



## **Data Analysis with R:**

Lecture Slides (all)

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#### How to deal with missing values in R? (1/3)

- In R, missing values are represented by the symbol NA (not available).
- Impossible values (e. g., dividing by zero) are represented by the symbol NaN (not a number).
- Ask yourself why a NA and / or NaN occurs!

#### How to deal with missing values in R? (2/3)

### Testing for Missing Values

```
vec1 <- c(1, 2, 3, NA)
is.na(vec1) # returns a vector (FALSE, FALSE, FALSE, TRUE)
# The TRUE indicates the position of the NA in vec1.</pre>
```

### Recoding Values to Missing

```
# recode specific values (e. g. 0.001) to missing for variable x # select rows where x is 0.001 and recode value in column x with NA tmp.row <- which(datx = 0.001) datx[tmp.row] <- NA
```

#### How to deal with missing values in R? (3/3)

Excluding Missing Values from specific function calls

```
a <- c(1, 2, NA, 3)
mean(a) # returns NA
mean(a, na.rm=TRUE) # returns 2</pre>
```

 Check for complete cases with function complete.cases(...)

```
# list rows of data that have missing values
dat[!complete.cases(dat),]
subdat <- dat[complete.cases(dat),]</pre>
```

 Create new dataset without missing data with function na.omit(...)

```
new.dat <- na.omit(dat)
```

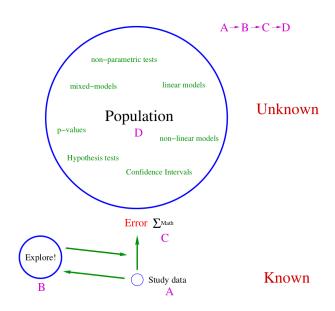
### How to check your data for plausibility?

- Ask yourself what can go wrong?
- Implausible values?
- Impossible values?
- Logical errors?



#### **Exercise 10**

#### **Overview**



#### **Basic Statistical Tests**

Study data is collected for a purpose - to answer one or more specific scientific questions. The classical way to perform a formal statistical analyses of these data is to formulate these research questions into statistical **hypothesis tests**.

In this section we will go through a simple example in detail to highlight some of the important concepts - the general approach for more complex analyses is exactly same. *Note: the precise technical details are much less important than the concepts!* 

## After six weeks will the mean weight of a chicken be more than 250 grams?

There are 71 observations in chickwts from which to answer this question. This can be formulated into a statistical hypothesis test. A hypothesis test has two parts, the null hypothesis and the alternative hypothesis. This is typically written as follows:

 $H_0: \mu \leq 250$  $H_A: \mu > 250$ 

where  $\mu$  is the mean weight in the **population** of chickens from which the sample of 71 chickens was drawn. Remember - we know the mean weight in the sample of chickens is greater than 250 it is the **population** of chickens which we are interested in.

## After six weeks will the mean weight of a chicken be at least 250 grams?

 $H_0: \mu \le 250$  $H_A: \mu > 250$ 

The null hypothesis  $(H_0)$  is the default situation, sometimes called the "state of nature". In a treatment-control trial,  $H_0$  is typically that the effect of the treatment is not different from the control. In this example our default position is that the mean weight of chickens is  $\leq$  250. This is called a single-sided hypothesis test.



We now analyse the 71 observations to see whether there is evidence to **REJECT** the null hypothesis  $H_0$ , and if the null hypothesis is rejected then we can conclude that the available evidence supports the alternative hypothesis.

```
t.test(chickwts$weight, mu = 250, alternative = "greater")
t.test(chickwts$weight, mu = 250, alternative = "less")
```

Note that hypothesis testing is concerned with finding evidence in support of the null hypothesis  $H_0$  - the default situation - rather than evidence in favour of the alternative hypothesis.

#### One Sample t-test

For the chicken weights data an appropriate formal analyses is to use a **one-sample t-test**, why this test is appropriate will be discussed later. This analysis involves calculating a simple summary statistic - called a *t*-statistic - which we do entirely from the observed data.

$$T_{obs} = \frac{\bar{x} - \mu}{s / \sqrt{n}}$$

where  $\bar{x}$  is the sample mean, s the sample standard deviation and  $\mu$  is the population mean in the null hypothesis which we wish to test for. We then look up the value of  $T_{obs}$  in a set of statistical tables/computer to see what the answer is to our research question.

#### Important concept - sampling

Why is 
$$T_{obs} = \frac{\bar{x} - \mu}{s / \sqrt{(n)}}$$
 called a *t*-statistic?

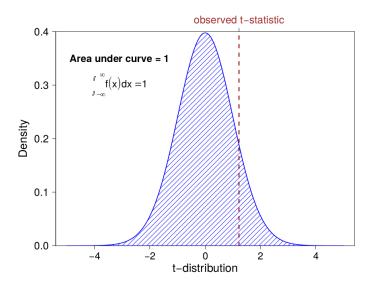
If another sample of 71 chickens from the same population were weighed then the values for  $\bar{x}$  and s would be different, and hence the value for  $T_{obs}$ . If this was repeated many times and a histogram/Q-Q/P-P plot produced of the values for  $T_{obs}$  then this would follow the shape of a known distribution - **student-***t* **probability distribution**. It is this piece of mathematics - knowing what the sampling distribution of  $T_{obs}$  is - which allows us to infer information about the population of chickens from which our original 71 chickens were sampled - without actually having to collect lots and lots of other samples of chickens! Mathematical theory is used to fill this data gap.

chickwts: t-test

$$T_{obs} = \frac{261.31 - 250}{78.07/\sqrt{71}} = 1.22$$

Put the values for the sample mean and standard deviation into the t-statistic formula along with the  $\mu=250$ . We now look up the value of this in a t-distribution reference table. All this calculation will be done for you in R but it is important to understand the general process as this is the same for hypothesis testing in other more complex analyses.

#### One Sample, one-sided, t-test



#### Important concept - p-values

- The result of a hypothesis test is usually communicated in the form of a p-value
- The interpretation of a p-value is of crucial importance it is the probability that the test statistic takes values at least as extreme as that observed assuming that H<sub>0</sub> is true
- Exactly what at least as extreme as refers to depends on the alternative hypothesis H<sub>A</sub>.
- This may sound rather abstract but it is usually obvious in practice

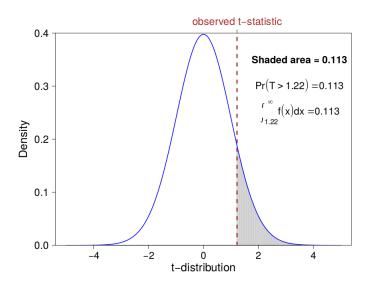
# After six weeks will the mean weight of a chicken be at least 250 grams?

 $H_0: \mu \leq 250$ 

 $H_A: \mu > 250$ 

The alternative hypothesis is  $\mu >$  250 so in this test **at least as extreme as** in the definition of a p-value is the probability of observing a t-statistic which is > 1.22 **assuming that**  $H_0$  **is true** - this is why 250 was used for  $\mu$  when calculating  $T_{obs}$ .

#### One Sample, one-sided, t-test



#### **Research Question**

The purpose of this hypothesis test analysis is to answer a very specific scientific question:

## After six weeks will the mean weight of a chicken be more than 250 grams?

So what is our answer?

The *p*-value for this hypothesis test is 0.113. Based on this value we can either **reject**  $H_0$  and conclude that the mean weight of chickens in the population is likely to be greater than 250 grams or else we can **accept**  $H_0$  where the mean chicken weight is less than 250 grams.

#### Research Question - be pragmatic with p-values

By convention a p-value of less than 0.05 is considered to provide reasonable evidence for rejecting  $H_0$ . A p-value of between 0.05 and 0.1 might be considered as weak evidence against  $H_0$ . Values of less that 0.01 are generally considered as very strong evidence for rejecting  $H_0$ . It is **always** best to provide a p-value in any analyses to let the reviewer/client see the strength of evidence rather than simply claiming statistically significant findings!

### **Communicating Results of Hypothesis Tests**

Transparency is essential - the devil can be in the detail - which at the very least should comprise:

- i. what hypothesis was being tested be clear and precise
- ii. what statistical test was used
- iii. what the p-value is
- iv. what the treatment effect is (more later).

This is particularly crucial if the analyses are to be given to someone *else* to then make a judgment on the scientific significance.

#### **Two-sided Tests: One Population**

## After six weeks will the mean weight of a chicken be equal to 250 grams?

This is now a two sided hypothesis test:

 $H_0: \mu = 250$ 

 $H_A: \mu \neq 250$ 

This time the hypothesis test is asking how much evidence is there in our sample data to conclude that in the population of all chickens the mean weight is not equal to 250 grams.

#### **Two-sided Tests: One Population**

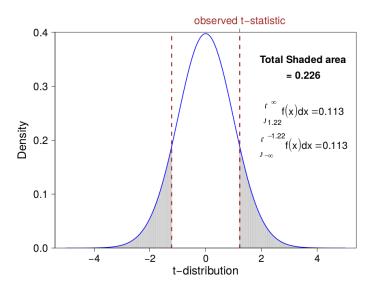


```
# t.test(chickwts$weight, mu = 250, alternative = "two.sided")
t.test(chickwts$weight, mu = 250)
##
##
   One Sample t-test
##
## data: chickwts$weight
## t = 1.2206, df = 70, p-value = 0.2263
## alternative hypothesis: true mean is not equal to 250
## 95 percent confidence interval:
## 242.8301 279.7896
## sample estimates:
## mean of x
## 261.3099
```

#### **Two-sided Tests**

A two-sided test is similar to a one-sided test - the key difference is in what is now defined as **at least as extreme** in the definition of the *p*-value. This time the alternative hypothesis refers to observing a value of **either**  $\bar{x} > 250$  or  $\bar{x} < -250$  **assuming that**  $H_0$  **is true**, which using the t-test approach is equivalent to the probability of observing  $T_{obs} > 1.22$  or  $T_{obs} < -1.22$  which we can again look up in reference tables.

#### One Sample, two-sided, t-test



#### **Two-sided Tests**

- The two-sided t-test has a p-value which is exactly double the single sided test
- Think! intuitively the p-value should be less for a single sided test as the research question you are asking is much narrower e.g. greater than 250 grams, as opposed to whether the mean chicken weight might be either less than 250 grams or greater than 250 grams.
  - $\rightarrow$  You are using the same amount of information (71 observations) to answer a narrower research question and so all else being equal you should expect a "more powerful" analyses (e.g. a lower *p*-value all else being equal)

#### **Exercise 11**



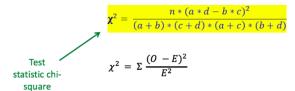
#### **Chi-square Test**

There are two very commonly used statistical tests for testing dependence between two categorical variables: Chi-squared test & Fisherâs exact test.

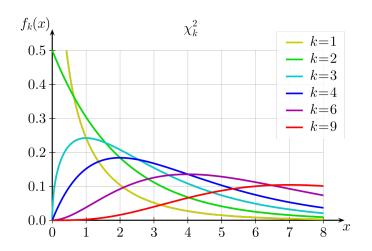
## To test independence of rows and columns

	Disease		
Risk Factor	+	-	Total
+	a	b	a+b
-	С	d	c+d
Total	a+c	b+d	n = a+b+c+d

• Assumptions: a, b, c, d must have at least 5 observations!



#### **The Chi-square Distribution**



#### **Exact Fisher Test: Permutation Test**

	Success	Failure	Total
Therapy	7	2	9
New Therapy	2	8	10
Total	9	10	19

7 2 8	P = 9!*10!*9!*10! / 19!*7!*2!*2!*8! = 0.01754
8 1 1 9	P = 9!*10!*9!*10! / 19!*8!*1!*1!*9! = 0.00097
9 0 0 10	P = 9!*10!*9!*10! / 19!*9!*0!*0!*10! = 0.00001

For a one-sided test: p = 0.01754+0.00097+0.00001 = 0.01852

#### **Exercise 12**



### Simulating in R



#### Continuous variable

```
n <- 100
rnorm(n, mean = 0, sd = 1)  # Normal distribution</pre>
```

#### Binary variable

```
rbinom(n, size = 1, prob = 0.4) # Binomial distribution
```

#### Count variable

```
rpois(n, lambda = 7)  # Poisson distribution
```

#### Other options

```
seq(from = 0, to = 100, by = 1)  # ID
sample(3:30, size = 50, replace = TRUE)  # herd size
rep(c(1, 2, 3), times = 5)
rep(c(1, 2, 3), each = 5)
```

• ...

## **Plotting in R**



## **Plotting in R**



- Continuous data
  - Histogram
  - Boxplot
- Nominal / Ordinal data
  - Barplot
  - Mosaicplot
  - Scatterplots

#### Exercise 13A and 13B



#### **Exercise 14**



### **Lecture Slides for Day 5**

#### Overview: ANOVA and linear models

- Introduction to ANOVA
- How to run an ANOVA in R
- Checking model assumptions in R
- Multiple comparisons options in R
- ANOVA as a special case of a linear model
- The simple linear regression model
- The multiple linear regression model
- Model selection: R<sup>2</sup> and AIC
- Two-way Interactions in R (Day 6)
- Confounding (Day 6)

#### **Hypothesis Testing - One Way ANOVA**

We have seen how to perform hypothesis tests when comparing two populations using the two sample t-test. In many analyses we may have multiple populations - for example suppose we have a treatment which has a number of different levels high/medium/low/placebo, or equivalently a number of different treatments. What then is the hypothesis we wish to test?

#### Is there a difference in the effect of the treatment?

 $H_0$ :  $\mu_1 = \mu_2 = \cdots = \mu_k$ 

 ${\it H}_{\it A}$  : at least one pair of  $\mu_1,\ldots,\mu_k$  are different

where  $\mu_1, \ldots, \mu_k$  denote the mean effect of treatment levels 1 through k.

#### One Way ANOVA

Analysis of variance, **ANOVA**, to analyze differences between group means. The observed variance in the outcome variable is partitioned into components attributable to different sources of variation.

### ANOVA estimates three sample variances (sum of squares)

- a total variance based on all observation deviations from the grand mean
- a variance based on the group mean deviations from the grand mean
- an (error) variance based on all the observation deviations from their group mean

$$\sum_{i} \sum_{j} (x_{ij} - \bar{x})^{2} = \sum_{i} n_{i} (\bar{x}_{i} - \bar{x})^{2} + \sum_{i} \sum_{j} (x_{ij} - \bar{x}_{i})^{2}$$

$$\uparrow \qquad \qquad \uparrow$$
Variance Variance

between groups

within groups

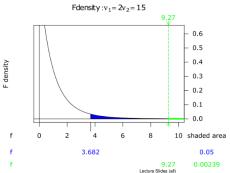
 $\bar{x}$ = grand mean  $\bar{x}_i$ = group mean

#### F-test / F-distribution

An **F-test**, a statistical test, in which the test statistic has an F-distribution under the null hypothesis is used to assess statistical significance in an ANOVA.

degrees of freedom

$$\mathsf{F} = \frac{variance\ between\ groups}{variance\ within\ groups}$$

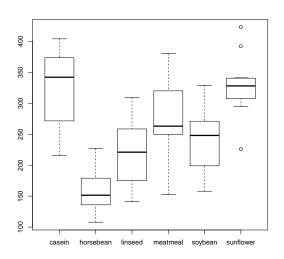


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#### ANOVA in R with chickwts

R

data(chickwts)
boxplot(chickwts\$weight ~ chickwts\$feed)



#### ANOVA in R with chickwts



```
# aov.mod <- aov(chickwts$weight ~ chickwts$feed)
aov.mod <- aov(weight ~ feed, data = chickwts)</pre>
# What objects can we extract from a anova model?
objects(aov.mod)
## [1] "assign"
                     "call"
                                     "coefficients" "contrasts"
## [5] "df.residual" "effects" "fitted.values" "model"
## [9] "qr"
                      "rank"
                                     "residuals" "terms"
## [13] "xlevels"
#
summary(aov.mod)
##
             Df Sum Sq Mean Sq F value Pr(>F)
## feed 5 231129 46226 15.37 5.94e-10 ***
## Residuals 65 195556 3009
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

#### Overview: Checking model assumptions

- mean(residuals) = 0
- Residuals are normally distributed (qqnorm, qqplot)
- Variance homoscedasticity (Bartlett & Levene's Test)
- Cook's distance: Influential data points
- Any pattern(s)?

### Checking model residuals: mean(residuals) = 0

# R

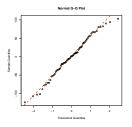
#### "Unexplained rest of the model"

```
# What are residuals?
chickwts$residuals <- residuals(aov.mod)
tapply(chickwts$weight, chickwts$feed, mean)
     casein horsebean linseed meatmeal sovbean sunflower
##
## 323.5833 160.2000 218.7500 276.9091 246.4286 328.9167
chickwts[c(1:3).]
    weight feed residuals
##
## 1 179 horsebean 18.8
## 2 160 horsebean -0.2
## 3 136 horsebean -24.2
# Save residuals to an objects and check mean of residuals
aov.mod.resid <- residuals(aov.mod)
mean(aov.mod.resid)
## [1] 7.573045e-16
```

# Checking model residuals: Residuals normally distributed "Unexplained rest of the model"



```
par(mfrow=c(1,1))
qqnorm(aov.mod.resid)
qqline(aov.mod.resid, col = "red", lwd = 3, lty = 2)
```



```
# Shapiro-Wilk test (dependent on sample size --> limited use)
shapiro.test(aov.mod.resid)

##
## Shapiro-Wilk normality test
##
## data: aov.mod.resid
## W = 0.98616, p-value = 0.6272
```

# Checking model residuals: Variance homoscedasticity (1/3) Hypothesis tests



```
# Bartlett Test
bartlett.test(chickwts$weight ~ chickwts$feed)

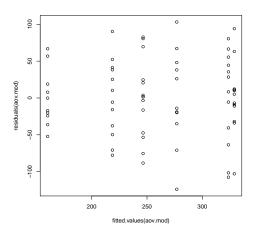
##
## Bartlett test of homogeneity of variances
##
## data: chickwts$weight by chickwts$feed
## Bartlett's K-squared = 3.2597, df = 5, p-value = 0.66

# Levene's Test
# library("Rcmdr")
# levene.test(chickwts$weight ~ chickwts$feed)
```

# Checking model residuals: Variance homoscedasticity (2/3) Graphical interpretation is better!



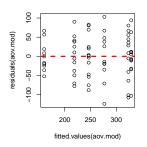
```
# Plot fitted against residual values
plot(fitted.values(aov.mod), residuals(aov.mod))
```

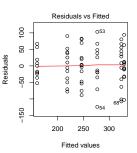


# Checking model residuals: Variance homoscedasticity (3/3) Graphical interpretation is better!



```
# Plot fitted against residual values
par(mfrow=c(1,2), pty="s", mar = c(10, 4, 1, 2))
plot(fitted.values(aov.mod), residuals(aov.mod))
abline(h = 0, col = "red", lwd = 3, lty = 2)
plot(aov.mod, which=1)
```

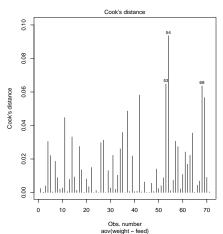




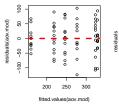
### Checking model residuals: Cook's distance



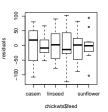
```
# Plot fitted against residual values
# Cut-off at 3
par(mfrow=c(1,1), pty="s", mar = c(5, 4, 4, 2))
plot(aov.mod, which=4)
```



#### **Checking for potential patterns**



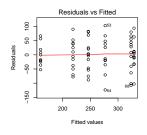


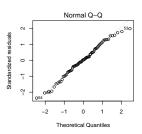


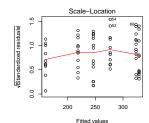
#### Plot anova objects

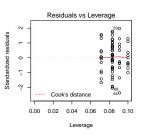
```
par(mfrow=c(2, 2))
plot(aov.mod)
```











### Multiple comparisons options in R



So far, we know there is difference between the feed types. However, we do not yet know which feed type differ. In principal, we could do multiple t-tests, BUT ... we would use our data several times. Classically, we choose an  $\alpha$ -level of 5 %, in cases of multiple comparisons we are facing the familywise error rate:

$$FWE \leq 1 - (1 - \alpha)^n,$$

where  $\alpha = 5$  % and n = number of comparisons.

```
alpha = 0.05

1 - (1 - alpha)^1  # n = 1  # [1] 0.05

1 - (1 - alpha)^5  # n = 5  # [1] 0.2262191

1 - (1 - alpha)^10  # n = 10  # [1] 0.4012631
```

### Multiple comparisons options in R



Hence, we are better of using one of the following procedures to adjust for multiple comparisons:

- **Bonferroni**: p-value correction by testing each individual hypothesis at a significance level of  $\frac{\alpha}{m}$ , where  $\alpha$  is the desired overall  $\alpha$  level and m is the number of hypotheses.
- **Dunnett**: Multiple comparisons of each group to a reference.
- Tukey Honest Significant Differences (Homogeneous subgroups): Multiple comparisons of all possible combinations.

• ..

### Multiple comparisons options in R: Bonferroni



```
aov.mod <- aov(weight ~ feed, data = chickwts)
pairwise.t.test(chickwts$weight, chickwts$feed, p.adj = "none")
##
   Pairwise comparisons using t tests with pooled SD
##
## data: chickwts$weight and chickwts$feed
##
            casein horsebean linseed meatmeal sovbean
##
## horsebean 2.1e-09 -
## linseed 1.5e-05 0.01522 -
## meatmeal 0.04557 7.5e-06 0.01348 -
## soybean 0.00067 0.00032 0.20414 0.17255 -
## sunflower 0.81249 8.2e-10 6.2e-06 0.02644 0.00030
##
## P value adjustment method: none
pairwise.t.test(chickwts$weight, chickwts$feed, p.adj = "bonferroni")
##
   Pairwise comparisons using t tests with pooled SD
##
## data: chickwts$weight and chickwts$feed
##
##
            casein horsebean linseed meatmeal sovbean
## horsebean 3.1e-08 -
## linseed 0.00022 0.22833 -
## meatmeal 0.68350 0.00011 0.20218 -
## soybean 0.00998 0.00487 1.00000 1.00000 -
## sunflower 1.00000 1.2e-08 9.3e-05 0.39653 0.00447
##
## P value adjustment method: bonferroni
```

#### Multiple comparisons options in R: Dunnett



```
library("multcomp")
# compares always to baseline levels (here: casein) --> saves degrees of freedom
dunnett <- glht(aov.mod, linfct = mcp(feed = "Dunnett"))</pre>
summary(dunnett)
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: aov(formula = weight ~ feed, data = chickwts)
##
## Linear Hypotheses:
                         Estimate Std. Error t value Pr(>|t|)
##
## horsebean - casein == 0 -163.383
                                      23.485 -6.957 < 0.001 ***
## linseed - casein == 0 -104.833 22.393 -4.682 < 0.001 ***
## meatmeal - casein == 0 -46.674 22.896 -2.039 0.16707
## sovbean - casein == 0 -77.155 21.578 -3.576 0.00314 **
## sunflower - casein == 0.5.333 22.393 0.238 0.99945
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

# Multiple comparisons options in R: Tukey Tukey Honest Significant Differences

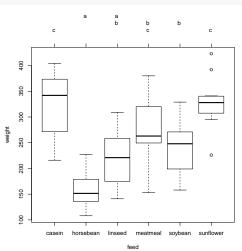


```
library("multcomp")
# compares all factor levels
tukey <- glht(aov.mod, linfct = mcp(feed = "Tukey"))</pre>
summarv(tukev)
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: aov(formula = weight ~ feed, data = chickwts)
##
## Linear Hypotheses:
##
                             Estimate Std. Error t value Pr(>|t|)
## horsebean - casein == 0
                             -163.383
                                          23.485 -6.957 < 0.001 ***
                             -104.833
## linseed - casein == 0
                                          22.393 -4.682 < 0.001 ***
## meatmeal - casein == 0
                            -46.674
                                          22.896 -2.039 0.33199
## soybean - casein == 0
                              -77.155
                                          21.578 -3.576 0.00836 **
## sunflower - casein == 0
                              5.333
                                          22.393
                                                   0.238 0.99989
                                          23,485
## linseed - horsebean == 0
                               58.550
                                                   2.493 0.14105
                                          23.966
## meatmeal - horsebean == 0
                              116.709
                                                   4.870 < 0.001 ***
## soybean - horsebean == 0
                              86.229
                                          22.710
                                                   3.797 0.00418 **
## sunflower - horsebean == 0
                              168.717
                                          23.485
                                                   7.184 < 0.001 ***
## meatmeal - linseed == 0
                               58.159
                                          22.896
                                                   2.540 0.12744
## soybean - linseed == 0
                              27.679
                                          21.578
                                                   1.283 0.79293
## sunflower - linseed == 0
                              110.167
                                          22.393
                                                   4.920 < 0.001 ***
## soybean - meatmeal == 0
                              -30.481
                                          22.100 -1.379 0.73881
## sunflower - meatmeal == 0
                              52.008
                                                   2.271 0.22035
                                          22.896
## sunflower - soybean == 0
                              82.488
                                          21.578
                                                   3.823 0.00380 **
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
stottia Halturkada,TertragriDotoch ស្រាប់ទោសទៅ reported -- single-step method) Lecture Slides (all)
```

# Multiple comparisons options in R: Tukey Tukey Honest Significant Differences with homogeneous subgroups



```
# summary(tukey) # standard display
tukey.cld <- cld(tukey) # letter-based display
# the cld(...) function sets up a compact letter display of all pair-wise comparisons
par(mfrow=c(1,1), mar=c(5, 4, 5, 2))
plot(tukey.cld)</pre>
```



#### **Exercise 15**



# **Introduction: ANOVA as a special case of a linear model Linear Modelling**

So far we have considered how to determine whether statistically significant differences exist between different "feed" groups (factors).

The explanatory variable has been categorical. We have not yet considered continuous explanatory variables.

ANOVA and linear regression are both a special cases of **linear** models.

#### Simple linear regression (1/3)

A **simple linear regression** fits a straight line through a set of data points. This straight line is fitted in a way that makes the sum of the squared residuals (the vertical distances between each data point and the fitted line) as small as possible.

#### Simple linear regression (2/3)

In a simple linear regression model for n data points  $(x_i, y_i)$ , i = 1, ...n the following equation is used:

$$y_i = \alpha + \beta x_i + \epsilon$$
,

#### where

- α: intercept or constant
- $\beta$ : slope, regression coefficient (effect size)
- $\epsilon$ : error, residuals

#### Simple linear regression (3/3)

The goal is to find the equation of the straight line

$$f(x) = y = \alpha + \beta x$$
,

which would provide a "best" fit for the data points (least square approach).

#### Assumptions of a linear regression

A **simple linear regression** is based on several assumptions which should be checked carefully.

- linearity of the relationship between the explanatory (independent) and the outcome (dependent) variable
- normality of the residuals
- independence
- constant variance (homoscedasticity)

### Linear regression model in R

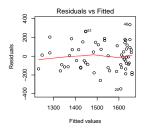


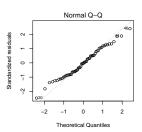
```
data(water)
# mod.hard <- lm(mortality ~ hardness, data = water)</pre>
mod.hard <- lm(water$mortality ~ water$hardness)</pre>
summary(mod.hard)
##
## Call:
## lm(formula = water$mortality ~ water$hardness)
##
## Residuals:
## Min 1Q Median 3Q Max
## -348.61 -114.52 -7.09 111.52 336.45
##
## Coefficients:
##
                 Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1676.3556 29.2981 57.217 < 2e-16 ***
## water$hardness -3.2261 0.4847 -6.656 1.03e-08 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 143 on 59 degrees of freedom
## Multiple R-squared: 0.4288, Adjusted R-squared: 0.4191
## F-statistic: 44.3 on 1 and 59 DF, p-value: 1.033e-08
```

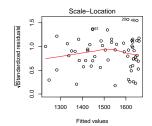
### **Checking linear model assumptions**

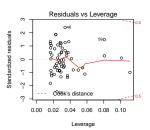
R

par(mfrow=c(2,2))
plot(mod.hard)









#### Linear model vs. t.test(...) in R



```
mod.loc <- lm(mortality ~ location, data = water)</pre>
coef(mod.loc)
##
     (Intercept) locationSouth
##
       1633.6000 -256.7923
t.test(water$mortality ~ water$location)
##
##
   Welch Two Sample t-test
##
## data: water$mortality by water$location
## t = 7.1427, df = 53.29, p-value = 2.584e-09
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## 184.6919 328.8928
## sample estimates:
## mean in group North mean in group South
##
              1633,600
                           1376.808
```

# The multiple linear model An extension of the simple linear model (1/2)

If several explanatory variables are of interest, instead of performing multiple simple regressions, a **multiple linear regression** or **multivariable** approach is appropriate.

- the same assumptions as for simple linear regressions should be checked
- collinearity might be an issue (see vif(...) function from package usdm)
- model comparison (AIC) (...discussed later)

# The multiple linear model An extension of the simple linear model (2/2)

Simple linear regression model:

$$y_i = \alpha + \beta x_i + \epsilon$$

Multiple linear regression model:

$$y_i = \alpha + \beta_1 x \mathbf{1}_i + \beta_2 x \mathbf{2}_i + \dots + \epsilon,$$
  

$$y_i = \alpha + \beta_1 x \mathbf{1}_i * \beta_2 x \mathbf{2}_i + \dots + \epsilon,$$
(1)

#### where

- α: intercept or constant
- β<sub>1</sub>: slope1, regression coefficient (effect size)
- β<sub>2</sub>: slope2, regression coefficient (effect size)
- $\beta_1 * \beta_2$ : interaction effect between  $x_1$  and  $x_2$
- $\epsilon$ : error, residuals

## The multiple linear model Interpretation of model coefficients

- α: intercept or constant
- β<sub>1</sub>: slope1, regression coefficient (effect size)
- β<sub>2</sub>: slope2, regression coefficient (effect size)
- $\beta_1 * \beta_2$ : interaction effect between  $x_1$  and  $x_2$
- $\epsilon$ : error, residuals
- → β<sub>1</sub> describes the number of units of a change in the outcome variable y as x<sub>1</sub> changes by one unit, x<sub>2</sub> being held constant.
- → β<sub>2</sub> describes the number of units of a change in the
   outcome variable y as x<sub>2</sub> changes by one unit, x<sub>1</sub> being held
   constant.

#### The multiple linear model in R



```
mod.hard.loc <- lm(mortality ~ hardness + location, data = water)
summary(mod.hard.loc)
##
## Call:
## lm(formula = mortality ~ hardness + location, data = water)
##
## Residuals:
## Min 10 Median 30
                                        Max
## -222.959 -77.281 7.143 90.751 307.836
##
## Coefficients:
##
               Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1695.4371 25.3285 66.938 < 2e-16 ***
## hardness -2.0341 0.4829 -4.212 8.93e-05 ***
## locationSouth -176.7108 36.8913 -4.790 1.19e-05 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 122.1 on 58 degrees of freedom
## Multiple R-squared: 0.5907, Adjusted R-squared: 0.5766
## F-statistic: 41.86 on 2 and 58 DF, p-value: 5.601e-12
```

#### **Model selection**



- Multiple R<sup>2</sup>: the larger, the better
- Akaike criterion (AIC): the smaller, the better

#### **Nested models**



Two models are nested if one of them is a particular case of the other one: the simpler model can be obtained by setting some coefficients of the more complex model to particular values.

```
mod.hard <- lm(mortality ~ hardness, data = water)  # mod 1
mod.loc <- lm(mortality ~ location, data = water)  # mod 2
mod.hard.loc <- lm(mortality ~ hardness + location, data = water) # mod 3</pre>
```

## Among the 3 above models ...

- which ones are nested?
- which ones are not nested?

## Multiple R<sup>2</sup>



 $R^2$  is a measure of fit quality:

$$R^2 = rac{ ext{SSRegression}}{ ext{SSTotal}}$$
 $R^2 = 1 - rac{ ext{SSError}}{ ext{SSTotal}}$ 

#### CAREFUL:

- The SSError always decreases as more predictors are added to the model.
- R<sup>2</sup> always increases and can be artificially large.

#### Akaike criterion (AIC)

- Model fit (R<sup>2</sup>) always improves with model complexity. We would like to strike a good balance between model fit and model simplicity.
- AIC combines a measure of model fit with a measure of model complexity: The smaller, the better.

For a given data set and a given model:

$$AIC = -2 \cdot \log(L) + 2p$$

L stands for the likelihood. p stands for the number of parameters in the models (penalizes complex models).

#### Model selection strategy for AIC

- Consider a number of candidate models. (They need not be nested.)
- Calculate their AIC.
- Choose the model(s) with the smallest AIC.
- ightarrow **CAREFUL**: The absolute value of AIC is meaningless. The relative AIC values, between models, is meaningful.

#### Model selection with AIC in R

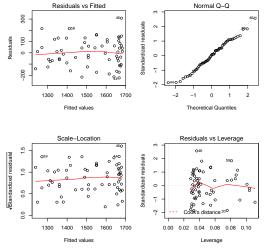


```
mod1 <- lm(mortality ~ hardness, data = water)</pre>
mod2 <- lm(mortality ~ location, data = water)</pre>
mod3 <- lm(mortality ~ hardness + location, data = water)
AIC(mod1, mod2, mod3)
## df AIC
## mod1 3 782.5692
## mod2 3 778.5186
## mod3 4 764.2366
round(AIC(mod1, mod2, mod3), 2)
## df AIC
## mod1 3 782.57
## mod2 3 778.52
## mod3 4 764.24
```

#### mod.hard.loc is the best!



 $\label{eq:mod3} $$ $$ \mbox{mod3} <-\mbox{lm(mortality $\tilde{\mbox{$^{\circ}$}}$ hardness $$+$ location, data = water)} $$ $$ \mbox{par(mfrow=c(2,2))} $$ $$ \mbox{plot(mod3)} $$$ 



#### **Exercise 16**

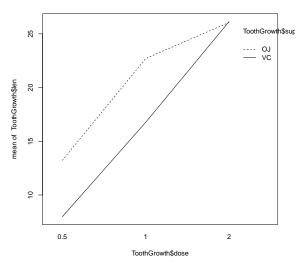


#### **Exercise 17**



#### **Interactions**





### Two-way Interactions in R (1/3)



#### There are three different interactions:

- Interaction between two categorical variables
- Interaction between one continuous and one categorical variables
- Interaction between two continuous variables

## Two-way Interactions in R (2/3)



## Model specification in R: Be aware, an interaction is never tested without its corresponding main effects included in the model.

```
# Interaction between two categorical variables
# mod.dose.supp.int <- lm(len ~ dose.fac + supp + dose.fac:supp,
# data = ToothGrowth)
mod.dose.supp.int <- lm(len ~ dose.fac * supp, data = ToothGrowth)
# summary(mod.dose.supp.int)</pre>
```

$$\begin{split} \textit{y} \sim \beta_{\text{baseline}((\text{dose==low}) \& (\text{supp==OJ}))} + \beta_{\text{dose==med}} + \\ \beta_{\text{dose==high}} + \beta_{\text{supp==VC}} \\ + \beta_{(\text{dose==med}) \& (\text{supp==VC})} \\ + \beta_{(\text{dose==high}) \& (\text{supp==VC})} \end{split}$$

#### Two-way Interactions in R (3/3)



```
# Interaction between two categorical variables
# mod.dose.supp.int <- lm(len ~ dose.fac + supp + dose.fac:supp,
# data = ToothGrowth)
mod.dose.supp.int <- lm(len ~ dose.fac * supp, data = ToothGrowth)
summarv(mod.dose.supp.int)
##
## Call:
## lm(formula = len ~ dose.fac * supp. data = ToothGrowth)
##
## Residuals:
##
     Min 1Q Median 3Q
                               Max
## -8.20 -2.72 -0.27 2.65 8.27
##
## Coefficients:
##
                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                      13.230 1.148 11.521 3.60e-16 ***
## dose.facmed
                       9.470
                                  1.624 5.831 3.18e-07 ***
                      12.830
## dose.fachigh
                                  1.624 7.900 1.43e-10 ***
                      -5.250 1.624 -3.233 0.00209 **
## suppVC
## dose.facmed:suppVC -0.680
                                  2.297 -0.296 0.76831
## dose.fachigh:suppVC
                      5.330
                                  2 297 2 321 0 02411 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.631 on 54 degrees of freedom
## Multiple R-squared: 0.7937, Adjusted R-squared: 0.7746
## F-statistic: 41.56 on 5 and 54 DF, p-value: < 2.2e-16
```

## Two-way Interactions in R: Interpretation of coefficients (1/2)



```
## (Intercept) dose.facmed dose.fachigh
## 13.23 9.47 12.83
## suppVC dose.facmed:suppVC dose.fachigh:suppVC
## -5.25 -0.68 5.33
```

$$y \sim eta_{
m baseline((dose==low)\ \&\ (supp==OJ))} + eta_{
m dose==med} + \ eta_{
m dose==high} + eta_{
m supp==VC} \ + eta_{
m (dose==med)\ \&\ (supp==VC)} \ + eta_{
m (dose==high)\ \&\ (supp==VC)}$$

### Two-way Interactions in R: Interpretation of coefficients (2/2)



```
coef(mod.dose.supp.int)

## (Intercept) dose.facmed dose.fachigh
## 13.23 9.47 12.83
## suppVC dose.facmed:suppVC dose.fachigh:suppVC
## -5.25 -0.68 5.33
```

- → dose.facmed:suppVC change in the slope between the low and med dose.fac group under the supplement type (supp) VC in comparison to the intercept. In other words, by changing the dose from low to med within the supplement group VC, the slope decreases by approx. -0.68.
- dose.fachigh:suppVC change in the slope between the low and high dose.fac group under the supplement type (supp) VC in comparison to the intercept. In other words, by changing the dose from low to high within the supplement group VC, the slope increases by approx. +5.33.

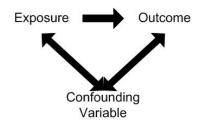
#### **Exercise 18**



#### Confounding



- Confounding variable is associated with the exposure and the outcome
- Confounding variable is not part of the causal path between exposure and the outcome



#### Some things to remember

- Not adjusting / controlling for a confounding variable may lead to bias results. It is good practice to present as well the crude (not adjusted ORs) and the adjusted ones. Adjustment is typically done if difference > 10%.
- Check also for interaction (= effect modification) e. g. using logistic regression.
- Do not adjust for a variable C if it is a common effect of E and D (collider) or if it is in the causal pathway of E and D.

## **Lecture Slides for Day 7**

#### Other topics

So far, we have covered some of the basic **tools** necessary for helping you to start to analyse your own study data.

Many things have been mentioned others have not been included some are worth mentioning as they are particularly common in some areas of veterinary research.

#### Normally distributed response variables

So far, we have only considered analyses where the response variable is a **continuous** measurement.

We now have a brief look at two other common types of **response** variables.

The methods we use are largely similar to what we have looked at previously.

## Data types in R



### numeric & integers

```
ToothGrowth$len[1:6]
## [1] 4.2 11.5 7.3 5.8 6.4 10.0
bacteria$week[1:6]
## [1] 0 2 4 11 0 2
```

### Data types in R



#### numeric & integers

```
ToothGrowth$len[1:6]
## [1] 4.2 11.5 7.3 5.8 6.4 10.0
bacteria$week[1:6]
## [1] 0 2 4 11 0 2
```

#### unordered factor with 2 levels

```
lung$sex[1:6]
## [1] female male female female male female
## Levels: female male
```

#### (un/ordered) factor (with more than 2 levels)

```
chickwts$feed[1:6]
## [1] horsebean horsebean horsebean horsebean horsebean
## Levels: casein horsebean linseed meatmeal soybean sunflower
```

### **Generalised Linear Modelling**

- random component:  $\mathbf{E}(\mathbf{Y}) = \mu$
- systematic component: covariates:  $\mathbf{x_1}, \mathbf{x_2}, \dots, \mathbf{x_p}$  produce a linear predictor  $\eta = \sum_1^p \mathbf{x_j} \beta_j$
- link between random and systematic components:  $\mu = \eta$ , e.g.
  - Logistic Regression:  $\eta_i = g(\mu_i), g(.)$  is logit function for binomial data
  - Poisson Regression:  $\eta_i = g(\mu_i), g(.)$  is exponential function for poisson data

## Logistic Regression: unordered factor with 2 levels as response

E. g. a logistic regression with one covariate (sex) fitting a model to  $\mathbb{P}(Y = TRUE) = \pi$ , e. g. presence of respiratory symptoms (experienced by the child over the previous 12 months), gives

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 \mathbf{x}_1$$

## Logistic Regression: unordered factor with 2 levels as response

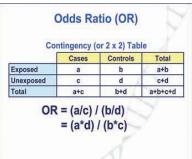
```
logreg.mod <- glm(respsymptoms ~ sex.
                 family = binomial, # description of the link function
                 data = lung)
summary(logreg.mod)
##
## Call:
## glm(formula = respsymptoms ~ sex, family = binomial, data = lung)
## Deviance Residuals:
      Min
               1Q Median 3Q
                                        Max
## -0.7440 -0.7440 -0.6915 -0.6915 1.7597
##
## Coefficients:
              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.1429 0.1276 -8.957 <2e-16 ***
## sexmale -0.1663 0.1901 -0.875 0.382
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
      Null deviance: 682.85 on 635 degrees of freedom
##
## Residual deviance: 682.08 on 634 degrees of freedom
## AIC: 686.08
##
## Number of Fisher Scoring iterations: 4
```

## Logistic Regression: unordered factor with 2 levels as response Interpretation of model coefficients

```
summary(logreg.mod)
##
## Call:
## glm(formula = respsymptoms ~ sex, family = binomial, data = lung)
##
## Deviance Residuals:
      Min
##
                10 Median
                                         Max
## -0.7440 -0.7440 -0.6915 -0.6915 1.7597
##
## Coefficients:
##
              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.1429 0.1276 -8.957 <2e-16 ***
## sexmale -0.1663 0.1901 -0.875 0.382
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
  (Dispersion parameter for binomial family taken to be 1)
##
      Null deviance: 682.85 on 635 degrees of freedom
##
## Residual deviance: 682.08 on 634 degrees of freedom
## ATC: 686.08
##
## Number of Fisher Scoring iterations: 4
cbind(logodds = coef(logreg.mod), odds = exp(coef(logreg.mod)))
                logodds
                             odds
## (Intercept) -1.1428851 0.3188976
## seymale
              -0.1662919 0.8467990
```

## Odds ratios versus log odds ratios

- The logistic regression model in R outputs the log odds ratios.
- exp(log odds ratio) = odds ratio
- An odds ratio (OR) is a measure of association between an exposure (here: sex) and an outcome (here: respsymptoms).
   The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.



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Lecture Slides (all)

# Logistic Regression: unordered factor with 2 levels as response Interpretation of model coefficients (cont'd)

The coefficient  $\beta_{\text{sex}}$  is the estimated amount by which the log odds of respsymptoms would in-/decrease if sex is male. The log odds of respsymptoms when sex is female is just above in the first row  $(\beta_{(\text{Intercept})})$ , reference level of sex).

#### Logistic Regression: probability of success as response

- A researcher is examining beetle mortality after 5 hours of exposure to carbon disulphide, at various levels of concentration of the gas.
- Beetles were exposed to gaseous carbon disulphide at various concentrations (in mg/L) for five hours (Bliss, 1935) and the number of beetles killed were noted.

## Logistic Regression: probability of success as response



```
logreg.mod <- glm(cbind(Num.Killed, Num.Surv) ~ Dose,</pre>
                 family = binomial, # description of the link function
                 data = beetle)
summarv(logreg.mod)
##
## Call:
## glm(formula = cbind(Num.Killed, Num.Surv) ~ Dose, family = binomial,
      data = beetle)
##
##
## Deviance Residuals:
##
      Min
                10 Median
                                  30
                                         Max
## -1.5941 -0.3944 0.8329 1.2592 1.5940
##
## Coefficients:
              Estimate Std. Error z value Pr(>|z|)
##
## (Intercept) -60.717 5.181 -11.72 <2e-16 ***
## Dose
               34 270
                            2 912 11 77 <2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
      Null deviance: 284.202 on 7 degrees of freedom
##
## Residual deviance: 11.232 on 6 degrees of freedom
## ATC: 41.43
##
## Number of Fisher Scoring iterations: 4
```

## Logistic Regression: probability of success as response Interpretation of model coefficients



```
summary(logreg.mod)
##
## Call:
## glm(formula = cbind(Num.Killed, Num.Surv) ~ Dose, family = binomial,
       data = beetle)
##
## Deviance Residuals:
##
       Min
                 1Q Median 3Q
                                            Max
## -1.5941 -0.3944 0.8329 1.2592 1.5940
##
## Coefficients:
               Estimate Std. Error z value Pr(>|z|)
##
## (Intercept) -60.717 5.181 -11.72 <2e-16 ***
               34.270 2.912 11.77 <2e-16 ***
## Dose
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
       Null deviance: 284.202 on 7 degrees of freedom
##
## Residual deviance: 11.232 on 6 degrees of freedom
## ATC: 41.43
##
## Number of Fisher Scoring iterations: 4
cbind(logodds = coef(logreg.mod), odds = exp(coef(logreg.mod)))
                 logodds
## (Intercept) -60.71745 4.273114e-27
## Dose 34.27033 7.645631e+14
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                                                       Lecture Slides (all)
```

# Logistic Regression: probability of success as response Interpretation of model coefficients (cont'd)



The coefficient  $\beta_{\text{Dose}}$  is the estimated amount by which the log odds of being killed would in-/decrease if Dose would increase by one unit. The log odds of being killed when Dose is 0 is just above in the first row ( $\beta_{\text{(Intercept)}}$ ).

#### Some things to remember

- Using generalised linear models (glm) is very similar to the previous models we have seen for normal data (lm) - things like residuals need to be checked for randomness, although this is more difficult with counts. Also the residuals being normally distributed is not relevant to these models.
- As always explore the data first visually and with tables before doing any formal analyses.

#### Short excursion to quantile regression

- When talking about regression, we almost exclusively model the conditional mean of a response given one or more explanatory variables.
- With the classical linear model we cannot skewed or otherwise non normal distribution as the corresponding quantiles from the linear model will be misleading. Therefore, we shift our attention to a completely distribution free approach that directly addresses conditional quantile modelling. (Text taken from Hothorn, Torsten, and Brian S. Everitt. A. handbook of statistical analyses using R. CRC press, 2014.)

## Short excursion to quantile regression

The data set sambia.csv (given on the switch drive) contains information on malnutrition of sambian children. The outcome variable is the z-score, which gives information on the severity of malnutrition.

The following covariates are considered:

zscore	Z-score of the child
sex	Sex of the child (0 = female, 1 = male)
age.child	Age of the child
work	Is the mother working $(0 = no, 1 = yes)$
age.mother.birth	Age of the mother at birth of child
bmi	BMI of the mother

A z-score indicates how many standard deviations an element is from the mean. A z-score can be calculated from the following formula.  $z=(X-\mu)/\sigma$  where z is the z-score, X is the value of the element,  $\mu$  is the population mean, and  $\sigma$  is the standard deviation.

Sonja Hartnack, Terence Odoch & Muriel Buri Lecture Slides (all)

#### Quantile regression in R



#### Quantile regression in R (cont'd)



```
# install.packages("quantreg")
library("quantreg")
## Loading required package: SparseM
##
## Attaching package: 'SparseM'
## The following object is masked from 'package:base':
##
##
      backsolve
##
## Attaching package: 'quantreg'
## The following object is masked from 'package:survival':
##
##
      untangle.specials
# tau = 2.5 %
rq.025 <- rq(zscore ~ sex + age.child + work + bmi + age.mother.birth,
             tau = 0.025,
             data = sambia)
# tau = 5 %
rq.05 <- rq(zscore ~ sex + age.child + work + bmi + age.mother.birth,
            tau = 0.05.
            data = sambia)
# tau = 50 %
rq.5 <- rq(zscore ~ sex + age.child + work + bmi + age.mother.birth,
           tau = 0.5.
           data = sambia)
```

# Quantile regression in R (cont'd) Comparison of the three models rq.025, rq.05, rq.5



```
rq_coef <- cbind(coef(rq.025), coef(rq.05), coef(rq.5))
rownames(rq_coef) <- rownames(summary(rq.5)$coefficients)</pre>
colnames(rq_coef) <- paste0("tau = ", c("0.025", "0.05", "0.5"))
round(rq_coef, 3)
##
                 tau = 0.025 tau = 0.05 tau = 0.5
## (Intercept)
                  -523.808 -491.812 -242.188
## sexmale
                      1.511 -3.816 -13.197
## age.child
                     -2.235 -2.496 -1.903
## workyes
                    -13.947 -16.280 -6.489
                       6.947 7.884 4.966
## bmi
## age.mother.birth
                       0.244
                                 0.465
                                         0.830
```

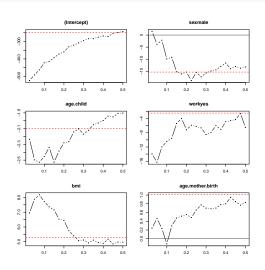
# Quantile regression in R (cont'd) Estimate rq for a grid of quantiles



## Quantile regression in R (cont'd) Plot results



plot(rq) # plot including line for the OLS coefficient (as estimated by lm)



## Quantile regression in R (cont'd) Plot results in more details



# A sequence of coefficient estimates for quantile regressions with # varying tau parameters is visualized along with associated confidence bands. plot(summary(rq))

