Kurs Bio144:
Datenanalyse in der Biologie

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Week 6: ANCOVA, Introduction to Linear Algebra
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Overview (todo: check)

- ANCOVA
- ANCOVA as special cases of a linear model
- Introduction to linear Algebra

Note:

ANCOVA = ANalysi of COVAriance (Kovarianzanalyse)

Course material covered today

- "The new Statistics with R" chapter 7 (ANCOVA)
- Stahel Script chapters 3.A (p. 43-45) and 3.4, 3.5 (p. 39-42)

Recap of ANOVA

- ANOVA is a method to test if the means of two or more groups are different.
- Post-hoc tests and contrasts, including correction for p-values, to understand the differences between the groups.
- Two-way ANOVA for factorial designs, interactions.
- Interpretation of the results.
- Model checking.
- ANOVA as a special case of linear regression with categorical covariates.

Recap of ANOVA II

To do, if needed.

Analysis of Covariance (ANCOVA)

An ANCOVA is an analysis of variance (ANOVA), including also at least one continuous covariate.

There, ANCOVA is a combination of ANOVA and regression, so in principle, there is nothing new to it...

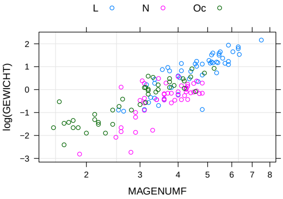
Given a categorical covariate x_i and a continuous covariate z_i. Then the ANCOVA equation (without interactions) is given as

$$y_i = \beta_0 + \beta_1 x_i^{(1)} + \ldots + \beta_k x_i^{(k)} + \beta_z z_i + e_i \ ,$$

where $x_i^{(k)}$ is the kth dummy variable ($x_i^{(k)}$=1 if ith observation belongs to category k, 0 otherwise).

Note: It is straightforward to add interactions $x_i^{(j)} z_i$ for all levels of x_i.

Example: Remember the earthworm study from week 3:
"Magenumfang" was used to predict "Gewicht" of the worm, including as covariate also the worm species.



Categorical and continuous covariates were used to predict a continuous outcome (the weight of the worm).

```
> r.lm <- lm(log(GEWICHT) ~  MAGENUMF + Gattung,d.wurm)
>  summary(r.lm)$coef
```

```
              Estimate Std. Error     t value     Pr(>|t|)
(Intercept) -2.5355459 0.22147279 -11.4485663 8.617670e-22
MAGENUMF     0.7118725 0.04528843  15.7186392 1.232126e-32
GattungN    -0.5151344 0.11009219  -4.6791186 6.760621e-06
GattungOc   -0.0907298 0.12791000  -0.7093254 4.793107e-01
```

Remember: What do the p-values ($p = 0.48$ and $p < 0.0001$) of the categorical covariate "Gattung" mean?

To understand if "Gattung" has an effect, **we need to carry out an F-test** (see slide 45, week 3). To this end, plot the ANOVA table:

```
> anova(r.lm)
```

```
Analysis of Variance Table

Response: log(GEWICHT)
           Df  Sum Sq Mean Sq F value    Pr(>F)
MAGENUMF    1 104.866 104.866  409.69 < 2.2e-16 ***
Gattung     2   7.177   3.589   14.02 2.842e-06 ***
Residuals 139  35.579   0.256
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

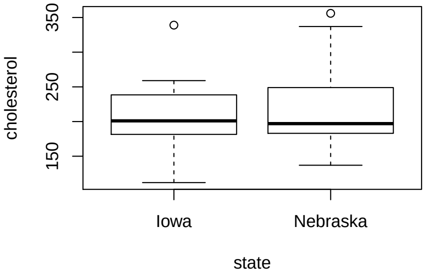
A new example: cholesterol levels

Example: Cholesterol levels [mg/ml] for 30 women from two US states, Iowa and Nebraska, were measured.

Question: Do these levels differ between the states?

Age (years) may be a relevant covariable.

```
> plot(cholesterol ~ state,data=d.chol)
```



The model includes state, age and the interaction of the two. The model equation is thus given as

$$y_i = \beta_0 + \beta_1(state)_i + \beta_2(age)_i + \beta_3(state)_i(age)_i + e_i \ , \quad e_i \sim \mathrm{N}(0, \sigma_e^2) \ .$$

```
> r.aov <- aov(cholesterol ~ age*state,data=d.chol)
> summary(r.aov)
```

```
            Df Sum Sq Mean Sq F value   Pr(>F)    
age          1  48976   48976  26.312 2.39e-05 ***
state        1   5456    5456   2.931   0.0988 .  
age:state    1    709     709   0.381   0.5425    
Residuals   26  48395    1861                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Interpretation?

Compare the results from the previous slide to the `lm()` output:

```
> r.lm <- lm(cholesterol ~ age*state,data=d.chol)
> summary(r.lm)$coef
```

```
                    Estimate Std. Error    t value    Pr(>|t|)
(Intercept)       35.8112138  55.116605  0.6497355 0.521562661
age                3.2381449   1.008827  3.2098104 0.003516155
stateNebraska     65.4865523  61.983368  1.0565181 0.300450053
age:stateNebraska -0.7177069   1.162845 -0.6171990 0.542471382
```

Compare the results for "state" also to the model without interaction:

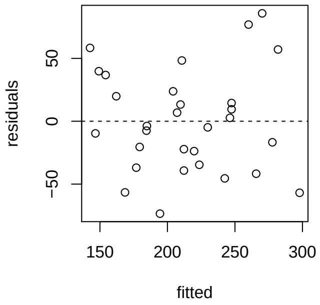
```
> r.lm2 <- lm(cholesterol ~ age + state,data=d.chol)
> summary(r.lm2)$coef
```

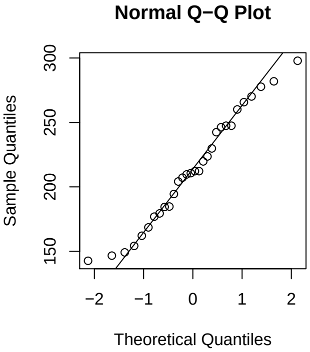
```
               Estimate Std. Error  t value     Pr(>|t|)
(Intercept)   64.489772 29.3024531 2.200832 3.648161e-02
age            2.697967  0.4959532 5.439963 9.361235e-06
stateNebraska 28.651025 16.5409806 1.732124 9.466292e-02
```

Note: the *p*-value for 'state' is now the same as on the previous slide (ANOVA table).

As always, some model checking is necessary:

```
> par(mfrow=c(1,2))
> plot(r.aov$fitted,r.aov$residuals,xlab="fitted",ylab="residuals")
> abline(h=0,lty=2)
> qqnorm(r.aov$fitted)
> qqline(r.aov$fitted)
```





$\rightarrow$ This seems ok.

A final word on ANCOVA

ANCOVA unifies several concepts that we approached in this course so far:

- Linear regression
- Categorical covariates
- Interactions (of continuous and categorical covariates)
- Analysis of Variance (ANOVA)

As such, it is a **special case of the linear model**.

An introduction to linear Algebra

Who has some knowledge of linear Algebra?

Overview

- The basics about
  - vectors
  - matrices
  - matrix algebra
  - matrix multiplication
- Why is linear Algebra useful?
- What does it have to do with data analysis and statistics?
- Regression equations in matrix notation.

Motivation

Why are vectors, matrices and their algebraic rules useful?

Example 1: The observations for a covariate $\mathbf{x}$ or the response $\mathbf{y}$ for all individuals $1 \leq i \leq n$ can be stored in a vector (vectors and matrices are always given in **bold** letters):

$$\mathbf{x} = \begin{pmatrix} x_1 \\ x_2 \\ \ldots \\ x_n \end{pmatrix} , \quad \mathbf{y} = \begin{pmatrix} y_1 \\ y_2 \\ \ldots \\ y_n \end{pmatrix} .$$

Example 2: Covariance matrices for multiple variables. Say we have $\mathbf{x}^{(1)}$ and $\mathbf{x}^{(2)}$. The covariance matrix is then given as

$$\begin{pmatrix} \text{Var}(\mathbf{x}^{(1)}) & \text{Cov}(\mathbf{x}^{(1)}, \mathbf{x}^{(2)}) \\ \text{Cov}(\mathbf{x}^{(1)}, \mathbf{x}^{(2)}) & \text{Var}(\mathbf{x}^{(2)}) \end{pmatrix} .$$

Example 3: The data (e.g. of some regression model) can be stored in a matrix:

$$\tilde{\mathbf{X}} = \left(\begin{array}{ccc} 1 & x_1^{(1)} & x_2^{(1)} \\ 1 & x_2^{(1)} & x_2^{(2)} \\ ... & ... & ... \\ 1 & x_n^{(1)} & x_n^{(2)} \end{array} \right) .$$

This is the so-called design matrix with a vector of 1's in the first column.

Example 4: A linear regression model can be written compactly using matrix multiplication:

$$\mathbf{y} = \tilde{\mathbf{X}} \cdot \tilde{\boldsymbol{\beta}} + \mathbf{e} \ ,$$

with $\tilde{\boldsymbol{\beta}}$ the vector of regression coefficients and $\boldsymbol{\epsilon}$ the vector of residuals.

Why do we discuss this topic in our course?

- Useful for compact notation.
- Enabels you to understand many statistical texts (books, research articles) that remain inaccessible otherwise.
- More advanced concepts often rely on linear algebra, e.g. principal component analysis (PCA) or random effects models.
- Often useful for efficient coding, e.g. in R, which helps to increase speed and to reduce error rates.
- Is part of a general education (Allgemeinbildung) ;-)

Matrices

An $n \times m$ Matrix is given as

$$\mathbf{A} = \left(\begin{array}{cccc} a_{11} & a_{12} & \dots & a_{1m} \\ a_{21} & a_{22} & \dots & a_{2m} \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \dots & a_{nm} \end{array} \right) ,$$

with rows $1 = 1, \dots, n$ and columns $j = 1, \dots, m$.

Quadratic matrix: $n = m$. Example:

$$\left(\begin{array}{ccc} 1 & 2 & 3 \\ 4 & 3 & 2 \\ 6 & 1 & 9 \end{array} \right)$$

Symmetric matrix: $a_{ij} = a_{ji}$. Example:

$$\begin{pmatrix} 1 & 2 & 3 \\ 2 & 3 & 4 \\ 3 & 4 & 5 \end{pmatrix} .$$

The diagonal of a quadratic matrix is given by $(a_{11}, a_{22}, \ldots, a_{nn})$.
Example: the diagonal of the above matrix is given as

$$(a_{11}, a_{22}, a_{33}) = (1, 3, 5) .$$

Diagonal matrix: A matrix that has entries $\neq 0$ only on the diagonal.
Example:

$$\begin{pmatrix} 1 & 0 & 0 \\ 0 & 3 & 0 \\ 0 & 0 & 5 \end{pmatrix} .$$

Transposing a matrix: Given a matrix $\mathbf{A}$. Exchange the rows by the columns and vice versa. This leads to the transposed matrix $\mathbf{A}^\top$:

$$\mathbf{A} = \begin{pmatrix} a_{11} & a_{12} & \dots & a_{1m} \\ a_{21} & a_{22} & \dots & a_{2m} \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \dots & a_{nm} \end{pmatrix} \quad \Rightarrow \quad \mathbf{A}^\top = \begin{pmatrix} a_{11} & a_{21} & \dots & a_{m1} \\ a_{12} & a_{22} & \dots & a_{m2} \\ \vdots & \vdots & & \vdots \\ a_{1n} & a_{2n} & \dots & a_{mn} \end{pmatrix} .$$

Examples (note also the change of dimensions):

$$\mathbf{A} = \begin{pmatrix} 1 & 2 & 3 & 4 \\ 5 & 6 & 7 & 8 \\ 9 & 10 & 11 & 12 \\ 13 & 14 & 15 & 16 \end{pmatrix} \quad \Rightarrow \quad \mathbf{A}^\top = \begin{pmatrix} 1 & 5 & 9 & 13 \\ 2 & 6 & 10 & 14 \\ 3 & 7 & 11 & 15 \\ 4 & 8 & 12 & 16 \end{pmatrix}$$

$$\mathbf{A} = \begin{pmatrix} 1 & 2 & 3 & 4 \\ 5 & 6 & 7 & 8 \end{pmatrix} \quad \Rightarrow \quad \mathbf{A}^\top = \begin{pmatrix} 1 & 5 \\ 2 & 6 \\ 3 & 7 \\ 4 & 8 \end{pmatrix}$$

- Transposing a matrix twice leads to the original matrix:

$$(\mathbf{A}^\top)^\top = \mathbf{A} \ .$$

- When a matrix is symmetric, then

$$\mathbf{A}^\top = \mathbf{A} \ .$$

  This is true in particular for diagonal matrices.

Vectors

A vector is nothing else than n numbers written in a column:

$$\mathbf{b} = \begin{pmatrix} b_1 \\ b_2 \\ \vdots \\ b_n \end{pmatrix}$$

Transposing a vector leads to a *row vector*:

$$\begin{pmatrix} b_1 \\ b_2 \\ \vdots \\ b_n \end{pmatrix}^\top = \begin{pmatrix} b_1 & b_2 & \dots & b_n \end{pmatrix}$$

Note: By definition (by default), a vector is always a column vector.

Addition and substraction

- Adding and substracting matrices and vectors is only possible when the objects have the same dimension.
- Examples: Elementwise addition (or substraction)

$$\begin{pmatrix} 1 & 2 & 3 \\ 4 & 5 & 6 \end{pmatrix} + \begin{pmatrix} 3 & 2 & 1 \\ 6 & 5 & 4 \end{pmatrix} = \begin{pmatrix} 4 & 4 & 4 \\ 10 & 10 & 10 \end{pmatrix}$$

$$\begin{pmatrix} 1 \\ 4 \end{pmatrix} - \begin{pmatrix} 3 \\ 9 \end{pmatrix} = \begin{pmatrix} -2 \\ -5 \end{pmatrix}$$

- But this addition is not defined:

$$\begin{pmatrix} 1 & 2 & 3 \\ 4 & 5 & 6 \end{pmatrix} + \begin{pmatrix} 3 & 6 \\ 2 & 5 \\ 1 & 4 \end{pmatrix} =$$

Multiplication by a scalar

Multiplication with a “number” (scalar) is simple: Multiply each element in a vector or a matrix.

Examples:

$$3 \cdot \begin{pmatrix} 1 & 2 & 3 \\ 4 & 5 & 6 \end{pmatrix} = \begin{pmatrix} 3 & 6 & 9 \\ 12 & 15 & 18 \end{pmatrix}$$

$$-2 \cdot \begin{pmatrix} 1 \\ 4 \\ -2 \end{pmatrix} = \begin{pmatrix} -2 \\ -8 \\ 4 \end{pmatrix}$$

Matrix multiplication

The multiplication of two matrices **A** and **B** is defined if

Number of columns in **A** = Number of rows in **B**.

It is easiest to explain matrix multiplication with an example:

$$\begin{pmatrix} 2 & 1 \\ -1 & 0 \\ 3 & 1 \end{pmatrix} \cdot \begin{pmatrix} 3 & 1 \\ 4 & -2 \end{pmatrix} = \begin{pmatrix} 2\cdot 3 + 1\cdot 4 & 2\cdot 1 + 1\cdot(-2) \\ (-1)\cdot 3 + 0\cdot 4 & (-1)\cdot 1 + 0\cdot(-2) \\ 3\cdot 3 + 1\cdot 4 & 3\cdot 1 + 1\cdot(-2) \end{pmatrix}$$

$$= \begin{pmatrix} 10 & 0 \\ -3 & -1 \\ 13 & 1 \end{pmatrix}$$

▸ Matrix multiplication app

Matrix multiplication rules I

Attention: Matrix multiplication does not follow the same rules as scalar multiplication!!!

- It can happen that $\mathbf{A} \cdot \mathbf{B}$ can be calculated, but $\mathbf{B} \cdot \mathbf{A}$ is not defined (see example on previous slide).
- In general: $\mathbf{A} \cdot \mathbf{B} \neq \mathbf{B} \cdot \mathbf{A}$, even if both are defined.
- It can happen that $\mathbf{A} \cdot \mathbf{B} = \mathbf{0}$ ($\mathbf{0}$ matrix), although both $\mathbf{A} \neq \mathbf{0}$ and $\mathbf{B} \neq \mathbf{0}$.
- The Assoziativgesetz holds: $\mathbf{A} \cdot (\mathbf{B} \cdot \mathbf{C}) = (\mathbf{A} \cdot \mathbf{B}) \cdot \mathbf{C}$.
- The Distributivgesetz holds:

$$\begin{aligned} \mathbf{A} \cdot (\mathbf{B} + \mathbf{C}) &= \mathbf{A} \cdot \mathbf{B} + \mathbf{A} \cdot \mathbf{C} \\ (\mathbf{A} + \mathbf{B}) \cdot \mathbf{C} &= \mathbf{A} \cdot \mathbf{C} + \mathbf{B} \cdot \mathbf{C} \end{aligned}$$

Matrix multiplication rules II

- Transposing inverts the order: $(\mathbf{A} \cdot \mathbf{B})^\top = \mathbf{B}^\top \cdot \mathbf{A}^\top$.
- The product $\mathbf{A} \cdot \mathbf{A}^\top$ is always symmetric.
- All these rules also hold for vectors, which can be interpreted as $n \times 1$ matrices:

$$\mathbf{a} \cdot \mathbf{b}^\top = \begin{pmatrix} a_1 b_1 & a_1 b_2 & \dots & a_1 b_m \\ a_2 b_1 & a_2 b_2 & \dots & a_2 b_m \\ \vdots & \vdots & & \vdots \\ a_n b_1 & a_n b_2 & \dots & a_n b_m \end{pmatrix}$$

If $\mathbf{a}$ and $\mathbf{b}$ have the same length:

$$\mathbf{a}^\top \cdot \mathbf{b} = \sum_i a_i b_i$$

Short exercises

Given vectors **a** and **b** and matrix **C**:

$$\mathbf{a} = \begin{pmatrix} 1 \\ -2 \\ 3 \\ 0 \end{pmatrix}, \quad \mathbf{b} = \begin{pmatrix} -2 \\ 4 \end{pmatrix}, \quad \mathbf{C} = \begin{pmatrix} 1 & 0 \\ -1 & 2 \end{pmatrix}$$

Calculate, if defined

- $\mathbf{a}^\top \cdot \mathbf{b}$
- $\mathbf{a} \cdot \mathbf{b}^\top$
- $\mathbf{C} \cdot \mathbf{a}$
- $\mathbf{C} \cdot \mathbf{b}$

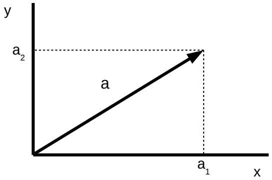
The length of a vector

The length of a vector $\mathbf{a}^\top = (a_1, a_2, \dots, a_n)$ is defined as $||\mathbf{a}||$ with

$$||\mathbf{a}||^2 = \mathbf{a}^\top \cdot \mathbf{a} = \sum_i a_i^2 \ .$$

This is basically the Pythagoras idea in 2, 3, ... n dimensions.

In 2 dimensions: $||\mathbf{a}||^2 = a_1^2 + a_2^2$:



Identity matrix (Einheitsmatrix)

The identity matrix (of dimension m) is probably the simples matrix that exists. It has 1's on the diagonal and 0's everywhere else:

$$\mathbf{I} = \begin{pmatrix} 1 & 0 & \ldots & 0 \\ 0 & 1 & \ldots & 0 \\ \vdots & \vdots & & \vdots \\ 0 & 0 & \ldots & 1 \end{pmatrix}$$

Multiplication with the identity matrix leaves a $m \times n$ matrix $\mathbf{A}$ unchanged:

$$\mathbf{I} \cdot \mathbf{A} = \mathbf{A} \cdot \mathbf{I} = \mathbf{A} \ .$$

Inverse matrix

Given a quadratic matrix $\mathbf{A}$ that fulfills

$$\mathbf{B} \cdot \mathbf{A} = \mathbf{I} \ ,$$

then $\mathbf{B}$ is called the inverse of $\mathbf{A}$ (and vice versa). One then writes

$$\mathbf{B} = \mathbf{A}^{-1} \ .$$

Note:

- In that case it also holds that $\mathbf{A} \cdot \mathbf{B} = \mathbf{I}$.
- Therefore: $\mathbf{A} = \mathbf{B}^{-1} \quad \Leftrightarrow \quad \mathbf{B} = \mathbf{A}^{-1}$

- The inverse of $\mathbf{A}$ may not exist. If it exists, $\mathbf{A}$ is regular, otherwise singular.

- $(\mathbf{A}^{-1})^{-1} = \mathbf{A}$.

- The inverse of a matrix product is given as

$$(\mathbf{A} \cdot \mathbf{B})^{-1} = \mathbf{B}^{-1} \cdot \mathbf{A}^{-1} \ .$$

- It is

$$(\mathbf{A}^\top)^{-1} = (\mathbf{A}^{-1})^\top \ .$$

  Therefore one may also write $\mathbf{A}^{-\top}$.

Linear equation system in matrix notation

Remember example 4 from slide 16: We said that a linear regression model can be written compactly using matrix multiplication:

$$\mathbf{y} = \tilde{\mathbf{X}} \cdot \tilde{\boldsymbol{\beta}} + \mathbf{e} \ .$$

Task: Verify this now, using a model with two variables $\mathbf{x}^{(1)}$ and $\mathbf{x}^{(2)}$ and

$$\mathbf{y} = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix} , \ \tilde{\boldsymbol{\beta}} = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{pmatrix} , \ \tilde{\mathbf{X}} = \begin{pmatrix} 1 & x_1^{(1)} & x_2^{(1)} \\ 1 & x_2^{(1)} & x_2^{(2)} \\ \dots & \dots & \dots \\ 1 & x_n^{(1)} & x_n^{(2)} \end{pmatrix} , \ \mathbf{e} = \begin{pmatrix} e_1 \\ e_2 \\ \vdots \\ e_n \end{pmatrix} .$$

It can be shown (see Stahel 3.4f,g) that the least-squares estimates $\hat{\boldsymbol{\beta}}$ can be calculated as

$$\hat{\boldsymbol{\beta}} = (\tilde{\mathbf{X}}^\top \tilde{\mathbf{X}})^{-1} \cdot \tilde{\mathbf{X}}^\top \cdot \mathbf{y}$$

Does this look complicated?

Let's test this in R ....

Doing linear algebra in R

Let us look at model $\mathbf{y} = \tilde{\mathbf{X}} \cdot \tilde{\boldsymbol{\beta}} + \mathbf{e}$ with coefficients $\beta_0 = 10, \beta_1 = 5, \beta_2 = -2$ and variables

i	$x_i^{(1)}$	$x_i^{(2)}$
1	0	4
2	1	1
3	2	0
4	3	1
5	4	4

Thus the model is given as

$$y_i = 10 + 5x_i^{(1)} + -2x_i^{(2)} + e_i \ , \text{for} \quad 1 \leq i \leq n \ .$$

Let us start by generating the "true" response, calculated as $\tilde{\mathbf{X}}\tilde{\boldsymbol{\beta}}$:

```
> x1 <- c(0,1,2,3,4)
> x2 <- c(4,1,0,1,4)
> Xtilde <- matrix(c(rep(1,5),x1,x2),ncol=3)
> Xtilde
```

```
     [,1] [,2] [,3]
[1,]    1    0    4
[2,]    1    1    1
[3,]    1    2    0
[4,]    1    3    1
[5,]    1    4    4
```

```
> beta <- c(10,5,-2)
> t.y <- Xtilde%*%beta
> t.y
```

```
     [,1]
[1,]    2
[2,]   13
[3,]   20
[4,]   23
[5,]   22
```

Matrix multiplication in R is done by the %*% symbol.

Next, we generate the vector containing the $e_i \sim N(0, \sigma_e^2)$ with $\sigma_e^2 = 1$:

```
> e <- runif(5,0,1)
```

which we add to the "true" $\mathbf{y} = \tilde{\mathbf{X}}\tilde{\beta}$ values, to obtain the "observed" values:

```
> t.Y <- t.y  + e
```

It is now possible to fit the model with `lm`:

```
> r.lm <- lm(t.Y ~ x1 + x2)
> summary(r.lm)$coef
```

```
             Estimate Std. Error   t value     Pr(>|t|)
(Intercept) 10.559927 0.15368505  68.71148 0.0002117402
x1           4.892453 0.05163983  94.74186 0.0001113893
x2          -1.948033 0.04364362 -44.63500 0.0005015590
```

Alternatively, we can use formula

$$\hat{\boldsymbol{\beta}} = (\tilde{\mathbf{X}}^\top \tilde{\mathbf{X}})^{-1} \tilde{\mathbf{X}}^\top \mathbf{y}$$

to find the parameter estimates:

```
> solve(t(Xtilde) %*% Xtilde)  %*%  t(Xtilde) %*% t.Y

          [,1]
[1,] 10.559927
[2,]  4.892453
[3,] -1.948033
```

- `solve()` calculates the inverse.
- `t()` gives the transposed.

Task: Do this calculation by yourself and verify for each step that the dimensions of the matrices and the vector are indeed fitting, so that this expression is defined.

Some R commands for matrix algebra

Ev?

Distribution of the β coefficients

See chapter 3.5 in Stahel: Linear algebra is useful to find the actual distribution of the regression coefficients $\boldsymbol{\beta}$.

No need to know this by heart – but please remember:

Finding the joint distribution for $\boldsymbol{\beta}$ is **much harder** without linear alebra.

Summary