Basic Biostatistics and Bioinformatics

Common statistical tests

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Illustration: Amrei Binzer-Panchal

Basic Biostatistics and Bioinformatics

A seminar series on the fundamentals

Organised by *SLUBI* and *Statistics at SLU*

Presentation of background and a practical exercise

Topic suggestions are welcome

SLUBI

- SLU bioinformatics center
- Weekly online drop-in (Wednesdays at 13.00)
- slubi@slu.se, https://www.slubi.se
- Alnarp: Lizel Potgieter (Dept. of Plant Breeding)

Statistics at SLU

- SLU statistics center
- Free consultations for all SLU staff
- statistics@slu.se
- Alnarp: Jan-Eric Englund and Adam Flöhr (Dept. of Biosystems and Technology)

Today

Some fundamental ideas

Type of response variable

Type of explanatory variable(s)

Method of data collection

Linear models as a unifying approach

Fundamental ideas

Simplification for the sake of generalization

Descriptive

- Summarizing collected data

Inferential

- Tests to identify underlying patterns

Predictive

- Projecting to non-observed cases

Population to sample

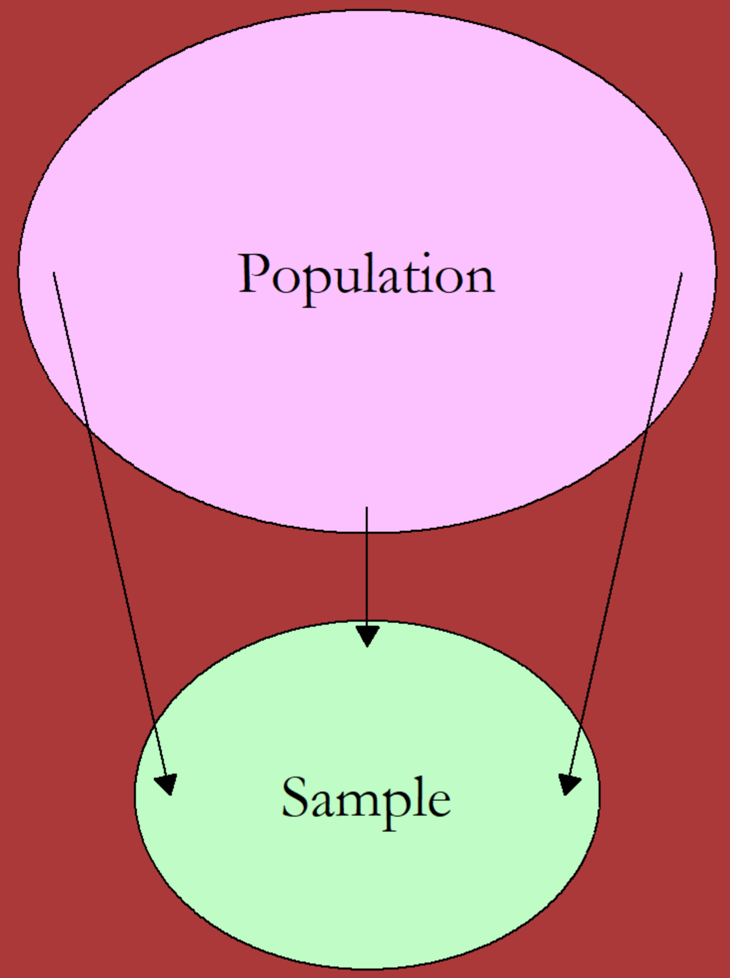
We generalize to some *population* of individual units

Seldom possible to observe full population

Draw a subset of individuals (a *sample*)

This sampling has some randomness too it

but given some assumptions about the population and the method of drawing a sample, we know what the sample *should* look like



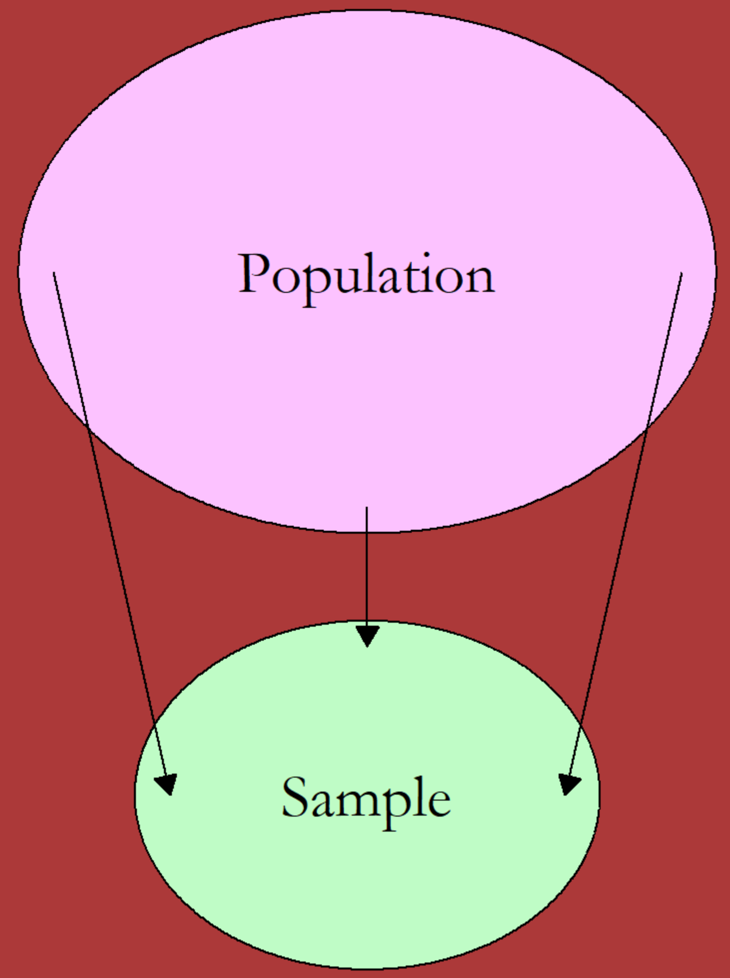
Sample to test

This population-sample setup is the basis of null hypothesis significance testing

Assume a certain population property (a *null hypothesis*)

Calculate the probability of getting the observed sample if that hypothesis is true (a *p-value*)

If that probability is small, the null hypothesis is assumed false (a *significant result*)



Choice of model/test

NHST is a general setup for inference

The exact type of test depends on the situation

Three main components

- Type of response variable
- Type of explanatory variables
- Method of data collection

Type of response variable

The response variable is whatever property we are measuring

Two connected matters

- The type of variable
- The distribution of the outcomes

Some typical categorisations

Quantitative or qualitative

- Quantitative: numerical
- Qualitative: non-numerical

Discrete or continuous numerical variables

- Discrete: subset of numbers as possible outcomes
  - Typically whole numbers (1, 2, 3 etc)
- Continuous: any decimal number is a possible outcome

Type of outcome

- Binary variables. Two possible outcomes
- Proportions. Outcomes between zero and one
  - Discrete case: number of successes of a total
  - Continuous case: any number in the 0-to-1-range

Measurements of scale

Also known as Stevens' typology

- Nominal
  - *Names*. Outcomes in categories
  - Two outcomes are either equal or different
- Ordinal
  - *Order*. Outcomes in ordered categories
  - One outcome is smaller, equal or greater than another
- Interval
  - Numerical outcome
  - The difference between two outcomes is meaningful
- Ratio
  - Numerical outcome
  - The ratio of two outcomes is meaningful

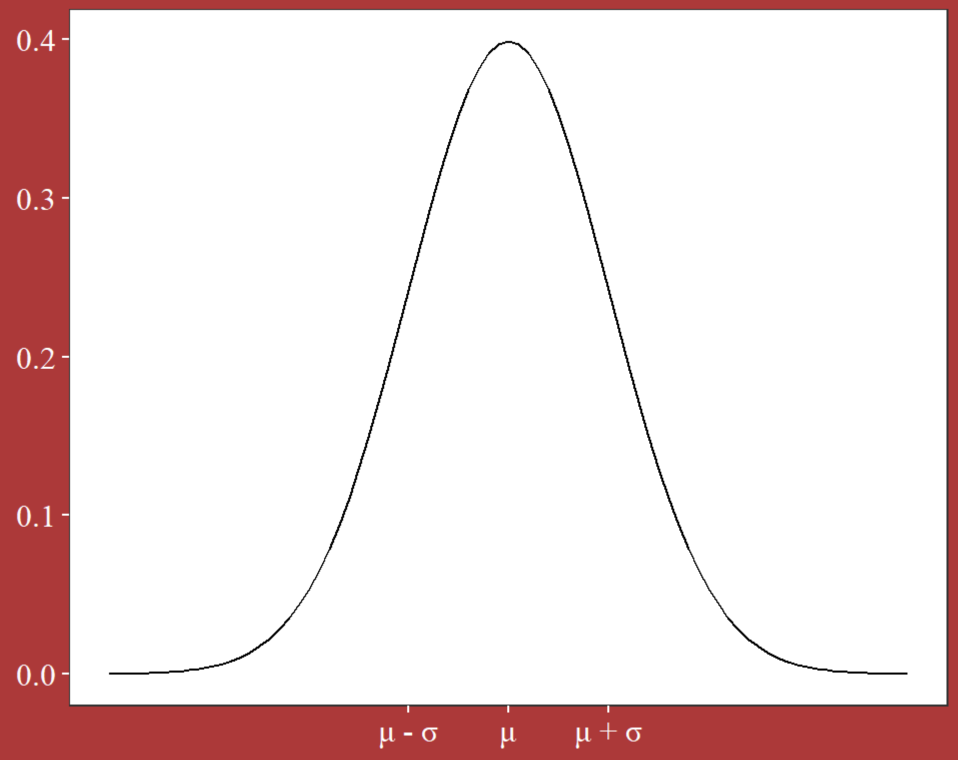
Distributions

The observations have some random variation

This randomness can be expressed with a probability distribution

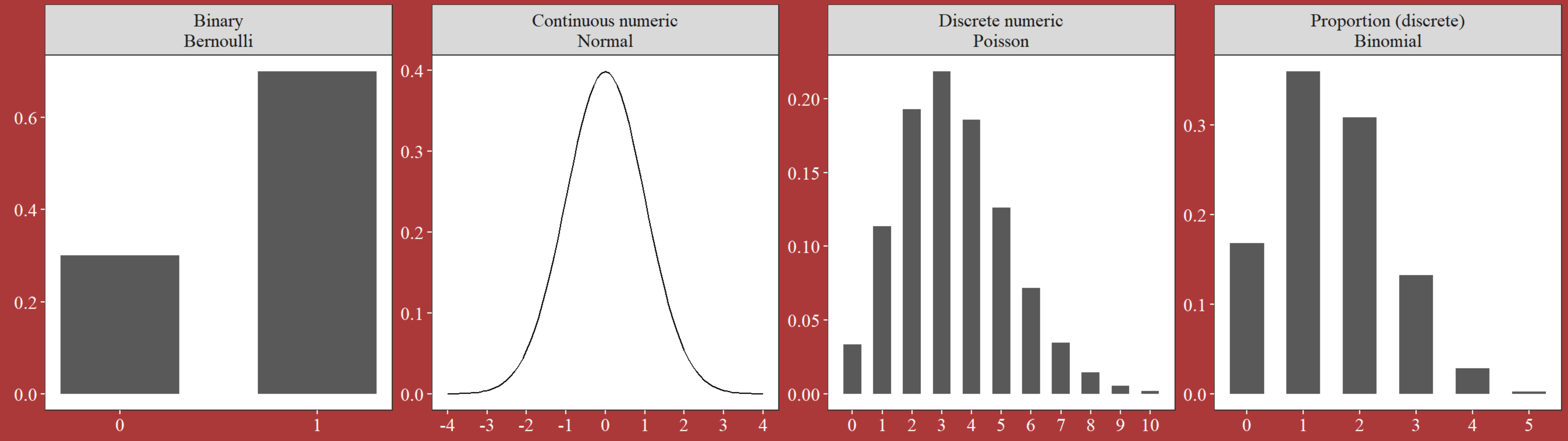
The distribution assigns a probability for each possible outcome

Distributions depend on *parameters*, estimated from collected data



The type of distribution depends on the type of variable

Variable type	**Scale**	**Common distribution**
Continuous numeric	Interval, ratio	Normal distribution
Discrete numeric	Interval, ratio	Poisson distribution
Binary	Nominal	Bernoulli distribution
Discrete proportion	Interval, ratio	Binomial distribution
Categorical	Nominal, ordinal	*Ad-hoc* distributions



Type of explanatory variable

The scientific question is typically to see how the response *depends* on another variable

Often called the *explanatory* or *independent* variable

Two distinct cases

- Categorical explanatory variable
  - The explanatory variable specifies a group
  - Want to test for a group difference
  - Test choice depends on number of groups
- Numerical explanatory variable
  - Want to test for a connection between explanatory variables and response variables

Data collection

Test choice also depends on the method of data collection

Most fundamental tests assume observations are independent random draws from the population

When comparing two treatment groups the data is often in *paired observations*

The observational units are divided into pairs and each treatment group is used once

This generalizes to multiple groups as *blocks*

The observational units are divided into blocks and each treatment group is used once per block

Observational units and pseudo-replicates

Another common collection trait is multiple observation on the same observational unit

- Multiple leafs from a plant
- Multiple soil samples from the same treatment plot
- Technical replicates of the same biological sample

This will result in non-independent observations

Sometimes referred to as pseudo-replicates

One can aggregate by taking the average (mean)

Choice of test

We get different test for different situations

The setup is always the same:

- The null hypothesis is that there are no differences / no connections between variables
- The p-value captures the probability of the observed data if that null is true
- A low p-value gives rejection of the null

In the background a *test-value* is calculated and the p-value is based on that value following a specific *test-distribution*

Tests are typically named after that distribution (t-tests, F-tests, χ^2-tests) or after an early user of the test

Table of basic tests

Response \ Explanatory	Categorical	Numerical
Nominal	χ^2	(Nominal regression)
Ordinal	χ^2	(Ordinal regression)
Binary	χ^2- or z-test	(Binary/logistic regression)
Interval/Ratio	t-test or F-test	Correlation or regression
Non-parametric	Kruskal-Wallis	Spearman correlation

Cases in brackets are not common introductory material

It is also possible to see the t-test (or F-test) for ordinal or non-normal numerical responses

This can be justified by the *central limit theorem*

Example data

We can create some random data in R

Three different explanatory variables

- one grouping with 2 levels,
- one grouping with 4 levels,
- and one numerical

Three different response variables:

- a nominal response,
- a binary response,
- and a numeric response

```r
library(tidyverse)
set.seed(250310)
dat <- tibble(group_1 = rep(c("A","B"), each = 40),
              group_2 = rep(c("a", "b", "c", "d"), each = 20),
              numeric_explanatory = runif(80),
              nominal_response = sample(c("a", "b", "c"), 80, replace = T),
              binary_response = sample(c("a", "b"), 80, replace = T),
              numeric_response = rnorm(80) + (group_1 == "A") + numeric_explanatory)
dat
```

```
## # A tibble: 80 × 6
##    group_1 group_2 numeric_explanatory nominal_response binary_response
##    <chr>   <chr>                 <dbl> <chr>            <chr>
##  1 A       a                    0.235  b                a
##  2 A       a                    0.310  b                b
##  3 A       a                    0.244  b                b
##  4 A       a                    0.568  b                a
##  5 A       a                    0.0716 b                b
##  6 A       a                    0.386  b                b
##  7 A       a                    0.509  b                b
##  8 A       a                    0.458  c                a
##  9 A       a                    0.399  c                b
## 10 A       a                    0.647  c                b
## # ℹ 70 more rows
## # ℹ 1 more variable: numeric_response <dbl>
```

Nominal or ordinal response

Categorical explanatory

Connection between variables can be tested with a χ^2-test on a cross-table

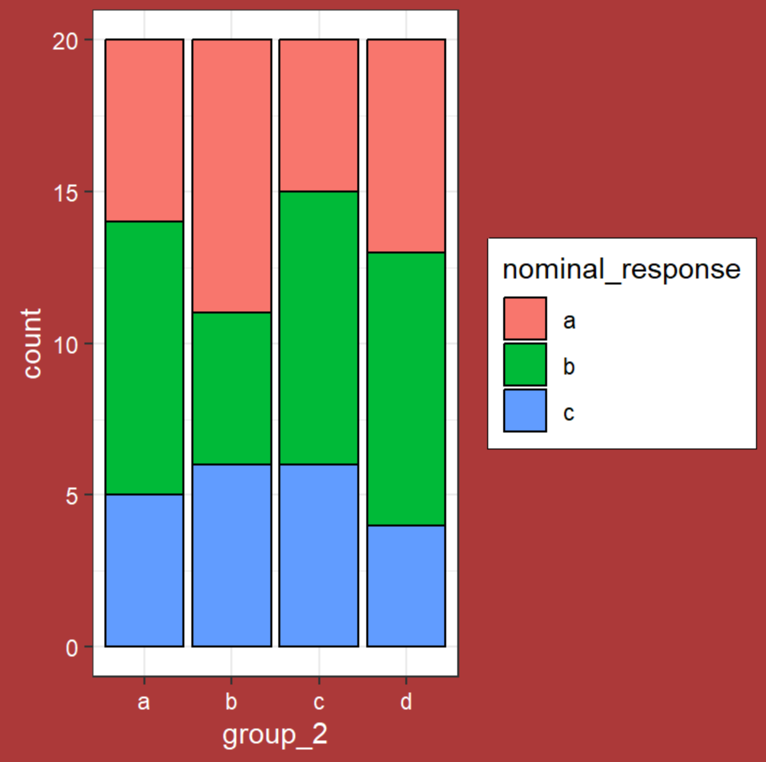
```r
table(dat$group_2, dat$nominal_response)
```

```
##
##      a b c
##    a 6 9 5
##    b 9 5 6
##    c 5 9 6
##    d 7 9 4
```

```r
chisq.test(table(dat$group_2, dat$nominal_response))
```

```
##
##  Pearson's Chi-squared test
##
## data:  table(dat$group_2, dat$nominal_response)
## X-squared = 3.3201, df = 6, p-value = 0.7677
```

```r
ggplot(dat, aes(group_2, fill = nominal_response)) +
  geom_bar(color = "black")
```



Binary response

Categorical explanatory with more than two categories

Connection between variables can be tested with a χ^2-test on a cross-table

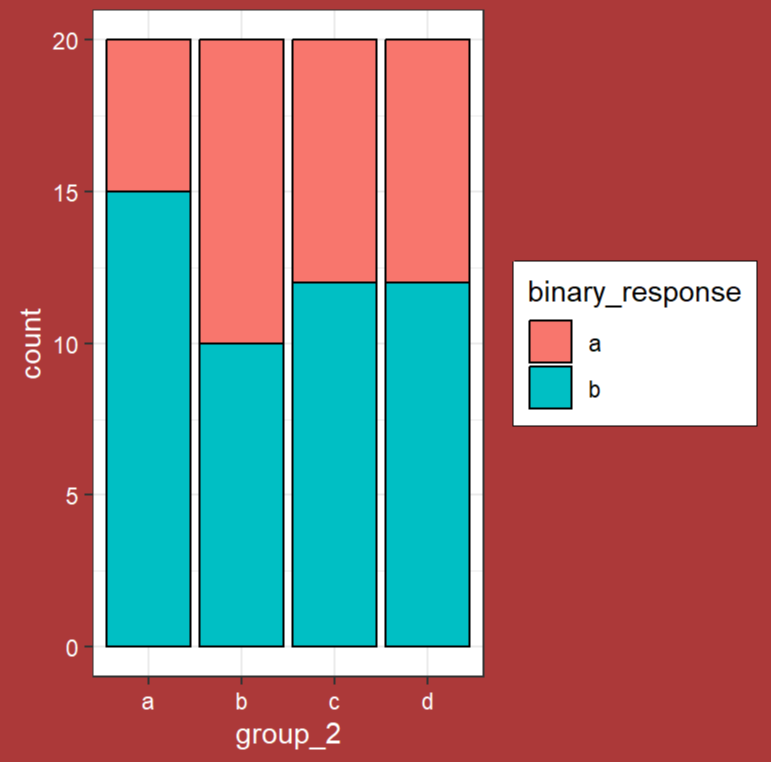
```
table(dat$group_2, dat$binary_response)
```

```
##
##      a  b
##   a  5 15
##   b 10 10
##   c  8 12
##   d  8 12
```

```
chisq.test(table(dat$group_1, dat$nominal_response))
```

```
##
##  Pearson's Chi-squared test
##
## data:  table(dat$group_1, dat$nominal_response)
## X-squared = 0.88095, df = 2, p-value = 0.6437
```

```
ggplot(dat, aes(group_2, fill = binary_response)) +
  geom_bar(color = "black")
```



Binary response

Categorical explanatory with two categories

Can be tested with a z-test

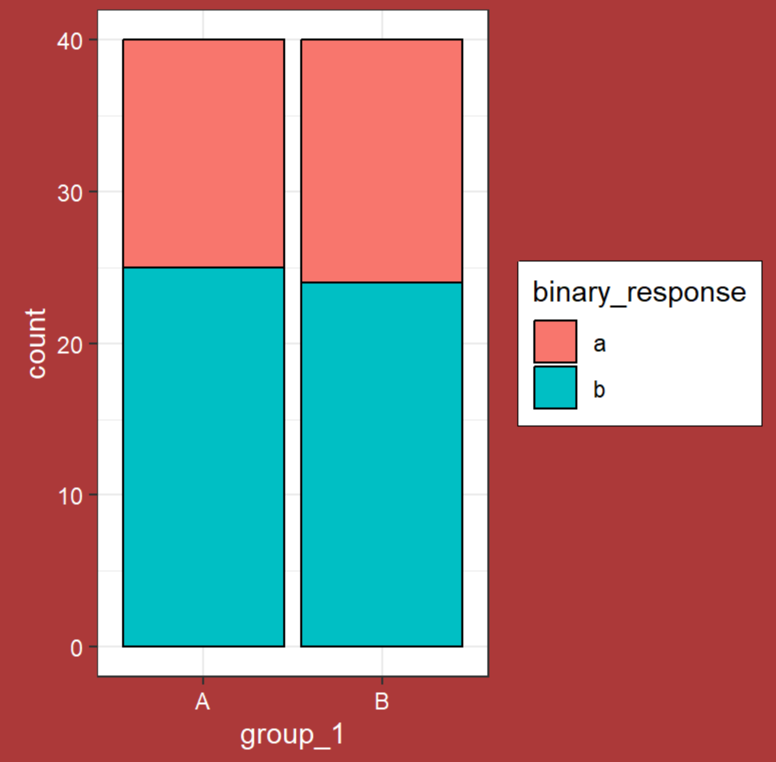
```
table(dat$group_1, dat$binary_response)
```

```
##
##      a  b
##   A 15 25
##   B 16 24
```

```
prop.test(x = c(15,16), n = c(40, 40))
```

```
##
##  2-sample test for equality of proportions with continuity correction
##
## data:  c(15, 16) out of c(40, 40)
## X-squared = 0, df = 1, p-value = 1
## alternative hypothesis: two.sided
## 95 percent confidence interval:
##  -0.2634416  0.2134416
## sample estimates:
## prop 1 prop 2
##  0.375  0.400
```

```
ggplot(dat, aes(group_1, fill = binary_response)) +
  geom_bar(color = "black")
```



Interval / ratio response

Categorical explanatory with two categories

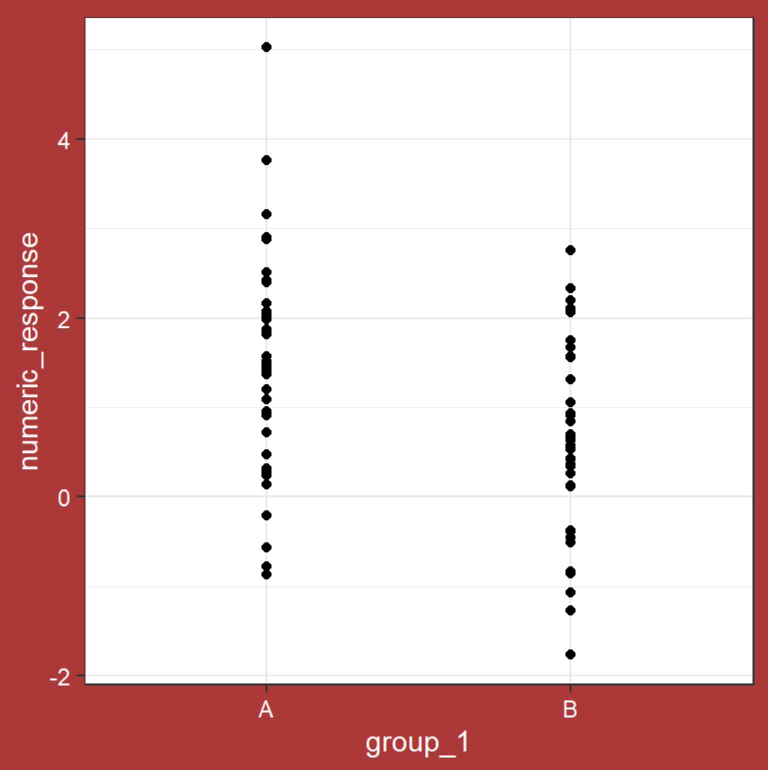
The two-sample t-test

Exact form depends on if data is paired or not

```
t.test(numeric_response ~ dat$group_1, dat)
```

```
##
##  Welch Two Sample t-test
##
## data:  numeric_response by dat$group_1
## t = 3.92, df = 76.365, p-value = 0.0001917
## alternative hypothesis: true difference in means between group A and gr
## 95 percent confidence interval:
##  0.4909192 1.5048311
## sample estimates:
## mean in group A mean in group B
##       1.5435224       0.5456472
```

```
ggplot(dat, aes(group_1, numeric_response)) +
  geom_point()
```



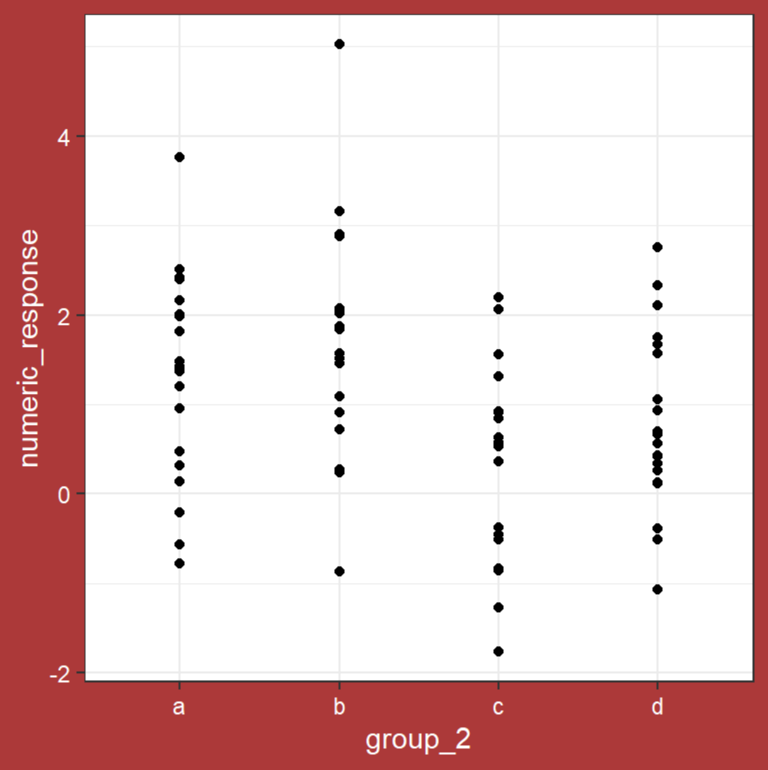
Categorical explanatory with more than two categories

F-test from an Anova model

```r
mod <- lm(numeric_response ~ group_2, dat)
anova(mod)
```

```
## Analysis of Variance Table
##
## Response: numeric_response
##           Df Sum Sq Mean Sq F value    Pr(>F)    
## group_2    3 24.438  8.1461  6.4114 0.000626 ***
## Residuals 76 96.564  1.2706                      
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
ggplot(dat, aes(group_2, numeric_response)) +
  geom_point()
```



Typically followed up with pairwise *post-hoc-tests*

```r
library(emmeans)
emmeans(mod, pairwise ~ group_2)
```

```
## $emmeans
##  group_2 emmean    SE df lower.CL upper.CL
##  a        1.314 0.252 76    0.812    1.816
##  b        1.773 0.252 76    1.271    2.275
##  c        0.300 0.252 76   -0.202    0.802
##  d        0.791 0.252 76    0.289    1.293
##
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate    SE df t.ratio p.value
##  a - b      -0.460 0.356 76  -1.290  0.5722
##  a - c       1.013 0.356 76   2.843  0.0287
##  a - d       0.523 0.356 76   1.466  0.4629
##  b - c       1.473 0.356 76   4.133  0.0005
##  b - d       0.982 0.356 76   2.756  0.0361
##  c - d      -0.491 0.356 76  -1.377  0.5175
##
## P value adjustment: tukey method for comparing a family of 4 estimates
```

Interval / ratio response

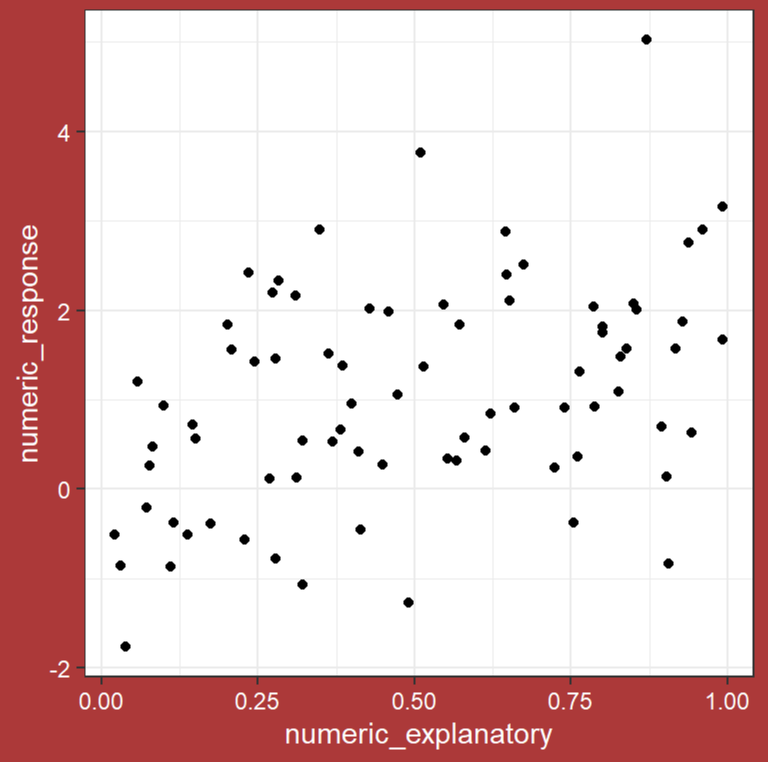
Numerical explanatory

Connection between two numerical variables can be tested with correlation or regression

```
cor.test(dat$numeric_explanatory,
         dat$numeric_response)
```

```
##
##  Pearson's product-moment correlation
##
## data:  dat$numeric_explanatory and dat$numeric_response
## t = 4.0504, df = 78, p-value = 0.0001198
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.2170323 0.5831725
## sample estimates:
##       cor
## 0.4168705
```

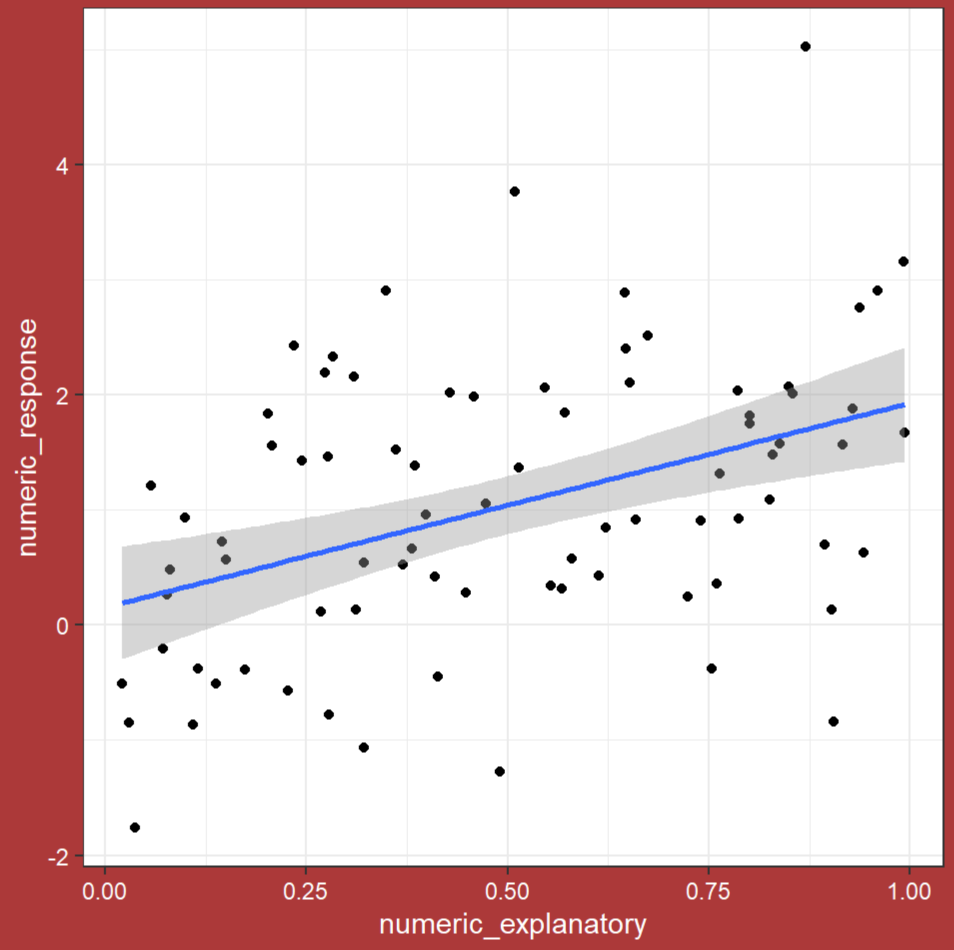
```
ggplot(dat, aes(numeric_explanatory, numeric_response)) +
  geom_point()
```



Alternatively as a regression

```
mod <- lm(numeric_response ~ numeric_explanatory, dat)
anova(mod)
```

```
## Analysis of Variance Table
##
## Response: numeric_response
##                     Df Sum Sq Mean Sq F value    Pr(>F)
## numeric_explanatory  1 21.028 21.0279  16.406 0.0001198 ***
## Residuals           78 99.974  1.2817
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



Non-parametric methods

Non-parametric methods are used when distribution assumptions are not met

Categorical explanatory

A Kruskal-Wallis test for overall differences

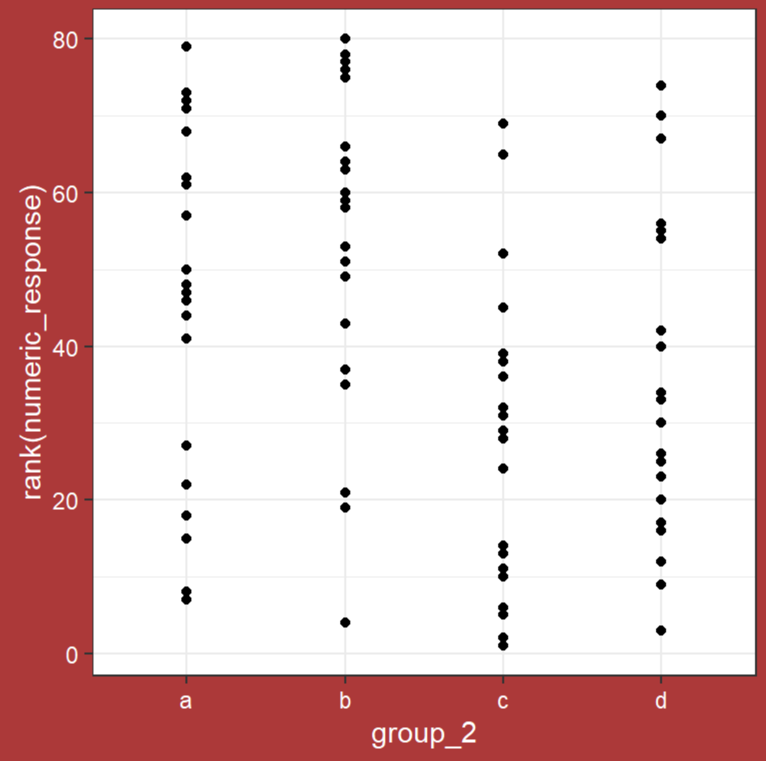
```
kruskal.test(numeric_response ~ group_2, dat)
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  numeric_response by group_2
## Kruskal-Wallis chi-squared = 14.464, df = 3, p-value = 0.002337
```

Gives if there are *any* differences

Pairwise testing can be done with a *Dunn test* or repeated Kruskal-Wallis tests

```
ggplot(dat, aes(group_2, rank(numeric_response))) +
  geom_point()
```



Numerical explanatory

The previously used correlation is the *Pearson* correlation

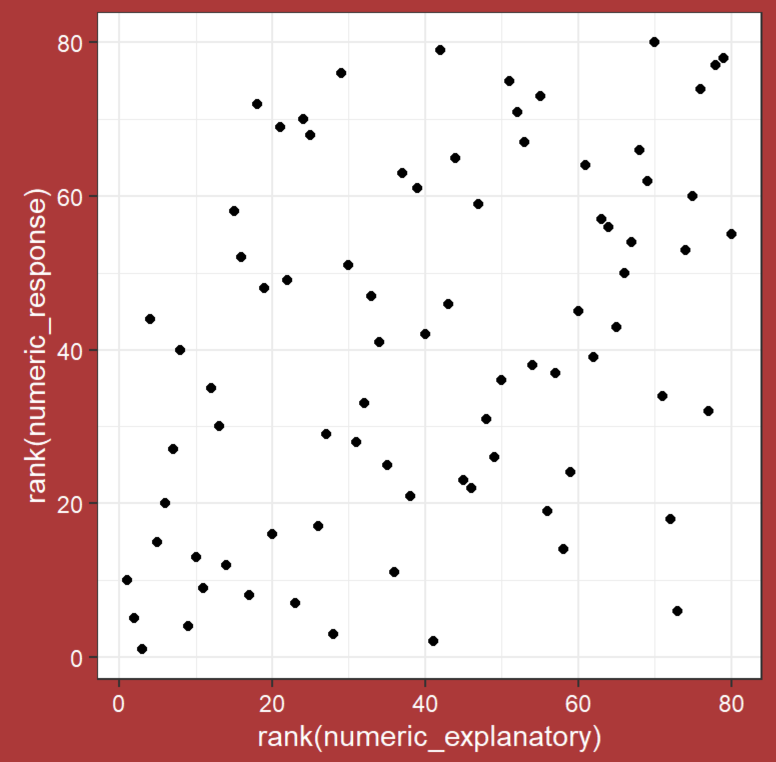
That test relies on an underlying normality assumption

Spearman correlation is a variant which drops that assumption

```
cor.test(dat$numeric_explanatory,
         dat$numeric_response,
         method = "spearman")
```

```
##
##  Spearman's rank correlation rho
##
## data:  dat$numeric_explanatory and dat$numeric_response
## S = 50542, p-value = 0.000198
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.4076184
```

```
ggplot(dat, aes(rank(numeric_explanatory),
                rank(numeric_response))) +
  geom_point()
```



Connections to linear models

Many basic tests are specific cases of *linear models*

A model where the response is the sum of additive terms

Each term is an explanatory variable x multiplied with some parameter β

$$y_i = \beta_0 + \beta_1 x_{1i} + \ldots + \beta_p x_{pi} + \varepsilon$$

Direct examples are the Anova models and multiple regression models

t-tests

A t-test comparing two groups (assuming equal variance) is equivalent to an Anova model

```
t.test(numeric_response ~ group_1, dat, var.equal = T)$p.value
```

```
## [1] 0.0001889247
```

```
mod <- lm(numeric_response ~ group_1, dat)
anova(mod)
```

```
## Analysis of Variance Table
##
## Response: numeric_response
##           Df  Sum Sq Mean Sq F value    Pr(>F)
## group_1    1  19.915  19.915  15.367 0.0001889 ***
## Residuals 78 101.087   1.296
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Frequency tests

χ^2-tests are equivalent to tests on a Poisson model, a kind of *GLM* - *generalized linear model*

```
chisq.test(table(dat$group_2, dat$nominal_response))$p.value
```

```
## [1] 0.7677293
```

```
dat_freq <- dat %>% count(group_2, nominal_response)
mod <- glm(n ~ group_2 * nominal_response, dat_freq, family = "poisson")
anova(mod, test = "Rao")
```

```
## Analysis of Deviance Table
##
## Model: poisson, link: log
##
## Response: n
##
## Terms added sequentially (first to last)
##
##
##                          Df Deviance Resid. Df Resid. Dev    Rao Pr(>Chi)
## NULL                                        11     5.7805
## group_2                   3   0.0000         8     5.7805 0.0000
## nominal_response          2   2.3059         6     3.4746 2.2750   0.3206
## group_2:nominal_response  6   3.4746         0     0.0000 3.3201   0.7677
```

Non-parametric tests

Non-parametric tests are similar, but not equivalent, to standard tests on the *ranks* of the numerical values

The rank is the order number of the variable

For an explanatory grouping

```
kruskal.test(numeric_response ~ group_1, dat)$p.value
```

```
## [1] 0.0004607553
```

```
t.test(rank(numeric_response) ~ group_1, dat)$p.value
```

```
## [1] 0.0002991131
```

For an explanatory numerical variable

Spearman correlation is the Pearson correlation between ranks

```
cor.test(dat$numeric_explanatory, dat$numeric_response, method = "spearman", exact = F)$p.value
```

```
## [1] 0.0001748424
```

```
cor.test(rank(dat$numeric_explanatory), rank(dat$numeric_response), method = "pearson")$p.value
```

```
## [1] 0.0001748424
```

Additional resources

Flowcharts

https://guides.library.lincoln.ac.uk/mash/choosing_a_test

https://www.med.soton.ac.uk/resmethods/statisticalnotes/which_test_flow.htm

Literature

Thulin - *Modern Statistics with R* https://www.modernstatisticswithr.com/

Lindelöv - *Common statistical tests are linear models* https://lindeloev.github.io/tests-as-linear/

The End

Thanks for your attention

3Bs will return in about two weeks