta)$, i.e. $65/70 = 0.93$

Statistical inference: hypothesis testing

Recall: basic setup

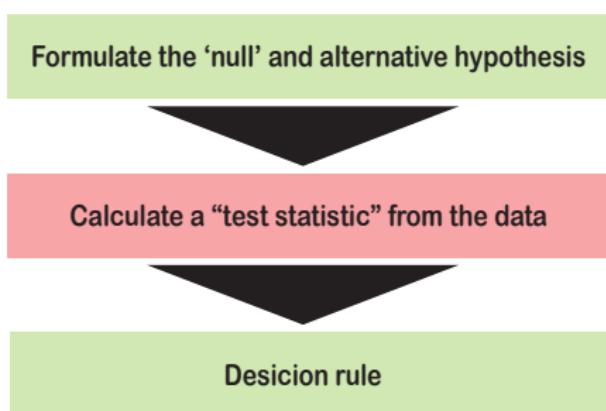
Formulate the ‘null’ and alternative hypothesis

Calculate a “test statistic” from the data

Decision rule

Statistical inference: hypothesis testing

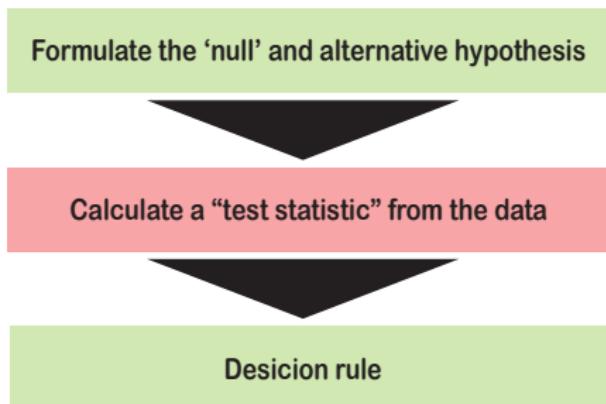
Recall: basic setup



A **test statistic** is a summary calculated from the data whose distribution is known (if H_0 is true)

Statistical inference: hypothesis testing

Recall: basic setup



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We will explore some example test statistics later today

Statistical inference: hypothesis testing

Recall: basic setup

Formulate the ‘null’ and alternative hypothesis

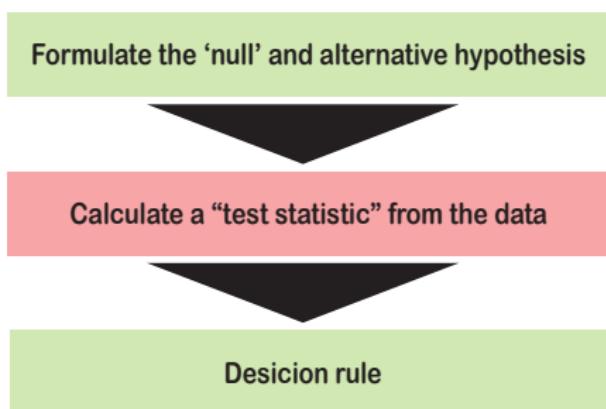
Calculate a “test statistic” from the data

Decision rule

We can also use a
p-value

Statistical inference: hypothesis testing

Recall: basic setup



We can also use a
p-value

What is a p-value?

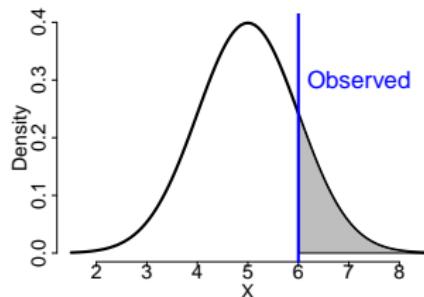
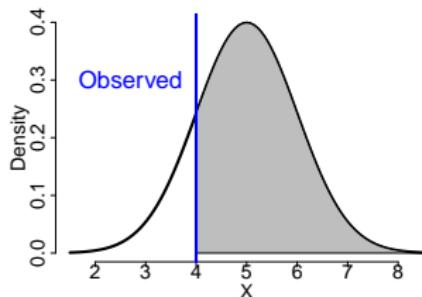
Statistical inference: what is a p-value?

A **p-value** is the probability of observing the current data (or more extreme) given than H_0 is true.

Statistical inference: what is a p-value?

A **p-value** is the probability of observing the current data (or more extreme) given than H_0 is true. For example

$$H_0 : \mu = 5 \text{ vs } H_1 : \mu > 5$$

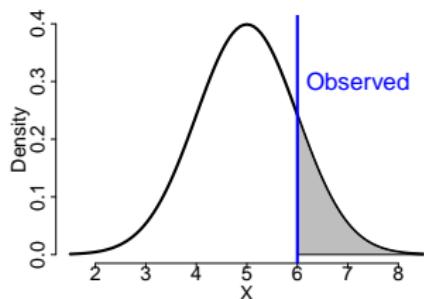
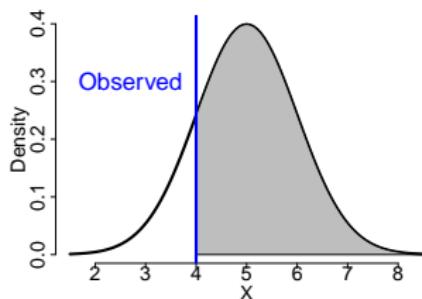


The p-value is equal to the gray area

Statistical inference: what is a p-value?

The smaller p-value, the stronger the evidence against H_0

$$H_0 : \mu = 5 \text{ vs } H_1 : \mu > 5$$



Statistical inference: what is a p-value? (shiny app)

This app illustrates how p-values work. In this example, each observation (dot) represents the measured body temperature of a person.

The left panel represents the distribution of the data *under the null*

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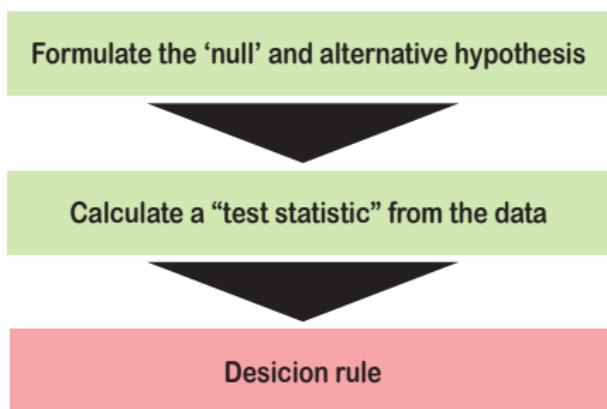
For any given observation: how do we calculate a p-value?

How do p-values behave when:

- ▶ the null hypothesis is true?
- ▶ the alternative hypothesis is true?

Statistical inference: decision rule of an hypothesis test

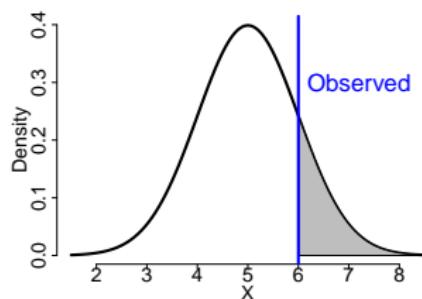
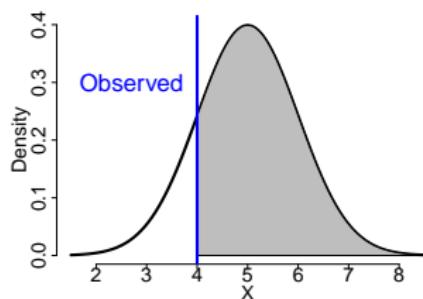
Recall: basic setup



Statistical inference: decision rule of an hypothesis test

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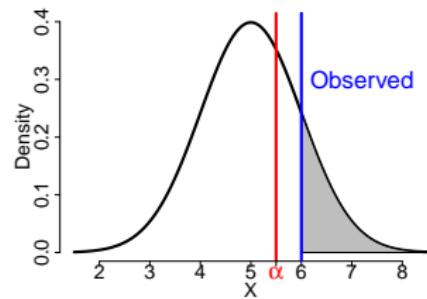
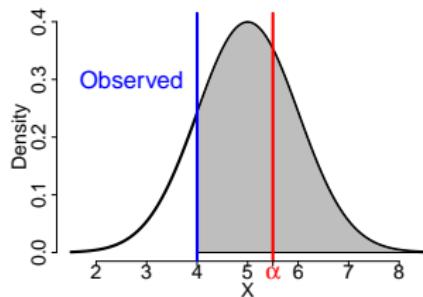


Where to place the cut-off?

Statistical inference: decision rule of an hypothesis test

The cut-off is typically set to control **type I error**

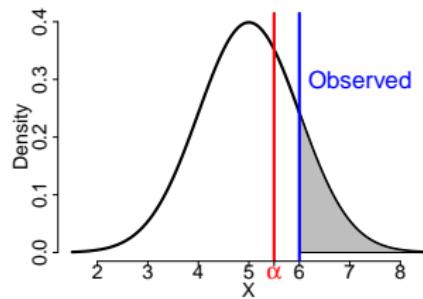
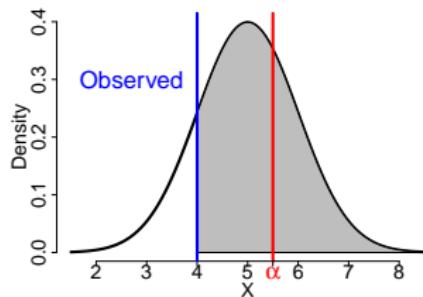
$$\alpha = P(\text{Reject } H_0 \text{ when } H_0 \text{ is true}) \quad (\text{typically } \alpha = 0.05)$$



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$$\alpha = P(\text{Reject } H_0 \text{ when } H_0 \text{ is true}) \quad (\text{typically } \alpha = 0.05)$$



Cut-off must be set before running the test!

Statistical inference: what test to use?

Standard statistical softwares (e.g. R) have a wide range of hypothesis tests built-in ⇒ easy to get numbers back!

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Before running a test it is important to recognise:

- ▶ What type of data is available?
- ▶ What is the relevant hypothesis?
- ▶ What assumptions underlie the test?

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- ▶ What assumptions underlie the test?

We will explore some examples in detail this afternoon ...

Acknowledgements

Part of the materials discussed today have been adapted from materials provided by

- ▶ Mark Dunning (CRUK-CI)
- ▶ Paul Kirk (MRC-BSU)