# Kurs Bio144:
# Datenanalyse in der Biologie

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Lecture 6: ANOVA

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# Overview

- One-way ANOVA
- Post-hoc tests and contrasts
- Two-way ANOVA
- ANOVA as special cases of a linear model

Note:

ANOVA = ANalysis Of VAriance (Varianzanalyse)

# Course material covered today

The lecture material of today is based on the following literature:

- Chapter 12 from Stahel book "Statistische Datenenalyse"
- "Getting Started with R" chapters 5.6 and 6.2

# ANOVA and ANCOVA

ANOVA = Varianzanalyse
ANCOVA = Kovarianzanalyse

Introduction by Sir R. A. Fisher (1890-1962). He worked at the agricultural research station in Rothamstead (England). AN(C)OVA are/were therefore traditionally used to analyze agricultural experiments.

Questions of AN(C)OVA:

- Generally: Are the means of two or more groups different?
- Example: Are different plant breeds different in important aspects (e.g., yields / Ertrag)?
- Example: What is the influence of different treatments on plants (Biology) or patients (Medicine)?

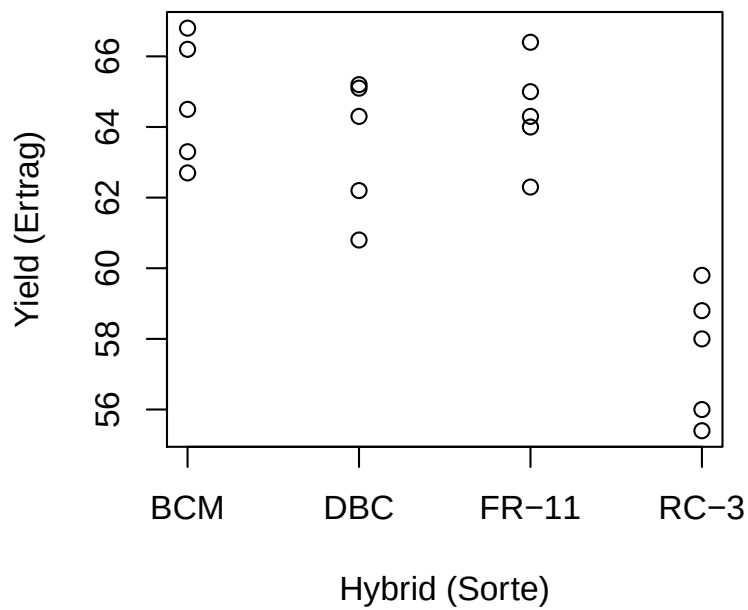
# Beispiel: Ertragspotential bei Hybrid-Mais mit erhöhter Pilzbrand-Resistenz

(Source: W. Blanckenhorn, UZH)

Es wurden 4 Hybrid-Mais-Sorten angebaut und ihr Körnerertrag ermittelt. Jede Sorte wurde an 5 Orten angepflanzt.

**Frage:** Unterscheiden sich die Hybrid-Mais-Sorten im Ertrag?

Achtung: Die Frage bezieht sich auf *irgendeinen* Unterschied. Präziser könnte man fragen, ob sich irgendeine der Sorten von den anderen unterscheidet?



**Naive idea:** To carry out pairwise $t$-tests between any two groups.

1. How many tests would this imply?
2. Why is this not a very clever idea?

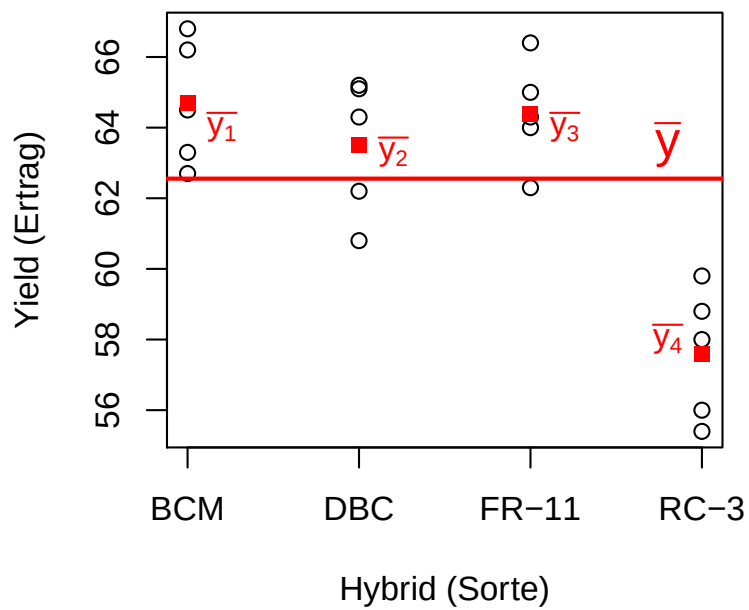
Please answer these questions here: http://www.klicker.uzh.ch/bkx

# Better idea

Formulate a model that is able to test simultaneously whether there is an overall difference between the groups. That is, ask only **one question!**

This leads us to the

**Idea of the ANOVA analysis:**
Compare the variability within groups ($MS_E$) to the variability between the group means ($MS_G$).



We formulate a model as follows:

$$y_{ij} = \mu_i + e_{ij} \ ,$$

where

- $y_{ij}$= "Ertrag der $j$-ten Pflanze der Sorte $i$"
- $\mu_i$="Mittlerer Ertrag der Sorte $i$"
- $e_{ij} \sim \mathrm{N}(0, \sigma_e^2)$ is an independent error term.

Typically, this is **rewritten as**

$$y_{ij} = \mu + \beta_i + e_{ij} \ ,$$

where $\mu + \beta_i = \mu_i$ from above, thus the **group mean** of group $i$.

# Single factor ANOVA (Einfaktorielle Varianzanalyse)

More generally, this leads us to the single factor ANOVA:

Assume we have $g$ groups and in each group $i$ there are $n_i$ measurements of some variable of interest, denoted as $y_{ij}$. The model is then given as

$$y_{ij} = \mu + \beta_i + e_{ij} \quad \text{for} \quad \begin{aligned} i &= 1, \ldots, g \ , \\ j &= 1, \ldots, n_i, \\ e_{ij} &\sim \mathrm{N}(0, \sigma_e^2) \quad i.i.d. \end{aligned} \tag{1}$$

- $\mu$ plays the role of the intercept $\beta_0$ in standard regression models.
- The estimation of $\mu$, $\beta_2$, $\ldots$, $\beta_g$ is again done by least squares minimization.
- The $e_{ij} \sim \mathrm{N}(0, \sigma_e^2) \quad i.i.d.$ assumption is again crucial, so model checking will be needed again.

Attention: Model (**??**) is overparameterized, thus an additional constraint is needed! Most popular:

- $\beta_1 = 0$ (treatment contrast; default in R).

  Interpretation: Group 1 is usually chosen such that it is some sort of reference group or reference level, for example a standard diet, while groups 2, 3, etc. corresponde to novel diets whose effect is tested in an experiment.

- $\sum_i \beta_i = 0$ (sum-to-zero contrast).

  Interpretation: The effects $\beta_1$, $\beta_2$ etc give the deviation from the population averaged effect.

# ANOVA as a special case of a linear model

The clou is: Model (??) is identical to the regression model with a factor covariate, see slides 35/36 from week 3.

**Interpretation: The levels of the factor are now the different group memberships.**

Thus (assuming $\beta_1 = 0$):

$$y_{ij} = \begin{cases} \mu + e_{ij}, & \text{for group 1} \\ \mu + \beta_2 + e_{ij}, & \text{for group 2} \\ \ldots & \\ \mu + \beta_g + e_{ij}, & \text{for group } g \ , \end{cases}$$

and $\hat{y}_{ij} = \overline{y}_{.i} = \mu + \beta_i$ can be interpreted as the predicted value.

# The ANOVA test: The $F$-test

**Aim of ANOVA**: to test *globally* if the groups differ. That is:

| | | | |
|---|---|---|---|
| $\mathbf{H_0}$ | : | $\mu_1 = \mu_2 = \ldots = \mu_g$ | or, equivalently $\beta_2 = \ldots = \beta_g = 0$ |
| $\mathbf{H_1}$ | : | At least two groups are different | |

Remember from slides 45/46 of week 3 (or Stahel script p.33): We have already used the $F$-test for categorical variables (see $F$-test for the earthworms, slide 47 of week 3). This was equivalent to testing if all $\beta$s that belong to a categorical variable are $=0$ at the same time.

$\rightarrow$ equivalent to test if the (categorical!) covariate is needed in the model.

This is the very same problem here, thus we need the $F$-test again!

# Variance decomposition

To derive the ingredients of the $F$-test, we look at the decomposition of variance (Remember this idea from week 3, slide 23):

$$\begin{aligned}
\text{total variability} &= \text{explained variability} + \text{residual variability} \\
SS_{total} &= SS_{\text{between groups}} + SS_{\text{within groups}} \\
\sum_{i=1}^{g}\sum_{j=1}^{n_i}(y_{ij}-\overline{y})^2 &= \sum_{i=1}^{g} n_i(\overline{y}_{.i}-\overline{y})^2 + \sum_{i=1}^{g}\sum_{j=1}^{n_i}(y_{ij}-\overline{y}_{.i})^2
\end{aligned}$$

Degrees of freedom:

$$n-1 = (g-1) + (n-g)$$

From this:

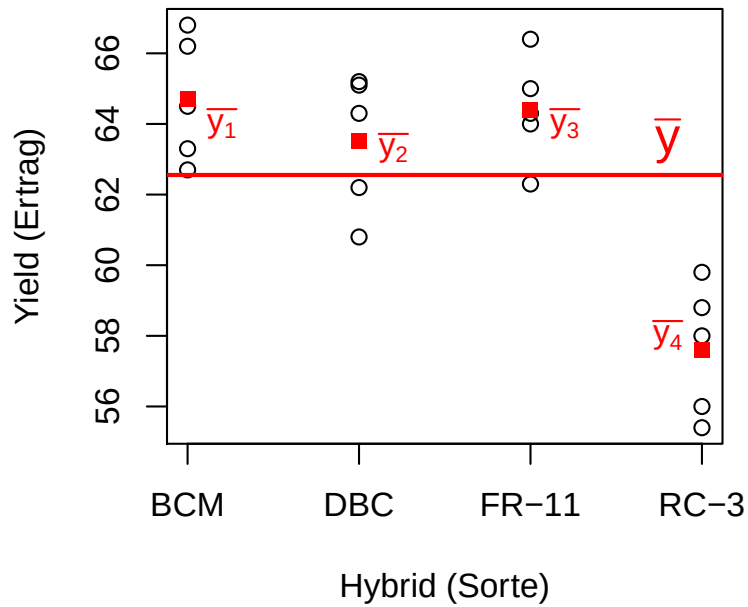
$$\left.\begin{aligned} MS_G &= \tfrac{SS_{\text{between}}}{g-1} \\ MS_E &= \tfrac{SS_{\text{within}}}{n-g} \end{aligned}\right\} \Rightarrow F = \frac{MS_G}{MS_E} \quad \text{is } \sim F_{g-1,n-g} \text{ distributed.}$$

# Interpretation of the $F$ statistic

- $MS_G$: Quantifies the variability **between** groups.
- $MS_E$: Quantifies the variability **within** groups.

**Example:**

- $MS_G$ captures the variability among the four means $(\overline{y}_1, \overline{y}_2, \overline{y}_3, \overline{y}_4)$
- $MS_E$ captures the variability of the $y_{ij}$ within the groups.



# Interpretation of the $F$ statistic II

Remember: $F = \frac{MS_G}{MS_E}$.

- $F$ increases
  - when the group means become more different, or
  - when the variability within groups decreases.
- On the other hand, $F$ decreases
  - when the group means become more similar, or
  - when the variability within groups increases.

$\rightarrow$ The larger $F$, the less likely is the data seen under $H_0$.

▸ ANOVA App

https://gallery.shinyapps.io/anova_shiny_rstudio/

# The ANOVA table

An overview of the results is typically given in an ANOVA table (Varianzanalysen-Tabelle):

| Variation | df | SS | MS = SS/df | F | $p$ |
|---|---|---|---|---|---|
| Between groups | $g-1$ | $SS_G$ | $MS_G$ | $\frac{MS_G}{MS_E}$ | $\Pr(F_{g-1,n-g} > \lvert F \rvert))$ |
| Within groups | $n-g$ | $SS_E$ | $MS_E$ | | |
| Total | $n-1$ | $SS_{\text{total}}$ | | | |

# Our first ANOVA: Hybrid-Mais example

| HYBRID | LOCATION | YIELD |
|---|---|---|
| FR-11 | NW | 62 |
| FR-11 | NE | 64 |
| FR-11 | C | 64 |
| FR-11 | SE | 65 |
| FR-11 | SW | 66 |
| BCM | NW | 63 |
| BCM | NE | 63 |
| BCM | C | 66 |
| BCM | SE | 67 |
| BCM | SW | 64 |

| HYBRID | LOCATION | YIELD |
|---|---|---|
| DBC | NW | 61 |
| DBC | NE | 64 |
| DBC | C | 65 |
| DBC | SE | 62 |
| DBC | SW | 65 |
| RC-3 | NW | 55 |
| RC-3 | NE | 56 |
| RC-3 | C | 60 |
| RC-3 | SE | 58 |
| RC-3 | SW | 59 |

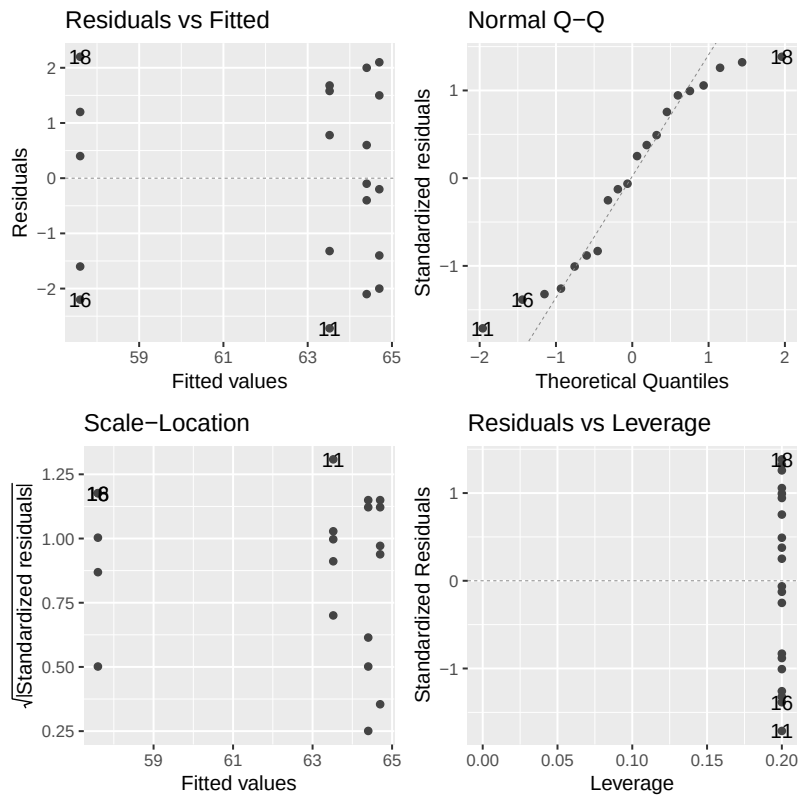
```
> glimpse(d.mais)

Observations: 20
Variables: 3
$ HYBRID   <fct> FR-11, FR-11, FR-11, FR-11, FR-11, BCM, BCM, BCM, BCM, BCM...
$ LOCATION <fct> NW, NE, C, SE, SW, NW, NE, C, SE, SW, NW, NE, C, SE, SW, N...
$ YIELD    <dbl> 62.3, 64.0, 64.3, 65.0, 66.4, 63.3, 62.7, 66.2, 66.8, 64.5...
```

# Hybrid-Mais example – Estimation

Using the `lm()` function in R and then look at the ANOVA table:

```
> r.mais <- lm(YIELD ~ HYBRID, d.mais)
```

As always, before looking at the results, let's do some model checking. This is identical to all we did so far, because we are still working with linear models!



**Always** when we needed to do an $F$-test and when categorical covariates were involved, the `anova()` table was required:

```
> anova(r.mais)

Analysis of Variance Table

Response: YIELD
          Df  Sum Sq Mean Sq F value    Pr(>F)
HYBRID     3 167.441  55.814  17.681 2.474e-05 ***
Residuals 16  50.508   3.157
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

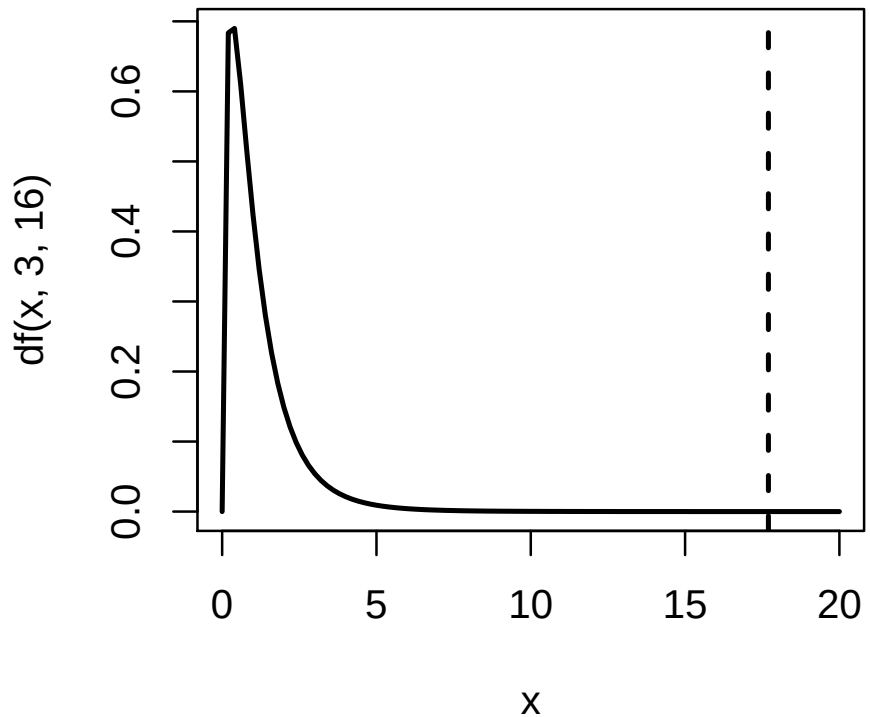
You can see that the value of $F = 17.68$ is $F$-distributed with 3 and 16 degrees of freedom, and the $p$-value of the test "$\beta_2 = \beta_3 = \beta_4$ ?" is $< 0.0001$.

Conclusion: There are differences among the four groups!

$\rightarrow$ This is equivalent to "The group variable is relevant for the model".

**Exercise:** Look at the table a bit closer. How are `Df`, `Sum Sq`, `Mean Sq`, `F value` and `Pr(<F)` related?

The $F$-distribution with 3 and 16 degrees of freedom, as well as the estimated value $F$=17.68:



What happens if you apply `summary()` to the `lm()` object?

```
> summary(r.mais)

Call:
lm(formula = YIELD ~ HYBRID, data = d.mais)

Residuals:
   Min     1Q Median     3Q    Max
 -2.72  -1.45   0.15   1.52   2.20

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  64.7000     0.7946  81.427  < 2e-16 ***
HYBRIDDBC    -1.1800     1.1237  -1.050    0.309
HYBRIDFR-11  -0.3000     1.1237  -0.267    0.793
HYBRIDRC-3   -7.1000     1.1237  -6.318 1.02e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.777 on 16 degrees of freedom
Multiple R-squared:  0.7683,        Adjusted R-squared:  0.7248
F-statistic: 17.68 on 3 and 16 DF,  p-value: 2.474e-05
```

The table contains the estimates of the intercept 64.70 ($\mu$ in ANOVA notation, $\beta_0$ in regression notation), and estimates for $\beta_2$, $\beta_3$, $\beta_4$ (while the reference was set to $\beta_1 = 0$).

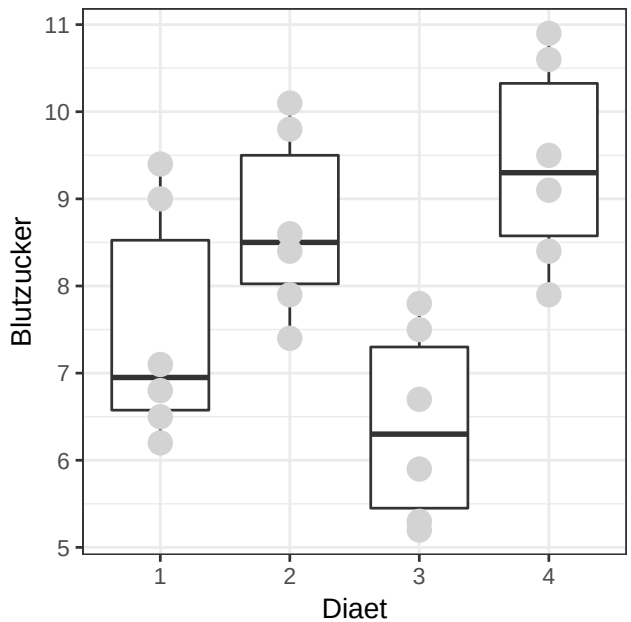
**Hint:** Check also the *F*-test on the last line...

# Exercise: Ernährung und Blutzucker

Remember example 3 from the first week:

24 Personen werden in 4 Gruppen unterteilt. Jede Gruppe erhält eine andere Diät (DIAET). Es werden zu Beginn und am Ende (nach 2 Wochen) die Blutzuckerwerte gemessen. Die Differenz wird gespeichert (BLUTZUCK).

**Frage:** Unterscheiden sich die Gruppen in der Veränderung der Blutzuckerwerte?



Interpret the results:

```
> d.blz <- mutate(d.blz,DIAET=as.factor(DIAET))
> anova(lm(BLUTZUCK ~ DIAET,d.blz))
```

```
Analysis of Variance Table

Response: BLUTZUCK
          Df Sum Sq Mean Sq F value   Pr(>F)
DIAET      3  31.56   10.52  7.5143 0.001476 **
Residuals 20  28.00    1.40
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Question: Why is `mutate()` needed first?

# Multiple comparisons, multiple tests

To remember:

- The $F$-Test is used to check whether any two group means differ.
- Using pairwise tests is not a very good idea (see slide **??**), because this leads to a multiple testing problem:

> When many tests are carried out, the probability to find a "significant" result **by chance** increases.

For instance, for four groups there are $4 \cdot 3/2 = 6$ pairwise combinations that could be tested.

$\rightarrow$ The probability to find *at least one result by pure chance under* $H_0$ is much higher than the 5% error level!!!

# Post-hoc tests

**Still:** If the test $\beta_2 = \ldots = \beta_g = 0$ is rejected, a researcher is then often interested

1. in finding the actual group(s) that deviate(s) from the others.
2. in estimates of the pairwise differences.

Several methods to circumvent the problem of too many "significant" test results (type-I error) have been proposed. The most prominent ones are:

- Bonferroni correction
- Tukey **h**onest **s**ignificant **d**ifferences (HSD) approach
- Fisher **l**east **s**ignificant **d**ifferences (LSD) approach

## Bonferroni correction

**Idea:** If a total of $m$ tests are carried out, simply divide the type-I error level $\alpha_0$ (often 5%) such that

$$\alpha = \alpha_0 / m \ .$$

## Tukey HSD approach

**Idea:** Take into account the distribution of *ranges* (max-min) and design a new test.

## Fisher's LSD approach

**Idea:** Adjust the idea of a two-sample test, but use a larger variance (namely the pooled variance of all groups).

Calculate the pairwise differences and tests with adjustments for the "Blutzucker" example:

Differences:

| | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 1.2 | | |
| 3 | -1.1 | -2.3 | |
| 4 | 1.9 | 0.7 | 3.0 |

Bonferroni $p$-values:

| | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.57 | | |
| 3 | 0.74 | 0.02 | |
| 4 | 0.07 | 1.00 | 0.002 |

Tukey HSD $p$-values:

| | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.32 | | |
| 3 | 0.40 | 0.01 | |
| 4 | 0.05 | 0.74 | 0.001 |

Fisher $p$-values:

| | 1 | 2 | 3 |
|---|---|---|---|
| 2 | 0.09 | | |
| 3 | 0.12 | 0.003 | |
| 4 | 0.01 | 0.32 | 3e-04 |

- Bonferroni $p$-values are the most conservative (largest $p$).
- Fisher $p$-values are the least conservative (smallest $p$).

# Other contrasts

Sometimes additional comparisons are of interest. For example, a new diet is to be compared to other, existing diets.

In the "Blutzucker" example, this could be, for instance:

**"Is diet 1 different from diets 2-4?"**

(Check also chapter 5.6.5 in GSWR)

# Choosing the reference level

Back to the Hybrid Mais example. R orders the levels alphabetically and takes the first level as reference level.

This can be changed manually:

```
> levels(d.mais$HYBRID)

[1] "BCM"   "DBC"   "FR-11" "RC-3"

> d.mais <- mutate(d.mais,HYBRID = relevel(HYBRID,ref="DBC"))
> anova(lm(YIELD ~ HYBRID, d.mais))

Analysis of Variance Table

Response: YIELD
          Df  Sum Sq Mean Sq F value    Pr(>F)
HYBRID     3 167.441  55.814  17.681 2.474e-05 ***
Residuals 16  50.508   3.157
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> summary(lm(YIELD ~ HYBRID, d.mais))$coef

            Estimate Std. Error    t value     Pr(>|t|)
(Intercept)    63.52  0.7945754 79.9420714 2.974727e-22
HYBRIDBCM       1.18  1.1236992  1.0501030 3.092739e-01
HYBRIDFR-11     0.88  1.1236992  0.7831277 4.449899e-01
HYBRIDRC-3     -5.92  1.1236992 -5.2683136 7.649526e-05
```

# Two-way ANOVA (Zweiweg-Varianzanalyse)

Example (from Hand et al. 1994 / Hothorn/Everitt "A Handbook of Statistical Analyses Using R"):
Experiment to study the weight gain of rats, depending on four diets. Protein amounts were either high or low, and the protein source was either beef or cereal. 10 rats for each diet were selected.

**Question:** How does diet affect weightgain?

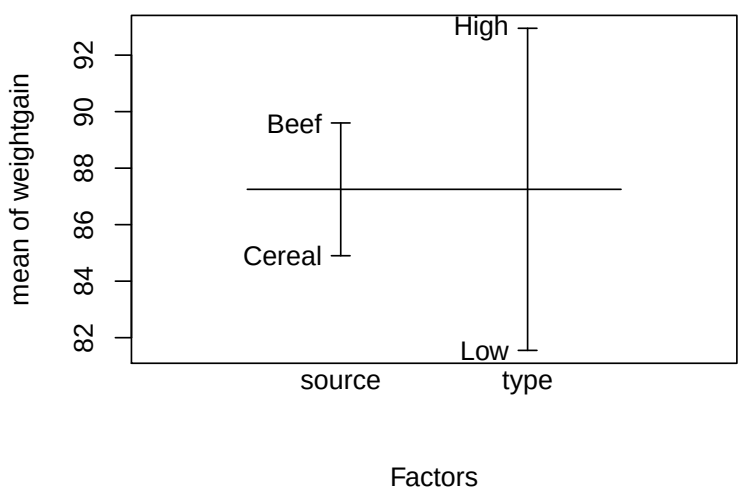
**Complication:** This is a factorial design (gekreuzte Faktoren), because each combination of protein source (beef/cereal) $\times$ level (high/low) is present ($2 \times 2$ groups).
Design:

| | beef | cereal |
|---|---|---|
| high | group 1 | group 2 |
| low | group 3 | group 4 |

Start by looking at means and standard deviations in the groups, as well as at a graphical description of the means:

```
> d.weightgain %>% group_by(source,type) %>% summarise(meanW = mean(weightgain),sdW = sd(weightgain))

# A tibble: 4 x 4
# Groups:   source [?]
  source type  meanW   sdW
  <fct>  <fct> <dbl> <dbl>
1 Beef   Low    79.2  13.9
2 Beef   High  100    15.1
3 Cereal Low    83.9  15.7
4 Cereal High   85.9  15.0
```

```
> plot.design(d.weightgain)
```




- Protein source (beef/cereal) seems less important than the amount (high/low).
- Variances seem to be equal in the four groups.

# Two-way ANOVA – The model

In the presence of a factorial design, the idea is to add separate effects $\beta_i$ (here $i = 1, 2$) and $\gamma_j$ (here $j = 1, 2$) for the $i$th level of the first factor and the $j$th level of the second factor:

Assume we have a factorial design with two factors $\beta_i$ and $\gamma_j$, then the $k$th outcome in the group of $i$ and $j$, $y_{ijk}$ is modelled as

$$y_{ijk} = \mu + \beta_i + \gamma_j + e_{ijk} \quad \text{with} \quad e_{ijk} \sim \mathrm{N}(0, \sigma_e^2) \quad i.i.d.$$

Again, additional constraints are needed!

- $\beta_1 = \gamma_1 = 0$ (treatment contrast; default in R).
- $\sum_i \beta_i = \sum_i \gamma_i = 0$ (sum-to-zero contrast).

# Two-way ANOVA in R

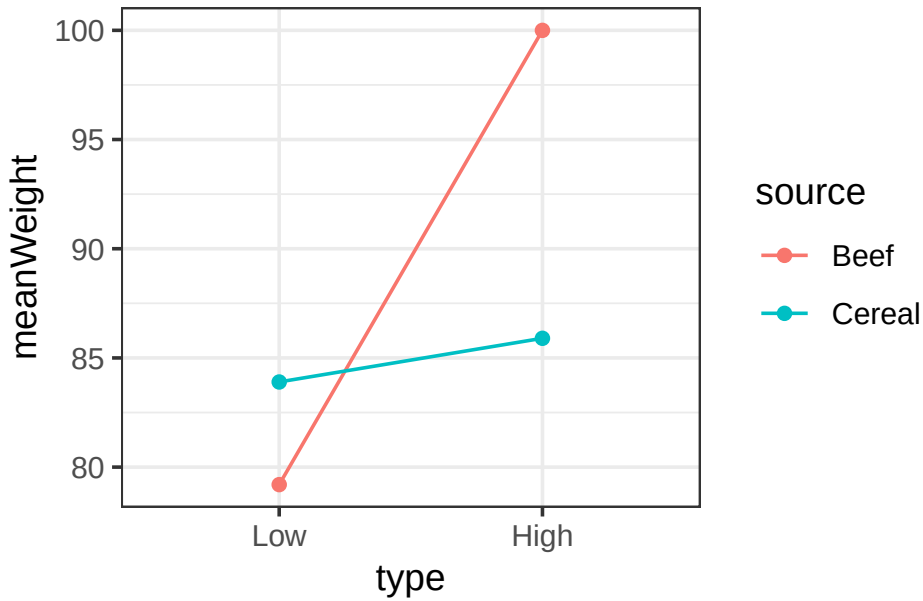
In R, a two-way ANOVA is as simple as one-way ANOVA, just add another variable:

```
> r.weight <- lm(weightgain ~ source + type, d.weightgain)
> anova(r.weight)

Analysis of Variance Table

Response: weightgain
          Df Sum Sq Mean Sq F value  Pr(>F)
source     1  220.9  220.90  0.9150 0.34501
type       1 1299.6 1299.60  5.3829 0.02596 *
Residuals 37 8933.0  241.43
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Interpretation: There seems to be a difference between low and high amounts of protein, but the source (beef/cereal) seems less relevant.

However: what if the additive model does not hold?

A so-called interaction plot helps to understand if the additive model is reasonable:




**Note:** if the additive model $\beta_i + \gamma_j$ holds, the lines would be parallel.

However, these lines are not parallel, indicating that there is an interaction between type and source!

In words: The type (low/how) has a different influence for the Beef and Cereal diets.

# Two-way ANOVA with interaction

- If the purely additive model is not correct, a more general model with an interaction term $(\beta\gamma)_{ij}$ may be used:

$$y_{ijk} = \mu + \beta_i + \gamma_j + (\beta\gamma)_{ij} + e_{ijk} \quad \text{with} \quad e_{ijk} \sim \mathrm{N}(0, \sigma_e^2) \quad i.i.d.$$

- As in linear regression, interactions allow for an interplay between the variables.
- In the rats experiment, increasing the amount from low to high has a different effect in the beef than in the cereal diet.
- Moreover: The plot on the previous slide shows that for the low amount of proteins case, the cereal diet leads to a larger average weight gain!

# Two-way ANOVA in R – Including an interaction

Let's include an interaction term in the rats example:

```
> r.weight2 <- lm(weightgain ~ source * type,d.weightgain)
> anova(r.weight2)

Analysis of Variance Table

Response: weightgain
            Df Sum Sq Mean Sq F value  Pr(>F)
source       1  220.9  220.90  0.9879 0.32688
type         1 1299.6 1299.60  5.8123 0.02114 *
source:type  1  883.6  883.60  3.9518 0.05447 .
Residuals   36 8049.4  223.59
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The coefficient estimates can be obtained as follows:

```
> summary(r.weight2)$coef

                    Estimate Std. Error    t value     Pr(>|t|)
(Intercept)             79.2   4.728577 16.7492235 1.416943e-18
sourceCereal             4.7   6.687218  0.7028333 4.866800e-01
typeHigh                20.8   6.687218  3.1104114 3.644273e-03
sourceCereal:typeHigh  -18.8   9.457155 -1.9879129 5.446757e-02
```

# Interpretation of the coefficients

This works in the same way as for categorical covariates in regression! To see this, let us estimate the means from the model. From the above output, we have [because of using treatment contrasts]:

$\hat{\beta}_{beef} = 0$, $\hat{\beta}_{cereal} = 4.7$,
$\hat{\gamma}_{low} = 0$, $\hat{\gamma}_{high} = 20.8$,
$(\hat{\beta\gamma})_{cereal\,/\,high} = -18.8$, $(\hat{\beta\gamma})_{beef\,/\,high} = (\hat{\beta\gamma})_{beef\,/\,low} = (\hat{\beta\gamma})_{cereal\,/\,low} = 0$.

Therefore:

| | | |
|---|---|---|
| Group 1: | beef / low | $\hat{y}_{beef,low} = 79.2 + 0 + 0 + 0 = 79.2$ |
| Group 2: | cereal / low | $\hat{y}_{cereal,low} = 79.2 + 4.7 + 0 + 0 = 83.9$ |
| Group 3: | beef / high | $\hat{y}_{beef,high} = 79.2 + 0 + 20.8 + 0 = 100$ |
| Group 4: | cereal / high | $\hat{y}_{cereal,high} = 79.2 + 4.7 + 20.8 - 18.8 = 85.9$ |

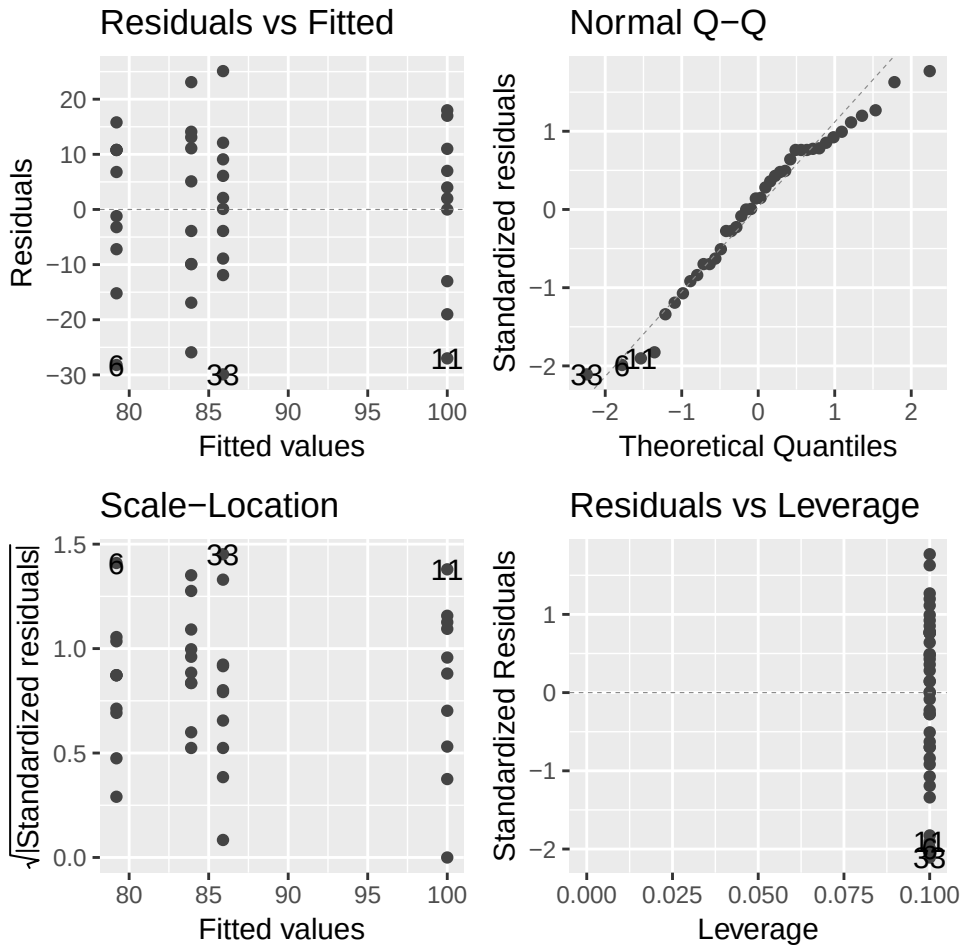
# A cautionary note

**Be careful:** In the presence of interactions, the $p$-values of the main effects can no longer be interpreted as before!

It is then required that separate "stratified" analyses are carried out. For example for "Beef" and "Cereal" protein sources:

```
> anova(lm(weightgain ~ type,subset(d.weightgain,source=="Beef")))

Analysis of Variance Table

Response: weightgain
          Df Sum Sq Mean Sq F value  Pr(>F)
type       1 2163.2 2163.20  10.253 0.00494 **
Residuals 18 3797.6  210.98
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> anova(lm(weightgain ~ type,subset(d.weightgain,source=="Cereal")))

Analysis of Variance Table

Response: weightgain
          Df Sum Sq Mean Sq F value Pr(>F)
type       1   20.0   20.00  0.0847 0.7744
Residuals 18 4251.8  236.21
```

And finally (what we should have done before checking the results!), the model diagnostics:



# Exercise:

In an experiment the influence of four levels of fertilizer (DUENGER) on the yield (ERTRAG) on 5 species (SORTE) of crops was investigated. For each DUENGER $\times$ ERTRAG level, 3 repeats were taken.

The data contain the following colums:

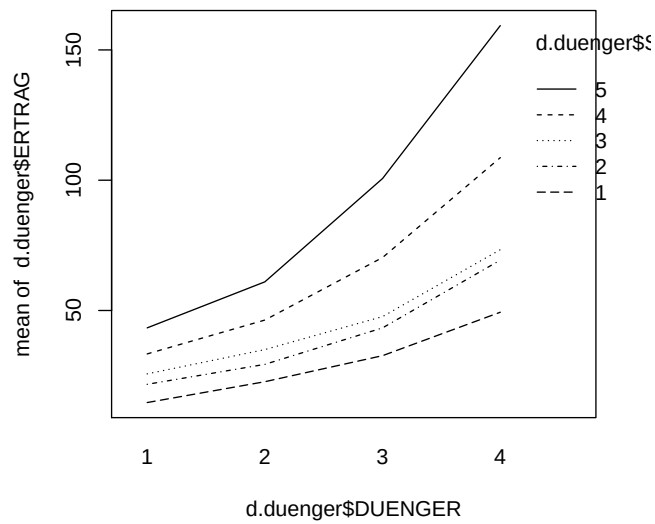
`DUENGER` (4 levels) `SORTE` (5 levels) `ERTRAG` (continuous)

The first 10 rows of the data:

```
> head(d.duenger,10)
```

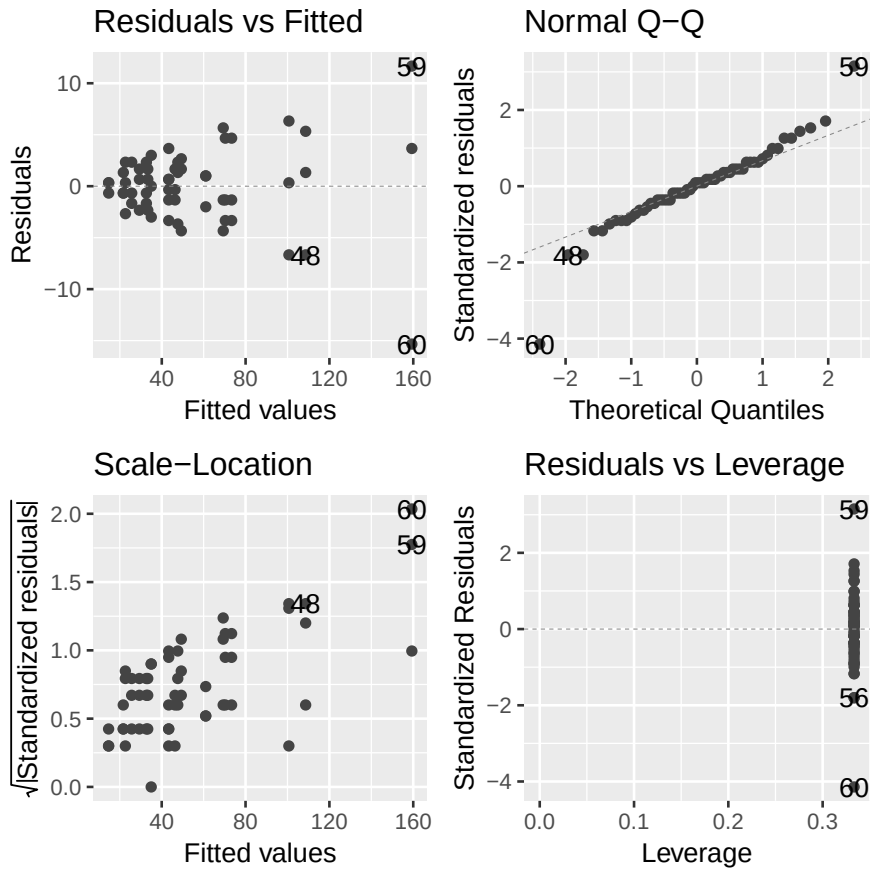
| | DUENGER | SORTE | ERTRAG |
|---|---|---|---|
| 1 | 1 | 1 | 14 |
| 2 | 1 | 1 | 15 |
| 3 | 1 | 1 | 15 |
| 4 | 2 | 1 | 20 |
| 5 | 2 | 1 | 25 |
| 6 | 2 | 1 | 23 |
| 7 | 3 | 1 | 35 |
| 8 | 3 | 1 | 31 |
| 9 | 3 | 1 | 32 |
| 10 | 4 | 1 | 52 |

And the interaction plot:



The interaction plot indicates that an interaction between SORTE and DUENGER is needed in the analysis. The results and residal plots are given here and on the next slide:

```
> d.duenger <- mutate(d.duenger,SORTE=as.factor(SORTE),DUENGER=as.factor(DUENGER))
> r.duenger <- lm(ERTRAG ~ DUENGER*SORTE,d.duenger)
```



```
> anova(r.duenger)

Analysis of Variance Table

Response: ERTRAG
              Df Sum Sq Mean Sq F value    Pr(>F)
DUENGER        3  35801 11933.5 580.707 < 2.2e-16 ***
SORTE          4  27805  6951.3 338.262 < 2.2e-16 ***
DUENGER:SORTE 12   7674   639.5  31.121 < 2.2e-16 ***
Residuals     40    822    20.6
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

What is the problem??

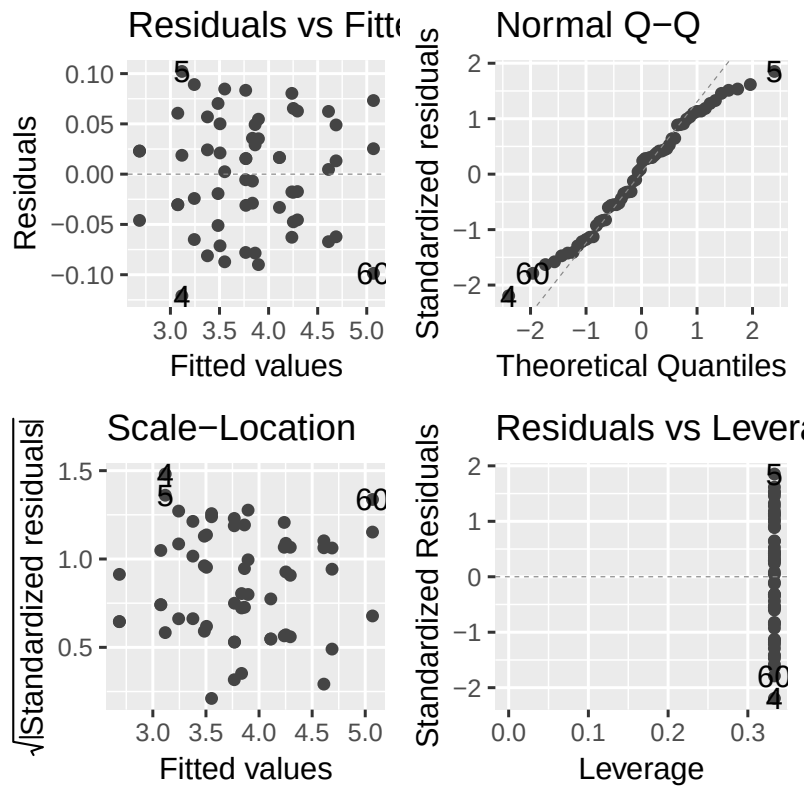
→ Look at the TA and the scale-location plots.

→ Ideas?

→ Interpretation?

# Log-transform the response (ERTRAG) and repeat the analysis:

```
> r.duenger2 <- lm(log(ERTRAG) ~ DUENGER*SORTE,d.duenger)
> anova(r.duenger2)

Analysis of Variance Table

Response: log(ERTRAG)
              Df  Sum Sq Mean Sq  F value Pr(>F)
DUENGER        3 11.6917  3.8972 854.0505 <2e-16 ***
SORTE          4  8.5202  2.1300 466.7851 <2e-16 ***
DUENGER:SORTE 12  0.0929  0.0077   1.6958 0.1045
Residuals     40  0.1825  0.0046
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



Btw, the summary table with coefficients looks horrible and the ***p*-values are not meaningful**! (why?)

| | Coefficient | 95%-confidence interval | *p*-value |
|---|---|---|---|
| Intercept | 2.69 | from 2.61 to 2.76 | $< 0.0001$ |
| DUENGER2 | 0.43 | from 0.32 to 0.54 | $< 0.0001$ |
| DUENGER3 | 0.80 | from 0.69 to 0.91 | $< 0.0001$ |
| DUENGER4 | 1.21 | from 1.10 to 1.32 | $< 0.0001$ |
| SORTE2 | 0.39 | from 0.28 to 0.50 | $< 0.0001$ |
| SORTE3 | 0.56 | from 0.45 to 0.67 | $< 0.0001$ |
| SORTE4 | 0.82 | from 0.71 to 0.93 | $< 0.0001$ |
| SORTE5 | 1.08 | from 0.97 to 1.19 | $< 0.0001$ |
| DUENGER2:SORTE2 | -0.13 | from -0.29 to 0.03 | 0.10 |
| DUENGER3:SORTE2 | -0.11 | from -0.26 to 0.05 | 0.18 |
| DUENGER4:SORTE2 | -0.049 | from -0.21 to 0.11 | 0.53 |
| DUENGER2:SORTE3 | -0.12 | from -0.28 to 0.04 | 0.13 |
| DUENGER3:SORTE3 | -0.18 | from -0.34 to -0.02 | 0.026 |
| DUENGER4:SORTE3 | -0.16 | from -0.32 to -0.00 | 0.046 |
| DUENGER2:SORTE4 | -0.10 | from -0.26 to 0.06 | 0.20 |
| DUENGER3:SORTE4 | -0.053 | from -0.21 to 0.10 | 0.50 |
| DUENGER4:SORTE4 | -0.03 | from -0.19 to 0.13 | 0.71 |
| DUENGER2:SORTE5 | -0.088 | from -0.25 to 0.07 | 0.27 |
| DUENGER3:SORTE5 | 0.044 | from -0.11 to 0.20 | 0.58 |
| DUENGER4:SORTE5 | 0.09 | from -0.07 to 0.25 | 0.25 |

Questions: Number of parameters? Degrees of freedom (60 data points)?

# Some summary remarks

- The $t$-test to compare the mean of two gropus is a special case of ANOVA.
- Even more, the $F$-test is a special case of the $t$-test: $F_{1,n} = t_n^2$.
- ANOVA is a special case of the linear regression model.
- ANOVA is often taught in separate lectures, although it could be integrated in a lecture on linear regression.
- ANOVA is traditionally most used to analyze experimental data.

# Appendix

# Illustration of $F_{1,n} = t_n^2$

Look again at the mercury example and include only one covariate (fish):

```
> r.lm <- lm(log10(Hg_urin) ~  fish,data=d.hg.m)
> summary(r.lm)

Call:
lm(formula = log10(Hg_urin) ~ fish, data = d.hg.m)

Residuals:
     Min       1Q   Median       3Q      Max
-1.01586 -0.37444  0.06843  0.27195  1.51199

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.77086    0.09446  -8.160 3.72e-11 ***
fish         0.02122    0.01406   1.509    0.137
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4721 on 57 degrees of freedom
Multiple R-squared:  0.03841,        Adjusted R-squared:  0.02154
F-statistic: 2.277 on 1 and 57 DF,  p-value: 0.1369
```

The F-value 2.28 has 1 and 57 degrees of freedom.

The $t$-statistic for `fish` is $t = 1.51$, also with 57 deg. of freedom.

$\rightarrow \quad t^2 = F.$