Analysis of Variance

The ASTA team

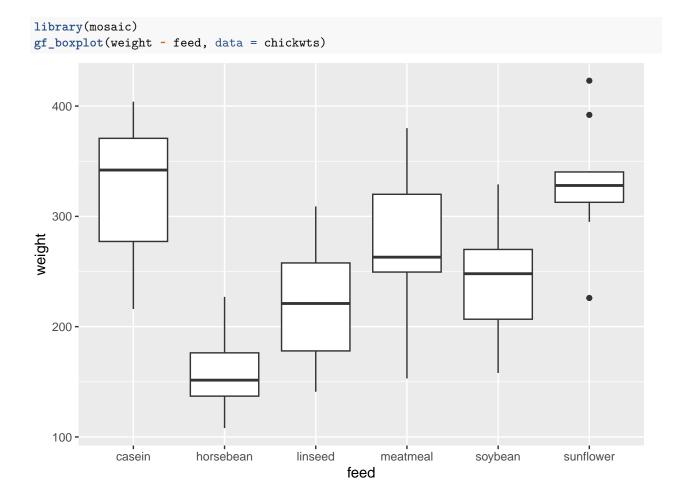
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1 One way analysis of variance

1.1 Example

- The data set chickwts is available in R, and on the course webpage.
- 71 newly hatched chicks were randomly allocated into six groups, and each group was given a different feed supplement.
- Their weights in grams after six weeks are given along with feed types, i.e. we have a sample with corresponding measurements of 2 variables:
 - weight: a numeric variable giving the chick weight.
 - feed: a factor giving the feed type.
- Always start with some graphics:



1.2 The ANOVA Model

- We measure the response y which in this case is weight.
- We want to study the effect of the factor x on y. In this case x = feed and divides the sample in g = 6 groups.
- The mean responses within the groups are denoted $\mu_1, \mu_2, \dots, \mu_q$.
- We will assume that
 - $-y = \mu_x + \epsilon$, when y is a response in group x
 - $-\epsilon$ are a sample from a population with mean zero and standard deviation σ .
 - The standard deviation for the population in each group is the same and equals σ
 - The response variable, y, is normal distributed within each group.
- The ANOVA test is a test of equal means for the different groups.

2 Estimation of mean values

2.1 Estimates

- Least squares estimates for population means $\hat{\mu}_x$ is given by the average of the response measurements in group x.
- For a given measured response y we let \hat{y} denote the model's prediction of y, i.e.

$$\widehat{y} = \widehat{\mu}_x$$

if y is a response for an observation in group x.

• We use mean to find the mean, for each group:

```
mean(weight ~ feed, data = chickwts)
```

```
## casein horsebean linseed meatmeal soybean sunflower ## 323.5833 160.2000 218.7500 276.9091 246.4286 328.9167
```

- We can e.g. see that $\hat{y} = 323.6$, when feed=casein but $\hat{y} = 160.2$, when feed=horsebean.
- Is it a significant difference?

2.2 Contrast coding

- In many cases there is a group corresponding to "no treatment" and we are interested in the effect of different treatments.
- In this example we only have different feeds, which are sorted in lexicographical order by R, so casein is the reference.
- We can specify the model via:
 - Intercept corresponding to the mean response for the reference (casein).
 - For each of the other groups we have a contrast, which measures the difference between the mean value for that group and the reference group.
- For a given contrast we can calculate standard error, t-score and p-value, and thereby investigate whether there is a difference between this group and the reference group.
- In Agresti this is referred to as using dummy variables.

2.3 Example

```
model <- lm(weight ~ feed, data = chickwts)</pre>
summary(model)
##
## lm(formula = weight ~ feed, data = chickwts)
##
## Residuals:
##
        Min
                       Median
                  1Q
                                     3Q
                                             Max
## -123.909 -34.413
                        1.571
                                38.170
                                        103.091
##
## Coefficients:
##
                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                  323.583
                              15.834 20.436 < 2e-16 ***
                              23.485 -6.957 2.07e-09 ***
## feedhorsebean -163.383
## feedlinseed
                 -104.833
                              22.393
                                      -4.682 1.49e-05 ***
## feedmeatmeal
                  -46.674
                              22.896
                                      -2.039 0.045567 *
## feedsoybean
                  -77.155
                              21.578
                                      -3.576 0.000665 ***
                              22.393
                                       0.238 0.812495
## feedsunflower
                    5.333
## Signif. codes:
                  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 54.85 on 65 degrees of freedom
## Multiple R-squared: 0.5417, Adjusted R-squared: 0.5064
## F-statistic: 15.36 on 5 and 65 DF, p-value: 5.936e-10
```

- We get information about contrasts and their significance:
- Intercept corresponding to case in has weight different from zero $(p < 2 \times 10^{-16})$ (of course, chickens grow a lot over 6 weeks)
- Weight difference between casein and horsebean is extremely significant (p= 2×10^{-9}).

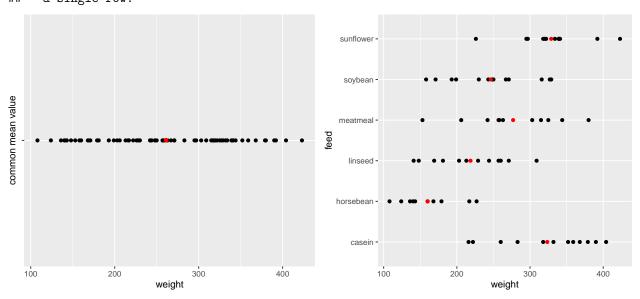
• There is no significant weight difference between casein and sunflower (p=81%).

3 Overall test for effect

3.1 Graphical representation of models

- We have two alternative explanations of the data.
- Simple model with one parameter (mean): "The feed type doesn't matter. The weight is just random around a common mean value".
- Complex model with six parameters (means): "The feed type is important. For each feed type we get a different mean value and the weights are random around these values."

Warning in geom_point(aes(x = red_dot), color = "red"): All aesthetics have length 1, but the data h
i Please consider using `annotate()` or provide this layer with data containing
a single row.



3.2 Hypotheses and test statistic

• Is the complex model significantly better (i.e. is there any effect of the explanatory grouping variable)? We can write the corresponding hypotheses in two different ways

 $H_0: \mu_1 = \mu_2 = \cdots = \mu_g$ against $H_a:$ At least 2 of the population means are different

Alternatively

 H_0 : All contrasts are equal to zero. H_a : At least one contrast is non-zero.

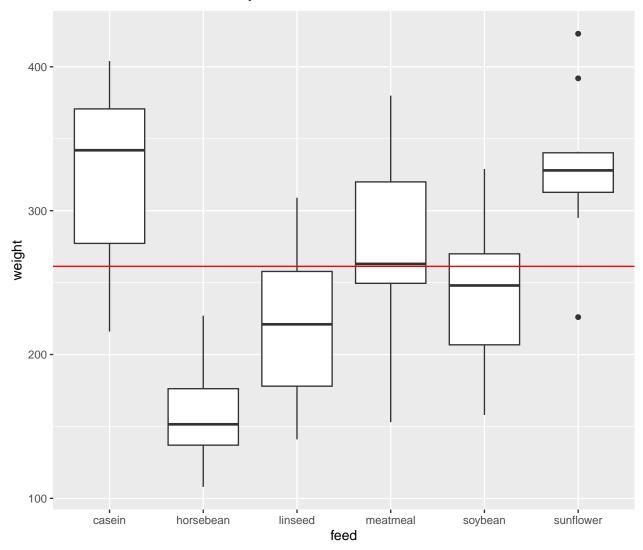
• We will (indirectly) use R^2 to do the test. If it is large, the complex model has good predictive power compared to the simple model. To judge significance we use

$$F_{obs} = \frac{(n-g)R^2}{(g-1)(1-R^2)} = \frac{(TSS-SSE)/(g-1)}{SSE/(n-g)}.$$

- Large values of R^2 implies large values of F_{obs} , which points to the alternative hypothesis.
- I.e. when we have calculated the observed value F_{obs} , then we have to find the probability that a new experiment would result in a larger value.
- TSS: error sum of squares if common mean. SSE: error sum of squares if different means.
- TSS-SSE: how much does error sum of squares increase if means are restricted to be equal.

3.3 Interpretation of F statistic - Variance between/within groups

- It can be shown that the numerator of F_{obs} is a measure of the variance between the groups, i.e. how much "boxes" vary around the total average (the red line).
- Likewise it can be shown the denominator of F_{obs} is a measure for the variance within groups, i.e. how "tall" the boxes in the boxplot are.



• The bigger deviations between the red line and the box means relative to the variation within boxes, the less we trust H_0 . This is measured by the F-test statistic, which can be stated as

$$F_{obs} = \frac{\text{variance between groups}}{\text{variance within groups}}$$

3.4 Example

Call:

```
model <- lm(weight ~ feed, data = chickwts)
summary(model)
##</pre>
```

lm(formula = weight ~ feed, data = chickwts)

```
##
## Residuals:
##
        Min
                  1Q
                       Median
                                    30
  -123.909
                        1.571
                                38.170
                                        103.091
##
            -34.413
##
## Coefficients:
##
                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                  323.583
                              15.834
                                      20.436 < 2e-16 ***
## feedhorsebean -163.383
                              23.485
                                      -6.957 2.07e-09 ***
## feedlinseed
                 -104.833
                              22.393
                                      -4.682 1.49e-05 ***
## feedmeatmeal
                  -46.674
                              22.896
                                      -2.039 0.045567 *
## feedsoybean
                  -77.155
                              21.578
                                      -3.576 0.000665 ***
## feedsunflower
                    5.333
                              22.393
                                       0.238 0.812495
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 54.85 on 65 degrees of freedom
## Multiple R-squared: 0.5417, Adjusted R-squared: 0.5064
## F-statistic: 15.36 on 5 and 65 DF, p-value: 5.936e-10
```

• The last line gives us the value of $F_{obs} = 15.36$ and the corresponding p-value (5.9×10^{-10}) . Clearly there is a significant difference between the types of feed.

4 Two way analysis of variance

4.1 Additive effects

- The data set ToothGrowth is available in $\mathbf R$ and on the webpage. For more info about this data, use ?ToothGrowth.
- The data describes the tooth length in guinea pigs where some receive vitamin C treatment and others are given orange juice in different dosage.
- A total of 60 observations on 3 variables.
 - [,1] len The tooth length
 - [,2] supp The type of the supplement (OJ or VC)
 - [,3] dose The dosage (LO, ME, HI)
- We will study the response len with the predictors supp and dose.
- At first we look at the model with additive effects

```
- len=\mu + "effect of supp"+ "effect of dose" + error
```

- This is also called the main effects model since it does not contain interaction terms.
- The parameter μ corresponds to the Intercept and is the mean tooth length in the reference group (supp OJ, dose LO).
- The effect of supp is the difference in mean when changing from OJ to VC.
- The effect of dose is the difference in mean when changing from LO to either ME or HI.

4.2 Dummy coding

- Let us introduce dummy variables:
 - $-s_C=1$ if supp VC and zero otherwise.
 - $-d_{M}=1$ if dose is ME and zero otherwise.

- $-d_H = 1$ if dose is HI and zero otherwise.
- Then we state the model

length =
$$\mu + \beta_1 s_C + \beta_2 d_M + \beta_3 d_H + \text{error.}$$

- Interpretation:
 - $-\mu$ is the expected tooth length when supp is OJ and dose is LO ($s_C = d_M = d_H = 0$)).
 - $-\beta_1$ is the effect of supplement OJ to VC ($s_C=1$).

MainEff <- lm(len ~ supp + dose, data = ToothGrowth)

- $-\beta_2$ is the effect of increasing dosage from LO to ME $(d_M=1)$.
- $-\beta_3$ is the effect of increasing dosage from LO to HI $(d_H=1)$.
- As a two-way table:

$$\begin{array}{ccccc} & LO & ME & HI \\ OJ & \mu & \mu + \beta_2 & \mu + \beta_3 \\ VC & \mu + \beta_1 & \mu + \beta_1 + \beta_2 & \mu + \beta_1 + \beta_3 \end{array}$$

4.3 Main effect model in R

• The main effects model is fitted by

```
summary(MainEff)
##
## Call:
## lm(formula = len ~ supp + dose, data = ToothGrowth)
##
## Residuals:
     Min
##
             1Q Median
                            3Q
                                 Max
## -7.085 -2.751 -0.800 2.446
                               9.650
##
## Coefficients:
##
              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 12.4550
                            0.9883
                                   12.603 < 2e-16 ***
## suppVC
               -3.7000
                            0.9883
                                   -3.744 0.000429 ***
                                    7.543 4.38e-10 ***
## doseME
                9.1300
                            1.2104
## doseHI
                15.4950
                            1.2104 12.802 < 2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 3.828 on 56 degrees of freedom
## Multiple R-squared: 0.7623, Adjusted R-squared: 0.7496
```

- The model has 4 parameters.
- The F test at the end compares with the (null) model with only one overall mean parameter.

4.4 Testing effect of supp

• Alternative model without effect of supp:

F-statistic: 59.88 on 3 and 56 DF, p-value: < 2.2e-16

```
doseEff <- lm(len ~ dose, data = ToothGrowth)</pre>
```

• We can compare R^2 to see if doseEff (Model 1) is sufficient to explain the data compared to MainEff (Model 2). This is done by converting to F-statistic:

$$F_{obs} = \frac{(R_2^2 - R_1^2)/(df_1 - df_2)}{(1 - R_2^2)/df_2} = \frac{(SSE_1 - SSE_2)/(df_1 - df_2)}{(SSE_2)/df_2}.$$

- $SSE_1 SSE_2$: increase in error sum of square when using Model 1 instead of Model 2
- In **R** the calculations are done using anova:

```
anova(doseEff, MainEff)
```

```
## Analysis of Variance Table
##
## Model 1: len ~ dose
## Model 2: len ~ supp + dose
## Res.Df RSS Df Sum of Sq F Pr(>F)
## 1 57 1025.78
## 2 56 820.43 1 205.35 14.017 0.0004293 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

• p-value is 0.004 hence we reject that supp does not have an effect. Thus we prefer Model 2.

4.5 Testing effect of dose

• Alternative model without effect of dose:

```
suppEff <- lm(len ~ supp, data = ToothGrowth)
anova(suppEff, MainEff)</pre>
```

```
## Analysis of Variance Table
##
## Model 1: len ~ supp
## Model 2: len ~ supp + dose
## Res.Df RSS Df Sum of Sq F Pr(>F)
## 1 58 3246.9
## 2 56 820.4 2 2426.4 82.811 < 2.2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1</pre>
```

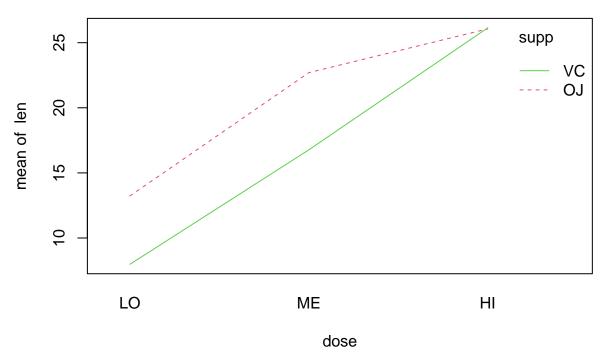
• p-value is ≈ 0 hence we reject that dose does not have an effect. Thus we prefer Model 2.

5 Interaction

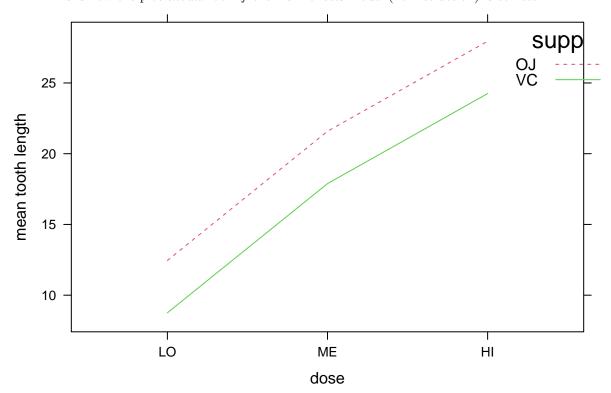
5.1 Example

- We will extend the model by introducing an interaction between supp and dose.
- Interaction plot:

```
with(ToothGrowth, interaction.plot(dose, supp, len, col = 2:3))
```



- For each of the supplement types we plot the average tooth length as a function of dosage.
- If the main effects model is correct then the difference between supplements is the same for all levels of dosage, i.e. the curves should be parallel except for noise.
- This does not seem to be the case.
- This is how the plot *should* look *if* the main effects model (no interaction) is correct:



• Parallel lines mean that effect of supplement does not depend on dose !

5.2 Dummy coding

• The extended model can be formulated as

$$\texttt{length} = \mu + \beta_1 s_C + \beta_2 d_M + \beta_3 d_H + \beta_4 s_C d_M + \beta_5 s_C d_H + \texttt{error}$$

- Interpretation:
 - μ is the expected tooth length for supp OJ and dose LO ($s_C = d_M = d_H = 0$).
 - $-\beta_1$ is the effect of changing from supp OJ to VC, dose is LO $(s_C=1,d_M=d_H=0)$.
 - $-\beta_2$ is the effect of increasing dose from LO to ME, when supp is OJ $(s_C=0,d_M=1)$.
 - $-\beta_3$ is the effect of increasing dose from LO to HI, when supp is OJ $(s_C=0,d_H=1)$.
 - $-\beta_4$ is an additional effect of both changing from supp OJ to VC and increasing dose from LO to ME $(s_C=1,d_M=1)$
 - β_5 is an additional effect of both changing from supp OJ to VC and increasing dose from LO to HI $(s_C=1,d_H=1)$
- As a two-way table:

$$\begin{array}{cccc} & LO & ME & HI \\ OJ & \mu & \mu + \beta_2 & \mu + \beta_3 \\ VC & \mu + \beta_1 & \mu + \beta_1 + \beta_2 + \beta_4 & \mu + \beta_1 + \beta_3 + \beta_5 \end{array}$$

- Further examples:
 - effect of changing from supp OJ to VC if dose is LO is $\mu + \beta_1 \mu = \beta_1$
 - effect of changing from supp OJ to VC if dose is ME is $\mu + \beta_1 + \beta_2 + \beta_4 \mu \beta_2 = \beta_1 + \beta_4$
 - effect of changing from supp OJ to VC if dose is HI is $\mu + \beta_1 + \beta_3 + \beta_5 \mu \beta_3 = \beta_1 + \beta_5$
 - if $\beta_4 = 0$ and $\beta_5 = 0$ the effect of changing from OJ to VC does not depend on dose

5.3 Example

• We fit the interaction model by changing plus to multiply in the model expression from before:

```
Interaction <- lm(len ~ supp*dose, data = ToothGrowth)</pre>
```

- Now we can think of an experiment with 6 groups corresponding to each combination of the predictors.
- Is added interaction significant ? we compare main effects model and more complex interaction model using anova:

```
anova(MainEff, Interaction)
```

```
## Analysis of Variance Table
##
## Model 1: len ~ supp + dose
## Model 2: len ~ supp * dose
## Res.Df RSS Df Sum of Sq F Pr(>F)
## 1 56 820.43
## 2 54 712.11 2 108.32 4.107 0.02186 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

• With a p-value of 2.1860269% there is a significant interaction supp:dose, i.e. the lack of parallel curves in the interaction plot is significant.

```
summary(Interaction)
```

```
##
## Call:
## lm(formula = len ~ supp * dose, data = ToothGrowth)
##
```

```
## Residuals:
##
      Min
              1Q Median
                             30
                                   Max
##
    -8.20 \quad -2.72 \quad -0.27
                           2.65
                                  8.27
##
## Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                   13.230
                                1.148 11.521 3.60e-16 ***
## suppVC
                                       -3.233 0.00209 **
                   -5.250
                                1.624
## doseME
                    9.470
                                1.624
                                        5.831 3.18e-07 ***
## doseHI
                                1.624
                                        7.900 1.43e-10 ***
                   12.830
## suppVC:doseME
                   -0.680
                                2.297
                                       -0.296 0.76831
## suppVC:doseHI
                    5.330
                                2.297
                                        2.321 0.02411 *
##
                   0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Signif. codes:
##
## 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
```

• Note the negative effect of changing from OJ to VC when dose is low is cancelled by the positive interaction parameter β_5 =suppVC:doseHI) meaning almost no difference between OJ and VC when dose is high (compare with interaction plot)

5.4 Hierarchical principle

- In presence of interaction effect it does not make sense to make tests for absence of main effects! Indeed each factor has an effect that just happens to vary depending on the other factor
- Hence start by investigating whether there is an interaction effect
- If yes: no further tests!
- If no: you may test main effects if relevant for your study