Chi-squared test and ANOVA

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Contents

At the last class, we used the t-test to determine if the mean value of a variable differed between two groups. The analysis of variance (ANOVA), which we will start discussing today, makes it possible to extend this comparison to several groups.

Before discussing ANOVA, we will present another test, the chi-squared test, which aims to detect an association between two categorical variables.

Objectives

- Use the chi-squared test to compare the frequencies of a categorical variable to a reference distribution, or to test the association between two categorical variables in a contingency table.
- Understand the principle of the analysis of variance and perform a one-way ANOVA.
- Determine the significant differences between treatments using Tukey's range test.

Compare the frequencies of a variable to a reference distribution

Example: To check if a die is balanced, we compile the result of 100 throws. The following table shows the number of times each number was obtained (its **frequency** f).

i	f_i
1	12
2	17
3	16
4	18
5	11
6	26
Total	**100**

Our null hypothesis is that the die is balanced, so there is an equal probability of getting each value ($p_i = 1/6$ for i from 1 to 6). If we multiply these probabilities by the total number of throws, we obtain the expected frequency ($\hat{f}_i$) for each number.

i	f_i	p_i	$\hat{f}_i$
1	12	1/6	16.7
2	17	1/6	16.7
3	16	1/6	16.7
4	18	1/6	16.7
5	11	1/6	16.7
6	26	1/6	16.7
Total	**100**		

Pearson's chi-squared test

For a variable with k categories, the value of the chi-squared (χ^2) statistic is calculated as:

$$\chi^2 = \sum_{i=1}^{k} \frac{(f_i - \hat{f}_i)^2}{\hat{f}_i}$$

The chi-squared statistic thus measures the sum of the deviations between the observed and expected frequencies (normalized by the expected value). When the $\hat{f}_i$ for each category are large enough (typically, 5 or more), this statistic roughly follows a χ^2_{k-1} distribution, where $k - 1 $is the number degrees of freedom.

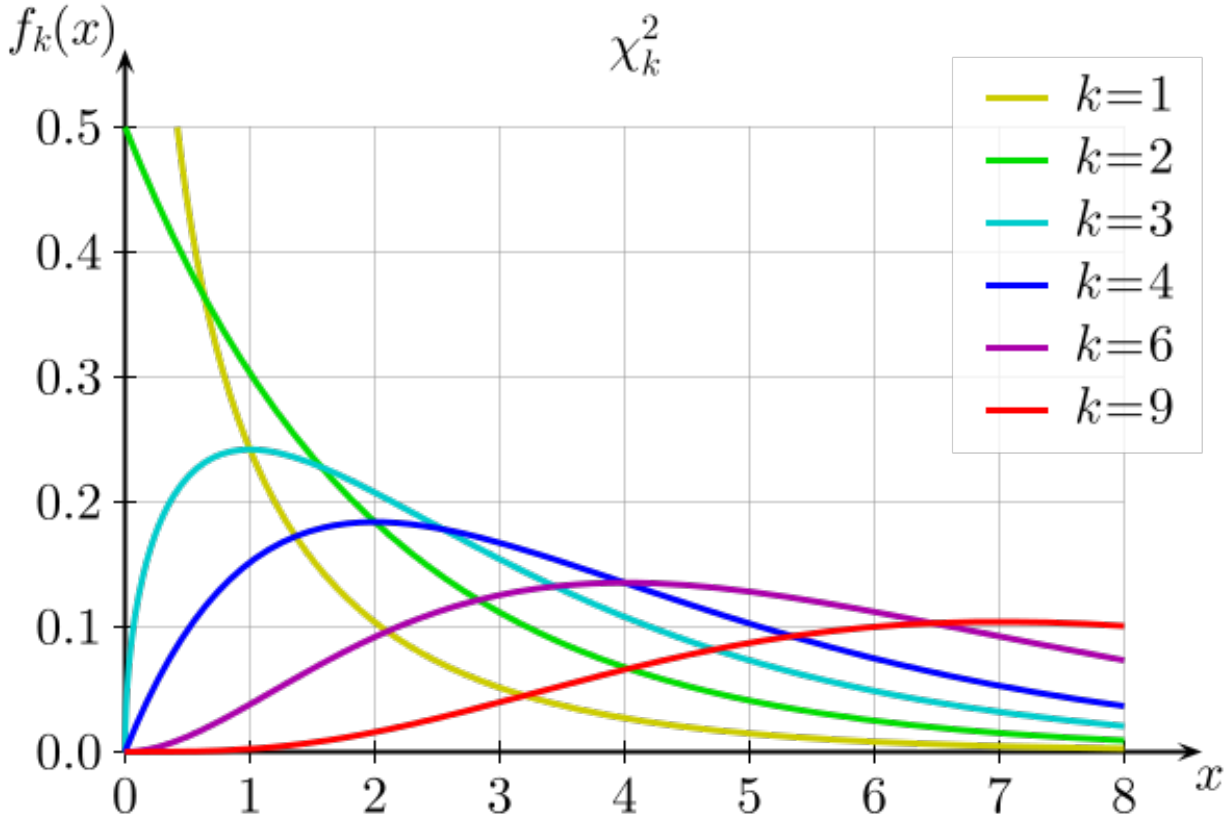
> *Reminder*: The degrees of freedom correspond to the number of independent data used in the calculation of a statistic. Here, the χ^2 is calculated from the deviations between observed and expected frequencies for k categories. However, since the sum of the deviations must equal 0 (because the total of f_i and $\hat{f}_i$ is the same) there are $k - 1$ independent deviations.

$$\sum_{i=1}^{k} f_i = \sum_{i=1}^{k} \hat{f}_i$$

, thus

$$\sum_{i=1}^{k} (f_i - \hat{f}_i) = 0$$

Here is the distribution of χ^2_k for different values of k:



In R, the `pchisq(q, df)` function gives the probability of getting a value less than or equal to q for a chi-squared distribution with df degrees of freedom. Let's calculate this probability for our example.

```
# Data
x <- c(12, 17, 16, 18, 11, 26)
n <- sum(x) # total

# Theoretical probabilities
p <- rep(1/6, 6)

# Calculate chi-squared statistic
khi2 <- sum((x - n*p)^2 / (n*p))
khi2
```

```
## [1] 8.6
```

```
pchisq(khi2, df = 5)
```

```
## [1] 0.8738776
```

Question: What is the p-value for this test? Is it a one-sided or two-sided test?

This is a one-sided test, since if the theoretical model was a bad fit, the sum of the deviations would be larger than expected. The p-value is `1 - pchisq(chi2, df = 5)`, which is about 0.126.

Rather than manually calculating the statistic, we can use the `chisq.test` function.

```
chisq.test(x, p = p)
```

```
##
##  Chi-squared test for given probabilities
##
## data:  x
## X-squared = 8.6, df = 5, p-value = 0.1261
```

Test of association between two categorical variables

Contingency table

Often, we do not have a reference distribution for a categorical variable, but we want to check if its distribution depends on the value of another categorical variable, in other words, if the two variables are *associated*.

For example, suppose the number of dead and live trees of three coniferous species (ABBA: balsam fir, PIGL: white spruce, PIMA: black spruce) were counted in a plot following a spruce budworm outbreak.

```
# rbind creates a matrix by binding vectors as rows
survie <- rbind(c(29, 11, 12), c(31, 29, 38))
rownames(survie) <- c("dead", "alive")
colnames(survie) <- c("ABBA", "PIGL", "PIMA")
survie
```

```
##       ABBA PIGL PIMA
## dead    29   11   12
## alive   31   29   38
```

This type of matrix is called a **contingency table**.

In our example, the two variables (survival and species) are associated if the mortality rate depends on the species. The null hypothesis represents the absence of association, that is, survival is *independent* of the species.

Chi-squared test for two variables

As in the previous section, we will calculate the χ^2 from the deviations between the observed (f_{ij}) and expected ($\hat{f_{ij}}$) frequencies.

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(f_{ij} - \hat{f_{ij}})^2}{\hat{f_{ij}}}$$

Here, r and c refer to the number of rows and columns in the table, respectively.

How to determine the expected frequencies $\hat{f_{ij}}$? Let's first calculate the totals in each row and column, as well as the grand total.

	ABBA	PIGL	PIMA	Total
dead	29	11	12	52
alive	31	29	38	98
Total	60	40	50	150

Let N_i be the total of the row i, N_j the total of the column j and N be the grand total. We estimate the probability of each category by the proportion of the grand total included in this category: $\hat{p_i} = N_i/N$ and $\hat{p_j} = N_j/N$.

The joint probability of two independent variables is the product of the probabilities of the variables taken separately, e.g.: (Prob. that the tree is a live fir) = (Prob. that the tree is a fir) x (Prob. that the tree is alive). Thus, the expected frequencies according to the null hypothesis are calculated as follows.

$$\hat{f_{ij}} = N\hat{p_i}\hat{p_j} = \frac{N_i N_j}{N}$$

If the null hypothesis is correct, then the χ^2 statistic follows a distribution with $(r-1) \times (c-1)$ degrees of freedom. In our example, $df = 2$. Indeed, since the expected frequencies are based on the totals of each row and each column, the sum of the deviations in each row and each column must be zero.

If we choose a threshold $\alpha = 0.05$ and then apply the `chisq.test` function to that matrix, we get a p-value of 0.01, meaning there is a significant association between the two variables.

```
chisq.test(survie)
```

```
##
##  Pearson's Chi-squared test
##
## data:  survie
## X-squared = 8.3669, df = 2, p-value = 0.01525
```

To specify the association, we can assign the result of the test to a variable `khi2` and inspect the expected frequencies (`khi2$expected`) and the residuals (`khi2$residuals`).

```
khi2 <- chisq.test(survie)
khi2$expected
```

```
##       ABBA     PIGL     PIMA
## dead  20.8 13.86667 17.33333
## alive 39.2 26.13333 32.66667
```

```
khi2$residuals
```

```
##            ABBA       PIGL       PIMA
## dead   1.797969 -0.7698235 -1.2810252
## alive -1.309697  0.5607636  0.9331389
```

The residuals are the standardized deviations:

$$\frac{f_{ij} - \hat{f_{ij}}}{\sqrt{\hat{f_{ij}}}}$$

The sum of the squared values of these deviations are equal to χ^2.

How can we interpret this matrix of residuals? Since there is an excess of dead fir (positive residual), the fir mortality rate is higher than predicted by the null hypothesis, whereas it is lower than expected for both spruces.

However, the rejection of the null hypothesis (independence between mortality and species) in the table does not tell us between which species the mortality rate varies significantly. Later this semester, we will see how a logistic regression estimates the probability of a binary result (e.g., survival) based on a categorical or continuous predictor.

Notes on using the chi-squared test

- The chi-squared test must always be done on the frequencies (number of observations), not on the proportions. Without knowing the size of the sample, the proportions themselves do not tell us if a

deviation is significant. For example, if two categories should be in equal proportions (50% / 50%), frequencies of 60 and 40 may represent a significant deviation, but not frequencies of 6 and 4.

- Since the chi-squared test approximates discrete data by a continuous distribution, it becomes less accurate as the sample size decreases. The test is therefore not recommended if one of the expected frequencies ($\hat{f}_{ij}$) is less than 5. In this case, we can use Fisher's exact test (`fisher.test` function in R), which calculates the exact probabilities of different contingency tables, assuming that the row and column totals are fixed.

```
tab <- matrix(c(4, 6, 8, 2), nrow = 2)
tab
```

```
##      [,1] [,2]
## [1,]    4    8
## [2,]    6    2
```

```
chisq.test(tab)
```

```
## Warning in chisq.test(tab): L'approximation du Chi-2 est peut-être incorrecte
```

```
##
##  Pearson's Chi-squared test with Yates' continuity correction
##
## data:  tab
## X-squared = 1.875, df = 1, p-value = 0.1709
```

```
fisher.test(tab)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  tab
## p-value = 0.1698
## alternative hypothesis: true odds ratio is not equal to 1
## 95 percent confidence interval:
##  0.01252647 1.65925396
## sample estimates:
## odds ratio
##  0.1841181
```

Analysis of variance (ANOVA)

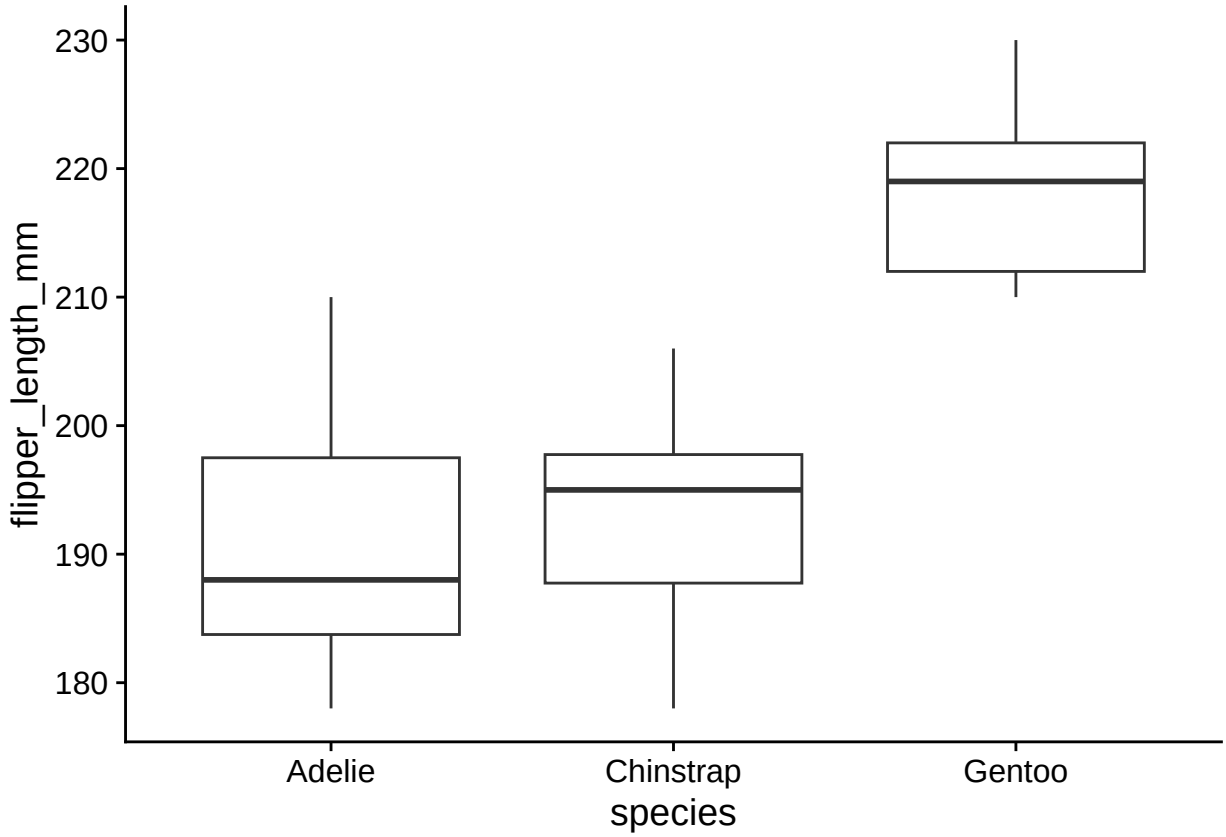
Suppose we want to compare the mean of a variable between several (>2) groups. We could compare the groups in pairs with t-tests (for example, A-B, B-C, and A-C for three groups), but as we saw in the previous class, performing multiple tests increases the probability of making a type I error.

For a sample divided into several groups, the analysis of variance (ANOVA) compares the variation between observations within each group to the variation between the groups. It thus makes it possible to globally test the null hypothesis according to which observations in each group come from populations with the same mean.

For example, let's take the `penguins` dataset from the *palmerpenguins* package, which contains measurements taken on different penguin species in the Palmer Archipelago of Antarctica. We select a random sample of 12 individuals per species with the `group_by` and `sample_n` functions of *dplyr*. The graph below shows the variation in flipper length by species for that sample.

```
library(palmerpenguins)
penguins_ech <- penguins %>%
```

```
    filter(!is.na(flipper_length_mm)) %>%
    group_by(species) %>%
    sample_n(12)
ggplot(penguins_ech, aes(x = species, y = flipper_length_mm)) +
    geom_boxplot()
```



Here, we have a numeric variable that varies based on a categorical variable (or factor) with three categories. A *one-way* ANOVA compares groups along one categorical variable.

One-Way ANOVA Model

Suppose we measure the variable y for l groups each including n observations. The difference between an observation k of the group i (y_{ik}) and the (theoretical) mean of that group (μ_i) is the residual ϵ_{ik}.

$$y_{ik} = \mu_i + \epsilon_{ik}$$

In the ANOVA model, we assume that residuals follow a normal distribution with mean 0 and a fixed standard deviation.

$$\epsilon_{ik} \sim N(0, \sigma)$$

The null hypothesis is that μ_i is the same for all groups. We can also represent the same model based on the grand mean, μ, and the deviation α_i between the mean of group i and μ.

$$y_{ik} = \mu + \alpha_i + \epsilon_{ik}$$

Sum of squared deviations

If $\bar{y}$ is the grand mean of the observed y and $\bar{y_i}$ is the mean of observations in group i, then we can prove the following relationship between sums of squared deviations.

$$\sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y})^2 = \sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y_i})^2 + \sum_{i=1}^{l}\sum_{k=i}^{n}(\bar{y_i}-\bar{y})^2$$

Since the last term doesn't depend on k, we can re-write the equation as:

$$\sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y})^2 = \sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y_i})^2 + \sum_{i=1}^{l}n(\bar{y_i}-\bar{y})^2$$

The term on the left is the total sum of squares (SST), the first term on the right is error (residual) sum of squares (SSE) and the second term on the right is the sum of squares between groups of factor A (SSA, the only factor in this case).

We thus obtain the equation $SST = SSE + SSA$, which decomposes the total sum of squares into two components: one due to the differences observed within each group (SSE) and the other due to the differences observed between groups (SSA).

Analysis of variance table

From the sums of squared deviations seen above, the mean squared deviations can be calculated by dividing each sum by the appropriate number of degrees of freedom.

- For the deviations from the grand mean, there are $nl-1$ degrees of freedom; since the sum of the deviations is zero, the last one is not independent from the others.
- For the residuals, there are $(n-1)l$ degrees of freedom, since there are $n-1$ independent deviations per group.
- For the differences between the group means and the grand mean, there are $l-1$ degrees of freedom.

Notice that the sum of the degrees of freedom is the same on both sides of the equation: $nl-1 = (n-1)l+(l-1)$.

The sum of squared deviations, degrees of freedom and mean squared deviations can be presented in an ANOVA table.

Component	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)
Factor A	$SSA = \sum_{i=1}^{l} n(\bar{y_i}-\bar{y})^2$	$l-1$	$MSA = \frac{SSA}{l-1}$
Residual	$SSE = \sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y_i})^2$	$(n-1)l$	$MSE = \frac{SSE}{(n-1)l}$
Total	$SST = \sum_{i=1}^{l}\sum_{k=i}^{n}(y_{ik}-\bar{y})^2$	$nl-1$	

Null hypothesis test

Recall the model for a one-way ANOVA:

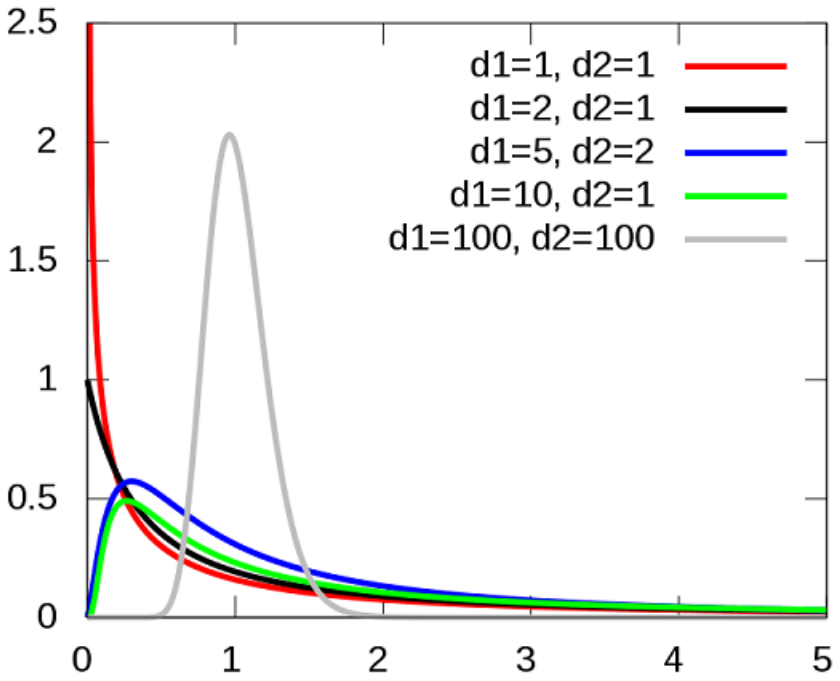
$$y_{ik} = \mu + \alpha_i + \epsilon_{ik}$$

$$\epsilon_{ik} \sim N(0,\sigma)$$

The mean of squared residuals (MSE) is an estimator of the variance in that model (σ^2).

If the null hypothesis is correct and there are no systematic differences between groups (that is, all α_i are equal to 0 in the model), then the mean of squared group differences (MSA) is also an estimator of σ^2. Indeed, according to the null hypothesis, the different groups are independent samples of the same population. In this case, the term MSA corresponds to the variance of $\bar{y}_i$ multiplied by n (the number of observations per group). The variance of a mean of n observations is precisely equal to σ^2/n, where σ^2 is the variance of the individual observations.

If MSA and MSE are two estimators of the same variance under the null hypothesis, then their ratio $F = MSA/MSE$ follows the F distribution. This distribution has two parameters (d_1 and d_2) corresponding to the degrees of freedom of MSA and MSE.



Conversely, if the null hypothesis is false, the value of MSA is expected to be higher than MSE, since systematic differences between groups will be added to the random variations in sampling. It is therefore a one-sided test: the p-value is the probability of a F ratio equal to or greater than the one observed in the data.

Assumptions of the ANOVA model

For the model on which the ANOVA is based to be valid, the residuals must be (1) independent between observations and follow (2) a normal distribution with (3) the same variance σ^2 in each group.

- The independence of the residuals requires, among other things, that the unmeasured factors that influence the response are distributed in a similar way for each group. For an experimental design, the random assignment of treatments helps to ensure this independence.
- Like the t-test, the ANOVA is robust to weak to moderate deviations from the normal distribution.
- Unlike the t-test, the equality of the group variances (**homoscedasticity**) is essential for the ANOVA. If this assumption is not met, we must transform the data or resort to a more complex model, as we will see later.

The calculations presented above apply only to a **balanced** sample, that is, where the number of observations

is the same in each group. For today's class, we will limit ourselves to balanced samples. In order to perform an unbalanced ANOVA in R, we must use linear regression methods that we will see in future courses.

Examples of one-way ANOVA

With the `aov` function in R, we perform an ANOVA of the flipper length by penguin species, from the sample of the `penguins` data frame chosen at the beginning of this section.

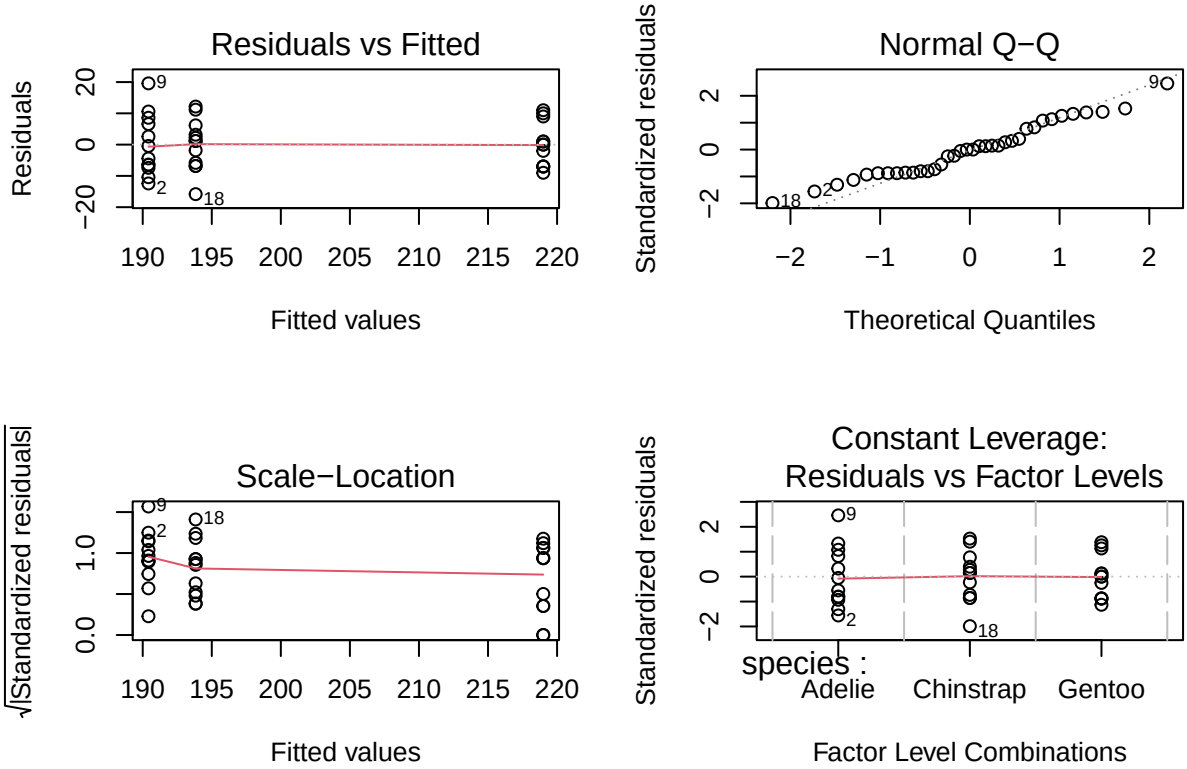
```
anova1 <- aov(flipper_length_mm ~ species, data = penguins_ech)
summary(anova1)
```

```
##              Df Sum Sq Mean Sq F value   Pr(>F)
## species       2   5848  2924.1   42.24 7.97e-10 ***
## Residuals    33   2285    69.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The `aov` function requires a description of the model in the `response ~ predictor` format, as well as the name of the data frame containing these variables (`data` argument). By applying the `summary` function to the result of an ANOVA, we obtain the ANOVA table as presented above, along with the value of the F statistic and its p-value (`Pr(>F)`). With a threshold of 0.05, the null hypothesis that species have the same mean flipper length would be rejected.

To verify that our data conforms to the assumptions of the model, we must consult the diagnostic plots, obtained by applying the `plot` function to the result.

```
plot(anova1)
```



The first two graphs are the most important here. The `Residuals vs. Fitted` plot shows the value of the residuals according to the estimated mean for each group; if the model is valid, there should be no visible trend and the residuals of the different groups should have a similar variance around zero, which is the case here. The second graph is a quantile-quantile plot to check if the residuals approximately follow a normal distribution.

To display the group means, we must call the `coef` function (for coefficients).

```
coef(anova1)
```

```
##      (Intercept) speciesChinstrap    speciesGentoo
##       190.416667         3.416667        28.583333
```

By default, the `(Intercept)` is the mean of the first group (here, the species *Adelie*), while the other coefficients indicate the difference between the other groups and that first mean. The mean for species *Chinstrap* is 190.4 + 3.4 = 193.8, while the mean for species *Gentoo* is 190.4 + 28.6 = 219.0. The result does not tell us which of these differences are significant.

If there are only two groups, the ANOVA is equivalent to a t-test between two samples with equal variance.

```
library(dplyr)
penguins_2sp <- filter(penguins_ech, species != "Gentoo")
anova2 <- aov(flipper_length_mm ~ species, data = penguins_2sp)
summary(anova2)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## species      1     70   70.04   0.881  0.358
## Residuals   22   1749   79.48
```

```
coef(anova2)
```

```
##      (Intercept) speciesChinstrap
##       190.416667         3.416667
```

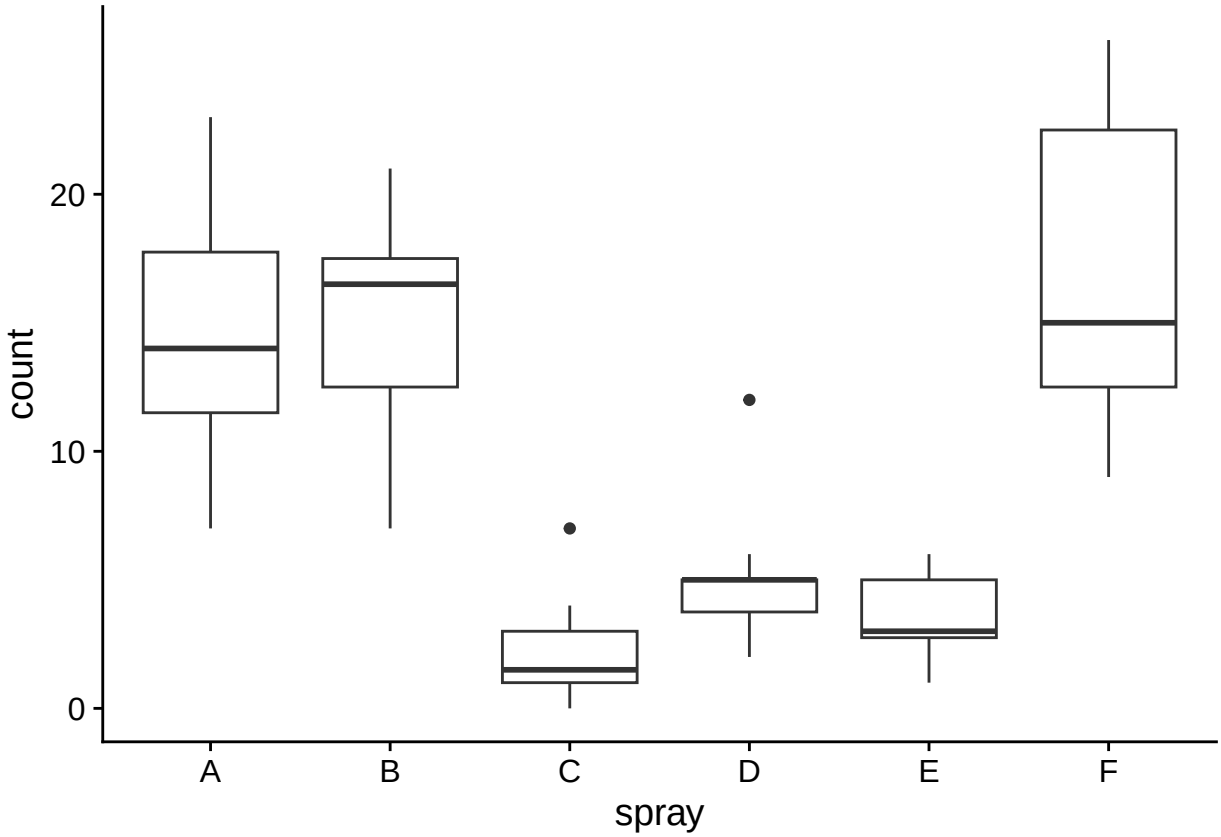
```
t.test(flipper_length_mm ~ species, data = penguins_2sp, var.equal = TRUE)
```

```
##
##  Two Sample t-test
##
## data:  flipper_length_mm by species
## t = -0.93874, df = 22, p-value = 0.3581
## alternative hypothesis: true difference in means between group Adelie and group Chinstrap is not equa
## 95 percent confidence interval:
##  -10.964779   4.131446
## sample estimates:
##    mean in group Adelie mean in group Chinstrap
##                190.4167                193.8333
```

The p-value and the estimated mean by species are the same for both tests.

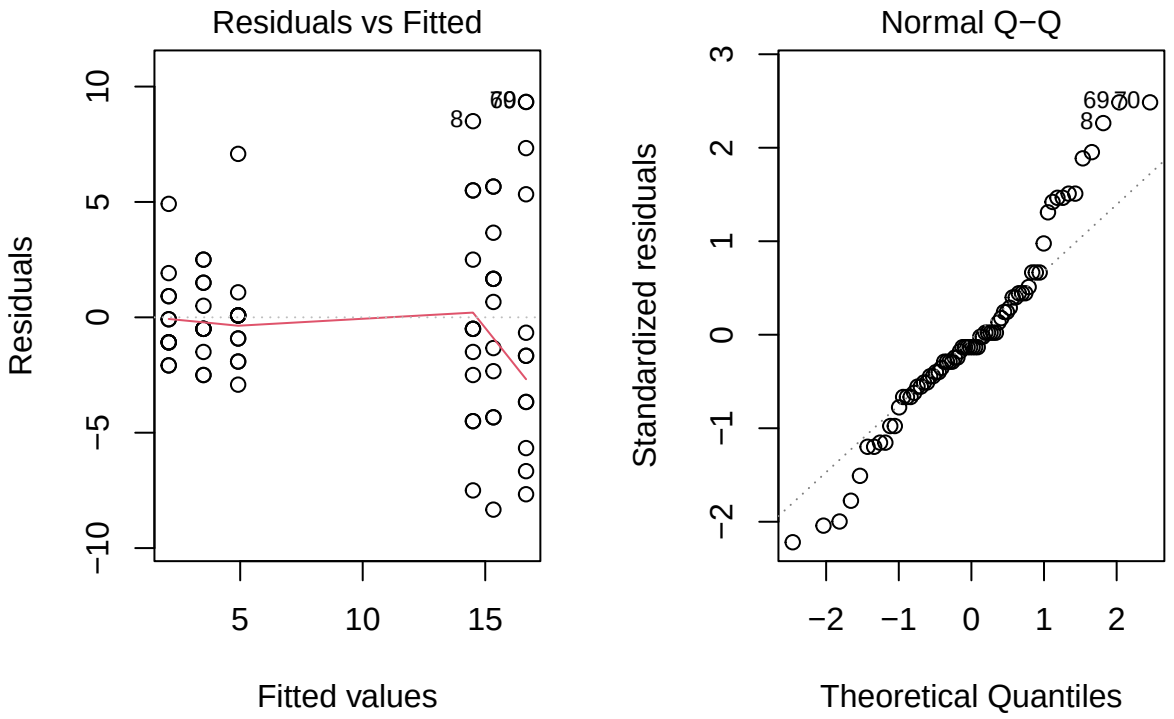
Now, let's consider the `InsectSprays` data frame that describes the number of insects (`count`) on plots treated with different insecticides (`spray`).

```
ggplot(InsectSprays, aes(x = spray, y = count)) +
    geom_boxplot()
```



Before looking at the ANOVA results, we inspect the first two diagnostic plots.

```
anova3 <- aov(count ~ spray, InsectSprays)
plot(anova3, which = 1:2)
```

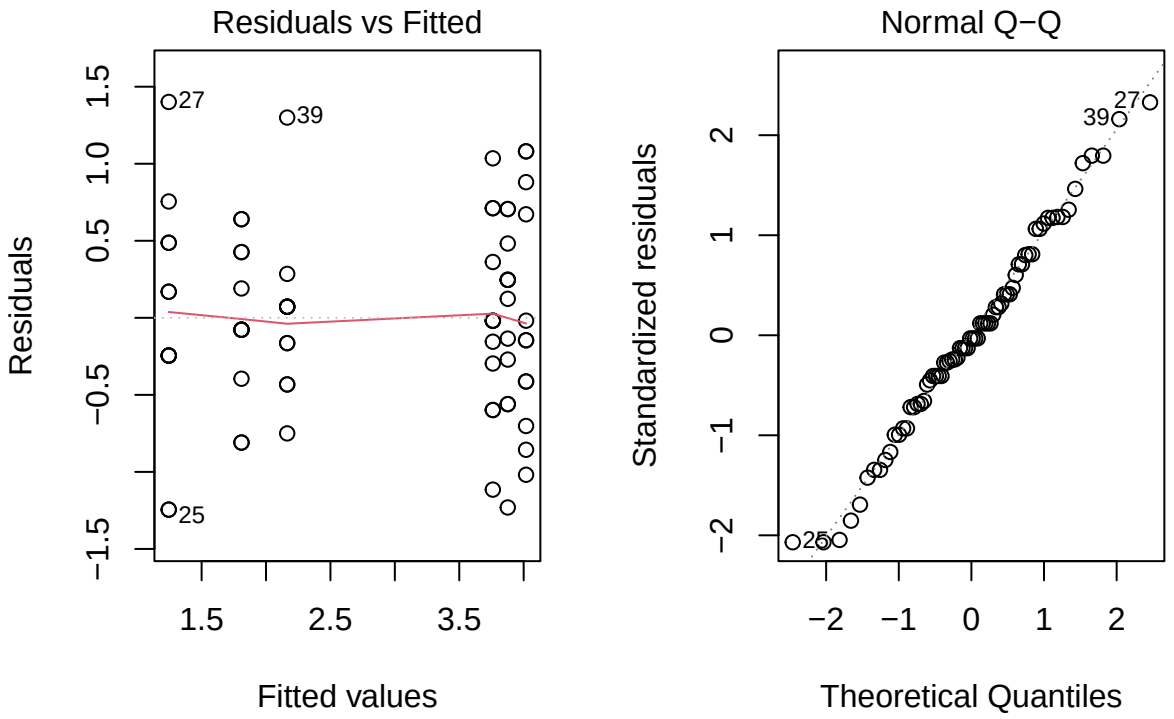


The graph of residuals vs. fitted values shows that the variance of the residuals is higher for the groups with higher means, which contradicts the assumption of equal variance. When this happens, the distribution of residuals also departs from normal, as can be seen in the quantile-quantile plot.

Square root transformation

An increase of the variance for groups with a higher mean occurs frequently when dealing with count data. For this type of data, taking the square root (`sqrt`) of the original variable can make the variances more homogeneous. Note that a logarithmic transformation is not possible when the data includes 0s.

```
anova3_racine <- aov(sqrt(count) ~ spray, InsectSprays)
plot(anova3_racine, which = 1:2)
```




```
summary(anova3_racine)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## spray        5  88.44  17.688    44.8 <2e-16 ***
## Residuals   66  26.06   0.395
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Instead of transforming the response variable (the number of insects), another option would be to choose a model that is not based on a normal distribution of the residuals. This is the approach taken by generalized linear models, which we will study later in the semester.

Multiple comparisons between groups

As we saw above, the rejection of the null hypothesis in ANOVA indicates that the observed differences would be unlikely if all groups had the same mean, but does not tell us which groups have significantly different means.

Tukey's range test is designed for this scenario. The test compares the groups in pairs with a statistic based on the t distribution, but which takes into account the multiple comparisons to ensure that the overall probability of Type I error remains below the desired threshold (usually 5%).

This test is implemented in R by the `TukeyHSD` function (for Honest Significant Difference). For example, we apply the Tukey test to the result of our first ANOVA, on the variation of the flipper length between penguin species.

```
anova1 <- aov(flipper_length_mm ~ species, data = penguins_ech)
tukey1 <- TukeyHSD(anova1)
```

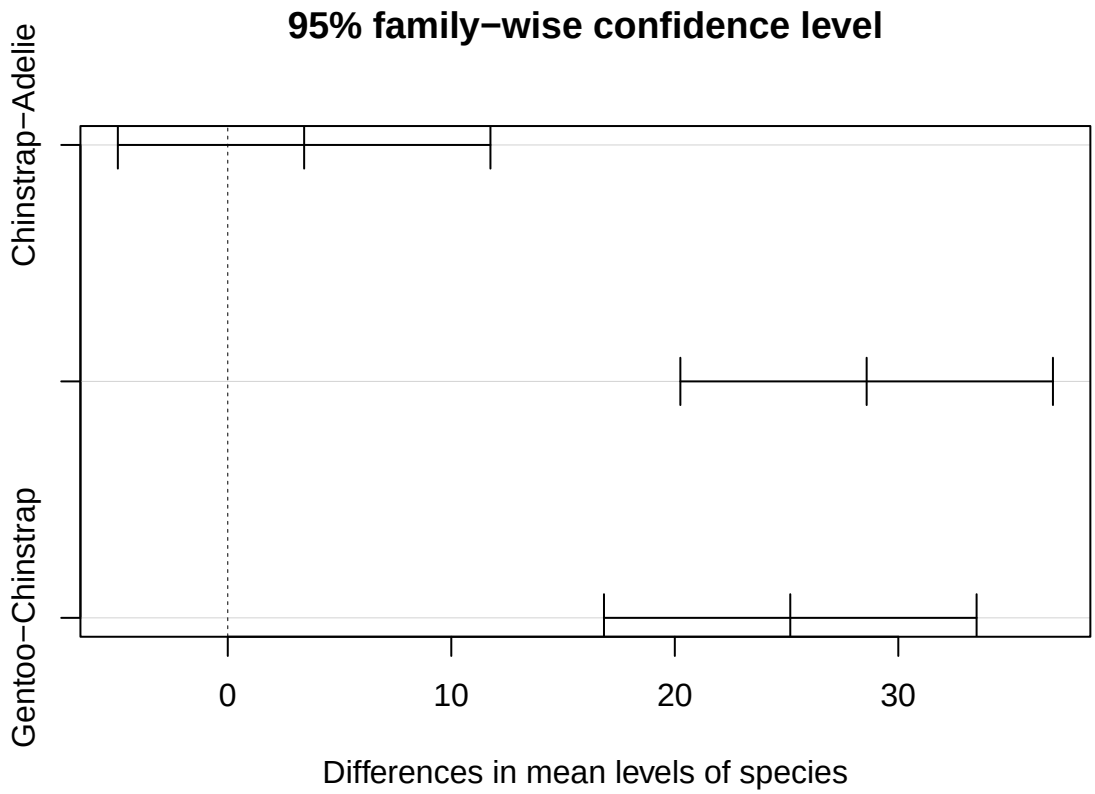
```
tukey1
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = flipper_length_mm ~ species, data = penguins_ech)
##
## $species
##                         diff      lwr      upr     p adj
## Chinstrap-Adelie  3.416667 -4.91840 11.75173 0.5784484
## Gentoo-Adelie    28.583333 20.24827 36.91840 0.0000000
## Gentoo-Chinstrap 25.166667 16.83160 33.50173 0.0000000
```

There seems to be a significant difference between *Gentoo* and the two other species.

The `plot` function produces a graph of the confidence intervals for Tukey's range test.

```
plot(tukey1)
```



Since this method targets a 5% probability of Type I error across all comparisons, each of the individual comparisons must have a $\alpha < 0.05$. In this case, the probability of Type II error (not detecting a significant difference) increases. We can therefore control the type I error rate in a multiple comparison problem, but at the cost of decreasing the power of the test when the number of comparisons to be made increases. Since ANOVA has greater power than multiple comparisons, it is even possible that none of the differences between two groups are significant, even if the ANOVA null hypothesis was rejected.

Summary

Chi-squared test

- A contingency table is a cross-tabulation of the number of observations (frequencies) according to two categorical variables.
- The chi-squared test is used to compare the frequencies of a categorical variable to a reference distribution, or to check the independence of two categorical variables in a contingency table.
- When the expected frequencies are very low (<5), the approximation the of chi-squared test must be replaced by a test that computes the exact probabilities of the contingency tables, like Fisher's exact test.

ANOVA

- The analysis of variance aims to determine if samples from different groups (e.g. treatments) are distributed with the same mean.
- It assumes that the residuals in each group are independent and follow a normal distribution with the same variance.
- If the ANOVA null hypothesis is rejected, Tukey's range test can be used to identify significant differences between groups.

Comparison with other models

More generally, we could say that the t-test and ANOVA deal with the effect of categorical predictors (e.g. different treatments) on a numerical response. In contrast, the chi-squared test aims to detect an association between two categorical variables; depending on the interpretation, one or the other can be considered the response.

Later this semester, we will focus on regression models. These are broader in scope because they link a response variable to categorical and numerical predictors. In particular, we will see that the t-test and ANOVA are examples of linear regression models.

	Categorical response	Numerical response
Categorical predictor	Chi-squared test	t-test (2 categories) or ANOVA (more than 2 categories)
Categorical or numerical predictor	Logistic regression	Linear regression

References

The content of this lecture is based on the course ECL7102 - Analyses et modélisation des données écologiques offered at UQAT during the fall 2021 session created by Philippe Marchand (formerly professor UQAT). The course content is available online at https://github.com/pmarchand1/ECL7102