Introduction to Statistical Analysis

Cancer Research UK Cambridge Institute - 17th of November 2023

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CANCER RESEARCH UK | Cambridge Institute

UNIVERSITY OF CAMBRIDGE

Timeline

- Morning (9.30-12.30)
  - Data types and descriptive statistics → Online quiz
  - Probability distributions and central limit theorem (CLT) → Simulations
  - Inferential statistics: estimation → Simulations
- Lunch
- Afternoon (13.00-17.00)
  - Inferential statistics: one-sample tests → Exercises
  - Inferential statistics: two-sample tests → Exercises

The Scope of Statistics

1. Study of populations

2. Study of variation

3. Study of methods of the reduction of data

1	0	0	0	0	1		1	0	0	0	0
2	0	0	0	0	1	0	1	0	0	0	1
3	0	0	0	0	1	1	1	0	0	0	1
4	0	0	0	1	0	0	1	0	0	1	0
5	0	0	0	1	0	1	1	0	0	1	0
6	0	0	0	1	1	0	1	0	0	1	1
7	0	0	0	1	1	1	1	0	0	1	1
8	0	1	0	0	0	0	1	0	1	0	0
9	0	1	0	0	0	1	1	1	1	0	0
10	0	1	0	0	1	0	1	1	1	0	1
11	0	1	0	0	1	1	1	1	1	1	1
12	0	1	1	1	0	0	1	1	1	1	0
13	0	1	1	0	0	1	1	1	0	1	0
14	0	1	1	1	1	0	1	1	0	1	1
15	0	1	1	1	1	1	1	1	0	0	1
16	1	0	0	0	0	0	1	1	0	0	0
17	1	0	0	0	0	1	0	1	0	0	0
18	1	0	0	0	1	0	0	1	0	0	1
19	1	0	0	0	1	1	0	1	0	0	1
20	1	0	0	1	0	0	0	1	0	1	0
21	1	0	0	1	0	1	0	1	1	1	0
22	1	0	0	1	1	0	0	1	1	1	1
23	1	0	0	1	1	1	0	1	1	0	1
24	1	1	0	0	0	0	0	1	1	0	0
25	1	1	0	0	0	1	0	0	1	0	0
26	1	1	0	0	1	0	0	0	1	0	1
27	1	1	0	0	1	1	0	0	1	1	1
28	1	1	0	1	0	0	0	0	1	1	0
29	1	1	0	1	0	1	0	0	0	1	0
30	1	1	0	1	1	0	0	0	0	1	1
31	1	1	0	1	1	1	0	0	0	0	1

R.A. Fisher, Statistical Methods for Research Workers - Introduction, First Edition 1925

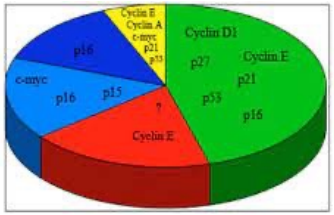
Data types

Complexity

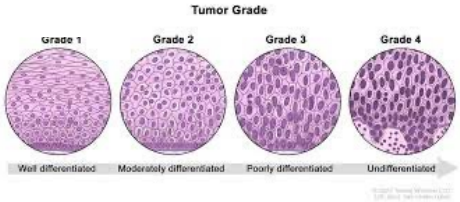
Qualitative

⇒ Binary/dichotomous

⇒ Nominal

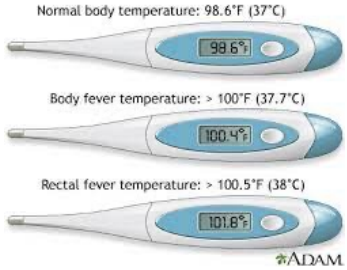


⇒ Ordinal



Quantitative

⇒ Interval scale (no true zero)



⇒ Ratio scale (true zero)

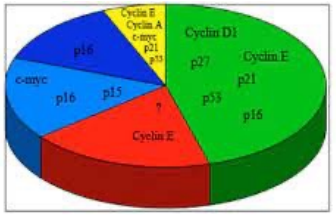
Data types

Complexity

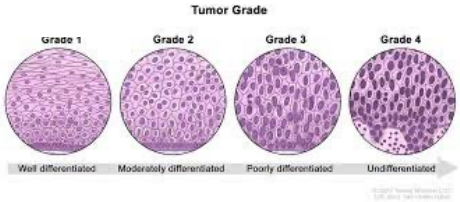
Qualitative

⇒ Binary/dichotomous

⇒ Nominal



⇒ Ordinal



Quantitative

⇒ Discrete

Number of metastases

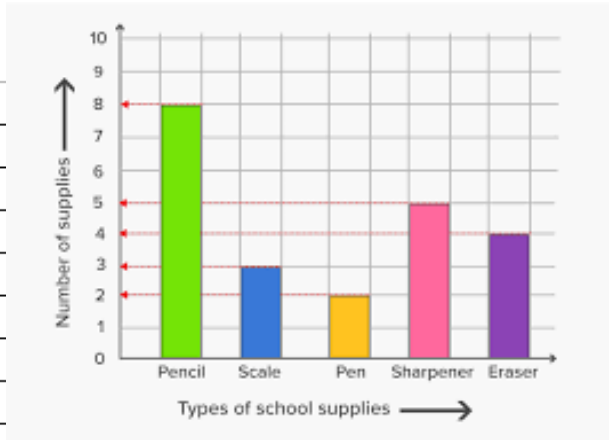
⇒ Continuous

Data analysis: descriptive statistics

Here, the data are analyzed on their own terms, essentially without extraneous assumptions.
The principal aim is the organization and summarization of the data in ways that bring out their main features and clarify their underlying structure.

Category	Sale	Percent
Category1	3500	25%
Category2	4100	29%
Category3	6350	46%
Category4	0	0%
Category5	0	0%
Total	**13950**	**100%**

Median

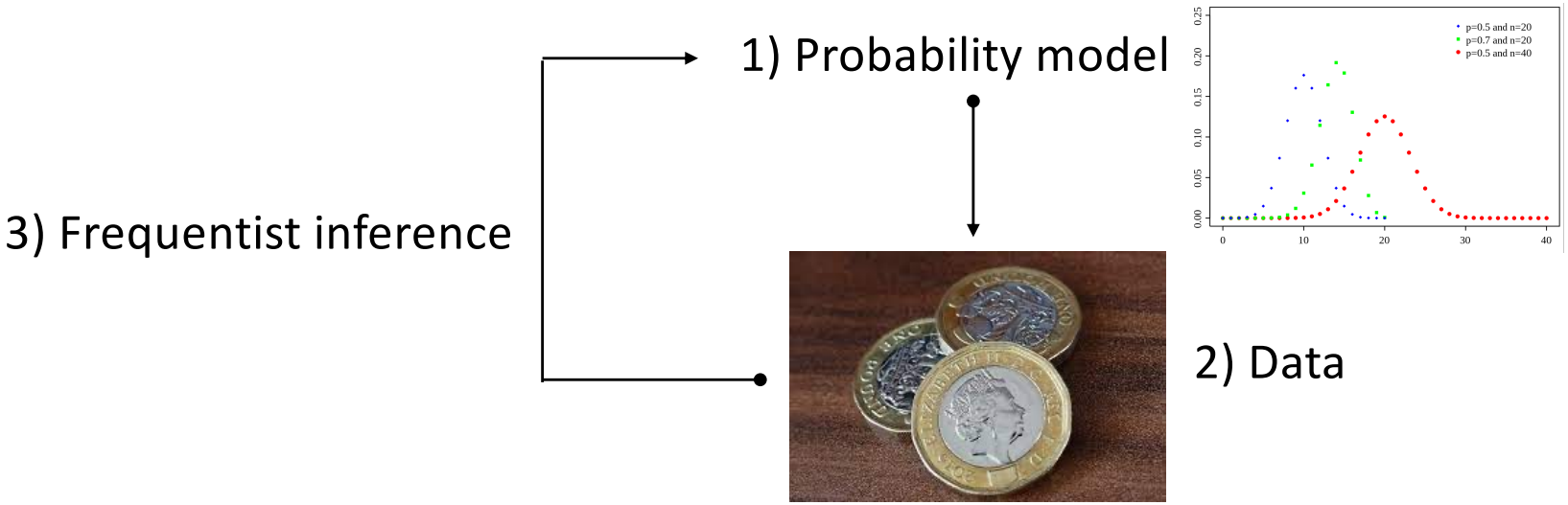


Range

E.L. Lehmann, George Casella, Theory of Point Estimation, Second Edition

Data analysis: frequentist inference

Here, the data are postulated to be the values taken on by random variables which are assumed to follow a joint probability distribution $\boldsymbol{\Phi}$.
The principal aim is to infer information about $\boldsymbol{\Phi}$.



E.L. Lehmann, George Casella, Theory of Point Estimation, Second Edition

…to be clear about frequentist inference

Population

Sample

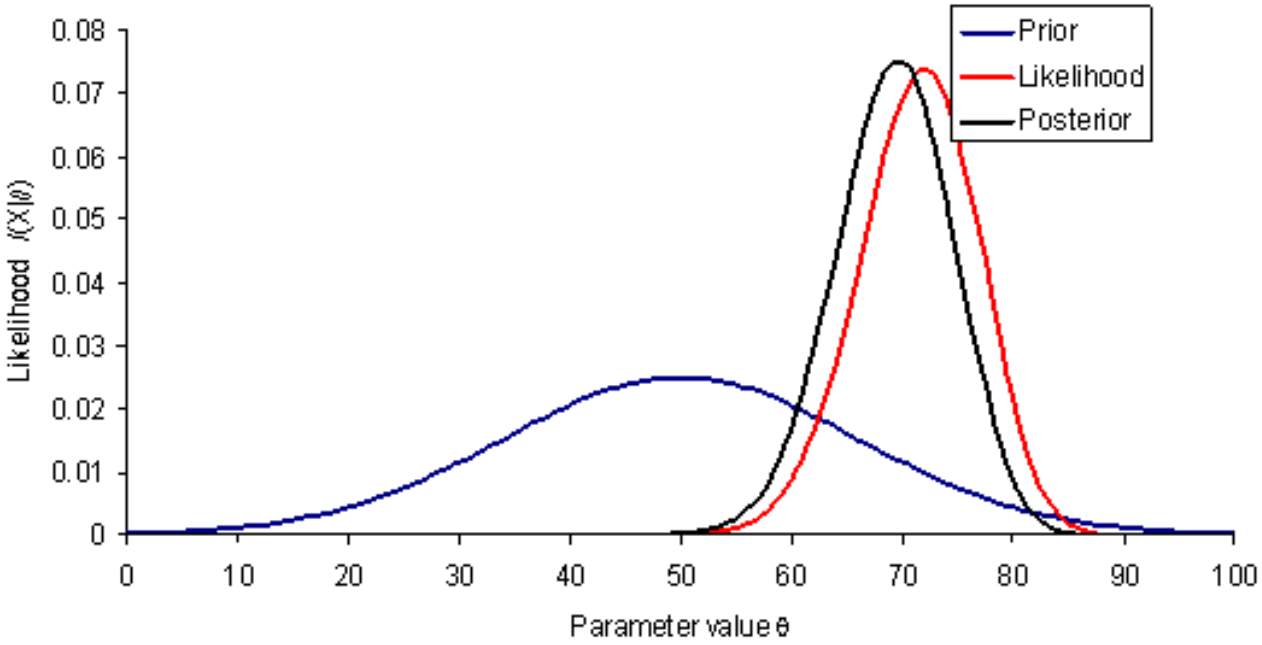
Data (x_1, x_2,…, x_n)

Inferential statistics (e.g., sample mean, std, p)

Distribution described by parameters (e.g., μ, σ, π)

Data analysis: bayesian inference

The basic paradigm of bayesian statistics



Initial beliefs concerning a parameter $\boldsymbol{\theta}$ of interest are expressed as **a prior distribution**

↓

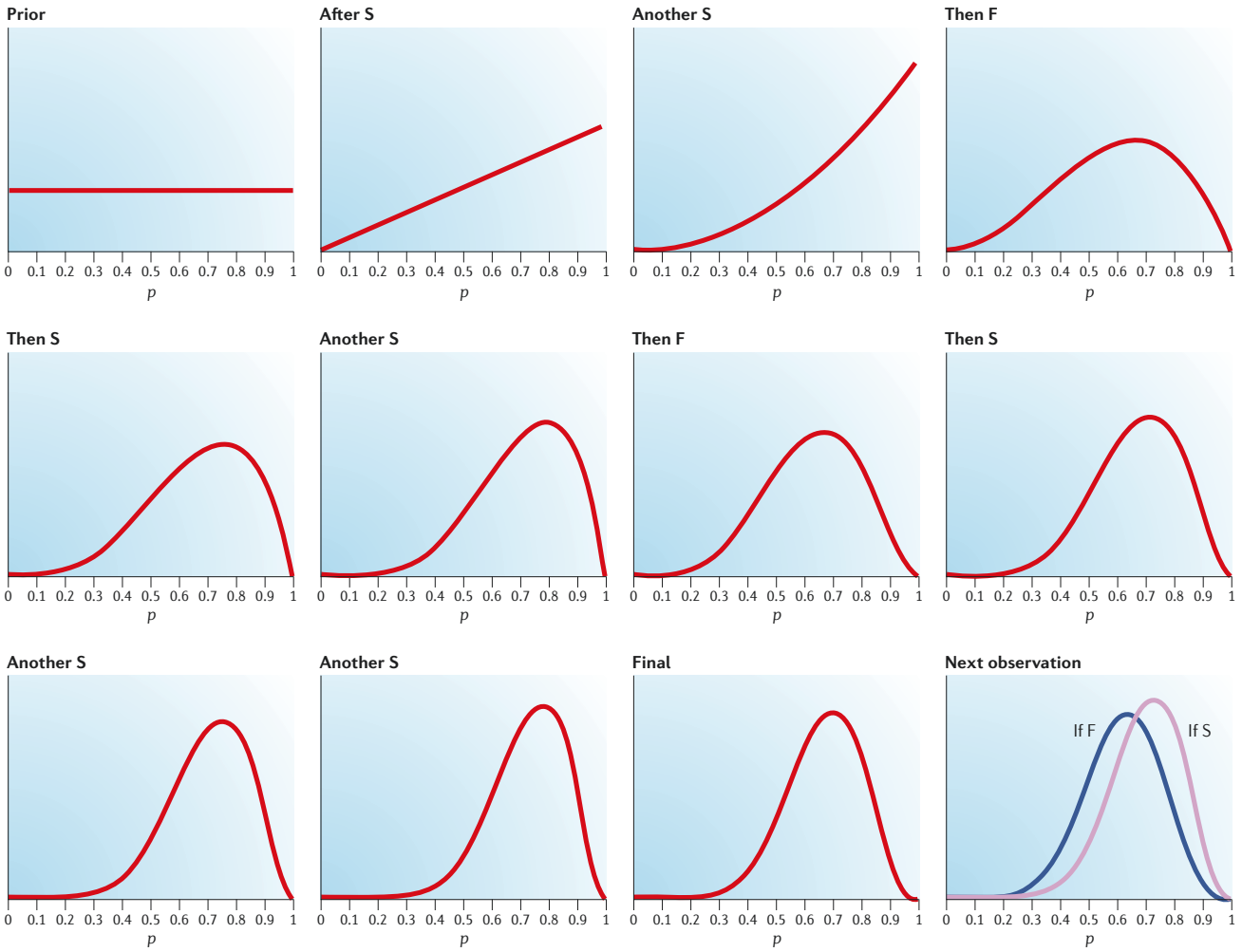
Evidence from further data is summarized by a **likelihood function** for the parameter $\boldsymbol{\theta}$

↓

Using Bayes theorem (i.e. normalized product of the prior and the likelihood) initial beliefs are updated to form the **posterior distribution**, on the basis of which conclusions on the parameter $\boldsymbol{\theta}$ should be drawn

D.J. Spiegehalter et al., Bayesian Approaches to Randomized Trials, *J.R. Statist. Soc.* A (1994) 157, *Part 3*, *pp.* 357-416

A practical example of bayesian inference



Berry DA. Bayesian clinical trials. Nat Rev Drug Discov. 2006 Jan;5(1):27-36. doi: 10.1038/nrd1927. PMID: 16485344.

Data analysis: bayesian inference

The recourse to the prior distribution on the parameters of a model is truly revolutionary. There is in fact a major step from the notion of an *unknown* parameter to the notion of a *random* parameter.

Christian P. Robert, The Bayesian Choice, Second Edition

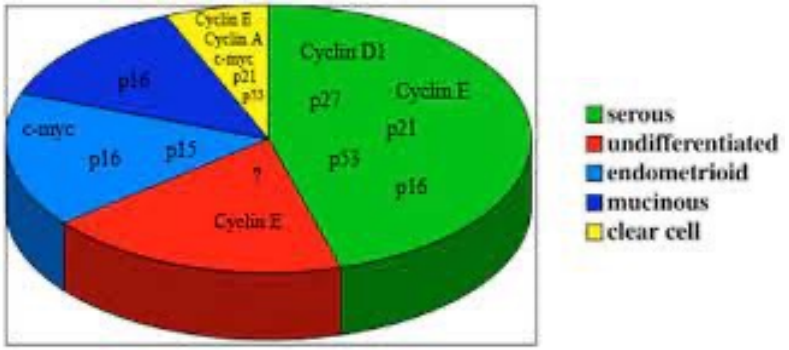
Descriptive statistics, univariate analysis

Nominal data

Histotype	N	%
Serous	48	41.7
Undifferentiated	22	19.1
Endometrioid	21	18.3
Mucinous	16	13.9
Clear cell	8	7.0

Serous is the mode.
The mode is the category that appears most often in a set of data values

Pie chart



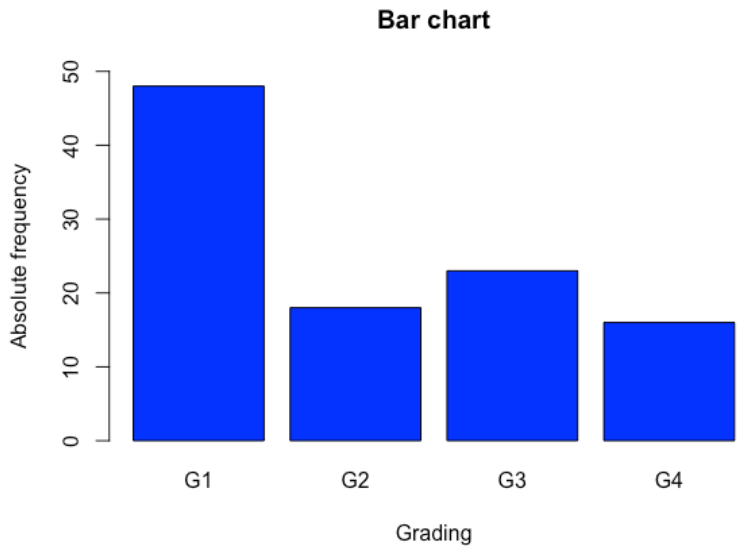
Descriptive statistics, univariate analysis

Ordinal data

Grading	N	%
G1	48	45.7
G2	18	17.1
G3	23	21.9
G4	16	15.2

→ G1 is the mode.

→ G2 is the median.
The median is the category separating the higher half from the lower half of a data sample



Descriptive statistics, univariate analysis

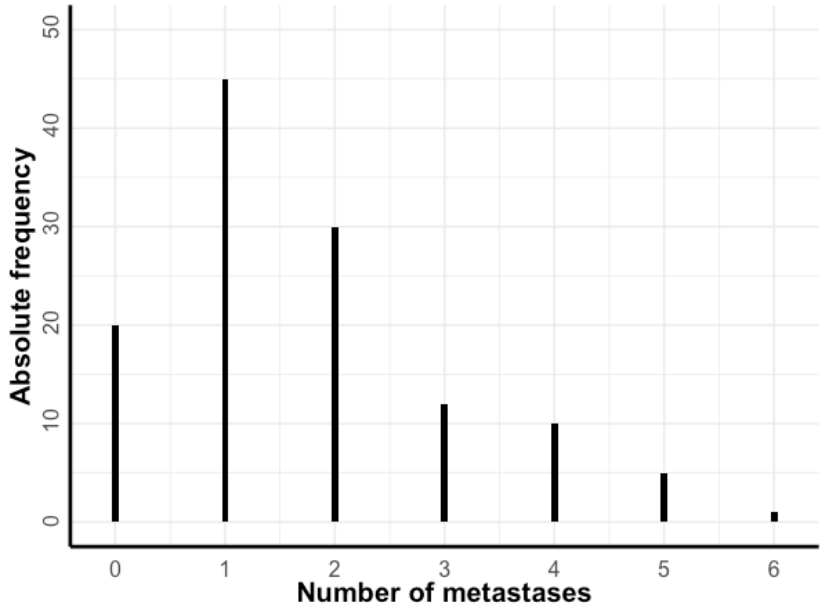
Discrete data

N. of metastases	N	%
0	20	16.3
1	45	36.6
2	30	24.4
3	12	9.8
4	10	8.1
5	5	4.1
6	1	0.8

→ 1 metastasis is the mode.

→ 1 metastatis is the median.

→ 1.7 is the mean number of metastases.



Descriptive statistics, univariate analysis

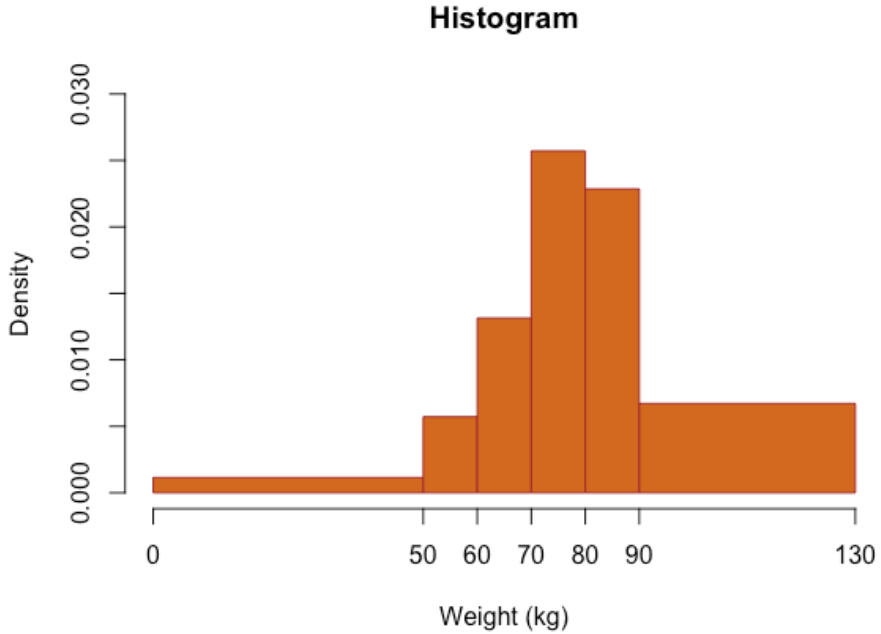
Continuous data

Weight (kg)	N (%)	N/10kg
0 -| 50	10 (5.7)	2
50 -| 60	10 (5.7)	10
60 -| 70	23 (13.1)	23
70 -| 80	45 (25.7)	45
80 -| 90	40 (22.9)	40
90 -| 130	47 (26.9)	11.8

→ 70 -| 80 is the modal interval.

→ 70 -| 80 is the median interval.

→ 81.4 kg is the mean weight of patients.



Descriptive statistics, univariate analysis

Quantitative data

Properties of the mean

- $\sum_i (a_i - \boldsymbol{\mu}) = 0$
- $\sum_i (a_i - \boldsymbol{\mu})^2 < \sum_i (a_i - x)^2,\ x \neq \boldsymbol{\mu}$
- Linearity
- Associative property

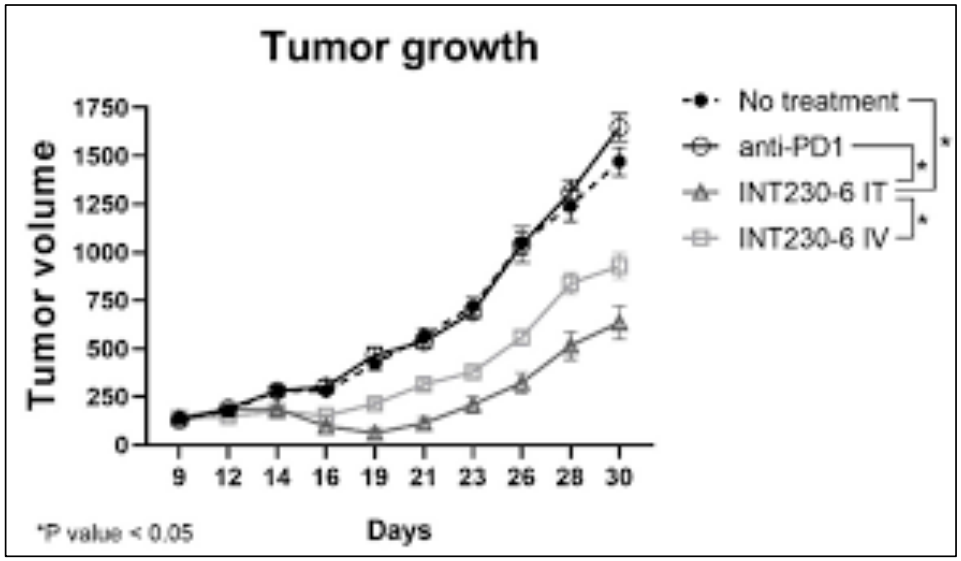
Descriptive statistics, univariate analysis

Quantitative data

Measures of variability

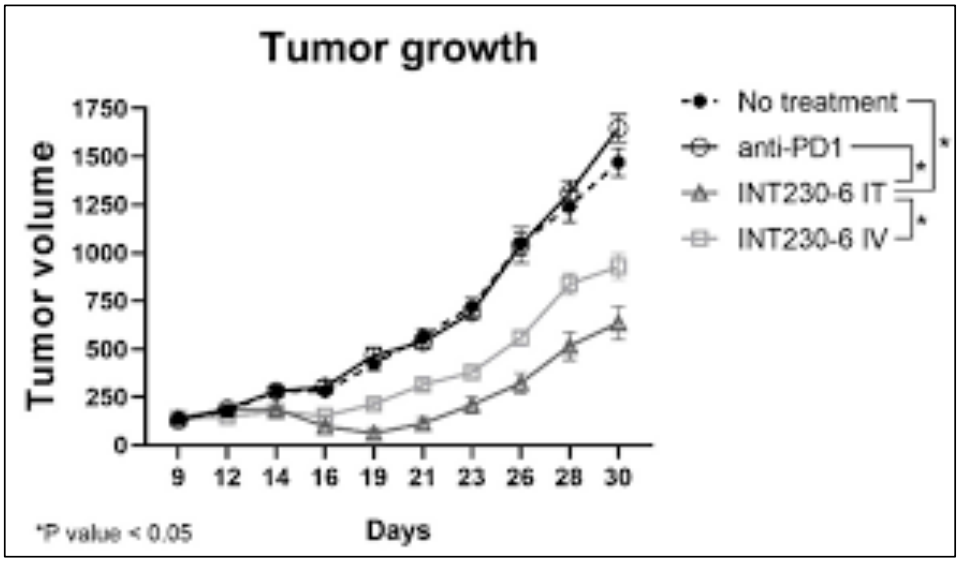
- $(\sum_i |a_i - M|)/n$, where M is a measure of central tendency
- $\sqrt{[(\sum_i |a_i - M|^2)/n]}$, if $M = \boldsymbol{\mu}$, it is called standard deviation ($\boldsymbol{\sigma}$)
- Interquartile range (IQR)

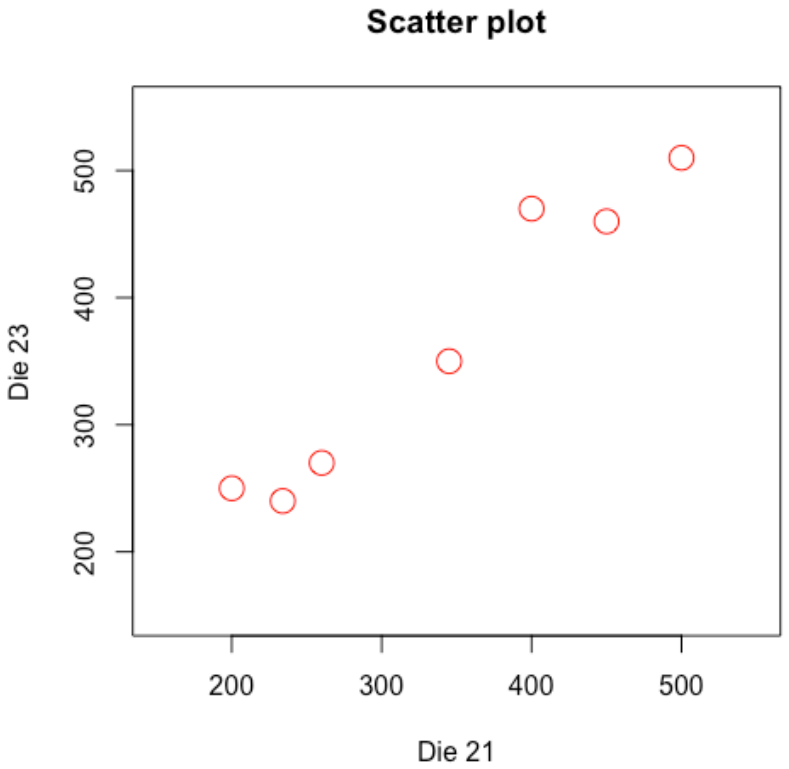
Descriptive statistics, bivariate analysis



ID mouse	Day 21, mm ^{3}	Day 23, mm ^{3}
M101	260	270
M102	234	240
M103	400	470
M104	345	350
M105	450	460
M106	200	250
M107	500	510

Descriptive statistics, bivariate analysis





Take home message:

Paired data are not independent. They correlate.

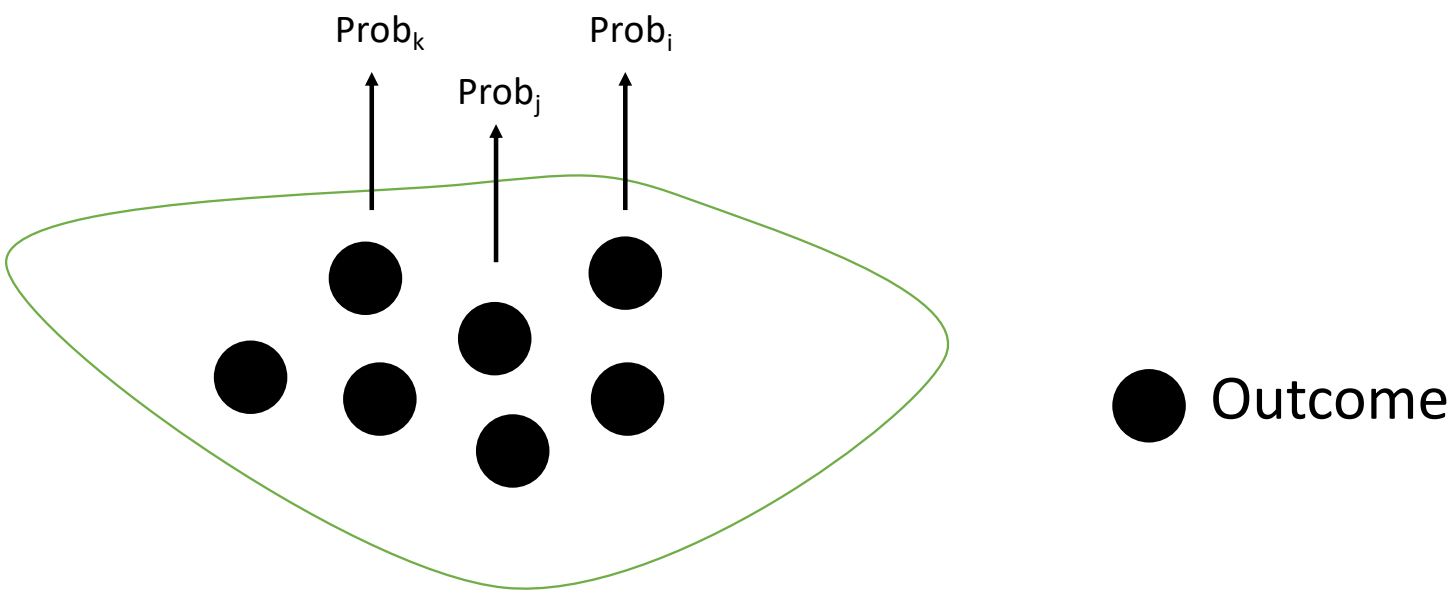
Online quiz

Exercises

http://bioinformatics-core-shared-training.github.io/IntroductionToStats

Probability distribution

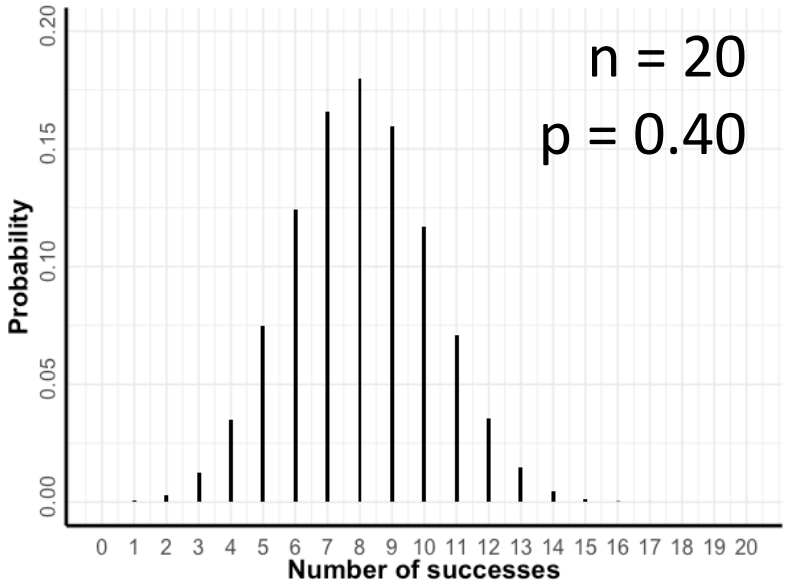
Def: In probability theory and statistics, a **probability distribution** is the *mathematical function* that gives the *probabilities* of occurrence of different possible *outcomes* for an experiment.



https://en.wikipedia.org/wiki/Probability_distribution

Binomial distribution

Def: the **binomial distribution** with parameters *n* and *p* is the discrete probability distribution of the number of successes in a sequence of *n* independent trials. Each trial (Bernoulli trial) has a binary outcome: success with probability *p* and failure with probability 1-*p*.

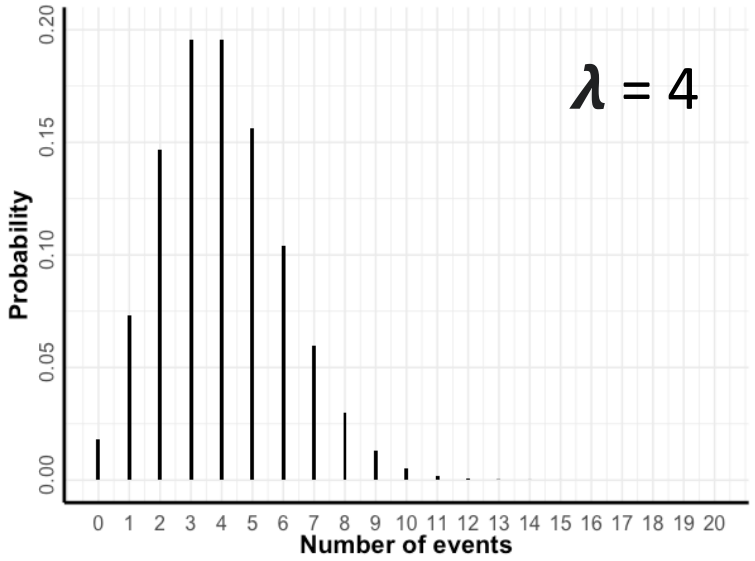


Assumptions of the binomial distribution

- The outcome of each Bernoulli trial is dichotomous
- Independence of outcomes: the outcome of each trial does not depend on the outcome of the other trials
- The probability of success is *p* for every trial

Poisson distribution

Def: the **Poisson distribution** with parameter λ is the discrete probability distribution of the number of events that occur randomly and uniformly in a fixed time interval or in a given area.



Assumptions of the Poisson distribution

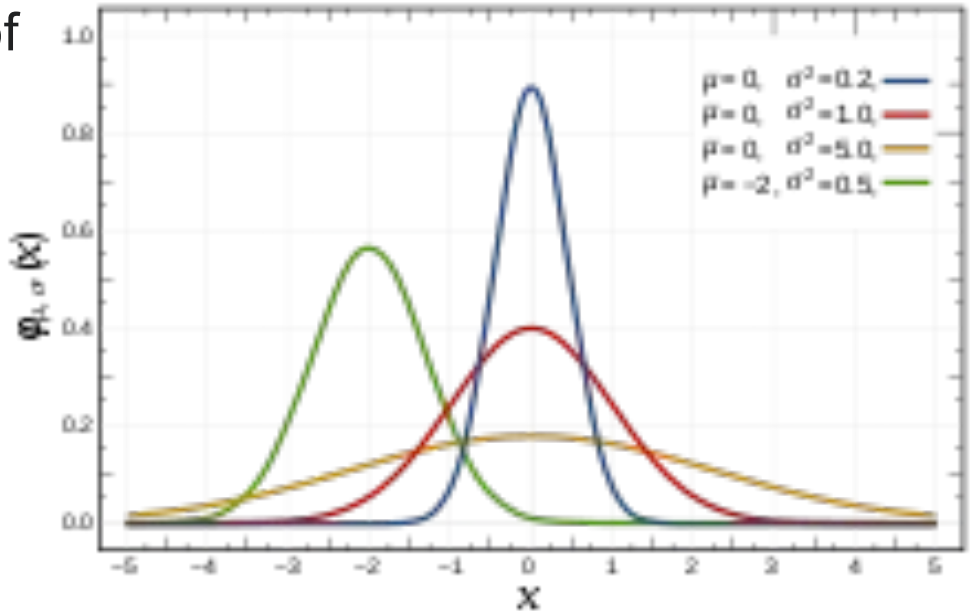
- The outcome is a count
- Independence of events: the occurrence of one event does not affect the probability that another event will occur
- Two events cannot occur at exactly the same instant in time or at the same point of the given area
- Events occur at a uniform rate over the entire time period or area. λ is the expected (mean) number of events per unit time/area

Normal distribution

Def: a **normal distribution** or **Gaussian distribution** is a type of continuous probability distribution. It is determined by two parameters:

1. the parameter μ is the mean or expectation of the distribution (and also its median and mode)

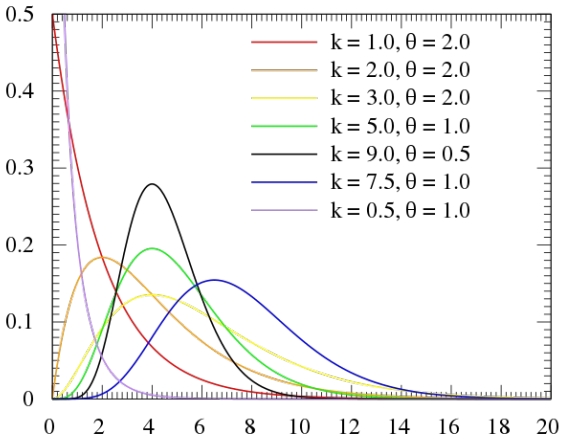
2. the parameter σ is its standard deviation.



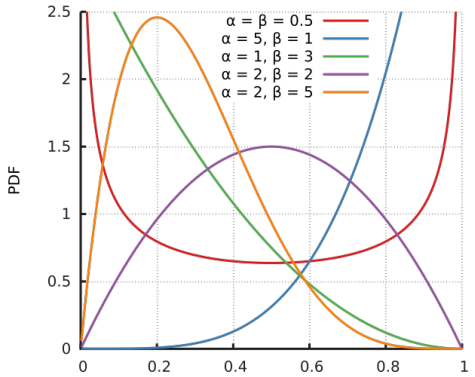
https://en.wikipedia.org/wiki/Normal_distribution

Other distributions

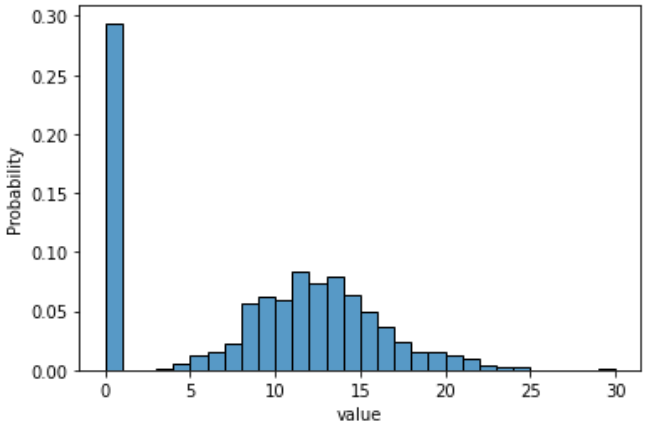
Gamma distribution



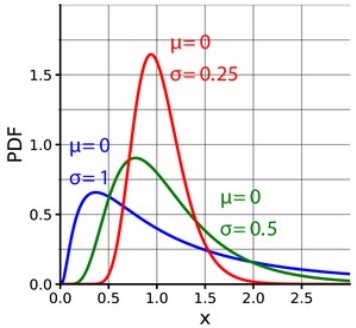
Beta distribution

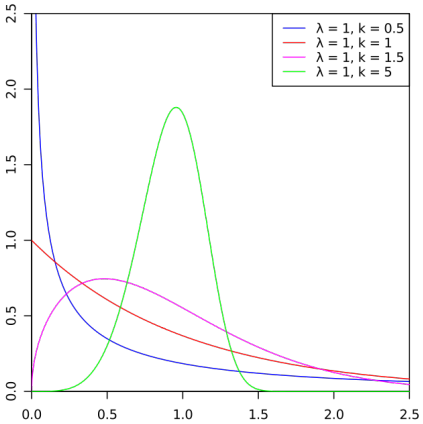


Zero-inflated Poisson distribution



Log-normal distribution





Weibull distribution

Central limit theorem (CLT)

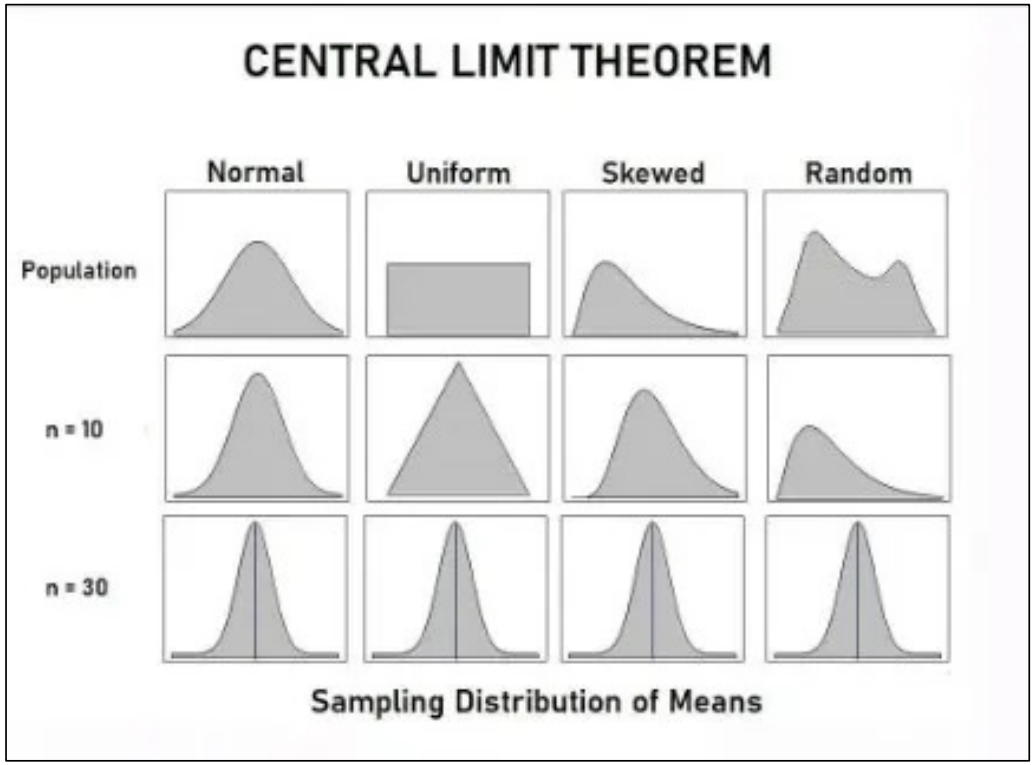
Let $X_1,\ldots,X_n$ be independent and identically distributed random variables with mean $\boldsymbol{\mu}$ and standard deviation $\boldsymbol{\sigma}$

The sample mean $\dot{x}$ is a statistic obtained by calculating the arithmetic average of the values of $X_1,\ldots,X_n$ in a sample

CLT: $\dot{x}$ is distributed as N ($\boldsymbol{\mu}$, $\boldsymbol{\sigma}/\sqrt{(n)}$) as the sample size n gets larger

Central limit theorem (CLT)

Central limit theorem



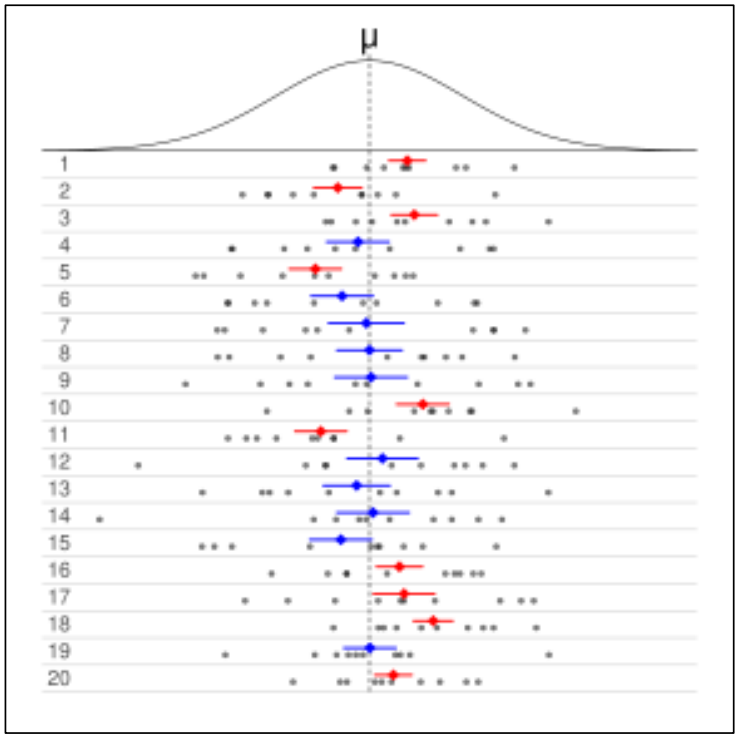
The usefulness of the CLT is that the distribution of sample means approaches normality regardless of the distribution of the population

Confidence intervals

In frequentist inference, a confidence interval (CI) is a **range of estimates** for an unknown parameter Θ.

It is computed at a designated confidence level (e.g., 95% CI). The confidence level represents the long run proportion of CIs that theoretically contain the true value of the parameter Θ.

For example, out of all intervals computed at the 95% level, 95% of them should contain the parameter's true value.



https://en.wikipedia.org/wiki/Confidence_interval

Confidence intervals for the normal distribution

Normal data, $\boldsymbol{\sigma}$ known: one sample z-confidence interval

Sample mean $\dot{x}$ is <u>exactly</u> distributed according to N ($\boldsymbol{\mu}$, $\boldsymbol{\sigma}/\sqrt{(n)}$)

95% CI = $\dot{x} \pm z_{0.975} \cdot \boldsymbol{\sigma}/\sqrt{(n)}$, where $z_{0.975} \simeq 1.96$

If you do not know $\boldsymbol{\sigma}$

Student's *t*-distribution

Let $x_1,\ldots,x_n$ be independent and identically distributed observations from a normal distribution with mean μ and std σ.

The sample mean and unbiased sample standard deviation are given by:

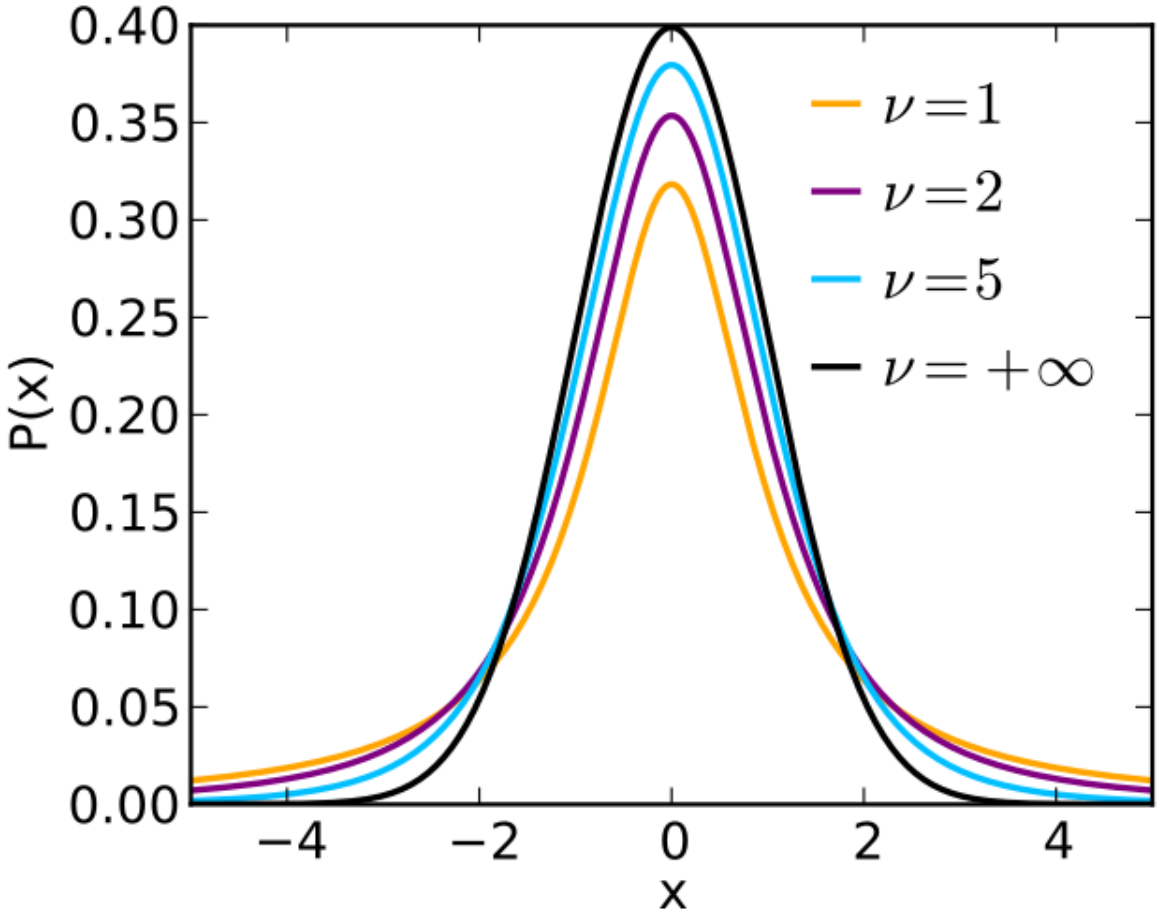
$\dot{x} = (x_1+\ldots+x_n)/n$ [biological signal collected in the sample]

$std^2 = (1/(n-1))\ \mathbf{\Sigma}_i\ (x_i - x_m)^2$ [noise collected in the sample]

$(\dot{x} - \mu) / (std / \sqrt{n}) \sim t_{n-1}$ is distributed according to a Student's *t*-distribution with n-1 degrees of freedom

The *t*-statistic has a probability distribution that not depends on the unknown σ

Student's *t*-distribution



Confidence intervals for the normal distribution

Normal data, $\boldsymbol{\sigma}$ unknown: one sample *t*-confidence interval

Sample mean $\dot{x}$ - $\boldsymbol{\mu}$ is <u>exactly</u> distributed according to [std/√(n)] · t_{n-1}

95% CI = $\dot{x} \pm t_{n-1,\,0.975} \cdot$ std/√(n). We use the t-tables to obtain these "critical" values

If data are not normally distributed…

Consequence of CLT

t-distribution methods are robust when the sample size is large ($n \geq 30$). The data should not have extreme outliers or evidence of severe skewness.

For small samples it is risky to use *t*-confidence intervals. Only use if you are sure the population is roughly normally distributed and the sample has no outliers and very little skew. Other methods (e.g. bootstrap) should be used.

Simulations

Exercises

http://bioinformatics-core-shared-training.github.io/IntroductionToStats/practical.html

Shiny web application

https://bioinformatics.cruk.cam.ac.uk/apps/stats/central-limit-theorem

Hypothesis Testing

- A hypothesis is a statement about the population(s)

  Example n.1: Carboplatin induced response in at least 70% of NSCLC patients

  Example n.2: The mean patient's blood pressure is the same in populations A and B

  Example n.3: The two populations A and B have the same height distribution

- The goal of a hypothesis test is to decide which of two complementary hypotheses is true

  Example n.1: H_0: RR < 0.70; H_1: RR $\geq$ 0.70

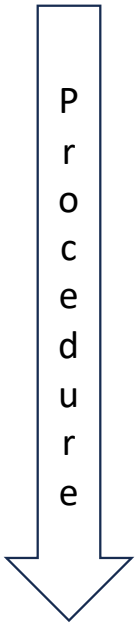
  Example n.2: H_0: $\boldsymbol{\mu}_1 = \boldsymbol{\mu}_2$; H_1: $\boldsymbol{\mu}_1 \neq \boldsymbol{\mu}$

  Example n.3: H_0: $D_A = D_B$; H_1: $D_A \neq D_B$

Hypothesis Testing

H_0: null hypothesis
H_1: alternative hypothesis

There is no symmetry between H_0 and H_1:



1st step: We assume H_0 to be true

2st step: The strength of evidence provided by the data against H_0 is measured

3st step: If a contradiction is found, H_1 is accepted.
If a contradiction is not found, the method of proof fails and the hypothesis H_0 could be either true or false

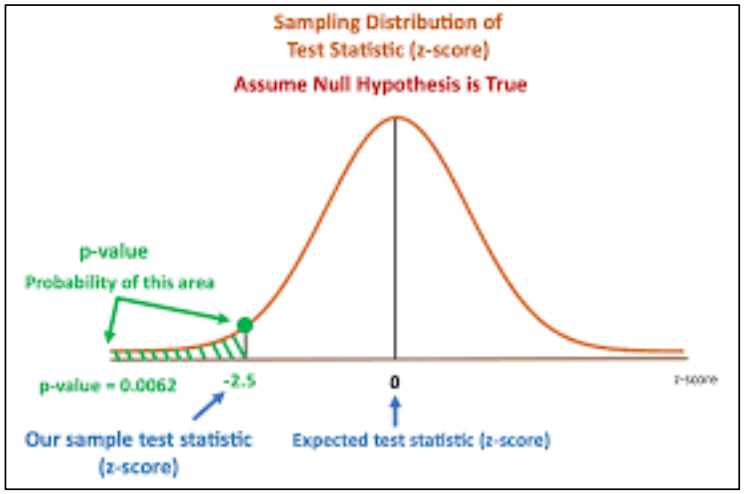
Evidence provided by the data

Procedure

Data: $x_1,\ldots,x_n$

Test statistic: $t_s = W(x_1,\ldots,x_n)$

Distribution of the test statistic under H_0:



The p-value is the statistical index used to measure the strength of evidence against H_0

Evidence provided by the data

H_0: $\theta = 0$, $\theta \in \{0, 1, 2\}$
H_1: $\theta = 1,2$

Distribution of the test statistic under H_0:

t_s	1	2	3	4
Prob (t_s | H_0)	0.980	0.005	0.005	0.010
P-value	1.00	0.01	0.01	0.020

An **α significance level** (e.g. 0.05) is simply a decision rule as to which p-values will cause one to reject the null hypothesis. In other words, it is merely a decision point as to how weird the data must be before rejecting the null model. If the p-value is less than or equal to α, the null is rejected. Implicitly, an α level determines what data would cause one to reject H0 and what data will not cause rejection. The α level rejection region is defined as the set of all data points that have a p-value less than or equal to α.

The two types of errors in hypothesis testing

		Decision	
		Accept H_0	Reject H_0
Truth	H_0	Correct decision	Type I error (α)
	H_1	Type II error (β)	Correct decision

1. If the hypothesis test incorrectly decides to reject H_0, then the test has made a Type I error (i.e. false positive decision)

2. If the hypothesis test incorrectly decides to not reject H_0, then the test has made a Type II error (i.e. false negative decision)

Statistical power

The power (1-β) of a hypothesis test is the probability to reject the null hypothesis (H_0) if H_1 is true

Distribution of the test statistic under H_0:

t_s	1	2	3	4
Prob (t_s | H_0)	0.980	0.005	0.005	0.010
P-value	1.00	0.010	0.010	0.020

Rejection region of the test with **α significance level= 0.05**

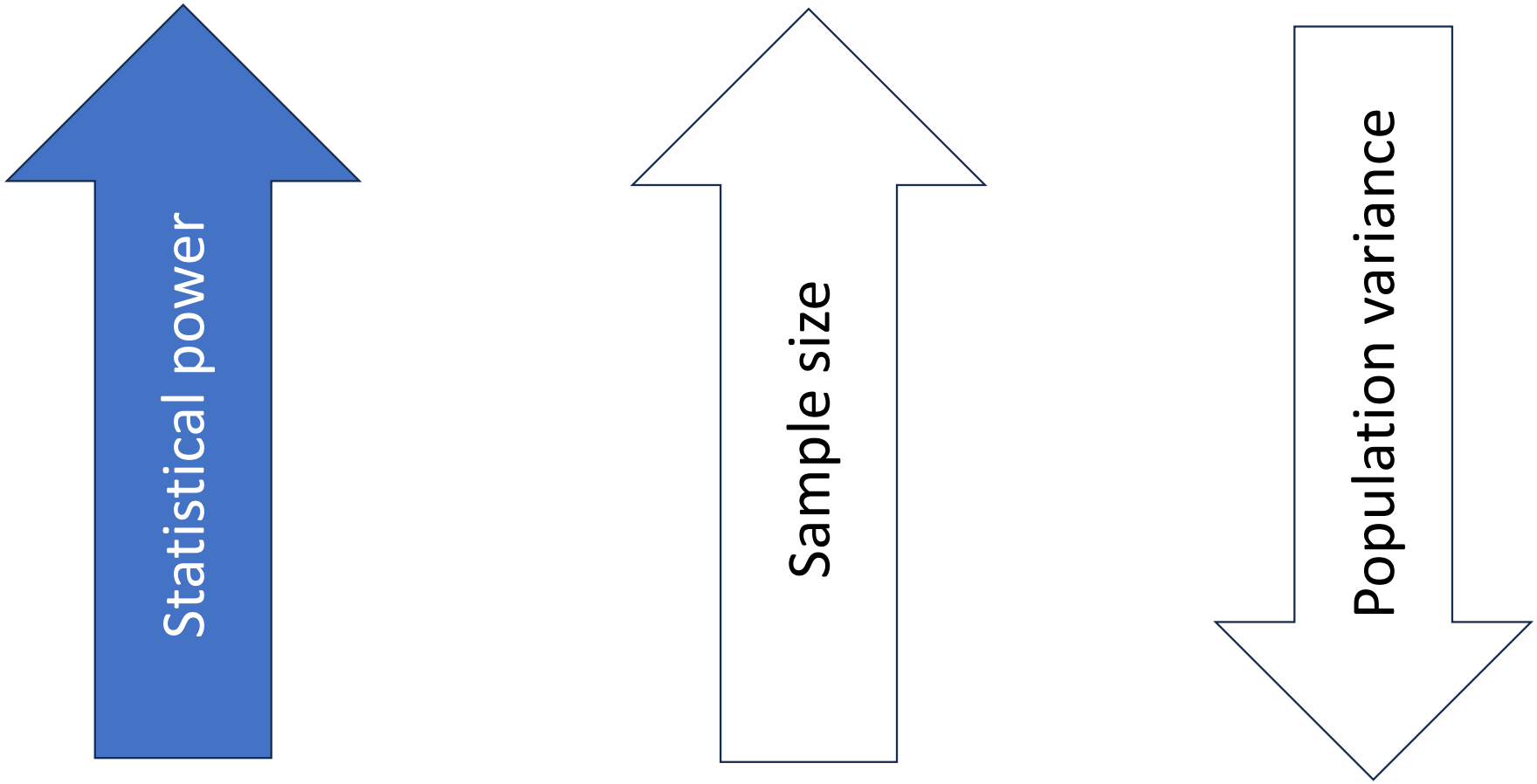
Distribution of the test statistic under H_1:

t_s	1	2	3	4
Prob (t_s | θ = 1)	0.100	0.200	0.200	0.500
Prob (t_s | θ = 2)	0.098	0.001	0.001	0.900

→ (1-β | **θ = 1**) = 0.900

→ (1-β | **θ = 2**) = 0.902

Statistical power



Multiple testing

Let m > 1 the number of null hypotheses $H_1,\ldots,H_m$ to be tested.

H_i	Not rejected	Rejected	Total
True	U	V	m_0
False	T	S	$m-m_0$
Total	W	R	m

Familywise error rate (FWER)

P(V>0), which is the probability of committing al least one Type I error.

Notes:

- If all hypothesis tests are conducted with a significance level of α, the FWER $\geq$ α
- the FWER reduces to the common Type I error rate for m=1.

Multiple testing

Controlling Familywise error rate (FWER)

- **Bonferroni method**

  Let $m > 1$ the number of null hypotheses $H_1,\ldots,H_m$ to be tested.
  Each p-value (p_i, $i=1,\ldots m$) is compared to the threshold α / m.
  Then FWER $\leq \alpha$.
  ✓ No assumption is requested

- **Sidak method**

  Let $m > 1$ the number of null hypotheses $H_1,\ldots,H_m$ to be tested.
  Each p-value (p_i, $i=1,\ldots m$) is compared to the threshold $\alpha_{SIDAK} = 1 - (1- \alpha)^{1/m}$.
  Then FWER $\leq \alpha$.
  ✓ Very strong assumption: hypothesis tests are independent

One-sample location tests

One-sample Student's *t*-test

Assumptions:

1. the data are continuous
2. sample data have been randomly sampled from a population
3. independent observations x_i, i=1,…,n
4. the population is normally distributed

Hypotheses to test:

H_0: mean of the population distribution $\boldsymbol{\mu} = \boldsymbol{\mu}_0$

H_1: $\boldsymbol{\mu} \neq \boldsymbol{\mu}_0$

Test statistic:

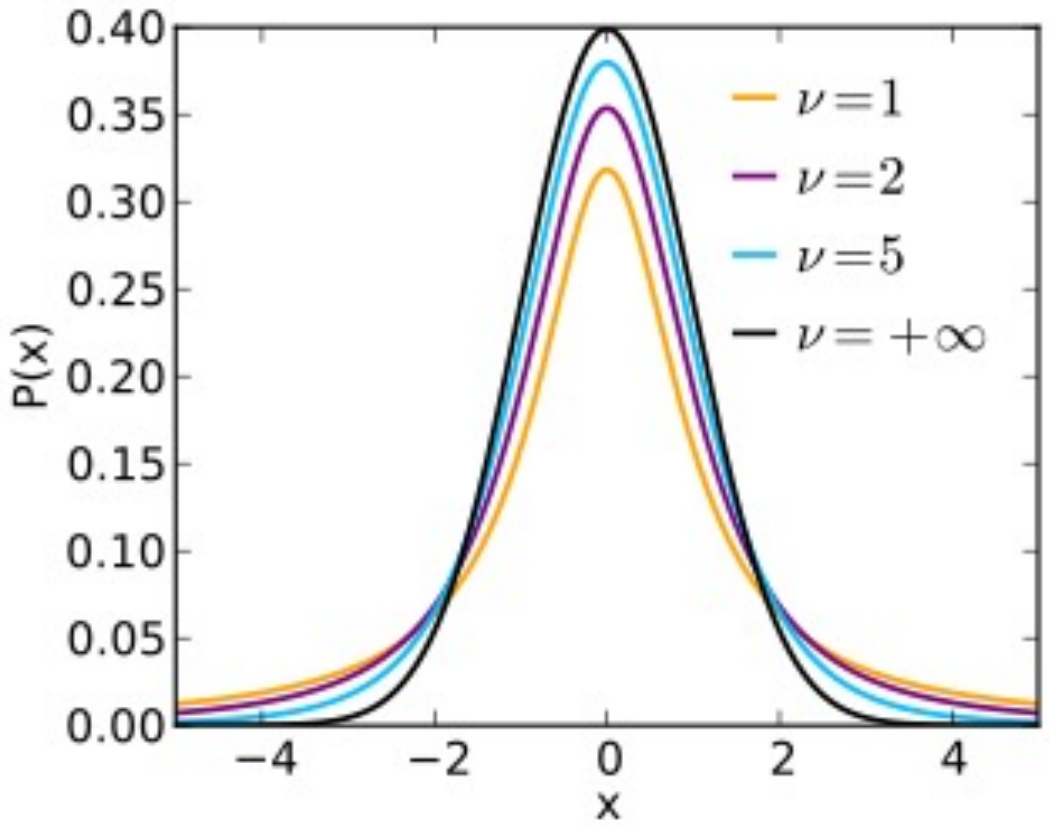
$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

ẋ = sample mean

s = sample standard deviation

One-sample Student's *t*-test

Distribution of the test statistic: *t*-distribution with n-1 degrees of freedom



Sign test

Assumptions:

1. the data are continuous
2. sample data have been randomly sampled from a population
3. independent observations x_i, i=1,…,n

Hypotheses to test:

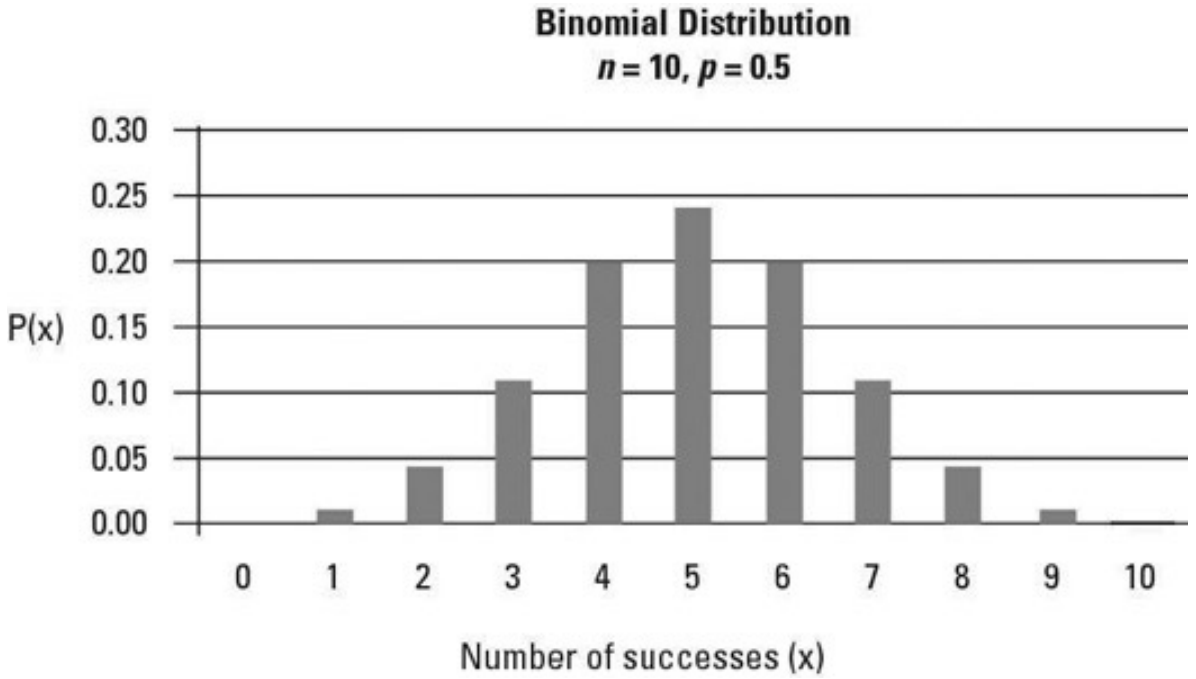
H_0: median of the population distribution $\boldsymbol{\theta} = \boldsymbol{\theta}_0$

H_1: $\boldsymbol{\theta} \neq \boldsymbol{\theta}_0$

Test statistic: number of values above (or below) $\boldsymbol{\theta}_0$

Sign test

Distribution of the test statistic: binomial distribution, X ~ Bin (n, 0.5)



In case of values equal to $\boldsymbol{\theta}$, discarde these values and apply the sign test only to the values above and below $\boldsymbol{\theta}$

Wilcoxon signed-rank test

Assumptions:

1. the data are continuous
2. sample data have been randomly sampled from a population
3. independent observations x_i, i=1,…,n
4. the population distribution is symmetric

Hypotheses to test:

H_0: median/mean of the population distribution $\boldsymbol{\theta} = \boldsymbol{\theta}_0$

H_1: $\boldsymbol{\theta} \neq \boldsymbol{\theta}_0$

Test statistic: sum of the positive signed ranks

Wilcoxon signed-rank test

n:	3
Raw data:	67, -12, 55
$\boldsymbol{\theta}$**:**	50
Absolute differences:	5, 17, 62
Signed ranks:	+1, +2, -3
Test statistic:	+3
Distribution of the test statistic:	P(+1,+2,+3) = P(+1,+2,-3) = P(+1,-2,+3) = P(+1,-2,-3) = P(-1,+2,+3) = P(-1,+2,-3) = P(-1,-2,+3) = (-1,-2,-3)= 1/8, hence…

Wilcoxon signed-rank test

Distribution of the test statistic:

Sum of signed ranks	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Probability	1/8	1/8	1/8	2/8	1/8	1/8	1/8

Two-sided p-value: 1.0
One-sided p-value: 5/8=0.625

At the significance level of 0.05, we can't reject the null hypothesis ($\boldsymbol{\theta}$=50)

Take home message

The Wilcoxon signed-rank test is more powerful than the sign test because it makes use of the magnitudes of the differences rather than just their sign.

It should be the preferred method, but it makes a stronger assumption: the distribution of the differences is symmetric.

In case this assumption is doubtful, the sign test should be used.
Graphical display is *recommended*

Take home message

The one-sample location tests could be used for paired data samples.

Each paired data is summarized by the difference and the one-sample location tests are applied to the differences.

Experimental unit	Paired data	Difference
1	23-55	-32
…	…	…
k	107-100	7

Exercises

http://bioinformatics-core-shared-training.github.io/IntroductionToStats/practical.html

Shiny web application

https://bioinformatics.cruk.cam.ac.uk/stats/shinystats/

Two-sample location tests

Two-sample Student's *t*-test

Assumptions:

1. data are continuous
2. random sampling from the two populations
3. independent observations x_i, i=1,…,n_1 and y_j, j=1,...,n_2
4. the two population distributions are normal
5. equal variances s_1^2 and s_2^2

Hypotheses to test:

H_0: $\mu_1 = \mu_2$

H_1: $\mu_1 \neq \mu_2$

Test statistic:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_p\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$$

where $S_p = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}}$

The statistic t has a Student's *t* distribution with n_1+n_2-2 degrees of freedom

Unequal variance *t*-test (i.e. Welch's *t*-test)

Assumptions:

1. data are continuous
2. random sampling from the two populations
3. independent observations x_i, i=1,…,n_1 and y_j, j=1,...,n_2
4. the two population distributions are normal

Hypotheses to test:

H_0: $\mu_1 = \mu_2$

H_1: $\mu_1 \neq \mu_2$

Test statistic:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

The statistic t has a Student's *t* distribution with degrees of freedom:

$$\nu \approx \frac{\left(\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}\right)^2}{\frac{s_1^4}{N_1^2 \nu_1} + \frac{s_2^4}{N_2^2 \nu_2}}.$$

where $\nu_i = n_i - 1$, i=1,2

Student's *t*-test and Welch's *t*-test

n_1	n_2	s_1	s_2	t-test *	Unequal *
11	11	1	1	0.052	0.051
11	11	4	1	0.064	0.054
11	21	1	1	0.052	0.051
11	21	4	1	0.155	0.051
11	21	1	4	0.012	0.046
25	25	1	1	0.049	0.049
25	25	4	1	0.052	0.048

* Type I error rate for the *t*-test and unequal variance *t*-test with nominal type I error of 0.05

When sample sizes are unequal, the Type I error probabilities of the Student's *t*-test is decidedly influenced by unequal variances. Similar results have been found for type II error probabilities and statistical power

Take home message

- Student's *t*-test is robust under violation of homogeneity of variance provided sample sizes are equal

- When sample size are unequal the type I error, type II error and statistical power of the Student's *t*-test are decidedly influenced by unequal variances

- Even when the variances are identical, the Welch's *t*-test performs well in terms of type I error, type II error and statistical power

Take home message

- Unless an argument based on logical, physical, or biological grounds can be made as to why the variances are very likely to be identical for the two populations, the Welch's *t*-test should be applied.

- It is *not recommended* to pre-test for equal variances and then choose between Student's *t*-test or Welch's *t*-test ⋆ .
  Graphical display is *recommended* to qualitatively evaluate the difference between sample variances.

⋆ Zimmerman DW. A note on preliminary tests of equality of variances. Br J Math Stat Psychol. 2004 May;57(Pt 1):173-81. doi: 10.1348/000711004849222. PMID: 15171807.

If the assumption of normality of the underlying populations is violated?

Wilcoxon rank-sum test

Assumptions: 1. data are ordinal or continuous
2. random sampling from the two populations
3. independent observations x_i, i=1,…,n_1 and y_j, j=1,...,n_2

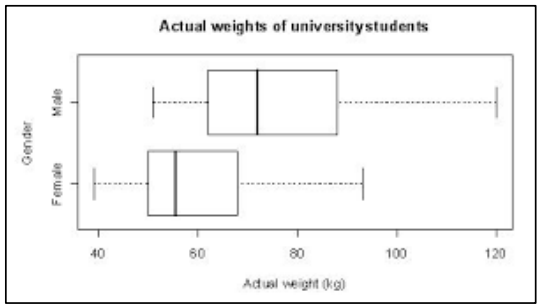
Hypotheses to test:

H_0: the population distributions are the same (G=F)

H_1: G ≠ F (two-sided H_1) or G < F * (one-sided H_1) or G > F ° (one-sided H_1)

* G is shifted to the left of F

° G is shifted to the right of F



Test statistic: sum of the ranks from one of the two groups

Calculation of the test statistic

ID mouse	Group	Outcome	Rank	Sum rank	Average rank	Sum of ranks	
1	A	0	1			Group A:	162.5
5	A	0	2	10	2.5	Group B:	302.5
8	A	0	3				
14	A	0	4				
6	A	1	5				
9	A	1	6				
11	A	1	7	45	7.5		
12	A	1	8				
15	A	1	9				
21	B	1	10				
2	A	2	11				
7	A	2	12	50	12.5		
24	B	2	13				
25	B	2	14				
3	A	3	15				
13	A	3	16				
16	B	3	17				
20	B	3	18	126	18		
23	B	3	19				
26	B	3	20				
29	B	3	21				
4	A	4	22				
17	B	4	23				
18	B	4	24	120	24		
19	B	4	25				
28	B	4	26				
22	B	5	27	55	27.5		
30	B	5	28				
27	B	7	29	29	29		
10	A	8	30	30	30		

Distribution of the test statistic

Group 1, ranks	**3,4,5**	**2,4,5**	**1,4,5**	**2,3,5**	**1,3,5**
Test statistic	12	11	10	10	9
Probability under H_0	0.1	0.1	0.1	0.1	0.1

Group 1, ranks	**2,3,4**	**1,3,4**	**1,2,4**	**1,2,3**	**1,2,5**
Test statistic	9	8	7	6	8
Probability under H_0	0.1	0.1	0.1	0.1	0.1

  $n_1 = 3$
  $n_2 = 2$

- Simulation: rank j as the same probability to be assigned to one group or the other

- For large samples, a normal approximation with known mean and variance can be applied

Parametric vs non-parametric tests

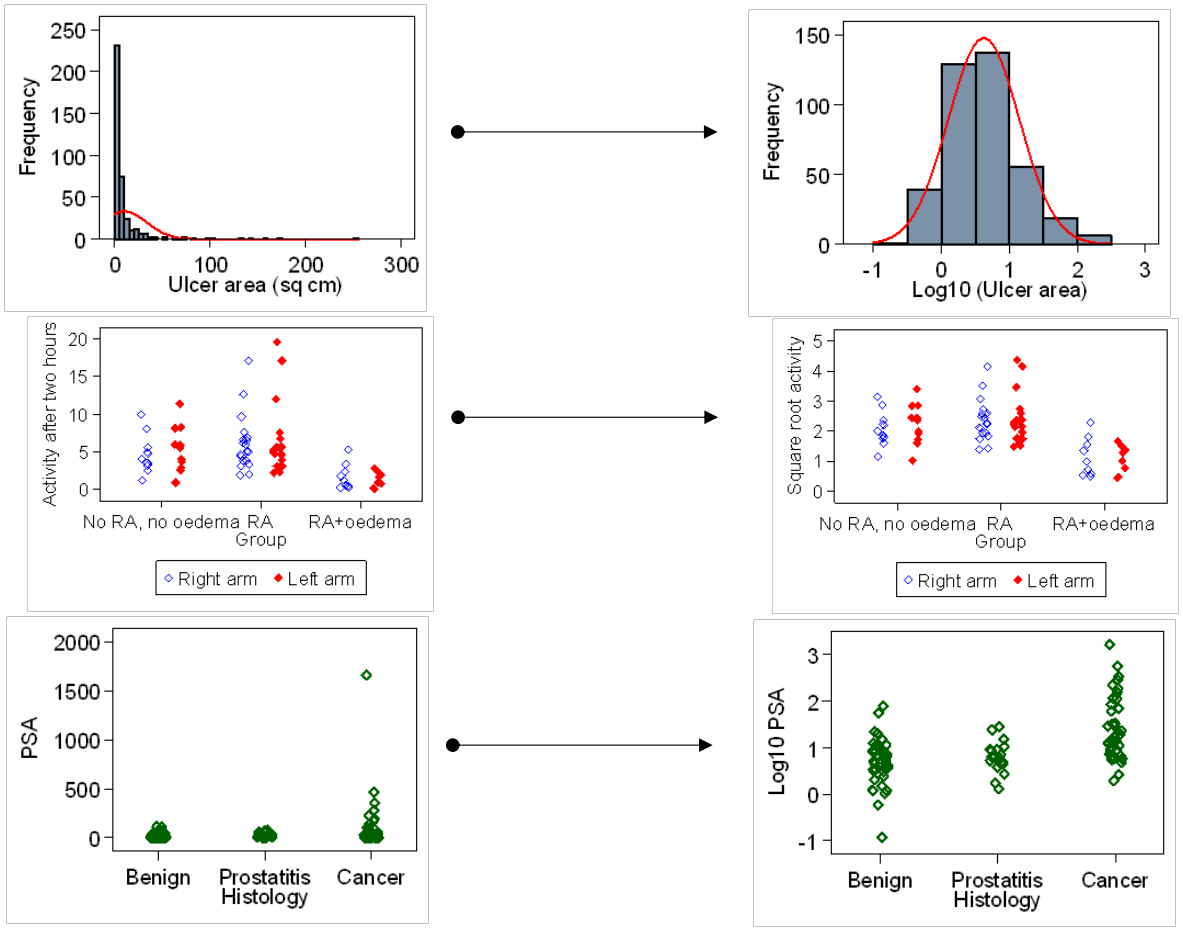
Situations which may suggest the use of non-parametric tests:

1. When one outcome has a distribution other than normal
2. When the data are ordered with many ties or are rank ordered
3. When the data has notable outliers
4. When there is a small sample size or very unequal groups.

Data transformations

We can transform the data mathematically…

- to make them fit the normality more closely
- to obtain more similar variances
- to handle outliers



The most common used transformations

We can transform the data mathematically into…

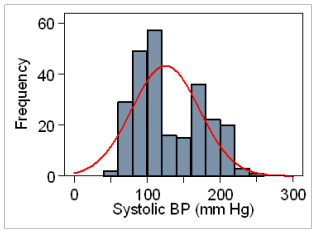
1. the logarithm ($x_i > 0$, i=1,…n)
2. the square root ($x_i \geq 0$, i=1,…n)
3. the reciprocal ($x_i > 0$, i=1,…n)

Take home message:

- These transformations could be useful to obtain normality, similar variance and handling outliers

- The best choice depends on the relationship between variability and mean. Graphical display of data is useful to choose the best transformation

- Not all data can be transformed successfully



Hypothesis to be tested after data transformations

Assumptions: 1. Student's *t*-test assumptions or
2. Welch's *t*-test assumptions

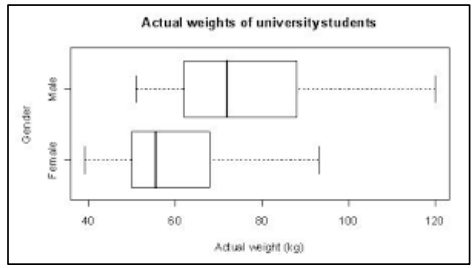
Hypotheses to test:

H_0: The population distributions are the same (G=F) * *

H_1: G ≠ F (two-sided H_1) or G < F * (one-sided H_1) or G > F ° (one-sided H_1)

* G is shifted to the left of F

° G is shifted to the right of F

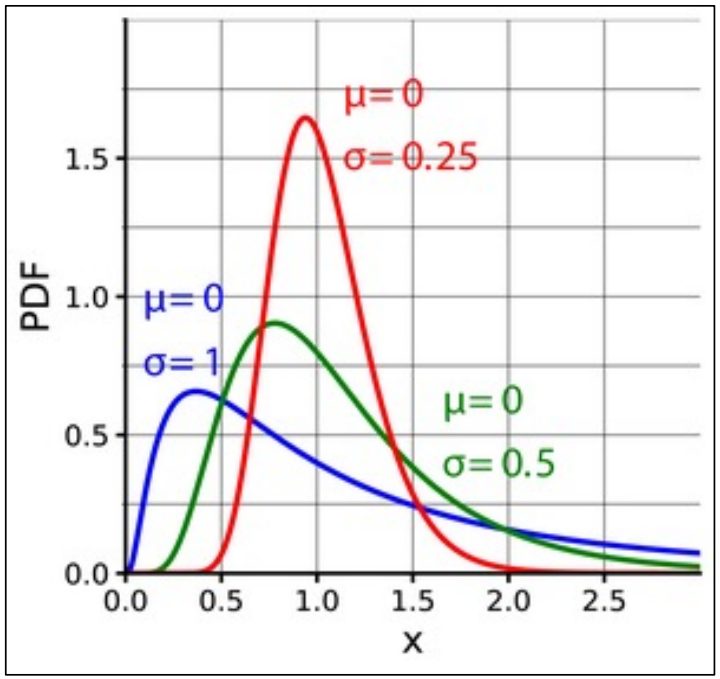


* * Previous data transformations are monotonic. Hence,
G=F on the natural scale if and only if G=F on the transformed scale

Test statistic: Student's test statistic or Welch's test statistic

Hypothesis to be tested after data transformations

Log-normal distribution



Properties of the log-normal distribution

- Mean log-normal: $\exp(\boldsymbol{\mu} + \boldsymbol{\sigma}^2 / 2)$
- Median log-normal: $\exp(\boldsymbol{\mu})$

Consequences

- If $\boldsymbol{\mu}_1 = \boldsymbol{\mu}_2$ then $\text{Median}_1 = \text{Median}_2$
- If $\boldsymbol{\mu}_1 = \boldsymbol{\mu}_2$ and $\boldsymbol{\sigma}_1 \neq \boldsymbol{\sigma}_2$ then $\text{Mean}_1 \neq \text{Mean}_2$

Exercises

http://bioinformatics-core-shared-training.github.io/IntroductionToStats/practical.html

Shiny web application

https://bioinformatics.cruk.cam.ac.uk/stats/shinystats/