

## Introduction to statistics

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## Who are we?

- Anders Tolver:
  - ▶ Course lecturer.
  - ▶ Associate professor.
  - ▶ Mathematical education, PhD in statistics from 2005.
  - ▶ Experience on applied statistics from DSL and from the former KU-LIFE.
  - ▶ Office 04.3.27, 3'rd floor E-building at HCØ (Nørre Campus).
- Phillip Bredahl Mogensen:
  - ▶ PhD student in statistics at KU-MATH.
  - ▶ Will be present at the exercise class in the afternoon.

## Welcome to everyone!

- Applied statistics using R via the RSTUDIO interface.
  - ▶ 6 course days with lectures and (computer) exercises.
  - ▶ Frequentist statistics with uni-variate endpoints.
  - ▶ Statistical models for categorical and continuous data.
- Lectures and exercises given jointly for two courses:
  - ▶ Applied Statistics (Master Course, 7.5 ECTS).
  - ▶ Statistical methods for the Biosciences, part I (PhD Course, 4.5 ECTS).
- Background:
  - ▶ Teaching level and course aims.
  - ▶ Data Science Laboratory

## Course material

- Computer software:
  - ▶ R: [www.r-project.org](http://www.r-project.org) + RStudio: [www.rstudio.com](http://www.rstudio.com)
- Main literature:
  - ▶ The slides!
  - ▶ The help pages in R.
  - ▶ Course book: **Martinussen, Skovgaard, Sørensen, "A first guide to statistical computations in R", Biofolia 2012.**
  - ▶ Sterne & Smith (2001), "Sifting the evidence—what's wrong with significance tests?", British Medical Journal, 226–231.
  - ▶ Gelman & Carlin (2014), "Beyond Power Calculations: Assessing Type S (Sign) and Type M (Magnitude) Errors", Perspectives on Psychological Science, 1–11.
- Also used:
  - ▶ Your old book on basic statistics.
  - ▶ Golemund, Wickham (2017), [R for Data Science](#), O'Reilly.
  - ▶ Wickham (2016), [ggplot2](#), Springer.

## Statistical software

Provides validity, reliability, reproducibility

**Programming:** R, SAS, Stata, MatLab, ...

**Menu based:** Excel, Graphpad Prism, SPSS, SAS Enterprise, JMP, Stata, R-commander, ...

Pros and Cons:

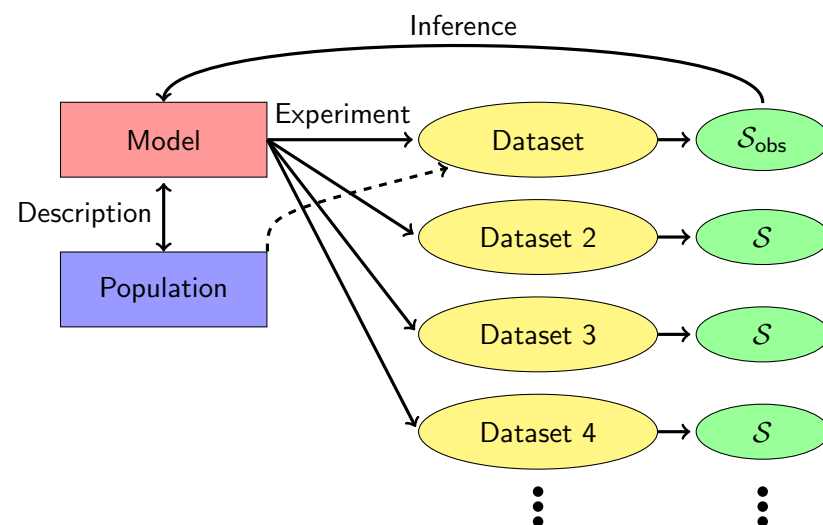
	Programming	Menu based
+	Full control, direct reproducibility	Good overview of models and possibilities
–	Syntax, commands, options, etc.	Mouse clicking, limited flexibility, reproducibility difficult

**RStudio:** An interface to R successfully encountering many of the cons in programming.

**R Markdown:** File format for making dynamic documents that integrate code, output, graphs and text.

## Model, data, statistic

Examples of statistics  $\mathcal{S}$ : estimator, confidence interval, test statistic, p-value



## Why do statistics?

Well, to answer four important questions

- 1 Is there an effect?
  - ▶ Answered by p-values.
- 2 Where is the effect?
  - ▶ Answered by p-values from post hoc analyses.
- 3 What is the effect?
  - ▶ Answered by confidence and prediction intervals.
- 4 Can the conclusions be trusted?
  - ▶ Answered by model validation.

Remarks:

- Often “effect” should be replaced by “association”.
- Statistical models are also used for other purposes: Which ones?

## Does feed concentrate increase milk yield?

An intervention study

- Does feeding concentrate to dairy cows have an effect on milk yield?



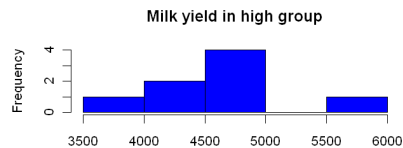
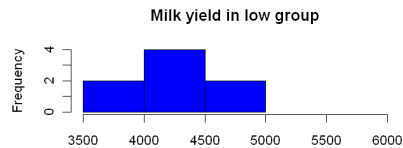
- Objective of the example:
  - ▶ Answer the posed question.
  - ▶ Learn basic concepts of hypothesis testing doing this.
  - ▶ First look at some R code.
- What has been learned?

## Does feeding concentrate influence milk yield?

Two groups of 8 cows, given low or high amounts of feed concentrate

Concentrate/day	Milk yield (kg) from week 1 to 36							
Low: 4.5 kg	4132	3672	3664	4292	4881	4287	4087	4551
High: 7.5 kg	3860	4130	5531	4259	4908	4695	4920	4727

Reference: V. Østergaard (1978)



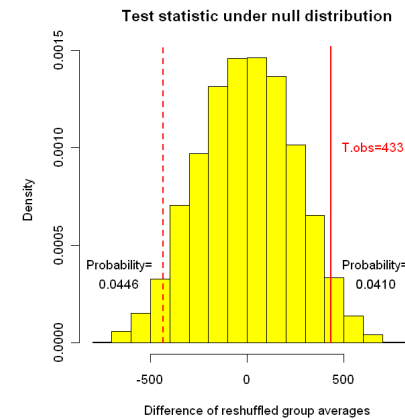
- Apparently there is an effect of feed concentrate.
- Confirmation by **falsification** of the **null hypothesis** of no effect.
- A **test statistic** summarizes the difference between groups, e.g.:

$$\begin{aligned}T_{\text{obs}} &= \text{mean}(\text{high}) - \text{mean}(\text{low}) \\&= 4629 - 4195 \\&= 433\end{aligned}$$

## Is the observed effect ( $T_{\text{obs}} = 433$ kg) significant?

Or might it be due to mere randomness?

- Null hypothesis  $\implies$  Observed difference of average milk yield due to random allocation of 16 cows into two groups.
- So let's redo a random allocation 10,000 times and inspect the differences of average milk yields. . .



The **p-value** is the probability of a more extreme test statistic than  $T_{\text{obs}}$ ,

$$\begin{aligned}p &= \text{Prob}(|T| \geq |T_{\text{obs}}|) \\&= 0.0410 + 0.0446 \\&= 0.0856\end{aligned}$$

## R code for the permutation test

For illustration only, in practice use e.g. `wilcox.test()`

```
# Read data and compute test statistic
low  <- c(4132,3672,3664,4292,4881,4287,4087,4551)
high <- c(3860,4130,5531,4259,4908,4695,4920,4727)
T.obs <- mean(high)-mean(low)

# Resample test statistic and compute p-values
T.resample <- replicate(10000,{
  permuted.cows <- c(low,high)[sample(1:16)]
  group.1      <- permuted.cows[1:8]
  group.2      <- permuted.cows[9:16]
  mean(group.1)-mean(group.2)
})
p.value.onesided <- mean(T.resample >= T.obs)
p.value.twosided <- mean(abs(T.resample) >= abs(T.obs))
```

## Summary of basic concepts

What to be learned from the milk yield example

- Statistical hypothesis tests distinguish **real effects** from **random variation**.
- Scientific hypothesis supported by **falsifying opposite hypothesis**.
- Test statistic measures discrepancy between data and null hypothesis.
- P-value = probability of larger discrepancy than the observed one.
- Small p-value  $\implies$  significance.
- The observed p-value, 0.0856, is not sufficiently small to claim statistical significance. What to do about that? (Multiple answers!)

## Conclusion from a hypothesis test

Remark: Statistical significance is not the same as importance

- p-value measures disagreement with  $H_0$ :

small p: disagreement=reject

large p: agreement=cannot reject (accept)

Conventional labelling (“.” in some R outputs):

$p > 0.05$ : NS	(non significant)
$0.05 < p < 0.10$ : .	(significant at 10% level)
$0.01 < p < 0.05$ : *	(significant at 5% level)
$0.001 < p < 0.01$ : **	(significant at 1% level)
$p < 0.001$ : ***	(significant at 0.1% level)

- Small p = strong evidence against  $H_0$ . If  $p = 0.2\%$ , say, then
  - ▶ either  $H_0$  is false
  - ▶ or  $H_0$  is true and we have been unlucky! (risk = 2/1000)
  - ▶ or we have tested too many hypothesis (say 1000)
  - ▶ or the model is wrong (conclusion cannot be trusted)

## Questions?

- 1 And then a break!
- 2 After the break we discuss the building blocks of a dataset: Observations, variables, and variable types.
  - ▶ Basically, this corresponds to **tidy data** in the *tidyverse* invented by Hadley Wickham.

## A dataset with 4 variables and 10 observations

Variables: body weight, liver weight, dose, dose in liver

- An experiment was carried out to investigate the accumulation of a certain drug in the liver.
- 10 rats were given a dose, approximately proportional to their bodyweight.
- After some time period the rats were slaughtered, their livers weighted and the drug dose in the liver was measured.
- On the next slide we should remember to discuss the interplay between body weight, liver weight, and dose.

## A dataset with 4 variables and 10 observations

Variables: body weight, liver weight, dose, dose in liver

body weight	liver weight	dose	dose in liver
176	6.5	0.88	0.42
176	9.5	0.88	0.25
190	9.0	1.00	0.56
176	8.9	0.88	0.23
200	7.2	1.00	0.23
167	8.9	0.83	0.32
188	8.0	0.94	0.37
195	10.0	0.98	0.41
176	8.0	0.88	0.33
165	7.9	0.84	0.38

The dataset.

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The variable “dose”.

## A dataset with 4 variables and 10 observations

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The 4'th observation.

## A dataset with 4 variables and 10 observations

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“dose in liver” is the **response variable**.

## Rows and Columns, Observations and Variables

Group I	Group II	Group III
243	206	241
251	210	258
275	226	270
291	249	293
347	255	328
354	273	
380	285	
392	295	
	309	

Table shows red cell folate levels ( $\mu\text{g/l}$ ).

Reference: Amess et al. (1978), Megaloblastic haemopoiesis in patients receiving nitrous oxide, Lancet, 339-342.

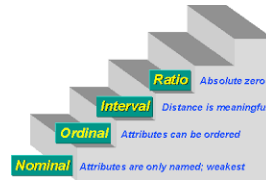
- Are these the same dataset?
- What are the variables? How many observations are there?

Group	Level
I	243
I	251
I	275
I	291
I	347
I	354
I	380
I	392
II	206
II	210
II	226
II	249
II	255
II	273
II	285
II	295
II	309
III	241
III	258
III	270
III	293
III	328

## Nominal, ordinal, interval, ratio

Four categories of variable types with increasing structural information

- Example of a **nominal** variable:
  - ▶ Color (red, green, purple).
- Example of an **ordinal** variable:
  - ▶ Status (healthy, slight symptoms, severe symptoms, dead).
- Example of an **interval** variable:
  - ▶ Temperature measured in degrees of Celsius.
- Examples of **ratio** variables:
  - ▶ Temperature measured in Kelvin.
  - ▶ Height (measured in cm).
  - ▶ Money on my bank account (measured in Danish kroner).
- Nominal and ordinal variables are subtypes of **categorical** variables.
- Interval and ratio variables are subtypes of **continuous** variables.



## Choosing the response variable

Recall the dataset from slides 15–19: body weight, liver weight, dose, dose in liver

An experiment with rats was carried out in order to investigate the accumulation of a certain drug in the liver. Each rat was given a dose of the drug, approximately proportional to their bodyweight. After a period the rats were slaughtered, their livers weighed and the drug dose in the liver was measured.

- “Dose in liver” is the response variable. But why?
  - ▶ This is what we are interested in.
  - ▶ This is what we want to predict knowing the other variables.
  - ▶ This is where the random variation matters to us.
- Quiz: Consider a dataset consisting of measurements of genes and the occurrence / non-occurrence of some disease. Would a geneticist and an epidemiologist agree on the choice of the response variable?
 

Hint: Geneticists study genes. Epidemiologists study diseases.

## Table of Variables

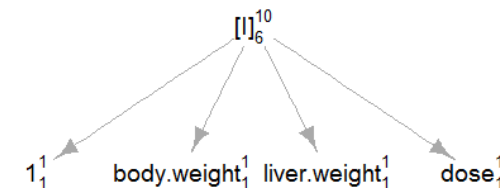
For “dose in liver” example on slides 15–19.

Variable	Type	Range	Usage
body weight	ratio	[165 ; 200]	fixed effect
liver weight	ratio	[6.5 ; 10.0]	fixed effect
dose	ratio	[0.83 ; 1.00]	fixed effect
dose in liver	ratio	[0.23 ; 0.56]	response

- For variables of type **categorical** you could state the *number of levels* and/or the *names of the levels*:
  - ▶ Separated by “,” or “<” for nominal and ordinal variables, respectively.
- Other possible usages of that we encounter later in the course are: *random effect*, *correlation effect*, *subject id*, *design parameter*.
- *Table-of-Variables* is a useful tool. The purpose is to guide the model building and the statistical analysis.

## Design Diagram

For “dose in liver” example on slides 15–19.



- *Design-Diagrams* is an extension of *Factor-Structure-Diagrams*.
- *Design-Diagrams* will be used through out this course to visualize the structure of experimental design.
- Note that the *response* variable doesn't appear in the basic diagram. But you may find the *number of observations*, *number of parameters*, and *degrees of freedom*.

## Table of Variables

Data example from PhD thesis by Dragana Vukasinovic

	winter	treat	DryMass
1	BW	8	0.0761
2	BW	8	0.0737
3	BW	8	0.0802
4	BW	8	0.0862
5	BW	8	0.0799
6	BW	8	0.0743
7	BW	8	0.0847
8	BW	8	0.0899
9	MW	-5	0.0817
10	MW	-5	0.0848
11	MW	-5	0.0840
12	MW	-5	0.0791
...			
36	EW	5	0.0737
37	EW	5	0.0767
38	EW	5	0.0684
39	EW	5	0.0942
40	EW	5	0.0853

Variable	Type	Range	Usage
winter	nominal	BW, MW, EW	fixed effect
treat	ordinal	-5 < +5 < +8	fixed effect
DryMass	continuous	[0.0737 ; 0.0942]	response

- Design-Diagram on next slide shows that the design is not balanced.
- The treatment +8 is only used BW, and the treatments -5 and +5 are only used MW and EW.

Meaning of the levels of the winter variable:

BW = "before winter",  
MW = "mid winter",  
EW = "end winter"

## Design Diagram

Data example from PhD thesis by Dragana Vukasinovic



- The **minumum** between *winter* and *treat* is a categorical variable with levels  
BW: +8      MW, EW: -5, +5.
- The factor **1** is the intercept.
- More on this later.

## Questions?

- 1 And then a break!
- 2 After the break I give a short introduction to R and RStudio.
- 3 And we discuss T-tests and data transformations (you might know much of this already).

## Introduction to R

The RStudio interface consists of  $4 = 2 \times 2$  subwindows

- Upper-left** The **editor**, where you write your R programs.
- Lower-left** The **console**, where code is executed and results are printed.
- Upper-right** Overview of **objects** (variables, vectors, matrices, data frames, lists, functions, "results", etc.) in the (global-) **environment**.
- Lower-right** Miscellaneous: overview of **working directory**, history of **plots**, administration of **packages**, and **help pages**.

- R is a full-scale objected oriented programming language.
- In R your data is typically stored in either **vectors**, **matrices**, or most commonly in **data frames** (*tidyverse* introduce specialization **tibbles**).
- Results from analyses are stored in associated objects (for well programmed functions):
  - ▶ E.g. a call to the `lm()` function results in an **lm-object**.
  - ▶ Such objects may be **printed**, **summarized** and/or **plotted**.

## Functions and R packages

R contains many predefined functions for doing statistical computations

- Standard functions: `mean()`, `sd()`, ...
  - ▶ These functions may be used without any further ado.
  - ▶ Includes so-called **generic** functions: `print()`, `summary()`, `plot()`, ...
- Functions from pre-installed packages: `MASS::boxcox()`, `cluster::agnes()`, ...
  - ▶ The package might be **loaded** in an R session: e.g. `library(MASS)`.
- Functions from other packages: `nlme::lme()`, `LabApplStat::DD()`, ...
  - ▶ The package must be **installed** once before it may be loaded / used: preferably done using the **Install Packages** button.
  - ▶ Ability to install packages is vital for the functionality of R.
  - ▶ Unfortunately, problems installing packages have become more prevalent (possible solutions: "Run as Administrator", `"install.packages(..., lib=.libPaths()[2])"`)

## Data example 1: Change in glucose level

One sample T-test

- For 8 diabetics the one-hour change in plasma glucose level after some glucose treatment was measured:

```
> change <- c(0.77, 5.14, 3.38, 1.44, 5.34, -0.55, -0.72, 2.89)
> mean(change)
[1] 2.21125
> sd(change)
[1] 2.36287
```
- Did the treatment change the plasma glucose level?
- Basic statistical concepts: Statistical model, null hypothesis, test statistic, p-value, confidence interval.

## Systematic effects vs. Random variation

- **Systematic effects:** Mean properties of the population. Often the object of interest.
  - ▶ For instance the expected life span of men and women, or the difference between the effect of two drugs.
- **Random variation:** The dispersion of the data points around the systematic properties.
  - ▶ Natural variation in the population.
  - ▶ Measurement errors.
  - ▶ Difference between a complex world and a simple model.
- **Hypothesis testing:** Are the systematic effects significant, or can they be explained by the random variation?

## Basic statistical concepts

Statistical model, null hypothesis, test statistic

- Models are described by parameters. In data example 1 these are the mean  $\mu$  and the standard deviation  $\sigma$ . And we have the model:

$$Y_1, \dots, Y_n \text{ i.i.d. } \mathcal{N}(\mu, \sigma^2)$$

- A statistical hypothesis is a simplifying statement about the model. Often formulated in terms of the parameters, i.e.

$$\text{Null hypothesis } H_0: \mu = 0, \quad \text{Alternative } H_A: \mu \neq 0$$

- Test statistic  $T$  is a function of the data. Actual value denoted  $t_{\text{obs}}$ .
  - ▶ If  $T$  measures **disagreement with  $H_0$**  and if  $t_{\text{obs}}$  is **too extreme**, then we reject  $H_0$ .
  - ▶ If the observed data is **conceived** as being random, then  $T$  becomes a random variable with a probability distribution.
  - ▶ Extremeness quantified by the **p-value**, calculated assuming  $H_0$  is true,

$$p = P(T \text{ more extreme than } t_{\text{obs}})$$



## One sample T-test

Testing in a normal sample:  $Y_1, \dots, Y_n$  i.i.d.  $\mathcal{N}(\mu, \sigma^2)$

- Given prefixed value  $\mu_0$ , often 0, we pose hypothesis  $H_0: \mu = \mu_0$ .

$$\text{Estimates: } \hat{\mu} = \bar{Y} = \frac{1}{n} \sum_{i=1}^n Y_i, \quad \hat{\sigma}^2 = s^2 = \frac{1}{n-1} \sum_{i=1}^n (Y_i - \bar{Y})^2$$

- Test statistic and p-value for one-sided test,  $H_A: \mu > \mu_0$ ,

$$T = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} \sim T_{\text{df}=n-1}, \quad p = P(T_{\text{df}=n-1} > t_{\text{obs}})$$

- Test statistic and p-value for two-sided test,  $H_A: \mu \neq \mu_0$ ,

$$T = \frac{\bar{Y} - \mu_0}{s/\sqrt{n}} \sim T_{\text{df}=n-1}, \quad p = P(|T_{\text{df}=n-1}| > |t_{\text{obs}}|)$$

---

Did treatment change plasma glucose level in data example 1?

$$t_{\text{obs}} = 2.21 \cdot \sqrt{8}/2.36 = 2.65, \quad p = 2 \cdot P(T_{\text{df}=7} > 2.65) = 0.03$$

## Data example 3: Phosphor concentration in lakes

Two independent samples, not necessarily of the same length

```
> lakes
# A tibble: 627 x 2
  location    phosphor
  <chr>      <dbl>
1 East-Denmark 255
2 East-Denmark 102.
3 East-Denmark 166.
4 East-Denmark 42.5
5 East-Denmark 102.
6 East-Denmark 60.6
7 East-Denmark 89.8
8 East-Denmark 182.
9 East-Denmark 243.
10 East-Denmark 30.9
# ... with 617 more rows
```

- Is there a difference between East-Denmark (235 observations) and West-Denmark (392 observations)?
- Let's write up the statistical model and do the analysis using R.

## Data example 2: Density of nerve cells

Two paired samples: Consider differences or possibly log ratios

Density of nerve cells measured at two sites of the intestine, midregion/mesentric region of jejunum ("tyndtarm"), for n=9 horses.

Sample statistics for diff:

horse    mid    mes    diff    mean = 7.33,    SD = 7.79

1    50.6    38.0    12.6

2    39.2    18.6    20.6

3    35.2    23.2    12.0

4    17.0    19.0    -2.0

5    11.2    6.6    4.6

6    14.2    16.4    -2.2

7    24.2    14.4    9.8

8    37.4    37.6    -0.2

9    35.2    24.4    10.8

Standard error  $SE = \frac{SD}{\sqrt{n}} = 2.60$ .

Test for population mean=0:

$$t_{\text{obs}} = \frac{7.33 - 0}{2.60} = 2.82$$

$$p = 2 \cdot P(T_{\text{df}=8} > 2.82) = 0.02$$

Conclusion?

## Statistical analysis of two independent normal samples

Statistical model:

First population  $\sim \mathcal{N}(\mu_1, \sigma_1^2)$ ,    Second population  $\sim \mathcal{N}(\mu_2, \sigma_2^2)$

Sequence of hypothesis [usually we skip (I) and simply use (IIb)]:

(I)  $H_0: \sigma_1 = \sigma_2$

(II)  $H_0: \mu_1 = \mu_2$

(IIa) Assuming equal standard deviations  $\sigma_1 = \sigma_2$ .

(IIb) Not assuming equal standard deviations.

Available statistical tests:

(I) `var.test()`, `bartlett.test()`, `lawstat::levene.test()`, `fligner.test()`, and many more.

► Don't do too many tests. Preferably only one test. Why?

(II) T-test, slightly different form in (IIa) and (IIb).

## Assumptions and Checking for Normality

All T-tests, and other "normal" models

### Assumptions

- ▶ The response variable (more precisely, the **error terms**) are normally distributed.
- ▶ Possibly *homogeneity of variance* (homoscedasticity), meaning that the variance of the response variable is constant over the observed range of some other variable. This is (I) on slide 36.

### Checking for Normality

- ▶ Visual inspection : QQ-plot.
- ▶ Goodness-of-fit tests: Shapiro-Wilks test, Kolmogorov-Smirnov test, Cramer-von Mises test, Anderson-Darling test.

Shapiro-Wilks and Kolmogorov-Smirnov tests are available in base R, the others in the package nortest.

## Data example 3: Using R

- Reading an Excel sheet.
- Validation of normality:
  - ▶ Graphical: `qqnorm(); abline(mean(),sd())`
  - ▶ Shapiro-Wilks test: `shapiro.test()`
  - ▶ Kolmogorov-Smirnov test: `ks.test(,"pnorm",mean(),sd())`

Statisticians often prefer the graphical method.

- Data transformation.
- The actual two sample T-test.
- Keyboard shortcuts (Windows): Ctrl-Enter, Ctrl-Shift-Enter, Ctrl-1, Ctrl-2

## Transforming data

Often a solution when normality assumptions fails

### Standard transformations (for $x > 0$ ):

- ▶ log transform:  $x \mapsto \log(x) = y$ .
- ▶ Square root transformation:  $x \mapsto \sqrt{x} = y$ .
- ▶ The inverse transformation:  $x \mapsto \frac{1}{x} = y$ .  
This transformation changes the order of the observations.
- ▶ Box-Cox transformation with index  $\lambda$ :

$$x \mapsto y_\lambda = \begin{cases} \frac{x^\lambda - 1}{\lambda} & , \lambda \neq 0 \\ \log(x) & , \lambda = 0. \end{cases}$$

Note the order of the observations is changed when  $\lambda < 0$ .

Some particular cases:

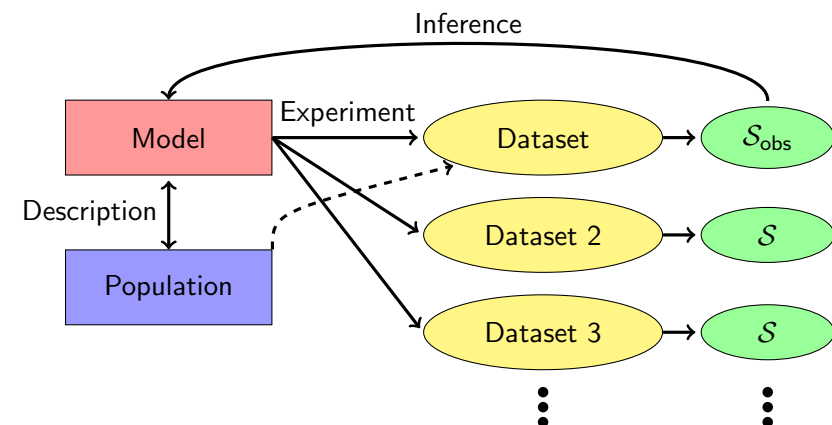
$\lambda = -1$	$\lambda = 0$	$\lambda = 0.33$	$\lambda = 0.5$	$\lambda = 1$
Inverse	log	cubic root	square root	no transformation

### Arcus sinus transformation (for $x \in [0, 1]$ ):

- ▶  $x \mapsto \arcsin(\sqrt{x})$ .
- ▶ May be appropriate when  $x$  measures the *proportion of successes out of a number of trials*.

## Today's summary: Model, data, statistic

Examples of statistics  $\mathcal{S}$ : estimator, confidence interval, test statistic, p-value



- What is the distribution of the p-value?
- Where do **standard deviation** and **standard error** reside?
- What is the interpretation of a confidence interval?

## Homework

- Exercise class November 18 from 12.45 to 15.35.
  - ▶ Use Zoom link on webpage
  - ▶ Exercise sheets: `ex_day1.pdf`, `ggplot2_exercise.pdf`
  - ▶ Exercises not completed at class should be completed at home.
- Before the lectures on November 25 you should read the papers:
  - ▶ Sterne & Smith (2001), “Sifting the evidence—what’s wrong with significance tests?”, *British Medical Journal*, 226–231.
  - ▶ Gelman & Carlin (2014), “Beyond Power Calculations: Assessing Type S (Sign) and Type M (Magnitude) Errors”, *Perspectives on Psychological Science*, 1–11.