



**University of  
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**MAKERERE UNIVERSITY**

# **Data Analysis with R: Day 5**

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# 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:  $R^2$  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?**

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

$$H_A : \text{at least one pair of } \mu_1, \dots, \mu_k \text{ are different}$$

where  $\mu_1, \dots, \mu_k$  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

$$\sum_i \sum_j (x_{ij} - \bar{x})^2 = \sum_i n_i (\bar{x}_i - \bar{x})^2 + \sum_i \sum_j (x_{ij} - \bar{x}_i)^2$$



Variance  
between groups



Variance  
within groups

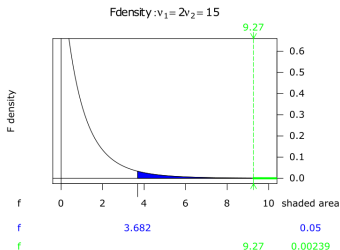
$\bar{x}$  = grand mean  
 $\bar{x}_i$  = group mean

## 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

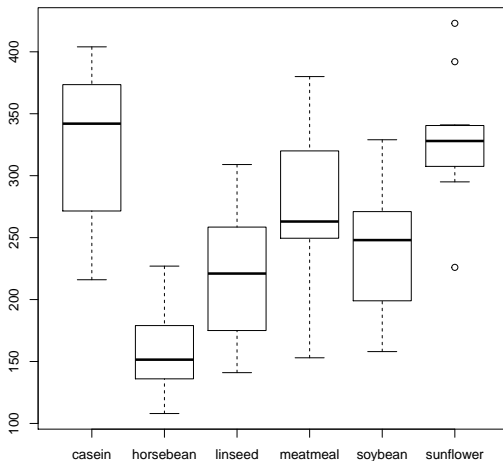
$$F = \frac{\text{variance between groups}}{\text{variance within groups}}$$



## ANOVA in R with chickwts



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



```
# aov.mod <- aov(chickwts$weight ~ chickwts$feed)
aov.mod <- aov(weight ~ feed, data = chickwts)
# 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

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

## Checking model residuals: $\text{mean}(\text{residuals}) = 0$



"Unexplained rest of the model"

```
# What are residuals?
chickwts$residuals <- residuals(aov.mod)
tapply(chickwts$weight, chickwts$feed, mean)

##      casein horsebean  linseed  meatmeal   soybean 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)

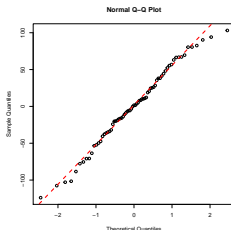
## [1] 7.573045e-16
```

# 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

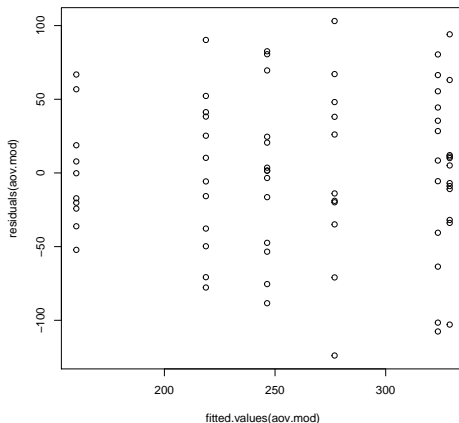
# Levene's Test
# library("Rcmdr")
# levene.test(chickwts$weight ~ chickwts$feed)
```

## Checking model residuals: Variance homoscedasticity (2/3)

### Graphical interpretation is better!



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

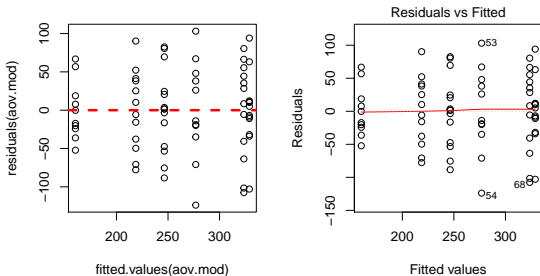


## Checking model residuals: Variance homoscedasticity (3/3)

### Graphical interpretation is better!



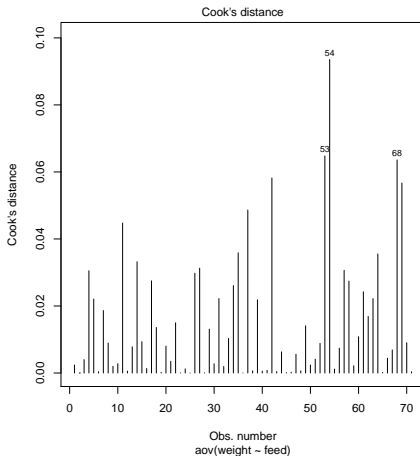
```
# Plot fitted against residual values
par(mfrow=c(1,2), pty="s", mar = c(1, 4, 1, 2))
plot(fitted.values(aov.mod), residuals(aov.mod))
abline(h = 0, col = "red", lwd = 3, lty = 2)
plot(aov.mod, which=1)
```



# Checking model residuals: Cook's distance

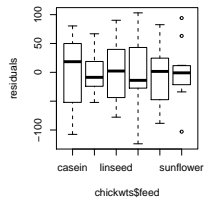
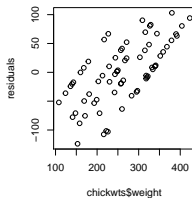
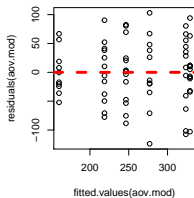


```
# Plot fitted against residual values  
# Cut-off at 3  
par(mfrow=c(1,1), pty="s", mar = c(5, 4, 4, 2))  
plot(aov.mod, which=4)
```



# Checking for potential patterns

```
par(mfrow=c(1,3), pty="s")
plot(fitted.values(aov.mod), residuals(aov.mod))
abline(h = 0, col = "red", lwd = 3, lty = 2)
# Plot residuals against variables from the model
plot(chickwts$weight, residuals(aov.mod), ylab = "residuals")
plot(chickwts$feed, residuals(aov.mod),
      xlab = "chickwts$feed", ylab = "residuals")
```

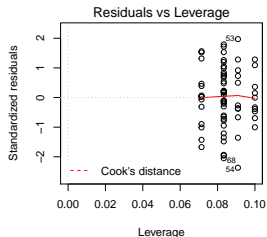
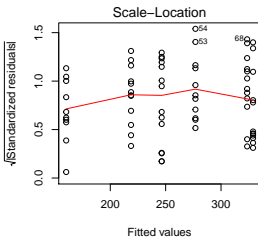
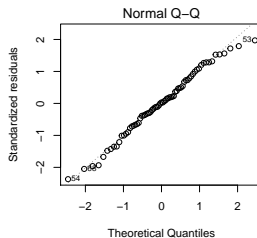
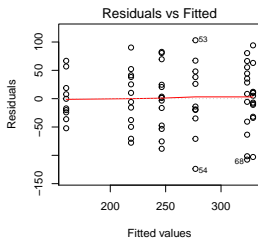




# Plot anova objects



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





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
```



Hence, we are better off 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  soybean
## 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  soybean
## 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"))
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 0.16699
## soybean - casein == 0   -77.155    21.578  -3.576 0.00316 **
## sunflower - casein == 0    5.333    22.393   0.238 0.99945
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 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"))
summary(tukey)

##
## 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 ***
## linseed - casein == 0     -104.833    22.393   -4.682 < 0.001 ***
## meatmeal - casein == 0     -46.674    22.896   -2.039 0.03220
## soybean - casein == 0      -77.155    21.578   -3.576 0.00844 **
## sunflower - casein == 0       5.333    22.393    0.238 0.99989
## linseed - horsebean == 0     58.550    23.485    2.493 0.14099
## meatmeal - horsebean == 0    116.709    23.966    4.870 < 0.001 ***
## soybean - horsebean == 0     86.229    22.710    3.797 0.00424 **
## sunflower - horsebean == 0  168.717    23.485    7.184 < 0.001 ***
## meatmeal - linseed == 0      58.159    22.896    2.540 0.12763
## soybean - linseed == 0       27.679    21.578    1.283 0.79295
## sunflower - linseed == 0    110.167    22.393    4.920 < 0.001 ***
## soybean - meatmeal == 0     -30.481    22.100   -1.379 0.73873
## sunflower - meatmeal == 0    52.008    22.896    2.271 0.22030
## sunflower - soybean == 0     82.488    21.578    3.823 0.00390 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

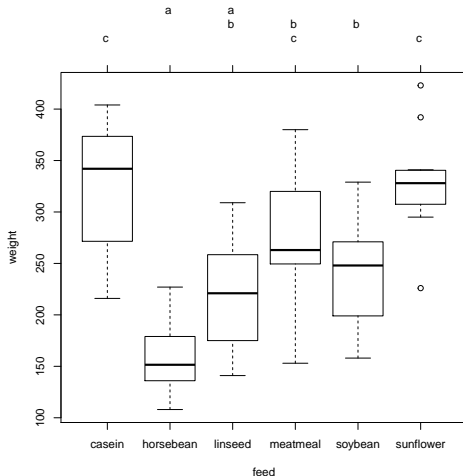
Scanned with CamScanner

# 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)
```



## Exercise 15





# Introduction: ANOVA as a special case of a linear model

## Linear Modelling

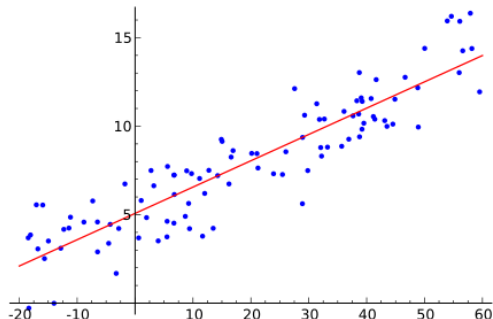
So far we have considered how to determine whether statistically significant differences exist between different "feed" groups (factors).

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  $(x_i, y_i)$ ,  $i = 1, \dots, n$  the following equation is used:

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

where

- $\alpha$ : 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.frame': 61 obs. of 4 variables:
## $ location : Factor w/ 2 levels "North","South": 2 1 2 1 1 1 1 2 1 2 ...
## $ town : chr "Bath" "Birkenhead" "Birmingham" "Blackburn" ...
## $ mortality: int 1247 1668 1466 1800 1609 1558 1807 1299 1637 1359 ...
## $ hardness : int 105 17 5 14 18 10 15 78 10 84 ...
```

```
data(water)
# mod.hard <- lm(mortality ~ hardness, data = water)
mod.hard <- lm(water$mortality ~ water$hardness)
summary(mod.hard)

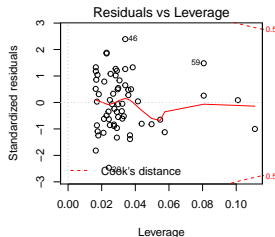
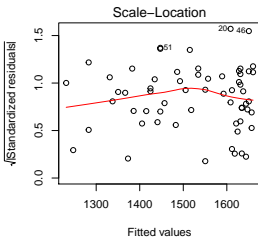
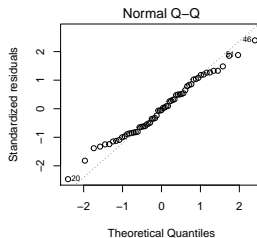
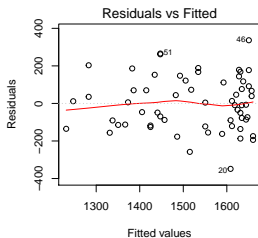
##
## Call:
## lm(formula = water$mortality ~ water$hardness)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -348.61 -114.52   -7.09   111.52   336.45
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   1676.3556    29.2981   57.217 < 2e-16 ***
## water$hardness    -3.2261     0.4847  -6.656 1.03e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Standard error: 143 on 59 degree of freedom
```

# Checking linear model assumptions



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



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



```
mod.loc <- lm(mortality ~ location, data = water)
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:

$$y_i = \alpha + \beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \epsilon, \quad (1)$$

$$y_i = \alpha + \beta_1 x_{1i} * \beta_2 x_{2i} + \dots + \epsilon,$$

where

- $\alpha$ : intercept or constant
- $\beta_1$ : slope1, regression coefficient (effect size)
- $\beta_2$ : 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

- $\alpha$ : intercept or constant
  - $\beta_1$ : slope1, regression coefficient (effect size)
  - $\beta_2$ : slope2, regression coefficient (effect size)
  - $\beta_1 * \beta_2$ : interaction effect between  $x_1$  and  $x_2$
  - $\epsilon$ : error, residuals
- 
- $\rightarrow \beta_1$  describes the number of units of a change in the outcome variable  $y$  as  $x_1$  changes by one unit,  $x_2$  being held constant.
  - $\rightarrow \beta_2$  describes the number of units of a change in the outcome variable  $y$  as  $x_2$  changes by one unit,  $x_1$  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       1Q   Median       3Q      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 '**' 0.01 '*' 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
```



- Multiple  $R^2$ : the larger, the better
- Akaike criterion (AIC): the smaller, the better



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
```

Among the 3 above models...

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



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

$$R^2 = \frac{\text{SSRegression}}{\text{SSTotal}} \quad (2)$$
$$R^2 = 1 - \frac{\text{SSError}}{\text{SSTotal}}$$

### CAREFUL:

- The SSError always decreases as more predictors are added to the model.
- $R^2$  always increases and can be artificially large.

## Akaike criterion (AIC)

- Model fit ( $R^2$ ) 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

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

where  $-2\log(L)$  is a function of the prediction error: the smaller, the better. Measures how the model fits the data.  $2p$  penalizes complex models: the smaller, the better.



## 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.

→ **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)
mod2 <- lm(mortality ~ location, data = water)
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
```

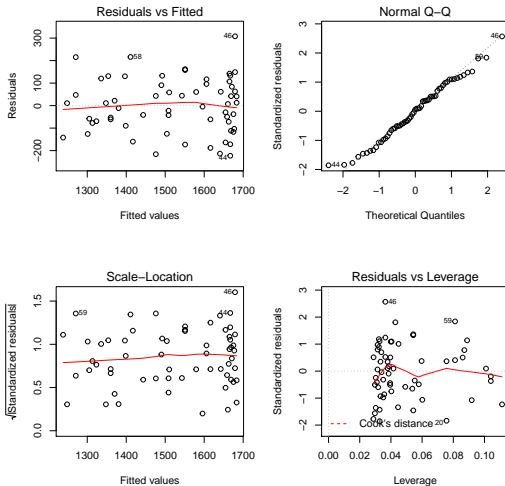
```
round(AIC(mod1, mod2, mod3), 2)
```

```
##      df      AIC
## mod1  3 782.57
## mod2  3 778.52
## mod3  4 764.24
```

## mod.hard.loc is the best!



```
mod3 <- lm(mortality ~ hardness + location, data = water)
par(mfrow=c(2,2))
plot(mod3)
```



## Exercise 16

