R workshop #2: multivariate regression analysis and factor interactions

Nicola Romanò

Introduction

Last year we have talked about linear models and their use to perform regression and analysis of variance (ANOVA). We only considered simple situations with one independent variable influencing the output variable (one-way ANOVA) or two factors (two-way ANOVA) that do not interact with each other. In the lectures we have now talked about interactions and how they change our interpretation of linear models. In this workshop we will have a look at how to deal with interactions in R.

Learning objectives

After completing this workshop you will be able to:

- Use linear models to perform multiple regression and analysis of variance with multiple factors
- Correctly interpret the output of a linear model
- Compare two models to choose the one that fits the data better
- Correctly interpret the results of your analysis in the presence of interactions

Section 1 - A refresher on linear models

We start this workshop with a little refresher of linear models. **This also includes some more details about linear models that we only briefly touched upon last year**, so please go through this carefully.
A linear model is a statistical model that relates the changes in a dependent variable (Y) with the changes in one or more independent variables ($X_1, X_2, ..., X_n$).

The general equation for such model is:
$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_n X_n + \epsilon$
Where:

- Y is our measured/outcome variables
- $X_1, ..., X_n$ are the factors (or predictors) that influence Y. They are generally the other variables in your dataset, or transformations/combination of them [1].

[1] For instance, we may have collected the weight of the subjects in our study, but use log(weight) as a predictor for our model. Or we may have collected two different values and use their ratio as a model parameter

- $\beta_1, ...\beta_n$ are the regression coefficients, scaling factors for the predictors.
- ϵ is the error, or residual. It represents the difference between what is explained by the model prediction, and what we have observed. It includes the effect of all the factors that we did not measure in our experimental setup, as well as measurement errors. We generally assume that it is normally distributed[2].

When we use R (or any other software!) to generate the model, what it does is estimating the coefficients β in such a way to minimise the error[3].

In this formula each predictor acts independently from the others. In other words, if we have two predictors, X_1 and X_2, the effect of X_1 on Y will always be the same, independently of the value of X_2. As we have seen in the lecture this is not always the case.

Simple regression

As a first example let's consider the dataset *pressure-workshop2.csv*. In this study the effect of a drug on reducing blood pressure (measured in mmHg) has been investigated on 150 patients of different age, weight (measured in kg), and sex.

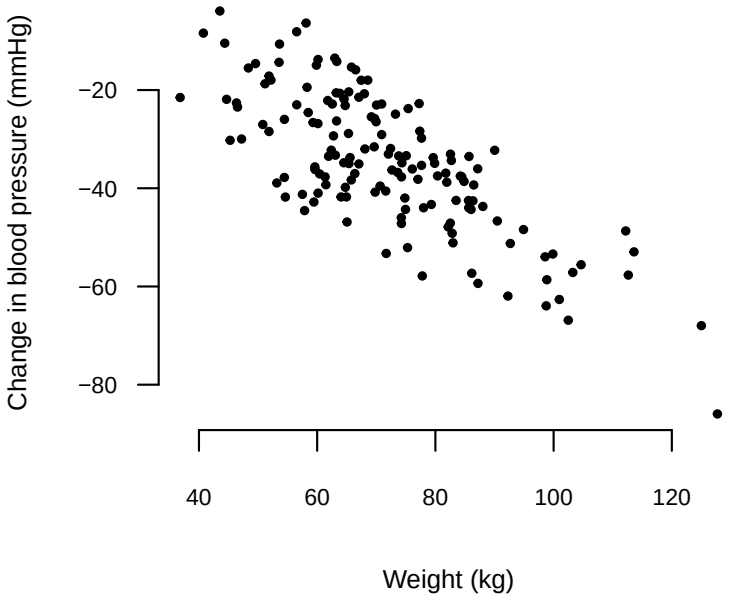
Start by familiarising with the data. How many men and women are there? What range of age and weight? Plot the various variables against each other and see if any particular patterns emerge [4].

Let's forget for a moment about the other variables and concentrate on the relation between Weight and Response; it looks like the largest effect is seen in heavier patients.

[2] Note that although having normally distributed residuals makes things easier... you can still have a good, usable model when residuals are not normally distributed, especially if deviation from normality are small. Mostly, it boils down to critical observation of your data and experience. Also, talking with a statistician is always a good idea!

[3] In the case of `lm`, this is called a least-square estimation. In statistics books and publications you may see the estimated parameters indicated as $\hat{\beta}$ (read as "beta hat"). This is to indicate that this is the result of an estimation, that is an approximation of the true value of β for the population, which remains unknown. We can obtain confidence intervals for these estimates by using `confint(model)`

[4] If you do not remember how to do that, see Workshop 1.



We can use a linear model to test whether such a relation exists.
As always, we start by stating our null hypothesis _____

_____

Do you remember how to perform a linear regression in R? Try it, if you don't remember see the following page!

```
model <- lm(Response ~ Weight, data = pressure)
```

This generates the model

$Response = \beta_0 + \beta_1 * Weight + \epsilon$

What are the assumption of this model? Do you remember how to verify that they are satisfied? [5]

This is one of the simplest linear models we can generate, where the value of the outcome depends on a single parameter. This is called *simple regression*.

Let's look at the output of the model

```
summary(model)
```

```
##
## Call:
## lm(formula = Response ~ Weight, data = pressure)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -19.190  -6.354   0.874   6.568  19.554
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 11.78143    3.32703   3.541 0.000533 ***
## Weight      -0.64852    0.04521 -14.346  < 2e-16 ***
## ---
## Signif. codes:
## 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 9.133 on 148 degrees of freedom
## Multiple R-squared:  0.5817, Adjusted R-squared:  0.5789
## F-statistic: 205.8 on 1 and 148 DF,  p-value: < 2.2e-16
```

The summary gives us a lot of information.

First of all, it tells us the parameters β (coefficients) that have been estimated by the model.

$\hat{\beta}_0 = 11.78$ and $\hat{\beta}_1 = -0.65$

Therefore

$\text{Response} = 11.78 - 0.65 * \text{Weight} + \epsilon$

This means that for any increase of 1 Kg in weight there is a decrease of 0.65 mmHg in blood pressure following the intake of the drug. The effect of weight on the response to the drug is statistically significant ($F_{1,148} = 205.8, p = 2 * 10^{-16}$)[6].

[5] Let's discuss this in the forum! I would say that for this case the assumptions are generally satisfied, what do you think?

[6] R also reports a p-value for the intercept; this is the result of a one sample t-test comparing the intercept to 0. In other words, in this case the intercept is statistically different from 0. The intercept is the value corresponding to a change in blood pressure where all of the factors (in this case weight) are equal to zero. Since a weight of 0 is not biologically meaningful we can ignore this value in this instance

Another important value is the coefficient of determination (R^2). This is a measure of how good the model is, or how much of the variation in the data it explains. R^2 is not a great way to compare two different models, since it depends on the number of parameters; that is, if we add an extra descriptor to our model, R^2 will always increase. For this reason, R reports also an "adjusted" version of it. In this case $adj.R^2 = 0.5789$; this means that our model describes/explains ~57.9% of the variability in our data, which is OK but not great. It means that there are other factors that we have not considered accounting for >40% of the variability! [7] So, what are these other factors?

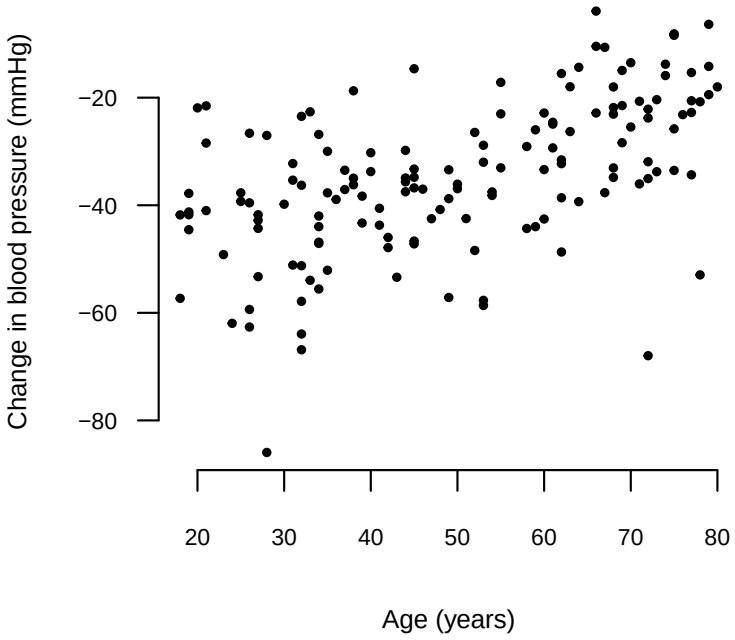
[7] What do you think is the maximum value of R^2? Why?

Multiple regression

Our dataset contains two other descriptors: Age and Sex. It is very biologically plausible that these would affect blood pressure, so we should add them to our model[8]. To keep things simple, we will start with Age, and consider gender later.

[8] Note that, although for the sake of simplicity we are adding these descriptors one at a time, in practice we would probably start from a complete model, including all of the descriptors that we measured. That is why we measured them, isn't it?

It is useful, at this point, to also plot the change in blood pressure against age.



We see a possible relation in the response to the drug depending on age. Let's incorporate age in our model.

```
model.2 <- lm(Response ~ Weight + Age, data = pressure)
```

This will generate a model that considers the effect of weight and

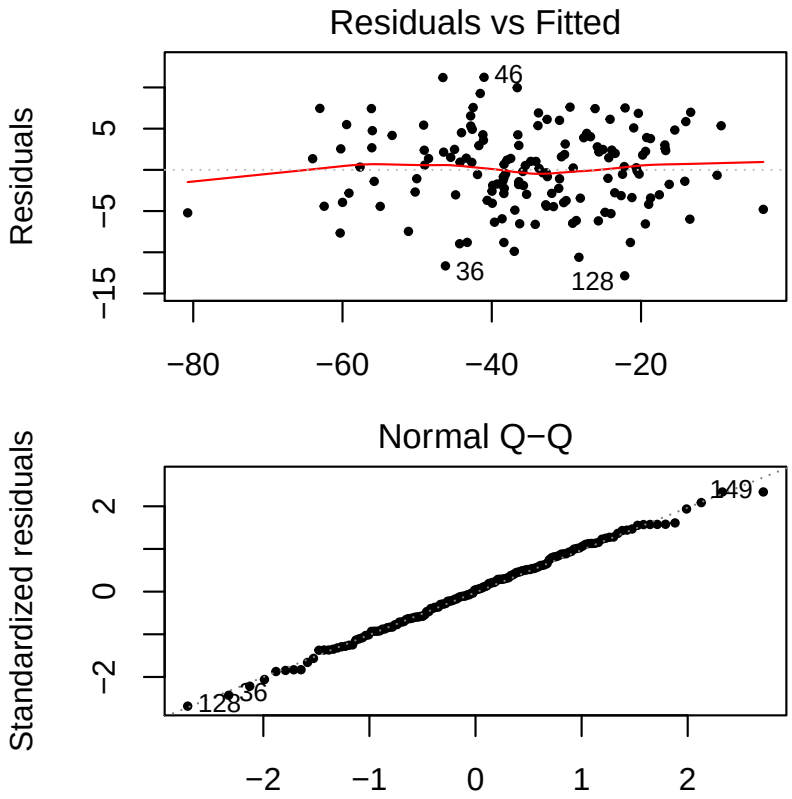
the effect of age, independently of each other [9]

What are the null hypotheses[10] that this model is testing?

Again, we want to check the assumptions of the model by using diagnostic plots.

[9] This means that the model will look at the effect of the weight of the individual on his/her response to the drug, independently of his/her age, and vice versa for age.

[10] There is more than one!!!



Since the diagnostic plots look good (equal variance throughout and normally distributed residuals), we can continue with this model.

```
summary(model.2)
```

```
##
## Call:
## lm(formula = Response ~ Weight + Age, data = pressure)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -12.8545  -3.3021   0.1972   3.1070  11.2320
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -8.60214    2.04592  -4.205 4.53e-05 ***
## Weight      -0.65779    0.02392 -27.501  < 2e-16 ***
## Age          0.42390    0.02169  19.541  < 2e-16 ***
## ---
## Signif. codes:
```

```
## 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.832 on 147 degrees of freedom
## Multiple R-squared:  0.8837, Adjusted R-squared:  0.8821
## F-statistic: 558.6 on 2 and 147 DF,  p-value: < 2.2e-16
```

You can interpret the result of this model just like you did for the previous one. It tells us that there is a statistically significant effect of weight ($p < 2 * 10^{-16}$) and of age ($p < 2 * 10^{-16}$) on the response to the drug ($F_{2,147} = 558.6$ [11]) Note that now the model explains 88% of the variability!

[11] Note that the F statistic reported by `summary` refers to the whole model. If you wanted to know the F statistic for specific components of the model you could run `anova(model.2)`, which would give you F and DF for the various descriptors of your model.)

Qualitative predictors and dummy variables

Let's now consider gender and add it to our model. Plot the data, do you think gender affects the response to the drug?

In this case, we are dealing with a discrete qualitative variable, with two levels, F and M. All that we said so far still applies, and `lm` is able to deal with this type of variables with no issue. However, the way we deal with these type of variables is slightly different.

```
model.3 <- lm(Response ~ Weight + Age + Sex, data = pressure)
```

This is modelling the following:

$\text{Response} = \beta_0 + \beta_1 * \text{Weight} + \beta_2 * \text{Height} + \beta_3 * D$

We introduce a new variable D, called a "dummy variable", that codes Sex in this way:

$$D = \begin{cases} 1, & \text{if Sex} = M \\ 0, & \text{otherwise} \end{cases}$$

By default R assigns 0 to the first level of the variable (the *reference level*, in this case F), and 1 to the second[12]

[12] Levels are ordered alphabetically; see Workshop 1 for how to change level ordering.

Therefore, for observations at the reference level (so from female subjects), the third term $\beta_3 * D$ will be 0; for male subjects that will be $\beta_3 * 1 = \beta_3$. Thus, β_3 represents the **difference between the response of a male and a female**, keeping all of the other factors constant.

Let's have a look at the summary of the model to better clarify this.

```
summary(model.3)
```

```
##
## Call:
## lm(formula = Response ~ Weight + Age + Sex, data = pressure)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -12.6250  -3.2145   0.1914   3.2024  11.0588
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -8.36394    2.11449  -3.956 0.000119 ***
## Weight      -0.66298    0.02645 -25.062  < 2e-16 ***
## Age          0.42224    0.02204  19.154  < 2e-16 ***
## SexM         0.41103    0.88467   0.465 0.642898
## ---
## Signif. codes:
## 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.845 on 146 degrees of freedom
## Multiple R-squared:  0.8839, Adjusted R-squared:  0.8815
## F-statistic: 370.5 on 3 and 146 DF,  p-value: < 2.2e-16
```

The output is not much different from what we had before. Does Sex have a statistically significant effect on the response? What percent of variance is explained by this model?[13]

Consider the estimates

$\beta_1 = -0.66;\ \beta_2 = 0.42;\ \beta_3 = 0.41$

These mean that:

- For every increase in 1 kg of weight, the response decreases of 0.66 mmHg (keeping age and sex the same)
- For every increase in 1 year of age, the response increases of 0.42 mmHg (keeping weight and sex the same)
- If the patient is male the response increases of 0.41 mmHg

So, what would you predict the response of a 50 year old male weighing 82 kg will be? Write your response on the forum[14].

[13] Note how, although minimally, R^2 has increased, since we have added an extra parameter, however adj. R^2 has decreased!

[14] Remember to include the intercept in your calculations as well!

Dummy variables for multiple levels

You may have realised at this point, that dummy variables are what R uses to code for groups or other discrete factors when doing an ANOVA!

In some cases, however, you will have more than two levels; the reasoning is the same, however multiple dummy variables will be used to define the different levels.

For example, suppose you measured the levels of LH in three different species of fish: mackerel, salmon, and trout.

You can code the species variable with two dummy variables (so, number of levels - 1) D_1 and D_2 such as:

$$D_1 = \begin{cases} 1, & \text{if Species} = \text{"Salmon"} \\ 0, & \text{otherwise} \end{cases}$$

$$D_2 = \begin{cases} 1, & \text{if Species} = \text{"Tuna"} \\ 0, & \text{otherwise} \end{cases}$$

Therefore:

Species	D_1	D_2
Mackerel	0	0
Salmon	1	0
Tuna	0	1

Our model may be something like:
$\text{LH} = \beta_0 + \beta_1 * D_1 + \beta_2 * D_2 + \epsilon$
where $\beta 1$ represents the difference between LH levels in salmons and mackerels, and $\beta 2$ the difference between LH levels in tuna and mackerel.

Section 2 - Choosing a model

Going back to our initial example, we have fitted three models:

1. $\text{Response} = \beta_0 + \beta_1 * \text{Weight} + \epsilon$
2. $\text{Response} = \beta_0 + \beta_1 * \text{Weight} + \beta_2 * \text{Age} + \epsilon$
3. $\text{Response} = \beta_0 + \beta_1 * \text{Weight} + \beta_2 * \text{Age} + \beta_3 * D_{male} + \epsilon$

We could argue that #2 is better than #1, as it explains a much larger percentage of the variance (88% vs 58%), but what about #3?

Is it correct to say that since Sex does not have a statistically significant effect on the response, and since the value of adjusted R^2 is lower (albeit by a very small amount) we should drop Sex from the model, and only consider Age and Weight as predictors? One way of deciding this is to use the `anova` function to compare the two models. This tests the null hypothesis that the most complex model does not fit the data better than the simpler one[15].

[15] To be more precise, what this actually does is test whether the extra estimated coefficients in the more complex model are not different from 0. Note that you can use this function only if all of the parameters of the smallest model are also present in the more complex model.

```
anova(model, model.2)
```

```
## Analysis of Variance Table
##
## Model 1: Response ~ Weight
## Model 2: Response ~ Weight + Age
##   Res.Df     RSS Df Sum of Sq      F    Pr(>F)
## 1    148 12346.1
## 2    147  3431.7  1    8914.4 381.85 < 2.2e-16 ***
## ---
## Signif. codes:
## 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

As expected, the p-value is very low, indicating that the second, more complex, model fits the data better than the first one, and should be preferred to it. See also how much the residual sum of squares (RSS) has decreased, indicating that the model is much closer to the real data (hence the residuals (and their squared sum) are smaller).

Conversely

```
anova(model.2, model.3)
```

```
## Analysis of Variance Table
##
## Model 1: Response ~ Weight + Age
## Model 2: Response ~ Weight + Age + Sex
##   Res.Df    RSS Df Sum of Sq      F Pr(>F)
## 1    147 3431.7
## 2    146 3426.7  1    5.0665 0.2159 0.6429
```

The p-value is 0.64, indicating that we cannot refute the null hypothesis, meaning that our third model (with Age, Weight, and Sex) is not better than the simpler model.

R also provides a convenient function, called `drop1`, that removes one predictor at a time from a larger model. You can see that this confirms what we have seen above.[16]

```
drop1(model.3, test = "F")
```

```
## Single term deletions
##
## Model:
## Response ~ Weight + Age + Sex
##        Df Sum of Sq     RSS    AIC  F value Pr(>F)
## <none>               3426.7 477.31
## Weight  1   14741.9 18168.5 725.52 628.1081 <2e-16 ***
## Age     1    8610.5 12037.2 663.77 366.8706 <2e-16 ***
## Sex     1       5.1  3431.7 475.53   0.2159 0.6429
## ---
## Signif. codes:
## 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

[16] Please, note that these are only very general guidelines. The choice of what to include in your model is not an easy one and different school of thoughts exist on whether it is better to always have a simpler model or a more complete one and often the answer is not straightforward. **The important thing is that any choice you make is based on a solid motivation.** Don't just stop at the p-value… think of what question you are asking and what your model tells you.

Section 3 - Interactions between factors

In the lectures you have learnt about interactions amongst factor in multiple regression. We will now see how to analyse interactions in R.

We will now consider the data in `fox_workshop2.csv`. This dataset[17] contains the litter size, as a measure of reproductive success, in two different population of Artic foxes, in relations to their age and location, as well as rodent availability[18] (which has been classified into low, medium, or high).

[17] Data for this example is fictional, but quite loosely based on work by Tannerfeldt and Angerbjörn (Oikos, 1998)

[18] Rodents are one source of food for Arctic foxes, but their number is fluctuating year-by-year in many regions

Figure 1: Sleepy arctic fox - Eric Kilby - CC BY-SA 2.0

As usual, we read the data and begin to explore it

```
foxes <- read.csv("fox-workshop2.csv")

summary(foxes)
```

```
##       Age          Location  RodentAvail   LitterSize
##  Min.   :1.00   Coastal:25   High  :17   Min.   : 1.00
##  1st Qu.:2.00   Inland :25   Low   :13   1st Qu.: 3.00
##  Median :2.00                Medium:20   Median : 5.50
##  Mean   :2.54                            Mean   : 5.72
##  3rd Qu.:3.00                            3rd Qu.: 7.75
##  Max.   :5.00                            Max.   :15.00
```

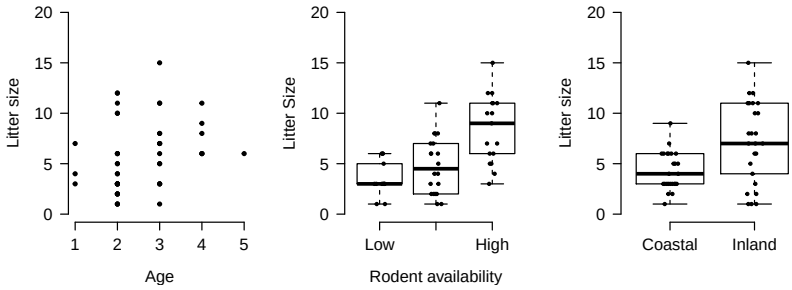
You may have noticed that the levels of the RodentAvail factor are in a slightly unusual order[19]. We can reorder it so Low is used as reference.

[19] Alphabetical!

```
foxes$RodentAvail <- factor(foxes$RodentAvail,
    levels = c("Low", "Medium", "High"))
```

We can then plot some of the relationships between variables[20].

[20] Can you reproduce these plots?



From a first inspection, it seems that reproductive success is somehow correlated to all of the other variables. We can use a linear model to study these relationships[21].

[21] Litter size is a count, therefore it is bounded to 0; we will learn later in the course that a linear model is not the best way to analyse these type of bounded data, but for the moment we can ignore this problem.

Just we did before, we can create a model using `lm`:

```
model <- lm(LitterSize ~ Age + Location + RodentAvail,
    data = foxes)

summary(model)
```

```
##
## Call:
## lm(formula = LitterSize ~ Age + Location + RodentAvail, data = foxes)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -3.898 -1.729  0.233  1.573  4.250
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        -2.6194     1.1996  -2.184   0.0342 *
## Age                 1.9827     0.3696   5.365 2.71e-06 ***
## LocationInland      1.5697     0.6259   2.508   0.0158 *
## RodentAvailMedium   1.3225     0.7810   1.693   0.0973 .
## RodentAvailHigh     5.8513     0.8489   6.892 1.47e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.043 on 45 degrees of freedom
## Multiple R-squared:  0.6738, Adjusted R-squared:  0.6448
## F-statistic: 23.24 on 4 and 45 DF,  p-value: 1.823e-10
```

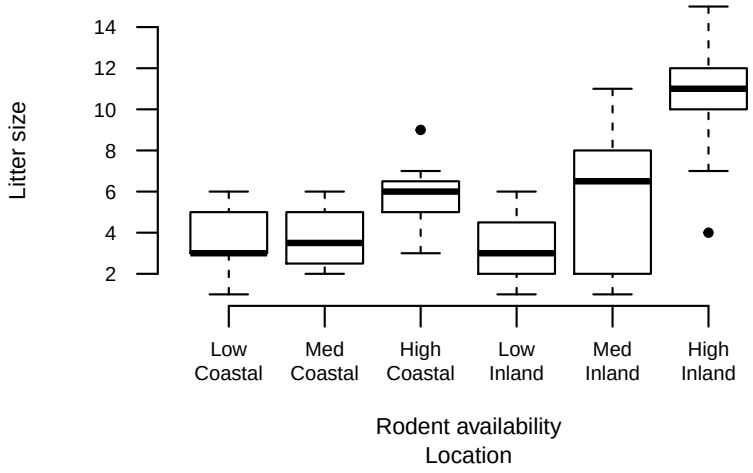
I will leave the point-by-point interpretation of the model's summary to you[22].

[22] Look at the intercept, what does a negative value mean there? Do you see any problems with that?

There is an important question now: is this the best model to describe our data? The adjusted R^2 value is 0.64, meaning that the model only explains about 64% of the data's variance.

Can we improve the model? The answer partly depends on the question we want to address. For example, one interesting question

that this model cannot currently answer is "Does rodent availability affect the litter size of both population of foxes in the same way?". This is a more specific and potentially more interesting question to ask, but slightly more complex to answer. We can start by going back to our plots. Let's consider this



Now, that is interesting! It looks like the two populations are not equal when considering the effect of rodent availability on reproductive success! In other words, there is an interaction between location and rodent availability in deremining litter size. This is not only interesting because it gives us the opportunity to look at how to analyse interactions in R... but also because it brings about other questions such as "why there is this difference?"[23].

We can modify our model to take this into account

```
model.2 <- lm(LitterSize ~ Age + Location + RodentAvail +
    RodentAvail:Location, data = foxes)
```

The `RodantAvail:Location` notation is used to indicate the interaction between the two factors. An alternative, and completely equivalent, way of indicating interactions is by using the $*$ sign.

```
model.2 <- lm(LitterSize ~ Age + Location * RodentAvail,
    data = foxes)
```

[23] For example, one explanation could be that coastal areas offer larger provisions of birds, that nest on the cliffs on the coast. These birds can be used by foxes as an alternative food source. Therefore foxes living on the coast have on average smaller litters every year, while foxes living inland have large litters in years when there is a lot of food available. Could you design an experiment to test this hypothesis?

```
summary(model.2)
```

```
##
## Call:
## lm(formula = LitterSize ~ Age + Location * RodentAvail, data = foxes)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.5903 -1.0377 -0.1342  1.0894  2.8658
##
## Coefficients:
##                                Estimate Std. Error t value Pr(>|t|)
## (Intercept)                     -3.0511     0.9171  -3.327 0.001805 **
## Age                              2.4634     0.2883   8.544 8.17e-11 ***
## LocationInland                  -2.6479     1.0468  -2.530 0.015168 *
## RodentAvailMedium                1.2585     0.7386   1.704 0.095597 .
## RodentAvailHigh                  3.2777     0.7648   4.286 0.000101 ***
## LocationInland:RodentAvailMedium 2.9610     1.2328   2.402 0.020707 *
## LocationInland:RodentAvailHigh   7.5482     1.3035   5.791 7.36e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.533 on 43 degrees of freedom
## Multiple R-squared:  0.8246, Adjusted R-squared:  0.8002
## F-statistic:  33.7 on 6 and 43 DF,  p-value: 1.019e-14
```

This is slightly more complex from what we have seen before, however it can be interpreted in a very similar manner. We see that the model now explains 80% of the variability in our data, an improvement compared to the previous model[24]! The model also tells us that there is a significant effect of age on litter size. $\hat{\beta}_{Age}$ tells us that for each increase of 1 year in age there is an increase in 2.4 in litter size[25]. It also tells us that there are significant interactions.

We can visualise these interactions by using an *interaction plot*, such as that provided by the `emmip` function in the package `emmeans`[26].

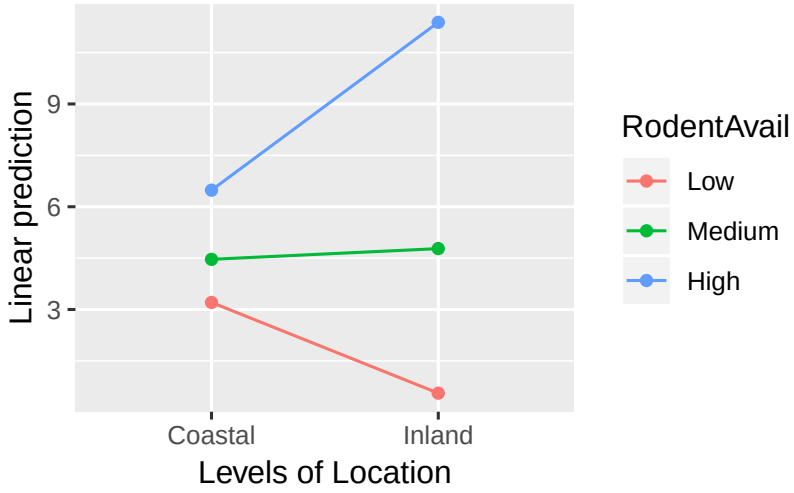
```
par(mar = c(4, 4, 1, 4), cex = 0.5, cex.lab = 1,
    cex.axis = 1)

library(emmeans)  # You need to have emmeans installed for this!
emmip(model.2, RodentAvail ~ Location)
```

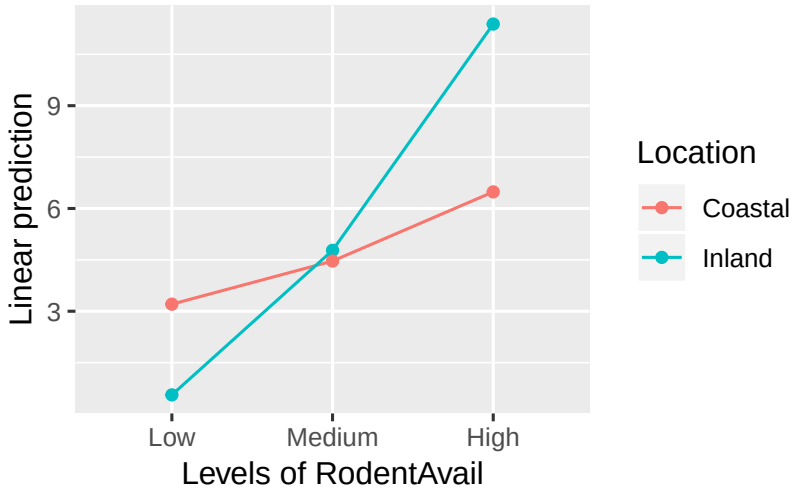
[24] You can also compare this model with the previous one using the `anova` function

[25] We need to be very careful when interpreting these coefficients. The model does not tell us why older animals have bigger litters. It may be directly because of age, or because older animals have previously had litters, and this has an effect on litter size!

[26] If you do not have the package emmeans installed, you can do so using `install.packages("emmeans")`. R also provides another function, called `interaction.plot`, that can produce the same graph)




```
emmip(model.2, Location ~ RodentAvail)
```



These graphs show the *estimated marginal means*, that is the means estimated by our model for each level of the factors we are considering.

Both graphs show the same information; since the lines are not running parallel to each other we can say that there is an interaction between the two factors. Rodent availability is influencing the inland population more than it is the coastal population.

And what should we make of the estimates for Location and RodentAvail[27]? Because we have a significant interaction, these coefficients become slightly less useful. $\hat{\beta}_2$ is the mean difference in litter size between foxes living inland and those living on the coast, **independently of rodent availability**[28]. However, since rodent availability affects this difference… we would generally ignore these two coefficient when interpreting our model. More formally, in the

[27] That is $\hat{\beta}_2 = 2.46$ and $\hat{\beta}_3 = -2.65$

[28] Can you interpret $\hat{\beta}_3$? Write your explanation in the forum

presence of interactions, we generally ignore the main effects (so the independent effects of each of the two interacting factors across the whole sample).

Finally, let's say that we want to know whether there is a statistical differece between the coastal and inland population, at the different level of RodentAvail. We can use the `emmeans` and `pairs` functions to do so. These function can perform pairwise comparison (just like the Tukey test did), also taking into account interactions. Rather than comparing all of the possible levels, we can specify specific differences (also called contrasts) that we are interested in; this will avoid, for instance, comparisons such as Inland/High vs Coastal/Low, which do not give us any particular biological information.[29].

[29] Still, in case we wanted to look at all possible comparisons... we could use `emmeans(model.2, pairwise ~ Location * RodentAvail)`. This will return all possible comparisons, without having to call pairs

```
marginals <- emmeans(model.2, ~Location * RodentAvail)
pairs(marginals, by = "RodentAvail")
```

```
## RodentAvail = Low:
##  contrast           estimate        SE df t.ratio p.value
##  Coastal - Inland  2.6479079 1.0467996 43   2.530  0.0152
##
## RodentAvail = Medium:
##  contrast           estimate        SE df t.ratio p.value
##  Coastal - Inland -0.3130441 0.7195572 43  -0.435  0.6657
##
## RodentAvail = High:
##  contrast           estimate        SE df t.ratio p.value
##  Coastal - Inland -4.9003370 0.7572678 43  -6.471  <.0001
```

In the calls to pairs we specify that we want to compare Location at different levels of RodentAvail. The output gives us the estimate of the difference (e.g.: for low rodent availability, the coastal foxes have on average 2.6 pups more than inland foxes), and the standard error associated with this estimate[30]. We also get a p-value for each of the contrasts. Remember, although the p-value tells us that the two conditions are different, it is probably more interesting to look at some measure of effect size (such as the estimate), which tells us about the biological significance of the result. Plot the data, look at the number and think! Would a difference in 0.01 pups/litter with a p-value of 0.02 be of any biological significance? Or, would you immediately dismiss a difference in 6 pups/litter because it was associated with a p-value of 0.09[31]?

[30] Remember, these estimates are based on the values of the $\hat{\beta}$ coefficients calculated for our model, but these are only estimations of the true population parameters

[31] Think of what that 0.09 means...

Finally, can you compare, for each location, the litter size at different level of rodent availability?

A final exercise

Finally, to consolidate what explained so far, consider the dataset `nerveConduction-workshop2.csv`. This contains measures of nerve conduction velocity in myelinated and unmyelinated fibres, in relation to their diameter.

Explore the dataset, and visually determine relationships between the various variables. Fit a linear model to explore the effect of Sex, Myelination and Diameter on conduction Velocity, exploring different interactions, and define what the various parameters estimated by your model mean. Which model best describes the data? What conclusions can you draw?