Experimental Design and Data Analysis, Lecture 3

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Lecture overview

1. two paired samples (normal and not normal)
   - permutation test
   - dependence in two paired samples
     - Pearson's correlation test
     - Spearman's rank correlation test

2. two independent samples (normal and not normal)
   - two samples t-test
   - Mann-Whitney test
   - Kolmogorov-Smirnov test

permutation tests for two paired samples

permutation test for two paired samples

Dependence in two paired samples

two independent samples

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Reminder: setting and design for two paired samples

Setting:

- An experiment with a numerical outcome measured according to two conditions per experimental unit;
- Interest is in a possible difference between the two outcomes per unit.

EXAMPLE Difference in average course grade for mathematical courses and informatics courses for BA-students at the VU.

EXAMPLE Difference in pain relief by an active drug and a placebo for patients.

Design (the standard paired samples design):

- Take a random sample of experimental units from the relevant population.
- Measure the two outcomes on each unit.

Idea of permutation technique

- Data $(X_1, Y_1), (X_2, Y_2), \ldots, (X_n, Y_n)$ must be two paired samples.

- In a permutation test we do not assume normality.

- We use any test statistic $T = T(X_1, Y_1, \ldots, X_n, Y_n)$ to test H_0: no difference between the distributions of X_j's and that of Y_j's. The choice of test statistics should express somehow the difference conjectured.

- Like in a bootstrap test, we simulate the distribution of T under H_0, using B surrogate T^*-values. Repeat B times (for $i = 1, \ldots, B$):
  - generate each (X_j^*, Y_j^*), $j = 1, \ldots, n$, by applying a random permutation of the original (X_j, Y_j), i.e., choose between (X_j, Y_j) and (Y_j, X_j) with equal probability;
  - next, compute $T_i^* = T(X_1^*, Y_1^*, \ldots, X_n^*, Y_n^*)$.

- Under H_0 of no difference between the distributions of X and Y within pairs permuting the labels should not change the distribution of T.

Permutation test in R: data input and graphics

Recall dataset ashina.txt (headache after drug or placebo for 16 subjects).

```
> ashina=read.table("ashina.txt",header=TRUE)
> boxplot(ashina[,1],ashina[,2],names=c("active","placebo"))
> plot(ashina[,1],ashina[,2])
```
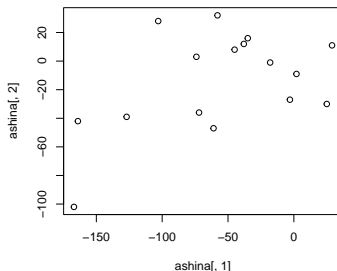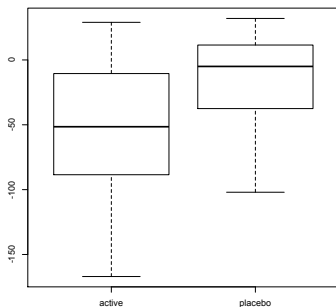

Based on the boxplots, we expect the active medicine to yield better pain relief.

permutation test for two paired samples
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Dependence in two paired samples
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two independent samples
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Permutation test in R: testing (1)

```
> mystat=function(x,y) {mean(x-y)}
> B=1000; tstar=numeric(B)
> for (i in 1:B) {
+ ashinastar=t(apply(cbind(ashina[,1],ashina[,2]),1,sample))
+   tstar[i]=mystat(ashinastar[,1],ashinastar[,2]) }
> myt=mystat(ashina[,1],ashina[,2])
```

Instead of computing all $2^{16} = 65536$ possible permutations, we generate 1000 randomly chosen permutations to estimate the distribution of our test statistic under H_0. The function apply applies a function to either all rows or all columns in a matrix (parameter 1 indicates rows), t(matrix) means transposition of matrix.

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Permutation test in R: testing (2)

Histogram of tstar

```
> myt
[1] -42.875
> hist(tstar)
> lines(rep(myt,2),c(0,20),
 + col="red",lwd=2)
> pl=sum(tstar<myt)/B
> pr=sum(tstar>myt)/B
> p=2*min(pl,pr); p
[1] 0.008
```
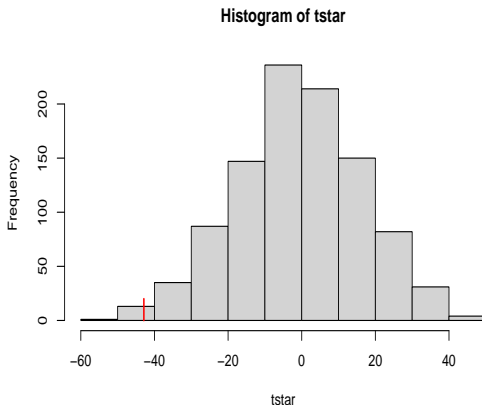

Conclusion: there is indeed a significant difference between the active drug and the placebo.

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Permutation test: discussion

- A permutation test for two paired samples can be performed with any test statistic that expresses difference between the X and Y within pairs. (The mean of differences $Z_i = X_i - Y_i$ is most common to consider, but one may as well consider the median of the Z_i's.)

- Alternatives to the permutation test for two paired samples are the sign test and the Wilcoxon signed rank test applied to the differences.

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Dependence in two paired samples

Dependence between two paired samples

Setting:

An experiment with two numerical outcomes (say X and Y) per experimental unit. Interest is in a possible dependence between the two outcomes per unit.

EXAMPLE Relation between shoe size and body mass index of a person.

EXAMPLE Relation between average course grade and number of students taking the course for courses at the VU.

EXAMPLE Relation between amount of precipitation and sun hours for different cities in Europe.

Design:

- Take a random sample of experimental units from the relevant population.
- Measure the two quantities on each unit. (The two outcomes are in principal related, because measured on the same experimental unit.)
- However, we possibly have measured unrelated quantities of the units and we want to test whether these quantities are correlated.

Pearson's correlation test

- Data $(X_1, Y_1), (X_2, Y_2), \ldots, (X_n, Y_n)$.
- The Pearson correlation test assumes normality of the both X_i's and Y_i's.
  (Rather, the asympt. normality of the sample correlation $\hat{\rho}$.)
- The test is based on the sample correlation coefficient (which estimates the "true" correlation $\rho = Cor(X, Y)$):

$$\hat{\rho} = \hat{\rho}_{X,Y} = \frac{\frac{1}{n-1}\sum_{i=1}^{n}(X_i - \bar{X}_N)(Y_i - \bar{Y}_n)}{\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(X_i - \bar{X}_n)^2)\frac{1}{n-1}\sum_{i=1}^{n}(Y_i - \bar{Y}_n)^2}}.$$

- We test the null hypothesis $H_0 : \rho = \rho_0 = 0$ that the correlation between the two populations is $\rho_0 = 0$. The test statistic is given by

$$T_\rho = \frac{\hat{\rho} - \rho_0}{\sqrt{(1 - \hat{\rho}^2)/(n-2)}} = \frac{\hat{\rho}}{\sqrt{(1 - \hat{\rho}^2)/(n-2)}},$$

which has under $H_0 : \rho = 0$ a t-distribution with $n - 2$ degrees of freedom.

Spearman's rank correlation test

- Data $(X_1, Y_1), (X_2, Y_2), \ldots, (X_n, Y_n)$.
- Spearman's rank correlation test does not assume normality. The test considers the ranks $R(X_i)$ and $R(Y_i)$ in the two samples, and compares the ordering of the ranks in the X_i and the Y_i.
- If the data are rank correlated, these sequences of ranks will run (approximately) in parallel or in opposite order.
- The test statistic is the sample correlation $\tilde{\rho}$ between the the rank vectors.
- We test the null hypothesis $H_0 : \rho_s = 0$. (Correlation of the rank variables.)

If all n ranks are distinct integers, the test statistic can be computed as $\tilde{\rho} = 1 - \frac{6 \sum_{i=1}^{n} d_i^2}{n(n^2-1)}$ where $d_i = R(X_i) - R(Y_i)$ is the difference between the two ranks of observations X_i and Y_i.

This test is useful in testing whether variable Y is a monotone transformation of variable X (or vice versa) in which case the true rank correlation is $\rho_s = 1$.
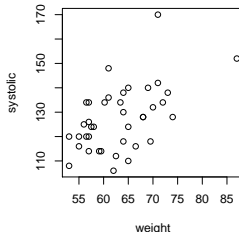
Correlation tests in R: example

Consider the data frame peruvians.txt, where the rows correspond to 39 Peruvian men that moved from a native culture to a modern society. Amongst others, years since migration, systolic and diastolic blood pressure, heart rate (column wrist), weight, length were measured.

```
> peruvians=read.table("peruvians.txt",header=TRUE); peruvians
   age migration weight length chin  arm calf wrist systolic diastolic
1   21         1   71.0   1629  8.0  7.0 12.7    88      170        76
2   22         6   56.5   1569  3.3  5.0  8.0    64      120        60
       [ some output deleted ]
39  54        40   87.0   1542 11.3 11.7 11.3    92      152        88
```

```
> attach(peruvians)
> plot(systolic~weight)
```

Based on this picture, we expect
dependence between systolic and weight.

Pearson's test in R: example

```
> cor.test(systolic,weight)

Pearson's product-moment correlation

data:  systolic and weight
t = 3.7164, df = 37, p-value = 0.0006654
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2463759 0.7186619
sample estimates:
      cor
0.5213643
```
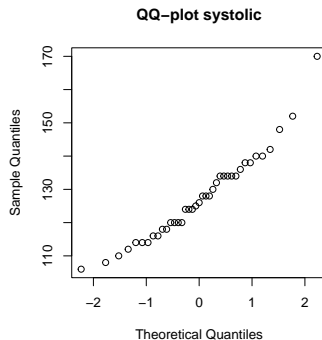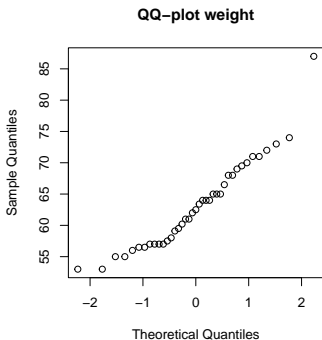
Conclusion: there is significant correlation, if normality is assumed.

The default for cor.test is Pearson's correlation test, based on normality.

Correlation tests: diagnostics

Check the normality assumption on the two samples:

```
> par(mfrow=c(1,2)); qqnorm(peruvians$weight,main="QQ-plot weight")
> qqnorm(peruvians$systolic,main="QQ-plot systolic")
```


QQ-plots show that normality is doubtful for the weight sample. Hence, we use the rank correlation test of Spearman (and not Pearson's correlation test).

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Spearman's test in R: example

```
> cor.test(systolic,weight,method="spearman")

        Spearman's rank correlation rho

data:  systolic and weight
S = 5322.352, p-value = 0.003119
alternative hypothesis: true rho is not equal to 0
sample estimates:
      rho
0.4613004

Warning message:
In cor.test.default(systolic, weight, method = "spearman") :
  Cannot compute exact p-values with ties
```

Conclusion: there is indeed significant rank correlation.

There is a warning about ties, which means that some values occur multiple times in weight and/or systolic. Therefore R uses an approximation for the p-value.

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two independent samples

Two independent samples: setting and design

Setting: an experiment with

- one numerical outcome per experimental unit,
- two independent groups of experimental units.

Interest is in a possible difference between the two populations. medskip

EXAMPLE Comparing the weight of newborn children in two countries.

EXAMPLE Total yield from an agricultural plot for two different fertilizers.

Design:

- Take a random sample of experimental units of size M from the first population and a random sample of size N from the second population;
- Measure the outcome on each unit.

The numbers M and N need not be the same.

t-test for two independent samples

- Data $(X_1, \ldots, X_M)$ and $(Y_1, \ldots, Y_N)$.
- The two samples t-test assumes that both samples $X_1, \ldots, X_M$ and $Y_1, \ldots, Y_N$ come from independent normal populations. Denote the mean of the first population by μ and the mean of the second by ν.
- We test about the relation between the population means μ and ν:

$$H_0 : \mu \left\{ \begin{array}{c} = \\ \leq \\ \geq \end{array} \right\} \nu \quad \text{versus} \quad H_1 : \mu \left\{ \begin{array}{c} \neq \\ > \\ < \end{array} \right\} \nu.$$

- The test statistic is

$$T = \frac{\overline{X}_M - \overline{Y}_N}{S_{N,M}}, \quad \text{which has the } t_{N+M-2}\text{-distribution under } H_0.$$

t-test in R: data input and graphics

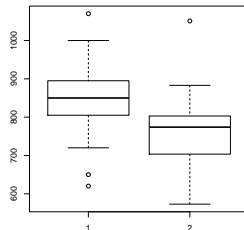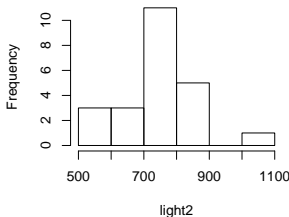Consider two data sets of measurements of the speed of light (minus 299000) by Michelson in 1879 and in 1882.

```
>light1=scan("light1.txt"); light2=scan("light2.txt")
> hist(light1); hist(light2); boxplot(light1,light2)
```
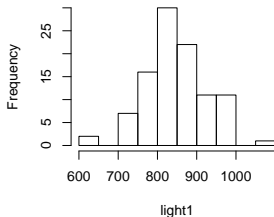
t-test in R: estimation and testing

The two samples t-test:

```
> t.test(light1,light2)

        Welch Two Sample t-test

data: light1 and light2
t = 4.0598, df = 27.754, p-value = 0.0003625
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  47.63387 144.73135
sample estimates:
mean of x mean of y
 852.4000 756.2174
```

Conclusion: $H_0$ of equal means is rejected.

By default t.test with two arguments performs the two samples $t$-test for independent samples.

## t-test in R: diagnostics

```
> qqnorm(light1)
> qqnorm(light2)
```


Normal Q–Q Plot

Normal Q–Q Plot
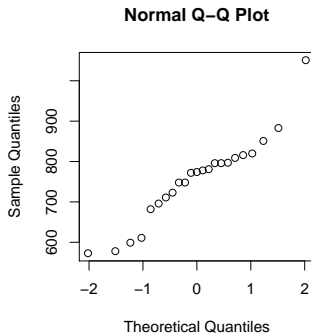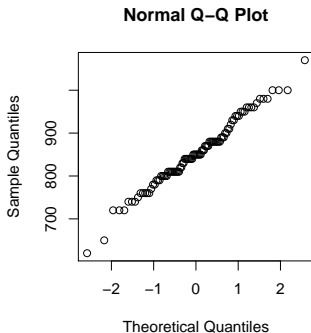
Normality of the second sample is actually doubtful.

Mann-Whitney test

- Data: two independent samples $(X_1, \ldots, X_M)$ and $(Y_1, \ldots, Y_N)$.
- The Mann-Whitney test assumes that the sample $X_1, \ldots, X_M$ stems from population F and sample $Y_1, \ldots, Y_N$ stems from population G.
- We test the null hypothesis $H_0 : F = G$ (the distributions are the same).
- The Mann-Whitney test is again based on ranks. It considers the M ranks $R_1, \ldots, R_M$ of $X_1, \ldots, X_M$ in the combined sample $(X_1, \ldots, X_M, Y_1, \ldots, Y_N)$ of length $M + N$. If $F = G$ these M rank numbers should lie randomly between 1 and $M + N$. The test statistic is

$$T = \sum_{i=1}^{M} R_i, \quad \text{the distribution of } T \text{ under } H_0 \text{ is (approximately) known.}$$

- Large values of T indicate that F is shifted towards the right from G, i.e. that X-values are bigger than Y-values.

If responses are continuous, a significant result of Mann-Whitney test shows a difference in medians, actually this test is only consistent against the alternative $H_1 : P(X > Y) \neq P(Y > X)$.

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Dependence in two paired samples
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Mann-Whitney test in R: testing

```
> wilcox.test(light1,light2)

        Wilcoxon rank sum test with continuity correction

data:  light1 and light2
W = 1829, p-value = 1.056e-05
alternative hypothesis: true location shift is not equal to 0
```

Conclusion: H_0 of equal medians is rejected. The underlying distribution of light1 is shifted to the right from that of light2.

When given two arguments wilcox.test will perform the Mann-Whitney test for two samples. The Mann-Whitney test is especially suited for detecting shift differences — differences in location — between two populations.

One-sided alternatives are also possible to test by the Mann-Whitney test. For example, to test whether the distribution of light1 is on the bigger values than the distribution of light2, we use wilcox.test(light1,light2,alt="g").
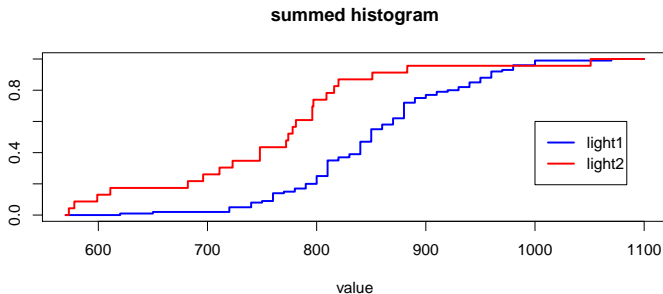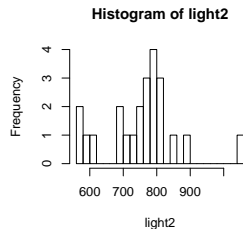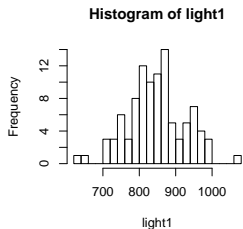
Kolmogorov-Smirnov test

- Data: two independent samples $(X_1, \ldots, X_M)$ and $(Y_1, \ldots, Y_N)$.
- The Kolmogorov-Smirnov test assumes that the sample $X_1, \ldots, X_M$ stems from distribution F_X and sample $Y_1, \ldots, Y_N$ stems from distribution F_Y.
- We test the null hypothesis $H_0 : F_X = F_Y$ (the distributions are the same).
- The Kolmogorov-Smirnov test is based on the maximal difference of the two empirical distribution functions for two samples.
- The test statistic computes the maximal vertical difference in empirical distribution functions (summed histograms). Its distribution under H_0 is known (e.g., in R).

The empirical distribution function for a sample $Z_1, \ldots, Z_n$ is defined as $\hat{F}_n(x) = \frac{\#(i : Z_i \le x)}{n}$ for all $x \in \mathbb{R}$. This is a non-decreasing from 0 to 1 step function making jumps of size $\frac{1}{n}$ in points $Z_{(1)}, \ldots, Z_{(n)}$.

Kolmogorov-Smirnov test in R: graphics

```
> hist(light1)
> hist(light2)
```


Histogram of light1

Histogram of light2

summed histogram

Testing in R by the Kolmogorov-Smirnov test

```
> ks.test(light1,light2)

        Two-sample Kolmogorov-Smirnov test

data:  light1 and light2
D = 0.5391, p-value = 3.803e-05
alternative hypothesis: two-sided

Warning message:
In ks.test(light1, light2) : cannot compute exact p-values with ties
```

A warning about ties again: R uses an approximation for computing the p-value.

Conclusion: $H_0 : F_X = F_Y$ is rejected (in fact, light1 is larger than light2).
One-sided alternatives are also possible to test by the Kolmogorov-Smirnov test. But take into account the **counterintuitive interpretation**: ks.test(x,y,alt="g") tests the alternative $F_X(x) \geq F_Y(x)$ which means that F_Y is shifted towards the right from F_X, i.e., that Y-values are bigger than X-values. For example, we suspect that light1 is larger than light2. To test this, ks.test(light1,light2,alternative="less") (or ks.test(light2,light1,alternative="greated").

To finish

Today we discussed: two samples tests (including permutation test); for paired and independent samples, for normal and not normal cases.

Next time: k samples, one way ANOVA.