

# Kurs Bio144: Datenanalyse in der Biologie

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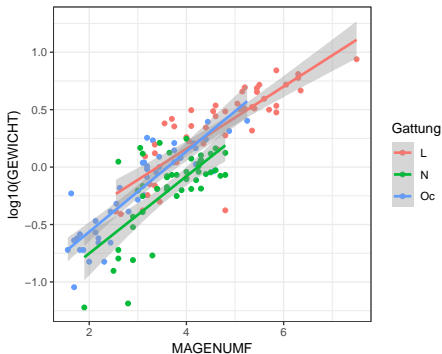
Lecture 6: ANOVA

25. March 2019

## Recap of muddiest point from last week

Main topic: Fitting and interpreting models with interactions.

Let's go back to the earthworm example, and fit a model that allows species-specific intercepts and slopes:



```

r.lm <- lm(log10(GEWICHT) ~ MAGENUMF * Gattung,d.wurm)
summary(r.lm)

##
## Call:
## lm(formula = log10(GEWICHT) ~ MAGENUMF * Gattung, data = d.wurm)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.75318 -0.12834  0.01742  0.12268  0.59732
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.92394    0.13402   -6.894 1.82e-10 ***
## MAGENUMF         0.27091    0.02816    9.620 < 2e-16 ***
## GattungN       -0.49990    0.21454   -2.330  0.0213 *
## GattungOc      -0.33921    0.17228   -1.969  0.0510 .
## MAGENUMF:GattungN  0.06516    0.05289    1.232  0.2200
## MAGENUMF:GattungOc 0.07894    0.04430    1.782  0.0769 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2185 on 137 degrees of freedom
## Multiple R-squared:  0.7652, Adjusted R-squared:  0.7566
## F-statistic: 89.29 on 5 and 137 DF,  p-value: < 2.2e-16

```

- Which are the interaction terms?
- Interpretation?

We have now actually fitted **three** models, one model for each species:

$$\text{L: } \hat{y}_i = -0.92 + 0.27 \cdot \text{MAGENUMF}$$

$$\text{N: } \hat{y}_i = -0.92 + (-0.50) + (0.27 + 0.07) \cdot \text{MAGENUMF}$$

$$\text{O: } \hat{y}_i = -0.92 + (-0.34) + (0.27 + 0.08) \cdot \text{MAGENUMF}$$

To remember:

- The “Gattung” terms in the model output are the **differences in intercepts** with respect to the reference level.
- The “MAGENUMF:Gattung” terms in the model output are the **differences in slopes** with respect to the reference level.

## Testing for an interaction term

If we want to find out if the interaction term for a factor covariate with more than two levels is relevant, we again need an  $F$ -test, that is, use the `anova()` function:

```
anova(r.lm)

## Analysis of Variance Table
##
## Response: log10(GEWICHT)
##              Df Sum Sq Mean Sq F value    Pr(>F)
## MAGENUMF      1 19.7790  19.7790 414.4743 < 2.2e-16 ***
## Gattung       2  1.3537   0.6768  14.1835 2.521e-06 ***
## MAGENUMF:Gattung 2  0.1729   0.0864   1.8112  0.1673
## Residuals    137  6.5377   0.0477
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Here,  $p = 0.167$ , thus there is not much evidence that the three species differ in their regression slopes.

# Overview for today

- 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 Datenanalyse”
- “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.

Central question of AN(C)OVA:

Are the means of two or more groups different?



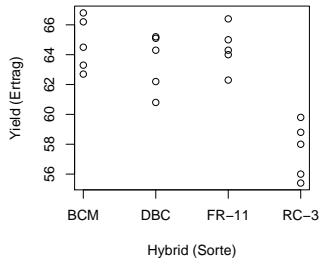
# Example: Yield of Hybrid-Mais breeds with increased resistance to “Pilzbrand”

(Source: W. Blanckenhorn, UZH)

Four different hybrid Mais breeds were grown to assess their yield. Each breed was grown at 5 different locations.

**Questions:** Are there differences in yield among the four hybrids?

**Attention:** The question is about *any* difference. More precisely, we could ask if any breed is different from any other.



We can test with ANOVA whether there are differences between the four breeds.

**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>

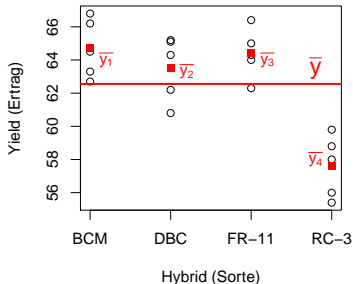
## Better idea

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:

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

where

- $y_{ij}$  = “Yield of the  $j^{\text{th}}$  plant of hybrid  $i$ ”
- $\mu_i$  = “Mean yield of hybrid  $i$ ”
- $\epsilon_{ij} \sim N(0, \sigma^2)$  is an independent error term.

Typically, this is **rewritten as**

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

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

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

- $\mu$  plays the role of the **intercept**  $\beta_0$  in standard regression models.
- The estimation of  $\mu$ , and the  $\beta$  coefficients is again done by **least squares minimization**.
- The  $\epsilon_{ij} \sim N(0, \sigma^2)$  *i.i.d.* assumption is again crucial, so **model checking** will be needed again.

Attention: Model (1) 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. correspond 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 clue is: Model (1) is identical to the regression model with a factor covariate, see slides 35/36 from lecture 4.

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

Thus (assuming  $\beta_1 = 0$ ):

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

# The ANOVA test: The $F$ -test

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

$$\mathbf{H}_0 : \mu_1 = \mu_2 = \dots = \mu_g \quad \text{or, equivalently} \quad \beta_2 = \dots = \beta_g = 0$$

$$\mathbf{H}_1 : \text{At least two groups are different}$$

Remember lectures 4 and 5: We have already used the  $F$ -test for categorical variables (see  $F$ -test for the earthworms in lecture 4 or cooking rule on slide 6 in lecture 5). This was equivalent to testing if all  $\beta_s$  that belong to a categorical variable are  $=0$  at the same time.

→ Equivalent to testing 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 lecture 4, slide 23):

total variability = explained variability + residual variability

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

Degrees of freedom:

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

From this:

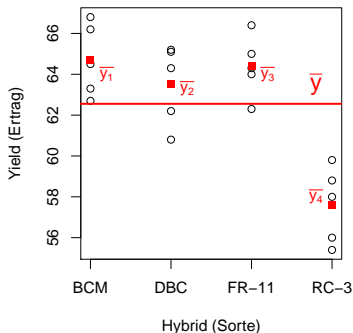
$$\left. \begin{aligned} MS_G &= \frac{SS_{\text{between}}}{g-1} \\ MS_E &= \frac{SS_{\text{within}}}{n-g} \end{aligned} \right\} \Rightarrow F = \frac{MS_G}{MS_E} \text{ is } \sim F_{g-1, n-g} \text{ distributed.}$$

# 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 ( $\bar{y}_1, \bar{y}_2, \bar{y}_3, \bar{y}_4$ )
- $MS_E$  captures the variability of the  $y_{ij}$  **within the groups**.



# Interpretation of the $F$ statistic II

- $F$  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.

→ The larger  $F$ , the less likely are the data seen under  $H_0$ .

► ANOVA App

[https://gallery.shinyapps.io/anova\\_shiny\\_rstudio/](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	$g - 1$	$SS_G$	$MS_G$	$\frac{MS_G}{MS_E}$	$\Pr(F_{g-1, n-g} >  F )$
Within groups	$n - g$	$SS_E$	$MS_E$		
Total	$n - 1$	$SS_{\text{total}}$			

## Our first ANOVA: Hybrid Mais example

HYBRID	LOCATION	YIELD	HYBRID	LOCATION	YIELD
FR-11	NW	62	DBC	NW	61
FR-11	NE	64	DBC	NE	64
FR-11	C	64	DBC	C	65
FR-11	SE	65	DBC	SE	62
FR-11	SW	66	DBC	SW	65
BCM	NW	63	RC-3	NW	55
BCM	NE	63	RC-3	NE	56
BCM	C	66	RC-3	C	60
BCM	SE	67	RC-3	SE	58
BCM	SW	64	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...
```

```
## $ LOCATION  <fct> NW, NE, C, SE, SW, NW, NE, C, SE, SW, NW, NE, C, SE, ...
```

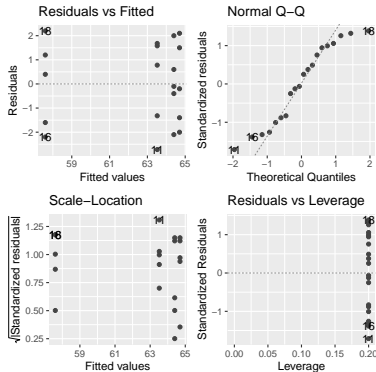
```
## $ YIELD     <dbl> 62.3, 64.0, 64.3, 65.0, 66.4, 63.3, 62.7, 66.2, 66.8,...
```

## Hybrid-Mais example – Estimation

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

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

Model checking 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 is 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
```

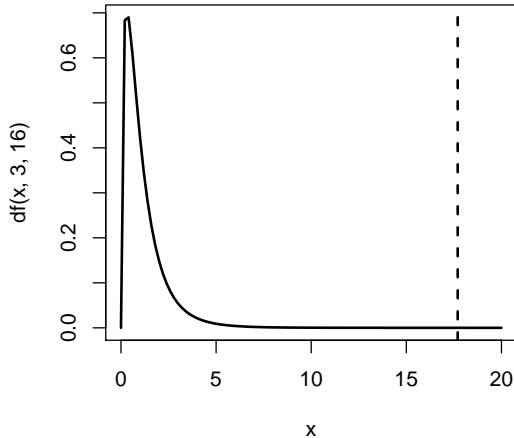
You can see that the value of  $F = 17.68$  is  $F$ -distributed with 3 and 16 degrees of freedom, and the  $p$ -value of the test " $\beta_2 = \beta_3 = \beta_4 = 0$ " is  $< 0.0001$ .

Conclusion: There are differences among the four groups!

→ 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
```

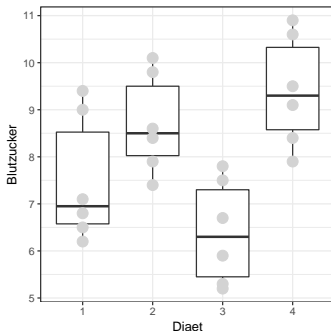
The table contains the estimates of the intercept 64.70 ( $\mu$  in ANOVA notation,  $\beta_0$  in regression notation), and estimates for  $\beta_2$ ,  $\beta_3$ ,  $\beta_4$  (while the reference was set to  $\beta_1 = 0$ ).

## 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 10), 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.

→ 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  $\beta_2 = \dots = \beta_g = 0$  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 **honest significant differences** (HSD) approach
- Fisher **least significant differences** (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	$3 \times 10^{-4}$

- 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 to 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).

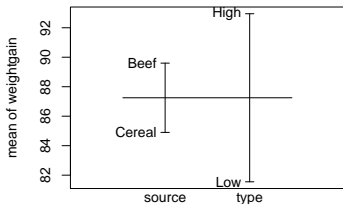
Design:

	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
```



- Protein source (beef/cereal) seems less influential 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

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

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:

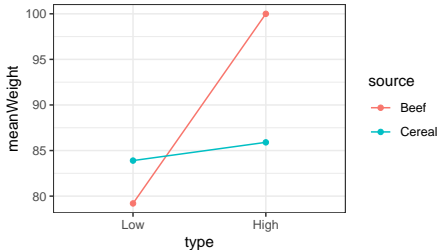
```
r.weight <- lm(weightgain ~ source + type, d.weightgain)
anova(r.weight)

## 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:

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

- 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:

$$\text{Group 1: beef / low} \quad \hat{y}_{beef,low} = 79.2 + 0 + 0 + 0 = 79.2$$

$$\text{Group 2: cereal / low} \quad \hat{y}_{cereal,low} = 79.2 + 4.7 + 0 + 0 = 83.9$$

$$\text{Group 3: beef / high} \quad \hat{y}_{beef,high} = 79.2 + 0 + 20.8 + 0 = 100$$

$$\text{Group 4: cereal / high} \quad \hat{y}_{cereal,high} = 79.2 + 4.7 + 20.8 - 18.8 = 85.9$$

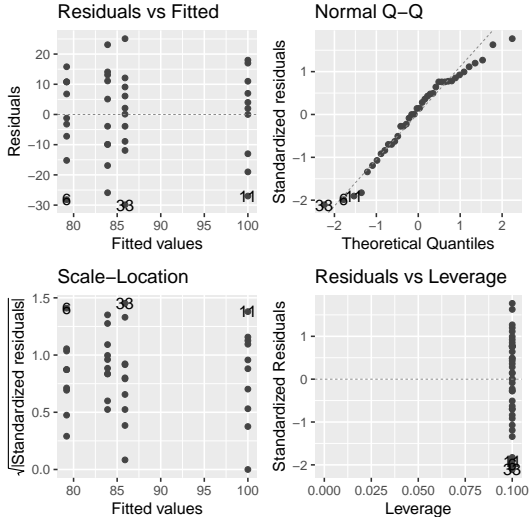
## A cautionary note

**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, 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 columns:

DUENGER (4 levels)

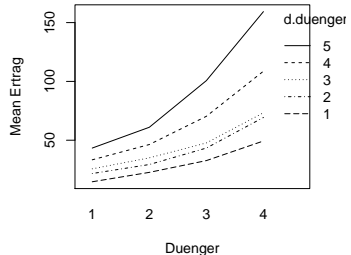
SORTE (5 levels)

ERTRAG (continuous)

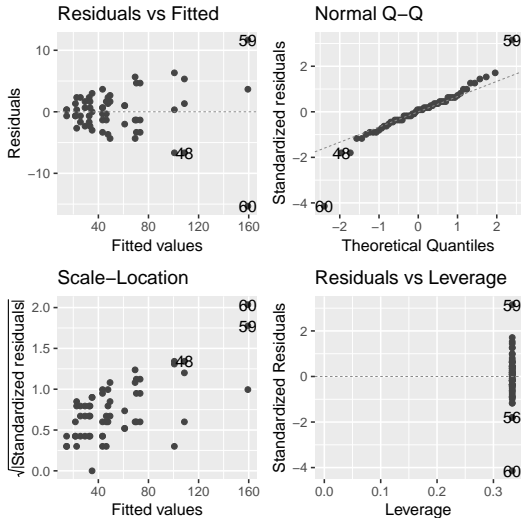
The first 10 rows of the data:

##	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 residual plots are given here and on the next slide:



```
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.

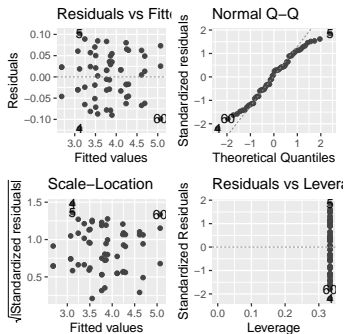
→ Ideas?

→ 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	<i>p</i> -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 groups 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.