

Data Science for Biologists - 5023Y

Philip Leftwich

2022-03-12

Contents

1	Introduction	9
1.1	Approach and style	9
1.2	Teaching	9
1.3	Introduction to R	10
1.4	Getting around on RStudio	11
1.5	Reading	11
1.6	Get Help!	12
2	Getting to know R: Week One	13
2.1	Your first R command	13
2.2	“true or false” data	16
2.3	Storing outputs	18
2.4	Writing scripts	20
2.5	Error	21
2.6	Functions	21
2.7	Packages	23
2.8	My first data visualisation	24
2.9	Quitting	25
3	Workflow Part One: Week Two	27
3.1	Meet the Penguins	27
3.2	The Question?	30
3.3	Preparing the data	30
3.4	Prepare the RStudio workspace	33
3.5	Get the data into R	36
3.6	Dataframes and tibbles	39
3.7	Clean and tidy	40
3.8	Summing up	44
4	Workflow Part Two: Week Three	47
4.1	Initial insights	47
4.2	Numbers and sex of the penguins	48
4.3	Distributions	51

4.4	More distributions	52
4.5	Data transformation	53
4.6	Developing insights	55
4.7	Relationship/differences	58
4.8	Sex interactions	60
4.9	Making our graphs more attractive	65
4.10	Quitting	67
5	ggplot2 A grammar of graphics: Week Four	69
5.1	Intro to grammar	69
5.2	Building a plot	70
5.3	Plot background	71
5.4	Aesthetics - aes()	72
5.5	Geometric representations - geom()	72
5.6	%>% and +	73
5.7	Colour	74
5.8	More layers	75
5.9	Facets	76
5.10	Co-ordinate space	77
5.11	Labels	79
5.12	Themes	80
5.13	Jitter	82
5.14	Boxplots	82
5.15	Density and histogram	84
5.16	More Colours	85
5.17	Patchwork	90
5.18	Test	91
5.19	Saving	92
5.20	Quitting	93
5.21	Summing up ggplot	93
6	Reports with Rmarkdown: Week Five	95
6.1	Background to Rmarkdown	97
6.2	Starting a new Rmd file	98
6.3	R Markdown parts	99
6.4	Images	104
6.5	Tables	105
6.6	Self-contained documents	105
6.7	Source files	106
6.8	Exercise - Make your own Rmd document	106
6.9	Summing up Rmarkdown	107
7	Version control with GitHub: Week Six	109
7.1	Let's Git it started	109
7.2	Set up GitHub	111

7.3	Exercise 1. Fork & clone an existing repo on GitHub, make edits, push back	111
7.4	Talking to GitHub	115
7.5	How to use version control - when to commit, push and pull	118
7.6	Exercise 2. GitHub Classrooms enabled R Projects with sub-folders	126
7.7	Find your classroom repos	129
7.8	Glossary-GitHub	130
7.9	Summing up GitHub	131
8	Dealing with data: dplyr: Week Eight	133
8.1	Let's get going	133
8.2	Introduction to dplyr	133
8.3	select	134
8.4	mutate	135
8.5	filter	136
8.6	arrange	137
8.7	Group and summarise	137
9	Dealing with data part 2: Week Nine	143
9.1	Expanding the data toolkit	143
9.2	Pipes	143
9.3	Strings	145
9.4	Dates and times	146
9.5	Pivot	148
9.6	Summing up	150
10	Deeper data insights part 1: Week Ten	153
10.1	Variables	153
10.2	Understanding Numerical variables	154
10.3	Descriptive statistics	159
10.4	Categorical variables	165
10.5	Summing up	169
11	Deeper data insights part 2: Week Eleven	171
11.1	Associations between numerical variables	171
11.2	Associations between categorical variables	177
11.3	Associations between Categorical-numerical variables	180
11.4	Complexity	182
11.5	Summing up	187
12	Introduction to Statistics - Spring Week One	189
12.1	Introduction to statistics	189
12.2	Darwin's maize data	190
12.3	Estimation	194
12.4	Summary	201

13 Keeping code DRY - Spring Week One	203
13.1 Functions	204
13.2 Iteration	213
13.3 More Practice	221
13.4 Summary	221
14 Introduction to Linear Models - Spring Week Two	223
14.1 Introduction to Linear Models	223
14.2 A linear model analysis for comparing groups	223
14.3 Confidence intervals	228
14.4 Answering the question	228
14.5 Assumption checking	231
14.6 Summary	235
15 Testing - Spring Week Three	237
15.1 It's t-time	237
15.2 Student's <i>t</i> -test	237
15.3 Effect sizes	243
15.4 Meta-analysis	246
15.5 Summary	248
16 Regression - Spring Week Four	251
16.1 Introduction to Regression	251
16.2 Linear regression	251
16.3 Data	252
16.4 Correlation	254
16.5 Regression in R	255
16.6 Assumptions	258
16.7 Prediction	264
16.8 Summary	266
17 ANOVA - Spring Week Five	267
17.1 ANOVA	267
17.2 Maize data	267
17.3 Two-way ANOVA	272
17.4 Summary	273
17.5 Practice	273
17.6 Importing the data	274
18 Multivariate linear models - Spring Week Six	279
18.1 Multivariate linear models	279
18.2 Factorial linear models	279
18.3 Balanced/Unbalanced designs	290
18.4 post-hoc	292
18.5 Continuous Linear Models	293
18.6 Summary	297

19 Complexity in Linear Models - Spring Week Seven	299
19.1 Designing a Model	299
19.2 Checking the data	300
19.3 Designing a model	304
19.4 Model checking & collinearity	305
19.5 Model selection	308
19.6 Posthoc	310
19.7 Write-up	310
19.8 Summary	312
19.9 Supplementary code	313
20 Generalized Linear Models	317
20.1 Maximum Likelihood and Generalized Linear Models	317
20.2 Checklist	317

Chapter 1

Introduction

1.1 Approach and style

This book is designed to accompany the module BIO-5023Y for those new to R looking for best practices and tips. So it must be both accessible and succinct. The approach here is to provide just enough text explanation that someone very new to R can apply the code and follow what the code is doing. It is not a comprehensive textbook.

A few other points:

This is a code reference book accompanied by relatively brief examples - not a thorough textbook on R or data science

This is intended to be a living document - optimal R packages for a given task change often and we welcome discussion about which to emphasize in this handbook

Top tips for the course:

DON'T worry if you don't understand everything

DO ask lots of questions!

1.2 Teaching

We have:

- one lecture per week
- one workshop per week

These are both timetabled in-person sessions, and you should check Timetabler for up to-date information on scheduling. However, all lessons can be accessed

remotely through Collaborate, and **everything** you need to complete workshops will be available on this site.

If you feel unwell, or cannot attend a session in-person because you need to self-isolate then don't worry you can access everything, and follow along in real time, or work at your own pace.

Questions/issues/errors can all be posted on our Yammer page.

1.2.1 Workshops

The workshops are the best way to learn, they teach you the practical skills you need to become an R wizard



Figure 1.1: courtesy of Allison Horst

1.3 Introduction to R

R is the name of the programming language itself and RStudio is a convenient interface., which we will be using throughout the course in order to learn how to organise data, produce accurate data analyses & data visualisations.

Eventually we will also add extra tools like GitHub and RMarkdown for data reproducibility and collaborative programming, check out this short (and very cheesy) intro video., which are collaboration and version control systems that we will be using throughout the course. More on this in future weeks.

By the end of this module I hope you will have the tools to confidently analyze real data, make informative and beautiful data visuals, and be able to analyse lots of different types of data.

The taught content this autumn will be given to you in several **worksheets**, these will be added to this dynamic webpage each week.

1.4 Getting around on RStudio

All of our sessions will run on cloud-based software. All you have to do is make a free account, and join our Workspace BIO-5023Y the sharing link is here.

Once you are signed up - you will see that there are two **Spaces**

- Your workspace
- BIO-5023Y

Make sure you are working in the class workspace - there is a limit to the hours/month on your workspace, so all assignments and project work should take place in the BIO-5023Y space.

Watch these short explainer videos to get used to navigating the environment.

1.4.1 An intro to RStudio

RStudio

Note - people often mix up R and RStudio. R is the programming language (the engine), RStudio is a handy interface/wrapper that makes things a bit easier to use.

1.4.2 Using R Studio Cloud

RStudio Cloud works in exactly the same way as RStudio, but means you don't have to download any software. You can access the hosted cloud server and your projects through any browser connection (Chrome works best), from any computer.

1.5 Reading

There are lots of useful books and online resources to help develop and improve your R knowledge. Throughout this webpage I will be adding useful resources for you.

The core textbook you might want to bookmark is R for Data Science (Hadley Wickham, 2020) but we will add others throughout the course, and their is a bibliography at the end which collects everything together!

1.6 Get Help!

There are a **lot** of sources of information about using R out there. Here are a few helpful places to get help when you have an issue, or just to learn more

- The R help system itself - we cover this in Week one Error
- Vignettes - type `browseVignettes()` into the console and hit Enter, a list of available vignettes for all the packages we have will be displayed
- Cheat Sheets - available at RStudio.com. Most common packages have an associate cheat sheet covering the basics of how to use them. Download/bookmark ones we will use commonly such as `ggplot2`, `Data transformation with dplyr`, `Data tidying with tidyr` & `Data import`.
- Google - I use Google constantly, because I continually forget how to do even basic tasks. If I want to remind myself how to round a number, I might type something like `R round number` - if I am using a particular package I should include that in the search term as well
- Ask for help - If you are stuck, getting an error message, can't think what to do next, then ask someone. It could be me, it could be a classmate. When you do this it is very important that you **show the code**, include the **error message**. "This doesn't work" is not helpful. "Here is my code, this is the data I am using, I want it to do X, and here's the problem I get".

Note - It may be daunting to send your code to someone for help. It is natural and common to feel apprehensive, or to think that your code is really bad. I still feel the same! But we learn when we share our mistakes, and eventually you will find it funny when you look back on your early mistakes, or laugh about the mistakes you still occasionally make!

Chapter 2

Getting to know R: Week One

Go to RStudio Cloud and enter the Project labelled **Week One** - this will clone the project and provide you with your own workspace.

Follow the instructions below to get used to the R command line, and how R works as a language.

2.1 Your first R command

In the RStudio pane, navigate to the console (bottom left) and type or copy the below it should appear at the >

Hit Enter on your keyboard.

```
10 + 20
```

You should now be looking at the below:

```
> 10 + 20  
[1] 30
```

The first line shows the request you made to R, the next line is R's response

You didn't type the > symbol: that's just the R command prompt and isn't part of the actual command.

It's important to understand how the output is formatted. Obviously, the correct answer to the sum $10 + 20$ is 30, and not surprisingly R has printed that out as part of its response. But it's also printed out this [1] part, which probably doesn't make a lot of sense to you right now. You're going to see that a lot.

You can think of [1] 30 as if R were saying “the answer to the 1st question you asked is 30”.

2.1.1 Typos

Before we go on to talk about other types of calculations that we can do with R, there’s a few other things I want to point out. The first thing is that, while R is good software, it’s still software. It’s pretty stupid, and because it’s stupid it can’t handle typos. It takes it on faith that you meant to type *exactly* what you did type. For example, suppose that you forgot to hit the shift key when trying to type +, and as a result your command ended up being $10 = 20$ rather than $10 + 20$. Try it for yourself and replicate this error message:

```
10 = 20
```

```
## Error in 10 = 20: invalid (do_set) left-hand side to assignment
```

What’s happened here is that R has attempted to interpret $10 = 20$ as a command, and spits out an error message because the command doesn’t make any sense to it. When a *human* looks at this, and then looks down at his or her keyboard and sees that + and = are on the same key, it’s pretty obvious that the command was a typo. But R doesn’t know this, so it gets upset. And, if you look at it from its perspective, this makes sense. All that R “knows” is that 10 is a legitimate number, 20 is a legitimate number, and = is a legitimate part of the language too. In other words, from its perspective this really does look like the user meant to type $10 = 20$, since all the individual parts of that statement are legitimate and it’s too stupid to realise that this is probably a typo. Therefore, R takes it on faith that this is exactly what you meant... it only “discovers” that the command is nonsense when it tries to follow your instructions, typo and all. And then it whinges, and spits out an error.

Even more subtle is the fact that some typos won’t produce errors at all, because they happen to correspond to “well-formed” R commands. For instance, suppose that not only did I forget to hit the shift key when trying to type $10 + 20$, I also managed to press the key next to one I meant do. The resulting typo would produce the command $10 - 20$. Clearly, R has no way of knowing that you meant to *add* 20 to 10, not *subtract* 20 from 10, so what happens this time is this:

```
10 - 20
```

```
## [1] -10
```

In this case, R produces the right answer, but to the wrong question.

2.1.2 More simple arithmetic

One of the best ways to get to know R is to play with it, it's pretty difficult to break it so don't worry too much. Type whatever you want into the console and see what happens.

If the last line of your console looks like this

```
> 10+
+
```

and there's a blinking cursor next to the plus sign. This means is that R is still waiting for you to finish. It "thinks" you're still typing your command, so it hasn't tried to execute it yet. In other words, this plus sign is actually another command prompt. It's different from the usual one (i.e., the > symbol) to remind you that R is going to "add" whatever you type now to what you typed last time. For example, type 20 and hit enter, then it finishes the command:

```
> 10 +
+ 20
[1] 30
```

Alternatively hit escape, and R will forget what you were trying to do and return to a blank line.

2.1.3 Try some maths

1+7

13-10

4*6

12/3

Raise a number to the power of another

5^4

As I'm sure everyone will probably remember the moment they read this, the act of multiplying a number x by itself n times is called "raising x to the n -th power". Mathematically, this is written as x^n . Some values of n have special names: in particular x^2 is called x -squared, and x^3 is called x -cubed. So, the 4th power of 5 is calculated like this:

$$5^4 = 5 \times 5 \times 5 \times 5$$

2.1.4 Perform some combos

Perform some mathematical combos, noting that the order in which R performs calculations is the standard one.

That is, first calculate things inside **B**rackets (), then calculate **O**rders of (exponents) ^, then **D**ivision / and **M**ultiplication *, then **A**ddition + and **S**ubtraction -.

Notice the different outputs of these two commands.

```
3^2-5/2
```

```
(3^2-5)/2
```

Similarly if we want to raise a number to a fraction, we need to surround the fraction with parentheses ()

```
16^1/2
```

```
16^(1/2)
```

The first one calculates 16 raised to the power of 1, then divided this answer by two. The second one raises 16 to the power of a half. A big difference in the output.

**Note - While the cursor is in the console, you can press the up arrow to see all your previous commands. You can run them again, or edit them. Later on we will look at scripts, as an essential way to re-use, store and edit commands.

2.2 “true or false” data

Time to make a sidebar onto another kind of data. A key concept in that a lot of R relies on is the idea of a ***logical value***. A logical value is an assertion about whether something is true or false. This is implemented in R in a pretty straightforward way. There are two logical values, namely **TRUE** and **FALSE**. Despite the simplicity, a logical values are very useful things. Let's see how they work.

2.2.1 Assessing mathematical truths

In George Orwell's classic book *1984*, one of the slogans used by the totalitarian Party was “two plus two equals five”, the idea being that the political domination

of human freedom becomes complete when it is possible to subvert even the most basic of truths.

But they didn’t have R. R will not be subverted. It has rather firm opinions on the topic of what is and isn’t true, at least as regards basic mathematics. If I ask it to calculate $2 + 2$, it always gives the same answer, and it’s not bloody 5:

```
2 + 2
```

Of course, so far R is just doing the calculations. I haven’t asked it to explicitly assert that $2 + 2 = 4$ is a true statement. If I want R to make an explicit judgement, I can use a command like this:

```
2 + 2 == 4
```

What I’ve done here is use the *equality operator*, `==`, to force R to make a “true or false” judgement.

**Note that this is a very different operator to the assignment operator `=` you saw previously. A common typo that people make when trying to write logical commands in R (or other languages, since the “`=` versus `==`” distinction is important in most programming languages) is to accidentally type `=` when you really mean `==`.

Okay, let’s see what R thinks of the Party slogan:

```
2+2 == 5
```

Take that Big Brother! Anyway, it’s worth having a look at what happens if I try to *force* R to believe that two plus two is five by making an assignment statement like $2 + 2 = 5$ or $2 + 2 <- 5$. When I do this, here’s what happens:

```
2 + 2 = 5
```

R doesn’t like this very much. It recognises that $2 + 2$ is *not* a variable (that’s what the “non-language object” part is saying), and it won’t let you try to “reassign” it. While R is pretty flexible, and actually does let you do some quite remarkable things to redefine parts of R itself, there are just some basic, primitive truths that it refuses to give up. It won’t change the laws of addition, and it won’t change the definition of the number 2.

That’s probably for the best.

2.3 Storing outputs

With simple questions like the ones above we are happy to just see the answer, but our questions are often more complex than this. If we need to take multiple steps, we benefit from being able to store our answers and recall them for use in later steps. This is very simple to do we can *assign* outputs to a name:

```
a <- 1+2
```

This literally means please *assign* the value of `1+2` to the name `a`. We use the **assignment operator** `<-` to make this assignment.

**Note the shortcut key for `<-` is Alt + - (Windows) or Option + - (Mac)

If you perform this action you should be able to do two things

- You should be able to see that in the top right-hand pane in the **Environment** tab there is now an **object** called `a` with the value of 3.
- You should be able to look at what `a` is by typing it into your Console and pressing Enter

```
a
```

```
> a  
[1] 3
```

You can now call this object at any time during your R session and perform calculations with it.

```
2 * a
```

```
[1] 6
```

What happens if we assign a value to a named object that already exists in our R environment??? for example

```
a <- 10  
a
```

```
[1] 10
```

You should see that the previous assignment is lost, *gone forever* and has been replaced by the new value.

We can assign lots of things to objects, and use them in calculations to build more objects.

```
b <- 5  
c <- a + b
```

Note that if you now change the value of b, the value of c does *not* change. Objects are totally independent from each other once they are made

```
b <- 7  
b  
c
```

Look at the environment tab again - you should see it's starting to fill up now!

**Note - RStudio will by default save the objects in its memory when you close a session. These will then be there the next time you logon. It might seem nice to be able to close things down and pick up where you left off, but it's actually quite dangerous. It's messy, and can cause lots of problems when we work with scripts later, so don't do this!!! To stop RStudio from saving objects by default go to the Preferences option and change "Save workspace to .RData on exit" to "Never". Instead we are going to learn how to use scripts to quickly re-run analyses we have been working on.

2.3.1 Choosing names

- Use informative variable names. As a general rule, using meaningful names like `orange` and `apple` is preferred over arbitrary ones like `variable1` and `variable2`. Otherwise it's very hard to remember what the contents of different variables actually are.
- Use short variable names. Typing is a pain and no-one likes doing it. So we much prefer to use a name like `apple` over a name like `pink_lady_apple`.
- Use one of the conventional naming styles for multi-word variable names. R only lets you use certain things as **legal** names. Legal names must start with a letter **not** a number, which can then be followed by a sequence of letters, numbers, ., or __. R does not like using spaces. Upper and lower case names are allowed, but R is case sensitive so `Apple` and `apple` are different.
- My favourite naming convention is `snake_case` short, lower case only, spaces between words are separated with a __. It's easy to read and easy to remember.

2.4 Writing scripts

Until now we have been typing words directly into the Console. This is fine for short/simple calculations - but as soon as we have a more complex, multi-step process this becomes time consuming, error-prone and *boring*. **Scripts** are a document containing all of your commands (in the order you want them to run), they are *repeatable, shareable, annotated records of what you have done*. In short they are incredibly useful - and a big step towards **open** and **reproducible** research.

To create a script go to File > New File > R Script.

This will open a pane in the top-left of RStudio with a tab name of **Untitled1**. In your new script, type some of the basic arithmetic and assignment commands you used previously. When you write a script, make sure it has all of the commands you need to complete your analysis, *in the order you want them to run*.

2.4.1 Commenting on scripts

Annotating your instructions provides yourself and others insights into why you are doing what you are doing. This is a vital aspect of a robust and reproducible workflow. And when you come back to a script, one week, one month or one year from now you will often wonder what a command was for. It is very, very useful to make notes for yourself, and its useful in case anyone else will ever read your script. Make these comments helpful they are for humans to read.

In R we signal a comment with the `#` key. Everything in the line after a `#` is ignored by R and won't be treated as a command. You should see that it is marked in a different colour in your script.

Put the following comment in your script. Try adding a few comments to your previous lines of code

```
# I really love R
```

2.4.2 Running your script

To run the commands from your script, we need to get it into the Console. You could select and copy/paste this into the Console. But there are a couple of faster shortcuts.

- Hit the Run button in the top right of the script pane. Pressing this will run the line of code the cursor is sitting on.
- Pressing Ctrl+Enter will do the same thing as hitting the Run button
- If you want to run the whole script in one go then press Ctrl+A then either click Run or press Ctrl+Enter

2.4.3 Saving your script

Our script now contains code and comments from our first workshop. We need to save it.

Alongside our data, our script is the most precious part of our analysis. We don't need to save anything else, any outputs etc. because our script can always be used to generate everything again. Note the colour of the script - the name changes colour when we have unsaved changes. Press the Save button or go to File > Save as. Give the File a sensible name like "Simple commands in R" and in the bottom right pane under **Files** you should now be able to see your saved script.

You could now safely quit R, and when you log on next time to this project, your script will be waiting for you.

2.5 Error

Things will go wrong eventually, they always do...

R is *very* pedantic, even the smallest typo can result in failure and typos are impossible to avoid. So we will make mistakes. One type of mistake we will make is an **error**. The code fails to run. The most common causes for an error are:

- typos
- missing commas
- missing brackets

There's nothing wrong with making *lots* of errors. The trick is not to panic or get frustrated, but to read the error message and our script carefully and start to *debug*...

... and sometimes we need to walk away and come back later!

2.6 Functions

Functions are the tools of R. Each one helps us to do a different task.

Take for example the function that we use to round a number to a certain number of digits - this function is called **round**

Here's an example

```
round(x = 2.4326782647, digits = 2)
```

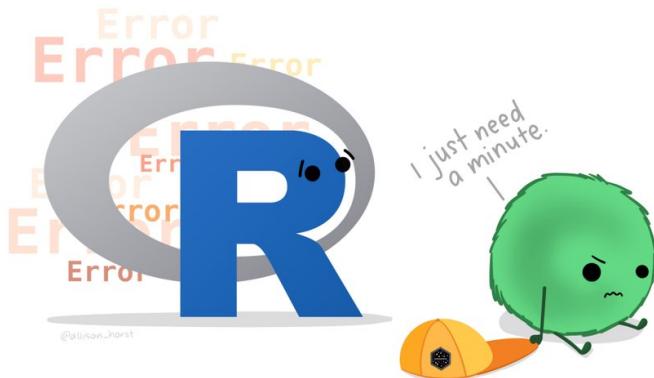


Figure 2.1: courtesy of Allison Horst

We start the command with the function name `round`. The name is followed by parentheses `()`. Within these we place the *arguments* for the function, each of which is separated by a comma.

The arguments

- `x = 2.4326782647`
- `digits = 2`

Arguments are the information we give to a function. These arguments are in the form `name = value` the name specifies the argument, and the value is what we are providing. That is the first argument `x` is the number we would like to round, it has a value of `2.4326782647`. The second argument `digits` is how we would like the number to be rounded and we specify `2`.

Ok put the above command in your script and add a comment with `#` as to what you are doing.

2.6.1 Storing the output of functions

What if we need the answer from a function in a later calculation. The answer is to use the assignment operator again.

Can you work out what is going on here? If so copy this into your R script and a `#comment` next to each line.

```
number_of_digits <- 2
my_number <- 2.4326782647
rounded_number <- round(x = my_number,
                         digits = number_of_digits)
```

2.6.2 More fun with functions

Check this out

```
round(2.4326782647, 2)
```

We don't *have* to give the names of arguments for a function to still work. This works because the function `round` expects us to give the number value first, and the argument for rounding digits second. *But* this assumes we know the expected ordering within a function, this might be the case for functions we use a lot. If you give arguments their proper names *then* you can actually introduce them in any order you want.

Try this:

```
round(digits = 2, x = 2.4326782647)
```

But this gives a different answer

```
round(2, 2.4326782647)
```

Are you happy with what is happening here? naming arguments overrides the position defaults

Ok what about this?

```
round(2.4326782647)
```

We didn't specify how many digits to round to, but we still got an answer. That's because in many functions arguments have `defaults` - the default argument here is `digits = 0`. So we don't have to specify the argument if we are happy for `round` to produce whole numbers.

How do we know argument orders and defaults? Well we get to know how a lot of functions work through practice, but we can also use the inbuilt R help. This is a function - but now we specify the name of another function to provide a help menu.

```
help(round)
```

2.7 Packages

An R package is a container for various things including functions and data. These make it easy to do very complicate protocols by using custom-built functions. Later we will see how we can write our own simple functions.

On RStudio Cloud I have already installed several add-on packages, all we need to do is use a simple function to load these packages into our workspace. Once this is complete we will have access to all the custom functions they contain.

Let's try that now:

```
library(ggplot2)
library(palmerpenguins)
```

Errors part 2 Another common source of errors is to call a function that is part of a package but forgetting to load the package. If R says something like “Error in function-name” then most likely the function was misspelled or the package containing the function hasn’t been loaded.

Packages are a lot like new apps extending the functionality of what your phone can do. To use the functionalities of a package they must be loaded *before* we call on the funcitons or data they contain. So the most sensible place to put library calls for packages is at the very **top** of our script. So let’s do that now.

2.8 My first data visualisation

Let’s run our first data visualisation using the functions and data we have now loaded - this produces a plot using functions from the `ggplot2` package (Wickham et al. (2021b)) and data from the `palmerpenguins` (Horst et al. (2020)) package. Use the `#` comments to add notes on what you are using each package for in your script.

Using these functions we can write a simple line of code to produce a figure. We specify the data source, the variables to be used for the x and y axis and then the type of visual object to produce, colouring them by the species.

Copy this into your console and hit Enter.

```
ggplot(data = penguins,aes(x = bill_length_mm, y = bill_depth_mm)) + geom_point(aes(co
```

**Note - you may have noticed R gave you a warning. Not the same as a big scary error, but R wants you to be aware of something. In this case that two of the observations had missing data in them (either bill length or bill depth), so couldn’t be plotted.

The above command can also be written as below, its in a longer style with each new line for each argument in the function. This style can be easier to read, and makes it easier to write comments with `#`. Copy this longer command into your `script` then run it by either highlighting the entire command or placing the cursor in the first line and then hit Run or Ctrl+Enter.

```
ggplot(data = penguins, # calls ggplot function, data is penguins
       aes(x = bill_length_mm, # sets x axis as bill length
           y = bill_depth_mm)) + # sets y axis value as bill depth
       geom_point(aes(colour=species)) # plot points coloured by penguin species
```

2.9 Quitting

- Make sure you have saved any changes to your R script - that's all you need to make sure you've done!
- If you want me to take a look at your script let me know
- If you spotted any mistakes or errors let me know
- Close your RStudio Cloud Browser
- Go to Blackboard to complete a short quiz!

Chapter 3

Workflow Part One: Week Two

Last week we got acquainted with some of the core skills associated with using R and RStudio.

In this workshop we work through the journey of importing and tidying data. Once we have a curated and cleaned dataset we can work on generating insights from the data.

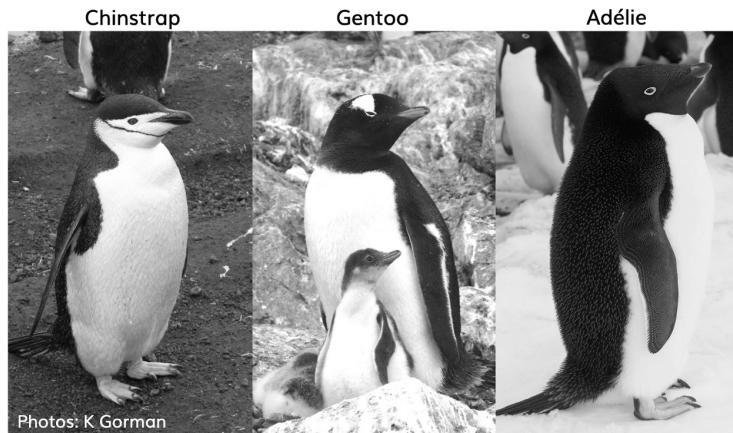
We are going to be working as though we are in the latter stages of a research project, where data has been collected, possibly over several years, to test against our hypotheses.

We have chosen to continue working with a dataset you have been introduced to already - the Palmer Penguins dataset. Previously we loaded a cleaned dataset, very quickly using an R package. Today we will be working in a more realistic scenario - uploading out dataset to our R workspace.

3.1 Meet the Penguins

This data, taken from the `palmerpenguins` (Horst et al. (2020)) package was originally published by Gorman et al. (2014).

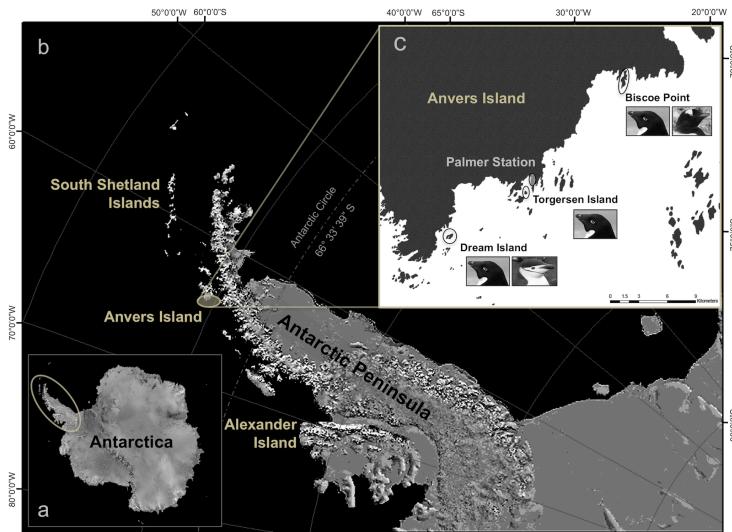
The `palmerpenguins` data contains size measurements, clutch observations, and blood isotope ratios for three penguin species observed on three islands in the Palmer Archipelago, Antarctica over a study period of three years.



These data were collected from 2007 - 2009 by Dr. Kristen Gorman with the Palmer Station Long Term Ecological Research Program, part of the US Long Term Ecological Research Network. The data were imported directly from the Environmental Data Initiative (EDI) Data Portal, and are available for use by CC0 license (“No Rights Reserved”) in accordance with the Palmer Station Data Policy. We gratefully acknowledge Palmer Station LTER and the US LTER Network. Special thanks to Marty Downs (Director, LTER Network Office) for help regarding the data license & use. Here is our intrepid package co-author, Dr. Gorman, in action collecting some penguin data:



Here is a map of the study site



3.1.1 Insights from data

This dataset is relatively simple, as there aren't too many variables to consider. But there are a reasonably large number of datapoints (individual penguins) making it possible to generate insights.

However, there are some specific and rather common problems in this data. Problems that we need to work through *before* we can start to make any visuals or try to draw any conclusions. There are some problems with the variable names, there are some problems with some of the values. There are problems that one of the response variables isn't actually encoded on the dataset (we have to make it).

Today we are going to

- Formulate clear research questions
- Import our dataset
- Learn how to prepare our RStudio Project workspace
- Learn how to clean, tidy and manipulate our data to allow tables, graphs and analyses to be run

Don't worry if you don't understand exactly what each function does at the moment, or struggle to remember every concept we are introduced to. We will cover these again, in lots more detail as we progress. The main point is to get familiar with our process for handling data and organising our projects.

3.2 The Question?

Imagine that you are a Penguin biologist. Chilly.

Imagine that you want to know more about the feeding habits of the different penguin species in the Antarctic. You also wish to characterise features such as bill morphology, and body size and compare them across species. Adelie and Chinstrap penguins are off-shore, shallow diving foragers, while Gentoo's feed closer inshore and are deep-divers. We might expect that we can find some features of Gentoo's that align with their different lifestyle/feeding habits.

With simple measuring techniques and identification skills we can sex the penguins, identify their species and take simple non-invasive measurements of features such as body size, flipper length and bill dimensions. You also recently read a paper about the ratios of different Nitrogen and Carbon isotopes in blood, and how these can be used to infer the types of prey that are forming an organism's diet.

3.2.1 Hypotheses

These hypotheses are fairly basic, we have not included directionality or specific expectations of the magnitude of the difference. This would come from doing more research on the subject area.

- The bill lengths/depths ratio to body size of Gentoo penguins will be different to Adelie and Chinstrap penguins.
- Gentoo penguins will have a different N and carbon isotope ratio than Adelie and Chinstrap penguins.

3.3 Preparing the data

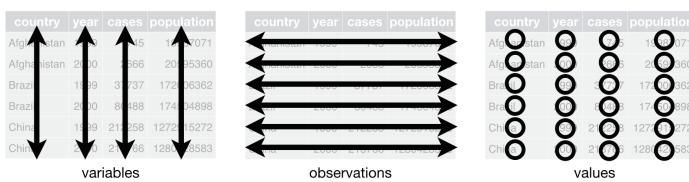
Imagine we have completed our practical study and have our data. The data is probably distributed in lots of places, originally notes collected in the field were probably on paper & notebooks. Then someone will have taken time to transcribe those into a spreadsheet. This will almost certainly have been done by typing all the data in by hand.

It is very important for us to know how we would like our data to be organised at the end. We are going to learn how to organise data using the *tidy* format¹. This is because we are going to use the **tidyverse** packages by Wickham et al. (2019). This is an opinionated, but highly effective method for generating reproducible analyses with a wide-range of data manipulation tools. Tidy data is an easy format for computers to read.

¹(<http://vita.had.co.nz/papers/tidy-data.pdf>)

3.3.1 Tidy data

Here ‘tidy’ refers to a specific structure that lets us manipulate and visualise data with ease. In a tidy dataset each *variable* is in one column and each row contains one *observation*. Each cell of the table/spreadsheet contains the *values*. One observation you might make about tidy data is it is quite long - it generates a lot of rows of data.



Typing data in, using any spreadsheet program (e.g. Excel, Google sheets), if we type in the penguin data, we would make each row contain one observation about one penguin. If we made a second observation about a penguin (say in the next year of the study) it would get a new row in the dataset. You are probably thinking this is a lot of typing and a lot of repetition - and you are right! But this format allows the computer to easily make summaries at any level.

If the data we input to R is “untidy” then we have to spend a little bit of time tidying, we will explore this more later.

Once data has been typed up into a spreadsheet and double/triple-checked against the original paper records, then they are saved as a ‘comma-separated values (CSV)’ file-type. These files are the simplest form of database, no coloured cells, no formulae, no text formatting. Each row is a row of the data, each value of a row (previously separate columns) is separated by a comma.

It is convenient to use something like Excel to type in our data - its much more usefully friendly than trying to type something in csv format. But we don’t save files in the Excel format because they have a nasty habit of formatting or even losing data when the file gets large enough². If you need to add data to a csv file, you can always open it in an Excel-like program and add more information.

It is possible to import data into R in an Excel format, but I recommend sticking with csv formats. Any spreadsheet can be easily converted with the *Save As..* command.

3.3.2 The dataset

For today’s workshop we want to acquire the dataset to work on it. We can retrieve the file we need from here (https://github.com/UEABIO/5023Y_

²<https://www.theguardian.com/politics/2020/oct/05/how-excel-may-have-caused-loss-of-16000-covid-tests-in-england>

	studyName	Sample Number	Species	Region	Island	Stage	Individual ID	Clutch Completion	Date Egg	Culmen Length (mm)	Curved Bill Length (mm)
2	PAL0708	1	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N1A1	Yes	11/11/2007	39.1	18.
3	PAL0708	2	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N1A2	Yes	11/11/2007	39.5	17.
4	PAL0708	3	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N2A1	Yes	16/11/2007	40.3	18.
5	PAL0708	4	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N2A2	Yes	16/11/2007	NA	NA
6	PAL0708	5	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N3A1	Yes	16/11/2007	NA	NA
7	PAL0708	6	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N3A2	Yes	16/11/2007	NA	NA
8	PAL0708	7	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N4A1	No	15/11/2007	NA	NA
9	PAL0708	8	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N4A2	No	15/11/2007	NA	NA
10	PAL0708	9	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N5A1	Yes	09/11/2007	NA	NA
11	PAL0708	10	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N5A2	Yes	09/11/2007	NA	NA
12	PAL0708	11	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N6A1	Yes	09/11/2007	NA	NA
									:		
1			studyName, Sample Number, Species, Region, Island, Stage, Individual ID, Clutch Completion, Date Egg, Culmen Length (mm), Curved Bill Length (mm)								
2			PAL0708, 1, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
3			PAL0708, 2, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
4			PAL0708, 3, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
5			PAL0708, 4, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
6			PAL0708, 5, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
7			PAL0708, 6, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
8			PAL0708, 7, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
9			PAL0708, 8, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
10			PAL0708, 9, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
11			PAL0708, 10, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
12			PAL0708, 11, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
13			PAL0708, 12, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
14			PAL0708, 13, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
15			PAL0708, 14, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
16			PAL0708, 15, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								
17			PAL0708, 16, Adelie Penguin (Pygoscelis adeliae), Anvers, Torgersen, "Adult, 1 Egg"								

Figure 3.1: Top image: Penguins data viewed in Excel, Bottom image: Penguins data in native csv format

Workshop/blob/main/data/penguins_raw.csv).

studyName	Sample Number	Species	Region	Island	Stage	Individual ID	Clutch Completion	Date Egg	Culmen Length (mm)	Curved Bill Length (mm)
PAL0708	1	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N1A1	Yes	11/11/2007	39.1	18.
PAL0708	2	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N1A2	Yes	11/11/2007	39.5	17.
PAL0708	3	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N2A1	Yes	16/11/2007	40.3	18.
PAL0708	4	Adelie Penguin (Pygoscelis adeliae)	Anvers	Torgersen	Adult, 1 Egg	N2A2	Yes	16/11/2007	NA	NA

Figure 3.2: Click on the copy raw contents button

We then need to

1. Select the copy raw contents button
2. Open a blank notepad
3. Paste the contents
4. Save the file as ‘penguin_data.csv’
5. Open the newly saved file in Excel and take a look

We can see a dataset with 345 rows (including the headers) and 17 variables

- **Study name:** an identifier for the year in which sets of observations were made
- **Region:** the area in which the observation was recorded
- **Island:** the specific island where the observation was recorded
- **Stage:** Denotes reproductive stage of the penguin
- **Individual ID:** the unique ID of the individual
- **Clutch completion:** if the study nest observed with a full clutch e.g. 2 eggs
- **Date egg:** the date at which the study nest observed with 1 egg
- **Culmen length:** length of the dorsal ridge of the bird's bill (mm)
- **Culmen depth:** depth of the dorsal ridge of the bird's bill (mm)
- **Flipper Length:** length of bird's flipper (mm)
- **Body Mass:** Bird's mass in (g)
- **Sex:** Denotes the sex of the bird
- **Delta 15N :** the ratio of stable Nitrogen isotopes 15N:14N from blood sample
- **Delta 13C:** the ratio of stable Carbon isotopes 13C:12C from blood sample

3.4 Prepare the RStudio workspace

Now we should have our question, we understand more about where the data came from, and we have our data in a CSV format.

The next step of our workflow is to have a well organised project space. RStudio Cloud does a lot of the hard work for you, each new data project can be set up with its own Project space.

We will define a project as a series of linked questions that uses one (or sometimes several) datasets. For example a coursework assignment for a particular module would be its own project, or eventually your final year research project.

A Project will contain several files, possibly organised into sub-folders containing data, R scripts and final outputs. You might want to keep any information (wider reading) you have gathered that is relevant to your project.

Open the Week Two - workflow project on RStudio Cloud.

Within this project you will notice there is already one file *.Rproj*. This is an R project file, this is a very useful feature, it interacts with R to tell it you are working in a very specific place on the computer (in this case the cloud

server we have dialed into). It means R will automatically treat the location of your project file as the ‘working directory’ and makes importing and exporting easier³.

3.4.1 Organise

Now we are going to organise our workspace, first its always a good first step to go to Tools > Project options and switch all of the options for saving and loading .Rdata to ‘No’

Then we create the following folders:

- data
- scripts
- figures



Make sure you type these **exactly** as printed here - remember that to R is case-sensitive so ‘data’ and ‘Data’ are two different things!

Having these separate subfolders within our project helps keep things tidy, means it’s harder to lose things, and lets you easily tell R exactly where to go to retrieve data.

Now do you remember where you saved the ‘penguin_data.csv’ file? I hope so!!! Go to the upload button in the Files tab of RStudio Cloud and tell it where the file is located on your local computer and upload it to the ‘data’ folder you have made in your Project.

3.4.2 Create a new R script

Let’s now create a new R script file in which we will write instructions and store comments for manipulating data, developing tables and figures. Use the File > New Script menu item and select an R Script.

Add the following:

```
# An analysis of the bill dimensions of male and female Adelie, Gentoo and Chinstrap penguins
# Data first published in Gorman, KB, TD Williams, and WR Fraser. 2014. "Ecological ...
```

Then load the following add-on package to the R script, just underneath these comments. Tidyverse isn’t actually one package, but a bundle of many different packages that play well together - for example it *includes ggplot2* which we used in the last session, so we don’t have to call that separately

³More on projects can be found in the R4DS book (<https://r4ds.had.co.nz/workflow-projects.html>)

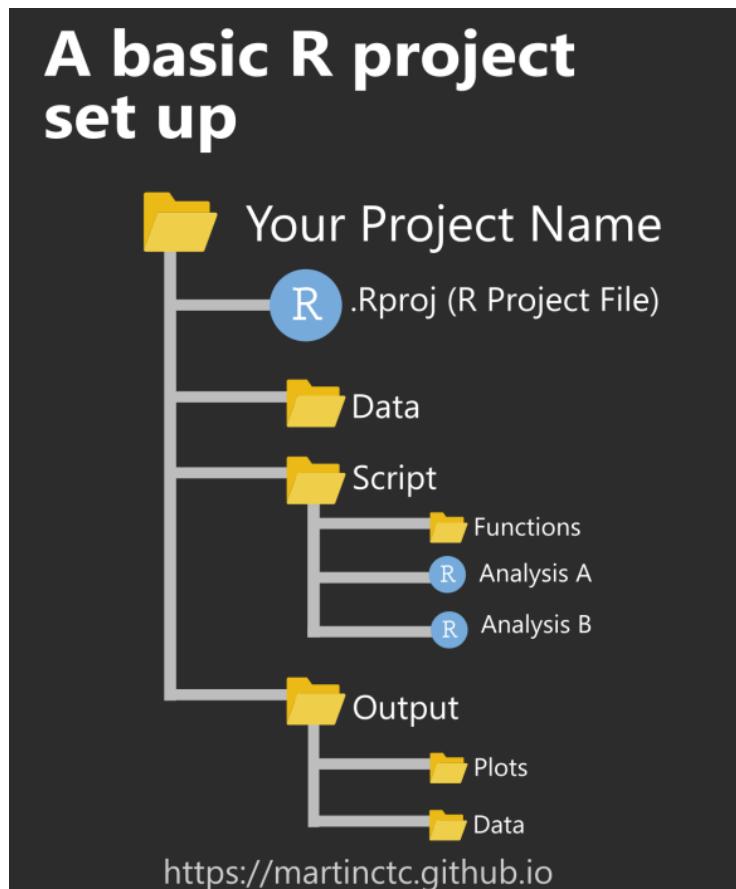


Figure 3.3: An example of a typical R project set-up

```
library(tidyverse) # tidy data packages
library(janitor) # cleaning variable names
library(lubridate) # make sure dates are processed properly
```

Now save this file in the scripts folder and call it *penguin_measurements.R*

3.5 Get the data into R

Now we are *finally* ready to import the data into R.

Add the following code to your script, then starting at line 1 - ask your script to run in order, library function first as the `read_csv()` function is from an add-on package `readr`

```
# read in the data from the data folder in my project
penguins <- read_csv("data/penguins_data.csv")
```

Error!

Houston we have a problem! We got an error!!! blah blah does not exist in current working directory

This usually happens if we told R to look in the wrong place, or didn't give it the correct name of what to look for. Can you spot what the mistake was?

Edit your existing line of script to replace it with the proper file name and run the command again.

```
# read in the data from the data folder in my project
penguins <- read_csv("data/penguins_data.csv")
```

```
Parsed with column specification:
cols(
  studyName = col_character(),
  `Sample Number` = col_double(),
  Species = col_character(),
  Region = col_character(),
  Island = col_character(),
  Stage = col_character(),
  `Individual ID` = col_character(),
  `Clutch Completion` = col_character(),
  `Date Egg` = col_character(),
  `Culmen Length (mm)` = col_double(),
  `Culmen Depth (mm)` = col_double(),
  `Flipper Length (mm)` = col_double(),
```

```

`Body Mass (g)` = col_character(),
Sex = col_character(),
`Delta 15 N (o/oo)` = col_double(),
`Delta 13 C (o/oo)` = col_double(),
Comments = col_character()
)

```

Great, no error this time, the `read_csv()` function has read the data and we assigned this data to the object `penguins`. If you look in the environment pane you should see the object `penguins`.

The lines printed in the R console tell us the names of the columns that were identified in the file and the type of variable R thinks it is

- `col_character()` means the column contains letters or words
- `col_double()` means the column contains numbers



Have a look - do all of these seem correct to you? If not we should fix these, and we will get onto that in a little bit.

3.5.1 View and refine

So we know our data is in R, and we know the columns and names have been imported. But we still don't know whether all of our values imported, or whether it captured all the rows.

There are lots of different ways to view and check data. One useful method is `glimpse`

```
# check the structure of the data
glimpse(penguins)
```

When we run `glimpse()` we get several lines of output. The number of observations “rows”, the number of variables “columns”. Check this against the csv file you have - they should be the same. In the next lines we see variable names and the type of data.

We should be able to see by now, that all is not well!!! `Body Mass (g)` is being treated as character (string), as is `Date Egg`, meaning R thinks these contain letters and words instead of numbers and dates.

Other ways to view the data

- type `penguins` into the Console
- type `view(penguins)` this will open a spreadsheet in a new tab

- type `head(penguins)` will show the first 10 lines of the data, rather than the whole dataset

**Note - `view()` lets you do cool stuff like reorder rows and quickly scroll through the dataset without affecting the underlying data.

3.5.2 Data management

We've imported the data, checked it and found some inconsistencies.

This is where we start to use some of the core functions from `tidyverse`.

If R thinks `Body Mass (g)` is a character variable, then it probably contains some words as well as numbers. So let's have a look at this and compare it to a variable which has been processed correctly

```
# get the first 10 rows of the Flipper Length and Body Mass variables
penguins %>%
  select(`Flipper Length (mm)`,
         `Body Mass (g)`) %>%
  head(10) # 10 rows
```

A tibble: 10 x 2

Flipper Length (mm)	Body Mass (g)
---------------------	---------------

181	3750
186	3800
195	3250
NA	na
193	3450
190	3650
181	3625
195	4675
193	3475
190	4250

1-10 of 10 rows

**Note - What is the `%>%` ??? It's known as a pipe. It sends the results of one line of code to the next. So the data `penguins` is sent to the `select` function which picks only those variables we want.

The result of this is then sent to the `head` function which with the argument for number of rows set to 10, prints the top 10 rows.

The other way to write this series of functions would be to use brackets and the rules of BODMAS:

```
head(select(penguins, `Flipper Length (mm)`, `Body Mass (g)`),10)
```

Hopefully you agree that the pipes make the code a lot more human-reader friendly. More on pipes later

So what's the problem with our data? in the Flipper length variable, missing observations have been correctly marked as `NA` signifying missing data. R can handle missing data just fine. However, in the Body mass variable, it looks as though someone has actually typed “na” in observations where the data is missing. Here R has failed to recognise an `NA` and has read it as a word instead. This is because `read_csv()` looks for gaps or `NA` but not “na”.

No problem this is an easy fix. Head back to your script and make the following edit the line for data importing

```
# read in the penguins data, specify that NA strings can be "na" or "NA"
penguins <- read_csv("data/penguin_data.csv", na=c("na", "NA"))
```

Now re-check your data using the same lines of code from before. All ok now?

3.6 Dataframes and tibbles

A quick sidebar on how R stores data. When we imported the data into R it was put into an object called a **tibble** which is a type of **dataframe**.

Let's have a quick go at making our own **tibble** from scratch.

Make a new script called ‘TibbleTrouble.R’ in the scripts folder as before.

At the top of this new script put

```
# just me messing about making tibbles

# libraries
library(tidyverse)

# make some variables, when we have a one dimensional object like this it is known as an atomic vector
person <- c("Mark", "Phil", "Becky", "Tony")
hobby <- c("kickboxing", "coding", "dog walking", "car boot sales")
awesomeness <- c(1,100,1,1)
```

The function `c` lets you ‘concatenate’ or link each of these arguments together into a single vector.

Now we put these vectors together, where they become the variables in a new tibble

```
# make a tibble
my_data <- tibble(person, hobby, awesomeness)
my_data
```

	person	hobby	awesomeness
	<chr>	<chr>	<dbl>
1	Mark	kickboxing	1
2	Phil	coding	100
3	Becky	dog walking	1
4	Tony	car boot sales	1

Have a go at messing about with your script and figure out what each of these does, add comments and save your script.

```
# Some R functions for looking at tibbles and dataframes I will comment next to each one

head(my_data, n=2)
tail(my_data, n=1)
nrow(my_data)
ncol(my_data)
colnames(my_data)
view(my_data)
glimpse(my_data)
str(my_data)
```

3.7 Clean and tidy

Back to your penguins script.

We have checked the data imported correctly, now its time to *clean and tidy* the data.

3.7.1 Tidy

In this example our data is already in `tidy` format i.e. one observation per row. We will cover what to do if data isn’t tidy later.

3.7.2 Clean

Here are a few things we might want to do to our data to make it ‘clean’.

- Refine variable names
- Format dates and times
- Rename some values
- Check for any duplicate records
- Check for any implausible data or typos
- Check and deal with missing values

**Note - Remember because we are doing everything in a script, the original data remains unchanged. This means we have data integrity, and a clear record of what we did to tidy and clean a dataset in order to produce summaries and data visuals

3.7.3 Refine variable names

Often we might want to change the names of our variables. They might be non-intuitive, or too long. Our data has a couple of issues:

- Some of the names contain spaces
- Some of the names contain brackets

Let’s correct these quickly

```
#clean all variable names to snake_case using the clean_names function from the janitor package
# note we are using assign <- to overwrite the old version of penguins with a version that has up
# this changes the data in our R workspace but not the original csv file

penguins <- penguins %>% #
  janitor::clean_names()

colnames(penguins) # quickly check the new variable names
```

**Note - in this example you can see that I put the name of the package `janitor`, in front of the function `clean_names`. But if I have already loaded the package with `library(janitor)` then this isn’t strictly necessary and `penguins %>% clean_names()` would also have worked just fine. So why do this? Remember when we loaded the tidyverse package - we got a warning about conflicts - this was because two of our packages `dplyr` and `stats` have functions that are different, but share the same name. But we can make sure we call the one we want by specifying which package we are calling a

function from. We don't need to do this very often, but it's good to know.

```
> library(tidyverse)
-- Attaching packages ----- tidyverse 1.3.0 --
v ggplot2 3.3.2     v purrr    0.3.4
v tibble   3.0.4     v dplyr    1.0.2
v tidyr    1.1.2     v stringr  1.4.0
v readr    1.3.1     v forcats 0.5.0
-- Conflicts ----- tidyverse_conflicts() --
x dplyr::filter() masks stats::filter()
x dplyr::lag()    masks stats::lag()
```

The `clean_names` function quickly converts all variable names into snake case. The N and C blood isotope ratio names are still quite long though, so let's clean those with `dplyr::rename()` where “new_name” = “old_name”.

```
# shorten the variable names for N and C isotope blood samples

penguins <- penguins %>%
  rename("delta_15n"="delta_15_n_o_oo", # use rename from the dplyr package
        "delta_13c"="delat_13_c_o_oo")
```

3.7.4 Dates

We can also see there is a `date_egg` variable. If you check it (using any of the new functions you have learned), you should see that it all looks like its been inputted correctly, but R is treating it as words, rather than assigning it a date value. We can fix that with the `lubridate` package. If we use the function `dmy` then we tell R this is date data in date/month/year format.

```
# use dmy from stringr package to encode date properly
penguins <- penguins %>%
  mutate(date_egg_proper=dmy(date_egg))
```



What is the deliberate mistake in my code comment above? By now you may have picked up there are the odd mistakes (possibly some non-deliberate ones) - to make sure you aren't just copy-pasting on autopilot.

Here we use the `mutate` function from `dplyr` to create a new variable called `date_egg_proper` based on the output of converting the characters in `date_egg` to date format. The original variable is left intact, if we had specified the “new” variable was also called `date_egg` then it would have overwritten the original variable.

3.7.5 Rename some values

Sometimes we may want to rename the values in our variables in order to make a shorthand that is easier to follow.

```
# use mutate and case_when for an if-else statement that changes the names of the values in a variable
penguins <- penguins %>%
  mutate(species = case_when(species == "Adelie Penguin (Pygoscelis adeliae)" ~ "Adelie",
                             species == "Gentoo penguin (Pygoscelis papua)" ~ "Gentoo",
                             species == "Chinstrap penguin (Pygoscelis antarctica)" ~ "Chinstrap")
```

3.7.6 Check for duplication

It is very easy when inputting data to make mistakes, copy something in twice for example, or if someone did a lot of copy-pasting to assemble a spreadsheet (yikes!). We can check this pretty quickly

```
# check for duplicate rows in the data
penguins %>%
  duplicated() %>% # produces a list of TRUE/FALSE statements for duplicated or not
  sum() # sums all the TRUE statements
```

[1] 0

Great!

3.7.7 Check for typos or implausible values

We can also explore our data for very obvious typos by checking for implausibly small or large values

```
# use summarise to make calculations
penguins %>%
  summarise(min=min(body_mass_g, na.rm=TRUE),
            max=max(body_mass_g, na.rm=TRUE))
```

The minimum weight for our penguins is 2.7kg, and the max is 6.3kg - not outrageous. If the min had come out at 27g we might have been suspicious. We will use `summarise` again to calculate other metrics in the future.

**Note - our first data insight, the difference the smallest adult penguin in our dataset is nearly half the size of the largest penguin.

We can also look for typos by asking R to produce all of the distinct values in a variable. This is more useful for categorical data, where we expect there to be only a few distinct categories

```
penguins %>%
  distinct(sex)
```

Here if someone had mistyped e.g. ‘FMALE’ it would be obvious. We could do the same thing (and probably should have before we changed the names) for species.

3.7.8 Missing values: NA

There are multiple ways to check for missing values in our data

```
# Get a sum of how many observations are missing in our dataframe
penguins %>%
  is.na() %>%
  sum()
```

But this doesn’t tell us where these are, fortunately the function `summary` does this easily

```
# produce a summary of our data
summary(penguins)
```

This provides a quick breakdown of the max and min for all numeric variables, as well as a list of how many missing observations there are for each one. As we can see there appear to be two missing observations for measurements in body mass, bill lengths, flipper lengths and several more for blood measures. We don’t know for sure without inspecting our data further, *but* it is likely that the two birds are missing multiple measurements, and that several more were measured but didn’t have their blood drawn.

We will leave the NA’s alone for now, but it’s useful to know how many we have.

We’ve now got a clean & tidy dataset!!

3.8 Summing up

3.8.1 What we learned

There was a lot of preparation here, and we haven’t really got close to make any major insights. But you have:

- Organised your project space
- Dealt with file formats
- Put data into a specific location and imported into R

- Checked the data import
- Cleaned and tidied the data

You have also been introduced to the `tidyverse` and two of its packages

- `readr` Wickham et al. (2021d)
- `dplyr` Wickham et al. (2021c)

As well as:

- `janitor` Firke (2021)
- `lubridate` Spinu et al. (2021)

3.8.2 Quitting



Remember to `save` your RScript before you leave. And ideally don't save your .Rdata!

- Close your RStudio Cloud Browser
- Go to Blackboard to complete this week's quiz!

Chapter 4

Workflow Part Two: Week Three

Last week we worked through the journey of importing and tidying data to produce a clean dataset.

It's important to remember what questions you have about the data collected, and to make an outline about what you want to do.



Now is a good time to think about what figures might we want to produce from our data?

We are mostly interested in observable ‘differences’ between our three penguin species. What sort of figures might illustrate that?

Sometimes it’s good to get a pen/pencil and paper - and sketch the figure you might want to make.

4.1 Initial insights

Let's start with some basic insights, perhaps by focusing on further questions about specific variables.

- How many penguins were observed?
- How many Adelie, Gentoo and Chinstrap penguins
- What is the distribution of morphologies such as bill length, body size, flipper length

Some of these are very simple in that they are summaries of single variables. Some are more complex, like evaluating the numbers of males and females which

are the two groups in the sex variable.

These are ‘safety-checking’ insights. You might already know the answers to some of these questions because you may have been responsible for collecting the data. Checking your answers against what you expect is a good way to check your data has been cleaned properly.

4.2 Numbers and sex of the penguins

```
# how many observations of penguins were made

penguins %>%
  summarise(n())

# but there are multiple observations of penguins across different years
# n_distinct deals with this

penguins %>%
  summarise(n_distinct(individual_id))
```

The answer we get is that there are 190 different penguins observed across our multi-year study.

This answer is provided in a tibble, but the variable name is an ugly composition of the functions applied, but we can modify the code

```
penguins %>%
  summarise(num_penguin_id=n_distinct(individual_id))
```

How about the number of penguins observed from each species?

```
penguins %>%
  group_by(species) %>% # ask for distinct counts in each species
  summarise(num_penguin_id=n_distinct(individual_id))
```

By adding the `group_by` function we tell R to apply any subsequent functions separately according to the group specified. Let’s do this again for male and female penguins

```
penguins %>%
  group_by(sex) %>% # ask for distinct counts in each species
  summarise(num_penguin_id=n_distinct(individual_id))
```

Now what about the number of female Gentoo penguins?

```
penguins %>%
  group_by(sex, species) %>% # ask for distinct counts in each species
  summarise(num_penguin_id=n_distinct(individual_id))
```

Now we have a table that shows each combination of penguin species by sex, we can see for example that 65 unique Male Adelie penguins were observed in our study. We can also see that for 6 Adelie and 5 Gentoo penguins, sex was not recorded.

****Note -** You have just had a crash course in using the `pipe` and `dplyr` to produce quick data summaries. Have a go at making some other summaries of your data, perhaps the numbers of penguins by island or region. Try some combinations.

4.2.1 Making a simple figure

Let's translate some of our simple summaries into graphs.

```
# make summary data and assign to an object with a sensible name we can use
penguin_species_sex <- penguins %>%
  group_by(sex, species) %>% # ask for distinct counts in each species
  summarise(num_penguin_id=n_distinct(individual_id)) %>%
  drop_na() # remove the missing data
```

```
# basic ggplot function to make a stacked barplot
penguin_species_sex %>%
  ggplot(aes(x=species,
             y=num_penguin_id,
             fill=sex))+
```

We will cover how `ggplot` works in more detail next week. But in brief, to get the graph we first use the `ggplot` function, we give `ggplot` the ‘aesthetic mappings’ of the plot, the values we wish to assign to the x axis, y axis and how we want to “fill in” the objects we will draw.

This first line of code will just draw a blank plot, with the *plus + sign* we signify that we want to add a new layer, this builds on top of the first layer and by specifying the function `geom_col` we request columns are layered onto the plot. This layer inherits all of the specifications of x,y and fill from `ggplot()`. And it produces a handy legend.

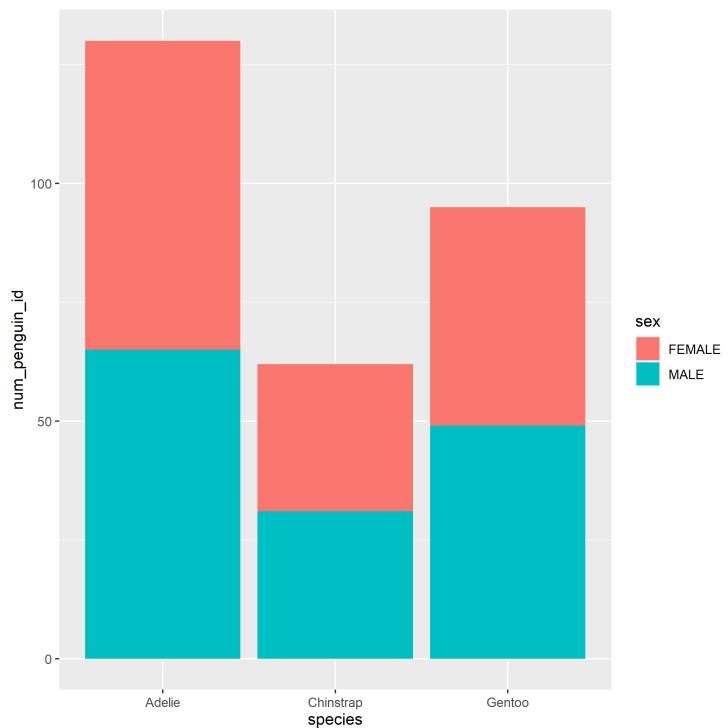


Figure 4.1: A first insight the number of male and female penguins of each species in our study

4.2.2 Challenge



Can you write and run a script, with appropriate comments, that produces a summary tibble and graph for the number of penguins of each species, on the three study islands?

4.3 Distributions

We now know how many penguins were surveyed. Let's move on to look at some distributions. One of our interests was the size of bill lengths, so let's look at the distribution of values in this variable.

Looking at frequency distributions is very *useful* because it shows the shape of the *sample distribution*, that shape is very important for the types of formal statistics we can do later.

Here is the script to plot frequency distribution, as before we pipe the data into ggplot. This time we only specify an x variable because we intend to plot a histogram and the y variable is always the count of observations. We then ask for the data to be presented in 50 equally sized bins of data. So in this case we have chopped the range of the x axis into 50 equal parts and counted the number of observations that fall within each one.

```
penguins %>%
  ggplot(aes(x=culmen_length_mm)) +
  geom_histogram(bins=50)
```

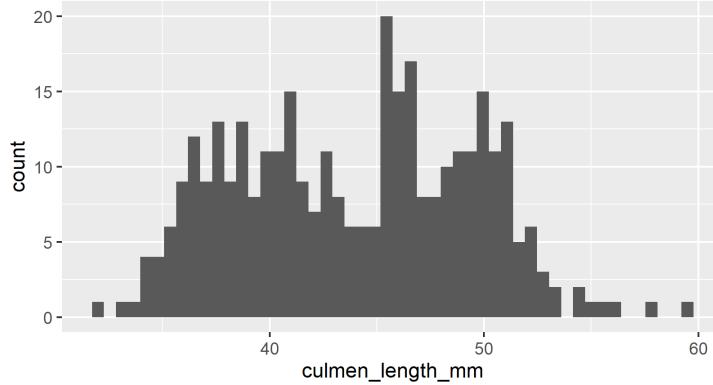


Figure 4.2: Frequency distribution of culmen length in penguins

** Note - Bins. Have a go at changing the value specified to the bins argument, and observe how the figure changes.

4.3.1 Insights



- Are you surprised at all by the distribution? We have drawn a continuous variable from a natural population, what did you expect the distribution to look like?
- Can you change the code for the histogram plot to produce distributions for each sex?
- How has this changed your interpretation of the distributions?

4.4 More distributions

From the figures you have made, you should be able to make some guesses about the means and medians of the data. But we can use `dplyr` to get more accurate answers.

```
# generate the mean, median and standard deviation of culmen length in three species of penguins
penguins %>%
  group_by(species) %>%
  summarise(mean_culmen_length=mean(culmen_length_mm),
            median_culmen_length=median(culmen_length_mm),
            sd_culmen_length=sd(culmen_length_mm))
```

Huh??? What's going on??? We get NAs because there are NAs in our dataset any calculation involving nothing produces nothing. Try this

```
# messing about with NA calculations
4+NA
3*NA
NA^2
(1+2+NA)/2
```

We need to remember to specify the argument to remove NA

```
# generate the mean, median and standard deviation of culmen length in three species of penguins
# specify the removal of NA values
penguins %>%
  group_by(species)
```

```
summarise(mean_culmen_length=mean(culmen_length_mm, na.rm=TRUE),
          median_culmen_length=median(culmen_length_mm, na.rm=TRUE),
          sd_culmen_length=sd(culmen_length_mm, na.rm=TRUE))
```

The mean and median values for each species are *very* similar, which indicates we do not have much *skew* in our data. This detail is important because statistical analyses make lots of assumptions about the underlying distributions of the data.

4.4.1 Initial conclusions

In these data we are already able to make some useful insights

- Fewer Chinstrap penguins were surveyed than Adelie or Gentoo's
- The average bill length for Adelie penguins is 38.8 mm, on average 8.7mm shorter than Gentoo's and 10mm shorter than Chinstrap's
- There does not appear to be much difference between Chinstrap and Gentoo bill lengths (on average 1.3mm)

These might appear to be modest insights - but have learned several data manipulation and summary techniques. We can also start to take a look at some of our initial hypotheses!!!



- How does this data stack up against the hypothesis about bill morphology we put forward last week?

To make more and conclusive insights we have a bit further to go, but I think you deserve a pat on the back

```
# R generate some praise
praise::praise()
```

4.5 Data transformation

In the previous section we made some basic insights into observation numbers, distributed by species, sex and location. We also started to gain core insights into some of our central hypotheses, but you have probably noticed we don't actually have a variable on the **relative bill lengths/depths**. Why is this important? Well we clearly saw there was a difference in bill lengths between our three species. But we haven't taken into account that some of these species might be very different in body size. Our measure of bill lengths as an indicator of feeding strategy, might be confounded by body size (a bigger penguin is likely to have a bigger bill).

We don't have a variable explicitly called body size. Instead we have to use "proxies" suitable proxies might be 'flipper length' or 'body mass'. Neither is perfect

- Flipper length
 - Pros: linked to skeletal structure, constant
 - Cons: relative flipper length could also vary by species
- Body mass
 - Pros: more of an indication of central size?
 - Cons: condition dependent, likely to change over the year

Let's take a look at the distribution of body mass among our three species.

```
# frequency distribution of body mass by species
penguins %>%
  ggplot(aes(x=body_mass_g, fill=species)) +
  geom_histogram(bins=50)
```

How does the distribution you have found compare with your insights on bill length? We can do this using the `cor_test` function from the package `rstatix` (Kassambara (2020)). This package contains a number of 'pipe-friendly' simple statistics functions

Add the `library()` for `rstatix` at the **top** of your RScript with an appropriate comment #

```
# a simple correlation test from the rstatix package
penguins %>%
  group_by(species) %>% # group by species
  cor_test(body_mass_g, culmen_length_mm) # correlation between body mass and bill len
```

We can see that these two variables 'co-vary' a lot, but this appears to be quite species specific. We can already make the insight that Chinstrap penguins appear to have the shortest bill length relative to body mass.

We can make a new variable that is the 'relative size of culmen length compared to flipper length'

```
# use mutate to produce a new variable that is a ratio of culmen length to flipper length
penguins <- penguins %>%
  mutate(relative_bill_length = culmen_length_mm/body_mass_g)
```

We are probably also interested in bill depth?

```
# use mutate to produce a new variable that is a ratio of culmen depth to flipper length
penguins <- penguins %>%
  mutate(relative_bill_depth = culmen_depth_mm/body_mass_g)

# check that these new variables have been included in the dataset
penguins %>%
  names()
```

4.6 Developing insights

First let's focus on the distributions of four variables of interest. Then we will progress on to look at their relationships and differences

- relative_bill_length
- relative_bill_depth
- delta_15n
- delta_13c

4.6.1 Distributions of the relative bill length

We will examine the shape of the *sample distribution* of the data again by using histograms.

```
# frequency distribution of relative bill length by species
# we already know we are interested in looking at the distributions 'within' each species
penguins %>%
  ggplot(aes(x=relative_bill_length, fill=species))+
  geom_histogram(bins=50)
```

Important questions, what shape is the distribution?

- First there must be a lower limit of zero (penguins cannot have negative length bills), does this lead to any truncating of the expected normal distribution bell curve? It doesn't look it.
- Is it symmetrical? Mostly.

If it's a little difficult to see - we can separate out these figures using the handy function `facet_wrap`

```
# frequency distribution of relative bill length by species
# we already know we are interested in looking at the distributions 'within' each species
penguins %>%
```

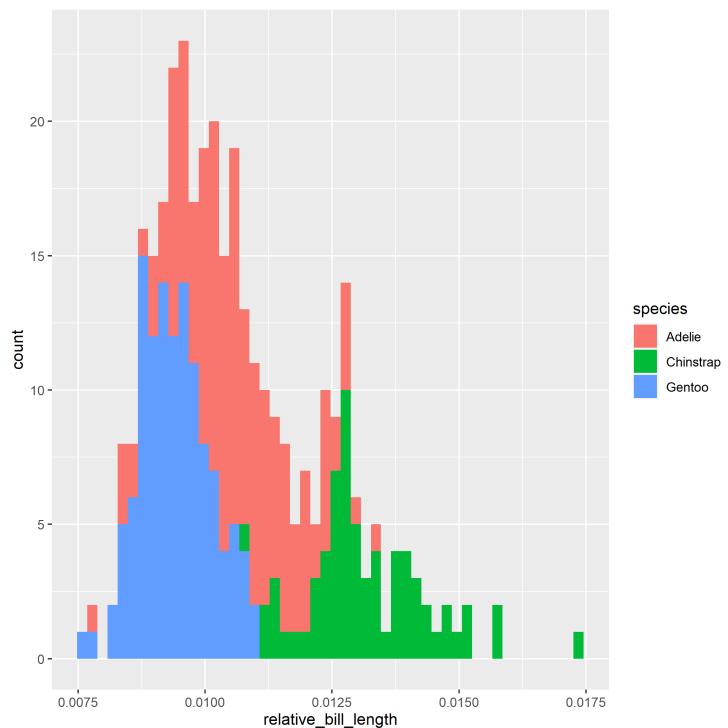
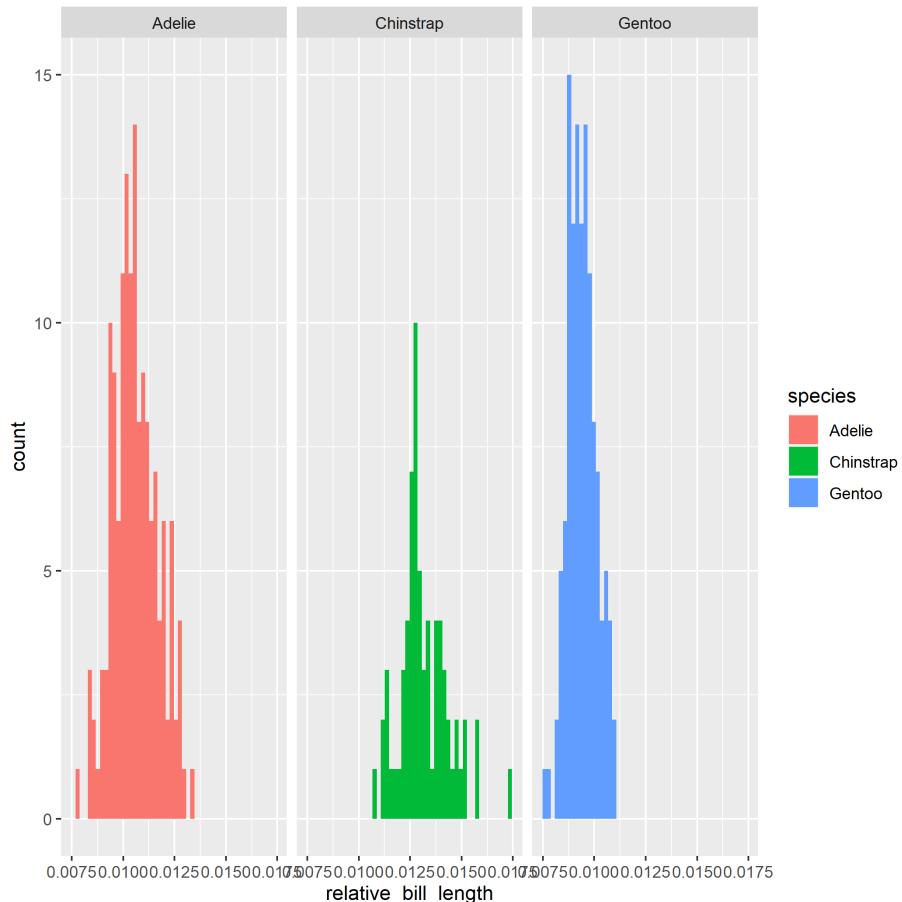


Figure 4.3: Frequency distribution of relative bill length in three species of penguins, Adelie, Chinstrap and Gentoo

```
ggplot(aes(x=relative_bill_length, fill=species))+  
  geom_histogram(bins=50)+  
  facet_wrap(~species) # facet wrap to look at the separate species more easily
```



\begin{figure}
\caption{Frequency distribution of relative bill length in three species of penguins, Adelie, Chinstrap and Gentoo - histograms split into three panes by facet_wrap} \end{figure}



Looking at these distributions, how do you think the mean & median values will compare in these three species? Think about it first - then run the code below.

```
# Mean and Median summaries  

penguins %>%
```

```

group_by(species) %>%
summarise(mean_relative_bill_length=mean(relative_bill_length, na.rm=TRUE),
median_relative_bill_length=median(relative_bill_length, na.rm=TRUE))

# A tibble: 3 x 3
  species   mean_relative_bill_length median_relative_bill_length
  <chr>           <dbl>                  <dbl>
1 Adelie        0.0106                 0.0105
2 Chinstrap     0.0132                 0.0129
3 Gentoo       0.00941                0.00939

```

We can see that the mean and median values are almost identical for each species. This indicates we *aren't* dealing with a lot of skew, this is important for when using statistics, which are based on a lot of assumptions like normal distribution.



Can you **repeat** these steps for the variable `relative_bill_depth`, `delta_15n` and `delta_13c` - add all appropriate comments and commands to your R Script.

4.7 Relationship/differences

Getting proper data insights involves looking for relationships or differences. Remember, if we have a manipulated variable in a well-designed experiment, we may be able to identify a causal effect. With a study without this manipulation, like this penguin study - we cannot be sure any relationships or differences are causal. We have to include some caution in our interpretations.

4.7.1 Differences

We have already looked at frequency distributions of the data, where it is possible to see differences. However we can use `ggplot` and `geom_point` to produce difference plots.

```

# specifying position with a jitter argument positions points randomly across the x axis
penguins %>%
  ggplot(aes(x=species,
             y=culmen_length_mm,
             colour=species))+ # some geoms use color rather than fill to specify colour
  geom_point(position=position_jitter(width=0.2))

```

**Note - try altering the width argument and see how it affects the output of the plot. We will do a deeper dive into `ggplot` later.

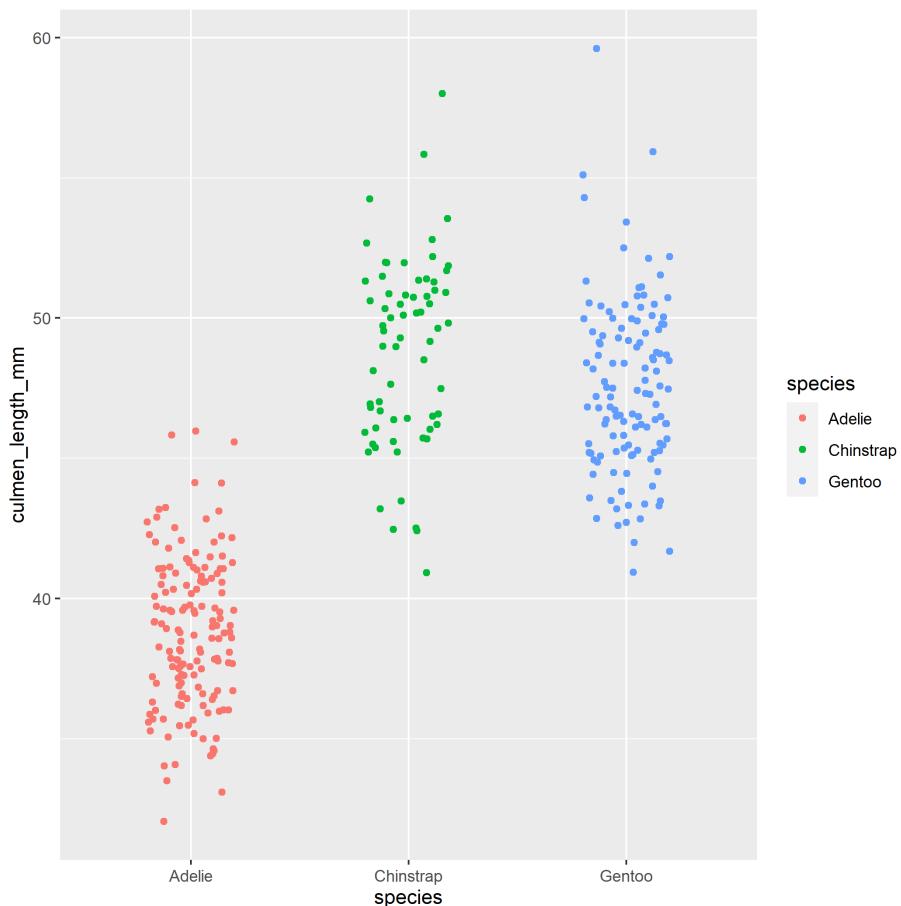


Figure 4.4: Differences in relative bill length of three different species of Antarctic Penguin.



Try producing these figures for the `culmen_depth_mm`, `delta_15n` and `delta_13c` variables as well.

What are your observations of the relative differences?

4.7.2 Associations

It might also be of interest to look at whether any of our variables of interest are strongly associated. For example what is the relationship between heavy carbon/heavy nitrogen isotopes?

```
penguins %>%
  ggplot(aes(x=delta_15n, y=delta_13c, colour=species)) +
  geom_point() +
  geom_smooth(method="lm", # produces a simple regression line
              se=FALSE)      # no standard error intervals
```

We can see here that the association between these isotopes varies a lot by different species, there is probably quite a complex relationship here. It also indicates we should probably investigate these two isotopes separately.

- Have a look at the relative bill length and depth relationships as well

4.8 Sex interactions

Now let's concern ourselves with interactions. We have *already* seen how our interpretation of certain variables can be heavily altered if we don't take into account important contexts - like morphology in relation to species.

Thinking about sensible interactions takes patience and good biological understanding, the payoff is it can produce unique insights.

Focusing on relative bill length, we have seen there are differences between species, however we have not considered the potential association of sex. In many species males and females are 'dimorphic' and this has the potential to influence our observations if:

- sex has a substantial/bigger effect on morphology than species
- uneven numbers of males/females were scored in our studies

Using `summarise group_by` and `n_distinct` you should quickly be able to check the numbers of males and females surveyed within each species.

```
# A tibble: 6 x 3
# Groups:   species [3]
  species   sex   num_penguin_id
```

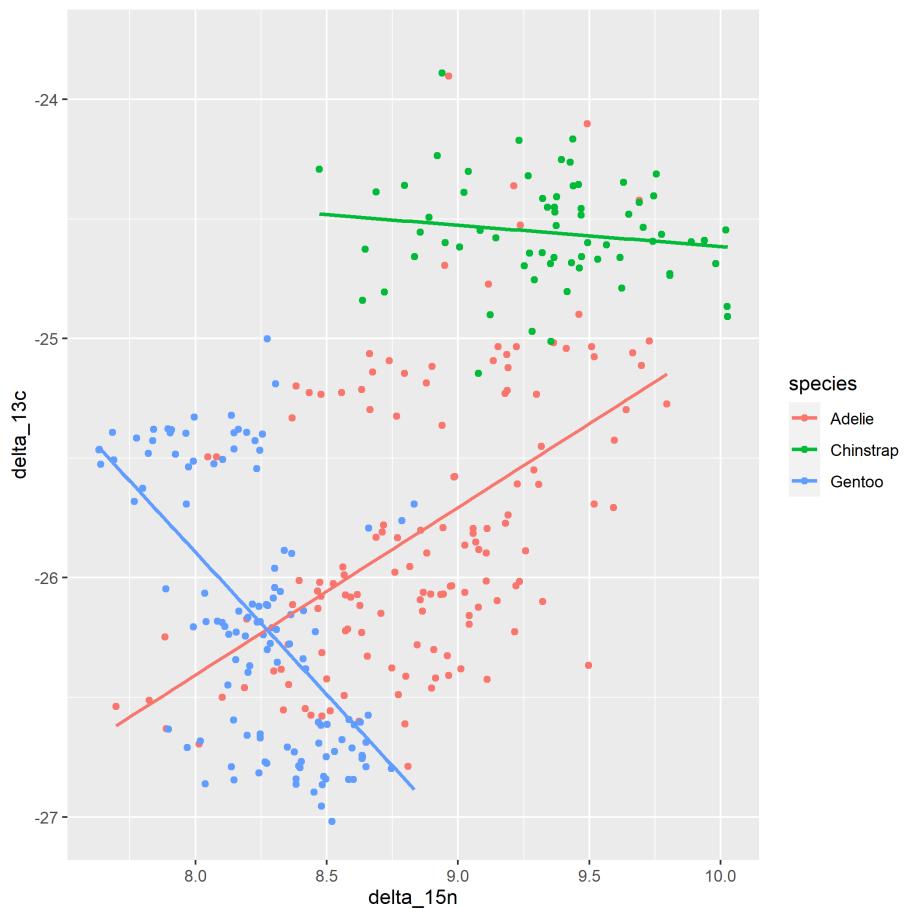


Figure 4.5: Asssocation between heavy Nitrogen and heavy Carbon isotopes in blood samples from three Antarctic Penguin species

	<chr>	<chr>	<int>
1	Adelie	FEMALE	65
2	Adelie	MALE	65
3	Chinstrap	FEMALE	31
4	Chinstrap	MALE	31
5	Gentoo	FEMALE	46
6	Gentoo	MALE	49

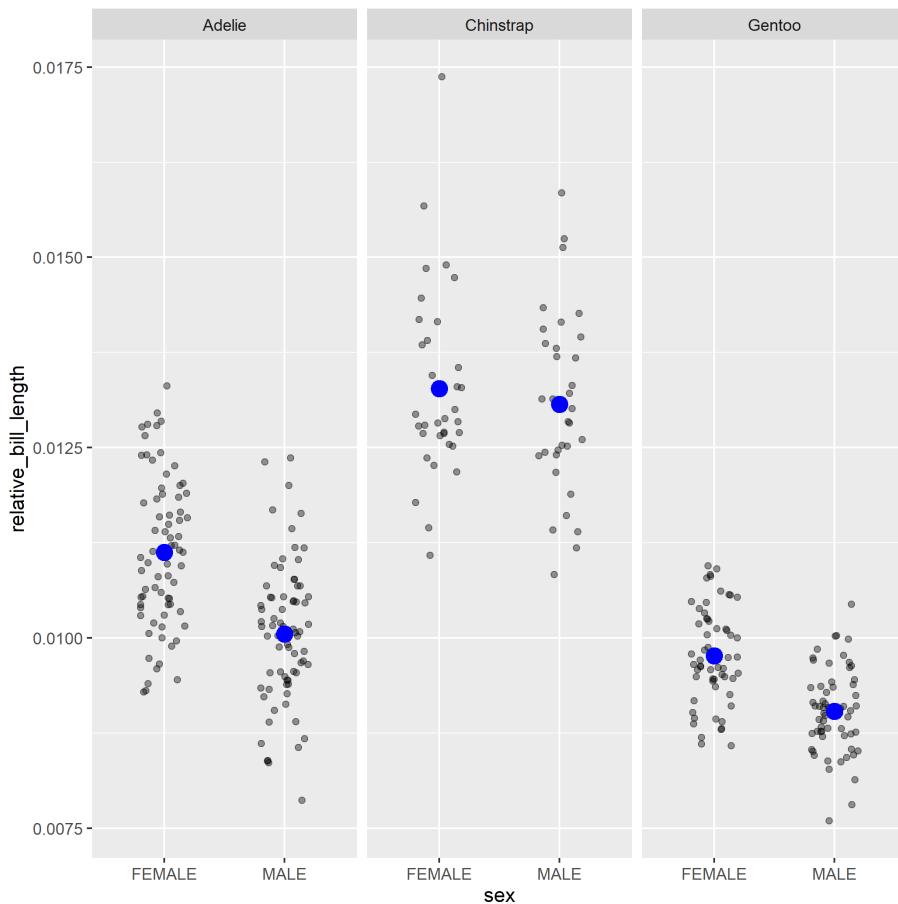
Looks like numbers are even, which is good. Again focusing on relative bill length let's generate some figures breaking down this variable by sex and species.

First let's generate some more simple summary stats - this time we are assigning it to an object to use later

```
penguin_stats <- penguins %>%
  group_by(sex, species) %>%
  summarise(mean_relative_bill_length=mean(relative_bill_length, na.rm=TRUE))
```

Now we are going to make our first figure which includes both raw and summary data. Copy and run the code below, and see if you can add comments next to the arguments you are unfamiliar with about what they might be doing.

```
penguins %>%
  drop_na(sex) %>%
  ggplot(aes(x=sex,
             y=relative_bill_length))+
  geom_jitter(position=position_jitter(width=0.2),
              alpha=0.4)+
  geom_point(data=penguin_stats, aes(x=sex,
                                      y=mean_relative_bill_length),
             size=4,
             color="blue")+
  facet_wrap(~species)
```



> Note - we could be producing something much simpler, like a box and whisker plot. It is often better to plot the data points rather than summaries of them

Imagine a line connects the two blue dots in each facet, these blue dots are the mean values. The slope of the line (if we drew them) would be downwards from females to males, indicating that on average females are larger. But the slope would not be very large. If we drew slope between *each* of the three female averages these would be much steeper. So we can say that size ‘on average’ varies more *between* species than *between sexes*.

See if you can make figures for all four of our variables of interest, then compare them to our initial hypotheses.

At this stage we do not have enough evidence to formally “reject” any of our null hypotheses, however we can describe the trends which appear to be present.

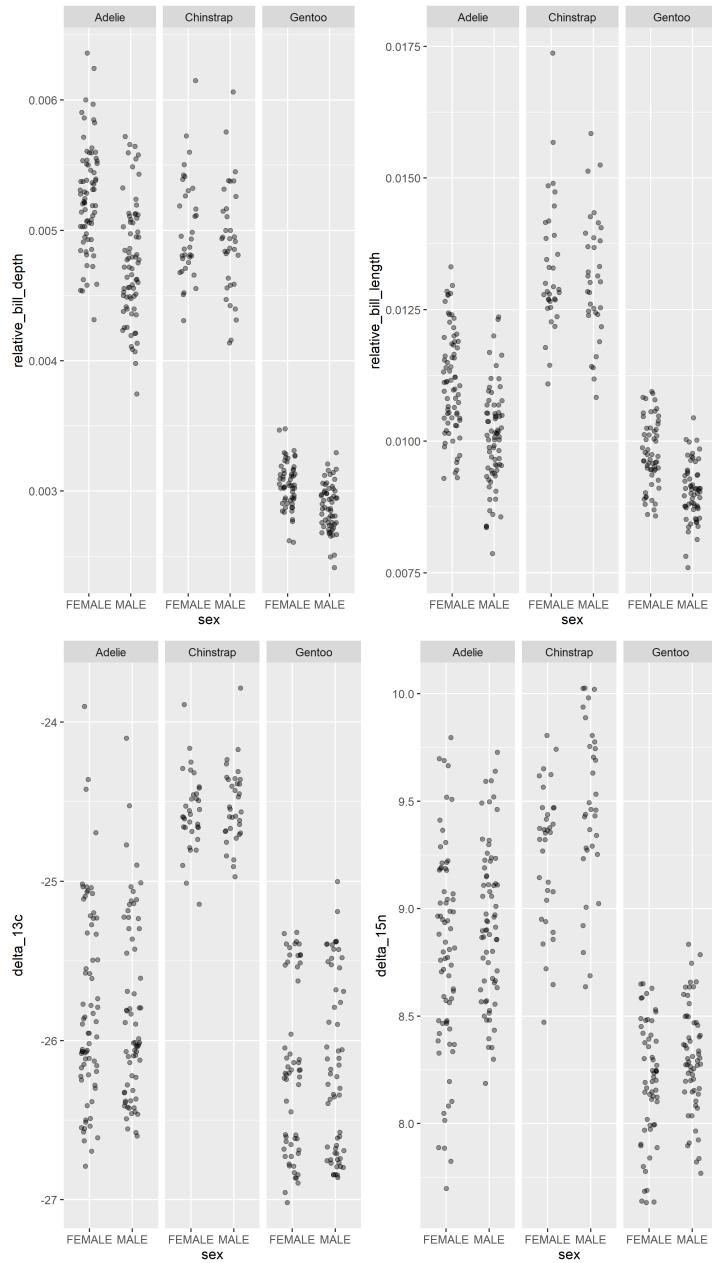


Figure 4.6: Difference in relative bill length and depth, and heavy carbon and nitrogen ratios, in male and female penguins from three different species - Adelie, Chinstrap and Gentoo

4.9 Making our graphs more attractive

We will dive into making attractive ggplots in more detail later. But let's spend a little time now fixing some of the more serious issues with our figures.

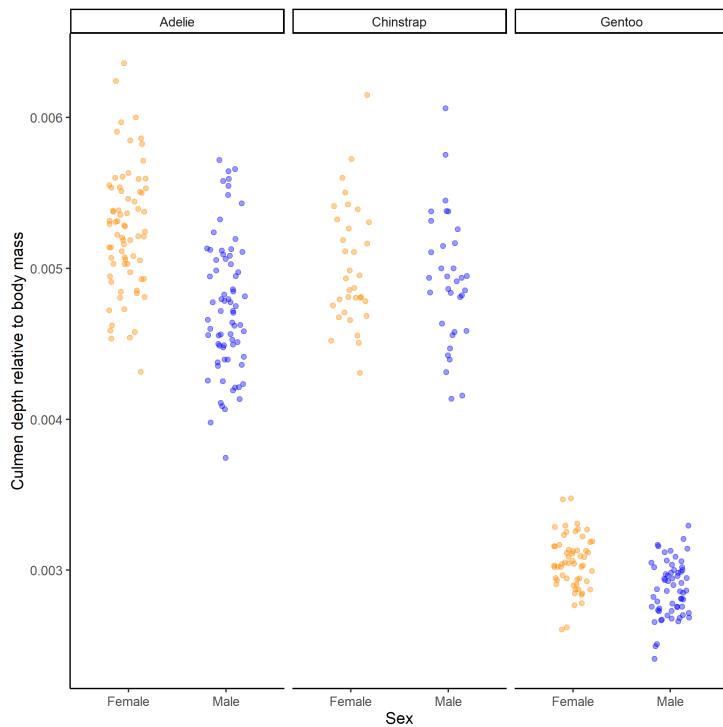
The aim with making a good figure, is that it is:

- Accurate - the figure must present the data properly, and not distort or mislead
- Beautiful - attractive figures will invite people to spend more time studying them
- Clear - a good figure should be able to stand on its own - see the answer or insight without prompting from the text

There are lots of things we could change here, but we will stick with the basics for now. We will:

- Change the axis labels
- Add some colours
- Remove the grey background

```
penguins %>%
  drop_na(sex) %>%
  ggplot(aes(x=sex,
             y=relative_bill_depth,
             colour=sex))+
  geom_jitter(position=position_jitter(width=0.2),
              alpha=0.4)+
  scale_color_manual(values=c("darkorange", "blue"))+
  facet_wrap(~species)+
  ylab("Culmen depth relative to body mass")+
  xlab("Sex")+
  scale_x_discrete(labels=c("Female", "Male"))+
  theme_classic()+
  theme(legend.position="none")
```



4.9.1 Saving your output

Now we have made a plot, that perhaps we think is worth saving. We can use the `ggsave` function. Just like when we imported data, when we output we specify a relative file path, this time we are saying where we want to ‘send’ the output. At the start of our project set-up we made a folder for figures to end up.

```
# save the last figure to a .png file in the figures folder
ggsave("figures/Jitter plot of relative culmen depth.png",
       dpi=300, # resolution
       width=7, # width in inches
       height=7)
```



Be careful here, if you ran this command again it would overwrite your previous file with a new output.

Check your Files tab - your image file should be saved in the appropriate folder

4.10 Quitting



Make sure you have saved your script!



Complete this week's Blackboard Quiz!

Chapter 5

ggplot2 A grammar of graphics: Week Four

5.1 Intro to grammar

The ggplot2 package is widely used and valued for its simple, consistent approach to making plots.

The ‘grammar’ of graphics relates to the different components of a plot that function like different parts of linguistic grammar. For example, all plots require axes, so the x and y axes form one part of the ‘language’ of a plot. Similarly, all plots have data represented between the axes, often as points, lines or bars. The visual way that the data is represented forms another component of the grammar of graphics. Furthermore, the colour, shape or size of points and lines can be used to encode additional information in the plot. This information is usually clarified in a key, or legend, which can also be considered part of this ‘grammar’.

The most common components of a ggplot are:

- aesthetics
- geometric representations
- facets
- coordinate space
- coordinate labels
- plot theme

We will cover each below.

The philosophy of ggplot is much better explained by the package author, Hadley Wickham (Wickham et al. (2021b)). For now, we just need to be aware that ggplots are constructed by specifying the different components that we want to display, based on underlying information in a data frame.

5.2 Building a plot

We are going to use the *simple* penguin data set contained in the `palmerpenguins` package (Horst et al. (2020)).



Make sure you have a folder structure for your project - scripts - data (won't be needed this week) - figures

Make sure your new script is in the scripts folder!

Throughout this practical you will be introduced to new R packages. Please remember to include all the `library()` calls at the TOP of your script.

And if you need to install a package, the command is `'install.packages("")'` - but DON'T include this in your script

```
library(palmerpenguins)
```

Let's check the first 6 rows of information contained in the data frame, using the `head()` function:

```
head(penguins)
```

```
# A tibble: 344 x 8
  species island   bill_length_mm bill_depth_mm flipper_length_mm body_mass_g sex
  <fct>   <fct>           <dbl>        <dbl>          <int>      <int> <fct>
1 Adelie  Torgersen     39.1         18.7          181       3750 male 
2 Adelie  Torgersen     39.5         17.4          186       3800 female
3 Adelie  Torgersen     40.3         18             195       3250 female
4 Adelie  Torgersen     NA            NA             NA        NA    NA
5 Adelie  Torgersen     36.7         19.3          193       3450 female
6 Adelie  Torgersen     39.3         20.6          190       3650 male 
7 Adelie  Torgersen     38.9         17.8          181       3625 female
8 Adelie  Torgersen     39.2         19.6          195       4675 male 
9 Adelie  Torgersen     34.1         18.1          193       3475 NA  
10 Adelie Torgersen      42            20.2          190       4250 NA 
# ... with 334 more rows
```

Here, we aim to produce a scatter plot

5.3 Plot background

To start building the plot, we first specify the data frame that contains the relevant data. We can do this in two ways

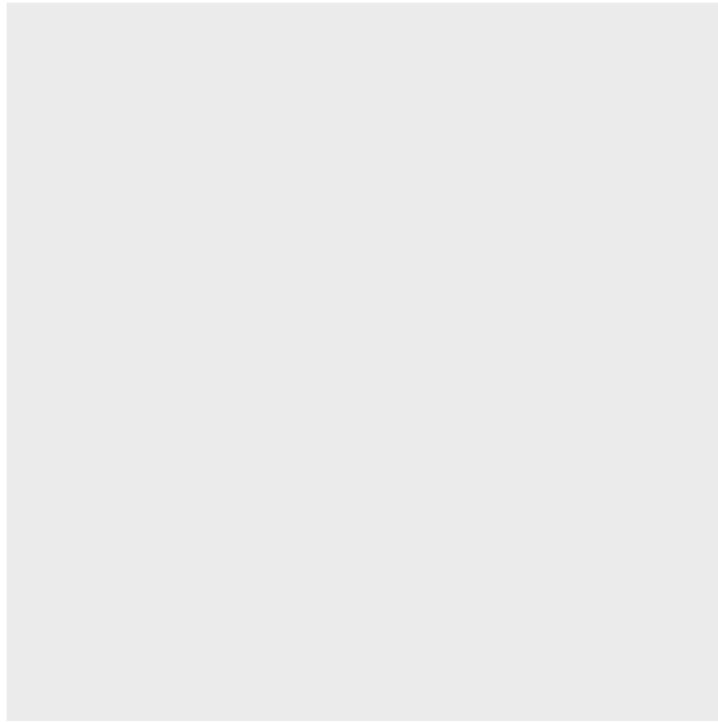
- 1) Here we are ‘sending the penguins data set into the `ggplot` function’:

```
# render the plot background
penguins %>%
  ggplot()
```

- 2) Here we are specifying the dataframe *within* the `ggplot()` function

```
ggplot(data=penguins)
```

The output is identical



> **Note -

Running this command will produce an empty grey panel. This is because we need to specify how different columns of the data frame should be represented in the plot.

5.4 Aesthetics - aes()

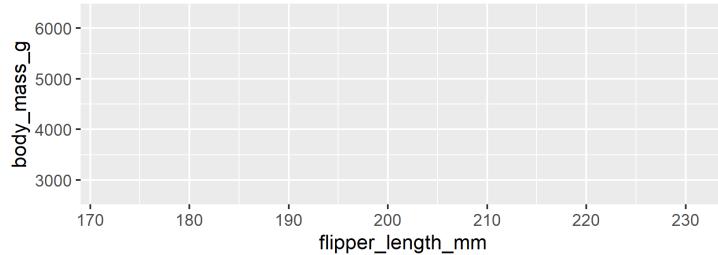
We can call in different columns of data from any dataset based on their column names. Column names are given as ‘aesthetic’ elements to the ggplot function, and are wrapped in the aes() function.

Because we want a scatter plot, each point will have an x and a y coordinate. We want the x axis to represent flipper length ($x = \text{flipper_length_mm}$), and the y axis to represent the body mass ($y = \text{body_mass_g}$).

We give these specifications separated by a comma. Quotes are not required when giving variables within aes().

**Note - Those interested in why quotes aren’t required can read about [non-standard evaluation] (<https://edwinth.github.io/blog/nse/>).

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g))
```



So far we have the grid lines for our x and y axis. ggplot knows the variables required for the plot, and thus the scale, but has no information about how to display the data points.

5.5 Geometric representations - geom()

Given we want a scatter plot, we need to specify that the geometric representation of the data will be in point form, using geom_point().

Here we are adding a layer (hence the + sign) of points to the plot. We can think of this as similar to e.g. Adobe Photoshop which uses layers of images that can be reordered and modified individually. Because we add to plots layer by layer **the order** of your geoms may be important for your final aesthetic design.

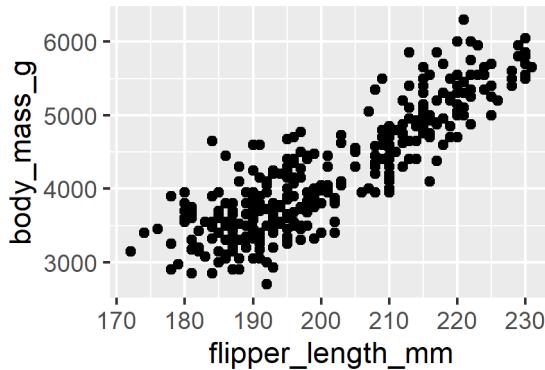
For ggplot, each layer will be added over the plot according to its position in the code. Below I first show the full breakdown of the components in a layer. Each layer requires information on

- data
- aesthetics
- geometric type
- any summary of the data
- position

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g)) +
  layer(
    geom="point",           # draw point objects
    stat="identity",        # each individual data point gets a geom (no summaries)
    position=position_identity()) # data points are not moved in any way e.g. we could specify j
```

This is quite a complicate way to write new layers - and it is more usual to see a simpler more compact approach

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g)) +
  geom_point() # geom_point function will always draw points, and unless specified otherwise the
```



Now we have the scatter plot! Each row (except for two rows of missing data) in the penguins data set now has an x coordinate, a y coordinate, and a designated geometric representation (point).

From this we can see that smaller penguins tend to have smaller flipper lengths.

5.6 %>% and +

ggplot2, an early component of the tidyverse package, was written before the pipe was introduced. The + sign in ggplot2 functions in a similar way to the

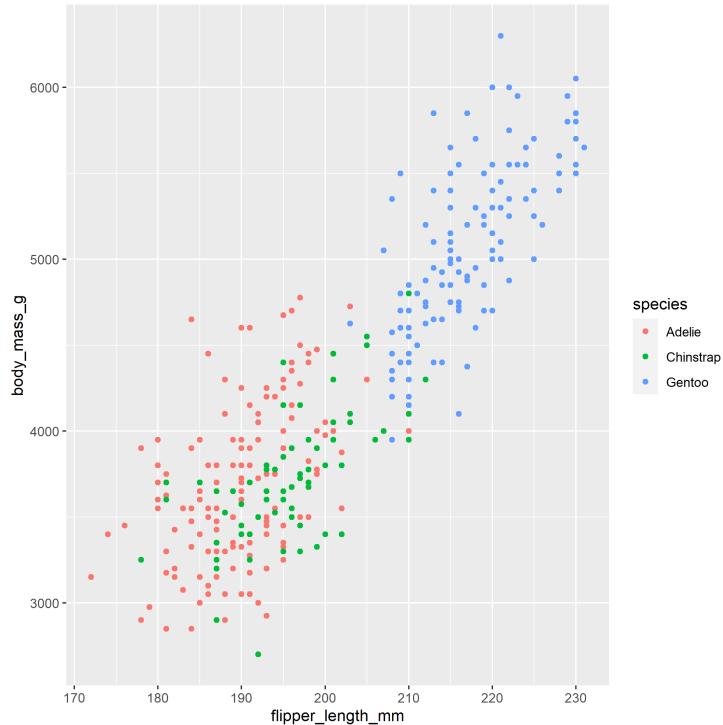
pipe in other functions in the tidyverse: by allowing code to be written from left to right.

5.7 Colour

The current plot could be more informative, to include information about the species of each penguin.

In order to achieve this we need to use aes() again, and specify which column we want to be represented as the colour of the points. Here, the aes() function containing the relevant column name, is given within the geom_point() function.

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g)) +
  geom_point(aes(colour=species))
```



**Note - you may (or may not) have noticed that the grammar of ggplot (and tidyverse in general) accepts British/Americanization for spelling!!!

So now we can see that the Gentoo penguins tend to be both larger and have

longer flippers

Remember to keep adding carriage returns (new lines), which must be inserted after the %>% or + symbols. In most cases, R is blind to white space and new lines, so this is simply to make our code more readable, and allow us to add readable comments.

5.8 More layers

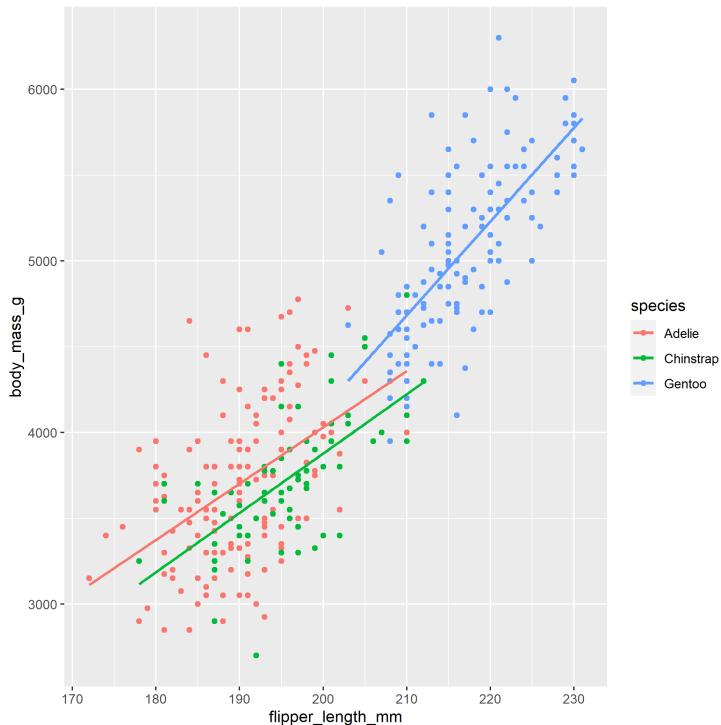
We can see the relationship between body size and flipper length. But what if we want to model this relationship with a trend line? We can add another ‘layer’ to this plot, using a different geometric representation of the data. In this case a trend line, which is in fact a summary of the data rather than a representation of each point.

The geom_smooth() function draws a trend line through the data. The default behaviour is to draw a local regression line (curve) through the points, however these can be hard to interpret. We want to add a straight line based on a linear model (‘lm’) of the relationship between x and y.

This is our **first** encounter with linear models in this course, but we will learn a lot more about them later on.

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g)) +
  geom_point(aes(colour=species)) +
  geom_smooth(method="lm",      #add another layer of data representation.
              se=FALSE,
              aes(colour=species)) # note layers inherit information from the top ggplot() function
```

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species)) + ### now colour is set here it will be inherited by ALL layers
  geom_point() +
  geom_smooth(method="lm",      #add another layer of data representation.
              se=FALSE)
```



> **Note - that

the trend line is blocking out certain points, because it is the ‘top layer’ of the plot. The geom layers that appear early in the command are drawn first, and can be obscured by the geom layers that come after them.



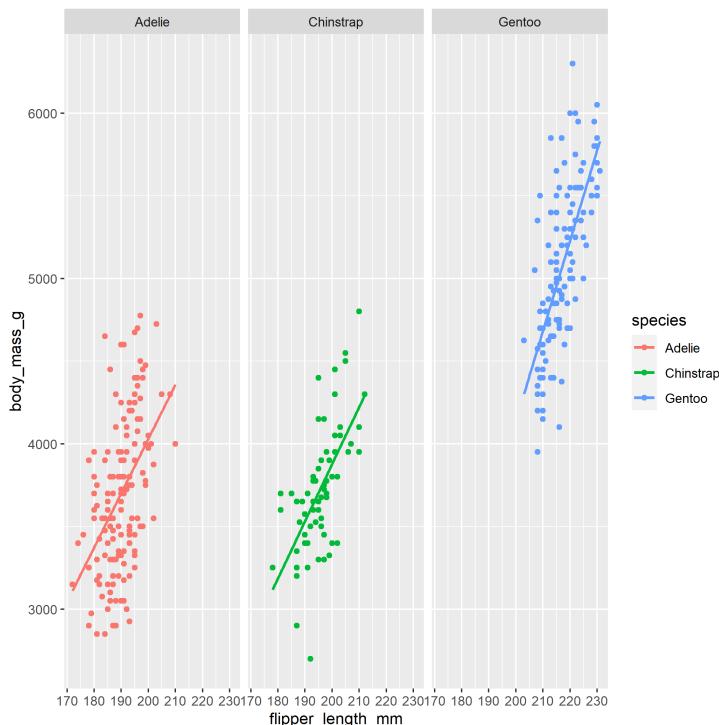
What happens if you switch the order of the `geom_point()` and `geom_smooth()` functions above? What do you notice about the trend line?

5.9 Facets

In some cases we want to break up a single plot into sub-plots, called ‘faceting’. Facets are commonly used when there is too much data to display clearly in a single plot. We will revisit faceting below, however for now, let’s try to facet the plot according to species. To do this we use the tilde symbol ‘~’ to indicate the column name that will form each facet.

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species))+
```

```
geom_point()+
  geom_smooth(method="lm",
              se=FALSE) +
  facet_wrap(~species)
```



**Note - the aesthetics and geoms including the regression line that were specified for the original plot, are applied to each of the facets.

5.10 Co-ordinate space

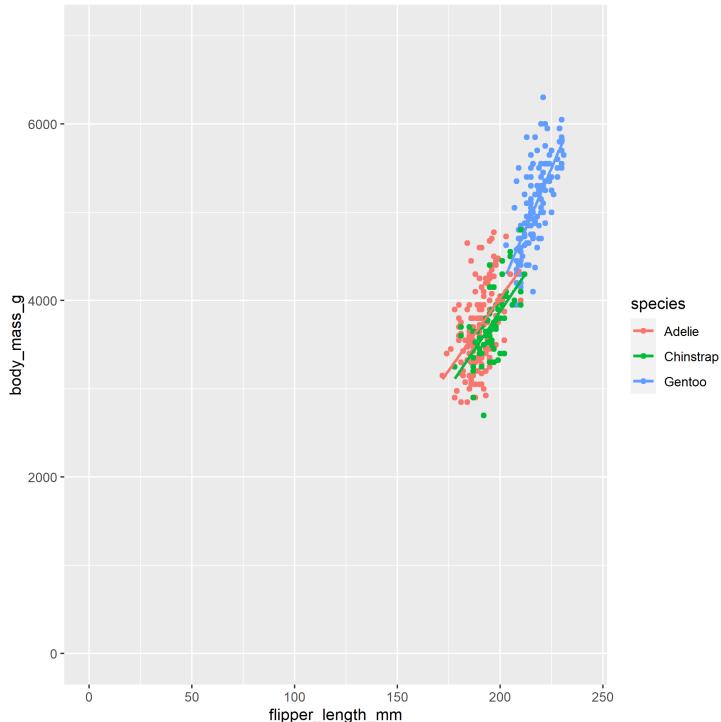
ggplot will automatically pick the scale for each axis, and the type of coordinate space. Most plots are in Cartesian (linear X vs linear Y) coordinate space.

For this plot, let's say we want the x and y origin to be set at 0. To do this we can add in xlim() and ylim() functions, which define the limits of the axes:

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species)) +
```

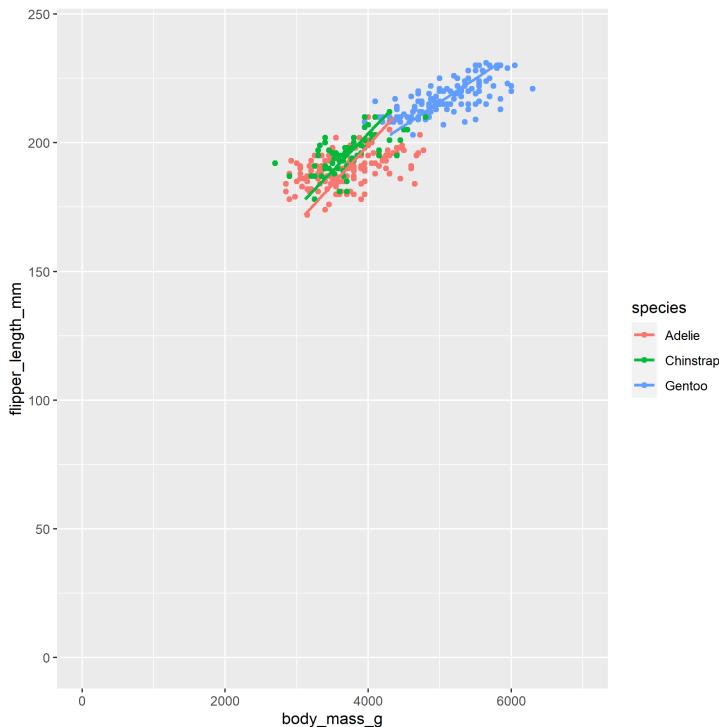
78 CHAPTER 5. GGPLOT2 A GRAMMAR OF GRAPHICS: WEEK FOUR

```
geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+
  xlim(0,240) + ylim(0,7000)
```



Further, we can control the coordinate space using coord() functions. Say we want to flip the x and y axes, we add coord_flip():

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species))+
  geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+
  xlim(0,240) + ylim(0,7000) +
  coord_flip()
```



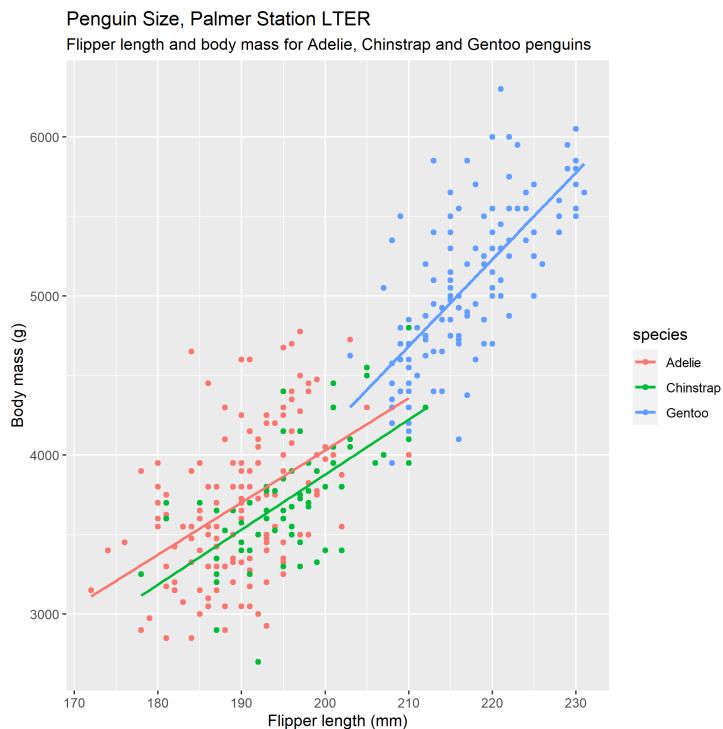
5.11 Labels

By default, the axis labels will be the column names we gave as aesthetics aes(). We can change the axis labels using the xlab() and ylab() functions. Given that column names are often short and can be cryptic, this functionality is particularly important for effectively communicating results.

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species)) +
  geom_point() +
  geom_smooth(method="lm",
              se=FALSE) +
  labs(x = "Flipper length (mm)",
       y = "Body mass (g)")
```

We can also add titles and subtitles

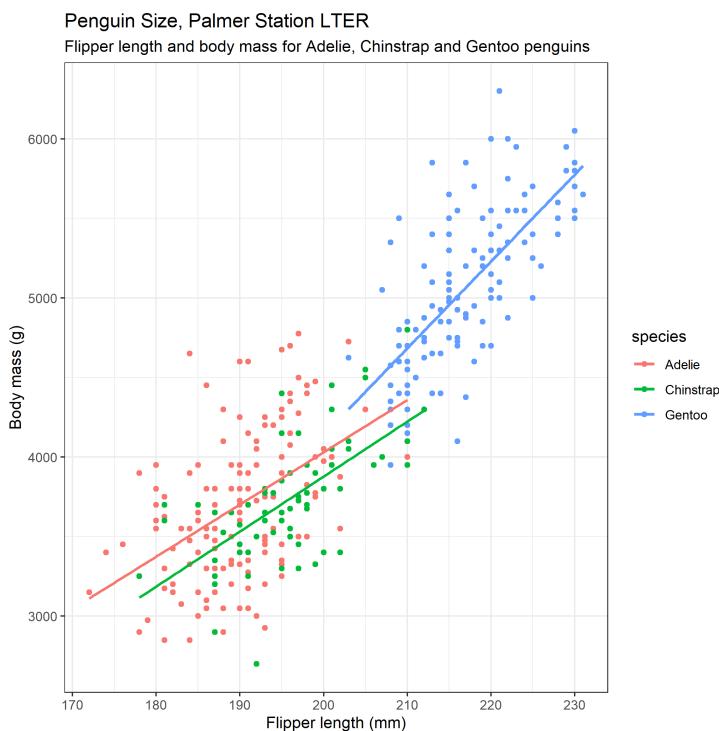
```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species))+
  geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+
  labs(x = "Flipper length (mm)",
       y = "Body mass (g)",
       title= "Penguin Size, Palmer Station LTER",
       subtitle= "Flipper length and body mass for Adelie, Chinstrap and Gentoo penguins")
```



5.12 Themes

Finally, the overall appearance of the plot can be modified using `theme()` functions. The default theme has a grey background which maximizes contrast with other contrasts. You may prefer `theme_classic()`, a `theme_minimal()` or even `theme_void()`. Try them out.

```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g,
             colour=species))+ 
  geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+ 
  labs(x = "Flipper length (mm)",
       y = "Body mass (g)",
       title= "Penguin Size, Palmer Station LTER",
       subtitle= "Flipper length and body mass for Adelie, Chinstrap and Gentoo penguins")+
  theme_bw()
```



**Note - there is a lot more customisation available through the `theme()` function. We will look at making our own custom themes in later lessons



You can try installing and running an even wider range of pre-built themes if you install the R package `ggthemes`.

First you will need to run the `install.packages("ggthemes")` command.

Remember this is one of the few times a command should NOT be written in your script but typed directly into the console. That's because it's rude to send someone a script that will install packages on their computer - think of library() as a polite request instead!

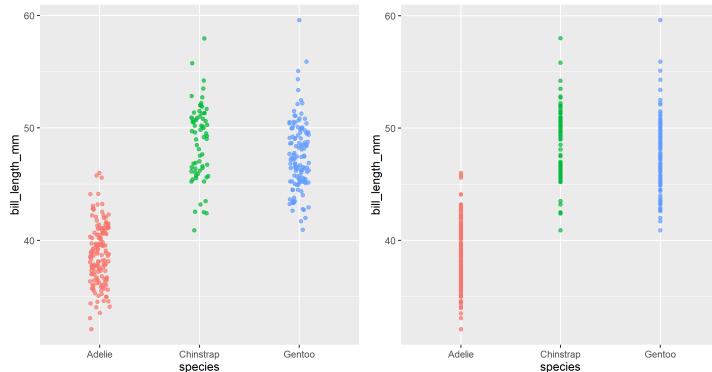
To access the range of themes available type `help(ggthemes)` then follow the documentation to find out what you can do.

5.13 Jitter

The `geom_jitter()` command adds some random scatter to the points which can reduce over-plotting. Compare these two plots:

```
ggplot(data = penguins, aes(x = species, y = bill_length_mm)) +
  geom_jitter(aes(color = species),
              width = 0.1, # specifies the width, change this to change the range of s
              alpha = 0.7, # specifies the amount of transparency in the points
              show.legend = FALSE) # don't leave a legend in a plot, if it doesn't add
```

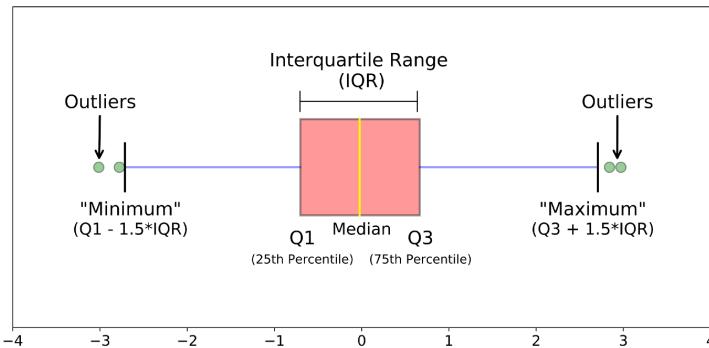
```
ggplot(data = penguins, aes(x = species, y = bill_length_mm)) +
  geom_point(aes(color = species),
             alpha = 0.7,
             show.legend = FALSE)
```



5.14 Boxplots

Box plots, or ‘box & whisker plots’ are another essential tool for data analysis. Box plots summarize the distribution of a set of values by displaying the

minimum and maximum values, the median (i.e. middle-ranked value), and the range of the middle 50% of values (inter-quartile range). The whisker line extending above and below the IQR box define $Q3 + (1.5 \times \text{IQR})$, and $Q1 - (1.5 \times \text{IQR})$ respectively. You can watch a short video to learn more about box plots here.



To create a box plot from our data we use (no prizes here) `geom_boxplot()`!

```
ggplot(data = penguins, aes(x = species, y = bill_length_mm)) +
  geom_boxplot(aes(fill = species), # note fill is "inside" colour and colour is "edges" - try it
               alpha = 0.7,
               width = 0.5, # change width of boxplot
               show.legend = FALSE)
```

The points indicate outlier values [i.e., those greater than $Q3 + (1.5 \times \text{IQR})$].

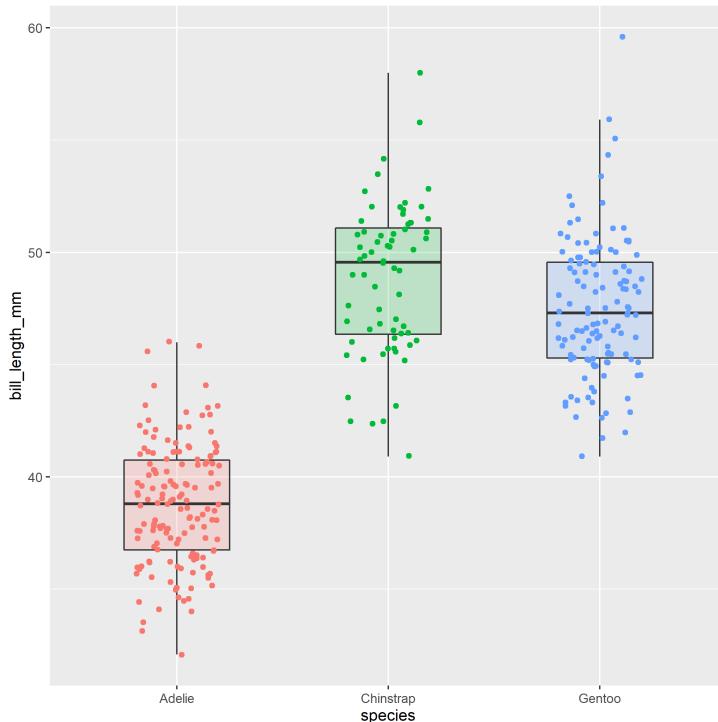
We can overlay a boxplot on the scatter plot for the entire dataset, to fully communicate both the raw and summary data. Here we reduce the width of the jitter points slightly.

```
ggplot(data = penguins, aes(x = species, y = bill_length_mm)) +
  geom_boxplot(aes(fill = species), # note fill is "inside" colour and colour is "edges" - try it
               alpha = 0.2, # fainter boxes so the points "pop"
               width = 0.5, # change width of boxplot
               outlier.shape=NA)+
  geom_jitter(aes(colour = species),
              width=0.2)+
  theme(legend.position = "none")
```



In the above example I switched from using `show.legend=FALSE` inside the `geom` layer to using `theme(legend.position="none")`. Why? This is an example of reducing redundant code. I would have to specify

`show.legend=FALSE` for every geom layer in my plot, but the theme function applies to every layer. Save code, save time, reduce errors!

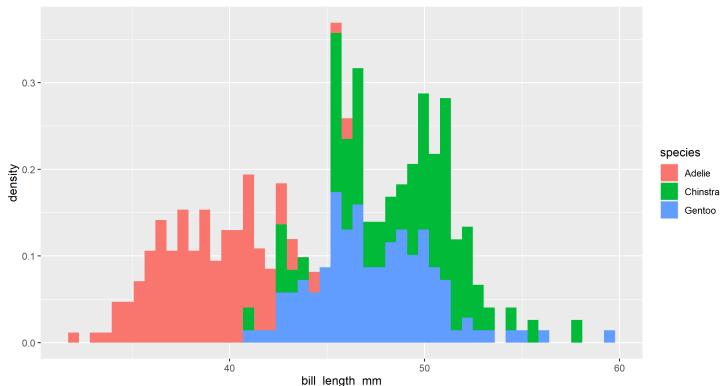


5.15 Density and histogram

Compare the following two sets of code

```
penguins %>%
  ggplot(aes(x=bill_length_mm, fill=species))+
  geom_histogram(bins=50)
```

```
penguins %>%
  ggplot(aes(x=bill_length_mm, fill=species))+
  geom_histogram(bins=50, aes(y=..density..))
```



At first you might struggle to see/understand the difference between these two charts. The shapes should be roughly the same.

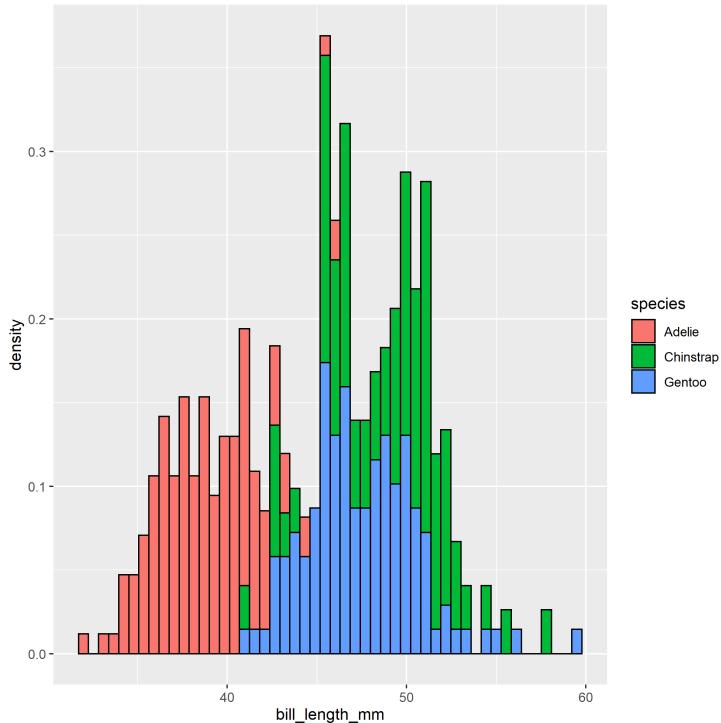
The first block of code produced a frequency histogram, each bar represents the actual number of observations made within each “bin”, the second block of code shows the “relative density” within each bin. In a density histogram the area under the curve for each sub-group will sum to 1. This allows us to compare distributions and shapes between sub-groups of different sizes. For example there are far fewer Adelie penguins in our dataset, but in a density histogram they occupy the same area of the graph as the other two species.

5.16 More Colours

There are two main differences when it comes to colors in `ggplot2`. Both arguments, color and fill, can be specified as single color or assigned to variables.

As you have already seen in this tutorial, variables that are inside the aesthetics are encoded by variables and those that are outside are properties that are unrelated to the variables.

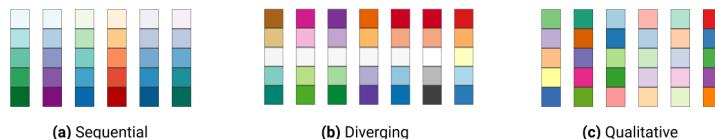
```
penguins %>%
  ggplot(aes(x=bill_length_mm)) +
  geom_histogram(bins=50,
    aes(y=..density.,
        fill=species),
    colour="black")
```



5.16.1 Assign colours to variables

You can specify what colours you want to assign to variables in a number of different ways.

In ggplot2, colors that are assigned to variables are modified via the `scale_color_*` and the `scale_fill_*` functions. In order to use color with your data, most importantly you need to know if you are dealing with a categorical or continuous variable. The color palette should be chosen depending on type of the variable, with sequential or diverging color palettes being used for continuous variables and qualitative color palettes for categorical variables:



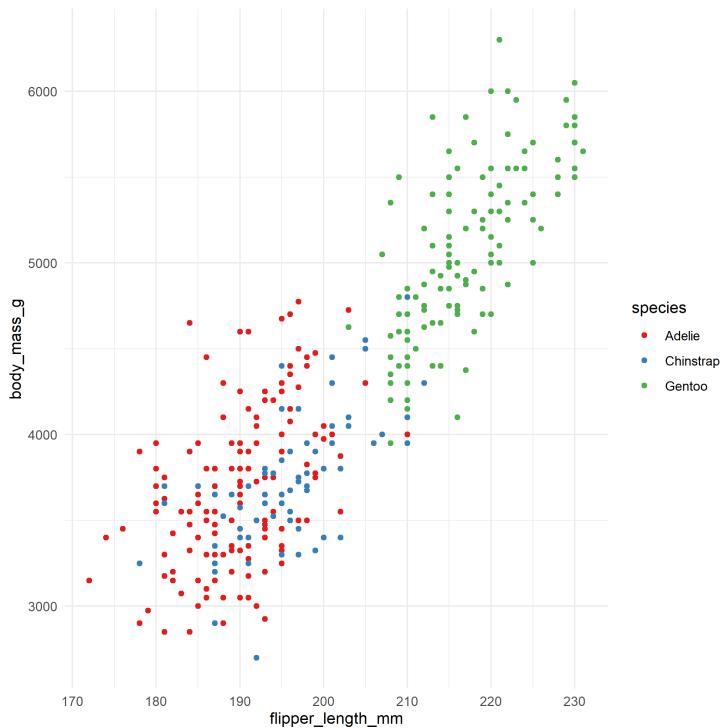
You can pick your own sets of colours and assign them to a categorical variable. The number of specified colours **has** to match the number of categories. You can use a wide number of preset colour names or you can use hexadecimals.

```
penguin_colours <- c("darkolivegreen4", "darkorchid3", "goldenrod1")

penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g))+
  geom_point(aes(colour=species))+
  scale_color_manual(values=penguin_colours)+
  theme_minimal()
```

You can also use a range of inbuilt colour palettes:

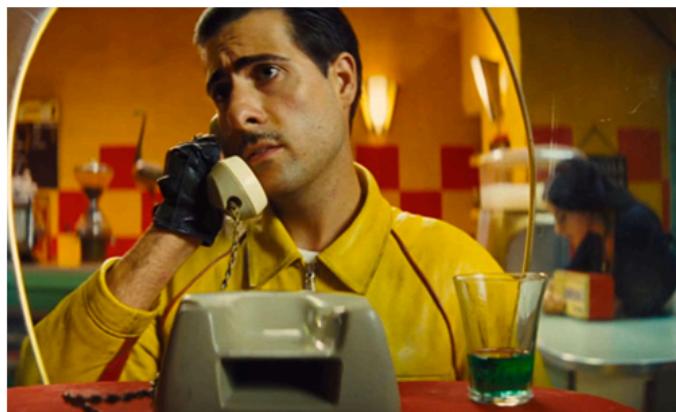
```
penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = body_mass_g))+
  geom_point(aes(colour=species))+
  scale_color_brewer(palette="Set1")+
  theme_minimal()
```





You can explore all schemes available with the command `RColorBrewer::display.brewer.all()`

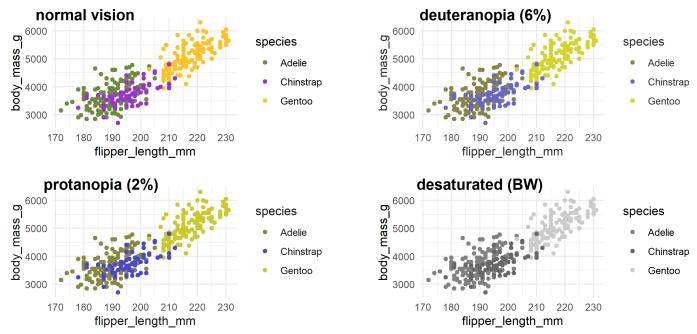
There are also many, many extensions that provide additional colour palettes. Some of my favourite packages include `ggsci`(Xiao (2018)) and `wesanderson`(Ram and Wickham (2018)).



5.16.2 Accessibility

It's very easy to get carried away with colour palettes, but you should remember at all times that your figures must be accessible. One way to check how accessible your figures are is to use a colour blindness checker Ou (2021)

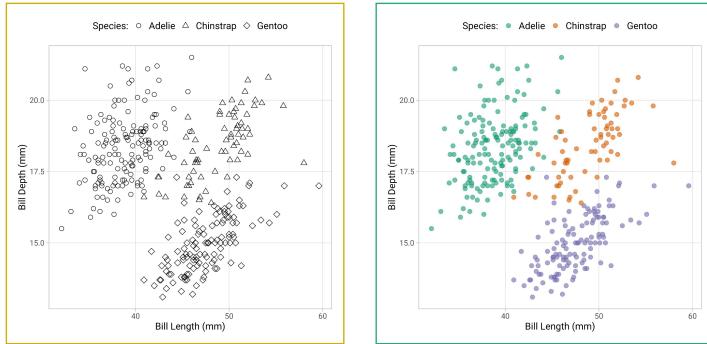
```
library(colorBlindness)
cvdPlot() # will automatically run on the last plot you made
```



5.16.3 Guides to visual accessibility

Using colours to tell categories apart can be useful, but as we can see in the example above, you should choose carefully. Other aesthetics which you can access in your geoms include `shape`, and `size` - you can combine these in complimentary ways to enhance the accessibility of your plots. Here is a hierarchy of “interpretability” for different types of data

Qualitative Nominal	Qualitative Ordinal	Quantitative Interval/Ratio
Position	Position	Position
Colour (Hue)	Pattern (Density)	Size (Length)
Pattern (Texture)	Colour (Lightness)	Angle
Connection	Colour (Hue)	Size (Area)
Pattern (Density)	Pattern (Texture)	Size (Volume)
Colour (Lightness)	Connection	Pattern (Density)
Symbol	Size (Length)	Colour (Lightness)
Size (Length)	Angle	Colour (Hue)
Angle	Size (Area)	Pattern (Texture)
Size (Area)	Size (Volume)	Connection
Size (Volume)	Symbol	Symbol



5.17 Patchwork

There are many times you might want to combine figures into multi-panel plots. Probably the easiest way to do this is with the `patchwork` package (Pedersen (2020)).

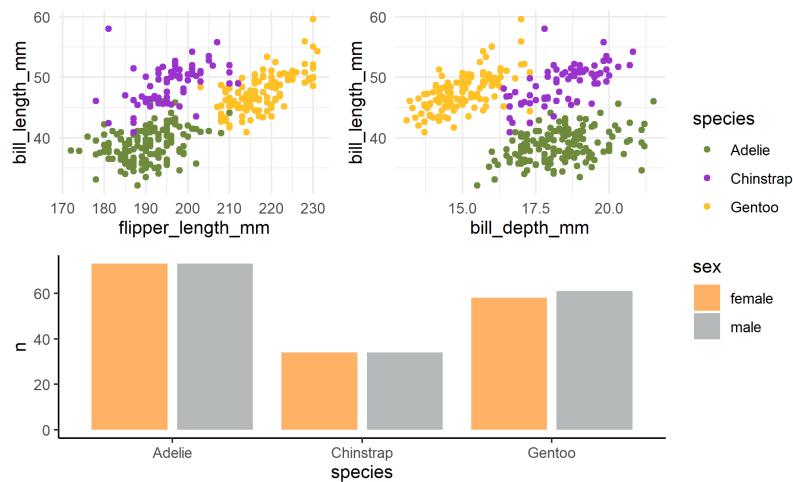
```
p1 <- penguins %>%
  ggplot(aes(x=flipper_length_mm,
             y = bill_length_mm))+
  geom_point(aes(colour=species))+
  scale_color_manual(values=penguin_colours)+
  theme_minimal()

p2 <- penguins %>%
  ggplot(aes(x=bill_depth_mm,
             y = bill_length_mm))+
  geom_point(aes(colour=species))+
  scale_color_manual(values=penguin_colours)+
  theme_minimal()

p3 <- penguins %>%
  group_by(sex,species) %>%
  summarise(n=n()) %>%
  drop_na(sex) %>%
  ggplot(aes(x=species, y=n)) +
  geom_col(aes(fill=sex),
           width=0.8,
           position=position_dodge(width=0.9),
           alpha=0.6)+

  scale_fill_manual(values=c("darkorange1", "azure4"))+
  theme_classic()
```

```
library(patchwork)  
  
(p1+p2)/p3+  
  plot_layout(guides = "collect")
```

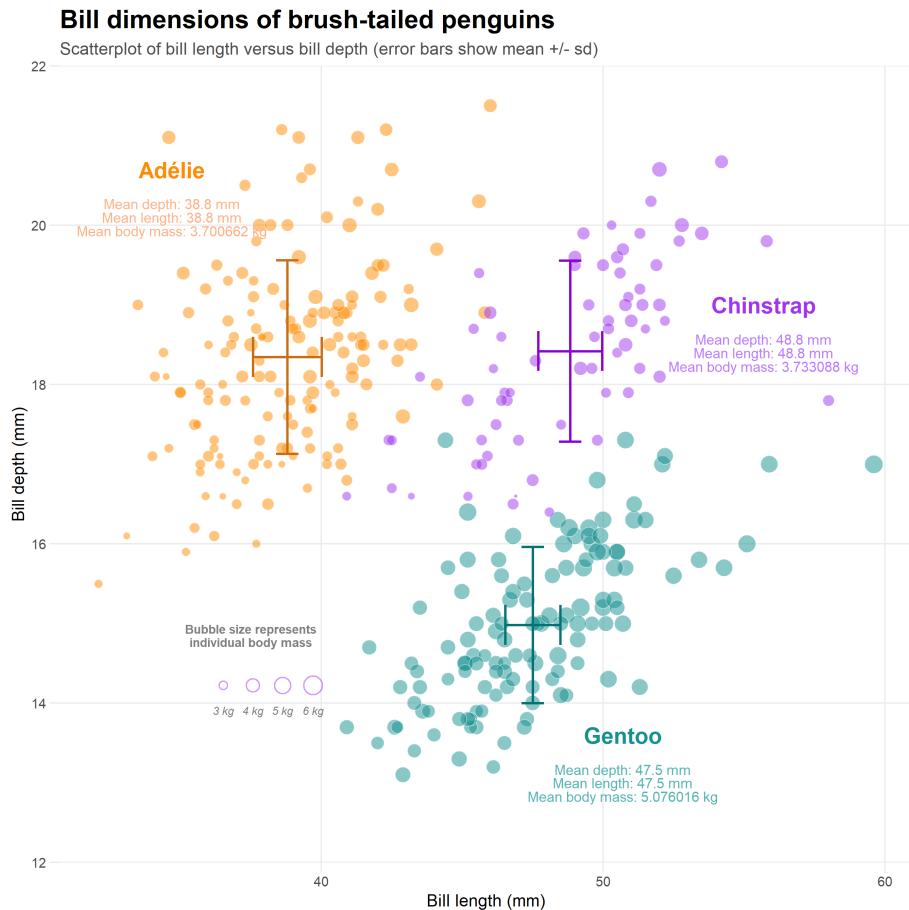


5.18 Test



Challenge - How close can you get to replicating the figure below?

Make sure to use the tips and links at the end of this chapter



5.19 Saving

One of the easiest ways to save a figure you have made is with the `ggsave()` function. By default it will save the last plot you made on the screen.

You should specify the output path to your **figures** folder, then provide a file name. Here I have decided to call my plot `plot` (imaginative!) and I want to save it as a .PNG image file. I can also specify the resolution (dpi 300 is good enough for most computer screens).

```
ggsave("figures/plot.png", dpi=300)
```

5.20 Quitting



Make sure you have saved your script!



Download your saved figure from RStudio Cloud and submit it to Blackboard “Week Four Test”

5.21 Summing up ggplot

5.21.1 What we learned

You have learned

- The anatomy of ggplots
- How to add geoms on different layers
- How to use colour, colour palettes, facets, labels and themes
- Putting together multiple figures
- How to save and export images

You have primarily worked with `tidyverse` and the package

- `ggplot2` Wickham et al. (2021b)

As well as:

- `colorBlindness` Ou (2021)
- `patchwork` Pedersen (2020)

5.21.2 Further Reading, Guides and tips

R Cheat Sheets

Wilke (2020) <https://clauswilke.com/dataviz/>

this book tells you everything you need to know about presenting your figures for accessibility and clarity

<https://www.cedricscherer.com/2019/08/05/a-ggplot2-tutorial-for-beautiful-plotting-in-r/>

an incredibly handy ggplot guide for how to build and improve your figures

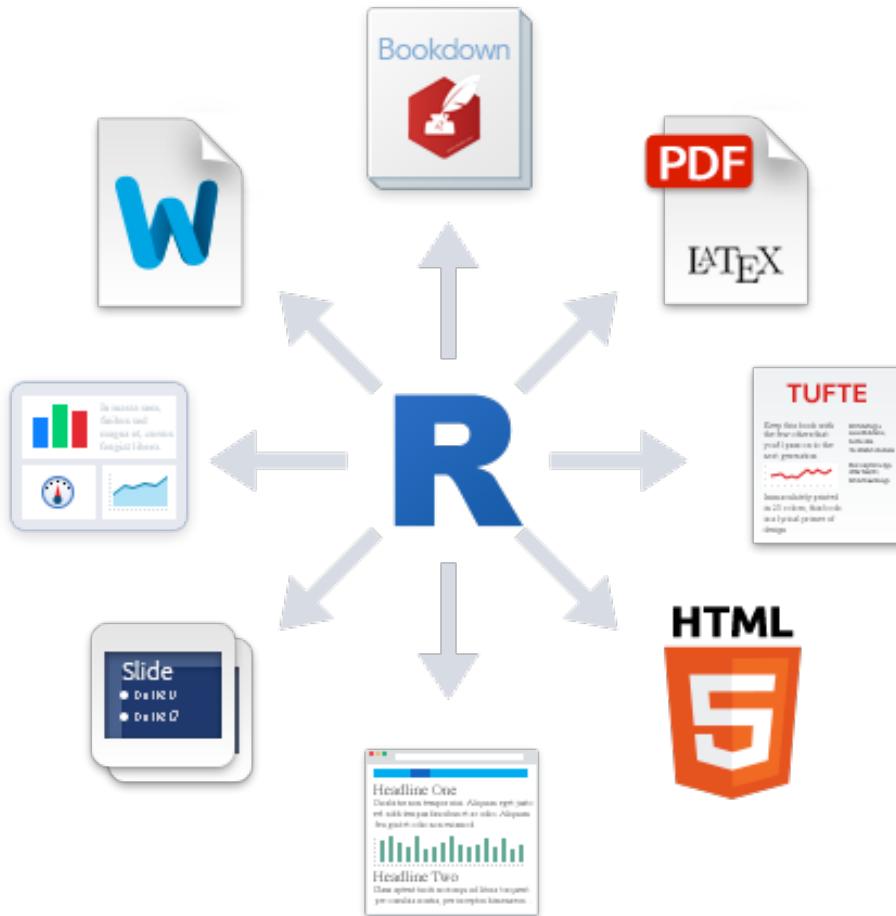
94 CHAPTER 5. GGPLOT2 A GRAMMAR OF GRAPHICS: WEEK FOUR

Hadley Wickham and Pedersen (2020) <https://ggplot2-book.org/>

the original Hadley Wickham book on ggplot2

Chapter 6

Reports with Rmarkdown: Week Five



R Markdown is a widely-used tool for creating automated, reproducible, and share-worthy outputs, such as reports. It can generate static or interactive outputs, in Word, pdf, html, powerpoint, and other formats.

An R Markdown script combines R code and text such that the script actually becomes your output document. You can create an entire formatted document, including narrative text (can be dynamic to change based on your data), tables, figures, bullets/numbers, bibliographies, etc.

Documents produced with Rmarkdown, allow analyses to be included easily - and make the link between raw data, analysis & and a published report *completely reproducible*.

With Rmarkdown we can make reproducible html, word, pdf, powerpoints or websites and dashboards¹

To make your Rmd publish - hit the knit button at the top of the doc

6.0.1 Format

- Go to RStudio Cloud and open **Week 5 - Rmarkdown**
- Follow the instructions carefully - and assemble your Rmarkdown file bit by bit - when prompted to ‘knit’ the document do it. We will then observe the results and might make changes.

6.1 Background to Rmarkdown

- Markdown is a “language” that allows you to write a document using plain text, that can be converted to html and other formats. It is not specific to R. Files written in Markdown have a ‘.md’ extension.
- R Markdown: is a variation on markdown that is specific to R - it allows you to write a document using markdown to produce text and to embed R code and display their outputs. R Markdown files have ‘.Rmd’ extension.
- rmarkdown - the package: This is used by R to render the .Rmd file into the desired output. Its focus is converting the markdown (text) syntax, so we also need...
- knitr: This R package will read the code chunks, execute it, and ‘knit’ it back into the document. This is how tables and graphs are included alongside the text.
- Pandoc: Finally, pandoc actually convert the output into word/pdf/powerpoint etc. It is a software separate from R but is installed automatically with RStudio.

¹(<https://rmarkdown.rstudio.com/lesson-9.html>)

Most of this process happens in the background (you do not need to know all these steps!) and it involves feeding the .Rmd file to knitr, which executes the R code chunks and creates a new .md (markdown) file which includes the R code and its rendered output. The .md file is then processed by pandoc to create the finished product: a Microsoft Word document, HTML file, powerpoint document, pdf, etc. When using RStudio Cloud - all of these features are pre-loaded - if you take your R journey further in the future and install a copy of R and RStudio on your own computer you might have to do a little setting up to get this working.



6.2 Starting a new Rmd file

In RStudio, if you open a new R markdown file, start with ‘File’, then ‘New file’ then ‘R markdown...’.

R Studio will give you some output options to pick from. In the example below we select “HTML” because we want to create an html document. The title and the author names are not important. If the output document type you want is not one of these, don’t worry - you can just pick any one and change it in the script later.

For now open the markdown file which I have made in the Markdown sub-folder.



The working directory for .rmd files is a little different to working with scripts.

With a .Rmd file, the working directory is wherever the Rmd file itself is saved.

For example if you have your .Rmd file in a subfolder ~/markdown-files/markdown.Rmd the code for `read_csv("data/data.csv")` within the markdown will look for a .csv file in a subfolder called data *inside* the `markdownfiles` folder and not the root project folder where the .RProj file lives.

So we have two options when using .Rmd files

- 1) Don’t put the .Rmd file in a subfolder and make sure it lives in the same directory as your .RProj file
- 2) Use the ‘here’ package to describe file locations - more later

6.3 R Markdown parts

An R Markdown document can be edited in RStudio just like a standard R script. When you start a new R Markdown script, RStudio tries to be helpful by showing a template which explains the different section of an R Markdown script.

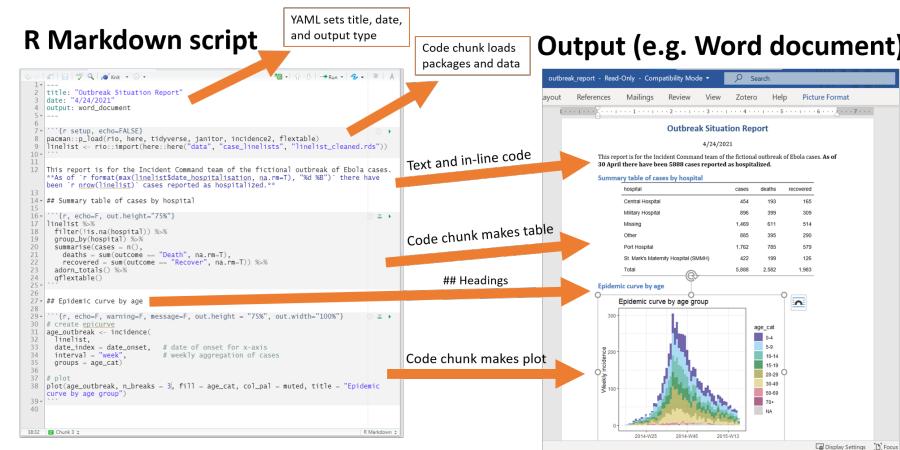
The below is what appears when starting a new Rmd script intended to produce an html output.

```

1 <!--
2   title: "Untitled"
3   author: "Phil Leftwich"
4   date: "19/10/2021"
5   output: html_document
6 ---
7
8 ---{r setup, include=FALSE}
9 knitr::opts_chunk$set(echo = TRUE)
10 -
11
12 ## R Markdown
13
14 This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For
15 using R Markdown see http://rmarkdown.rstudio.com.
16 When you click the **Knit** button a document will be generated that includes both content as well as the output of any R
17 code chunks you embed in the document. You can embed an R code chunk like this:
18
19 ---{r cars}
20 summary(cars)
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

```

As you can see, there are three basic components to any Rmd file: YAML, Markdown text, and R code chunks.



6.3.1 YAML

Referred to as the ‘YAML metadata’ or just ‘YAML’, this is at the top of the R Markdown document. This section of the script will tell your Rmd file **what type of output to produce**, formatting preferences, and other metadata such as document title, author, and date. In the example above, because

we clicked that our default output would be an html file, we can see that the YAML says output: `html_document`. However we can also change this to say `powerpoint_presentation` or `word_document` or even `pdf_document`.



Can you edit the YAML in the Rmarkdown file in the markdown folder to have your name as author, today's date and the title of the file should be called "Darwin's Maize Plants".

6.3.2 Text

This is the narrative of your document, including the titles and headings. It is written in the “markdown” language, which is used across many different software.

Below are the core ways to write this text. See more extensive documentation available on R Markdown “cheatsheets” at the RStudio website².

6.3.2.1 New lines

Uniquely in R Markdown, to initiate a new line, enter *two spaces** at the end of the previous line and then Enter/Return.

6.3.2.2 Text emphasis

Surround your normal text with these characters to change how it appears in the output.

Underscores (`_text_`) or single asterisk (`*text*`) to *italicise*

Double asterisks (`**text**`) for **bold** text

Back-ticks (`'text'`) to display text as `code`

The actual appearance of the font can be set by using specific templates (specified in the YAML metadata).

6.3.2.3 Titles and headings

A hash symbol in a text portion of a R Markdown script creates a heading. This is different than in a chunk of R code in the script, in which a hash symbol is a mechanism to comment/annotate/de-activate, as in a normal R script.

Different heading levels are established with different numbers of hash symbols at the start of a new line. One hash symbol is a title or primary heading. Two hash symbols are a second-level heading. Third- and fourth-level headings can be made with successively more hash symbols.

²(<https://www.rstudio.com/resources/cheatsheets/>)

```
# First-level heading / title
## Second level heading
### Third-level heading
```

6.3.2.4 Bullets and numbering

Use asterisks (*) to create a bullet list. Finish the previous sentence, enter two spaces, Enter/Return twice, and then start your bullets. Include a space between the asterisk and your bullet text. After each bullet enter two spaces and then Enter/Return. Sub-bullets work the same way but are indented. Numbers work the same way but instead of an asterisk, write 1), 2), etc. Below is how your R Markdown script text might look.

Here are my bullets (there are two spaces after this colon):

- * Bullet 1 (followed by two spaces and Enter/Return)
- * Bullet 2 (followed by two spaces and Enter/Return)
 - * Sub-bullet 1 (followed by two spaces and Enter/Return)
 - * Sub-bullet 2 (followed by two spaces and Enter/Return)

6.3.3 Code Chunks

Sections of the script that are dedicated to running R code are called “chunks”. This is where you may load packages, import data, and perform the actual data management and visualisation. There may be many code chunks, so they can help you organize your R code into parts, perhaps interspersed with text. To note: These ‘chunks’ will appear to have a slightly different background colour from the narrative part of the document.

Each chunk is opened with a line that starts with three back-ticks, and curly brackets that contain parameters for the chunk ({}). The chunk ends with three more back-ticks.



You can create a new chunk by typing it out yourself, by using the keyboard shortcut “Ctrl + Alt + i” (or Cmd + Shift + r in Mac), or by clicking the green ‘insert a new code chunk’ icon at the top of your script editor.

Some notes about the contents of the curly brackets {}:

They start with ‘r’ to indicate that the language name within the chunk is R

After the r you can optionally write a chunk “name” – these are not necessary but can help you organise your work. Note that if you name your chunks, you

should ALWAYS use unique names or else R will *complain* when you try to render.

The curly brackets can include other options too, written as tag=value, such as:

- eval = FALSE to not run the R code
- echo = FALSE to not print the chunk's R source code in the output document
- warning = FALSE to not print warnings produced by the R code
- message = FALSE to not print any messages produced by the R code
- include = either TRUE/FALSE whether to include chunk outputs (e.g. plots) in the document
- out.width = and out.height = - size of ouput e.g. out.width = "75%"
- fig.align = "center" adjust how a figure is aligned across the page
- fig.show='hold' if your chunk prints multiple figures and you want them printed next to each other (pair with out.width = c("33%", "67%").

A chunk header must be written in one line

Try to avoid periods, underscores, and spaces. Use hyphens (-) instead if you need a separator.

Read more extensively about the knitr options here³.

There are also two arrows at the top right of each chunk, which are useful to run code within a chunk, or all code in prior chunks. Hover over them to see what they do.

6.3.4 Here

The package `here` Müller (2020) and its function `here()` make it easy to tell R where to find and to save your files - in essence, it builds file paths.

This is how `here()` works within an R project:

- When the `here` package is first loaded within the R project, it places a small file called ".here" in the root folder of your R project as a "benchmark" or "anchor"
- In your scripts, to reference a file in the R project's sub-folders, you use the function `here()` to build the file path in relation to that anchor
- To build the file path, write the names of folders beyond the root, within quotes, separated by commas, finally ending with the file name and file extension as shown below

³(<https://yihui.org/knitr/options/>)

- here() file paths can be used for both importing and exporting

So when you use here wrapped inside other functions for importing/exporting (like read_csv or ggsave) if you include here you can still use the RProject location as the root directory when ‘knitting’ Rmarkdown files, even if your markdown is tidied away into a separate markdown folder.



Can you take the code below and put it into an R code chunk?

Try your first “knit” to make a document.

```
library(here)
library(tidyverse)

darwin <- read_csv(here("data", "darwin.csv"))
darwin
```

Rmarkdown

```
library(here)

## here() starts at /cloud/project

library(tidyverse)

## — Attaching packages — tidyverse 1.3.0 —

## ✓ ggplot2 3.3.5    ✓ purrr   0.3.4
## ✓ tibble  3.0.6    ✓ dplyr   1.0.2
## ✓ tidyr   1.1.2    ✓ stringr 1.4.0
## ✓ readr   1.3.1    ✓ forcats 0.5.0

## — Conflicts — tidyverse_conflicts() —
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()   masks stats::lag()

darwin <- read_csv(here("data", "darwin.csv"))

## Parsed with column specification:
## cols(
##   pot = col_character(),
##   pair = col_double(),
##   Cross = col_double().
```



Hmmm looks a little messy.

Can you edit the R chunk so that the code and any warnings/messages are invisible. But the code output is still printed?

Once you have made your edits try hitting ‘knit’ again.

6.3.4.1 Global options

For global options to be applied to all chunks in the script, you can set this up within your very first R code chunk in the script. This is very handy if you know you will want the majority of your code chunks to behave in the same way. For instance, so that only the outputs are shown for each code chunk and not the code itself, you can include this command in the R code chunk (and set this chunk to `include=false`):

```
knitr::opts_chunk$set(echo = FALSE)
```

6.3.4.2 In-text code

You can also include minimal R code within back-ticks. Within the back-ticks, begin the code with “r” and a space, so RStudio knows to evaluate the code as R code. See the example below.

```
' r Sys.Date()'
```

When typed in-line within a section of what would otherwise be Markdown text, it knows to produce an r output instead 2022-03-12

The example above is simple (showing the current date), but using the same syntax you can display values produced by more complex R code (e.g. to calculate the min, median, max of a column). You can also integrate R objects or values that were created in R code chunks *earlier* in the script.

```
average_height <- darwin %>%
  summarise(mean_self=mean(Self),
            mean_cross=mean(Cross))
```

The average height of the self-crossed plants is ‘ r average_height [,1]‘cm while the outcrossed plants are ‘ r average_height [,2]‘cm, so on average the outcrossed plants are ‘ r average_height [,2]- average_height [,1]‘cm taller.



Can you include the above code block and make sure it runs without the code being visible? Underneath include the text - it should produce the code outputs in the knitted doc. Try it!

6.4 Images

You can include images in your R Markdown in several ways:

```
knitr::include_graphics("path/to/image.png")
```

```
knitr::include_graphics("../data/images/darwin.png")
# .../ is necessary here because in order to organise the file path starting in the markdown folder
```

Alternatively we could use the `here()` function - which means it doesn't matter where our markdown file 'lives'.

```
knitr::include_graphics(here::here("path", "to", "image.png"))
```

```
knitr::include_graphics(here("data", "images", "darwin.png"))
```



Can you get your document to knit with this new image included?

6.5 Tables

To create and manage able objects, we first pass the data frame through the `kable()` function. The package `kableExtra` Zhu (2020) gives us lots of extra styling options.⁴



Can you get this working? Add the library call for `kableExtra` to the first chunk of your Rmd file, then make a chunk for the below at the bottom of your file and hit knit to test.

```
darwin %>%
  summarise(mean_self=mean(Self),
            mean_cross=mean(Cross)) %>%
  kbl(caption="Table 1. example table caption") %>%
  kable_styling(bootstrap_options = "striped", full_width = F, position = "left")
```

6.6 Self-contained documents

For a relatively simple report, you may elect to organize your R Markdown script such that it is “self-contained” and does not involve any external scripts.

Everything you need to run the R markdown is imported or created within the Rmd file, including all the code chunks and package loading. This “self-contained” approach is appropriate when you do not need to do much data processing (e.g. it brings in a clean or semi-clean data file) and the rendering of the R Markdown will not take too long.

⁴(https://cran.r-project.org/web/packages/kableExtra/vignettes/awesome_table_in_html.html)

In this scenario, one logical organization of the R Markdown script might be:

- Set global knitr options
- Load packages
- Import data
- Process data
- Produce outputs (tables, plots, etc.)
- Save outputs, *if applicable* (.csv, .png, etc.)

6.7 Source files

One variation of the “self-contained” approach is to have R Markdown code chunks “source” (run) other R scripts.

This can make your R Markdown script less cluttered, more simple, and easier to organize. It can also help if you want to display final figures at the beginning of the report.

In this approach, the final R Markdown script simply combines pre-processed outputs into a document.

```
source(here("scripts", "your-script.R"))
```



Can you try it for yourself? There is a pre-written script in your R project, just use the source command to read in this script - then you can call objects made externally - in this case a penguin plot - put the code block in and hit knit.

```
source(here("scripts", "penguin script.R"))
scat ### object generated in penguin script
```

6.8 Exercise - Make your own Rmd document

The data found in “darwin.csv” was originally collected by Charles Darwin and published in *The effects of cross and self-fertilisation in the vegetable kingdom*, in 1876. In this publication he described how he produced seeds of *Zea mays* (maize) that were fertilised with pollen from the same individual (inbreeding) or by crossing to a different plant (outbreeding). Pairs of seeds were then taken from self-fertilised and crossed plants and germinated in pots, and the height of the young seedlings measured as a proxy for their fitness. Darwin wanted to know whether inbreeding reduced their fitness.



Can you make your first reproducible document?

- Write a short background on this subject 250-300 words on the data and the experimental hypothesis (use some of the information above)
- Write a results section on this data including:
 - A figure of the individual data points
 - A summary results table with the mean and s.d. for the plants
 - Write a summary of the results where the figures and table are referenced but you *must* describe the results too. e.g.

“Results are shown in Figure 1” is **not ok**

But *in your own words* describe the direction and effect size of any differences and then refer to Figures and tables

“The mean height of self-crossed plants is x and the mean height of crossed plants is y , meaning on average self-crossed plants are... which may indicate that ... (Figure 1, Table 1)”

- knit your rmarkdown as a **pdf** and submit to Blackboard.



Remember your code blocks must be in the right order!!!

6.9 Summing up Rmarkdown

6.9.1 What we learned

You have learned

- How to use markdown
- How to embed R chunks, to produce code, figures and analyses
- How to organise projects with **here**
- How to knit to pdf and html outputs
- How to make simple tables

You have used

- **knitr** as part of Rmarkdown
- **kableExtra** Zhu (2020)
- **here** Müller (2020)

6.9.2 Further Reading, Guides and tips

R Cheat Sheets

Xie (2015) Dynamic documents with Rmarkdown

The fully comprehensive guide

(https://rmarkdown.rstudio.com/articles_intro.html)

(https://rmarkdown.rstudio.com/authoring_quick_tour.html)

Chapter 7

Version control with GitHub: Week Six

7.1 Let's Git it started

Git is a **version control system**. Originally built to help groups of developers work collaboratively on big software projects. It helps us manage our RStudio projects - with tracked changes.

Git and GitHub are a big part of the data science community. We can use GitHub in a number of ways

- 1) To source code and repurpose analyses built by others for our own uses
- 2) Manage our analysis projects so that all parts of it:
 - Data
 - Scripts
 - Figures
 - Reports

Are version controlled and open access

- 3) Version control lets you recover from any mistakes & your analysis is *backed up* externally
- 4) When you come to publish any reports - your analysis is accessible to others
- 5) Build up your own library of projects to show what you can do in Data Science

Watch this video *before* or *after* today's session

7.1.1 Will this be fun?

No.

Using GitHub and version control is a bit like cleaning your teeth. It's not exactly fun, but it's good for you and it promotes excellent hygiene. It also only takes about 2 minutes.

When we talk about projects on GitHub we refer to Repositories / repos.

Repos on GitHub are the same unit as an RStudio Project - it's a place where you can easily store all information/data/etc. related to whatever project you're working on.

The way in which we will set up our RStudio Projects will now have a few extra steps to it

- Make a new GitHub repository (or *fork* an existing one)
- Make a New project on RStudio Cloud - selecting the *from GitHub Repository* option
- Clone your GitHub repo into an RStudio Project
- Make sure RStudio and Github can *talk* to each other
- Go about your normal business
- When it comes to *saving* your files, you will also periodically make a **commit** - this takes a multi-file *snapshot* of your *entire* project
- At the end of a session **push** your changes to GitHub.

These changes to working with RStudio will feel a little different at first, but will quickly become routine - and are a big step forward in your Data Science skills.

7.1.2 The Payoff

- **A portfolio** build up a library of data science projects you can show off
- **be keen** track the development of R packages on GitHub
- **version control** keep a safe archive of all your edits and changes
- **play with others** easy ways to collaborate on data science projects

For the full rundown on how to use Git and R you can't go wrong with checking out Bryan (2012)

7.2 Set up GitHub

First things first you will need to set yourself up with a GitHub account.

Head to GitHub and sign up for a free account.



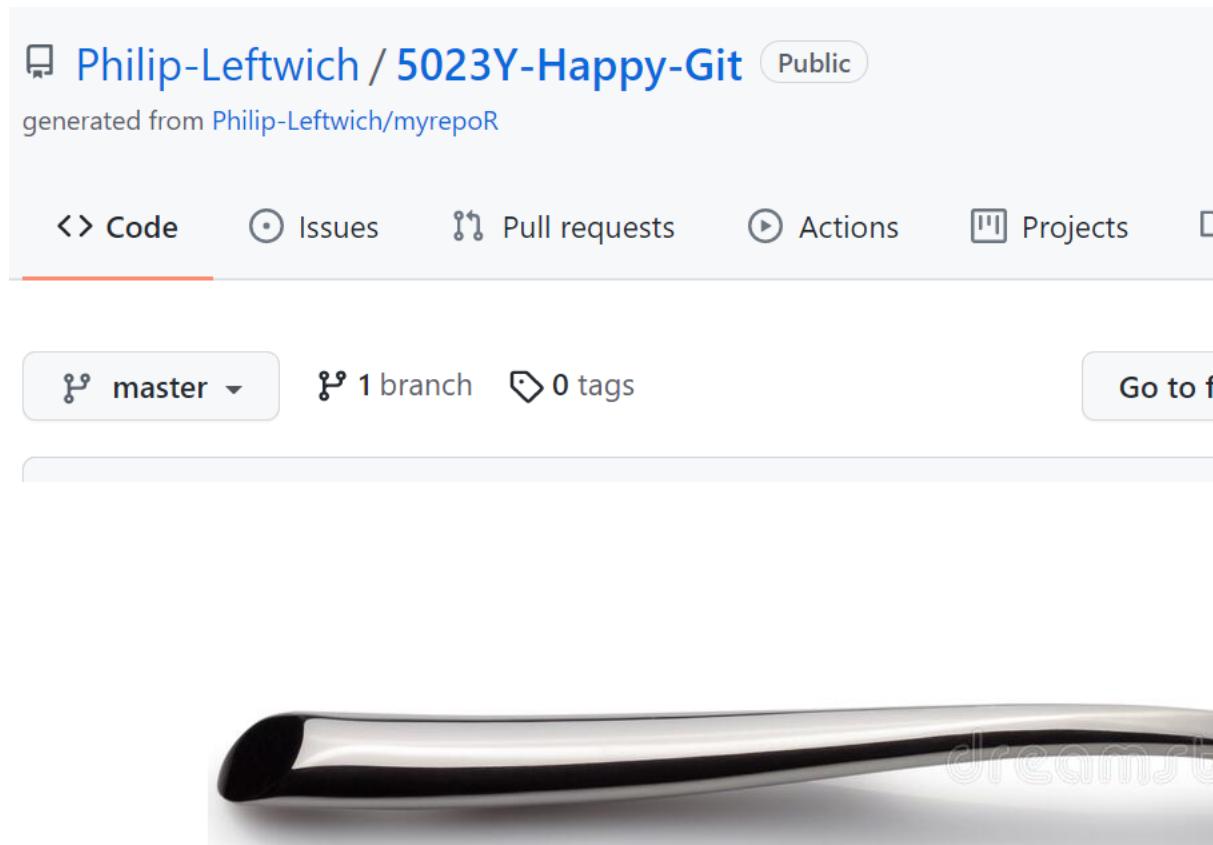
Make a careful note of

- The username you choose
- Use the same email you have signed up to RStudio Cloud with
- Note your password carefully!

7.3 Exercise 1. Fork & clone an existing repo on GitHub, make edits, push back

- a. Go to github.com and log in (you need your own account - for sign up with your uea.ac.uk e-mail)
- b. In the Search bar, look for repo **Philip-Leftwich/5023Y-Happy-Git**
- c. Click on the repo name, and look at the existing repo structure
- d. **FORK** the repo

7.3.1 What the hell is a fork?



A fork is when you generate a *personal* copy of another user's repository (7.8).

- e. Press Clone/download and copy the URL, then create a **new** project in RStudio Cloud selecting the **New project from Git repository** option - make sure you are in the 5023Y Workspace

7.3. EXERCISE 1. FORK & CLONE AN EXISTING REPO ON GITHUB, MAKE EDITS, PUSH BACK113

The screenshot shows a web-based project management interface. At the top, there is a header with a menu icon, the text "BIO-5023Y", and "Philip T Leftwich". Below the header, a blue button labeled "Projects" is highlighted. To its right are "Members" and "About" buttons. The main content area is titled "Your Projects". It includes a search bar with dropdown menus for "List" (set to "Your projects") and "Sort" (set to "By date created"). Below this, a project card is displayed for "Untitled Project". The card shows an "RStudio Project" icon, the creation date "Created Oct 20, 2021 1:36 PM", and a lock icon.

f. Open the some_cool_animals.Rmd document, and the accompanying html

g. Add *your name* to the top of the document

h. BUT WAIT. We have forgotten to add a great image and facts about a very important species - Baby Yoda, including an image (the file is in the repo, and the info to add is below).

FACTS



- Also known as “The Child”
 - likes unfertilised frog eggs & control knobs
 - strong with the force
-
- i. Once you’ve added Grogu, knit the Rmd document to update the html
 - j. Add your Git credentials go to section (7.4)
 - k. Stage, Commit & Push all files (7.8)

Staged - pick those files which you intend to bind to a commit

Commit - write a short descriptive message, binds changes to a single commit
(7.5.1)

Push - “Pushes” your changes from the local repo to the remote repo on GitHub
(7.5.2)

1. On GitHub, refresh and see that files are updated. Cool! Now you’ve used something someone else has created, customized it, and saved your updated version.

7.4 Talking to GitHub

Getting set up to talk to GitHub can seem like a pain. Eventually when you work on your own computer - with a copy of R & RStudio installed - you will only have to do this once. For now when we use RStudio Cloud - it looks like we have to do this **once per project**. It only takes a few seconds and you should put these commands **directly into your console**.

Run this first line **in your console** and put in your GitHub username and the e-mail connected to your GitHub account.

**Note - you might not have to do this first step if you go to your RStudio Cloud profile > Authentication and select Github Enabled & Private repo access also enabled

```
usethis::use_git_config(user.name = "Jane Doe", user.email = "jane@example.org")
```

Then you need to give RStudio Cloud your GitHub Personal Access Token, which you can retrieve by going to Settings > Develop Settings > Generate Token (7.8)

Select all the “scopes” and name your token.

[Settings / Developer settings](#)

GitHub Apps

OAuth Apps

Personal access tokens

Personal access tokens

Tokens you have generated that can be used to

Make a note of this because you will need it whenever you set up a new project you want to talk to GitHub. GitHub recently removed password authentication in favour of PATs, but RStudio Cloud doesn't seem to have updated this yet - that's ok though - just enter this line of code - then copy+paste your PAT when prompted. - Option set/replace these credentials.

```
gitcreds::gitcreds_set()
```

If you forget your PAT - that's ok - you can't retrieve it - but you can just generate a new one.

7.4.1 See changes

The first and most immediate benefit of using GitHub with your RStudio Project is seeing the changes you have made since your last commit.

The RStudio Git pane lists every file that's been added, modified or deleted. The icon describes the change:

-  You've changed a file
-  You've added a new file Git hasn't seen before
-  You've deleted a file

You can get more details on the changes that have been made to each file by right-clicking and selecting diff  . This opens a new window highlighting the differences between your current file and the previous commit.

Show Staged Unstaged Context 5 line Stage All Discard All

		how to solve, SO should be your first resource. It's highly likely you've had exactly the same problem as you, and there will be a whole bunch of answers to choose from.
i	68	68
i	69	RStudio provides many tools to make your day-to-day use of git easier. However, there are a huge number of git commands, and they're not all available in the IDE. That means you'll need to run a handful of command-line tools (from the RStudio console), especially when you're setting up, dealing with merges, or trying to get out of jams. The easiest way to get to a shell is Tools > Terminal. It's important to be familiar with using git from the command line, because if you want to search for problems, you'll need to know what the standard git commands do.
l	69	RStudio provides many tools to make your day-to-day use of git easier. However, there are a huge number of git commands, and they're not all available in the IDE. That means you'll need to run a handful of command-line tools (from the RStudio console), especially when you're setting up, dealing with merges, or trying to get out of jams. The easiest way to get to a shell is Tools > Terminal. It's important to be familiar with using git from the command line, because if you want to search for problems, you'll need to know what the standard git commands do.
70	70	
71		## Initial set up
	71	## Initial set up {#git-init}
72	72	
73	73	If you've never used git or github before, you'll need to do some setup:
74	74	
75	75	1. Install git:
76	76	
77		* Windows: < http://msysgit.github.io/ >
78		* OS X: < http://code.google.com/p/git-osx-installer/ >

The background colours tell you whether the text has been added (green) or removed (red). (If you're colourblind you can use the line numbers in the two

columns at the far left as a guide).

7.5 How to use version control - when to commit, push and pull

Repositories (repos) on GitHub are the same unit as an RStudio Project - it's a place where you can easily store all information/data/etc. related to whatever project you're working on.

When we create a Repository in GitHub and have it communicating with a Project in RStudio, then we can get (**pull**) information from GitHub to RStudio, or **push** information from RStudio to GitHub where it is safely stored and/or our collaborators can access it. It also keeps a *complete history* of updated versions that can be accessed/reviewed by you and your collaborators at any time, from anywhere, as long as you have the internet.

I have mentioned the term **commit** a few times (7.8). The fundamental unit of work in Git is a commit. A commit takes a snapshot of your code at a specified point in time.

You create a commit in two stages:

1. You **stage** files, telling Git which changes should be included in the next commit.

2. You **commit** the staged files, describing the changes with a message.

To create a new commit, **save** your files, then select files for inclusion by *staging* them, tick the checkbox and then select the commit box

7.5. HOW TO USE VERSION CONTROL - WHEN TO COMMIT, PUSH AND PULL119

The screenshot shows the RStudio interface with two main panels: the top panel displays the Git interface and the bottom panel displays the file browser.

Top Panel (Git Interface):

- Header: akulkar8, test
- Tab bar: Environment, History, Connections, **Git** (highlighted)
- Buttons: Diff, Commit (highlighted with a red box), Pull, Push, Refresh, Gear
- Status bar: master
- Table:

Staged	Status	Path
<input checked="" type="checkbox"/>	A	.gitignore
<input checked="" type="checkbox"/>	A	Step_1.PNG
<input checked="" type="checkbox"/>	A	test.R
<input checked="" type="checkbox"/>	A	test.Rproj

Bottom Panel (File Browser):

- Header: Files, Plots, Packages, Help, Viewer
- Toolbar: New Folder, Upload, Delete, Rename, More
- Path: Home > test
- Table:

	Name	Size	Modified
	..		
<input type="checkbox"/>	.gitignore	40 B	Jul 2, 2018, 7:29 AM
<input type="checkbox"/>	.Rhistory	0 B	Jul 2, 2018, 8:47 AM
<input type="checkbox"/>	README.md	45 B	Jul 2, 2018, 7:29 AM
<input type="checkbox"/>	test.Rproj	205 B	Jul 2, 2018, 12:13 PM
<input type="checkbox"/>	Step_1.PNG	173.3 KB	Jul 2, 2018, 12:15 PM
<input type="checkbox"/>	test.R	0 B	Jul 2, 2018, 12:16 PM

A new window will open - and you will see the diffs in the bottom pane, and all the files you have selected for the latest commit.

7.5.1 Commit

You now need to write a **commit message**. This should be short but meaningful

7.5. HOW TO USE VERSION CONTROL - WHEN TO COMMIT, PUSH AND PULL121

The screenshot shows the RStudio: Review Changes interface. At the top, there's a header bar with the R logo, the title "RStudio: Review Changes - Google Chrome", and a secure connection indicator. Below the header is a navigation bar with tabs for "Changes" (selected), "History", and "master". There are also buttons for "Stage", "Revert", and "Ignore". Underneath the navigation bar is a table with columns for "Staged", "Status", and "Path". The table lists four files: ".gitignore", "Step_1.PNG", "test.R", and "test.Rproj", all marked as "A" (Added). The "Path" column shows the full file paths. Below the table is a code editor window. The "Show" dropdown is set to "Staged". The code editor displays the following R configuration file content:

```
@@ -0,0 +1,13 @@
1 Version: 1.0
2
3 RestoreWorkspace: Default
4 SaveWorkspace: Default
5 AlwaysSaveHistory: Default
6
7 EnableCodeIndexing: Yes
8 UseSpacesForTab: Yes
9 NumSpacesForTab: 2
10 Encoding: UTF-8
11
12 RnwWeave: Sweave
13 LaTeX: pdfLaTeX
```

Describe the **why**, not the what. Git stores all the associated differences between commits, the message doesn't need to say exactly what changed. Instead it should provide a summary that focuses on the **reasons** for the change. Make this understandable for someone else!

Once you click commit a new window will open that summarises your commit - and you can close this

7.5. HOW TO USE VERSION CONTROL - WHEN TO COMMIT, PUSH AND PULL123

RStudio: Review Changes - Google Chrome

Secure | https://rstudio.cos.gmu.edu/?view=review_changes

Changes History

Your branch is ahead of 'origin/master' by 1 commit.

Staged Status ▲ Path

Git Commit

```
>>> git commit -F /tmp/RtmpcZZ9bM/git-commit-message-a2564dbda297.txt
[master 40a88c3] First commit
 4 files changed, 17 insertions(+)
  create mode 100644 .gitignore
  create mode 100644 Step_1.PNG
  create mode 100644 test.R
  create mode 100644 test.Rproj
```

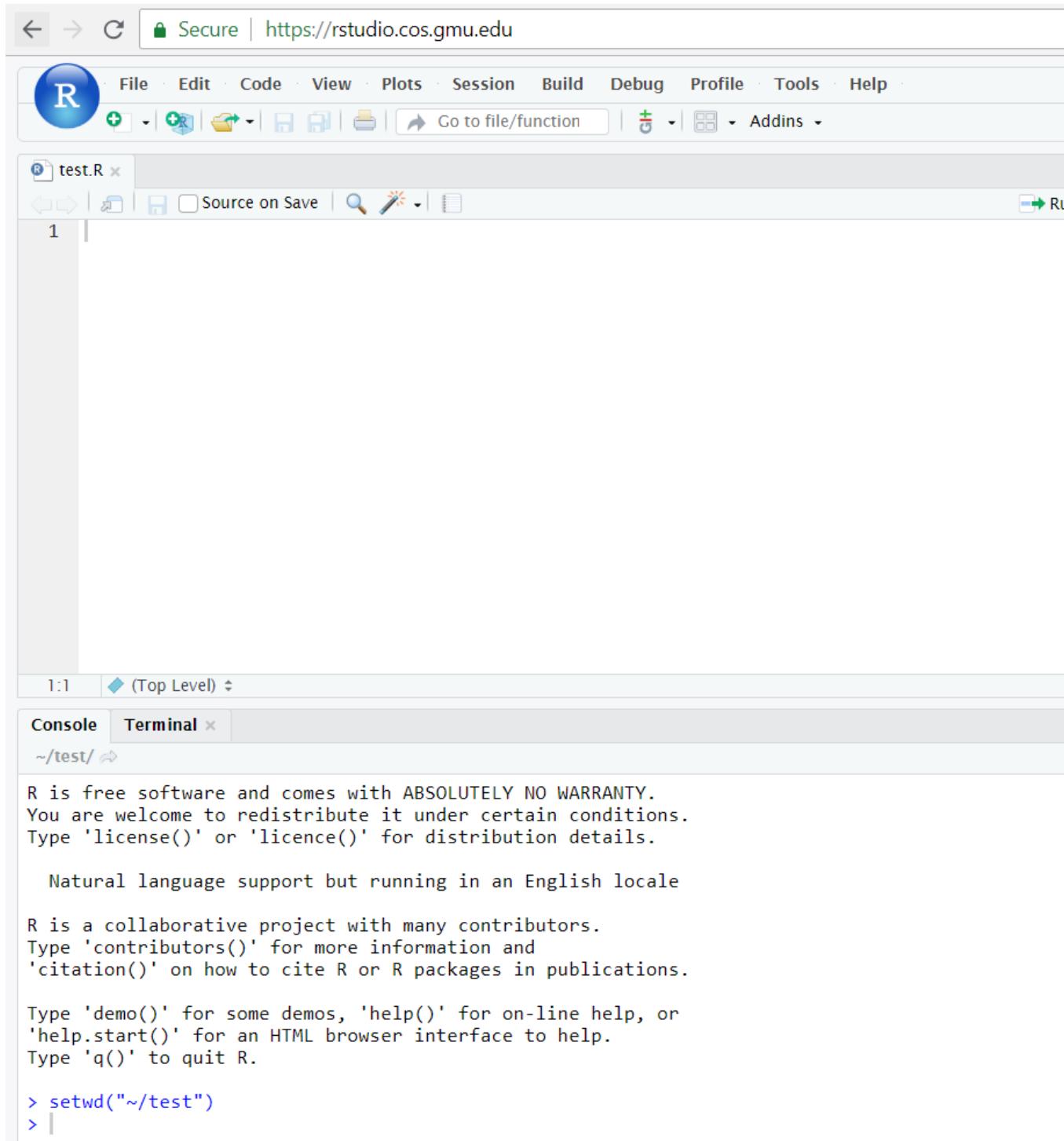
Show Staged Unstaged

7.5.2 Push

At the moment, all of your commits are *local*, in order to send them to GitHub you have to select **Push** at this point your github credentials need to be in place - if you get prompted to provide these, close the windows and follow the steps here (7.4) before trying again.

Your git pane will be empty at this point - but there is a little message at the top detailing how many commits you are *ahead* of the master repo on GitHub.

7.5. HOW TO USE VERSION CONTROL - WHEN TO COMMIT, PUSH AND PULL125



The screenshot shows the RStudio interface with the following details:

- Header:** Secure | https://rstudio.cos.gmu.edu
- Toolbar:** File, Edit, Code, View, Plots, Session, Build, Debug, Profile, Tools, Help.
- File Explorer:** Shows a file named "test.R".
- Source Editor:** A blank document window labeled "1".
- Console Tab:** Active tab, showing the R startup message:

```
R is free software and comes with ABSOLUTELY NO WARRANTY.  
You are welcome to redistribute it under certain conditions.  
Type 'license()' or 'licence()' for distribution details.  
  
Natural language support but running in an English locale  
  
R is a collaborative project with many contributors.  
Type 'contributors()' for more information and  
'citation()' on how to cite R or R packages in publications.  
  
Type 'demo()' for some demos, 'help()' for on-line help, or  
'help.start()' for an HTML browser interface to help.  
Type 'q()' to quit R.
```
- Console Input:** The command `> setwd("~/test")` is visible at the bottom of the console window.

7.5.3 A couple of general tips:



- Pull at the start of **every session** this retrieves the master repo from GitHub - which you update at the end of every session. This helps prevent *conflicts*
- **Commit/push** in small, meaningful increments and do this often. You can make **multiple** commits in a session - and **always push at the end of the session**
- In this way your GitHub Repo becomes the **master copy** of your project.

7.5.4 Turn back time with Git

"If I could turn back time,
 If I could find a way,
 I'd take back those commits that have hurt you,
 And you'd stay" - Cher

Once you start using Git with RStudio - you get a whole bunch of different options for undoing changes, correcting mistakes and turning back time to previous versions!

- To undo changes between commits you can select the diff option and remove lines one-by-one
- Right-click on a file in the Git pane and select the revert option will undo all changes between this and the previous commit **beware** cannot be undone.

If you don't catch a mistake right away you can select the history button and **pull** previous commits from GitHub

7.6 Exercise 2. GitHub Classrooms enabled R Projects with subfolders

GitHub Classrooms is a way for me to set repos as assignments - when you accept an assignment on GitHub Classroom it *automatically* forks a private repo for you.

You should make regular commits and pushes to save your work as you go - and I will be grading your project repositories on GitHub classrooms when you do your assignment work.



When you accept an assignment on GitHub classrooms - the repo won't appear on your main profile, this is because it belongs to our class rather than you. You can always find it by searching through your Organisations - **but** it's probably easiest just to make a bookmark/make a note of your unique URL for each assignment.

- a. Follow this invite link
- b. You will be invited to sign-in to Github (if not already) & to join the UEABIO organisation
- c. Clone your assignment to work locally in RStudio Cloud - 5023Y Workspace
- d. In your local project folder, create subfolders 'data' and 'figures', 'scripts', 'markdown' (**Note** use the `dir.create` commands in the console)
- e. Drop the file `disease_burden.csv` into the 'data' subfolder
- f. Open a new .R script
- g. Attach the `tidyverse`, `janitor`, and optionally `here` packages
- h. Read in the `infant_mortality.csv` data
- i. Stage, commit & push at this point - notice that the empty folder 'final_graphs' doesn't show up (won't commit an empty folder) - **you will have to set up your git credentials again**
- j. Back in the script, write a short script to read and clean the data.

This script is pre-written, it puts the data in tidy format, cleans names, makes sure year is treated as date data and filters four countries of interest.

Assign this to a new object

```
library(tidyverse)
library(lubridate)
library(janitor)
library(plotly)

infant_mortality <- read_csv("data/infant_mortality.csv")

subset_infant_mortality <- infant_mortality %>%
  pivot_longer(cols="1960":"2020",
               names_to="year",
               values_to="infant_mortality_rate") %>%
  mutate(year=lubridate::years(year)) %>% # set ymd format
```

```

mutate(year=lubridate::year(year)) %>% # extract year
janitor::clean_names() %>% # put names in snake case
filter(country_name %in%
      c("United States",
        "Japan",
        "Afghanistan",
        "United Kingdom")) # extract four countries

# View(subset_infant_mortality)

# subset the date according to (US,UK, Japan = lowest infant death rates, Afghanistan =

```

- k. Make a ggplot plotting the infant mortality rate by country

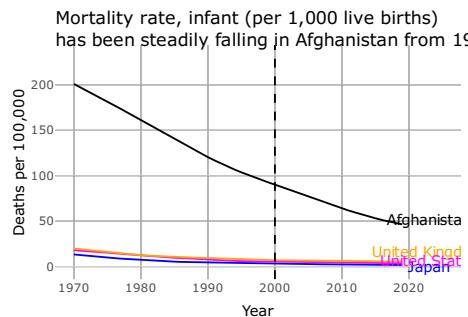
HINT - use `geom_line()` and remember to separate countries by colour

```

ggplot(data = subset_infant_mortality) +
  geom_line(aes(x = year,
                y = infant_mortality_rate,
                color = country_name))

```

- l. Update your graph with direct labels (using `annotate`) and vertical or horizontal lines with `geom_vline` or `geom_hline`.



- m. Use `ggsave()` to write your graph to a .png in the ‘final_graphs’ subfolder

```
ggsave("figures/infant mortality graph.png", plot=mortality_figure, dpi=900, width = 7, height =
```

- n. Save, stage, commit

- o Let’s do one more cool and fun thing! And make an interactive version of our plot using `plotly` Sievert et al. (2021) just for fun!

```
ggplotly(mortality_figure, tooltip = c("infant_mortality_rate"))
## uses plotly package
```

- p Now save, stage, commit & `push`

- q. Check that changes are stored on GitHub

(**NOTE** this will be in your organisations rather than repos)

Make sure you finish both exercises before next week to become a GitHub pro!!!!!!!

7.7 Find your classroom repos

When you work with GitHub classrooms your repos become part of our organisation UEABIO. If you want to find your repos on GitHub then you can use the direct URL (if you noted it), or head to (<https://github.com/UEABIO>) - you should only be able to see repos that belong to **you**.

The screenshot shows a GitHub repository page for the organization "UEABIO". The URL in the address bar is "github.com/UEABIO". The page features the GitHub logo and a search bar. Below the header, there is a large green square icon with a white checkered pattern. The repository name "UEABIO" is displayed in bold black text. A horizontal navigation bar below the main content includes links for "Overview", "Repositories" (with a count of 93), "Packages", "People" (with a count of 1), and "Team".

7.8 Glossary-GitHub

Terms	Description
clone	A clone is a copy of a repository that lives on your computer instead of on a web server.
commit	A commit, or revision, is an individual change to a file (or set of files). When you commit changes, they become part of the repository's history.
commit message	Short, descriptive text that accompanies a commit and communicates the changes made.
fork	A fork is a personal copy of another user's repository that lives on your account.
Git	Git is an open source program for tracking changes in text files. It was written by Linus Torvalds.
GitHub Classroom	GitHub Classroom automates repository creation and access control, making it easy to teach Git.
Markdown	Markdown is an incredibly simple semantic file format, not too dissimilar from plain text.
pull	Pull refers to when you are fetching in changes and merging them. For instance, when you pull changes from a remote repository, you are pulling changes into your local repository.
push	To push means to send your committed changes to a remote repository on GitHub.
README	A text file containing information about the files in a repository that is typically named "README.md".
repository	A repository (repo) is the most basic element of GitHub. They're easiest to interact with via the GitHub interface.
RMarkdown	RMarkdown is a package and filetype that are deeply embedded with RStudio.
personal access token	A token that is used in place of a password when performing Git operations over HTTP.

7.9 Summing up GitHub

7.9.1 What we learned

You have learned

- How to fork and clone GitHub Projects
- How to use GitHub classrooms
- How to make RStudio and GitHub talk to each other
- How to use version control, with stage, commit and push

You have used

- `gitcreds` Csárdi (2020)
- `usethis` Wickham et al. (2021a)

7.9.2 Further Reading, Guides and tips

Bryan (2012) <https://happygitwithr.com/>

The definitive guide

Chapter 8

Dealing with data: `dplyr`: Week Eight

8.1 Let's get going

You need to accept the latest assignment from Github Classrooms.

We will be using this project until the end of term, so make sure you make regular commits, and push your work at the end of each session.

8.2 Introduction to `dplyr`

In chapters 3 and 4 we demonstrated a brief data analysis on the `palmerpenguins` dataset. We ran through several pipelines, but without going into huge amounts of detail on each section.

In this chapter we are going to get really well acquainted with `dplyr` Wickham et al. (2021c).

We will look at several core functions:

- `select` (get some columns)
- `filter` (get some rows)
- `arrange` (order the rows)
- `mutate` (make new columns)
- `group_by` (add grouping information)
- `summarise` (calculate summary information)

You should make careful notes about these functions and what they do. Build scripts with carefully added notes that you can use for future work

As you work through this (and other chapters) - make notes, if you take a break, set up a commit, or reach the end of a session - push your work to Github.

Make sure you are using the R Cheat Sheets for dplyr

8.3 select

Start by setting up the packages you will need for this session

```
library(tidyverse)
library(lubridate)

penguins <- read_csv("data/penguins_simple.csv")
```

**** Note** - if you don't have these packages available, then you will need to run `install.packages("tidyverse")` - you should do this in the *console* and **not** your script.

We use `select` to *select variables* from a dataframe or tibble. for example:

```
data_set %>%
  select(variable_1, variable_2)

** Note this is pseudocode an example, not something you should run.
```

- The data is piped into the first argument position of the `select` function
- We then include the arguments where each one is the name of a variable in the dataset

For example if we actually run this:

```
penguins %>%
  select(flipper_length_mm)
```

To look at flipper length - we should note a couple of things.

- Variable name is **not** in quotes
- The original data is unchanged e.g. `penguins`. Unless we assign the results of `select` with the assignment arrow (`<-`), the results will just be printed in the console
- The order you ask for the variables in `select` determines the order of the columns in any new dataset

- `select` returns a tibble
- If we want to keep most variables, but *remove one*, use the minus operator

```
penguins <- penguins %>%
  select(-flipper_length_mm)
```



In the above option we just *overwrote* the penguin dataset with a new version that *does not* contain flipper length. Be careful - the above code will throw an error message if you try to run it again (there is no longer a flipper length variable to remove). If you want to *undo* this, the easiest way is to re-run your script to just before this line.

You can also use `select` to keep sets of consecutive variables together

```
penguins %>%
  select(species:flipper_length_mm)
```

There are also a number of helper functions like `starts_with`, `ends_with`, `contains`, `matches`. So if we want to keep all the variables that start with “b”

```
penguins %>%
  select(starts_with("b"))
```

8.4 mutate

Adding or creating new variables is a common task, we might want to make a log transformation, subtract one value from another or use `mutate` to add a new date variable

```
penguins <- penguins %>%
  mutate(date_proper=dmy(date))
```

Sometimes we want to change the values being used. For example we might want to change “male” and “female” to some abbreviation like “M” and “F”. (Probably not, but it’s an example!).

```
penguins %>%
  mutate(sex=case_when(sex == "male" ~ "M",
                       sex == "female" ~ "F"))
```

Things to know about `mutate`:

- Unless we assign the results of `mutate` back to an object (`<-`) the changes won't be saved
- We can use newly created variables in further calculations *within* the same `mutate`

8.5 filter

`filter` is used to subset observations in a data frame. We might want to see the observations where `flipper_length_mm` is larger or smaller than a fixed value. Or we might want to only see the data from female penguins, or a particular species.

```
penguins %>%
  filter(sex == "female",
         species == "Adelie",
         flipper_length_mm < 180)
```

In this example we've created a subset of `penguins` that only includes the five observations where flipper length is less than 180mm in female, Adelie penguins.

We can also set an either/or filter, for example if we only want small & large flipper lengths.

```
penguins %>%
  filter(species == "Adelie",
         flipper_length_mm < 180 | 
         flipper_length_mm > 200)
```

This creates a subset of Adelie penguins, that only includes 14 observations where flipper length is less than 180mm or greater than 200mm.

The vertical bar `|` is understood by R as OR.

The alternative is to look at values *between* two amounts

```
penguins %>%
  filter(species == "Adelie",
         between(flipper_length_mm, 180, 200))
```

8.6 arrange

We use `arrange` to reorder the rows of our data. Instances where we ‘need’ to do this are rare. But we may often want to reorder rows so that we can understand the data more easily

```
penguins_ordered <- penguins %>%
  arrange(sex, body_mass_g)

# arrange data first by sex - all females then all males, within each sex order body mass from low to high
penguins_ordered %>%
  select(penguin_id, sex, body_mass_g)
# view just a few variables
```

By default `arrange` sorts from low to high (and alphabetically) - we can reverse this if we wrap the variable names in the function `desc`

```
penguins_reverse_ordered <- penguins %>%
  arrange(desc(sex, body_mass_g))

penguins_reverse_ordered %>%
  select(penguin_id, sex, body_mass_g)

# we can also apply this to specific variables
penguins_reverse_ordered <- penguins %>%
  arrange(sex, desc(body_mass_g))
```

8.7 Group and summarise

Very often we want to make calculations about groups of observations, such as the mean or median. We are often interested in comparing responses among groups. For example, we previously found the number of distinct penguins in our entire dataset

```
penguins %>%
  summarise(n_distinct(penguin_id))
```

Now consider when the groups are subsets of observations, as when we find out the number of penguins in each species and sex

```
penguins %>%
  group_by(species, sex) %>%
  summarise(n_distinct(penguin_id))
```

We are using `summarise` and `group_by` a lot! They are very powerful functions:

- `group_by` adds *grouping* information into a data object, so that subsequent calculations happen on a *group-specific* basis.
- `summarise` is a data aggregation function that calculates summaries of one or more variables, and it will do this separately for any groups defined by `group_by`

8.7.1 Using `summarise`

```
penguins %>%
  summarise(mean_flipper_length = mean(flipper_length_mm, na.rm=TRUE),
            mean_bill_length = mean(bill_length_mm, na.rm=TRUE))
```

**Note - we provide informative names for ourselves on the left side of the `=`.

There are a number of different calculations we can use including:

- `min` and `max` to calculate minimum and maximum values of a vector
- `mean` and `median`
- `sd` and `var` calculate standard deviation and variance of a numeric vector

We can use several functions in `summarise`. Which means we can string several calculations together in a single step

```
penguins %>%
  summarise(n=n(),
            num_penguins = n_distinct(penguin_id),
            mean_flipper_length = mean(flipper_length_mm, na.rm=TRUE),
            prop_female = sum(sex == "female", na.rm=TRUE) / n())
```

**Note - we have placed each argument on a separate line. This is just stylistic, it makes the code easier to read.

8.7.2 Summarize all columns

We can use the function `across` to count up the number of NAs in every column (by specifying `everything`).

```
penguins %>%
  summarise(across(.cols = everything(),
                   .fns = ~sum(is.na(.)))) %>%
  glimpse()
```

It has two arguments, `.cols` and `.fns`. The `.cols` argument lets you specify column types, while the `.fns` argument applies the required function to all of the selected columns.

```
# the mean of ALL numeric columns in the data, where(is.numeric) hunts for numeric columns

penguins %>%
  summarise(across(.cols = where(is.numeric),
                   .fns = ~mean(., na.rm=TRUE)))
```

The above example calculates the means of any & all numeric variables in the dataset.

The below example is a slightly complicated way of running the `n_distinct` for `summarise`. The `.cols()` looks for any column that contains the word “penguin” and runs the `n_distinct()` command of these

```
# number of distinct penguins, as only one column contains the word penguin

penguins %>%
  summarise(across(.cols = contains("penguin"),
                   .fns = ~n_distinct(.))) %>%
  glimpse()
```

8.7.3 group_by summary

The `group_by` function provides the ability to separate our summary functions according to any subgroups we wish to make. The real magic happens when we pair this with `summarise` and `mutate`.

In this example, by grouping on the individual penguin ids, then summarising by n - we can see how many times each penguin was monitored in the course of this study.

```
penguin_stats <- penguins %>%
  group_by(penguin_id) %>%
  summarise(num=n())
```

**Note - the actions of group_by are powerful, but group_by on its own doesn't change the visible structure of the dataframe.

8.7.4 More than one grouping variable

What if we need to calculate by more than one variable at a time?

```
penguins_grouped <- penguins %>%
  group_by(sex, species)
```

We can then calculate the mean flipper length of penguins in each of the six combinations

```
penguin_summary <- penguins_grouped %>%
  summarise(mean_flipper = mean(flipper_length_mm, na.rm=TRUE))
```

Now the first row of our summary table shows us the mean flipper length (in mm) for female Adelie penguins. There are eight rows in total, six unique combinations and two rows where the sex of the penguins was not recorded(NA)

8.7.4.1 using group_by with mutate

When `mutate` is used with a grouped object, the calculations occur by ‘group’. Here’s an example:

```
centered_penguins <- penguins %>%
  group_by(sex, species) %>%
  mutate(flipper_centered = flipper_length_mm-mean(flipper_length_mm, na.rm=TRUE))
```

Here we are calculating a **group centered mean**, this new variable contains the *difference* between each observation and the mean of whichever group that observation is in.

8.7.5 remove group_by

On occasion we may need to remove the grouping information from a dataset. This is often required when we string pipes together, when we need to work using a grouping structure, then revert back to the whole dataset again

Look at our grouped dataframe, and we can see the information on groups is at the top of the data:

```
# A tibble: 344 x 10
# Groups:   sex, species [8]
  species island bill_length_mm bill_depth_mm flipper_length_mm body_mass_g
```

```
<chr> <chr>      <dbl>      <dbl>      <dbl>      <dbl>
1 Adelie Torge~     39.1       18.7      181       3750
2 Adelie Torge~     39.5       17.4      186       3800
3 Adelie Torge~     40.3        18       195       3250
```

```
centered_penguins %>%
  ungroup()
```

Look at this output - you can see the information on groups has now been removed from the data.

Chapter 9

Dealing with data part 2: Week Nine

9.1 Expanding the data toolkit

In this chapter we go through some more miscellaneous topics around dealing with data.

9.2 Pipes

As you have seen we often link our dplyr verbs together with `pipes`. Using one function after another e.g. `mutate` then `group_by` and perhaps `summarise`. The pipe `%>%` allows us to make “human-readable” an logical strings of instructions.

The two below examples of pseudocode would be read identically by R - but which one is easier for you to understand?

`%>%`

```
leave_house(get_dressed(get_out_of_bed(wake_up(me, time =
"8:00"), side = "correct"), pants = TRUE, shirt = TRUE), car
= TRUE, bike = FALSE)

me %>%
  wake_up(time = "8:00") %>%
  get_out_of_bed(side = "correct") %>%
  get_dressed(pants = TRUE, shirt = TRUE) %>%
  leave_house(car = TRUE, bike = FALSE)
```

One way we can try and breakdown the complexity of multiple nested brackets is to make lots of “intermediate” R objects:

```
penguins_grouped <- group_by(penguins, species, sex)

penguins_grouped_full <- drop_na(penguins_grouped, sex)

penguins_flipper_length <- summarise(penguins_grouped_full, mean=mean(flipper_length_mm))

penguins_flipper_length
```

**Note - nothing wrong with this, but look at how many R objects are cluttering up your environment tab which you will probably never use again!

Or...

```
penguins %>%
  group_by(., species, sex) %>%
  drop_na(., sex) %>%
  summarise(., mean=mean(flipper_length_mm))
```

**Note - check for yourself whether the outcome is identical

The pipe is becoming increasingly popular - because it makes code so much more readable. The newest versions of base R (4.1 and above) have even incorporate their own version of a pipe `|>` (<https://michaelbarrowman.co.uk/post/the-new-base-pipe/>).

But how does the pipe actually work? Well whenever you include a pipe in your code, it signals to take everything on the left-hand side of the pipe and place it as an “argument” in the code which then comes on the right hand side. The `.` in the code above shows you the placeholder where the argument is placed.

When you use the pipe (as you have been doing previously), you don’t actually need to include the `.` for most functions. If you don’t put it in, the pipe will simply place it into the *first argument* position. For this reason you will see that all dplyr functions have the first argument as the data.

```
penguins %>%
  group_by(species, sex) %>%
  drop_na(sex) %>%
  summarise(mean=mean(flipper_length_mm))
```

9.3 Strings

Datasets often contain words, and we call these words “strings”. Often these aren’t quite how we want them to be, but we can manipulate these as much as we like. Functions in the package `stringr` Wickham (2019), are fantastic. And the number of different types of manipulations are endless

```
str_replace_all(names(penguins), c("e"= "E"))
```

9.3.1 separate

Sometimes a string might contain two pieces of information in one. This does not confirm to our tidy data principles. But we can easily separate the information with `separate` from `tidyverse` Wickham (2021)

First we produce some made-up data

```
df <- tibble(label=c("a-1", "a-2", "a-3"))
#make a one column tibble
df
```

```
df %>%
  separate(label, c("treatment", "replicate"), sep="-")
```

We started with one variable called `label` and then split it into two variables, `treatment` and `replicate`, with the split made where - occurs. The opposite of this function is `unite()`

9.3.2 More stringr

Check out (<https://stringr.tidyverse.org/index.html>) Wickham (2019)

```
penguins %>%
  mutate(species=str_to_upper(species))
```

```
penguins %>%
  mutate(species=str_remove_all(species, "e"))
```

We can also trim leading or trailing empty spaces with `str_trim`. These are often problematic and difficult to spot e.g.

```
df2 <- tibble(label=c("penguin", " penguin", "penguin "))
df2
```

We can easily imagine a scenario where data is manually input, and trailing or leading spaces are left in. These are difficult to spot by eye - but problematic because as far as R is concerned these are different values. We can use the function `distinct` to return the names of all the different levels it can find in this dataframe.

```
df2 %>%
  distinct()
```

If we pipe the data through the `str_trim` function to remove any gaps, then pipe this on to `distinct` again - by removing the whitespace, R now recognises just one level to this data.

```
df2 %>%
  mutate(label=str_trim(label, side="both")) %>%
  distinct()
```

A quick example of how to extract partial strings according to a pattern is to use `grepl`. Combined with `filter` it is possible to subset dataframe by searching for all the strings that match provided information, such as all the penguin IDs that start with “N1”

```
penguins %>%
  filter(grepl("N1", penguin_id)) %>%
  distinct(penguin_id)
```

9.4 Dates and times

Dates and times are difficult.

There's a lot of different ways to write the same date

13-10-2019

10-13-2019

13-10-19

13th Oct 2019

2019-10-13

This variability makes it difficult to tell our software how to read the information, luckily we can use `lubridate` Spinu et al. (2021). We can tell R the order of our date (or time) data.

Back in the last chapter (8.4), we used `mutate` and `lubridate` to convert date data which had been parsed as a string, into date format with the function `dmy()`. Depending on how we interpret the date ordering in a file, we can use `ymd`, `ydm`, `mdy` etc.

If you get a warning that some dates could not be parsed, then you might find the date date has been inconsistently entered into the dataset.

```
penguins %>%
  select(date, date_proper) %>%
  head()
```

Once we have established our date data, we are able to perform calculations. Such as the date range across which our data was collected. Try this with our variable in string form and it will provide the alphabetical order instead.

```
penguins %>%
  summarise(min_date=min(date_proper),
            max_date=max(date_proper))
```

9.4.1 Calculations with dates

How many times was each penguin measured, and across what total time period?

```
penguins %>%
  group_by(penguin_id) %>%
  summarise(min=min(date_proper),
            max=max(date_proper),
            difference = max-min,
            n=n())
```

Cool we can also convert intervals such as days into weeks, months or years with `dweeks(1)`, `dmonths(1)`, `dyears(1)`.

As with all cool functions, you should check out the RStudio cheat sheet for more information. Date type data is common in datasets, and learning to work with it is a uesful skill.

```
penguins %>%
  group_by(penguin_id) %>%
```

```
summarise(min=min(date_egg_proper),  
          max=max(date_egg_proper),  
          difference = (max-min)/dyears(1),  
          n=n()) %>%  
arrange(desc(difference))
```

9.5 Pivot

In the previous chapters we discussed the principles of tidy data (3.3.1), tidy data is easy to analyse, but not always the format you will find data in.

The most likely format you will find data in is a “wide format”, where we have columns of related data. Luckily `tidyverse` contains a number of **pivot** functions

wide

id	x	y	z
1	a	c	e
2	b	d	f

Let's start with some dummy data - we will make a dataframe of three penguins and the number of three types of prey they are observed to eat over a week:

```
penguin_id <- c("N1A1", "N1A2", "N2A1")
krill <- c(70, 20, 43)
fish <- c(5, 19, 4)
squid <- c(11, 5, 0)

df3 <- tibble(penguin_id, krill, fish, squid)
df3
```

This dataset is perfectly legible, but does not conform to tidy data principles, each row is not a unique observation, but instead contains three observations of types of prey eaten. Most of the time, we can make data “tidy” but pivoting wide data into a long format. In this example we should rearrange the data to produce a longer dataframe with fewer columns. We should make a new column for prey “type” and a column for “amount”

```
df3_long <- df3 %>%
  pivot_longer(cols=(krill:squid), names_to = "prey_type", values_to = "consumption_per_week")
df3_long
```

It then becomes extremely easy to plot this data:

```
df3_long %>%
  ggplot(aes(x=penguin_id, y=consumption_per_week, fill=prey_type)) +
  geom_bar(stat="identity", position=position_dodge())
```

Once again, there's an RStudio cheatsheet for this, so check it out!

You may very occasionally need to use `pivot_wider()` to generate tidy data, but it's unusual, the above scenario is the most likely messy data you will encounter!

9.6 Summing up

9.6.1 What we learned

You have learned:

The most common data manipulation functions you will need to import and tidy data, and the way in which they operate.

- How to create new columns as products of functions or calculations
- How to select and filter data to access the subsets you are interested in

- How to apply functions to data groups
- How the pipe works, and why we use it
- How to deal with data subtypes like strings and dates
- How to reshape data with pivot

You have used `tidyverse` and the functions made available from:

- `dplyr` Wickham et al. (2021c)
- `tidyr` Wickham (2021)

You have also used two packages, which are also installed as part of the tidyverse, but have to be called with separate `library()` functions

- `lubridate` Spinu et al. (2021)
- `stringr` Wickham (2019)

9.6.2 Further Reading, Guides and tips

R Cheat Sheets

Chapter 10

Deeper data insights part 1: Week Ten

In these last chapters on generating data insights we are going to cover

- Types of variable
- Exploring numeric variables
- Exploring categorical variables
- Dealing with missing data

Before we dive into this it is important to understand where this data has come from, is it a single study? Is it a lab experiment, fieldwork survey, exploratory work or from a carefully designed study?

You should also play close attention to the data, and remind yourself **frequently** how many variables do you have and what are their names? How many rows/observations do you have?

10.1 Variables

10.1.1 Numerical

You **should** already be familiar with the concepts of numerical and categorical data. **Numeric** variables have values that describe a measure or quantity. This is also known as quantitative data. We can subdivide numerical data further:

- **Continuous numeric variables.** This is where observations can take any value within a range of numbers. Examples might include body mass (g), age, temperature or flipper length (mm). While in theory these values can have any numbers, within a dataset they are likely bounded (set within

a minimum/maximum of observed or measurable values), and the accuracy may only be as precise as the measurement protocol allows.

- **Discrete numeric variables** Observations are numeric but restricted to *whole values* e.g. 1,2,3,4,5 etc. These are also known as **integers**. Discrete variables could include the number of individuals in a population, number of eggs laid etc. Anything where it would make no sense to describe in fractions e.g. a penguin cannot lay 2 and a half eggs. Counting!

10.1.2 Categorical

Values that describe a characteristic of data such as ‘what type’ or ‘which category’. Categorical variables are mutually exclusive - one observation should not be able to fall into two categories at once - and should be exhaustive - there should not be data which does not *fit* a category (not the same as NA - not recorded). Categorical variables are qualitative, and often represented by non-numeric values such as words. It’s a bad idea to represent categorical variables as numbers (R won’t treat it correctly). Categorical variables can be defined further as:

- **Ordinal variables** Observations can take values that can be logically ordered or ranked. Examples include - activity levels (sedentary, moderately active, very active); size classes (small, medium, large).
- **Nominal variables** Observations that can take values that are not logically ordered. Examples include Species or Sex in the Penguins data.

It is important to order Ordinal variables in their logical order value when plotting data visuals or tables. Nominal variables are more flexible and could be ordered in whatever pattern works best for your data (perhaps you could order them according to the values of another numeric variable).



Don't use numbers to describe categorical information e.g. (Adelie = 1, Gentoo = 2, Chinstrap = 3). This can be done, but it isn't usually very sensible. It's clearer to use the words themselves, and helps when making tables and graphs later.

It's easy to assign levels to categorical data:

Example:

```
penguins <- penguins %>% mutate(species = factor(species, levels = c("Adelie", "Gentoo", "Chinstrap")))
```

10.2 Understanding Numerical variables

Let's take a look at some of our variables

```
glimpse(penguins)

## # Rows: 344
## # Columns: 9
## # $ species      <chr> "Adelie", "Adelie", "Adelie", "Adelie", "Adelie", "A-
## # $ island       <chr> "Torgersen", "Torgersen", "Torgersen", "Torgersen", ~
## # $ bill_length_mm <dbl> 39.1, 39.5, 40.3, NA, 36.7, 39.3, 38.9, 39.2, 34.1, ~
## # $ bill_depth_mm  <dbl> 18.7, 17.4, 18.0, NA, 19.3, 20.6, 17.8, 19.6, 18.1, ~
## # $ flipper_length_mm <dbl> 181, 186, 195, NA, 193, 190, 181, 195, 193, 190, 186-
## # $ body_mass_g    <dbl> 3750, 3800, 3250, NA, 3450, 3650, 3625, 4675, 3475, ~
## # $ sex            <chr> "male", "female", "female", NA, "female", "male", "f-
## # $ date           <chr> "11/11/2007", "11/11/2007", "16/11/2007", "16/11/200-
## # $ penguin_id     <chr> "N1A1", "N1A2", "N2A1", "N2A2", "N3A1", "N3A2", "N4A-
```

We can see that bill length contains numbers, and that many of these are fractions, but only down to 0.1mm. By comparison body mass all appear to be discrete number variables. Does this make body mass an integer? The underlying quantity (bodyweight) is clearly continuous, it is clearly possible for a penguin to weigh 3330.7g but it might *look* like an integer because of the way it was measured. This illustrates the importance of understanding the type of variable you are working with - just looking at the values isn't enough.

On the other hand, how we choose to measure and record data *can* change the way it is presented in a dataset. If the researchers had decided to simply record small, medium and large classes of bodyweight, then we would be dealing with ordinal categorical variables. These distinctions can become less clear if we start to deal with multiple classes of ordinal categories - for example if the researchers were measuring body mass to the nearest 10g. It might be reasonable to treat these as integers...

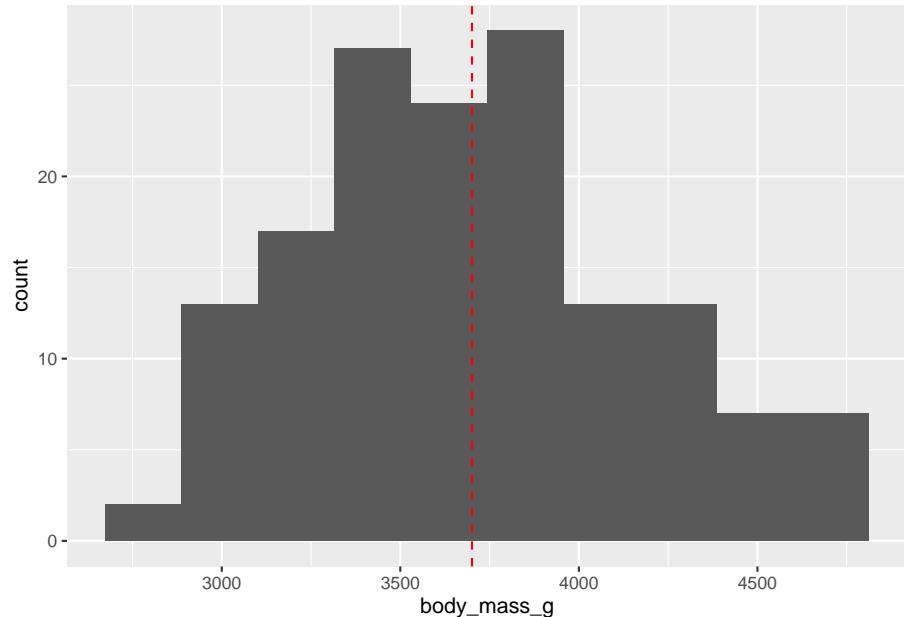
10.2.1 Graphing a numeric variable

```
adelie_penguins <- penguins %>%
  filter(species=="Adelie")

adelie_summary <- adelie_penguins %>%
  summarise(mean=mean(body_mass_g,
                      na.rm=TRUE))

ggplot() +
  geom_histogram(data= adelie_penguins,
                 aes(x=body_mass_g),
                 bins=10) + #remember it is a good idea to try multiple bins of data
  geom_vline(data=adelie_summary,
```

```
aes(xintercept=mean),
colour="red",
linetype="dashed")
```



Using this distribution, we can see that the data appears to fit a normal/gaussian distribution - mean body mass is slightly under 3750g. Penguins smaller than 3000g are rare.

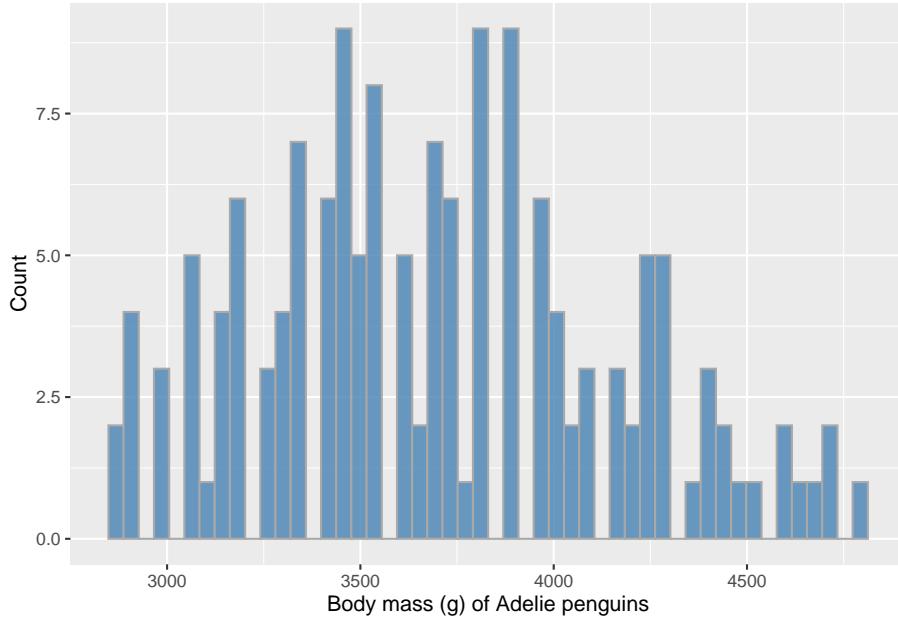
10.2.2 Insights about body mass

The histogram gives us a nice summary of the sample distribution of the body_mass_g variable. It reveals (1) the most common values, (2) the range of values and (3) the shape of the distribution. Remember it's a very good idea to play with the number of bins in order to see whether this changes the shape of the histogram.

Let's construct the histogram again with 30 bins. As well we will make some other tweaks.

```
ggplot()+
  geom_histogram(data=adelie_penguins,
    aes(x=body_mass_g),
    bins=50, # fifty bins
    fill="steelblue",
```

```
      colour="darkgrey",
      alpha=0.8) +
  labs(x="Body mass (g) of Adelie penguins",
       y = "Count")
```



Changing the colours and labels is purely aesthetic, and not needed *at all* for data exploration, but look how pretty!

What is important is that increasing the number of bins indicates that their *might* be two peaks in our data. We should think carefully about the other variables (probably categorical) that could be used to subset our data to investigate this. This requires careful thought about our data - in this instance - it seems sensible to try and see whether splitting the data by sex accounts for this

```
adelie_penguins %>%
  drop_na(sex) %>%
  ggplot() +
  geom_histogram(aes(x=body_mass_g,
                     fill=sex),
                 bins=50, # fifty bins
                 colour="darkgrey",
                 alpha=0.8,
                 position="identity") +
  labs(x="Body mass (g) of Adelie penguins",
```

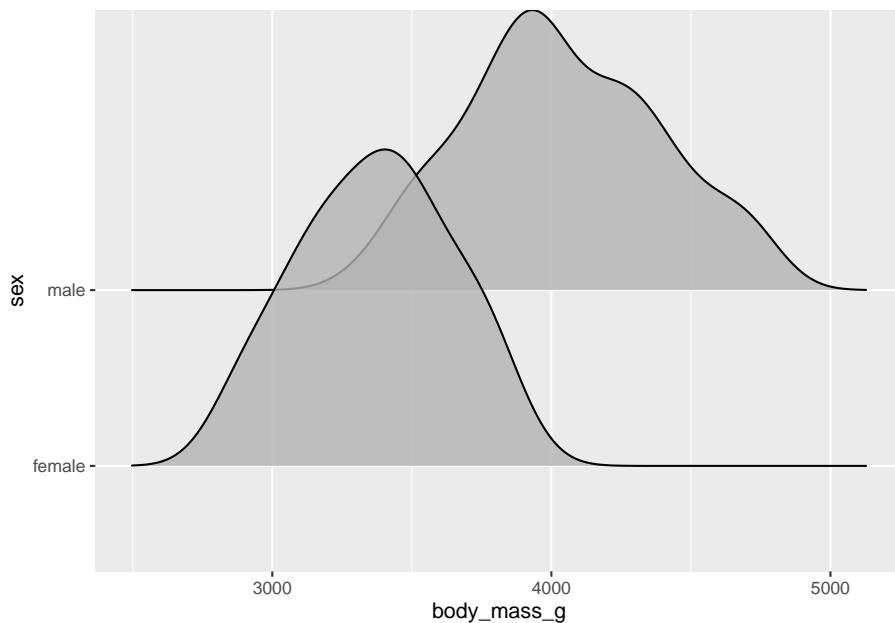
```
y = "Count")
```

Let's finish this section by looking at density. This is sensible to use when we have “large datasets”, and also allows us to make comparisons between groups with different sample sizes.

```
adelie_penguins %>%
  drop_na(sex) %>%
  ggplot() +
  geom_density(aes(x=body_mass_g,
                    fill=sex),
               colour="darkgrey",
               alpha=0.8,
               position="identity") +
  labs(x="Body mass (g) of Adelie penguins",
       y = "Count")
```

Or we can use one of my favourite packages `ggridges` Wilke (2021) which let's us separate out different groups along the y-axis.

```
adelie_penguins %>%
  drop_na(sex) %>%
  ggplot() +
  ggridges::geom_density_ridges(aes(x=body_mass_g,
                                    y=sex),
                                alpha=0.8)
```



10.3 Descriptive statistics

We have, so far, been spending our time describing the properties of data by examining graphs. Now we can start to build accurate and specific terms to the descriptions of our data.

- **central tendency** describes the typical (central) value of a distribution. The most well known description of central tendency is the arithmetic mean, however you should be comfortable with the idea that the median may be a better representation of the central tendency for some data distributions
- **dispersion** describes how a distribution is spread out. Dispersion measures the variability or scatter of a variable. If one distribution is more dispersed than another, this means that in some sense it encompasses a wider range of values. Basic statistics courses often tend to focus on variance and the standard deviation as two ways to measure/summarise dispersion. However, the interquartile range is another method often used in exploratory analysis.

In the next section we will use the median and interquartile ranges as effective measures of central tendency and dispersion - we will use graphics for this - and more specifically look at boxplots.

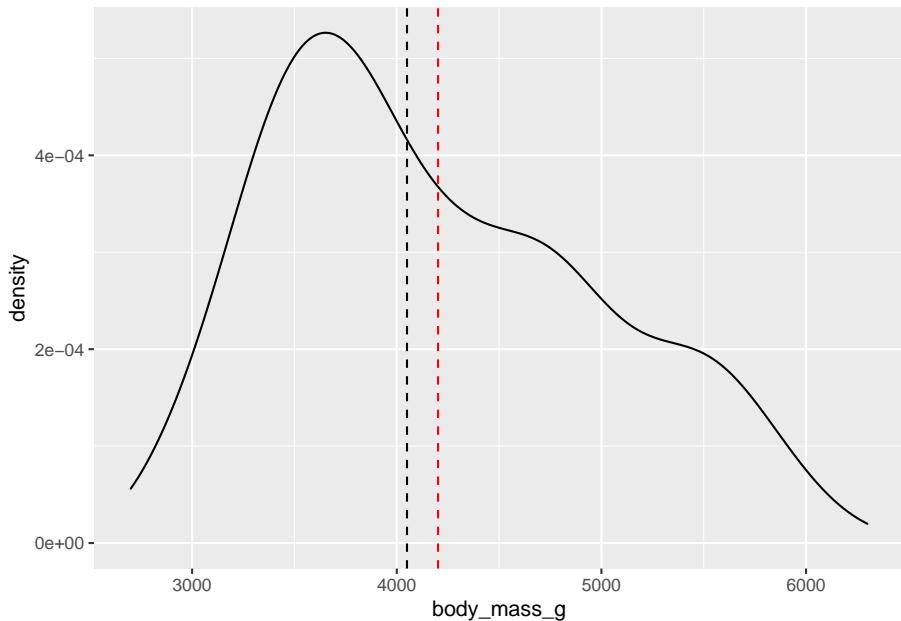
10.3.1 Central tendency

We can find both the mean and median easily with the summarise function.

```
penguin_body_mass_summary <- penguins %>%
  summarise(mean_body_mass=mean(body_mass_g, na.rm=T),
            median_body_mass=median(body_mass_g, na.rm=T))
```

```
penguin_body_mass_summary
```

```
## # A tibble: 1 x 2
##   mean_body_mass median_body_mass
##             <dbl>          <dbl>
## 1         4202.        4050
```



If we do this for the entire penguins dataset - we can clearly see that the mean value has been significantly “right-shifted” by the long tail of the data distribution. However, this is much less apparent for the median. In this way we can say that the median is less sensitive to the distribution of the data than the mean is.

10.3.2 Dispersion

Dispersion (how spread out the data is) is an important component towards understanding any numeric variable. Important measures for statistics are **variance** and **standard deviation**. These are both non-negative, smaller values indicate observations tend to be similar in value, while high values indicate these

observations are more spread out. We can quickly calculate these with `var` and `sd` - but they probably aren't the best place to *start* exploring your data from.

Variance is not an intuitive measure - calculated from squaring the deviation of each data point from the sample mean - it gives no overall insight into the shape of the distribution, and it is on a different scale to the original measurements. Standard deviation is the square root of the variance. This means it is on the same scale as the observation, making it easier to interpret, but just like variance it doesn't provide much insight into the overall shape of the distribution, and like the mean can be affected by outliers.

When trying to understand data it can be simpler and easier to use a measure that does not suffer from these issues outlined above. Instead we can use the **interquartile range**.

The interquartile range (IQR) is the range that contains the “middle 50%” of our data sample. This is given as the difference between the third and first quartiles. The reason we like to use the IQR is that the more spread out the data is, the larger the IQR. It will also indicate the shape of the distribution and is less affected by outliers than variance.

We can use the `IQR` function to find the interquartile range of the body mass variable

```
penguins %>%
  summarise(IQR_body_mass = IQR(body_mass_g, na.rm=TRUE))
```

The IQR is also useful when applied to the summary plots ‘box and whisker plots’. We can also calculate the values of the IQR margins, and add labels with `scales` Wickham and Seidel (2020).

```
penguins %>%
  summarise(q_body_mass = quantile(body_mass_g, c(0.25, 0.5, 0.75), na.rm=TRUE),
            quantile = scales::percent(c(0.25, 0.5, 0.75))) # scales package allows easy conversion
```

	q_body_mass	quantile
1	3550	25%
2	4050	50%
3	4750	75%

We can see for ourselves the IQR is obtained by subtracting the body mass at the 75% quantile from the 25% quantile ($4750 - 3550 = 1200$).

10.3.3 Visualising dispersion

```
penguins %>%
  ggplot() +
  geom_boxplot(aes(x = "",  

                    y = body_mass_g),  

                fill = "darkorange",  

                colour = "steelblue",  

                width = 0.4) +  

  labs(x = "Bodyweight",  

       y = "Mass (g)") +  

  theme_minimal()
```

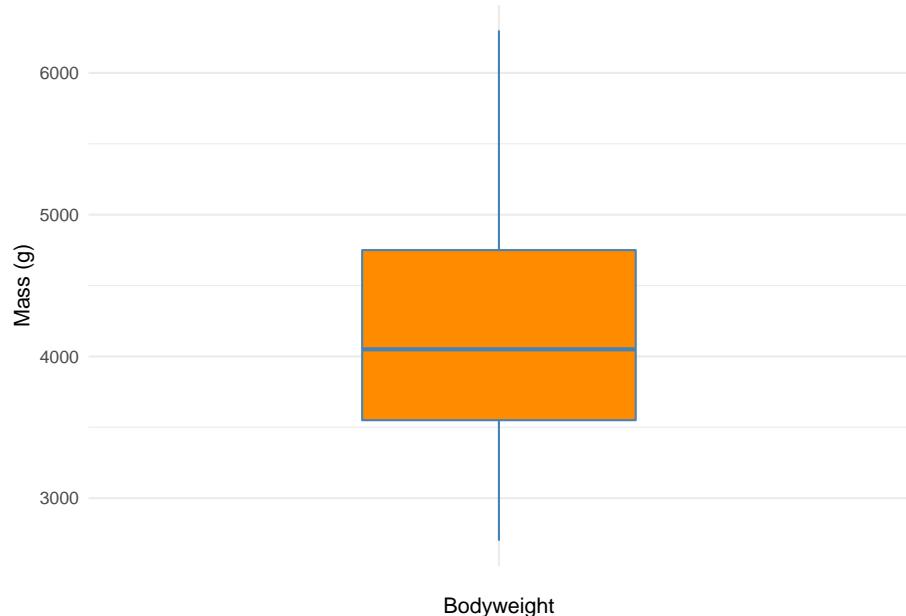


Figure 10.1: A boxplot of the body mass variable showing the median and IQR

**Note - we forced ggplot2 to hide the tick mark label on the x axis by coding a dummy label with x = " "

We now have several compact representations of the body_mass_g including a histogram, boxplot and summary calculations. You can *and should* generate the same summaries for your other numeric variables. These tables and graphs provide the detail you need to understand the central tendency and dispersion of numeric variables.

10.3.4 Combining histograms and boxplots

```

library(patchwork) # put this at the TOP of your script

penguins_na_sex <- penguins %>%
  drop_na(sex)

colours <- c("darkorange", "cyan") # set colour scheme here to save on repeating code
lims <- c(3000,6000) # set axis limits here to save on repeating code

p1 <- ggplot(data = penguins_na_sex,
  aes(x = species,
      y = body_mass_g,
      fill = sex))+ 
  geom_boxplot()+
  scale_fill_manual(values = colours)+ 
  scale_y_continuous(limits=lims)+ 
  labs(x="",
       y="")+
  coord_flip()# rotate box plot 90 degrees
  theme_minimal()+
  theme(legend.position="none")

p2 <- ggplot(data = penguins_na_sex,
  aes(x = body_mass_g,
      y = species,
      fill = sex))+ 
  ggridges::stat_density_ridges(quantile_lines = TRUE)+ 
  scale_fill_manual(values = colours)+ 
  scale_x_continuous(limits=lims)+ 
  labs(y="",
       x = "Body Mass (g)")+
  theme_minimal()

(p1/p2) # patchwork command to layer one plot above the other

```

10.3.5 Missing values

We first met `NA` back in Chapter 3.7.8 and you will hopefully have noticed, either here or in those previous chapters, that missing values `NA` can really mess up our calculations. There are a few different ways we can deal with missing data:

- `drop_na()` on everything before we start. This runs the risk that we lose **a lot** of data as *every* row, with an `NA` in *any column* will be removed

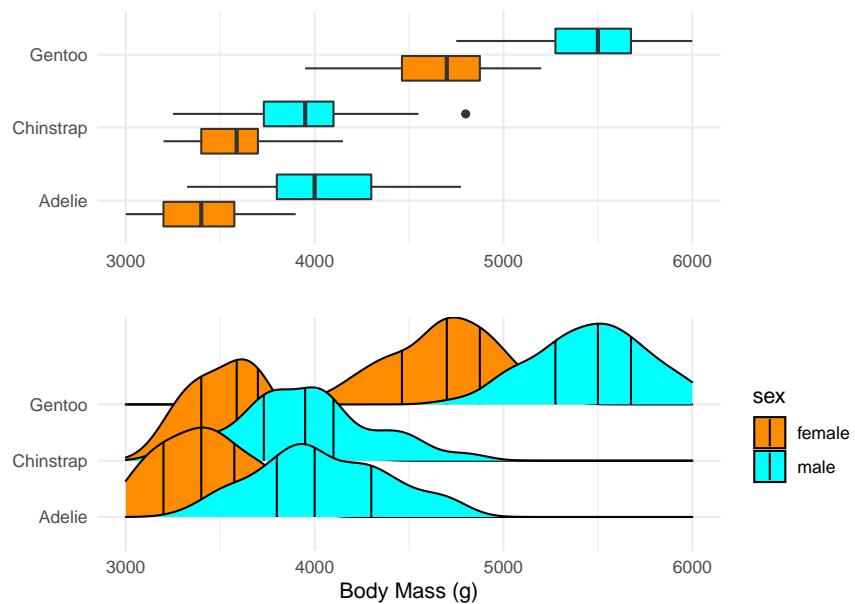


Figure 10.2: This figure shows the use of patchwork to combine two ggplots, demonstrating the boxplot and geom density figures show the same distributions of the data

- `drop_na()` on a particular variable. This is fine, but we should approach this cautiously - if we do this in a way where we write this data into a new object e.g. `penguins <- penguins %>% drop_na(body_mass_g)` then we have removed this data forever - perhaps we only want to drop those rows for a specific calculation - again they might contain useful information in other variables.
- `drop_na()` for a specific task - this is a more cautious approach **but** we need to be aware of another phenomena. Is the data **missing at random**? You might need to investigate *where* your missing values are in a dataset. Data that is truly **missing at random** can be removed from a dataset without introducing bias. However, if bad weather conditions meant that researchers could not get to a particular island to measure one set of penguins that data is **missing not at random** this should be treated with caution. If that island contained one particular species of penguin, it might mean we have complete data for only two out of three penguin species. There is nothing you can do about incomplete data other than be aware that data not missing at random could influence your distributions.

10.4 Categorical variables

Ok that was a lot of information - well done - have some praise.

```
praise::praise()
# have some praise
```

Now let's look at categorical variables -remember these can be **ordinal** or **nominal**. We don't have anything we would classify as ordinal data in the penguins dataset.

We can look at species with the function `distinct`:

```
penguins %>%
  distinct(species)
```

```
# A tibble: 3 x 1
  species
  <chr>
1 Adelie
2 Gentoo
3 Chinstrap
```

We can clearly see there would be no sense to applying an order to these three categories, we can order them in whatever way suits best when presenting our data.

10.4.1 Summaries

```
penguins %>%
  count(species, sort=TRUE)
```

```
# A tibble: 3 x 2
  species     n
  <chr>    <int>
1 Adelie     152
2 Gentoo     124
3 Chinstrap   68
```

It might be useful for us to make some quick data summaries here

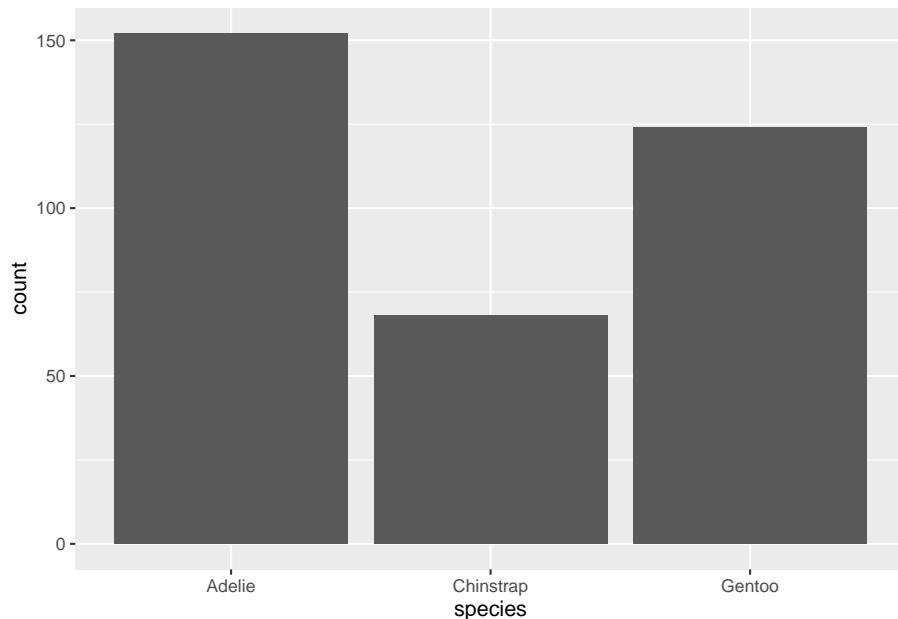
```
prob_obs_species <- penguins %>%
  count(species, sort=TRUE) %>%
  mutate(prob_obs = n/sum(n))

prob_obs_species
```

```
## # A tibble: 3 x 3
##   species     n prob_obs
##   <chr>    <int>    <dbl>
## 1 Adelie     152    0.442
## 2 Gentoo     124    0.360
## 3 Chinstrap   68    0.198
```

So about 44% of our sample is made up of observations from Adelie penguins. When it comes to making summaries about categorical data, that's about the best we can do, we can make observations about the most common categorical observations, and the relative proportions.

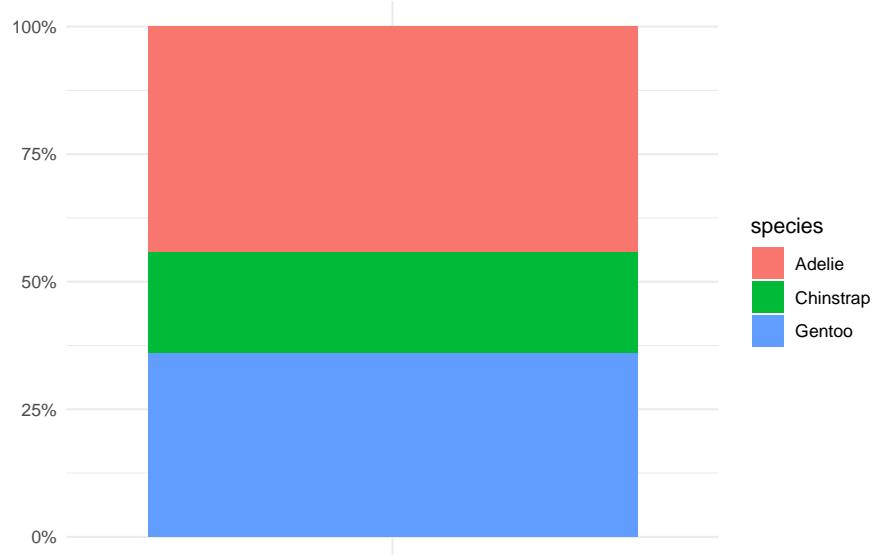
```
penguins %>%
  ggplot() +
  geom_bar(aes(x=species))
```



This chart is ok - but can we make anything better?

We could go for a stacked bar approach

```
penguins %>%
  ggplot(aes(x="",
              fill=species))+ # specify fill = species to ensure colours are defined by species
  geom_bar(position="fill")+ # specify fill forces geom_bar to calculate percentages
  scale_y_continuous(labels=scales::percent)+ #use scales package to turn y axis into percentages
  labs(x="",
       y="")+
  theme_minimal()
```

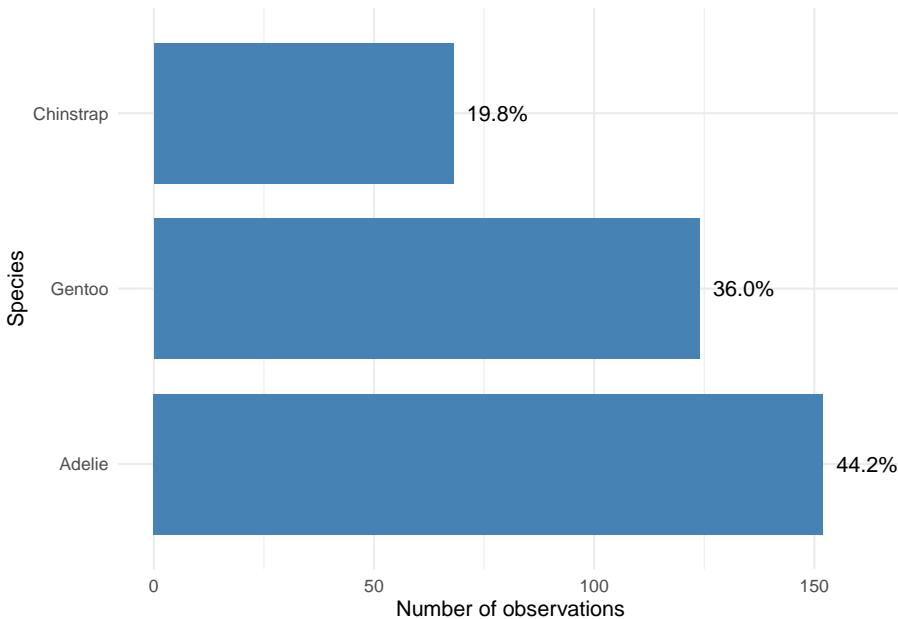


This graph is OK but not great, the height of each section of the bar represents the relative proportions of each species in the dataset, but this type of chart becomes increasingly difficult to read as more categories are included. Colours become increasingly samey, and it is difficult to read where on the y-axis a category starts and stops, you then have to do some subtraction to work out the values.

The best graph is then probably the first one we made - with a few minor tweaks we can rapidly improve this.

```
penguins %>%
  mutate(species=factor(species, levels=c("Adelie",
                                         "Gentoo",
                                         "Chinstrap"))) %>% # set as factor and provide
  ggplot()+
  geom_bar(aes(x=species),
           fill="steelblue",
           width=0.8)+
  labs(x="Species",
       y = "Number of observations")+
  geom_text(data=prob_obs_species,
            aes(y=(n+10),
                x=species,
                label=scales::percent(prob_obs)))+
  coord_flip()
```

```
theme_minimal()
```



10.5 Summing up

In this chapter we have really focused on single variables, understanding variable types and their distributions. We learned

- About different types of data
- How to estimate central tendencies
- Dispersions of numeric and categorical variables
- How to visualise metrics

You have primarily used `tidyverse` packages, but also:

- `ggridges` Wilke (2021)
- `scales` Wickham and Seidel (2020)

In the next chapter we will look at generating insights around the relationships between variables. Now is a good time to review earlier chapters such as Chapter 3, and think about how we ask questions of our data.

10.5.1 Reward

Haven't you done well???

Enjoy this stupid reward from ggcats

**Note - ggcats is sadly not available on CRAN library (yet) if you want to install your own version you can, you just need the package 'remotes' - then to follow the instructions on the ggcats github page.

```
library(Ecdat)
data(incomeInequality)

library(ggcats)
library(gganimate)

dat <-
  incomeInequality %>%
  select(Year, P99, median) %>%
  rename(income_median = median,
         income_99percent = P99) %>%
  pivot_longer(cols = starts_with("income"),
               names_to = "income",
               names_prefix = "income_")

dat$cat <- rep(NA, 132)

dat$cat[which(dat$income == "median")] <- "nyancat"
dat$cat[which(dat$income == "99percent")] <- rep(c("pop_close", "pop"), 33)

ggplot(dat, aes(x = Year, y = value, group = income, color = income)) +
  geom_line(size = 2) +
  ggtitle("ggcats, a core package of the memeverse") +
  geom_cat(aes(cat = cat), size = 5) +
  xlab("Cats") +
  ylab("Cats") +
  theme(legend.position = "none",
        plot.title = element_text(size = 20),
        axis.text = element_blank(),
        axis.ticks = element_blank()) +
  transition_reveal(Year)
```

Chapter 11

Deeper data insights part 2: Week Eleven

In the previous chapter we looked at individual variables, and understanding the different types of data. We made numeric and graphical summaries of the distributions of features within each variable. This week we will continue to work in the same space, and extend our understanding to include relationships between variables.

Understanding the relationship between two or more variables is often the basis of most of our scientific questions. These might include comparing variables of the same type (numeric against numeric) or different types (numeric against categorical). In this chapter we will see how we can use descriptive statistics and visuals to explore associations

11.1 Associations between numerical variables

11.1.1 Correlations

A common measure of association between two numerical variables is the **correlation coefficient**. The correlation metric is a numerical measure of the *strength of an association*

There are several measures of correlation including:

- **Pearson's correlation coefficient** : good for describing linear associations
- **Spearman's rank correlation coefficient**: a rank ordered correlation
 - good for when the assumptions for Pearson's correlation is not met.

Pearson's correlation coefficient r is designed to measure the strength of a linear (straight line) association. Pearson's takes a value between -1 and 1.

- A value of 0 means there is no linear association between the variables
- A value of 1 means there is a perfect *positive* association between the variables
- A value of -1 means there is a perfect *negative* association between the variables

A *perfect* association is one where we can predict the value of one variable with complete accuracy, just by knowing the value of the other variable.

We can use the `cor` function in R to calculate Pearson's correlation coefficient.

```
library(rstatix)

penguins %>%
  cor_test(bill_length_mm, bill_depth_mm)

## # A tibble: 1 x 8
##   var1           var2       cor statistic      p conf.low conf.high method
##   <chr>          <chr>     <dbl>     <dbl>    <dbl>    <dbl>    <chr>
## 1 bill_length_mm bill_depth_mm -0.24     -4.46  1.12e-5  -0.333  -0.132 Pears~
```

This tells us two features of the association. It's *sign* and *magnitude*. The coefficient is negative, so as bill length increases, bill depth decreases. The value -0.22 indicates that only about 22% of the variation in bill length can be explained by changes in bill depth (and *vice-versa*), suggesting that the variables are not closely related.

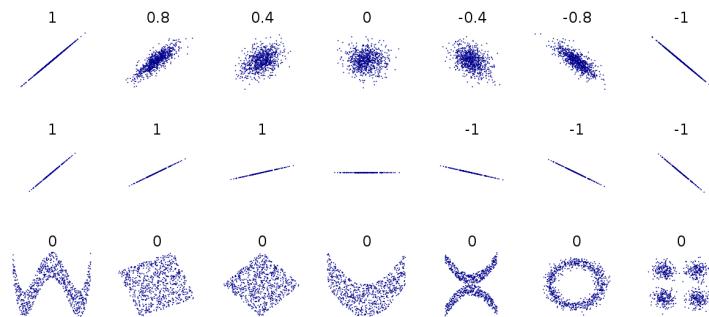


Figure 11.1: Different relationships between two numeric variables. Each number represents the Pearson's correlation coefficient of each association

- Because Pearson's coefficient is designed to summarise the strength of a linear relationship, this can be misleading if the relationship is *not linear*

e.g. curved or humped. This is why it's always a good idea to plot the relationship *first* (see above).

- Even when the relationship is linear, it doesn't tell us anything about the steepness of the association (see above). It *only* tells us how often a change in one variable can predict the change in the other *not* the value of that change.

This can be difficult to understand at first, so carefully consider the figure above.

- The first row above shows differing levels of the strength of association. If we drew a perfect straight line between two variables, how closely do the data points fit around this line.
- The second row shows a series of *perfect* linear relationships. We can accurately predict the value of one variable just by knowing the value of the other variable, but the steepness of the relationship in each example is very different. This is **important** because it means a perfect association can still have a small effect.
- The third row shows a series of associations where there is *clearly* a relationship between the two variables, but it is also not linear so would be inappropriate for a Pearson's correlation.

11.1.2 Non-linear correlations

So what should we do if the relationship between our variables is non-linear? Instead of using Pearson's correlation coefficient we can calculate something called a **rank correlation**.

Instead of working with the raw values of our two variables we can use rank ordering instead. The idea is pretty simple if we start with the lowest value in a variable and order it as '1', then assign labels '2', '3' etc. as we ascend in rank order. We can see a way that this could be applied manually with the function `dense_rank` from `dplyr` below:

```
penguins %>% select(bill_length_mm,
                      bill_depth_mm) %>%
  drop_na() %>%
  mutate(rank_length=dense_rank((bill_length_mm)),
        rank_depth=dense_rank((bill_depth_mm)))

## # A tibble: 342 x 4
##   bill_length_mm bill_depth_mm rank_length rank_depth
##       <dbl>          <dbl>      <int>      <int>
## 1       39.1         18.7        43          57
## 2       39.5         17.4        46          44
## 3       40.3         18.0        52          50
```

```

## 4      36.7      19.3      23      63
## 5      39.3      20.6      45      75
## 6      38.9      17.8      41      48
## 7      39.2      19.6      44      66
## 8      34.1      18.1       5      51
## 9      42        20.2      66      72
## 10     37.8      17.1      32      41
## # ... with 332 more rows

```

Measures of rank correlation then are just a comparison of the rank orders between two variables, with a value between -1 and 1 just like Pearson's. We already know from our Pearson's correlation coefficient, that we expect this relationship to be negative. So it should come as no surprise that the highest rank order values for bill_length_mm appear to be associated with lower rank order values for bill_depth_mm.

To calculate Spearman's ρ 'rho' is pretty easy, you can use the cor functions again, but this time specify a hidden argument to `method="spearman"`.

```

penguins %>%
  cor_test(bill_length_mm, bill_depth_mm, method="spearman")

## # A tibble: 1 x 6
##   var1           var2      cor statistic      p method
##   <chr>          <chr>    <dbl>    <dbl>    <dbl> <chr>
## 1 bill_length_mm bill_depth_mm -0.22  8145268. 0.0000351 Spearman

```

What we can see in this example is that Pearson's r and Spearman's ρ are basically identical.

11.1.3 Graphical summaries between numeric variables

Correlation coefficients are a quick and simple way to attach a metric to the level of association between two variables. They are limited however in that a single number can never capture the every aspect of their relationship. This is why we visualise our data.

We have already covered scatter plots and `ggplot2()` extensively in previous chapters, so here we will just cover some of the different ways in which you could present the nature of a relationship

```

length_depth_scatterplot <- ggplot(penguins, aes(x= bill_length_mm,
                                               y= bill_depth_mm)) +
  geom_point()

length_depth_scatterplot

```

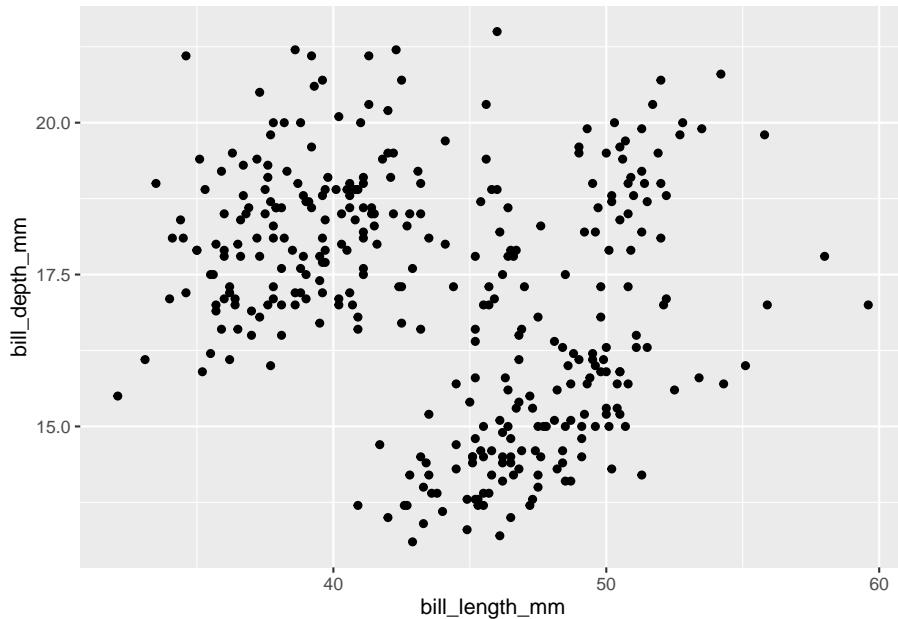


Figure 11.2: A scatter plot of bill depth against bill length in mm

**Note - Remember there are a number of different options available when constructing a plot including changing alpha to produce transparency if plots are lying on top of each other, colours (and shapes) to separate subgroups and ways to present third numerical variables such as setting aes(size=body_mass_g).

```
library(patchwork) # package calls should be placed at the TOP of your script

bill_depth_marginal <- penguins %>%
  ggplot()+
  geom_density(aes(x=bill_depth_mm), fill="darkgrey")+
  theme_void()+
  coord_flip() # this graph needs to be rotated

bill_length_marginal <- penguins %>%
  ggplot()+
  geom_density(aes(x=bill_length_mm), fill="darkgrey")+
  theme_void()

layout <- "
```

```

AA#
BBC
BBC"
# layout is easiest to organise using a text distribution, where ABC equal the three p

# layout <- "
# AAA#
# BBB
# BBB"
# BBB

bill_length_marginal+length_depth_scatterplot+bill_depth_marginal+ # order of plots is
plot_layout(design=layout) # uses the layout argument defined above to arrange the s

```

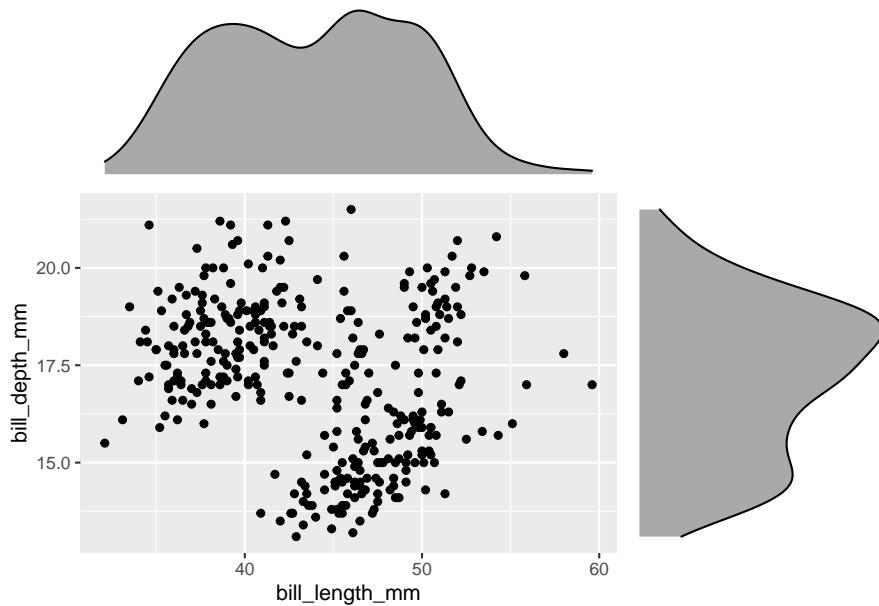


Figure 11.3: Using patchwork we can easily arrange extra plots to fit as marginals - these could be boxplots, histograms or density plots

These efforts allow us to capture details about the spread and distribution of both variables **and** how they relate to each other. This figure provides us with insights into

- The central tendency of each variable

- The spread of data in each variable
- The correlation between the two variables

11.2 Associations between categorical variables

Exploring associations between different categorical variables is not quite as simple as the previous numeric-numeric examples. Generally speaking we are interested in whether different combinations of categories are uniformly distributed or show evidence of clustering leading to *over- or under-represented* combinations. The simplest way to investigate this is to use `group_by` and `summarise` as we have used previously.

```
island_species_summary <- penguins %>%
  group_by(island, species) %>%
  summarise(n=n(),
            n_distinct=n_distinct(penguin_id)) %>%
  ungroup() %>% # needed to remove group calculations
  mutate(freq=n/sum(n)) # then calculates percentage of each group across WHOLE dataset

island_species_summary

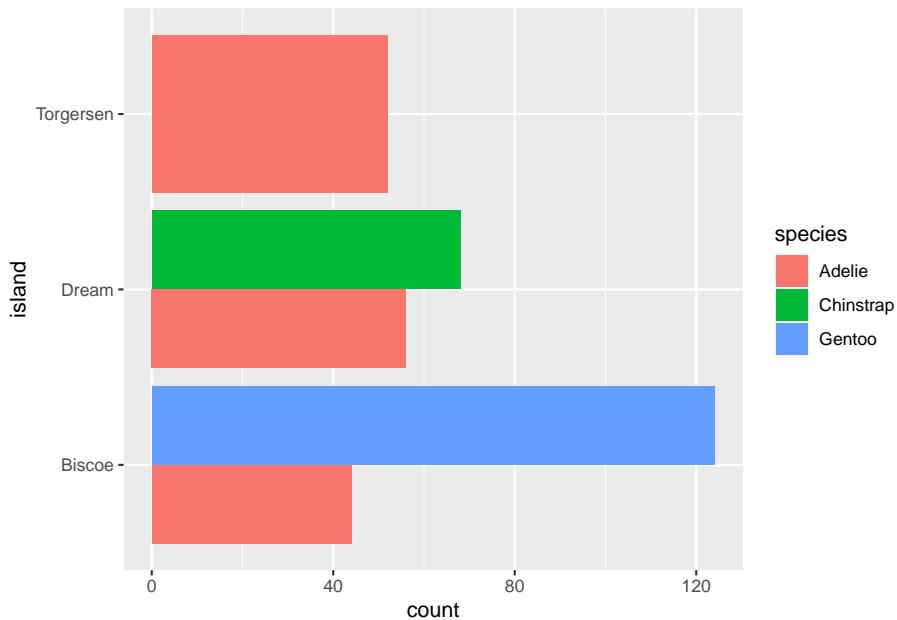
## # A tibble: 5 x 5
##   island   species     n n_distinct   freq
##   <chr>    <chr>     <int>      <int> <dbl>
## 1 Biscoe   Adelie     44        44  0.128
## 2 Biscoe   Gentoo    124       94  0.360
## 3 Dream    Adelie     56        56  0.163
## 4 Dream    Chinstrap   68        58  0.198
## 5 Torgersen Adelie     52        52  0.151

**Note - remember that group_by() applies functions which comes after it in a group-specific pattern.
```

What does the above tell us, that 168 observations were made on the Island of Biscoe, with three times as many Gentoo penguin observations made as Adelie penguins (remeber this is observations made, not individual penguins). When we account for penguin ID we see there are around twice as many Gentoo penguins recorded. We can see there are no Chinstrap penguins recorded on Biscoe. Conversely we can see that Gentoo penguins are **only** observed on Biscoe. The island of Dream has two populations of Adelie and Chinstrap penguins of roughly equal size, while the island of Torgensen appears to have a population comprised only of Adelie penguins.

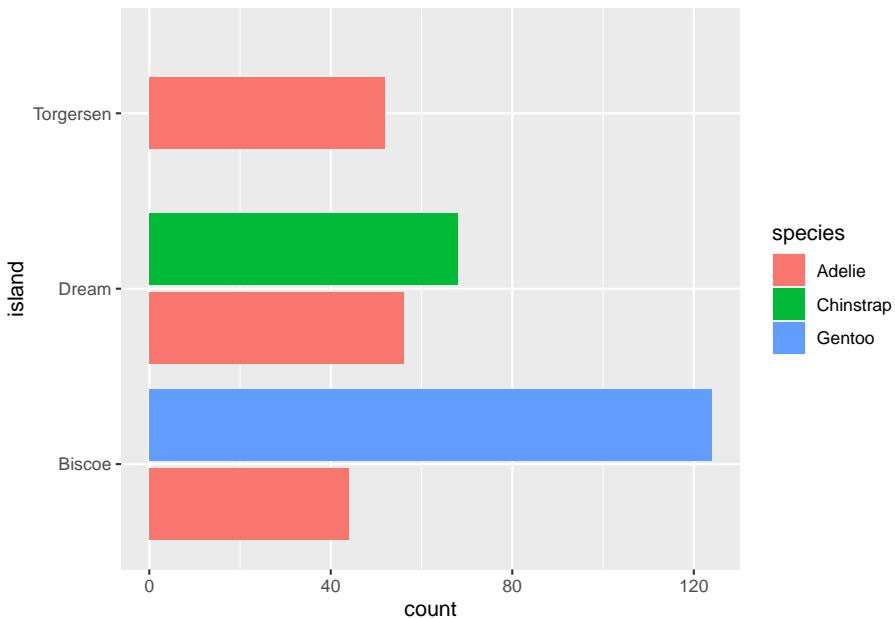
We could also use a bar chart in ggplot to represent this count data.

```
penguins%>%
  ggplot(aes(x=island, fill=species))+
  geom_bar(position=position_dodge())+
  coord_flip()
```



This is fine, but it looks a bit odd, because the bars expand to fill the available space on the category axis. Luckily there is an advanced version of the `position_dodge` argument.

```
penguins%>%
  ggplot(aes(x=island, fill=species))+
  geom_bar(position=position_dodge2(preserve="single"))+
  #keeps bars to appropriate widths
  coord_flip()
```



> **Note the default for bar charts would have been a stacked option, but we have already seen how that can produce graphs that are difficult to read.

An alternative approach would be to look at the ‘relative proportions’ of each population in our overall dataset. Using the same methods as we used previously when looking at single variables. Let’s add in a few aesthetic tweaks to improve the look.

```
penguins %>%
  ggplot(aes(x=island, fill=species)) +
  geom_bar(position=position_dodge2(preserve="single")) +
  #keeps bars to appropriate widths
  labs(x="Island",
       y = "Number of observations") +
  geom_text(data=island_species_summary, # use the data from the summarise object
            aes(x=island,
                y= n+10, # offset text to be slightly to the right of bar
                group=species, # need species group to separate text
                label=scales::percent(freq) # automatically add %
                ),
            position=position_dodge2(width=0.8)) + # set width of dodge
  scale_fill_manual(values=c("cyan",
                            "darkorange",
                            "purple"))
  )+
```

```
coord_flip()+
theme_minimal()+
theme(legend.position="bottom") # put legend at the bottom of the graph
```

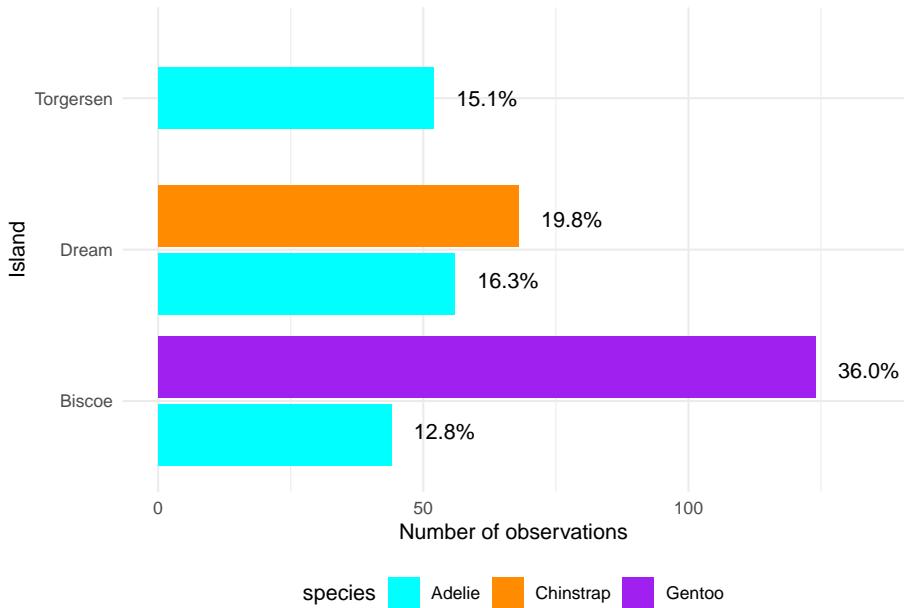
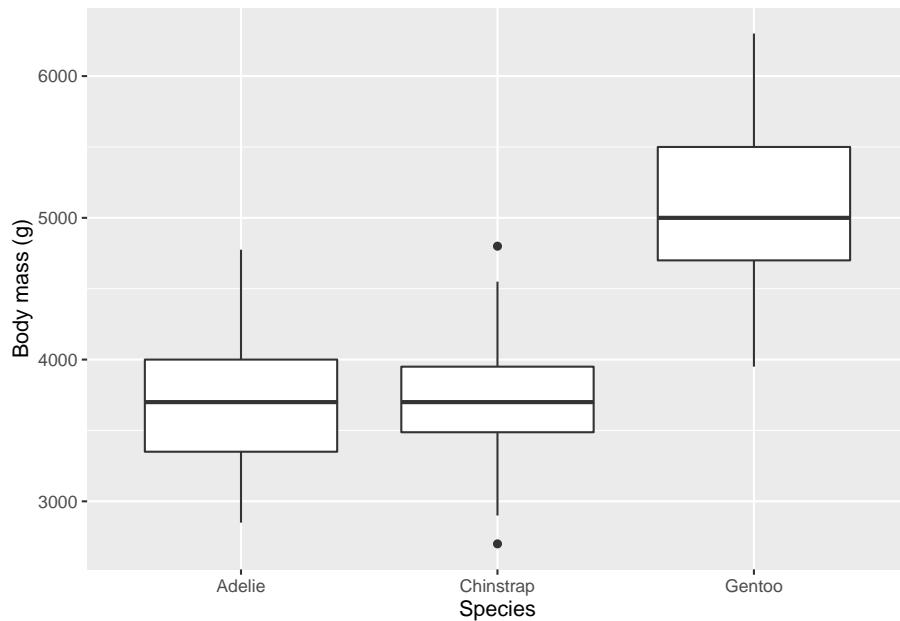


Figure 11.4: A dodged barplot showing the numbers and relative proportions of data observations recorded by penguin species and location

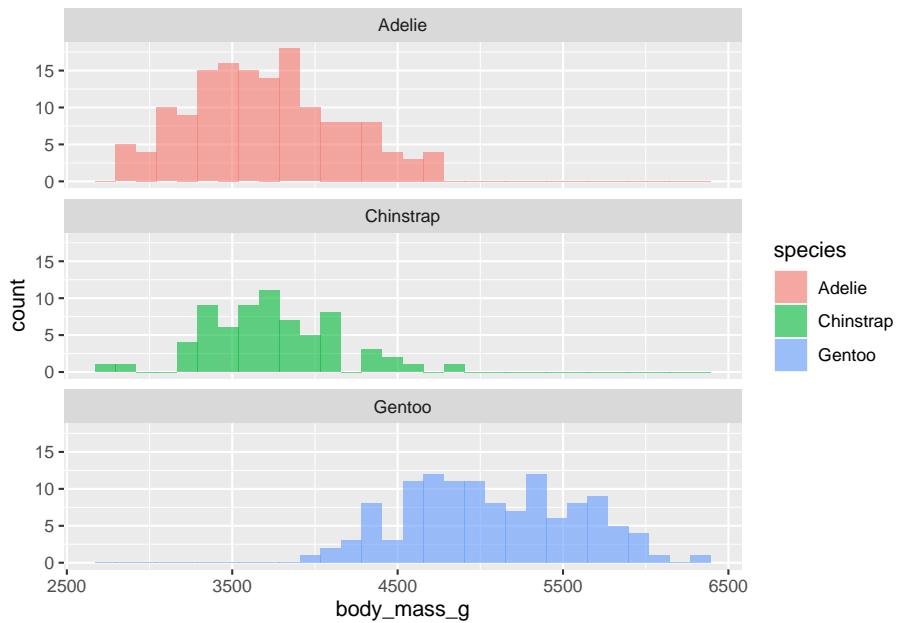
11.3 Associations between Categorical-numerical variables

```
penguins %>%
  ggplot(aes(x=species,
             y=body_mass_g))+
  geom_boxplot()+
  labs(y="Body mass (g)",
       x= "Species")
```

11.3. ASSOCIATIONS BETWEEN CATEGORICAL-NUMERICAL VARIABLES 181



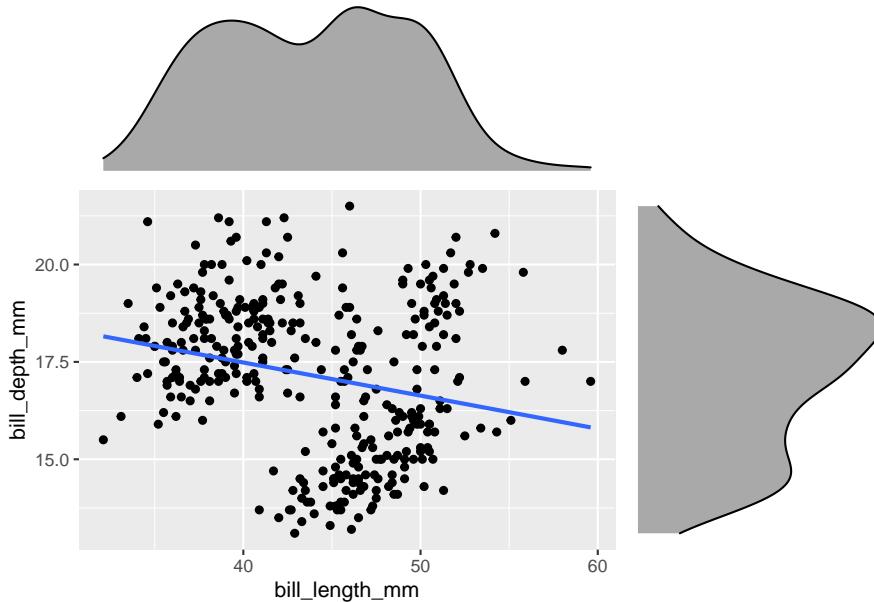
```
penguins %>%
  ggplot(aes(x=body_mass_g,
             fill=species))+
  geom_histogram(alpha=0.6,
                 bins=30,
                 position="identity")+
  facet_wrap(~species,
             ncol=1)
```



11.4 Complexity

11.4.1 Simpson's Paradox

Remember when we first correlated bill length and bill depth against each other we found an overall negative correlation of -0.22. However, this is because of a confounding variable we had not accounted for - species.



This is another example of why carefully studying your data - and carefully considering those variables which are likely to affect each other are studied or controlled for. It is an entirely reasonable hypothesis that different penguin species might have different bill shapes that might make an overall trend misleading. We can easily check the effect of a categorial variable on our two numeric variables by assigning the aesthetic colour.

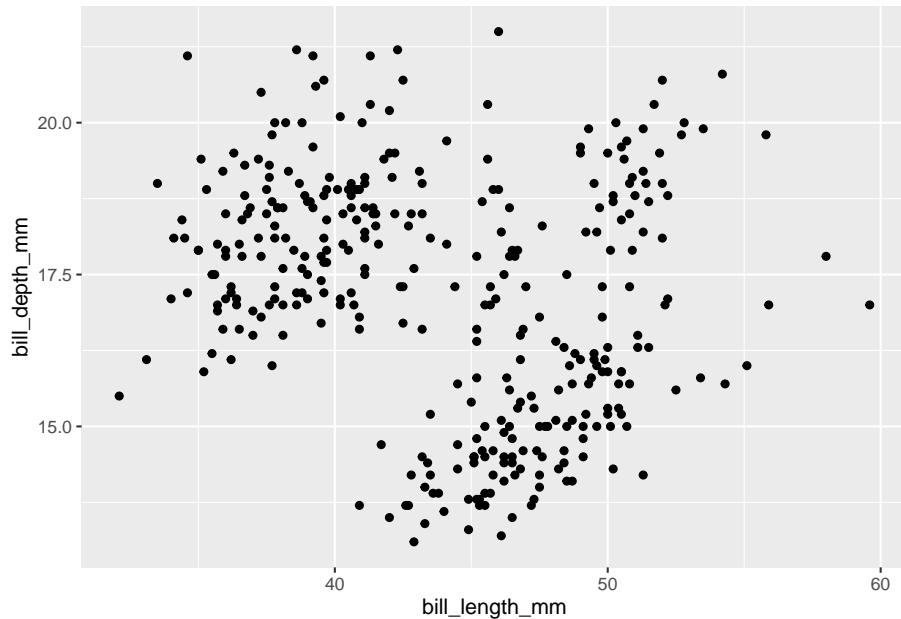
```

colours <- c("cyan",
           "darkorange",
           "purple")

length_depth_scatterplot_2 <- ggplot(penguins, aes(x= bill_length_mm,
                                                 y= bill_depth_mm,
                                                 colour=species)) +
  geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+
  scale_colour_manual(values=colours)+ 
  theme_classic()+
  theme(legend.position="none")+
  labs(x="Bill length (mm)",
       y="Bill depth (mm)")

length_depth_scatterplot

```



```

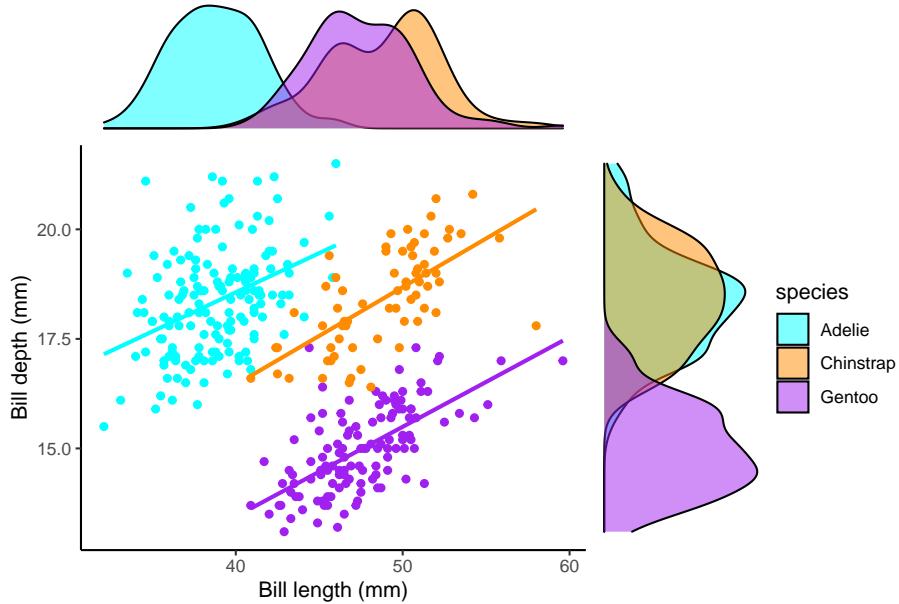
bill_depth_marginal_2 <- penguins %>%
  ggplot()+
  geom_density(aes(x=bill_depth_mm,
                    fill=species),
               alpha=0.5)+
  scale_fill_manual(values=colours)+
  theme_void()+
  coord_flip() # this graph needs to be rotated

bill_length_marginal_2 <- penguins %>%
  ggplot()+
  geom_density(aes(x=bill_length_mm,
                    fill=species),
               alpha=0.5)+
  scale_fill_manual(values=colours)+
  theme_void()+
  theme(legend.position="none")

layout2 <- "
AAA#
BBC
BBC
BBC"

```

```
bill_length_marginal_2+length_depth_scatterplot_2+bill_depth_marginal_2+ # order of plots is important
plot_layout(design=layout2) # uses the layout argument defined above to arrange the size and position of the plots
```



We now clearly see a striking reversal of our previous trend, that in fact *within* each species of penguin there is an overall positive association between bill length and depth.

This should prompt us to re-evaluate our correlation metrics:

```
penguins %>%
  group_by(species) %>%
  cor_test(bill_length_mm, bill_depth_mm)
```

```
## # A tibble: 3 x 9
##   species  var1    var2      cor statistic      p conf.low conf.high method
##   <chr>    <chr>    <chr>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl> <chr>
## 1 Adelie  bill_le~ bill_de~  0.39     5.19  6.67e- 7    0.247  0.519 Pears-
## 2 Chinstrap bill_le~ bill_de~  0.65     7.01  1.53e- 9    0.492  0.772 Pears-
## 3 Gentoo   bill_le~ bill_de~  0.64     9.24  1.02e-15   0.526  0.737 Pears-
```

We now see that the correlation values for all three species is >0.22 - indicating these associations are much closer than previously estimated.

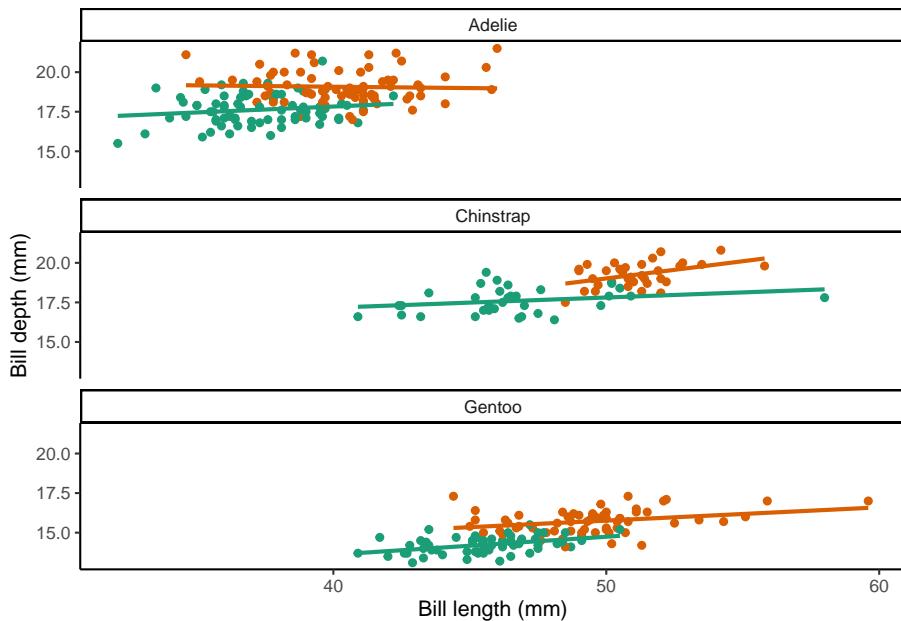
11.4.2 Three or more variables

In the above example therefore, we saw the importance of exploring relationships among more than two variables at once. Broadly speaking there are two ways top do this

1. Layer an extra aesthetic mapping onto ggplot - such as size, colour, or shape
2. Use facets to construct multipanel plots according to the values of a categorical variable

If we want we can also adopt both of these approaches at the same time:

```
penguins %>%
  drop_na(sex) %>%
  ggplot(aes(x= bill_length_mm,
             y= bill_depth_mm,
             colour=sex)) + # colour aesthetic set to sex
  geom_point()+
  geom_smooth(method="lm",
              se=FALSE)+
  scale_colour_manual(values=c("#1B9E77", "#D95F02"))+ # pick two colour scheme
  theme_classic()+
  theme(legend.position="none")+
  labs(x="Bill length (mm)",
       y="Bill depth (mm)")+
  facet_wrap(~species, ncol=1) # specify plots are stacked split by species
```



Here we can see that the trends are the same across the different penguin sexes. Although by comparing the slopes of the lines, lengths of the lines and amounts of overlap we can make insights into how “sexually dimorphic” these different species are e.g. in terms of beak morphology do some species show greater differences between males and females than others?

11.5 Summing up

This is our last data handling workshop. We have built up towards being able to discover and examine relationships and differences among variables in our data. You now have the skills to handle many different types of data, tidy it, and produce visuals to generate insight and communicate this to others.

A note of caution is required - it is very easy to spot and identify patterns.

When you do spot a trend, difference or relationship, it is important to recognise that you may not have enough evidence to assign a reason behind this observation. As scientists it is important to develop hypotheses based on knowledge and understanding, this can help (sometimes) with avoiding spurious associations.

Sometimes we may see a pattern in our data, but it has likely occurred due to random chance, rather than as a result of an underlying process. This is where formal statistical analysis, to quantitatively assess the evidence, assess probability and study effect sizes can be incredibly powerful. We will delve into these exciting topics next term.

That's it! Thank you for taking the time to get this far. Be kind to yourself if you found it difficult. You have done incredibly well.

Have some more praise!!!!

```
praise::praise()  
[1] "You are spectaculaR!"
```

Chapter 12

Introduction to Statistics - Spring Week One

12.1 Introduction to statistics

Welcome back! Last term we worked on developing our data manipulation, organisation, reproducibility and visualisation skills.

This term we will be focusing more on *statistics*. We actually did quite a lot of *descriptive statistics* work last term. Every time we summarised or described our data, by calculating a **mean**, **median**, **standard deviation**, **frequency/count**, or **distribution** we were carrying out *descriptive statistics* that helped us understand our data better.

We are building on this to develop our skills in *inferential statistics*. Inferential statistics allow us to make generalisations - taking a descriptive statistics from our data such as the **sample mean**, and using it to say something about a population parameter (i.e. the **population mean**).

For example we might measure the heights of some plants that have been outcrossed and inbred and make some summaries and figures to construct an average difference in height (this is **descriptive**). Or we could use this to produce some estimates of the general effect of outcrossing vs inbreeding on plant heights (this is **inferential**).

In fact that gives me an idea...

Head to Blackboard and check out today's assignment from GitHub Classrooms

12.2 Darwin's maize data

Loss of genetic diversity is an important issue in the conservation of species. Declines in population size due to over exploitation, habitat fragmentation lead to loss of genetic diversity. Even populations restored to viable numbers through conservation efforts may suffer from continued loss of population fitness because of inbreeding depression Katherine Ralls and Frankham (2020).

Charles Darwin even wrote a book on the subject “*The Effects of Cross and Self-Fertilisation in the Vegetable Kingdom*” Darwin (1876). In this he describes how he produced seeds of maize (*Zea mays*) that were fertilised with pollen from the same individual or from a different plant. The height of the seedlings that were produced from these were then measured as a proxy for their evolutionary fitness.

Darwin wanted to know whether inbreeding reduced the fitness of the selfed plants - this was his **hypothesis**. The data we are going to use today is from Darwin’s original dataset.

```
library(tidyverse)

darwin <- read_csv("data/darwin.csv")

# A tibble: 6 x 5
# ...1 pot pair type height
# <dbl> <chr> <dbl> <chr>   <dbl>
1     1 I      1 Cross   23.5
2     2 I      1 Self    17.4
3     3 I      2 Cross   12
4     4 I      2 Self    20.4
5     5 I      3 Cross   21
6     6 I      3 Self    20
```



Before you go any further - make sure your basic R project set up is up to scratch. Do you know where the data file is saved? Have you got separate subfolders set up within your project?

You should have a script set up to put your work from today into - use this to write instructions and store comments. Use the File > New Script menu item and select an R Script.

12.2.1 Description

The first thing we should know by now is to always start by exploring our data. If you want to stretch yourself, see if you can perform a basic data check **without** prompts.

If you need a little help with remembering what to check, but would like to try and adapt scripts go to 3.5.1 from workflow 1.

Click-me for answer - If you got completely stuck, or had a go but want to check your answers then click here:

```
# check the structure of the data
glimpse(darwin)

# check data is in a tidy format
head(darwin)

# check variable names
colnames(darwin)

# check for duplication
darwin %>%
  duplicated() %>%
  sum()

# check for typos - by looking at impossible values
darwin %>%
  summarise(min=min(height, na.rm=TRUE),
            max=max(height, na.rm=TRUE))

# check for typos by looking at distinct characters/values
darwin %>%
  distinct(pot)

darwin %>%
  distinct(pair)

darwin %>%
  distinct(type)

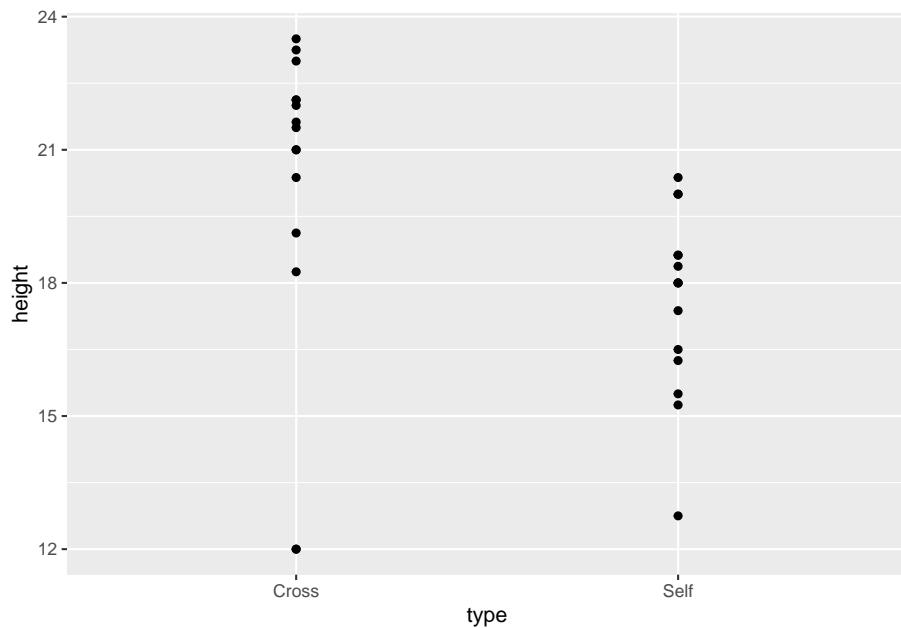
# missing values
darwin %>%
  is.na() %>%
  sum()

# quick summary
summary(darwin)
```

12.2.2 Visualisation

Now seems like a good time for our first data visualisation.

```
darwin %>%
  ggplot(aes(x=type,
             y=height))+
  geom_point()
```



```
# you could also substitute (or combine) other geoms including
# geom_boxplot()
# geom_violin()
# Why not have a go and see what you can make?
```

The graph clearly shows that the average height of the ‘crossed’ plants is greater than that of the ‘selfed’ plants. But we need to investigate further in order to determine whether the signal (any apparent differences in mean values) is greater than the level of noise (variance within the different groups).

The variance appears to be roughly similar between the two groups - though by making a graph we can now clearly see that in the crossed group, there is a *potential* outlier with a value of 12.

12.2.3 Comparing groups

As we have seen previously we can use various tidy functions to determine the mean and standard deviations of our groups.

```
darwin %>%
  group_by(type) %>%
  summarise(mean=mean(height),
            sd=sd(height))
```

```
# A tibble: 2 x 3
  type    mean     sd
  <chr> <dbl> <dbl>
1 Cross   20.2  3.62
2 Self    17.6  2.05
```

**Note - You should (re)familiarise yourself with how (and why) we calculate standard deviation.

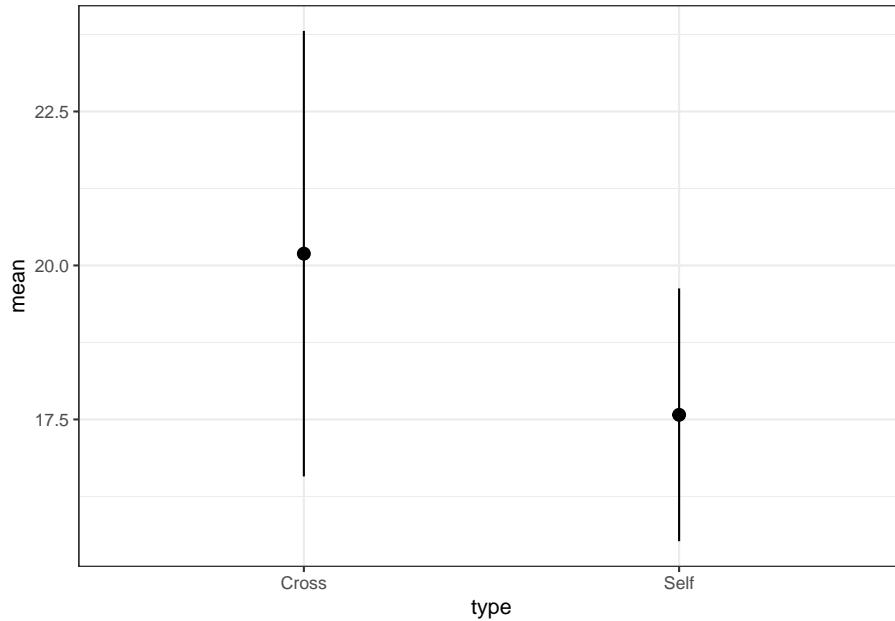
Summary statistics like these could be presented as figures or tables. We normally reserve tables for **very** simple sets of numbers, and this instance we could present both.

```
# make a new object
darwin_summary <-darwin %>%
  group_by(type) %>%
  summarise(mean=mean(height),
            sd=sd(height))

# make a summary plot
darwin_summary %>%
  ggplot(aes(x=type,
             y=mean))+
  geom_pointrange(aes(ymin=mean-sd, ymax=mean+sd))+
```

Table 12.1: Summary statistics of crossed and selfed maize plants

type	mean	sd
Cross	20.19167	3.616945
Self	17.57500	2.051676



```
# put this at top of script
library(kableExtra)

# use kable extra functions to make a nice table (could be replaced with kable() if needed)
darwin_summary %>%
  kbl(caption="Summary statistics of crossed and selfed maize plants") %>%
  kable_styling(bootstrap_options = "striped", full_width = T, position = "left")
```

Descriptive statistics and careful data checking are often skipped steps in the rush to answer the **big** questions. However, description is an essential part of early phase analysis.

12.3 Estimation

In the section above we concentrated on description. But the hypothesis Darwin aimed to test was whether ‘inbreeding reduced the fitness of the selfed plants’. To do this we will use the height of the plants as a proxy for fitness and explicitly

address whether there is a **difference** in the mean heights of the plants between these two groups.

Our goal is to:

- Estimate the mean heights of the plants in these two groups
- Estimate the mean *difference* in heights between these two groups
- Quantify our *confidence* in these differences

12.3.1 Differences between groups

Darwin's data used match pairs - each pair shared one parent. So that in pair 1 the same parent plant was either 'selfed' or 'crossed' to produce offspring. This is a powerful approach to experimental design, as it means that we can look at the differences in heights across each of the 15 pairs of plants - rather than having to infer differences from two randomly derived groups.

In order to calculate the differences in height between each pair we need to do some data wrangling - **can you complete the below to produce the required output?**

```
# pivot data to wide format then subtract Selfed plant heights from Crossed plant heights

darwin_wide <- darwin %>%
  pivot_wider(names_from=type, values_from=height, id_cols=pair) %>%
  mutate(difference=-----)

# A tibble: 15 x 4
  pair Cross  Self difference
  <dbl> <dbl> <dbl>      <dbl>
1     1   23.5  17.4      6.12
2     2   12    20.4     -8.38
3     3   21    20        1
4     4   22    20        2
```

We now have the difference in height for each pair, we can use this to calculate the **mean difference** in heights between paired plants, and the amount of variance (as standard deviation)

```
difference_summary <- darwin_wide %>%
  summarise(mean=mean(difference),
            sd=sd(difference),
            n=n())

difference_summary
```

```
# A tibble: 1 x 2
  mean     sd
  <dbl> <dbl>
1 2.62  4.72
```



What we have just calculated is the average difference in height between these groups of plants and the standard deviation of the difference Moving forward we will be working a lot with estimating our confidence in differences between groups

12.3.2 Standard error of the difference

Remember standard deviation is a *descriptive* statistic - it measures the variance within our dataset - e.g. how closely do datapoints fit to the mean. However for *inferential* statistics we are more interested in our confidence in our estimation of the mean. This is where standard error comes in.

We can think of error as a standard deviation of the mean. The mean we have calculated is an estimate based on one sample of data. We would expect that if we sampled another 30 plants these would have a different sample mean. Standard error describes the variability we would expect among sample means if we repeated this experiment many times. So we can think of it as a measure of the confidence we have in our estimate of a **true population mean**.

$$SE = \frac{s}{\sqrt{n}}$$

As sample size increases the standard error should reduce - reflecting an increasing confidence in our estimate.

We can calculate the standard error for our sample by applying this equation to our `difference_summary` object, can you complete this?

```
difference_summary %>%
  mutate(se=-----)
```

```
# A tibble: 1 x 4
  mean     sd     n     se
  <dbl> <dbl> <int> <dbl>
1 2.62  4.72    15  1.22
```

Our estimate of the mean is not really very useful without an accompanying measuring of *uncertainty* like the standard error, in fact estimates of averages or differences should **always** be accompanied by their measure of uncertainty.

Click me - With the information above, how would you present a short sentence describing the average difference in height?**

... the average difference in height was 2.62 ± 1.22 inches (mean \pm SE).

With a mean and standard error of the difference in heights between inbred and crossed plants - how do we work out how much confidence we have in their being a difference between our **population means**?

Standard error is a measure of uncertainty, the larger the standard error the more *noise* around our data and the more uncertainty we have. The smaller the standard error the more confidence we can have that our difference in means is *real*.

- Null hypothesis - there is no difference in the mean height of self vs crossed plants
- Alternate hypothesis - inbreeding reduces the fitness of the selfed plants, observed as selfed plants on average being smaller than crossed plants



A statistical way of thinking about our inferences is in terms of confidence around keeping or rejecting the null hypothesis. The (alternate) hypothesis is simply one that contradicts the null.

When we decide whether we have enough confidence that a difference is real (e.g. we could reject the null hypothesis), we cannot ever be 100% certain that this isn't a false positive (also known as a Type I error). More on this later

12.3.3 Normal distribution

As we should remember from last term (and before), the normal distribution is the bell-shaped curve. It is defined by two parameters:

- the mean
- the standard deviation

The mean determines where the centre/peak of the bell curve is, the standard deviation determines the width of the bell (how long the tails are).

Large standard deviations produce wide bell curves, with short peaks. Small standard deviations produce narrow bell curves with tall peaks.

The bell curve occurs frequently in nature, most circumstances where we can think of a continuous measure coming from a population e.g. human mass, penguin flipper lengths or plant heights.

As a *probability* distribution, the area within the curve sums to the whole population (e.g. the probability that the curve contains every possible measurement is 1). Known proportions of the curve lie within certain distances from the

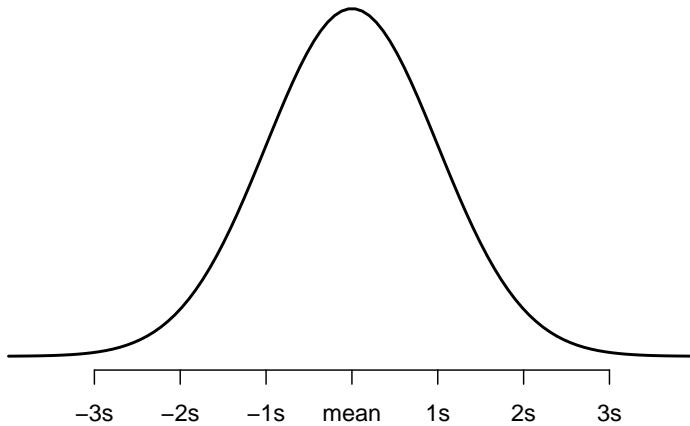
198CHAPTER 12. INTRODUCTION TO STATISTICS - SPRING WEEK ONE

centre e.g. 67.8% of observations should have values within one standard deviation of the mean. 95% of observations should have values within two standard deviations of the mean. This *idealised* normal distribution is presented below:

```
#Create a sequence of 100 equally spaced numbers between -4 and 4
x <- seq(-4, 4, length=100)

#create a vector of values that shows the height of the probability distribution
#for each value in x
y <- dnorm(x)

#plot x and y as a scatterplot with connected lines (type = "l") and add
#an x-axis with custom labels
plot(x,y, type = "l", lwd = 2, axes = FALSE, xlab = "", ylab = "")
axis(1, at = -3:3, labels = c("-3s", "-2s", "-1s", "mean", "1s", "2s", "3s"))
```

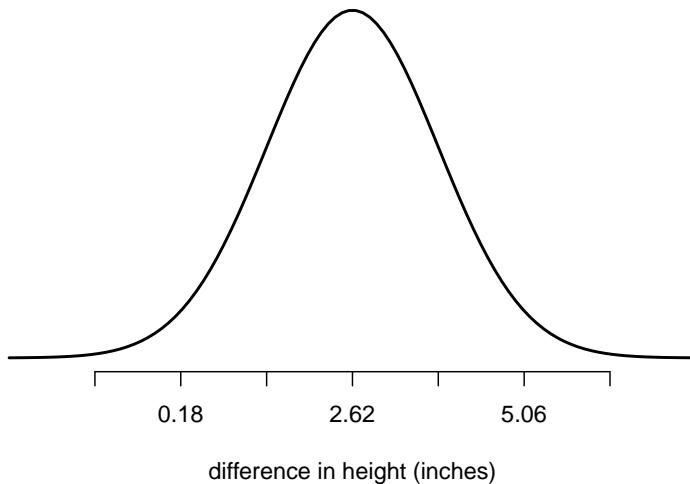


How do we convert this information into how likely we are to observe a difference of 2.62 inches in plant heights if the ‘true’ difference between crosses and selfed plants is *zero*?

The **central limit theorem** states that if you have a population with mean and standard deviation, and take sufficiently large random samples from the population, then the distribution of the sample means will be approximately normally distributed. Standard error then is our measure of variability around our sample mean, and we will assume that we can apply a normal distribution

to our ability to estimate the mean (*we will revisit this assumption later*).

So if we now center our bell curve on the estimate of the mean (2.62), then just over two thirds of the area under the curve is ± 1.22 inches. 95% of it will be within ± 2 standard errors, and 99.8% will be within ± 3 standard errors.



Taking a look at this figure we can ask ourselves where is zero on our normal distribution? One way to think about this is, if the true difference between our plant groups is zero, how surprising is it that we estimated a difference of 2.62 inches?

If zero was close to the center of the bell curve, then our observed mean would not be surprising at all (**if the null hypothesis is true**). However in this case it is not in the middle of the bell. It falls in the left-hand tail, and it is $>$ than two standard deviations from our estimated mean.

We can describe this in two ways:

- We estimate that if we ran this experiment 100 times then >95 of our experiments would estimate a mean difference between our plant groups that is > 0 .
- This is also usually taken as the minimum threshold needed to *reject* a null hypothesis. We can think of the probability of estimating this mean difference, if our true mean difference was zero, as $p < 0.05$.

You will probably be very used to a threshold for null hypothesis rejection of $\alpha = 0.05$, but this only the very lowest level of confidence at which we can pass a

200CHAPTER 12. INTRODUCTION TO STATISTICS - SPRING WEEK ONE

statistical test. If we increase the severity of our test so that the minimum we require is $\alpha = 0.001$ or 99% confidence, we can see that we no longer believe we have enough confidence to reject the null hypothesis (0 is within 3 s.d. of our estimated mean).

12.3.4 Confidence Intervals

Because ± 2 standard errors covers the central 95% of the area under the normal curve, we refer to this as our 95% confidence interval. We can calculate confidence intervals for any level, but commonly we refer to standard error (68% CI), 95% and 99%.

We can work out the 95% confidence interval range of our estimated mean as follows:

```
lowerCI <- 2.62-(2*1.22)
```

```
upperCI <- 2.62+(2*1.22)
```

```
lowerCI
```

```
upperCI
```

A common mistake it to state that we are 95% confident that the ‘true’ mean lies within our interval. But *technically* it refers to the fact that if we kept running this experiment again and again the intervals we calculate would capture the true mean in 95% of experiments. So really we are saying that we are confident we would capture the true mean in 95% of our experiments.

How might we write this up?



The maize plants that have been cross pollinated were taller on average than the self-pollinated plants, with a mean difference in height of 2.62 [0.18, 5.06] inches (mean [95% CI]).

Note that because it is possible to generate multiple types of average and confidence interval, we clearly state them in our write up. The same would be true if you were presenting the standard error (\pm S.E.) or the standard deviation (\pm S.D.) or a median and interquartile range (median [\pm IQR]).

The above is a good example of a simple but clear write-up because it clearly describes the *direction* of the difference, the *amount* and the *error* in our estimate.

12.4 Summary

Statistics is all about trying to interpret whether the signal (of a difference or trend) is stronger than the amount of noise (variability). In a sample the standard deviation is a strong choice for estimating this *within* a dataset. The standard deviation of the sampling distribution of the mean is known as the standard error. The standard error (of the mean) is a measure of the precision we have in our estimate of the mean. Thanks to the central limit theorem and normal distribution we can use the variability in our estimate to calculate confidence intervals, and decide whether our signal of an effect is strong enough to reject a null hypothesis (of no effect or no difference).

Next time we will start working with linear models - this approach allows us to estimate means and intervals in a more sophisticated (and automated) way.

202CHAPTER 12. INTRODUCTION TO STATISTICS - SPRING WEEK ONE

Chapter 13

Keeping code DRY - Spring Week One

Keep working in the same project workspace as Estimates because there's a file you need, but I would advise you start a new script to work on iteration.

So what the flip is **DRY** code anyway? It stands for **Don't Repeat Yourself**. It's aimed at reducing the repetition and number of lines of code in your scripts.

So what the flip is **DRY** code anyway? It stands for **Don't Repeat Yourself**. It's aimed at reducing the repetition and number of lines of code in your scripts.

Why is this useful?

- If you keep repeating the same/similar lines of code over and over then your script will become very large
- Changes or updates have to be manually applied to each and every time you have used a line of code in your script
- Mistakes can easily start to creep in

There are two broad ways we can DRY out our code

- 1) Use Functions - functions contain code instructions, and we can re-use them for similar processes simply by changing the arguments. In R you can use pre-built functions and start building your own!
- 2) Use Iteration - Apply functions to loop/repeat over different groups or lists.

13.1 Functions

Most of the time when we work in R, we will use functions; either pre-written functions or ones we write ourselves. **Functions** make it easy to use sets of code instructions repeatedly (without filling our scripts with the code underlying the function) and help us carry out multiple tasks in a single step without having to go through the details of how the steps are executed.

13.1.1 Common functions

You have been using functions in R from week one, we type the name of the function followed by parentheses e.g. `read_csv()`. Within the parentheses is where we will specify the function input and options, separated by commas `,`. One function you will use a lot is the **combine function** `c()`, which as the name suggests lets you concatenate different types of values into a **vector**.



If you are struggling to remember what a vector is, you might want to go back to our intro to R pages for a brief refresher on data types.

- Complete the script below to combine any set of numerical values using `c()`.
- Then use the `sum()` function to add them together

```
my_combined_values <- c(__,__)
sum(my_combined_values)
```

13.1.2 Write your own function

R makes it easy to create user defined functions by using `function()`. Here is how it works:

- Give your function an object name and assign the function to it, e.g. `my_function_name <- function()`.
- Within the parentheses you specify inputs and options just like how pre-written functions work, e.g. `function(input_data)`.
- Next, put all the code you want your function to execute inside curly brackets like this: `function(input_data){code to run}`
- Use `return()` to specify what you want your function to output once it is done running the code.

Use the following instructions to complete the function in the window below:

- I've started writing a function for you that will sum up values and take the square root of the sum. To take the square root, we use the `sqrt()` function.
- Complete this function by filling in `input_data` for the `sum()`, and then filling in the remaining empty parentheses with the appropriate object names.

*Now create an object containing a set of numerical values and call it `my_combined_values`. Then try out your new function on this object, which will compute the square root of the object's sum.

```
# Use the instructions above to complete the function below
my_function_name <- function(input_data){
  s <- sum( )
  ss <- sqrt( )
  return( )
}

# Create a new object and try out your new function
my_combined_values <- c(,)

my_function_name(my_combined_values)
```

Solution

```
# Use the instructions above to complete the function below
my_function_name <- function(input_data){
  s <- sum(input_data)
  ss <- sqrt(s)
  return(ss)
}

# Create a new object and try out your new function
my_combined_values <- c(,,)

my_function_name(my_combined_values)
```

A general rule of thumb. If you end up repeating a line of code more than three times in a script - you should write a function to do the work instead. And write clear comments on its use! Why?

It reduces the numbers of lines of code in your script, it reduces the amount of repetition in the code, if you need to make changes you can change the function without having to hunt through all of your code.

A really good way to organise your functions is to organise them into a separate

script to the rest of your analysis. Write functions in a separate script and use source("scripts/functions.R")

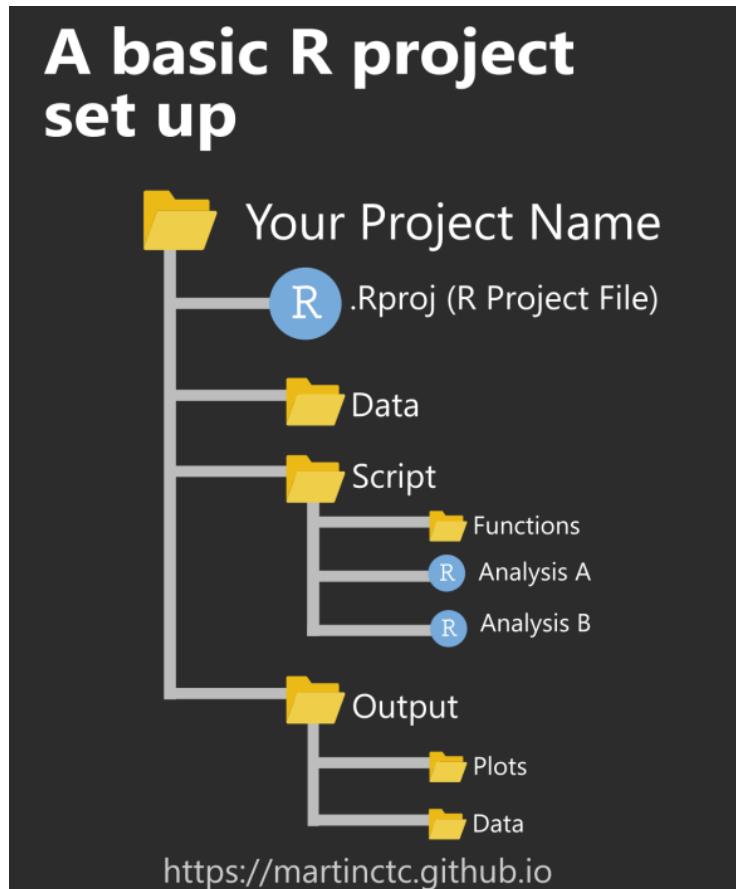


Figure 13.1: An example of a typical R project set-up

It might surprise you to know that there is no prebuilt function for standard error in base R, but we can build our own!

```
se <- function(x) {
  se <- sd(x)/(sqrt(length(x)))
  return(se)
}
```

```
# The seq R function generates a sequence of numeric values
x <- seq(8, 20, length=100)
```

```
# use the mean function to calculate average for x
mean(x)

# use our custom function to calculate se for x
se(x)

# could be used in the group_by and summarise pipes we are used to using
darwin_wide %>%
  summarise(mean=mean(difference),
            sd=sd(difference),
            n=n(),
            se=se(difference))
```



Functions can basically be given any name you want. However, you may accidentally overwrite existing functions if you give them the same name, so try and make them unique.

13.1.3 More functions

This is an example of a very simple function that just prints the string “Hello World” whenever you type the function `say_hello()`

```
say_hello <- function(){
  paste("Hello World")
}

say_hello()

# Notice the argument section is blank, what happens when you try to put something in the bracket
# say_hello("x")
```

Now lets try a similar function, but we include an argument:

```
say_morning <- function(x){
  paste("Good morning", x)
}

# what happens when you try to run this function
say_morning()
```

```
# what about this one?
say_morning("Phil")
```

So that was an example where we included an argument for our function. However, you are probably used to the idea that many functions have “default” values for arguments, and we can easily set these.

```
say_morning_default <- function(name="you"){
  paste("Good morning", name)
}
# what happens when you put a "string" in the brackets? What happens when you leave it
say_morning_default()
```

13.1.4 Exercise

We are going to try and write a custom function called `find_largest_male()`, it will be used to identify the largest male *Drosophila* from a small dataset.

```
# Make some fake data into a tibble

vial <- 1:10
sex <- (rep(c("male","female"),5))
weight_mg <- rnorm(10, mean=0.2, sd=0.01)

dros_weight <- tibble(vial, sex, weight_mg)
```

13.1.4.1 Step 1.

What functions would you use to extract the heaviest male from this dataset? Try and think that through first, then see if you can rework it into a function.

Click-me for A solution

```
find_largest_male <- function(df){
  dros_male <- filter(df, df[,2]=="male")
  head(arrange(dros_male, desc(dros_male[,3])),n=1)
}

# Note that this uses dplyr functions, so this would need to be loaded for this to work
```

13.1.4.2 Step 2.

Now we have a basic function we can work to refine and extend it. What if we made a function that could pick the largest Drosophila overall, or male or female depending on what we need?

Click-me for A solution

```
find_largest_fly <- function(df, n=1, s=c("male", "female"))>{
  drossex <- filter(df, sex %in% s )
  head(arrange(drossex, desc(drossex[,3])),n=n)
}
```

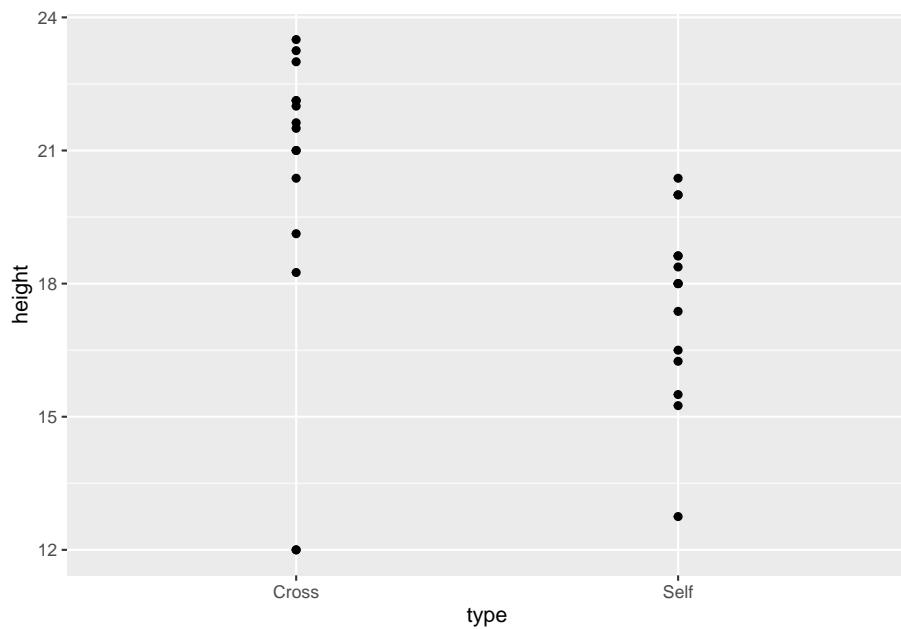
13.1.5 Custom ggplot themes

It is often the case that we start to default to a particular ‘style’ for our figures, or you may be making several similar figures within a research paper. Creating custom functions can extend to making our own custom ggplot themes. You have probably already used theme variants such as `theme_bw()`, `theme_void()`, `theme_minimal()` - these are incredibly useful, but you might find you still wish to make consistent changes.

Here is a plot we made in the previous chapter

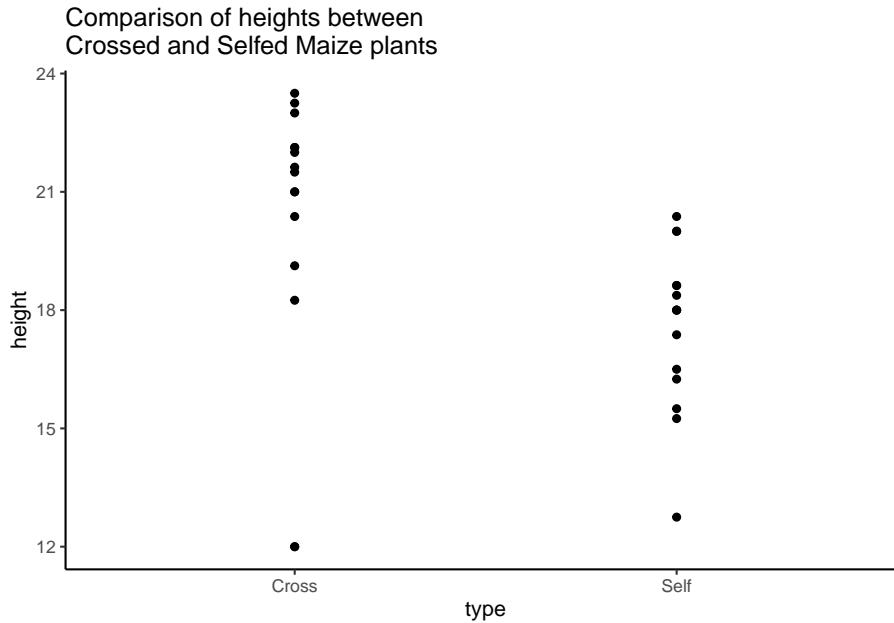
```
plot <- darwin %>%
  ggplot(aes(x=type,
             y=height))+
  geom_point()

plot
```



With the addition of a title and `theme_classic()` we can improve the style quickly

```
plot+  
  ggtitle("Comparison of heights between \nCrossed and Selfed Maize plants") +  
  theme_classic()
```



But I **still** want to make some more changes, rather than do this work for one figure, and potentially have to repeat this several times for subsequent figures, I can decide to make a new function instead.

```
# custom theme sets defaults for font and size, but these can be changed without changing the function
theme_custom <- function(base_size=14, base_family="serif"){
  theme_classic(base_size = base_size,
                base_family = base_family,
                ) +
  # update theme minimal
  theme(
    # specify default settings for plot titles - use rel to set titles relative to base size
    plot.title=element_text(size=rel(1.5),
                           face="bold",
                           family=base_family),
    #specify defaults for axis titles
    axis.title=element_text(
      size=rel(1.2),
      family=base_family),
    # specify position for y axis title
    axis.title.y=element_text(margin = margin(r = 10, l= 10)),
    # specify position for x axis title
    axis.title.x = element_text(margin = margin( t = 10, b = 10)),
    # set major y grid lines
    )}
```

```

panel.grid.major.y = element_line(colour="gray", size=0.5),
# add axis lines
axis.line=element_line(),
# Adding a 0.5cm margin around the plot
plot.margin = unit(c(0.5, 0.5, 0.5, 0.5), units = , "cm"),
# Setting the font for the legend text
legend.text = element_text(face = "italic"),
# Removing the legend title
legend.title = element_blank(),
# Setting the position for the legend - 0 is left/bottom, 1 is top/right
legend.position = c(0.9, 0.8)
)

}

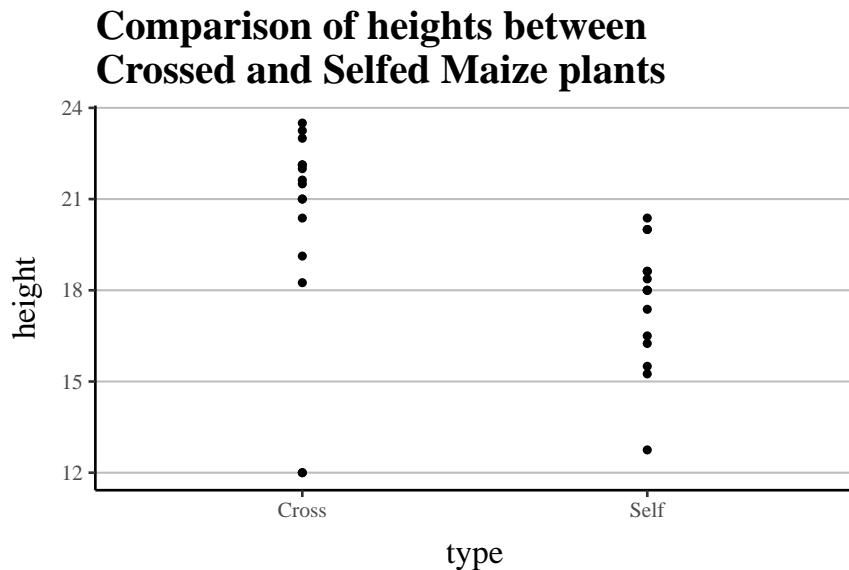
```

With this function set, I can now use it for as many figures as I wish. To use it in the future I should probably save it in a unique script, with a clear title and comments for future use.

```

plot+
ggtitle("Comparison of heights between \nCrossed and Selfed Maize plants")+
theme_custom()

```



13.1.6 Writing Packages

You should be familiar by now with the idea that R packages add data and functions to your workspace. They are bundles of code that anyone can write, most commonly you will be downloading packages from CRAN. However development stage packages can also be downloaded directly from GitHub.

In the previous section we walked through some very basic writing of new functions. We can save these as R scripts and move them from project to project. Or we could choose to write them into a documented R package. This tutorial shows you just how to do that. Writing packages is a useful thing to do, even if you think the only person that will ever use them is yourself, as you then more easily access these functions across different workspaces and projects.

13.2 Iteration

We've seen how to write a function and how they can be used to create concise reusable operations that can be applied multiple times in a script without having to copy and paste, but where functions really come into their own is when combined with iteration. Iteration is the process of running the same operation on a group of objects, further minimising code replication.

13.2.1 For Loops

A for loop has three core parts:

- 1) The sequence of items to iterate through
- 2) The operations to conduct per item in the sequence
- 3) The container for the results (optional)

The basic syntax is: `for (item in sequence) {do operations using item}`. Note the parentheses and the curly brackets. The results could be printed to console, or stored in a container R object.

```
for(i in list){
  # PERFORM SOME ACTION
}
```

A simple for loop **example** is below. For every number in the vector add 2. There is no *container object* here, the results of the function are printed directly into the console.

```
for (num in c(1,2,3,4,5)) {
  print(num + 2)
}
```

the SEQUENCE is defined (numbers 1 to 5) and loop is opened with '{'
The OPERATIONS (add two to each sequence number and print)
The loop is closed with "}'"

```
[1] 3
[1] 4
[1] 5
[1] 6
[1] 7
```

So let's make a slightly more complicated function - first we are making a new tibble, first we have four vectors - made of 10 numbers each randomly generated to be roughly close to a 0 mean with a s.d. of 1. Then we combine them to make a tibble

```
# a simple tibble
df <- tibble(
  a = rnorm(10),
  b = rnorm(10),
  c = rnorm(10),
  d = rnorm(10)
)
```

Each vector randomly generated so the actual averages will be slightly different

```
median(df$a)
# [1] 0.3085154
median(df$b)
# [1] 0.5429483
median(df$c)
# [1] -0.5137691
median(df$d)
# [1] 0.04415608
```

So the above code works, but it is repetitive, applying the same function again and again. Below we have a simple for loop

```
output <- vector("double", ncol(df)) # 1. output having a predefined empty list of the
                                         #   columns
for (i in seq_along(df)) {           # 2. sequence - determines what to loop over - here
  output[[i]] <- median(df[[i]])      # 3. body - each time the loop runs it allocates
}                                     #   a new element to the output vector
output
# [1] 0.30851540 0.54294832 -0.51376911 0.0441560
```

13.2.2 Exercise for For Loops

This part of the exercise is a real world example of using simple `for()` loops to create graphs. This data comes from the Living Planet Index, which holds data on various vertebrate species collected from 1974 to 2014.

First we should import the data:

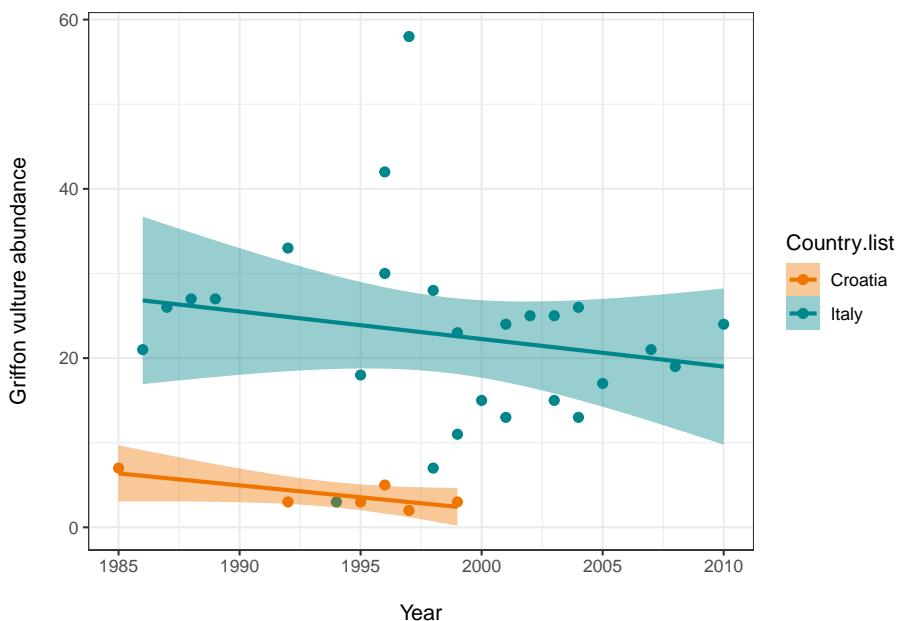
```
LPI <- read_csv("data/LPI_data_loops.csv")
```

Here is an example of a graph we can make from the data looking at Griffon Vulture changes in numbers over time.

```
vultureITCR <- LPI %>%
  filter(Common.Name == "Griffon vulture / Eurasian griffon") %>%
  filter(Country.list==c("Croatia", "Italy"))

vulture_scatter <- ggplot(vultureITCR, aes(x = year, y = abundance, colour = Country.list)) +
  geom_point(size = 2) +
  # Changing point size
  geom_smooth(method = lm, aes(fill = Country.list)) +
  # Adding a linear model fit and colour-coding by country
  scale_fill_manual(values = c("#EE7600", "#00868B")) +
  # Adding custom colours
  scale_colour_manual(values = c("#EE7600", "#00868B"),
                      # Adding custom colours
                      labels = c("Croatia", "Italy")) +
  # Adding labels for the legend
  ylab("Griffon vulture abundance\n") +
  xlab("\nYear") +
  theme_bw()

vulture_scatter
```



We can use custom themes (like the one you made earlier) to quickly update figures

```
vulture_scatter+theme_custom()
```

Now let's take a look at using functions and loops to help us build figures.

```
LPI_UK <- filter(LPI, Country.list == "United Kingdom")

# Pick 4 species and make scatterplots with a simple regression model fits that show how their abundance has changed over time

# Careful with the spelling of the names, it needs to match the names of the species in the LPI dataset

house_sparrow <- filter(LPI_UK, Common.Name == "House sparrow")
great_tit <- filter(LPI_UK, Common.Name == "Great tit")
corn_bunting <- filter(LPI_UK, Common.Name == "Corn bunting")
meadow_pipit <- filter(LPI_UK, Common.Name == "Meadow pipit")
```

So now we have four separate R objects holding data from four bird species, our standard approach might then be to make four figures looking at abundance over time.

```

house_sparrow_scatter <- ggplot(house_sparrow, aes (x = year, y = abundance)) +
  geom_point(size = 2, colour = "#00868B") +
  geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
  theme_custom() +
  labs(y = "Abundance\n", x = "", title = "House sparrow")

great_tit_scatter <- ggplot(great_tit, aes (x = year, y = abundance)) +
  geom_point(size = 2, colour = "#00868B") +
  geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
  theme_custom() +
  labs(y = "Abundance\n", x = "", title = "Great tit")

corn_bunting_scatter <- ggplot(corn_bunting, aes (x = year, y = abundance)) +
  geom_point(size = 2, colour = "#00868B") +
  geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
  theme_custom() +
  labs(y = "Abundance\n", x = "", title = "Corn bunting")

meadow_pipit_scatter <- ggplot(meadow_pipit, aes (x = year, y = abundance)) +
  geom_point(size = 2, colour = "#00868B") +
  geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
  theme_custom() +
  labs(y = "Abundance\n", x = "", title = "Meadow pipit")

```

If we want to look at all four plots at once we can use the layout functions from the package `patchwork`.

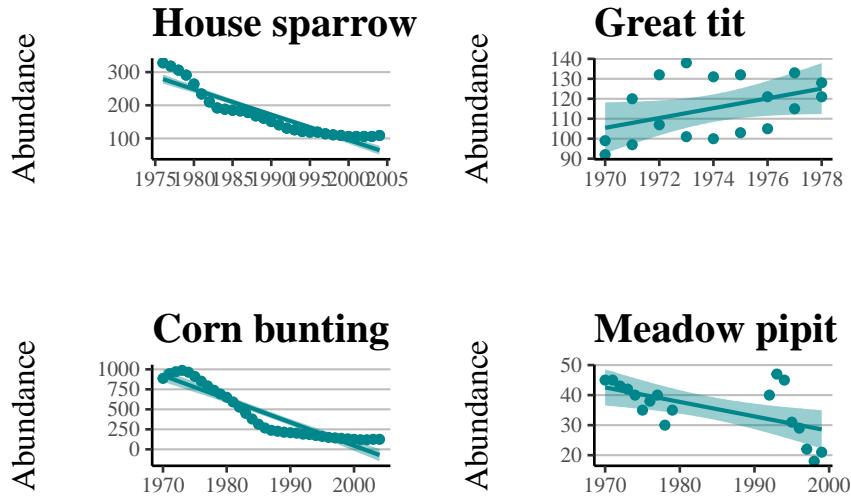
```

# put at the top of your script
library(patchwork)

layout <- "AABB
          CCDD"

house_sparrow_scatter+
  great_tit_scatter+
  corn_bunting_scatter+
  meadow_pipit_scatter+
  plot_layout(design=layout)

```



This is ok, but arguably still requires a lot of code repetition. We have used the same lines of code four times to recreate four plots that are functionally the same. If we want to make any changes to the look of our plots we have to make four separate edits & mistakes can easily creep in.

If we want to apply a loop, then the easiest thing is to first make our objects into an R list:

```
Sp_list <- list(house_sparrow, great_tit, corn_bunting, meadow_pipit)
```

Then loop down the length of our list:

```
my_plots <- list(length(Sp_list))

for (i in 1:length(Sp_list)) {
  # For every item along the length of Sp_list we want R to perform the following func
  data <- as.data.frame(Sp_list[i])
  # Create a dataframe for each species
  sp.name <- unique(data$Common.Name)
  # Create an object that holds the species name, so that we can title each graph
  plot <- ggplot(data, aes (x = year, y = abundance)) +
    # Make the plots and add our customised theme
    geom_point(size = 2, colour = "#00868B") +
    geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
```

```

theme_custom() +
labs(y = "Abundance\n", x = "", title = sp.name)

# makes a list of all the plots generates
my_plots[[i]] <- plot

# prints each plot out as it is made
print(plot)
}

```

So now we have a new object `my_plots` which is a list containing the four plots. This loop allowed us to code the details of our figures once, then iterate across four different groups.

```

wrap_plots(my_plots) +
  plot_layout(design=layout)
#wrap_plots function from patchwork can take a list of ggplots

```

What if you want to write a loop to save all four plots at once - can you modify the script to do this?

```

for (i in 1:length(Sp_list)) {
  # For every item along the length of Sp_list we want R to perform the following functions
  data <- as.data.frame(Sp_list[i])
  # Create a dataframe for each species
  sp.name <- unique(data$Common.Name)
  # Create an object that holds the species name, so that we can title each graph
  plot <- ggplot(data, aes (x = year, y = abundance)) +
    # Make the plots and add our customised theme
    geom_point(size = 2, colour = "#00868B") +
    geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +
    theme_custom() +
    labs(y = "Abundance\n", x = "", title = sp.name)

  if(i %% 1==0){    # The %% operator is the remainder, this handy if line prints a number every
    print(i)
  }
  # use paste to automatically add filename
  ggsave(plot, file=paste("figure/", sp.name, ".png", sep=''), dpi=900)
}

```

13.2.3 Iteration in R

Loops are a fundamental part of programming in many languages - that's why they have been introduced as a concept with examples here. However in R, Loops are used much less often than in other languages. That is because R is a 'vector based language', something we often take for granted. But what does that mean?

Here's an example - in the below written for R if we make a vector then apply a function to it, then it is applied to each element of the function, this is something we take for granted in R, but simply doesn't exist in languages like C++

```
x <- c(2,4,6,8,10)

x/2

# [1] 1 2 3 4 5
```

Instead for C++ we would be required to write a loop, in order to explicitly request that the division is applied to each element of the vector

```
for(i in seq_along(x)){
  x[i] = x[i]/2
}
x

# [1] 1 2 3 4 5
```

A lot of functions in R are already vectorised and you don't even have to think about this concept. When we want more control over how this vectorization is applied, we have used the function `group_by()`. It allows us to subset our data into hidden vectors, then command R to perform subsequent `dplyr` functions on each group separately. Because of the combined functionality of R's vectorization and `dplyr`'s data tools you might find there is little need for loops. When we *do* wish to apply a function that isn't vectorize in the way we want - then tidyverse has an answer to this as well - purrr Henry and Wickham (2020)

There are also a few other advantages to purrr - you can use it with pipes `%>%`, it handles errors better than normal for loops, and the syntax is quite clean and simple! If you are using a for loop, you can probably do it more clearly and succinctly with purrr!

Remember purrr is a very tidyverse focused method of iteration, so understanding for loops can be useful if you end up learning other programming languages in the future.

`purrr` is more focused on the function, and less obviously focused on the looping aspect, as you might be able to see below.

This is a really good intro to learning how to purrr - try some of the

simple exercises here before trying the below

Now you have a simple grasp of `purrr` you should see that we have a list containing species objects, and an anonymous function `~ggplot` on the right. We can use this to quickly make four plots, just like we did with our for loops.

the `map()` function takes a list or vector as the first argument, and a function (prefixed by `~`) as the second argument, it will then apply the function to each element of the list or vector. It will then return the output as new list. Simple!

```
my_plots_2 <- map(Sp_list, ~ggplot(data, aes (x = year, y = abundance)) +  
  geom_point(size = 2, colour = "#00868B") +  
  geom_smooth(method = lm, colour = "#00868B", fill = "#00868B") +  
  theme_custom() +  
  labs(y = "Abundance\n", x = "", title = sp.name))  
  
wrap_plots(my_plots_2)+plot_layout(design=layout)
```

13.3 More Practice

If you want to play with basic coding more, try some of these other tutorials, if you try them, let me know how you get on!

Importing Hospital Records

Bioinformatics and Lists

13.4 Summary

Making functions and iterations are advanced R skills, and can often seem daunting. I do not expect you to HAVE to implement these for this course, but I do want to give you an insight into some core programming skills that you might be interested in and want to develop.

Below are some links you may find useful

- RStudio education cheat sheet for `purr`
- R4DS - intro to programming
- Coding club
- EpiR handbook

Chapter 14

Introduction to Linear Models - Spring Week Two

14.1 Introduction to Linear Models

In the last chapter we conducted a simple analysis of Darwin's maize data using R. We worked out confidence intervals 'by hand'. This simple method allowed us to learn more about analysis, estimates, standard error and confidence. But it is also slow, and it relied on assumptions of a z-distribution.

Instead we will work through a much more efficient way to carry out comparisons, we will use the functions in R that let us perform a linear model analysis

14.1.1 Packages

```
library(tidyverse)
library(GGally)
library(emmeans)
```

14.2 A linear model analysis for comparing groups

R has a general function `lm()` for fitting linear models, this is part of base R (does not require the tidyverse packages). We will run through a few different iterations of the linear model increasing in complexity. We will often want to fit several models to our data, so a common way to work is to fit a model and

224CHAPTER 14. INTRODUCTION TO LINEAR MODELS - SPRING WEEK TWO

assign it to a named R object, so that we can extract data from when we need it.

In the example below I have called the model `lsmodel0`, short for “least-squares model 0”, this is because the linear-model uses a technique called **least-squares** we will explore what this means later.

```
lsmodel0 <- lm(formula=height~1, data=darwin)

## optional - the tidyverse way
# remember when we use non-dplyr functions (like lm), we need to put a . where the data

# lsmodel0 <- darwin %>%
#           lm(height~1, data=.)
#
```

The first argument of the `lm()` function is `formula` (we won’t write this out in full in the future) - and this specifies we want to analyse a **response** variable (`height`) as a function of an **explanatory** variable using the *tilde* symbol (`-`).

The simplest possible model ignores any explanatory variables, instead the `1` indicates we just want to estimate an intercept. Without explanatory variables this means the formula will just estimate the overall mean height of **all** the plants in the dataset.

```
broom::tidy(lsmodel0)

# could also use summary(lsmodel0) which is the base R function

# the base R summary output has a lot of information and is good for understanding you

# A tibble: 1 x 5
  term      estimate std.error statistic p.value
  <chr>       <dbl>     <dbl>      <dbl>    <dbl>
1 (Intercept)   18.9      0.581     32.5  2.27e-24
```

The output above is called the *table of coefficients*. The 18.9 is the *estimate of the model coefficient* (in this case it is the overall mean), together with its standard error. The first row in any R model output is always labelled the ‘Intercept’ and the challenge is usually to workout what that represents. In this case we can prove that this is the same as the overall mean as follows:

```
mean(darwin$height)
```

```
[1] 18.88333
```

This simple model allows us to understand what the `lm()` function does.

14.2.1 Compare means

What we really want is a linear model that analyses the *difference* in average plant height as a function of pollination type. We can use the `lm()` function to fit this as a linear model as follows:

```
lsmodel1 <- lm(height ~ type, data=darwin)

# note that the following is identical

# lsmodel1 <- lm(height ~ 1 + type, data=darwin)
```

Now the model formula contains the pollination type in addition to an intercept.

```
broom::tidy(lsmodel1)
```

term	estimate	std.error	statistic	p.value
<chr>	<dbl>	<dbl>	<dbl>	<dbl>
1 (Intercept)	20.2	0.759	26.6	2.05e-21
2 typeSelf	-2.62	1.07	-2.44	2.14e- 2

Some things to notice about the above:

- 1) The intercept value has changed! So what does it represent now?
- 2) The label of the second row is ‘typeSelf’

But what does this mean? Think about it and see if you can figure it out for yourself before clicking the reveal

Click-me for answer

The label of the second row ‘typeSelf’ is produced by combining the variable type, with one of the factors (Self). As there are only two levels in type, it stands to reason that the intercept must represent typeCross. So the coefficient in the label intercept is the average height of the 15 maize plants in the crossed treatment.

So what about the second row? A common mistake is to think that this must refer to the height of the Self plants. However, this cannot be true as the value is *negative*. Instead what it actually refers to is the **difference in the mean height of the two groups**. This focuses then on the question which we are asking: is there a difference in the height of plants that result from cross pollination when compared to plants that have been self pollinated?

226CHAPTER 14. INTRODUCTION TO LINEAR MODELS - SPRING WEEK TWO

This linear model indicates the average height of Crossed plants is 20.2 inches, and Selfed plants are an average of 2.6 inches *shorter*.

You can confirm this for yourself:

```
darwin %>%
  group_by(type) %>%
  summarise(mean=mean(height))
```

Let's take a look at a fuller summary of our model, to see what else we can determine

```
summary(lsmodel1)
```

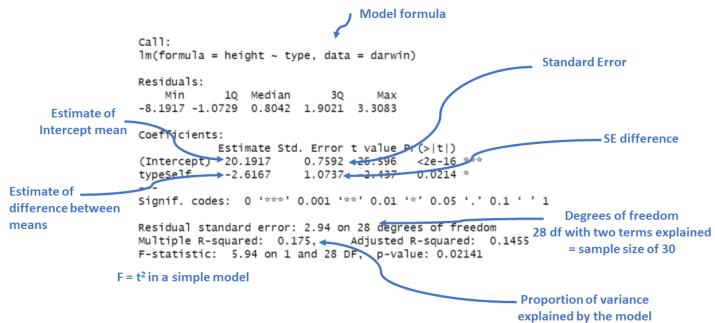
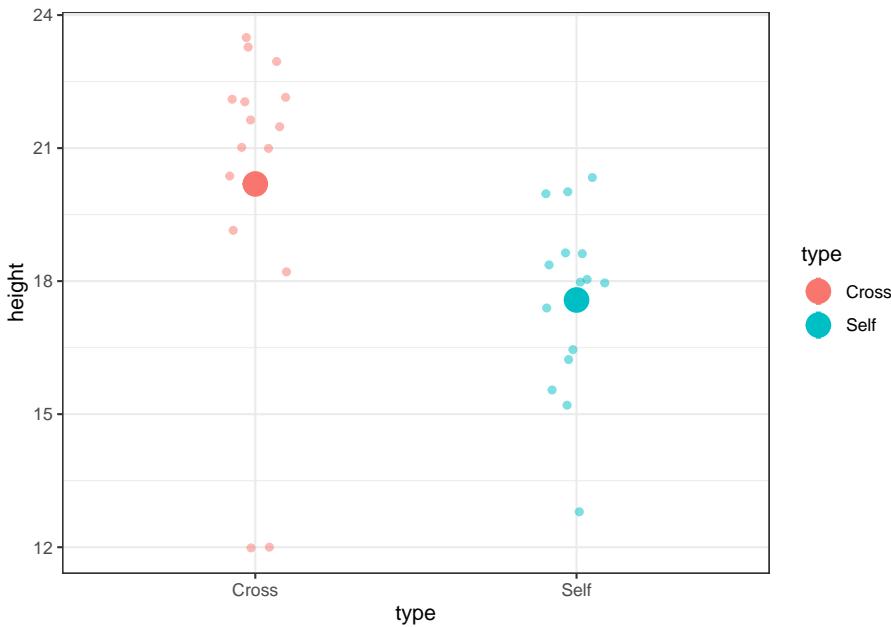


Figure 14.1: Annotation of the summary function output

We could use the information from this model to superimpose the calculated means onto a plot.

```
darwin %>%
  ggplot(aes(x=type,
             y=height,
             colour=type))++
  geom_jitter(alpha=0.5,
              width=0.1)++
  stat_summary(fun=mean,
              size=1.2)++
  theme_bw()
```



14.2.2 Standard error of the difference

The output of the model also gives the standard errors of the values (estimates). In the first row the intercept calculates a mean and the standard error of the mean (SEM). As the second row gives the mean *difference* in this column it gives the *standard error of the difference* between the two means (SED). We have already seen how to calculate SEM, but what about SED?

$$SED = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

** Note - The subscripts 1 and 2 indicate the two groups (self and cross).

Our linear model analysis doesn't actually calculate the individual variances for the two groups. Instead it uses a 'pooled' variance approach. This assumes the variance is roughly equal across all groups, and allows us to take advantage of the larger sample size through 'pooling', to generate a more accurate SED. However, this does assume that variance is *roughly equal* in the two groups. In fact last week when we calculated standard deviation, we saw this was not the case. So we must check this assumption in our model (more on this later).

14.3 Confidence intervals

```
broom::tidy(lsmodel1, conf.int=T)

## # A tibble: 2 x 7
##   term      estimate std.error statistic p.value conf.low conf.high
##   <chr>     <dbl>     <dbl>     <dbl>     <dbl>     <dbl>     <dbl>
## 1 (Intercept) 20.2      0.759    26.6  2.05e-21    18.6     21.7
## 2 typeSelf    -2.62     1.07     -2.44  2.14e- 2    -4.82    -0.417

# or from base R
# confint(lsmodel1)
```

Because this follows the same layout as the table of coefficients, the output intercept row gives a 95% CI for the height of the crossed plants and the second row gives a 95% interval for the *difference in height between crossed and selfed plants*. The lower and upper bounds are the 2.5% and 97.5% of a *t*-distribution (more on this later).

It is this difference in height in which we are specifically interested. Our summary models have generated *p*-values but we have conspicuously ignored these for now. Instead we are going to continue to focus on using confidence intervals to answer our question.

14.4 Answering the question

Darwin's original hypothesis was that self-pollination would reduce fitness (using height as a proxy for this). The null hypothesis is that there is no effect of pollination type, and therefore no difference in the average heights. We must ask ourselves if our experiment is consistent with this null hypothesis or can we reject it? If we choose to reject the null hypothesis, with what level of confidence can we do so?

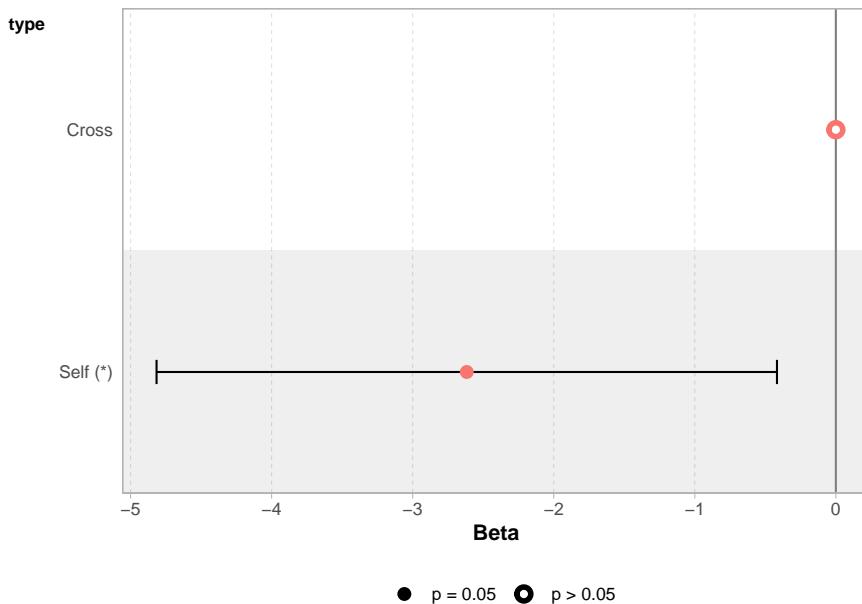
To do this, we can simply whether or not the predicted value of our null hypothesis (a difference of zero) lies inside the 95% CI for the difference of the mean.

If our confidence intervals contain zero (or no difference), then we cannot establish a difference between our sample difference in height (-2.62 inches) from the null prediction of zero difference, given the level of variability (noise) in our data.

In this case we can see that the upper and lower bounds of the confidence intervals do not cross zero. The difference in height is consistent with Darwin's alternate hypothesis of inbreeding depression.

The GGally package has a handy `ggcoef_model()` function, that produces a graph of the estimate mean difference with an approx 95% CI. As we can see reject the null hypothesis at a 95% confidence level.

```
GGally::ggcoef_model(lsmodel1,
                      show_p_values=FALSE,
                      conf.level=0.95)
```



If we increase the level of confidence (from 95% to 99%, roughly 2 SE to 3 SE), then we may find that we cannot reject the null hypothesis at a higher threshold of confidence. Try altering the `conf.level` argument above for yourself to see this in action.

We can also include this argument in the `tidy()` function if we wish to:

```
broom::tidy(lsmodel1, conf.int=T, conf.level=0.99)
```

14.4.1 Getting the other treatment mean and standard error

One limitation of the table of coefficients output is that it doesn't provide the mean and standard error of the other treatment level (only the difference between them). If we wish to calculate the "other" mean and SE then we can get R to do this.

230CHAPTER 14. INTRODUCTION TO LINEAR MODELS - SPRING WEEK TWO

```
darwin %>%
  mutate(type=factor(type)) %>%
  mutate(type=fct_relevel(type, c("Self", "Cross"))) %>%
  lm(height~type, data=.) %>%
  broom::tidy()
```

```
# A tibble: 2 x 5
  term      estimate std.error statistic p.value
  <chr>     <dbl>    <dbl>     <dbl>    <dbl>
1 (Intercept) 17.6     0.759    23.1  8.48e-20
2 typeCross    2.62     1.07     2.44  2.14e- 2
```

After releveling, the self treatment is now taken as the intercept, and we get the estimate for its mean and standard error

We could also use the package `emmeans` and its function `emmeans()` to do a similar thing

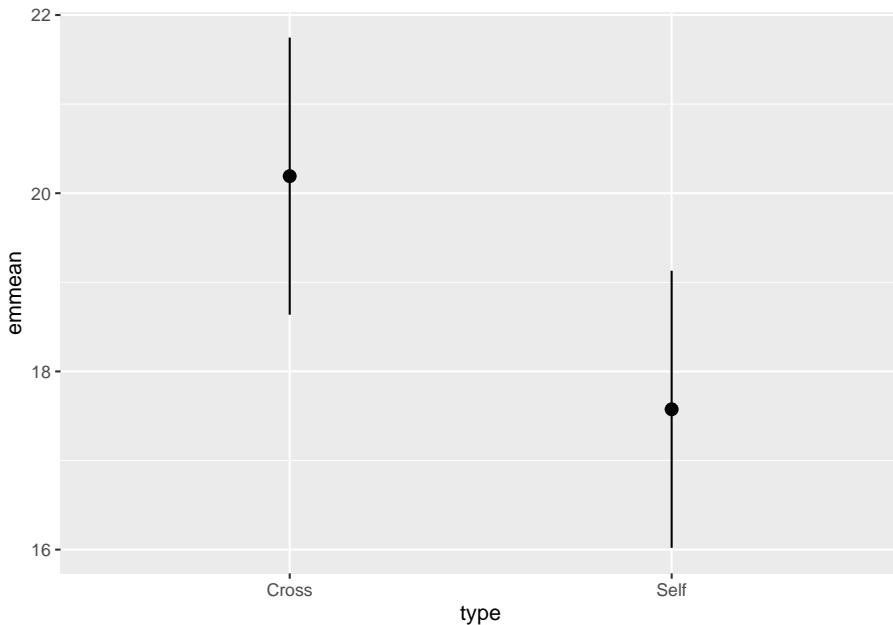
```
means <- emmeans::emmeans(lsmodel1, specs = ~type)
```

```
type  emmean    SE df lower.CL upper.CL
Cross  20.2 0.759 28     18.6     21.7
Self   17.6 0.759 28     16.0     19.1
```

Confidence level used: 0.95

The advantage of `emmeans` is that it provides the mean and standard error estimates of all levels from the model at once (e.g. it relevels the model multiple times behind the scenes).

```
means %>%
  as_tibble() %>%
  ggplot(aes(x=type,
             y=emmean))+ 
  geom_pointrange(aes(
    ymin=lower.CL,
    ymax=upper.CL))
```



Notice that no matter how we calculate the estimated SE (and therefore the 95% CI) of both treatments is the same. This is because as mentioned earlier the variance is a pooled estimate, e.g. variance is not being calculated separately for each group. The only difference you should see in SE across treatments will be if there is a difference in sample size between groups.

**Note - also notice how the Confidence intervals of the estimated means strongly overlap, there is a difference between the two SEMs and the SED we have calculated. So overlapping error bars cannot be used to infer significance.

Because of this pooled variance, there is an assumption that variance is equal across the groups, this and other assumptions of the linear model should be checked. We cannot trust our results if the assumptions of the model are not adequately met.

14.5 Assumption checking

So now we have been through the main parts of a linear model analysis, we have results and inferences. But we need to check whether the assumptions of the model are adequately met so that we know whether our analysis can be trusted.

In this first part we are going to check two of our assumptions:

- 1) that the residual/unexplained variance in our data is approximately normal

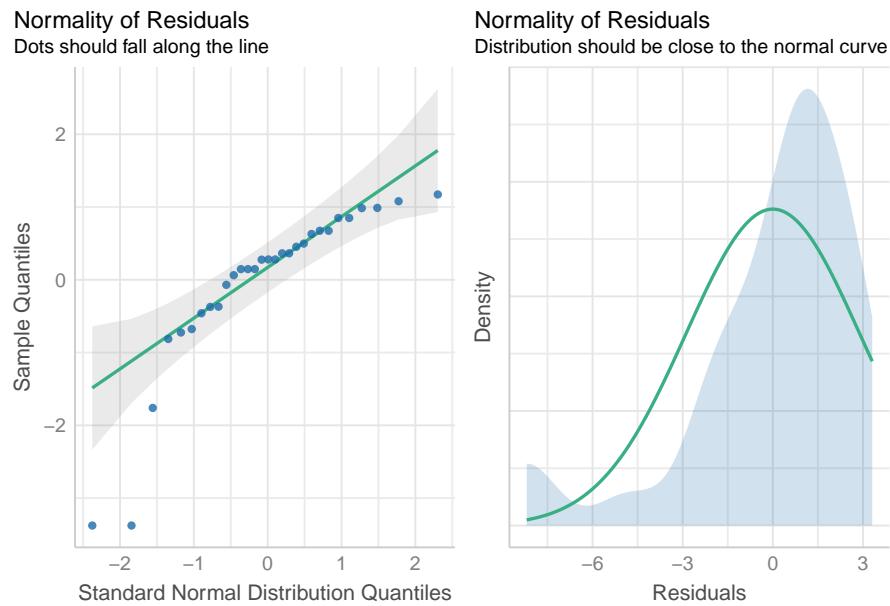
- 2) that the residual/unexplained variance is approximately equal between our groups

Residuals are the differences between the observed values and the fitted values produced by the model - in this case the heights of the plants against the treatment means. The assumption of a normal distribution applies because the linear model uses this to calculate the standard errors (and therefore confidence intervals). The assumption of equal variance applies because of this pooled variance approach (e.g. we have two treatments with 15 replicates - but by pooling the variance across treatments we have a sample size of 30).

Several functions exist to check assumptions of linear models, and the easiest way to do this is to make graphs. We can do this in several ways, in base R with the `plot()` function, and by using the package `performance` and its `check_model()` function.

14.5.1 Normal distribution

```
performance::check_model(lsmodel1, check=c("normality", "qq"))
```



```
plot(lsmodel1, which=c(2,2))
```

14.5.1.1 What is a Quantile-Quantile (QQ) plot?

A QQ plot is a classic way of checking whether a sample distribution is the same as another (or theoretical distribution). They look a bit odd at first, but they are actually fairly easy to understand, and very useful! The qqplot distributes your data on the y-axis, and a theoretical normal distribution on the x-axis. If the residuals follow a normal distribution, they should meet to produce a perfect diagonal line across the plot.

Watch this video to see QQ plots explained

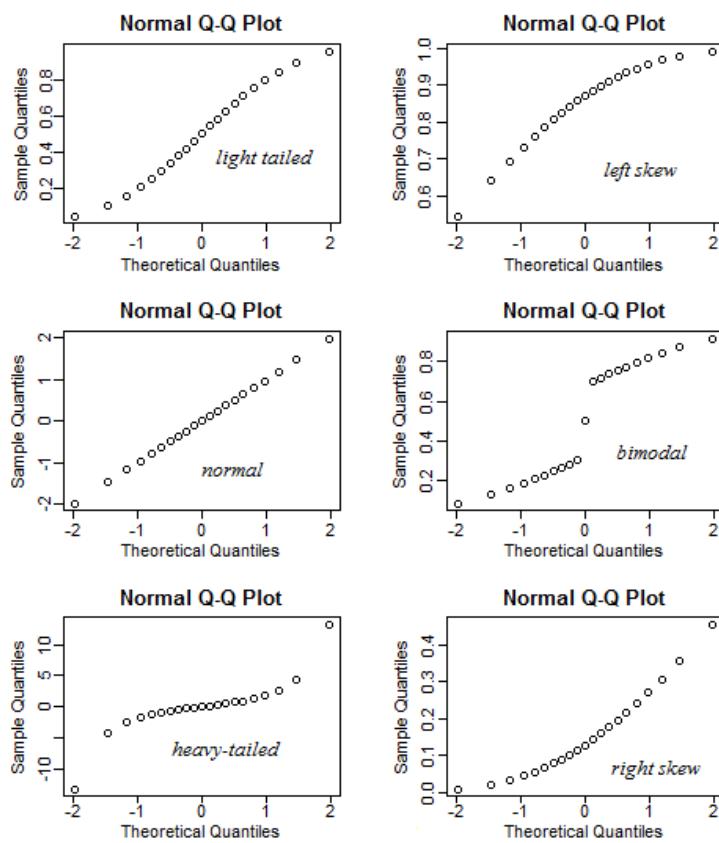


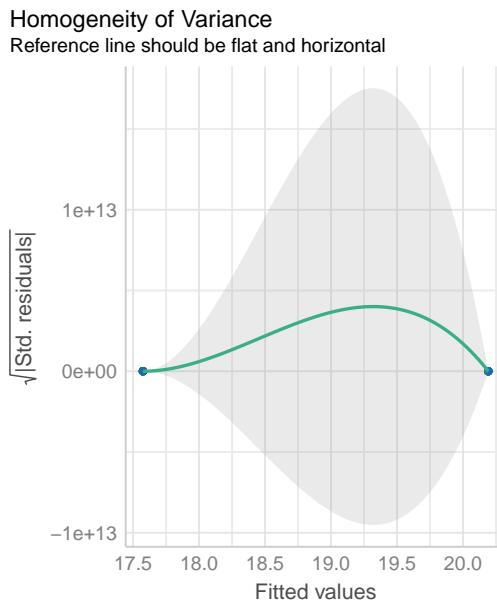
Figure 14.2: Examples of qqplots with different deviations from a normal distribution

In our example we can see that *most* of our residuals can be explained by a normal distribution, except at the extreme low end of our data. This is not surprising, as we already identified some potential outliers.

So the fit is not perfect, but it is also not terrible!

14.5.2 Equal variance

```
performance::check_model(lsmodel1, check="homogeneity")
```



```
plot(lsmodel1, which=c(1,3))
```

In order to assess if variances are equal we can plot the residuals (variance) of our data against the fitted (predicted) values. If the residuals were zero, this would mean there is no error, and our data exactly matches our estimates. In reality, there will always be residual error, but as long as it is evenly distributed between treatments this is ok.

The `check_models` plot provides what we call ‘standardized residuals’ where we divide the residual error by the standard deviation.

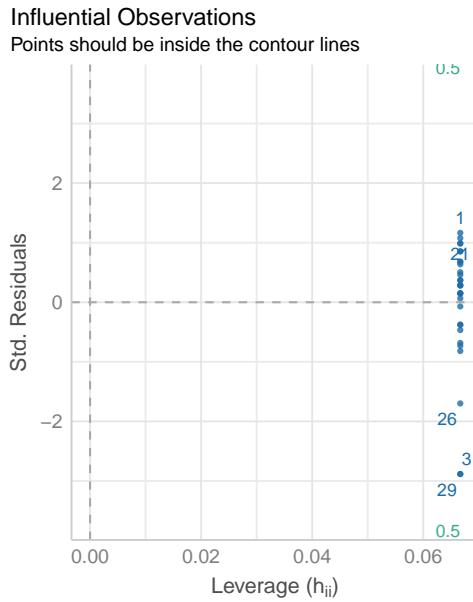
In this instance we can see that the higher fitted values (Cross treatment) appears to be more variable than the lower fitted values. Again, this is not too bad, but not perfect. Again, this is probably being influence at least partially by the potential outliers.

14.5.3 Outliers

We have talked a lot about the potential effect of outliers, we can see that they are potentially affecting our estimates of error/variance. However, we should

also check how much of an effect they might be having on the model estimates (means). This is where formal outlier tests are useful.

```
performance::check_model(lsmodel1, check="outliers")
```



```
plot(lsmodel1, which=c(4,4))
```

The value which the data points are being measured against is called Cook's distance. This is a measure of how much 'leverage' a single data point is exerting on the model, if it is too high, it may be having an outsized effect on the estimates. The `check_model()` function gives contours to indicate whether data points fall inside or outside the margins for affecting fit, but a rough estimate for an acceptable Cook's Distance is either $\frac{4}{N}$ or $\frac{4}{df}$, but you are unlikely to have to calculate this by hand.

14.6 Summary

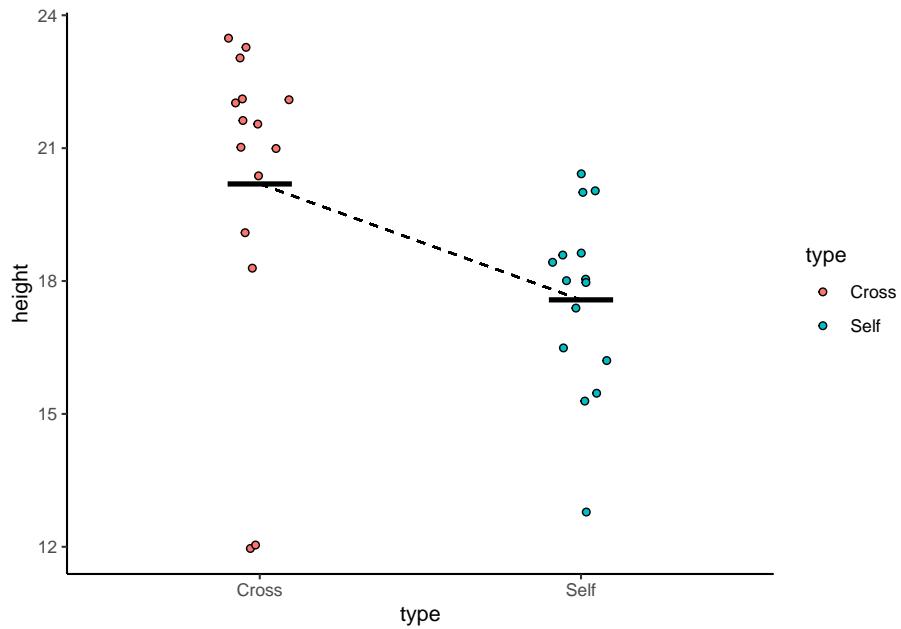
So what can we determine from this analysis? Our model is not perfect, however it is reasonably good. However, we have not addressed an important part of our experimental design yet. The fact that the plants are paired, so in this example we have basically carried out a Student's t-test, not a paired t-test. Later we will add pair as another explanatory variable and see how this affects our model.

236CHAPTER 14. INTRODUCTION TO LINEAR MODELS - SPRING WEEK TWO

So remember a linear model sets one factor level as the ‘intercept’ estimates its mean, then draws a line from the first treatment to the second treatment, the slope of the line is the difference in means between the two treatments.

The difference in means is always accompanied by a standard error of the difference (SED), and this can be used to calculate a 95% confidence interval. If this confidence interval does not contain the intercept value, we can reject the null hypothesis that there is ‘no effect’.

Linear models make a variety of assumptions, including that the noise (residual differences) are approximately normally distributed, with roughly equal (homogenous) variance.



Chapter 15

Testing - Spring Week Three

15.1 It's t-time

In the last chapter we used linear models to calculate estimates, estimates of mean difference and confidence intervals. As we can set our confidence intervals to whatever threshold we choose - reporting these without P should be sufficient for any estimation we choose. It's the interesting bit, reporting the direction and effect size of any relationship or difference at whatever confidence threshold we want.

P -values by comparison, are boring, no-one actually cares about P -values, but you would be forgiven for thinking they are the most important thing about statistics from the way we often see them presented. And so, inevitably, you will be asked to supply a P -value for many of your lab reports, dissertations (and maybe future scientific papers). Luckily significance tests come parcelled in with the coefficients of our linear models.

15.2 Student's t -test

The Student's t -test uses the t -distribution, a small-sample size version of the normal distribution, where tails are fatter if the degrees of freedom are small. There are two basic types of t -test which we have encountered with our linear models.

- The one sample t -test: takes the mean of a sample and compares it with the null hypothesis of zero

`lm(y ~ 1)`

```
lm (height~1)
```

- The two sample t -test which compares the difference between the means of two samples against a null hypothesis of no difference between the means of the two populations.

The general equation for calculating t is:

$$t = \frac{\text{difference}}{\text{SE}}$$

SO the calculation of the t -value is essentially counting the standard errors, our rough rule of thumb is that the estimate of the difference is about twice as large as the standard error at a confidence interval of 95%, and about three times as large as the standard error at a confidence interval of 99%.

This is very *approximate* and becomes less robust at smaller sample sizes, because when sample sizes are **large** the t -distribution is roughly equal to a normal (z) distribution. However, when sample sizes are small the t -distribution has a shorter and wider distribution (we need larger standard errors to capture our 95% confidence interval).

```
### base R

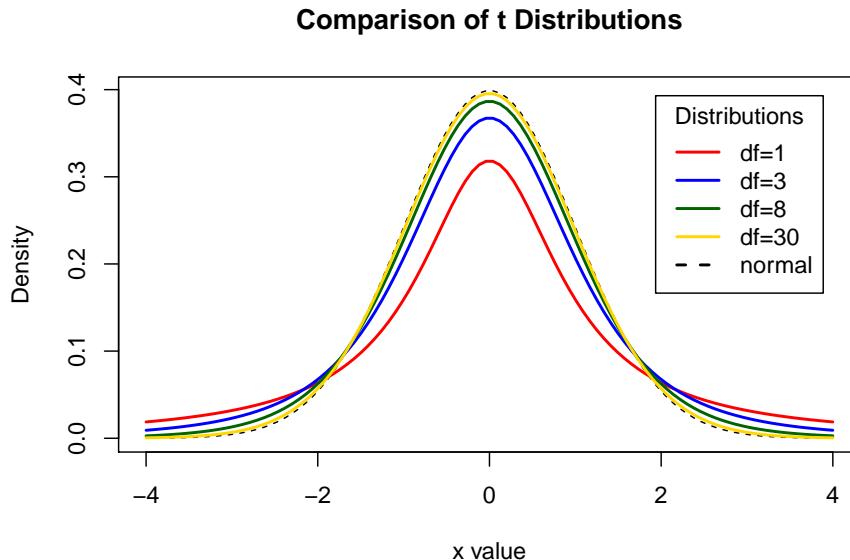
x <- seq(-4, 4, length=100)
hx <- dnorm(x)

degf <- c(1, 3, 8, 30)
colors <- c("red", "blue", "darkgreen", "gold", "black")
labels <- c("df=1", "df=3", "df=8", "df=30", "normal")

plot(x, hx, type="l", lty=2, xlab="x value",
      ylab="Density", main="Comparison of t Distributions")

for (i in 1:4){
  lines(x, dt(x,degf[i]), lwd=2, col=colors[i])
}

legend("topright", inset=.05, title="Distributions",
       labels, lwd=2, lty=c(1, 1, 1, 1, 2), col=colors)
```



```
#### with purrr

x <- seq(-4, 4, length=100)
z_dist <- dnorm(x)

values <- tibble(x,z_dist)

# map_dfc combines values returned into a dataframe
t <- map_dfc(degf, ~dt(x, .x))
colnames(t) <- degf

combined <- cbind(values,t)

combined %>%
  pivot_longer(cols=!x, names_to="distribution") %>%
  mutate(distribution=factor(distribution, levels=c("z_dist", "1", "3", "8", "30"))) %>%
  mutate(distribution=fct_recode(distribution, "z distribution" = "z_dist", "df = 1" = "1", "df = 3" = "3", "df = 8" = "8", "df = 30" = "30"))
  ggplot(aes(x=x, y=value, colour=distribution))+
  geom_line(linetype="dashed")+
  theme_classic()
```

A potential source of confusion when discussing t is that there are two values that must be considered. There is *critical t* the value which must be **exceeded** for the test to be significant (e.g. generates a P value that is less than our predefined

α). The critical value for t is defined by the df . Then there is the *observed* value of t , this is the value returned by your statistical test, calculated by $\frac{difference}{SE}$. When *observed t > critical t* the result can be declared significantly different at that threshold for α .

We are spending a lot of time with t because it is the default value generated by our linear model outputs, instead of assuming a normal distribution and using z . Recall that for a z distribution the critical value for $P = 0.05$ is $1.96 * SE$ (or roughly double the Standard Error), and actually if we look at the figure above, we can see that even at only 8 df the distribution of t is starting to look pretty close to a z distribution. The values for critical t at each degree of freedom up to 30 are presented below

```
df <- c(1:30)

# map_dbl forces returned values to be a single vector of numbers (rather than a list)
critical_t <- map_dbl(df, ~qt(p=0.05/2, df=.x, lower.tail=FALSE))

tibble(df,critical_t) %>%
  ggplot(aes(x=df, y=critical_t)) +
  geom_point() +
  geom_line() +
  geom_hline(aes(yintercept=1.96), linetype="dashed", colour="red") +
  labs(x= "Degrees of Freedom",
       y= expression(paste("Critical value of ", italic("t"))))

[1] 12.706205  4.302653  3.182446  2.776445  2.570582  2.446912  2.364624  2.306004  2
[11] 2.200985  2.178813  2.160369  2.144787  2.131450  2.119905  2.109816  2.100922  2
[21] 2.079614  2.073873  2.068658  2.063899  2.059539  2.055529  2.051831  2.048407  2
```

So far we have used a mixture of the base R `summary()` function and the broom package tibbles produced by `broom::tidy()`. Summary is the most common way to investigate a model result, but it is its own specific type of R object (e.g. not a dataframe or a tibble), which is why tidying the results into a dataframe like structure can be useful.

Using either method we can see that they include t -tests for the coefficient, summary explicitly calls them t , while `tidy()` refers to them generically as the ‘statistic’

```
lsmodel1 <- lm(height ~ type, data = darwin)
```

```
summary(lsmodel1)
broom::tidy(lsmodel1)
```

```
# A tibble: 2 x 5
  term      estimate std.error statistic p.value
  <chr>     <dbl>     <dbl>     <dbl>    <dbl>
1 (Intercept) 20.2      0.759    26.6  2.05e-21
2 typeSelf    -2.62     1.07     -2.44  2.14e- 2
```

The linear model summary will automatically apply a test to every row of our table, sometimes these are the important *apriori* tests defined by our hypothesis, and sometimes they are not.

As an example the *second* row of our table is a test we planned to perform, it tests our null hypothesis by comparing the average observed difference in plant heights between cross and self-pollinated plants, it calculates the average difference (estimate), the amount of uncertainty (std. error), then calculates an *observed t* value and determines the probability of observing an effect of at least this size (at this sample size) if the null hypothesis is true.

However, the first row also performs a *t*-test, it tests a null hypothesis that the intercept (here the mean height of the cross-pollinated plants) is zero. This was not a comparison we intended to make, nor is it likely that this test is particularly useful.

Anyway the observed difference in plant heights was 2.62 inches \pm 1.07, which produces an observed value of *t* as:

```
tidy_model1 <- broom::tidy(lsmodel1)

tidy_model1[[2,2]] / tidy_model1[[2,3]]
```

Which could be the same as the value for *t* in our model summary.

```
[1] -2.437113
```

15.2.1 Paired t

The structure of our linear model so far has produced the output for a standard two-sample Student's *t*-test. However, when we *first* calculated our estimates by hand - we started by making an average of the paired differences in height. To generate the equivalent of a paired *t*-test, we simply have to add the factor for pairs to our linear model formula

```
# note I have made pair a factor - pair 2 is not greater than pair 1 - so it doesn't make sense to
# use the standard t-test

lm(height ~ type + factor(pair), data = darwin) %>%
  broom::tidy()

# A tibble: 16 x 5
```

term	estimate	std.error	statistic	p.value
<chr>	<dbl>	<dbl>	<dbl>	<dbl>
1 (Intercept)	21.7	2.44	8.93	0.000000375
2 typeSelf	-2.62	1.22	-2.15	0.0497
3 factor(pair)2	-4.25	3.34	-1.27	0.223
4 factor(pair)3	0.0625	3.34	0.0187	0.985
5 factor(pair)4	0.563	3.34	0.169	0.869
6 factor(pair)5	-1.69	3.34	-0.506	0.621
7 factor(pair)6	-0.375	3.34	-0.112	0.912
8 factor(pair)7	-0.0625	3.34	-0.0187	0.985
9 factor(pair)8	-2.62	3.34	-0.787	0.444
10 factor(pair)9	-3.06	3.34	-0.918	0.374
11 factor(pair)10	-0.625	3.34	-0.187	0.854
12 factor(pair)11	-0.687	3.34	-0.206	0.840
13 factor(pair)12	-0.937	3.34	-0.281	0.783
14 factor(pair)13	-3	3.34	-0.899	0.384
15 factor(pair)14	-1.19	3.34	-0.356	0.727
16 factor(pair)15	-5.44	3.34	-1.63	0.125

The table of coefficients suddenly looks a lot more complicated! This is because **now** the intercept is the height of the crossed plant from pair 1:

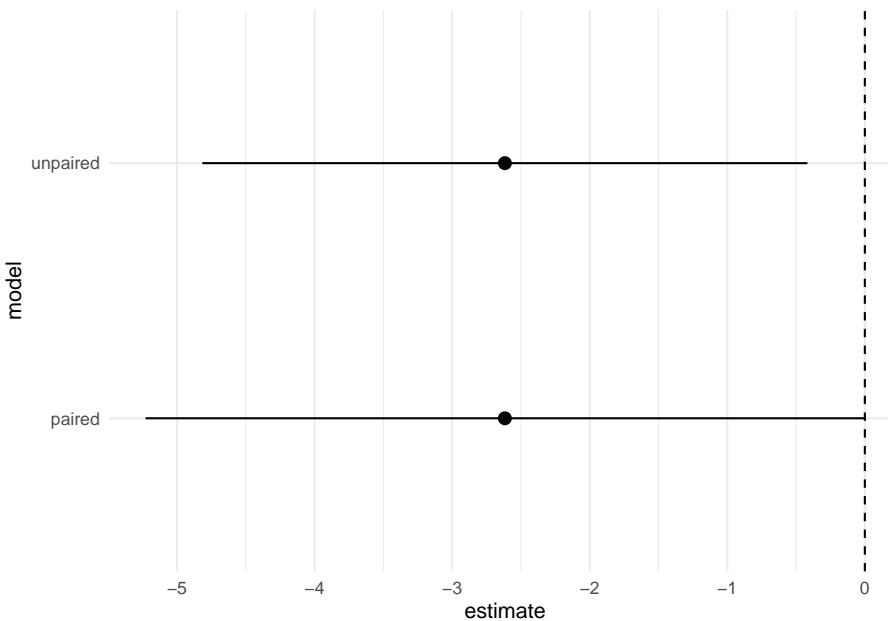
- The second row now compares the mean heights of Crossed and Selfed plants when they are in the same pair
- rows three to 16 compare the average difference of each pair (Crossed and Selfed combined) against pair 1

Again the linear model computes every possible combination of t -statistic and P -value, however the only one we care about is the difference in Cross and Self-pollinated plant heights. If we ignore the pair comparisons the second row gives us a *paired t*-test. ‘What is the difference in height between Cross and Self-pollinated plants when we hold pairs constant?’

For completeness let’s generate the confidence intervals for the *paired t*-test.

```
lm(height ~ type + factor(pair), data = darwin) %>%
  broom::tidy(., conf.int=T) %>%
  slice(1:2)
```

We can see that estimate of the mean difference is identical but the 95% confidence intervals are now slightly different. So in this particular version we have actually increased our level of uncertainty by including the pair parameter.



What does this mean for choosing the right model? Click-me for answer

In future sessions we will work through model building and simplification, in this case we had a good *a priori* reason to include pair in our initial model, there are then simple tests we can do to see if it is safe to remove it, if it doesn't appear to be adding to our explanation of the difference in heights between self and cross-fertilised plants.

15.3 Effect sizes

We have discussed the importance of using confidence intervals to talk about effect sizes. When our 95% confidence intervals do not overlap the intercept, this indicates we have difference in our means which is significant at $\alpha = 0.05$. More interestingly than this it allows us to talk about the ‘amount of difference’ between our treatments, the lower margin of our confidence intervals is the smallest/minimum effect size. On the response scale of our variables this is very useful, we can report for example that there is *at least* a 0.43 inch height difference between self and crossed fertilised plants at $\alpha = 0.05$.

However we may also see reports describing “large”, “medium” or “small” effect sizes, how do we know if we are looking at a result with a “large” effect size? The answer is to use a method of standardisation. Interestingly there are lots of these, but we will look at one of the most common **Cohen’s D**. It is simply a measure of standardising effect sizes across experiments according to the

Table 15.1: Cohen's D

Effect size	d
very small	0.01
small	0.20
medium	0.50
large	0.80
very large	1.20
huge	2.00

standard deviations of the differences.

For the unpaired *t*-test and then the paired *t*-test (lsmodel1, lsmodel2), see if you can use the following equations to manually calculate *d*.

Unpaired *t*-test - the difference in means divided by the pooled standard deviation:

$$\frac{\text{difference}}{\sqrt{\frac{(s_1^2 + s_2^2)}{2}}}$$

Paired *t*-test:

$$\frac{t}{\sqrt{(N)}}$$

What do you calculate as the effect size for these two analyses? Click-me for answer

unpaired *t*-test: 0.92

paired *t*-test: 0.45

There is also a package with functions to help you calculate a range of common standardised effect size measures - it also provides confidence intervals on a standardised scale (where 0 would be no effect):

```
library(effectsize)

t_to_d(2.437, df_error = 28, paired = F)

t_to_d(2.456, df_error=27, paired=T)
```

15.3.1 Power

You don't necessarily need to report effect sizes in a write-up, it is fairly rare to see them included in a paper. It will be more useful for you to report confidence intervals on the original measurement scale. But it does provide two important pieces of information.

- 1) All experiments/statistical analyses will become *statistically significant* if you make the sample size large enough. In this respect it shows how misleading a significant result can be. It is not that interesting if a result is statistically significant, but the effect size is tiny. Even without reporting d you can start thinking about whether confidence intervals indicate your result is *interesting*.
- 2) Type 2 errors. Statistical tests provide you with the probability of making a Type 1 error (rejecting the null hypothesis incorrectly) in the form of P . But what about Type 2 errors? Keeping the null hypothesis, when we should be rejecting it? Or not finding an effect.

The probability of making a Type 2 error is known as $1 - \beta$, where β refers to your statistical 'power'. Working out statistical power is very straightforward for simple tests, and then becomes rapidly more difficult as the complexity of your analysis increases... but it is an important concept to understand.

There are two potential uses for Power analysis

- 1) Working out what the statistical power of your analysis was *post hoc* to determine how likely it was you missed an effect (but see [this](#) useful paper).
- 2) Working out what sample size you *need* before an experiment to make sure you reach the desired power. Often a common β value is 0.8, in much the same way that a common α is 0.05, it is an arbitrary target, but here means we can tolerate a risk of failing to reject the null when we should have in 20% of our experiments that do not produce a significant result.

```
library(pwr)

# for n, d, sig.level and power - put values for three of the arguments and it will return the value of power
pwr.t.test(n = NULL, d = NULL, sig.level = 0.05, power = NULL,
           type = c("two.sample"),
           alternative = c("two.sided"))
```

Now you know how the `pwr.t.test()` function works - Can you make a simple iteration to check the power for a lower effect size (d) of 0.2 for a two-sided t-test at sample sizes from 0-1000 increasing by 10 each time?

```

sample_size <- seq(0,1000, by=10)

output <- list(length(sample_size))

for (i in 1:length(sample_size)) {

  sample <- pwr.t.test(n=sample_size[i], d=0.2, sig.level=0.05)
  output[[i]] <- sample

  if(i %% 1==0){      # The %% operator is the remainder, this handy if line prints
    print(i)
  }
}

sample_list <- as.list(sample_size)

names(output) <- sample_size

# now you should be able to call any sample size and check statistical power!

# output$`^30`
```

15.4 Meta-analysis

The repeatability of results is a key part of the scientific method. Unfortunately there is often an emphasis in the literature on ‘novel findings’, which means that unusual/interesting results that happen to reach statistical significance may be more likely to be published. The reality is that we know if we set an $\alpha = 0.05$, that we run the risk of rejecting the null hypothesis incorrectly in 1 in 20 of our experiments.

It is **not possible** for you to know from a single experiment whether you have found a genuine effect or made a Type 1 error. However, over time as experiments are eventually repeated the literature builds up allowing us to synthesise the evidence. Let’s try that now & imagine a scenario where Darwin’s experiment has been repeated another 20 times.

In the example below we have made a for loop that assumes we ‘know’ the true mean difference of crossed and fertilised plants and the standard deviation of the ‘population’(we have taken Darwin’s experimental data for this). The for loop then creates 20 new sampling experiments, and calculates the estimated mean difference for each experiment

```

set.seed(1234)

myList <- vector("list", 20)
y <- tibble()

for (i in 1:length(myList)) {

  x <- rnorm(n=12, mean=2.6, sd=2.83)
  data <- tibble(x)
  temp <- lm(x~1, data=data) %>%
    broom::tidy(conf.int=T)
  y <- rbind(y,temp)

}

y$"experiment number" <- rep(1:20)

# the new dataframe y contains the results of 20 new experiments

```

Using this newly generated data how many experiments found a significant difference and how many did not? - What would you conclude from this?

```

y %>%
  mutate(`p value < 0.05` = if_else(p.value > 0.049, "non-significant", "significant")) %>%
  group_by(`p value < 0.05`) %>%
  summarise(`number of experiments`=n())

## # A tibble: 2 x 2
##   `p value < 0.05` `number of experiments`
##   <chr>                  <int>
## 1 non-significant          6
## 2 significant              14

```

In my example nearly a third of the experiments did not find a statistically significant difference. A less formal review of the research might tally these *P*-values and conclude that there are inconsistent results in the literature.

A better way would be to look at the estimates and calculated confidence intervals

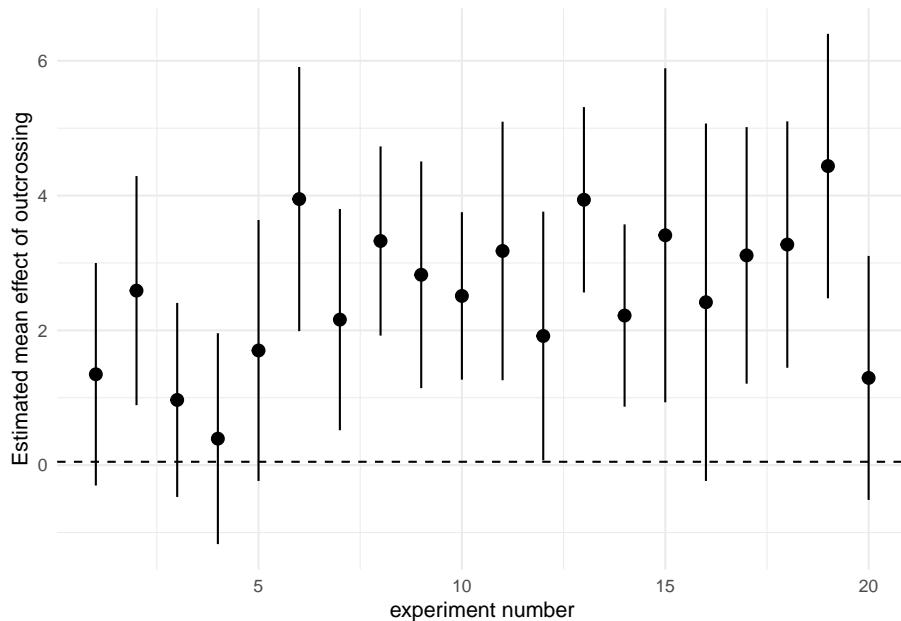
Using this newly generated data compare the estimates and confidence intervals? - What do you conclude from this?

```
y %>%
  ggplot(aes(x=`experiment number`, y=estimate))+
  geom_pointrange(aes(ymin = conf.low, ymax=conf.high))+
```

labs(y = "Estimated mean effect of outcrossing")+

```
geom_hline(linetype="dashed", yintercept=0.05)+
```

```
theme_minimal()
```



By illustrating this visually, it is clearer to see that the results are not really inconsistent, the negative effects of inbreeding depression are clear to see in all of the experiments - we are simply observing the effect of sampling error.

All 20 studies showed the effect of inbreeding depression, and all the experiments have identical levels of uncertainty. We can clearly see that estimates and intervals are a substantial improvement in the way we report experiments, and that they make comparisons across repeated studies more valuable.

15.5 Summary

This chapter finally allowed us to calculate P -values and test statistical significance for our experiments using linear models. We also compared the linear model structures for producing a paired vs. unpaired t -test.

However we also learned to appreciate the potential issues around making Type 1 and Type 2 errors, and how an appreciation of confidence intervals and stan-

dardised effect sizes can be used to assess these.

A single experiment is never definitive, and a reliance on reporting P -values is uninformative and can be misleading. Instead reporting estimates and confidence intervals allows us to report our levels of uncertainty, and provides results which are more informative for comparative studies.

Chapter 16

Regression - Spring Week Four

16.1 Introduction to Regression

So far we have used linear models for analyses between *two* ‘categorical’ explanatory variables e.g. *t*-tests. But what about when we have a ‘continuous’ explanatory variable? For that we need to use a regression analysis, luckily this is just another ‘special case’ of the linear model, so we can use the same `lm()` function we have already been using, and we can interpret the outputs in the same way.

16.2 Linear regression

Much like the *t*-test we have generating from our linear model, the regression analysis is interpreting the strength of the ‘signal’ (the change in mean values according to the explanatory variable), vs the amount of ‘noise’ (variance around the mean).

We would normally visualise a regression analysis with a scatter plot, with the explanatory (predictor, independent) variable on the x-axis and the response (dependent) variable on the y-axis. Individual data points are plotted, and we attempt to draw a straight-line relationship through the cloud of data points. This line is the ‘mean’, and the variability around the mean is captured by calculated standard errors and confidence intervals from the variance.

The equation for the linear regression model is:

$$y = a + bx$$

You may also note this is basically identical to the equation for a straight fit line $y = mx + c$.

Here:

- y is the predicted value of the response variable
- a is the regression intercept (the value of y when $x = 0$)
- b is the slope of the regression line
- x is the value of the explanatory variable

This formula explains the mean, you would need to include the unexplained residual error as a term to include our measure of uncertainty

$$y = a + bx + e$$

The regression uses two values to fit a straight line. First we need a starting point, known as the regression intercept. For categorical predictors this is the mean value of y for one of our categories, for a regression this is the mean value of y when $x = 0$. We then need a gradient (how the value of y changes when the value of x changes). This allows us to draw a regression line.

A linear model analysis estimates the values of the intercept and gradient in order to predict values of y for given values of x .

16.3 Data

Here we are going to use example data from the Australian forestry industry, recording the density and hardness of 36 samples of wood from different tree species. Wood density is a fundamental property that is relatively easy to measure, timber hardness, is quantified as the ‘the amount of force required to embed a 0.444” steel ball into the wood to half of its diameter’.

With regression, we can test the biological hypothesis that wood density can be used to predict timber hardness, and use this regression to predict timber hardness for new samples of known density.

Timber hardness is quantified using the ‘Janka scale’, and the data we are going to use today comes from an R package ‘SemiPar’ - once loaded you will then need to call the data using another function `data()` (note this is different to `PalmerPenguins` where the data was immediately available).

```
library(tidyverse)
library(SemiPar)
data(janka)
```

You should **ALWAYS** check your data before starting a new analysis
- write yourself a short checking script - then **CLICK HERE** to see
what I did

```
# check the structure of the data
glimpse(janka)

# check data is in a tidy format
head(janka)

# check variable names
colnames(janka)

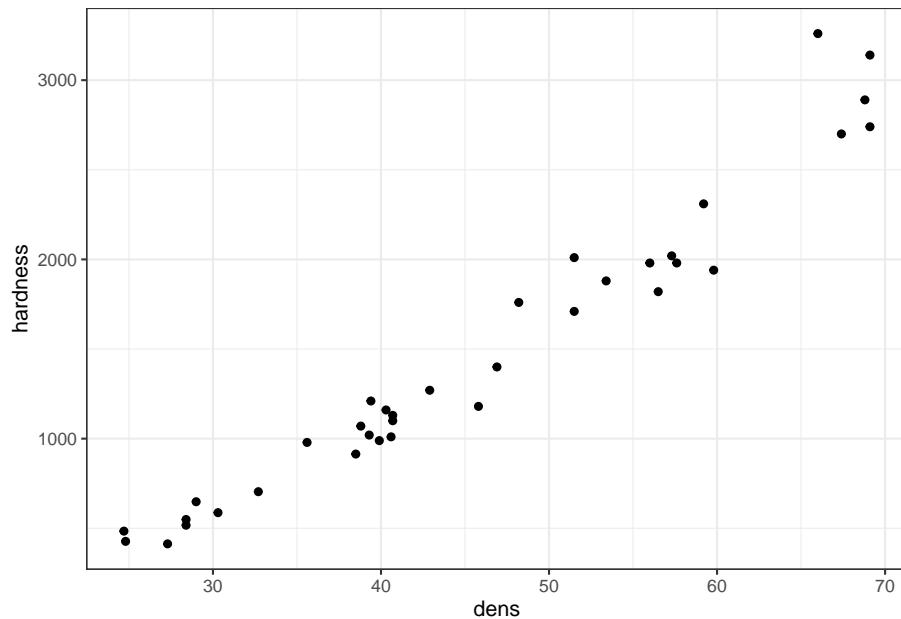
# check for duplication
janka %>%
  duplicated() %>%
  sum()

# check for typos - by looking at impossible values
janka %>%
  summarise(min_dens=min(dens, na.rm=TRUE),
            max_dens=max(dens, na.rm=TRUE),
            min_hard=min(hardness, na.rm=TRUE),
            max_hard=max(hardness, na.rm=TRUE))

# missing values
janka %>%
  is.na() %>%
  sum()

# quick summary
summary(janka)
```

```
janka %>%
  ggplot(aes(x=dens, y=hardness))+
  geom_point()+
  theme_bw()
```



16.4 Correlation

Wood density and timber hardness appear to be positively related, and the linear appears to be fairly linear. We can look at a simple strength of this association between dens and hardness using correlation

```
# base r
# cor() does not have a data option so need to use the with() function
with(janka, cor(dens, hardness))

# tidyverse
library(rstatix)

janka %>%
  cor_test(dens, hardness)
```

[1] 0.9743345

Correlation coefficients range from -1 to 1 for perfectly negative to perfectly positive linear relationships. The relationship here appears to be strongly positive. Correlation looks at the **association** between two variables, but we want to go further - we are arguing that wood density *causes* higher values of timber hardness. In order to test that hypothesis we need to go further than correlation and use regression.

16.5 Regression in R

We can fit the regression model in exactly the same way as we fit the linear model for Darwin's maize data. The *only* difference is that here our predictor variable is continuous rather than categorical.

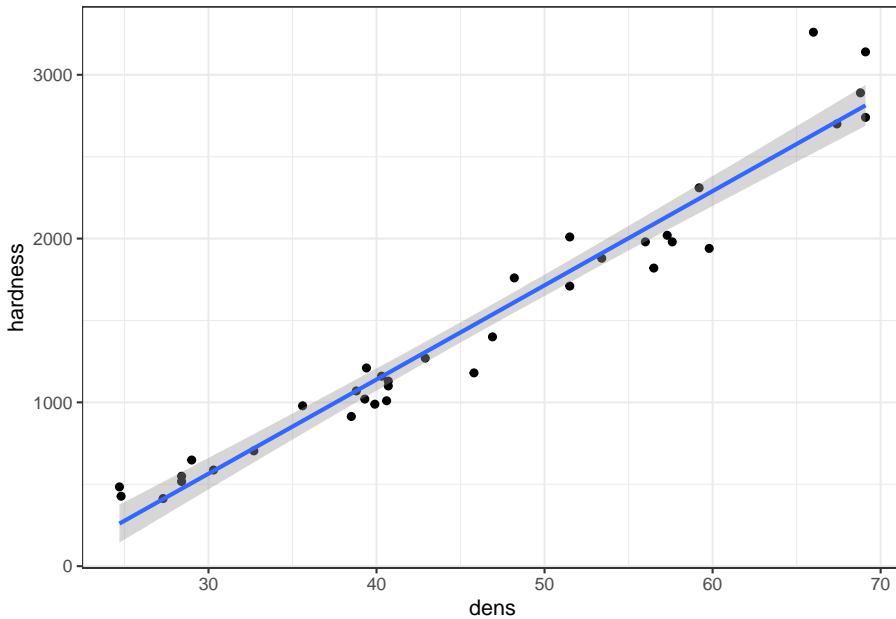
**Note - be careful when ordering variables here on the left of the ‘tilde’ is the response variable, on the right is the predictor. Get them the wrong way round and it will reverse your hypothesis.

```
janka_ls1 <- lm(hardness ~ dens, data = janka)
```

This linear model will estimate a ‘line of best fit’ using the method of ‘least squares’ to minimise the error sums of squares (the average distance between the data points and the regression line).

We can add a regression line to our ggplots very easily with the function `geom_smooth()`.

```
janka %>%
  ggplot(aes(x=dens, y=hardness)) +
  geom_point() +
  geom_smooth(method="lm") +
  theme_bw()
```



The blue line represents the regression line, and the shaded interval is the 95% confidence interval band. What do you notice about the width of the interval band as you move along the regression line? [Click-me for answer](#)

The 95% confidence interval band is narrowest in the middle and widest at either end of the regression line. But why?

When performing a linear regression, there are **two** types of uncertainty in the prediction.

First is the prediction of the overall mean of the estimate (ie the center of the fit). The second is the uncertainty in the estimate calculating the slope.

So when you combine both uncertainties of the prediction there is a spread between the high and low estimates. The further away from the center of the data you get (in either direction), the uncertainty of the slope becomes a large and more noticeable factor, thus the limits widen.

16.5.1 Summary

```
janka_ls1 %>%
  broom::tidy()

# base R summary(janka_ls1)
```

This output should look very familiar to you, because it's the same output produced for the analysis of the maize data. Including a column for the coefficient estimates, standard error, *t*-statistic and *P*-value. The first row is the intercept, and the second row is the difference in the mean from the intercept caused by our explanatory variable.

In many ways the intercept makes more intuitive sense in a regression model than a difference model. Here the intercept describes the value of *y* (timber hardness) when *x* (wood density) = 0. The standard error is standard error of this calculated mean value. The only wrinkle here is that that value of *y* is an impossible value - timber hardness obviously cannot be a negative value (anti-hardness??). This does not affect the fit of our line, it just means a regression line (being an infinite straight line) can move into impossible value ranges.

One way in which the intercept can be made more valuable is to use a technique known as 'centering'. By subtracting the average (mean) value of *x* from every data point, the intercept (when *x* is 0) can effectively be right-shifted into the centre of the data.

Try it for yourself, use your data manipulation skills to 'center' the values of x then fit a new linear model. [Click-me for answer](#)

```

dens_mean <- janka %>%
  summarise(mean_dens=mean(dens))
# 45.73333

janka %>%
  mutate(centered_dens = dens-dens_mean[,1]) %>%
  lm(hardness ~ centered_dens, data = .) %>%
  broom::tidy()

```

term	estimate	std.error	statistic	p.value
<chr>	<dbl>	<dbl>	<dbl>	<dbl>
1 (Intercept)	1469.	30.5	48.2	7.10e-33
2 centered_dens	57.5	2.28	25.2	1.33e-23

Note how the estimate for row 2 - the effect of density on timber hardness **has not** changed, but the intercept now represents the estimated mean timber hardness for the mean wood density e.g. at a density of $\rho = 45.73$ the average timber hardness on the janka scale is 1469.

16.5.2 the second row

The second row is labelled ‘dens’. Density is our explanatory variable, and the slope is estimated against it. So if 57.5 is the value of the regression slope (with its standard error) - then the timber hardness is predicted to **increase** by 57.5 on the janka scale for every unit change of density.

According to our model summary, this estimated change in the mean is statistically significant - so for this effect size and sample size it is unlikely that we would observe this relationship if the null hypothesis (that we cannot predict timber hardness from wood density) were true.

16.5.3 Confidence intervals

Just like with the maize data, we can produce upper and lower bounds of confidence intervals:

```

broom::tidy(janka_ls1, conf.int=T, conf.level=0.95)

# base r
# confint(lsmodel1)

# A tibble: 2 x 7
  term      estimate std.error statistic p.value conf.low conf.high
  <chr>      <dbl>     <dbl>     <dbl>     <dbl>     <dbl>
1 (Intercept) -1160.     109.    -10.7  2.07e-12   -1381.    -940.

```

Table 16.1: R squared effect size

Effect size	r^2
small	0.1
medium	0.3
large	0.5

2 dens	57.5	2.28	25.2	1.33e-23	52.9	62.1
--------	------	------	------	----------	------	------

Here we can say that at $\alpha = 0.05$ we think there is at least a 52.9 unit increase on the janka scale for every unit increase in density (ρ). Because our 95% confidence intervals do not span 0, we know that there is a significant relationship at $\alpha = 0.05$.

16.5.4 Effect size

With a regression model, we can also produce a standardised effect size. The estimate and 95% confidence intervals are the amount of change being observed, but just like with the maize data we can produce a standardised measure of how strong the relationship is. This value is represented by R^2 : the proportion of the variation in the data explained by the linear regression analysis. This is a more sensible measure of effect size than Cohen's d which is a standardised measure of *difference* according to standard deviation.

The value of R^2 can be found in the model summaries as follows

```
janka_ls1 %>%
  broom::glance()

# base r
# summary(janka_ls1)

# A tibble: 1 x 12
  r.squared adj.r.squared sigma statistic p.value    df logLik    AIC    BIC deviance d
        <dbl>         <dbl>   <dbl>     <dbl>    <dbl> <dbl> <dbl> <dbl> <dbl>    <dbl>
1     0.949       0.948   183.     637. 1.33e-23      1   -238.  481.  486.  1139366.
```

16.6 Assumptions

Regression models make ALL the same assumptions as all linear models - that the unexplained variation around the regression line (the residuals) is approximately normally distributed, and has constant variance. And we can check this in the same way.

Remember, the residuals are the *difference* between the observed values and the fitted values predicted by the model. Or in other words it is the *vertical* distance between a data point and the fitted value on the regression line. We can take a look at this with another function in the `broom` package `augment()`. This generates the predicted value for each data point according to the regression, and calculates the residuals for each data point.

```
augmented_ls1 <- janka_ls1 %>%
  broom::augment()
```

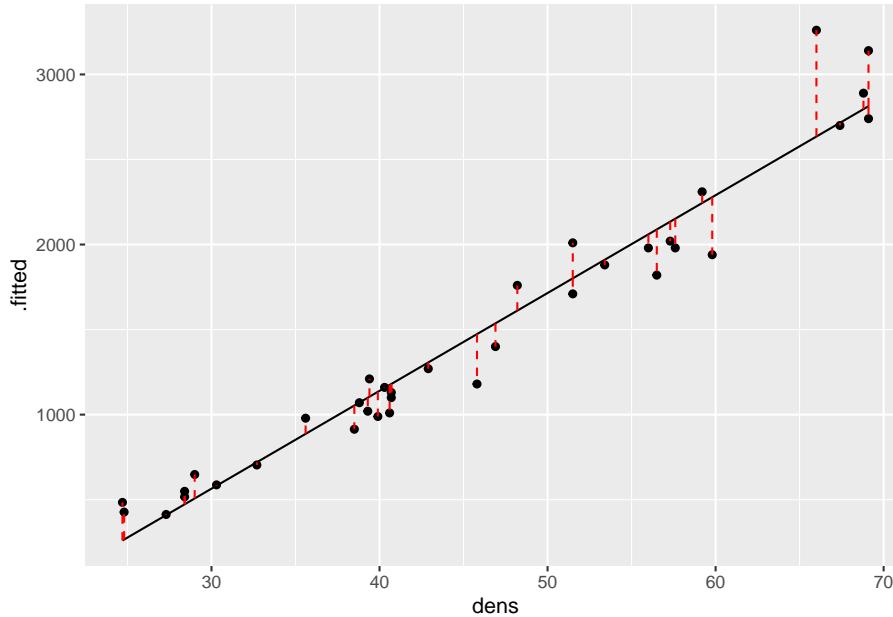
```
# base R

predicted(janka_ls1)

resid(janka_ls1)
```

If we plot this, with a black fitted regression line and red dashed lines representing the residuals:

```
augmented_ls1 %>%
  ggplot(aes(x=dens, y=.fitted)) +
  geom_line() + geom_point(aes(x=dens, y=hardness)) + geom_segment(aes(x=dens, xend=dens, y=.
```



We can use this augmented data to really help us understand what residual variance looks like, and how it can be used to diagnose our models. A perfect model would mean that all of our residual values = 0, but this is incredibly unlikely to ever occur. Instead we would like to see

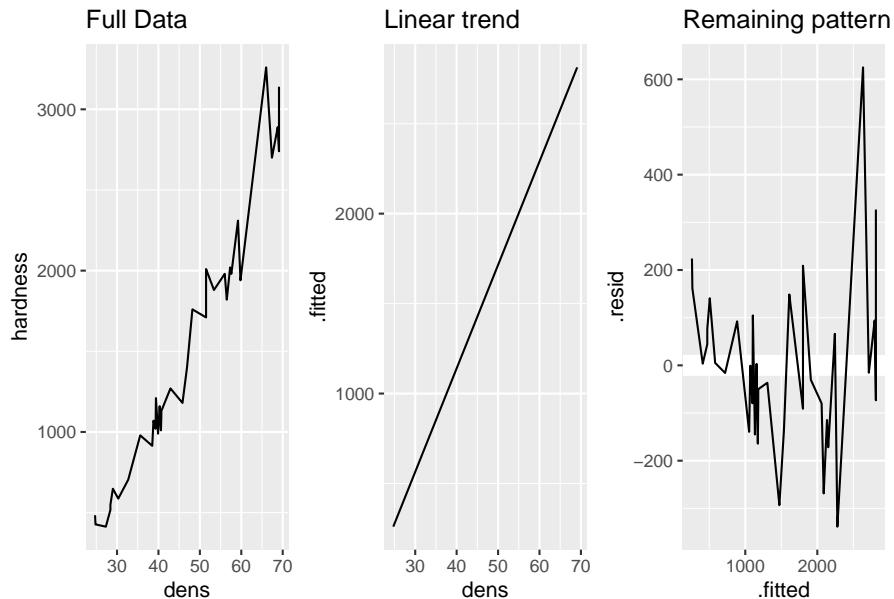
- 1) that there is a ‘normal distribution’ to the residuals e.g. more residuals close to the mean, and fewer further away in a rough z-distribution.
- 2) We also want to see homogeneity of the residuals e.g. it would be a bad model if the average error was greater at one end of the model than the other. This might mean we have more uncertainty in the slope of the line for large values over small values or vice versa.

```
# A line connecting all the data points in order
p1 <- augmented_ls1 %>%
  ggplot(aes(x=dens, y=hardness)) +
  geom_line() +
  ggtitle("Full Data")

# Plotting the fitted values against the independent e.g. our regression line
p2 <- augmented_ls1 %>%
  ggplot(aes(x=dens, y=.fitted)) +
  geom_line() +
  ggtitle("Linear trend")

# Plotting the residuals against the fitted values e.g. remaining variance
p3 <- augmented_ls1 %>%
  ggplot(aes(x=.fitted, y=.resid)) +
  geom_hline(yintercept=0, colour="white", size=5) +
  geom_line() +
  ggtitle("Remaining pattern")

library(patchwork)
p1+p2+p3
```



CHALLENGE - the above is an example of functional, but repetitive code - could you make a function that reduces the amount of code needed?

HINT - to make sure your arguments for a ggplot are passed properly use this structure `x=.data[[x]]`

[CLICK HERE](#) for a solution

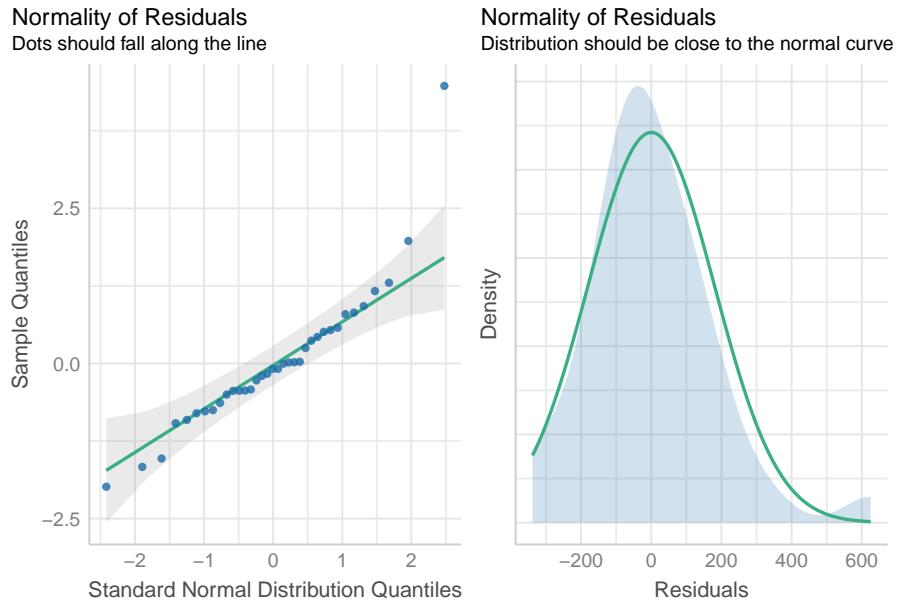
```
model_plot <- function(data=augmented_ls1, x="dens", y="hardness", title="Full data"){
  ggplot(aes(x=.data[[x]], y=.data[[y]]), data=data)+
    geom_line()+
    theme_bw()+
    ggtitle(title)
}

p1 <- model_plot()
p2 <- model_plot(y=".fitted", title="Linear prediction")
p3 <- model_plot(y=".resid", title="Remaining pattern")
```

16.6.1 Normal distribution

We can use the same model diagnostic plots as we used for the maize data. Here you can see it is mostly pretty good, with just one or two data points outside of the confidence intervals

```
performance::check_model(janka_ls1, check=c("normality", "qq"))
```



```
plot(janka_ls1, which=c(2,2))
```

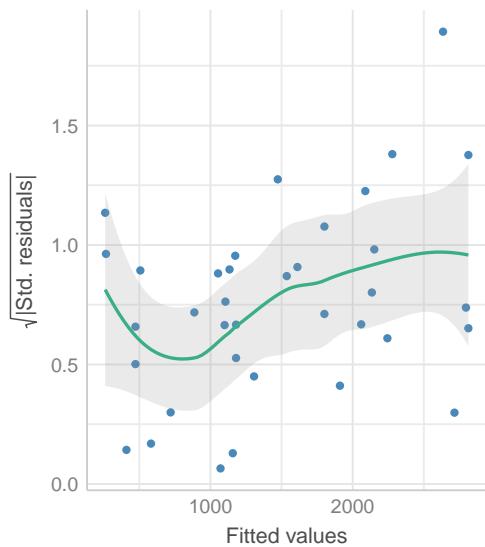
16.6.2 Equal variance

We can use the same model diagnostic plots as we used for the maize data. You should see that this is similar to the p3 plot we constructed manually. With the plot we constructed earlier we had the ‘raw’ residuals as a function of the fitted values. The plot we have produced now is the ‘standardized residuals’ - this is the raw residual divided by the standard deviation.

Both plots suggests that the residuals **do not** have constant variance, broadly speaking the amount of variance y increases as x increases. This means we have less confidence in our predictions at high values of density. Later we will see what we can do to improve the fit of this model

```
performance::check_model(janka_ls1, check="homogeneity")
```

Homogeneity of Variance
Reference line should be flat and horizontal

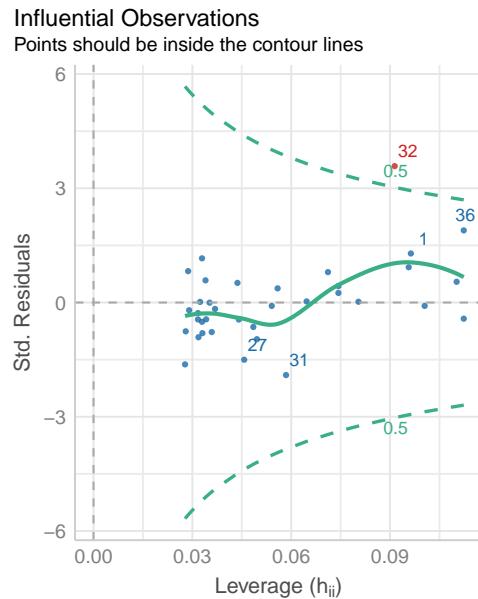


```
plot(janka_ls1, which=c(1,3))
```

16.6.3 Outliers

Here we can see there is just one potential outlier.

```
performance::check_model(janka_ls1, check="outliers")
```



```
plot(janka_ls1, which=c(4,5))
```

16.7 Prediction

Using the coefficients of the intercept and the slope we can make predictions on new data. The estimates of the intercept and the slope are:

```
coef(janka_ls1)
```

(Intercept)	dens
-1160.49970	57.50667

Now imagine we have a new wood samples with a density of 65, how can we use the equation for a linear regression to predict what the timber hardness for this wood sample should be?

[Click me for answer](#)

$$y = a + bx$$

```
# a + bx
-1160.49970 + 57.50667 * 65
```

Rather than work out the values manually, we can also use the coefficients of the model directly

```
coef(janka_ls1)[1] + coef(janka_ls1)[2] * 65
(Intercept)
2577.434
```

But most of the time we are unlikely to want to work out predicted values by hand, instead we can use functions like `predict()` and `augment()`

```
# base R
predict(janka_ls1, newdata=list(dens=c(22,35,65)))

# broom package
newdata <- tibble(dens=c(22,35,65))

broom::augment(janka_ls1, newdata=newdata)

# A tibble: 3 x 2
  dens .fitted
  <dbl>    <dbl>
1     22     105.
2     35     852.
3     65    2577

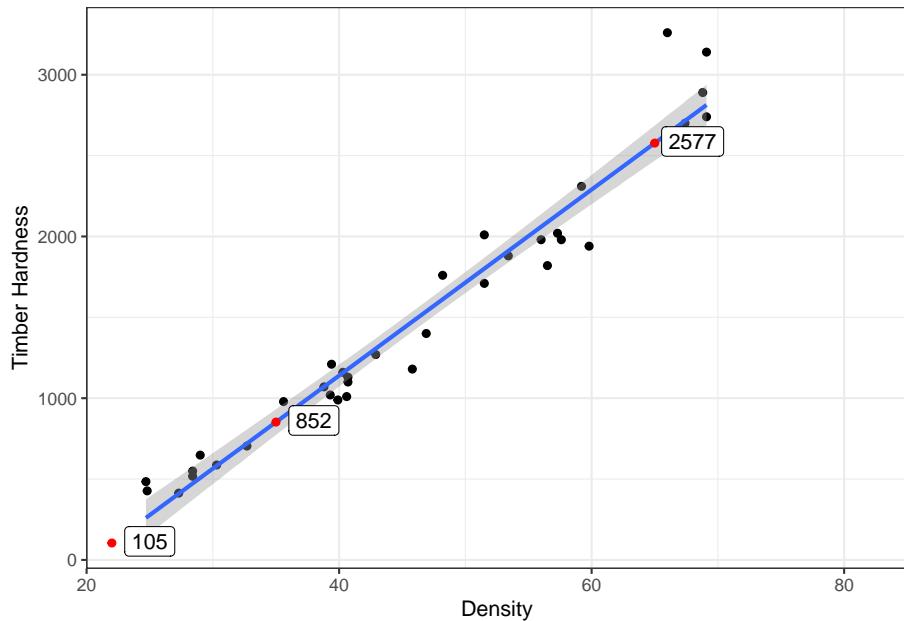
broom::augment(janka_ls1, newdata=newdata, se=TRUE)

broom::augment(janka_ls1, newdata=newdata, interval="confidence")
```

CHALLENGE - can you plot the three new predicted values onto an existing figure to recreate the below? [CLICK HERE](#) for a solution

```
janka %>%
  ggplot(aes(x=dens, y=hardness)) +
  geom_point() +
```

```
geom_smooth(method="lm")+
  geom_point(data=pred_newdata, aes(y=.fitted, x=dens), colour="red")
```



16.8 Summary

Linear model analyses can extend beyond testing differences of means in categorical groupings to test relationships with continuous variables. This is known as linear regression, where the relationship between the explanatory variable and response variable are modelled with the equation for a straight line. The intercept is the value of y when $x = 0$, often this isn't that useful, and we can use ‘mean-centered’ values if we wish to make the intercept more intuitive. As with all linear models, regression assumes that the unexplained variability around the regression line, is normally distributed and has constant variance.

Once the regression has been fitted it is possible to predict values of y from values of x , the uncertainty around these predictions can be captured with confidence intervals.

Chapter 17

ANOVA - Spring Week Five

17.1 ANOVA

So far we have used linear models for analyses between *two* ‘categorical’ explanatory variables e.g. *t*-tests, and for regression with *two* ‘continuous’ variables. However, as designs become more complicated, the number of comparisons can quickly become overwhelming, working with estimates and intervals alone can become harder.

As designs become more elaborate the number of pairwise *t*-tests rapidly increases, and therefore our risk of false positives (Type I errors). It is therefore useful to have a complementary approach that first asks if there is any support for a difference between the different means *before* diving into multiple comparisons. This approach is called *analysis of variance* (ANOVA), and although it tends to be associated with categorical data, we will see in the following chapters that the ANOVA is just another type of linear model, so this approach can also be extended to included continuous variables.

17.2 Maize data

Our simple linear model for the maize data was:

```
lsmodel1 <- lm(height ~ type, data = darwin)
```

This structure of fitting a linear model, with one explanatory variable is also known as a one-way ANOVA. The general strategy of the ANOVA is to quantify the overall variability in the data set and then to divide it into the variability between and within the groups. We can also refer to this as the ‘signal-to-noise’ ratio, or the variability explained by the slope of the linear model, vs unexplained variance.

The more of the variation explained by our fitted linear model, the more confident we can be that we have detected a real effect in our estimates of mean differences. This method of fitting a model is called ordinary least squares.

The least squares model first quantifies the total amount of variation (total sum of squares, SST) by measuring the difference from the individual data points to a reference point (usually the ‘grand’ mean, see below).

Next the model quantifies a fitted slope, it aims to produce a slope that produces the least amount of squared residuals to the data points (sum of squares of the regression/ANOVA, SSR/A).

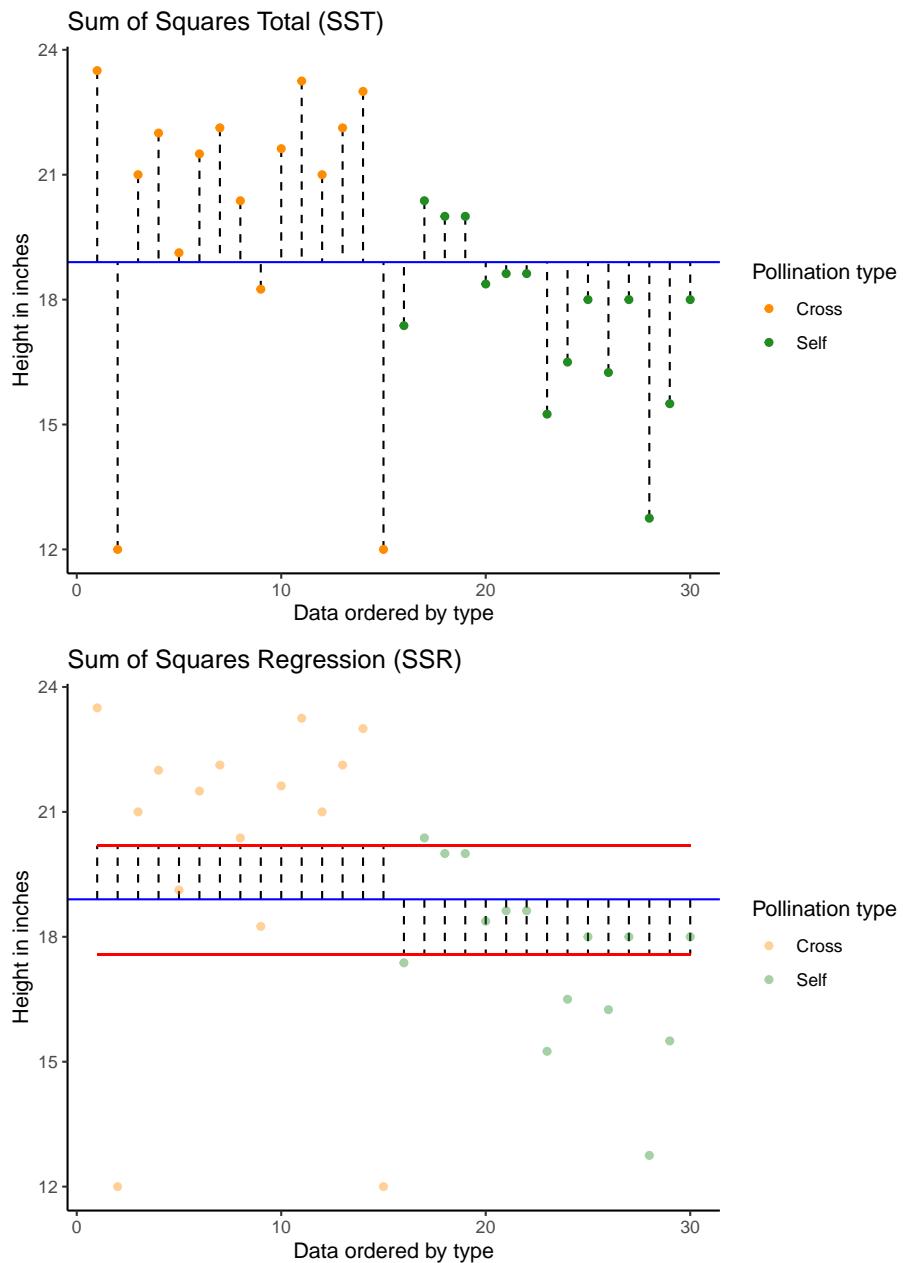
This then leaves the residual unexplained variation (sum of squares of the error, SSE).

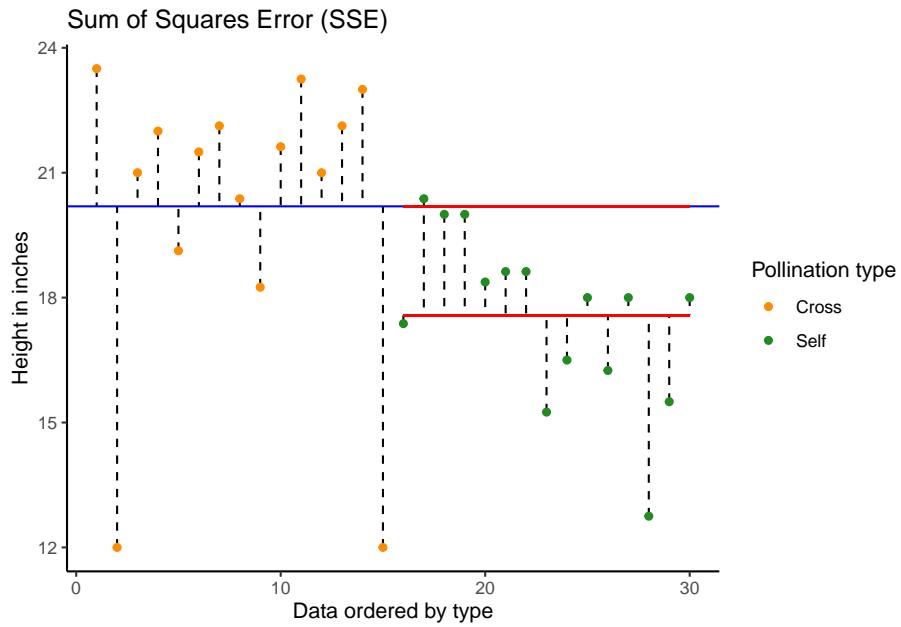
In this way the model is splitting the overall variability (SST) into signal (SSR) and noise (SSE):

$$SST = SSR + SSE$$

Remember:

- SST = the sum of squared differences between the data points and the grand mean
- SSR = the sum of the squared differences between the grand mean and the predicted position on the linear model
- SSE = the sum of the squared differences between the predicted position and the observed position





Note - Least squares quantifies top) total least squares, middle) treatment least squares, bottom) error sum of squares (SST, SSR, SSE). The vertical lines measure the distances, which are then squared and summed. SST is calculated by measuring to the intercept, SSR is calculated by measuring the distance of the estimates to the intercept, SSE is calculated by measuring the distance to the estimates

17.2.1 The ANOVA table

If we want to get the ANOVA table for a linear model we can use the `anova()` function:

```
anova(lsmodel1)
```

Analysis of Variance Table

```
Response: height
  Df  Sum Sq Mean Sq F value Pr(>F)
type      1  51.352  51.352  5.9395 0.02141 *
Residuals 28 242.083   8.646
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Compare this to the output you get when you use the `summary()` function. You should see that the F -value P -value and df are all exactly the same. The ANOVA

table actually has *far* less information in it, as it contains no information about the biology (estimates in difference, or uncertainty such as standard errors etc.). It is amazing how many times you will see these tables reported, even though they contain the *least* interesting part of the analysis.

BUT they can help us simplify effects where there are more than two levels, but we need to learn how to use them appropriately.

The ANOVA table has six columns and two rows of information.

The first column contains the information source, here the signal/regression (first row) and error/noise (second row).

The second column gives the degrees of freedom, in the first row this is the number of predictors(treatments) ($k - 1$, including intercept), and in the second row the residual degrees of freedom ($N - k$).

The third column is the sum of squares in the first row this is the SSR, and the second row SSE (remember that SST = SSR + SSE).

The fourth column is the *mean* sum of squares MSR = SSR/(k-1) & MSE = SSE/(N-k). These are the average amounts of variability per treatment level.

It's worth stopping briefly at this point and remembering that we have effectively pooled the SSE from *both* treatments to generate the MSE. Linear models always use this pooled variance approach, it can give them greater power for detecting effects with smaller sample sizes, but is the reason why we need homogeneity of variance as an assumption of our model.

The fifth column is our *F* statistic (the signal-to-noise ratio). It is calculated by dividing the treatment variance by the residual error variance

$$F = \frac{SSR/(k - 1)}{SSE/(N - k)} = \frac{MSR}{MSE}$$

In our example $F = 5.9$, which means that the estimated signal is nearly six times larger than the estimated noise.

We can then use this *F* value with our sample size and treatment levels to calculate the probability of observing this ratio of signal to noise if the null hypothesis that there were no effect of treatment is true.

The probability value can be looked up in an ANOVA table, and is calculated from an *F* distribution. The *F*-test takes sample size into account, and the probability assigned takes into account the degrees of freedom of both the signal and noise.

We can see this if we use the R function `pf()` that this should recreate the exact *P*-value we see in our ANOVA table.

The first three arguments here are the *F*-value, degrees of freedom for the signal, and noise. The last argument is to set this as a two directional test.

```
pf(5.9395, 1, 28, lower.tail=FALSE)
```

It is common to see P -values reported with no supporting information (naked P -values). But it is impossible to interpret these without knowing: what test they came from, what the observed value of the test was, and the numbers of the degrees of freedom. So the good (and conventional) way to report this result as an ANOVA would be:

The height of the cross-pollinated plants was significantly taller than the height of the self-pollinated plants ($F_{1,28} = 5.9$, $P = 0.02$).

BUT we know that this way of reporting, emphasises the statistical tests and not the underlying biology. The report doesn't tell us anything about the heights of the plants, the estimated difference between them, or any measure of the uncertainty around this.

The self pollinated maize plants measured an average of 17.6 [16-19.1] (mean[95% CI]) inches high, while the cross-pollinated plants had a mean height of 20.2 [18.6-21.7] inches - a difference of 2.6 [-0.4-4.8] inches (one-way ANOVA: $F_{1,28} = 5.9$, $P = 0.02$).

17.3 Two-way ANOVA

Two way ANOVA (as you might guess) includes **two** explanatory variables. Usually these are treatments of interest, but for this example we will stick with including the pair variable.

```
lsmodel2 <- lm(height ~ type + as.factor(pair), data = darwin)
```

And we can look at this table using the `anova()` function again

```
anova(lsmodel2)
```

Analysis of Variance Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	*					
type	1	51.352	51.352	4.6139	0.0497	*					
as.factor(pair)	14	86.264	6.162	0.5536	0.8597						
Residuals	14	155.820	11.130								

Signif. codes:	0	'***'	0.001	'**'	0.01	'*'	0.05	'.'	0.1	' '	1

Note how some of the degrees of freedom which were initially in the residuals, have now been 'used' by the new term in the more complex model. And that some of the SSE has now been used by SSR explained by the pair term.

As we should already have been able to predict from the last time that we looked at this model structure, the pairing doesn't really do anything. If pairing did 'nothing' we would an F ratio ~ 1 . However, we can see that is actually quite a bit lower than this, and this implies a 'negative variance component' - it has actually increased the relative proportion of SSE compared to SSR here.

This is quite surprising and might indicate a poorly designed experiment. Darwin's design is quite elegant and well-balanced, but it is possible he didn't randomise his samples effectively.

17.4 Summary

ANOVA tables can be built for any linear model. The tables partition the variance into signal(s) and noise, which can be compared using an F -test. For complex analyses where many pairwise comparisons could be performed, an initial F -test can provide the initial evidence for whether there are any differences at all, reducing the risk of overtesting and the false positives that can be generated.

17.5 Practice

Now is the time to try and put your analysis skills into action. Below are instructions for importing data and setting up a new project to answer the question, does temperature affect frogspawn development? This is your chance to practice

- Setting up and organising a project that talks to GitHub
- Importing, checking and tidying a dataset
- Producing a linear model to try and analyse the observations
- Produce a report that contains
 - An informative figure with explanatory legend
 - A very brief Results section

Push your project to GitHub Classrooms, and I will provide feedback on your work

There are code solutions hidden below, I would like you to use these ONLY if you get completely stuck, do your best to completed and submit this assignment BEFORE you check your answers.

17.5.1 Our question

How does frogspawn hatching time vary with temperature?

Imagine we ran a manipulative experiment.

A manipulative study is one in which the experimenter changes something about the experimental study system and studies the effect of this change.

We collected newly-layed frogspawn from a pond in the Italian Alps and we brought them back to the lab, where we divided them into 60 water containers. 20 of the containers' water temperature was kept to 13°C, 20 containers were kept to 18°C and the remaining 20 containers were kept to 25°C. Having a high number of replicates increases our confidence that the expected difference between groups is due to the factor we are interested in. Here, temperature.

We monitored each water container and we recorded hatching times (days until hatching of eggs) in a spreadsheet (here called frogs_messy_data.csv).

- Our response variable is Hatching_time.
- Our explanatory variable is Temperature, with 3 levels: 13°C, 18°C and 25°C.

We want to compare the means of 3 independent groups (13°C, 18°C and 25°C temperature groups) and we have one continuous response variable (Hatching time in days) and one categorical explanatory variable (Temperature). One-way ANOVA is the appropriate analysis!

17.5.2 Hypothesis

Always make a hypothesis and prediction, before you delve into the data analysis.

A hypothesis is a tentative answer to a well-framed question, referring to a mechanistic explanation of the expected pattern. It can be verified via predictions, which can be tested by making additional observations and performing experiments.

This should be backed up by some level of knowledge about your study system.

In our case, knowing that frogspawn takes around 2-3 weeks to hatch under optimal temperatures (15-20°C), we can hypothesize that the lower the temperature, the longer it will take for frogspawn to hatch. Our hypothesis can therefore be: mean frogspawn hatching time will vary with temperature level. We can predict that given our temperature range, at the highest temperature (25°C) hatching time will be reduced.

17.6 Importing the data

The data can be retrieved from GitHub Classrooms, you should set up a new project in the shared workspace on RStudio Cloud.

You will then need to organise your Github credentials and make sure you can commit and push.

Organise your project workspace and set up a new analysis script.

EXERCISE With the information above can you **import** and **tidy** the data? Hint: as well as checking the data, you may need to use *pivot_longer* to put the data in tidy format.

```
frogs <- read_csv("data/frogs_messy_data.csv")
```

```
frogs <- frogs %>%
  rename("13" = Temperature13,
        "18" = Temperature18,
        "25" = Temperature25,
        frogspawn_id = `Frogspawn sample id`) %>%
  pivot_longer(`13`:`25`, names_to="temperature", values_to="days") %>%
  drop_na(days)
```

EXERCISE - With the data tidied can you produce a **linear model**, check its assumptions and interpret its findings?**

```
lsmodel_frogs1 <- lm(days ~ temperature, data = frogs)
```

```
# summary(lsmodel_frogs1)
```

```
# anova(lsmodel_frogs1)
```

```
# broom::tidy(lsmodel_frogs1, conf.int = T)
```

```
# plot(lsmodel_frogs1)
```

```
performance::check_model(lsmodel_frogs1,
                           check = c("qq", "outliers", "homogeneity"))
```

EXERCISE - Can you produce a suitable **Figure** for this dataset?

There are multiple ways to present the same data, equally valid, but emphasising different concepts. For example this first figure uses a boxplot and data points to illustrate the differences. Remember the median and IQR are essentially descriptive statistics, not inferential.

Whereas the next figure, uses the **emmeans()** package to produce estimate means and confidence intervals from the **lm()** and therefore is the produce of inferential statistics, this figure illustrates the estimates of our model rather than the parameters of our sample.

Neither method is ‘best’.

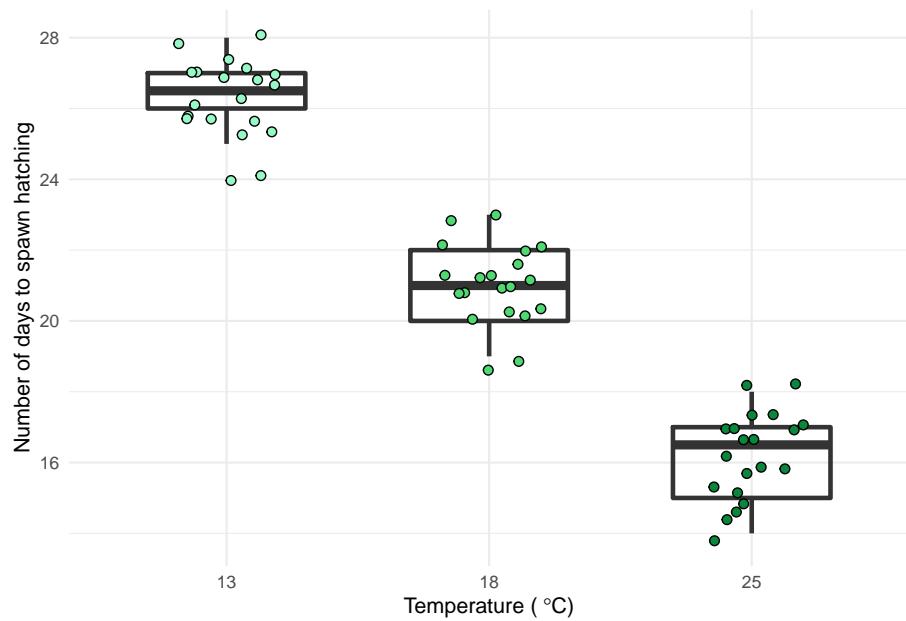


Figure 17.1: Frogspawn hatching times at 13, 18 and 25 degrees Celsius. Boxplot displays median, hinges are first and third quartiles, whiskers extend from the hinge to 1.5X the interquartile range. Points represent individual frogspawns.

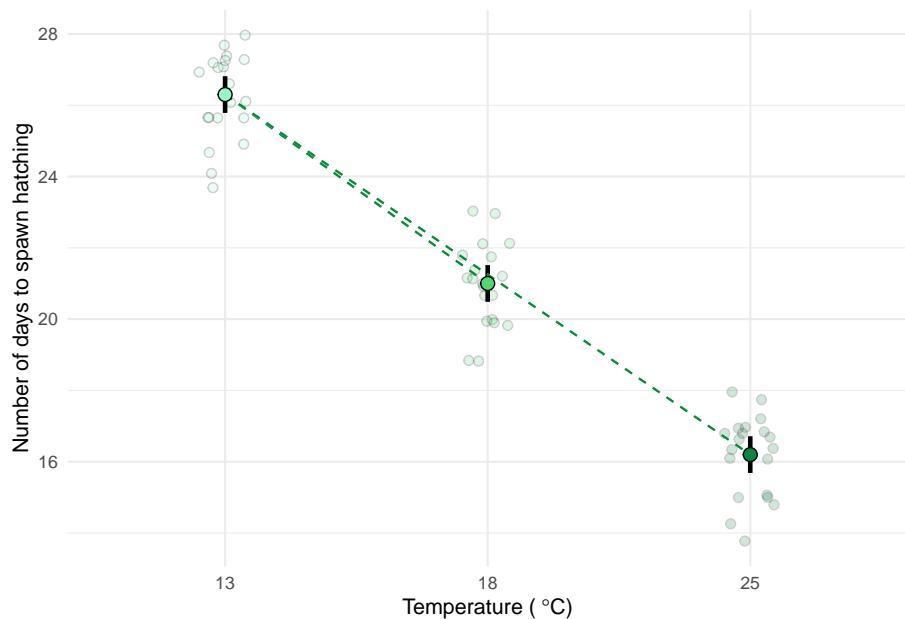


Figure 17.2: Time to hatching is inversely related to temperature in frogspawn. Circles represent estimated mean hatching times with 95% confidence intervals from a one-way ANOVA ($F_{1,28} = 385.9$, $P < 0.001$). Dashed lines indicate the slope of the mean difference between 13-18 degrees and 13-25 degrees Celsius. Faded points represent individual data points.

EXERCISE - Can you write a summary of the **Results**?

Increasing temperatures had a clear effect on reducing the time taken for frogspawn to hatch (one-way ANOVA: $F_{2,57} = 385.9, P < 0.001$). At 13° C the mean time to hatching was 26.3 days [25.8-26.8 95% CI], this reduced by an average of 5.3 days [4.57 - 6.02] at 18° C and by 10.1 days [9.37 - 10.82] at 25° C.

END

Chapter 18

Multivariate linear models - Spring Week Six

18.1 Multivariate linear models

So far we have worked almost exclusively with single explanatory variables either continuous (regression) or categorical (t-test or ANOVA). But what about analyses where we have more than one possible explanatory variable? Extra terms can easily be incorporated into linear models in order to test this.

18.2 Factorial linear models

The example data is the response of above ground plant biomass production of grassland plots in relation to two resource addition treatments. The addition of Fertiliser to the soil and the addition of Light to the grassland understorey. If biomass is limited by the nutrients in the soil, we would expect the addition of fertiliser to increase production. If biomass is limited by low levels of light caused by plant crowding we would expect an addition of light to increase biomass.

```
biomass <- read_csv("data/biomass.csv")
```

You should first check the data and look at the top rows, we can see that this shows the application or non-application of fertiliser and light additions, because *all four* possible combinations are present, this is known as a *fully factorial design*:

- control (F- L-, no added light or fertiliser)

- fertiliser (F+ L-)
- light (F- L+)
- addition of both (F+ L+)

Close inspection of the dataset shows that the same data is presented in *two* ways:

- Column 1 shows the status of Fertiliser only (F +/-) (two levels)
- Column 2 shows the status of Light only (L +/-) (two levels)
- Column 3 shows whether fertiliser **and** light have been applied (four levels, a combination of the previous two columns)
- Column 4 shows the biomass

EXERCISE -Check the data

```
# check the structure of the data
glimpse(biomass)

# check data is in a tidy format
head(biomass)

# check variable names
colnames(biomass)

# check for duplication
biomass %>%
  duplicated() %>%
  sum()

# check for typos - by looking at impossible values
biomass %>%
  summarise(min=min(Biomass.m2, na.rm=TRUE),
            max=max(Biomass.m2, na.rm=TRUE))

# check for typos by looking at distinct characters/values
biomass %>%
  distinct(Fert)

biomass %>%
  distinct(Light)

biomass %>%
  distinct(FL)
```

```
# missing values
biomass %>%
  is.na() %>%
  sum()

# quick summary
summary(biomass)
```

18.2.1 Data summary

So we have 64 observations with four variables, the last column is the response variable (biomass) and the other three columns are *two* different ways of indicating the experimental design.

- If we use column 3 - we are treating the design as though it is a one-way ANOVA (with four levels)
- If we use columns 1 & 2 - we are treating this as a *factorial design*

We will look at the two different ways to analyse this data then, and the pros and cons of these two approaches.

18.2.2 One-way ANOVA

Plotting the data and superimposing mean values produces the graph shown above. Now we are confident there is a difference in our sample means, we can begin a linear model to quantify this and test it against a null hypothesis.

If we use the FL column as a predictor then this is equivalent to a four-level one way ANOVA.

```
ls_1 <- lm(Biomass.m2 ~ FL, data = biomass)
summary(ls_1)

Call:
lm(formula = Biomass.m2 ~ FL, data = biomass)

Residuals:
    Min      1Q  Median      3Q     Max 
-233.619 -42.842   1.356  67.961 175.381 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 355.79     23.14  15.376 < 2e-16 ***
FLF-L+       30.12     32.72   0.921  0.36095  

```

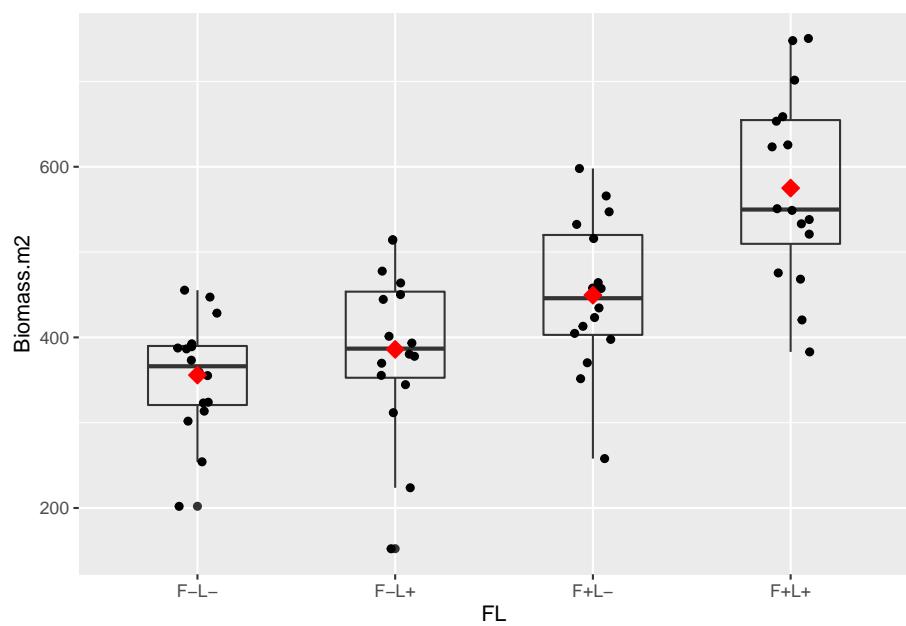


Figure 18.1: Boxplot and individual biomass values (black points) with treatment means (red diamonds)

```

FLF+L-      93.69     32.72    2.863  0.00577 ** 
FLF+L+     219.23     32.72    6.699 8.13e-09 *** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 

Residual standard error: 92.56 on 60 degrees of freedom
Multiple R-squared:  0.4686,   Adjusted R-squared:  0.442 
F-statistic: 17.63 on 3 and 60 DF,  p-value: 2.528e-08

```

We can see that the control treatment (F- L-) is the intercept with a mean of 356g and standard error of 23g. The Light treatment adds an average of 30g to the biomass (but this close to the standard error of this estimated mean difference - 32.72g). In contrast the addition of fertiliser adds 93g to the biomass.

If we wanted to get the precise confidence intervals we could use `broom::tidy(ls_1, conf.int = T)` or `confint(ls1)`.

We can visualise these differences with a coefficient plot, and see clearly that adding light by itself produce a slight increase in the mean biomass of our samples **but** the 95% confidence interval includes zero, so we don't have any real confidence that this is a consistent/true effect.

```
GGally::ggcoef_model(ls_1,
                      show_p_values=FALSE,
                      signif_stars = FALSE,
                      conf.level=0.95)
```

But what about the fourth level - combined fertiliser and light treatments? If there is no interaction (combined effect) between light and fertiliser then we would expect the average biomass difference caused by the light and the average biomass difference caused by fertiliser to *add together* to approximately the value found in the combined treatment.

In other words if one treatment has an effect size of A , and another treatment has an effect size of B , then ANOVA predicts that in combination the effect size of the combined treatments is $A + B$.

```
# combine the average mean differences of the light effect and fertiliser effect
coef(ls_1)[2] + coef(ls_1)[3]
```

```
# compare this to the average difference of the combined treatment
coef(ls_1)[4]
```

```
predicted combined effect = 123.82
```

```
observed combined effect = 219.2
```

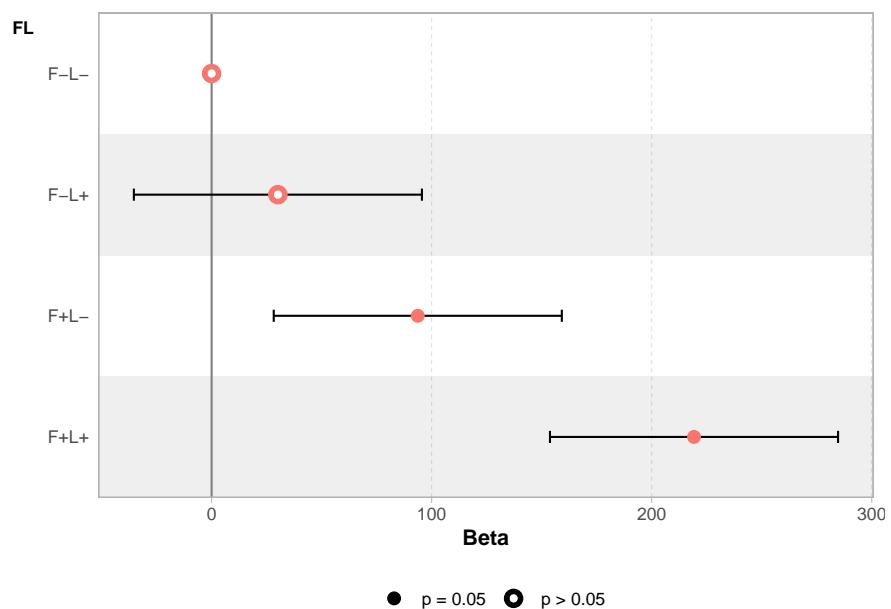


Figure 18.2: Effects of light and fertiliser treatments on biomass relative to an untreated control (error bars = 95% CI)

In this example we can see clearly that combining the separate treatments **does not** add up to the combined value. The mean biomass of the combined treatment is well above the additive prediction.

The mean biomass of the combined treatment is well above what we would expect from the additive effects alone. This suggests there may be a *positive interaction* (that light and fertiliser treatments produce a sum effect that is greater than could be predicted by looking at their individual effects).

Using our one-way ANOVA design we are able to accurately estimate the mean difference between the controlled group and the combined treatment group. **But** we cannot really say anything concrete about the *strength of the interaction effect* e.g. is their a true interaction? how confident can we be in this effect? How does the strength of the interaction effect compare to the main effects?

In contrast to the one-way ANOVA approach, a factorial design lets us test and compare additive effects and interaction effects

18.2.3 Testing for interactions

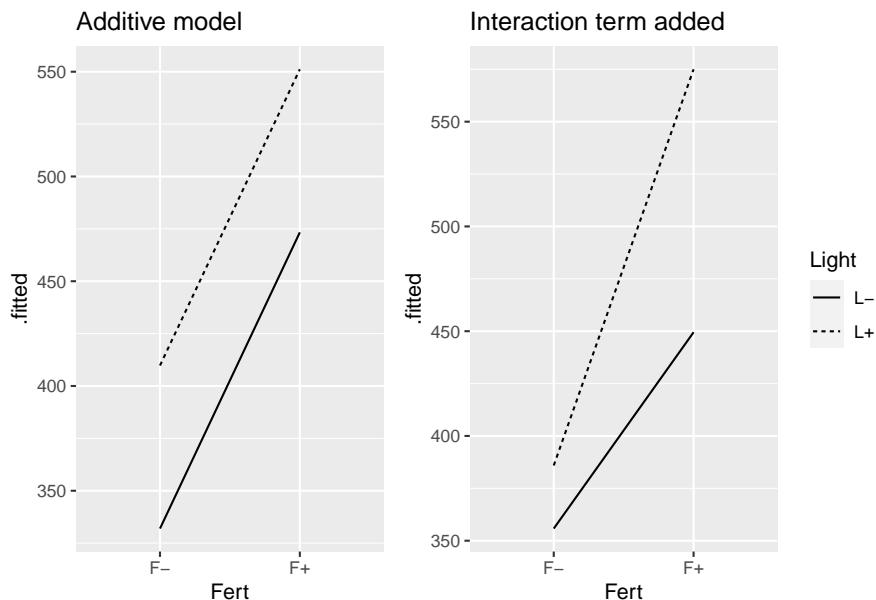
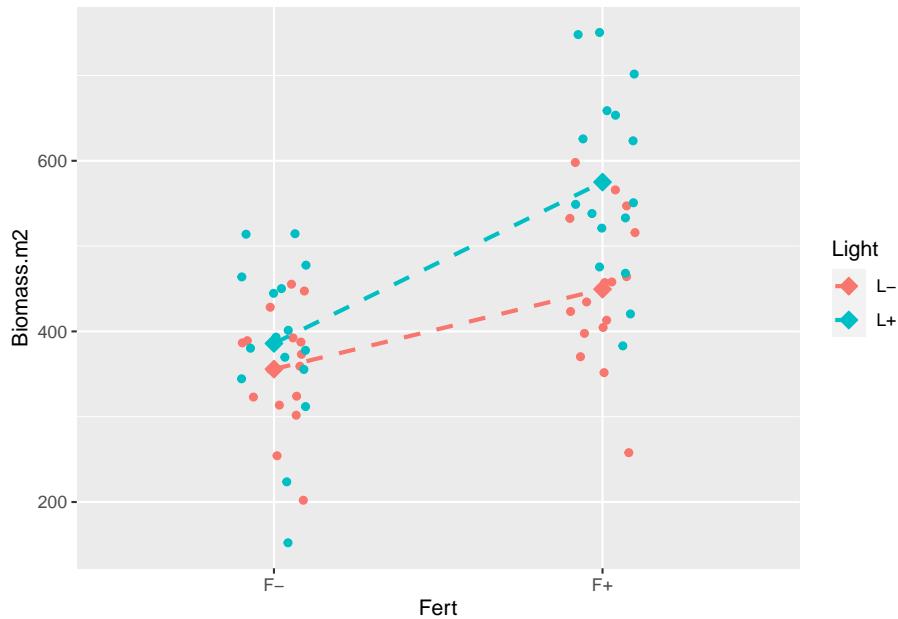


Figure 18.3: A comparison of an additive model vs an interaction model

EXERCISE - Plot the data and determine whether you think there is any sign of an interaction effect? - Use `stat_summary()` to make summary points and lines.

```
biomass %>% ggplot(aes(x=Fert, y=Biomass.m2, colour = Light, fill = Light, group = Light)) +
  geom_jitter(width=0.1) +
  stat_summary(
    geom = "point",
    fun = "mean",
    size = 3,
    shape = 23
  )+stat_summary(
    geom = "line",
    fun = "mean",
    size = 1, linetype = "dashed"
)
)
```



To produce an interactive model, we need to use the *separate* factors for fertiliser and light rather than the single `f1` factor. When we make this model, we include the main effects of `Light` and `Fert`, if the additive model explains sufficient variance then this will be enough, but if we suspect an interaction term we add this as `Light:Fert` written out as follows:

```
ls_2 <- lm(Biomass.m2 ~ Fert + # main effect
            Light + # main effect
            Fert:Light, # interaction term
            data = biomass)
```

```
summary(ls_2)
```

```
Call:
lm(formula = Biomass.m2 ~ Fert + Light + Fert:Light, data = biomass)

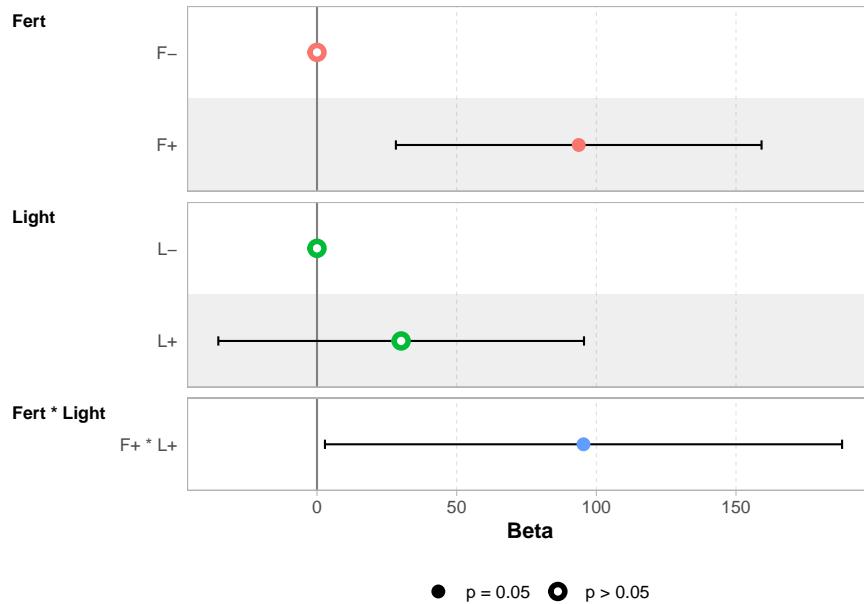
Residuals:
    Min      1Q  Median      3Q     Max 
-233.619 -42.842   1.356  67.961 175.381 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 355.79     23.14   15.376 < 2e-16 ***
FertF+       93.69     32.72    2.863  0.00577 **  
LightL+       30.13     32.72    0.921  0.36095    
FertF+:LightL+ 95.41     46.28    2.062  0.04359 *  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 92.56 on 60 degrees of freedom
Multiple R-squared:  0.4686,    Adjusted R-squared:  0.442 
F-statistic: 17.63 on 3 and 60 DF,  p-value: 2.528e-08
```

Notice the estimates of the mean differences are the same as the previous one-way ANOVA model. The fourth line now indicates *how much of an effect the two factors interacting changes the mean*. Also note the standard error is larger, there is less power to accurately estimate an interaction over a main effect.

```
GGally::ggcoef_model(ls_2,
                      show_p_values=FALSE,
                      signif_stars = FALSE,
                      conf.level=0.95)
```



When we use a factorial combination, the last line of the table of coefficients estimates the size of the interaction effect at around 95g. So if combining the light and fertilisation treatments produced a biomass equivalent to their additive predictions, the estimate of the interaction would be *zero*. Instead it is **95g more than we would expect from the additive effects alone**. This means in order to work out the estimated biomass for the treatment of light and fertiliser we must sum the additive effects of Light+ Fert and the interaction effect Light:Fert.

18.2.4 Model estimates and confidence intervals

When we compare this to our one-way ANOVA model, we must add the single terms and the interaction term, this should add up to the combined treatment from our first model:

```
# model 1
coef(ls_1)[4]

# model 2
coef(ls_2)[2] + coef(ls_2)[3] + coef(ls_2)[4]
```



Don't make the mistake of looking at the main effect of light treatment, and reporting there is no effect because the uncertainty intervals cross

zero. The main effect of light gives the average effect across the two fertiliser treatments. However, because of the interaction effect, we know this doesn't tell the whole story - whether light has an effect depends on whether fertiliser has been applied.

So, if you have a significant interaction term, you must consider the main effects, regardless of whether they are significant on their own.

18.2.5 ANOVA tables

So you have made a linear model with an interaction term, how do you report whether this interaction is significant? How do you report the main effect terms when there is an interaction present?

1. Start with the interaction

In previous chapters we ran the `anova()` function directly on our linear model. This works well for simple & 'balanced' designs (there are equal sample sizes in each level of a factor), but can be misleading with 'unbalanced' designs or models with complex interactions. In order to report an F statistic for the interaction effect, we need to carry out an F -test of two models, one *with* and one *without* the interaction effect. This is easy with the function `drop1()`

```
drop1(ls_2, test = "F")
```

Single term deletions

```
Model:
Biomass.m2 ~ Fert + Light + Fert:Light
Df Sum of Sq    RSS    AIC F value    Pr(>F)
<none>          513998 583.43
Fert:Light  1      36409 550407 585.81  4.2501 0.04359 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

This F -test is testing the *null hypothesis* that there is no true interaction effect. The significance test rejects the null hypothesis (just). The `drop1()` function also provides the **Akaike information criterion (AIC)**, an alternative method of model selection (more on this later).

We could now write this up as follows:

There was an interactive effect of light and fertiliser treatments (ANOVA $F_{1,60} = 4.25$, $P = 0.044$) in which combining treatments produced substantially more biomass (95.4g [95% CI: 2.8 - 188]) than expected from the additive effects alone (Fertiliser 93.7g [28.2 - 159.2], Light 30.1g [-35.3 - 95.6]).

Do not make the mistake of *just* reporting the statistics, the interesting bit is the size of the effect (estimate) and the uncertainty (confidence intervals).



Remember to check the assumptions of your full model

2. Main effects

A good thing about the `drop1()` function is that if there are interactions in the model, it stops there. This is because if the interaction effect is significant, then the main effects must be included, even if they aren't significant on their own. We can make further models to test the main effects **but** these are less important because we already know the interaction term provides the main result.

If we decide to include reports of the main effect then estimates and confidence intervals should come from the *full* model, but we need to produce an interaction free model to produce accurate *F*-values (especially for unbalanced designs, see below).

```
# we have to remove the interaction term before we can keep using drop1()

ls_3 <- lm(Biomass.m2 ~ Fert + Light, data = biomass)

drop1(ls_3, test = "F")
```

Single term deletions

```
Model:
Biomass.m2 ~ Fert + Light
      Df Sum of Sq    RSS    AIC F value    Pr(>F)
<none>          550407 585.81
Fert     1    319889 870296 613.13   35.452 1.395e-07 ***
Light    1    96915  647323 594.19   10.741  0.001732 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



You can use this reduced model to get F values, BUT reports of estimates should come from the full model.

18.3 Balanced/Unbalanced designs



In an unbalanced design when you run the `anova()` function on your model, the order in which your variables were included can have an effect e.g.

`lm(Fert + Light)` would give a different anova table to
`lm(Light + Fert)`

In the examples above, you are unlikely to find much of a difference between running the `drop1()` function or `anova()` on the complex or reduced models. This is because these designs are ‘balanced’ (equal numbers in each level of a predictor). When designs are not balanced then the order matters when we use `anova()` - this is because the sum of squares is calculated sequentially (in the order of the formula), and so we could get different results depending on the order in which we assemble predictors into our model!!!!!!!

18.3.1 Practice

Let’s start by making a deliberately unbalanced dataset

```
# make three vectors and combine them into a new tibble

height <- c(50, 57, 91, 94, 102, 110, 57, 71, 85, 105, 120)
size <- c(rep("small", 2), rep("large", 4), rep("small", 3), rep("large", 2))
treatment <- c(rep("Control", 6), rep("Removal", 5))

unbalanced <- tibble(height, size, treatment)

unbalanced
```

EXERCISE - Produce a linear model testing size and treatment against height. Try making two models swapping the order for including the two predictors and compare them with `anova()**`

```
model_1 <- lm(height ~ treatment + size, data = unbalanced)
anova(model_1)
```

```
model_2 <- lm(height ~ size + treatment, data = unbalanced)
anova(model_2)
```

```
> anova(model_1)
Analysis of Variance Table

Response: height
          Df Sum Sq Mean Sq F value    Pr(>F)
treatment   1   35.3   35.3  0.3725  0.5586
size         1 4846.0 4846.0 51.0676 9.746e-05 ***
Residuals   8   759.2   94.9
```

```

---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> anova(model_2)
Analysis of Variance Table

Response: height
  Df Sum Sq Mean Sq F value    Pr(>F)
size      1 4291.2 4291.2 45.2208 0.0001489 ***
treatment 1  590.2   590.2  6.2193 0.0372980 *
Residuals 8  759.2    94.9
---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

```

Compare these two anova tables. Same data, same predictors, but a different conclusion just because of the order the terms were included in the model!

EXERCISE - Now compare your two models with the drop1() function

```
drop1(model_1)
drop1(model_2)
```

Single term deletions

```

Model:
height ~ treatment + size
  Df Sum of Sq    RSS    AIC
<none>             759.2 52.577
treatment  1      590.2 1349.3 56.904
size       1     4846.0 5605.2 72.569
Single term deletions
```

Because the drop1 function drops one term from the model, then adds it back in and drops a new one it doesn't matter what order they were included.

18.4 post-hoc

In this example it is unnecessary to spend time looking at pairwise comparisons between the four possible levels, the interesting finding is to report the strength of the interaction effect. **But** it is possible to generate estimated means, and produce pairwise comparisons with the emmeans() package

```
emmeans::emmeans(ls_2, specs = pairwise ~ Light + Fert + Light:Fert)
# including the argument pairwise in front of the ~ prompts the post-hoc pairwise comp
```

```
# $emmeans contains the estimate mean values for each possible combination (with confidence intervals)
# $contrasts contains tukey test post hoc comparisons between levels

$emmeans
Light Fert emmean    SE df lower.CL upper.CL
L-   F-      356 23.1 60     310     402
L+   F-      386 23.1 60     340     432
L-   F+      449 23.1 60     403     496
L+   F+      575 23.1 60     529     621

Confidence level used: 0.95

$contrasts
contrast      estimate    SE df t.ratio p.value
(L- F-) - (L+ F-)    -30.1 32.7 60 -0.921  0.7940
(L- F-) - (L- F+)    -93.7 32.7 60 -2.863  0.0287
(L- F-) - (L+ F+)   -219.2 32.7 60 -6.699 <.0001
(L+ F-) - (L- F+)    -63.6 32.7 60 -1.943  0.2215
(L+ F-) - (L+ F+)   -189.1 32.7 60 -5.779 <.0001
(L- F+) - (L+ F+)   -125.5 32.7 60 -3.836  0.0017

P value adjustment: tukey method for comparing a family of 4 estimates
```

18.5 Continuous Linear Models

The previous section looked at an interaction between two categorical variables, we can also examine interactions between a factor and a continuous variable. Often referred to as ANCOVA.

```
pollution <- read_csv("data/pollution.csv")
```

The data is from an experimental study of the effects of low-level atmospheric pollutants and drought on agricultural yields. The experiment aimed to see how the yields soya bean (William variety), were affected by stress and Ozone levels. Your task is to first determine whether there is any evidence of an interaction effect, and if not report the effects of the two predictors separately.

EXERCISE -Explore the data

```
# check the structure of the data
glimpse(pollution)
```

```

# check data is in a tidy format
head(pollution)

# check variable names
colnames(pollution)

# check for duplication
pollution %>%
  duplicated() %>%
  sum()

# check for typos - by looking at impossible values
# quick summary

summary(biomass)

# check for typos by looking at distinct characters/values
pollution %>%
  distinct(Stress)

# missing values
biomass %>%
  is.na() %>%
  sum()

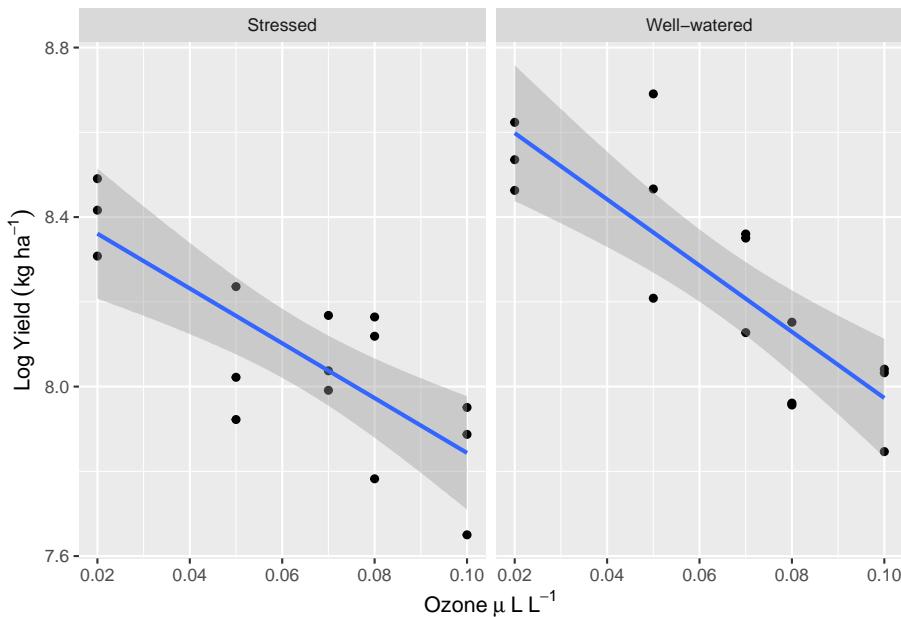
```

EXERCISE - Visualise the data

```

pollution %>%
  ggplot(aes(x = O3, y = William)) +
  geom_point() +
  geom_smooth(method = "lm") +
  facet_wrap(~ Stress)

```



EXERCISE - Model the data

```
William_ls1 <- lm(William ~ O3 + Stress + O3:Stress, data = pollution)
```

```
William_ls1 %>%
  broom::tidy(conf.int = T)
```

term	estimate	std.error	statistic	p.value	conf.low	conf.high
(Intercept)	8.49	0.0975	87.1	1.34e-33	8.29	8.69
O3	-6.47	1.40	-4.61	9.29e- 5	-9.35	-3.59
StressWell-watered	0.264	0.138	1.92	6.64e- 2	-0.0192	0.548
O3:StressWell-watered	-1.35	1.98	-0.680	5.03e- 1	-5.42	2.73

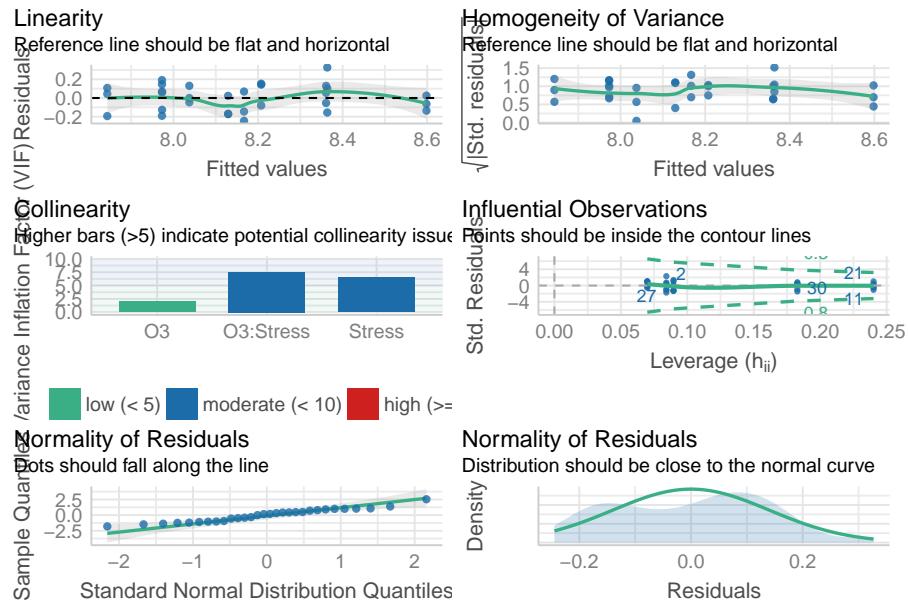
term	estimate	std.error	statistic	p.value	conf.low	conf.high
(Intercept)	8.49	0.0975	87.1	1.34e-33	8.29	8.69
O3	-6.47	1.40	-4.61	9.29e- 5	-9.35	-3.59
StressWell-watered	0.264	0.138	1.92	6.64e- 2	-0.0192	0.548
O3:StressWell-watered	-1.35	1.98	-0.680	5.03e- 1	-5.42	2.73

It looks as though there is no strong evidence here for an interaction effect, but before we proceed any further we should check that the model is a good fit for

296CHAPTER 18. MULTIVARIATE LINEAR MODELS - SPRING WEEK SIX

our data.

```
performance::check_model(William_ls1)
```



Everything looks pretty good, so now we could go ahead and simplify our model.

```
drop1(William_ls1, test = "F")
```

Single term deletions

```
Model:
William ~ O3 + Stress + O3:Stress
Df Sum of Sq    RSS      AIC F value Pr(>F)
<none>          0.56985 -110.91
O3:Stress     1  0.010129  0.57998 -112.38  0.4621 0.5026
```

```
William_ls2 <- lm(William ~ O3 + Stress, data = pollution)
```

```
# A tibble: 3 x 7
  term            estimate std.error statistic p.value conf.low conf.high
  <chr>           <dbl>     <dbl>     <dbl>     <dbl>     <dbl>     <dbl>
1 (Intercept)     8.53      0.0733    116. 5.17e-38   8.38      8.68
2 O3              -7.14      0.981     -7.28 7.89e- 8  -9.15     -5.13
3 StressWell-watered 0.178     0.0535     3.33 2.55e- 3   0.0682     0.288
```

```
drop1(William_ls2, test = "F")

Model:
William ~ O3 + Stress
      Df Sum of Sq    RSS     AIC F value    Pr(>F)
<none>          0.57998 -112.379
O3      1   1.13812 1.71810 -81.799  52.983 7.893e-08 ***
Stress  1   0.23764 0.81762 -104.077 11.063  0.002547 **
```

EXERCISE - Write up the results

I hypothesised that plants under stress might react more negatively to pollution than non-stressed plants, however when tested I found no evidence of a negative interaction effect between stress and Ozone levels ($F_{1,27} = 0.4621$, $P = 0.5$). Individually however, while well-watered plants had higher yields than stressed plants (mean 0.178 [95% CI: 0.0682 - 0.288]) ($F_{1,28} = 11$, $P = 0.003$), there was a much larger effect of Ozone, where every unit increase ($\mu\text{L L}^{-1}$) produced a mean decrease in yield of 7.14 kg ha^{-1} [5.13 - 9.15] ($F_{1,28} = 53$, $P < 0.001$).

18.6 Summary

- Always have a good hypothesis for including an interaction
- When models have significant interaction effects you must always consider the main terms even if they are not significant by themselves
- Report F values from interactions as a priority
- **IF** interactions are significant then estimates should come from the full model, while F-values should come from a reduced model (for main effects). **IF** interaction terms are not significant they can be removed (model simplification).
- Use drop1() to avoid mistakes when using an unbalanced experiment design
- Always report estimates and effect sizes - this is the important bit - and easy to lose sight of when models get more complicated

END

Chapter 19

Complexity in Linear Models - Spring Week Seven

19.1 Designing a Model

We are introduced to the fruitfly dataset Partridge and Farquhar (1981)¹. From our understanding of sexual selection and reproductive biology in fruit flies, we know there is a well established ‘cost’ to reproduction in terms of reduced longevity for female fruitflies. The data from this experiment is designed to test whether increased sexual activity affects the lifespan of male fruitflies.

The flies used were an outbred stock, sexual activity was manipulated by supplying males with either new virgin females each day, previously mated females (Inseminated, so remating rates are lower), or provide no females at all (Control). All groups were otherwise treated identically.

```
fruitfly <- readRDS("data/fruitfly.RDS")
```

- **type**: type of female companion (virgin, inseminated, control(partners = 0))
- **longevity**: lifespan in days
- **thorax**: length of thorax in micrometres (a proxy for body size)
- **sleep**: percentage of the day spent sleeping

¹<https://nature.com/articles/294580a0>

19.1.1 Hypothesis

Before you start any formal analysis you should think clearly about the sensible parameters to test. In this example, we are *most* interested in the effect of sexual activity on longevity. But it is possible that other factors may also affect longevity and we should include these in our model as well, and we should think **hard** about what terms might reasonably be expected to *interact* with sexual activity to affect longevity.

Question - Which terms and interactions do you think we should include in our model?

In this exercise I have just asked you to try and think logically about suitable predictors. For a more formal investigation you should support this with evidence where possible

- type - should definitely be included.
- thorax - the size of the flies could determine longevity. Carreira et al (2009)²
- sleep - sleep could easily help determine longevity. Thompson et al (2020)³
- type:sleep - the amount that sleep (rest) helps promote longevity could change depending on how much activity the fly engages in when awake. Chen et al (2017)⁴

Other interactions *could* be included but you should have a strong reason for them.

19.2 Checking the data

You should now import, clean and tidy your data. Making sure it is in tidy format, all variables have useful names, and there are no mistakes, missing data or typos.

Based on the variables you have decided to test you should start with some simple visualisations, to understand the distribution of your data, and investigate visually the relationships you wish to test.

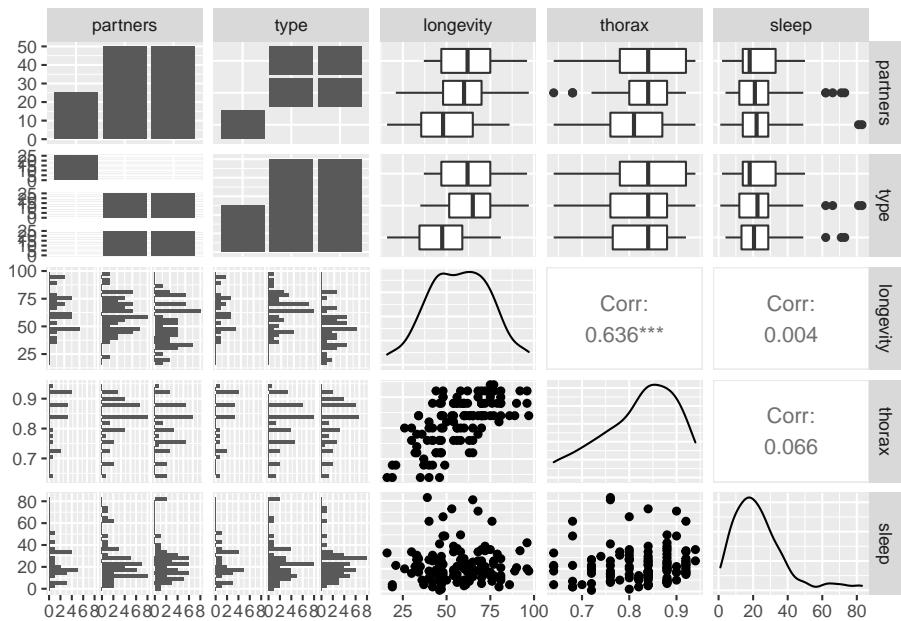
This is full two-by-two plot of the entire dataset, but you should try and follow this up with some specific plots.

```
GGally::ggpairs(fruitfly)
```

²<https://www.nature.com/articles/hdy2008117>

³<https://journals.biologists.com/bio/article/9/9/bio054361/225803/Sleep-length-differences-are-associated-with>

⁴<https://www.nature.com/articles/s41467-017-00087-5#:~:text=In%20this%20study%2C%20we%20show,but%20arous>



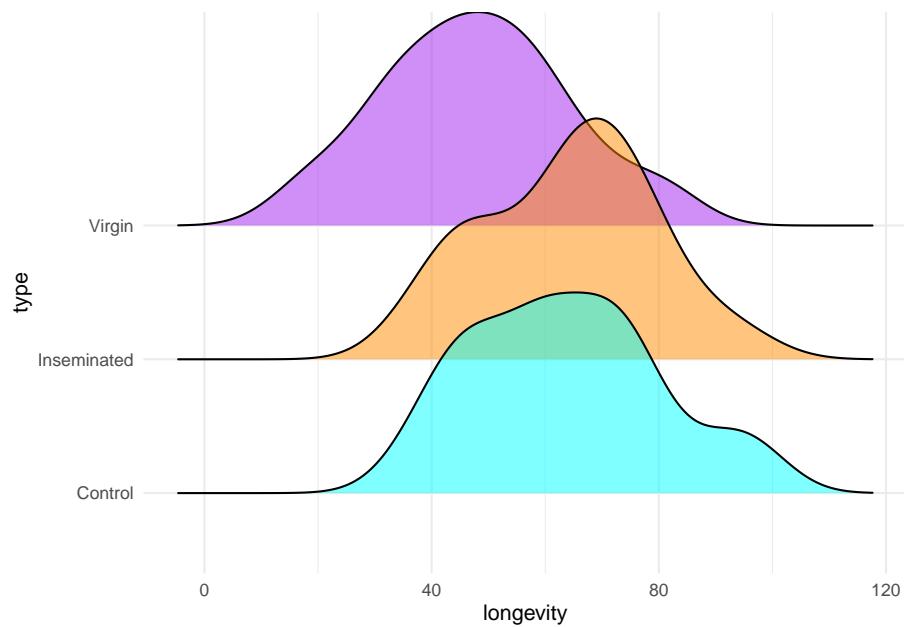
EXERCISE - Think carefully about the plots you should make to investigate the potential differences and relationships you wish to investigate - examples hidden behing this dropdown

In this first figure - we can investigate whether there is an obvious difference in the longevities of males across the three treatments

```
library(gggridges)

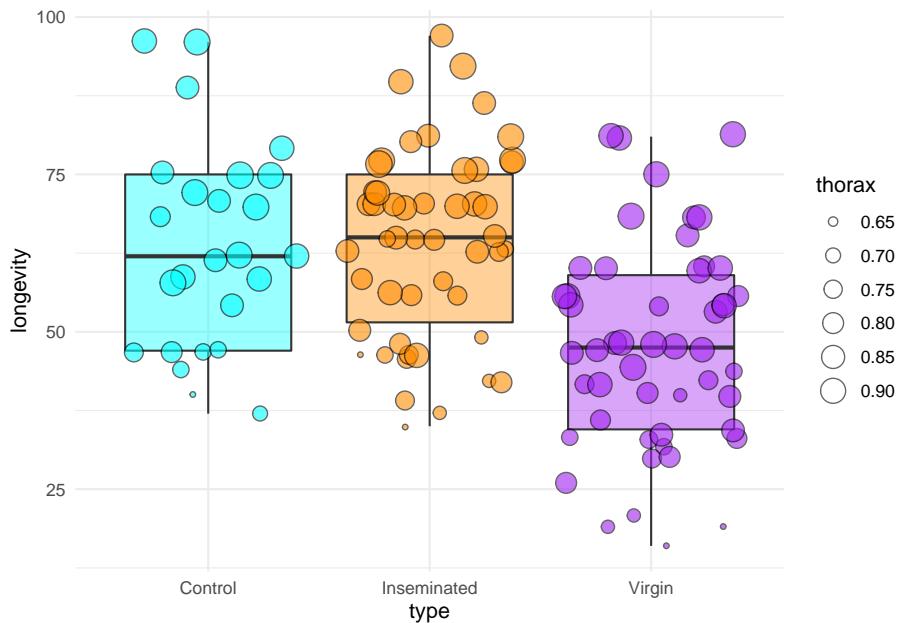
colours <- c("cyan", "darkorange", "purple")

fruitfly %>%
  ggplot(aes(x = longevity, y = type, fill = type))+
  geom_density_ridges(alpha = 0.5)+
  scale_fill_manual(values = colours)+
  theme_minimal()+
  theme(legend.position = "none")
```



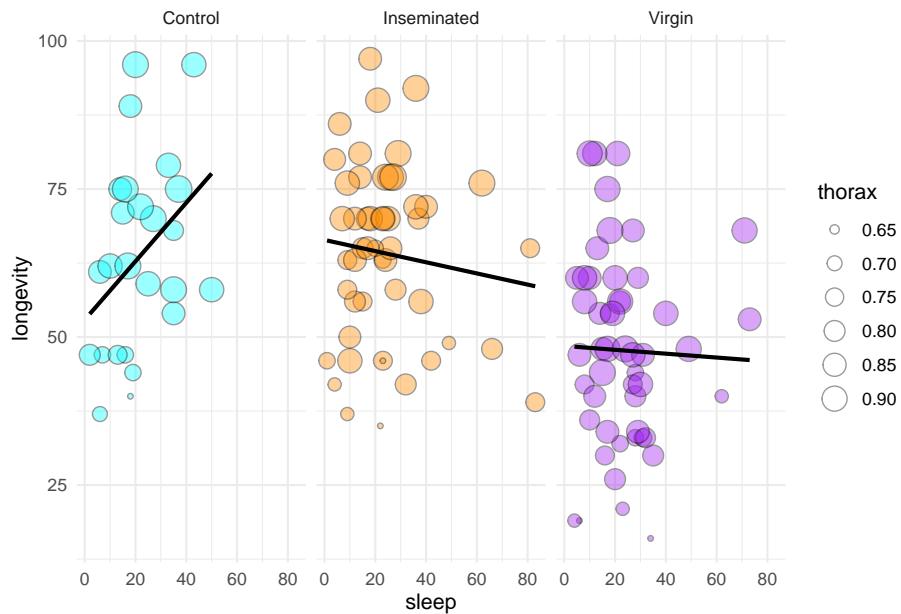
With careful thought we can construct figures that help us investigate the distribution of multiple potential predictors at once. Here we can overlay body size onto the three treatments. Here it does look as though larger flies have a longer lifespan than smaller flies. Is this trend enough of a deviation to reject a null hypothesis of no effect?

```
fruitfly %>%
  ggplot(aes(x=type, y = longevity, fill = type))+
  geom_boxplot(alpha = 0.4)+
  geom_jitter(aes(size = thorax),
              alpha = 0.6,
              width = 0.4,
              shape = 21)+
  scale_fill_manual(values = colours)+
  guides(fill = "none",
         colour = "none")+
  theme_minimal()
```



We are also interested in the potential effect of sleep on activity, we can construct a scatter plot of sleep against longevity, while including body size as a covariate. In these faceted plots - do the data points appear to follow the trendline? Are the trendlines moving in the same direction? Investigating these can help us determine is there much evidence of a potential effect of sleep, is there evidence for whether this might be an additive effect or one which interacts with the the three treatments?

```
fruitfly %>%
  ggplot(aes(x=sleep, y = longevity, fill = type)) +
  geom_point(aes(size = thorax,
                 fill = type),
             shape = 21,
             alpha = 0.4) +
  geom_smooth(method = "lm", colour = "black",
             se = FALSE) +
  scale_fill_manual(values = colours) +
  guides(fill = "none",
         colour = "none") +
  facet_wrap(~ type) +
  theme_minimal()
```



19.3 Designing a model



When you include an interaction term, the numbers produced from this are how much **more** or **less** the mean estimate is than if you just combined the main effects.

```
# a full model
flyls1 <- lm(longevity ~ type + thorax + sleep + type:sleep, data = fruitfly)
```

```
# A tibble: 7 x 7
  term            estimate std.error statistic p.value conf.low conf.high
  <chr>          <dbl>     <dbl>     <dbl>    <dbl>    <dbl>    <dbl>
1 (Intercept)   -57.5      11.4     -5.07  1.52e- 6  -80.0    -35.0
2 typeInseminated    7.99     5.34      1.50  1.37e- 1   -2.59    18.6
3 typeVirgin     -10.9      5.47     -1.99  4.86e- 2   -21.7   -0.0664
4 thorax         143.       13.4      10.6   6.53e-19   116.     169.
5 sleep          0.0904    0.189     0.480  6.32e- 1   -0.283    0.464
6 typeInseminated:sleep -0.197    0.208     -0.944 3.47e- 1   -0.609    0.216
7 typeVirgin:sleep -0.112     0.217     -0.519 6.05e- 1   -0.541    0.317
```

Note because we have included an interaction effect the number of terms is quite long and takes more consideration to understand. We can see for the individual estimates that it does not appear that

the interaction is having a strong effect (estimate) and this does not appear to be different from a null hypothesis of no interaction effect. But we we should use an F test to look at the overall effect to be sure.

Question - From the model summary table could you say what the mean longevity of a male with a 0.79mm thorax, that sleeps for 22% of the day and is paired with virgin females would be?

```
# intercept
coef(flyls1)[1] +
# 1*coefficient for virgin treatment
coef(flyls1)[3] +
# 0.79 * coefficient for thorax size
(coef(flyls1)[4]*0.79) +
# 22 * coefficient for sleep
(coef(flyls1)[5]*22) +
# 22 * 1 * coefficient for interaction
(coef(flyls1)[7]*22*1)
```

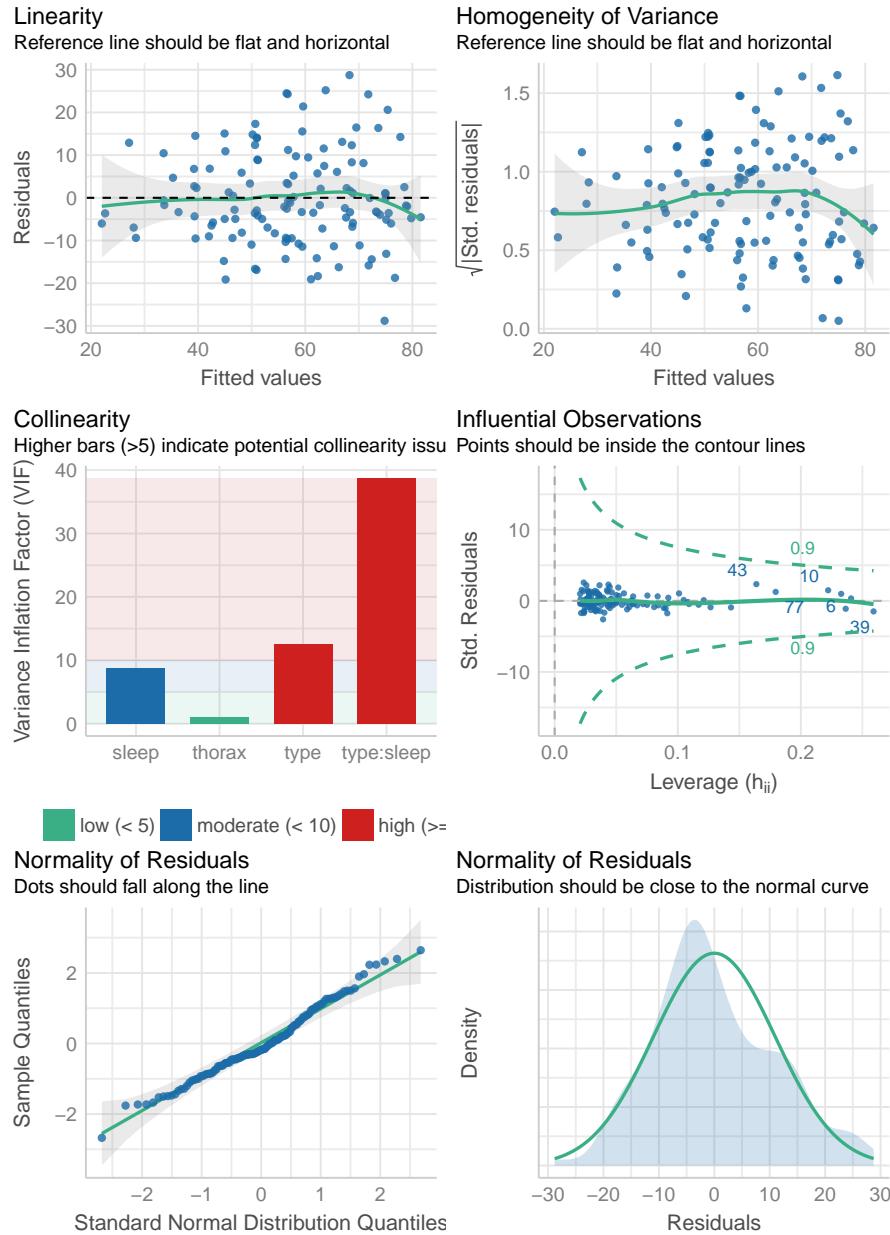
43.66344 days

19.4 Model checking & collinearity

Before we start playing with the terms in our model, we should check to see if this is even a good way of fitting and measuring our data. We should check the assumptions of our model are being met.

```
performance::check_model(flyls1)
```

306CHAPTER 19. COMPLEXITY IN LINEAR MODELS - SPRING WEEK SEVEN



Question - IS the assumption of homogeneity of variance met?

- Mostly - the reference line is fairly flat (there is a slight curve).
- It looks as though there might be some increasing heterogeneity with larger values, though very minor.

VERDICT, pretty much ok, should be fine for making inferences.

With a slight curvature this could indicate that you *might* get a better fit with a transformation, or perhaps that there is a missing variable that if included in the model would improve the residuals. In this instance I wouldn't be overly concerned. See here for a great explainer on interpreting residuals⁵.

Question - ARE the residuals normally distributed?

Yes - the QQplot looks pretty good, a very minor indication of a right skew, but nothing to worry about.

Interpreting QQ plots

Question - IS there an issue with Collinearity?

This graph clearly shows there **is** collinearity. But this is not unusual when we include an *interaction term*, if we see evidence of collinearity in terms that are not part of an interaction **then** we should take another look⁶.

What can you do about collinearity in main effects? 1) Nothing 2) Transform 3) Drop one of the terms.

The `check_performance()` function produces a visual summary of a Variance Inflation Factor produced from the `vif()` function. This is a measure of the standard error of each estimated coefficient. If this is very larger (greater than 5 or 10), this indicates the model has problems estimating the coefficient. This does not affect model predictions, but makes it more difficult to determine the estimate change from a predictor.

```
car::vif(flyls1)

      GVIF Df GVIF^(1/(2*Df))
type     12.479  2        1.880
thorax   1.053  1        1.026
sleep    8.751  1        2.958
type:sleep 38.749  2        2.495
```

19.4.1 Data transformations

The most common issues when trying to fit simple linear regression models is that our response variable is not normal which violates our modelling assumption. There are two things we can do in this case:

- Variable transformation e.g `lm(sqrt(x) ~ y, data = data)`
 - Can sometimes fix linearity

⁵<https://www.qualtrics.com/support/stats-iq/analyses/regression-guides/interpreting-residual-plots-improve-regression/>

⁶https://easystats.github.io/performance/reference/check_collinearity.html

Table 19.1: Common Box-Cox Transformations

lambda value	transformation
0.0	$\log(Y)$
0.5	\sqrt{Y}
1.0	Y
2.0	$Y^{\wedge}1$

- Can sometimes fix non-normality and heteroscedasticity (i.e non-constant variance)
- Generalized Linear Models (GLMs) to change the error structure (i.e the assumption that residuals need to be normal - see next week.)

19.4.2 BoxCox



The BoxCox gets its name from its two inventors, George Box and David Cox. Implemented by the MASS package, when applied to a linear model it systematically applies transformations by raising the y variable to a power (lambda).

The R output for the `boxcox()` function plots a maximum likelihood curve (with a 95% confidence interval - drops down as dotted lines) for the best transformation for fitting the data to the model.

```
# run this, pick a transformation and retest the model fit
MASS::boxcox(flyls1)
```

Question - Does the fit of the model improve with a square root transformation?

Not really, despite the suggestion that a sqrt transformation would improve the model, residual fits are not really any better - so we might as well stick with the original scale.

19.5 Model selection

```
# use drop1 function to remove top-level terms
drop1(flyls1, test = "F")
```

Single term deletions

Model:

	longevity ~ type + thorax + sleep + type:sleep	Df	Sum of Sq	RSS	AIC	F value	Pr(>F)					
<none>			14994	612.39								
thorax	1	14347.5	29342	694.31	112.9088	<2e-16 ***						
type:sleep	2	130.1	15125	609.47	0.5121	0.6006						

	Signif. codes:	0	'***'	0.001	'**'	0.01	'*'	0.05	'.'	0.1	' '	1

Based on this ANOVA table, we do not appear to have a strong rationale for keeping the interaction term in the model (AIC or F-test). Therefore we can confidently remove the interaction, simplifying our model and making interpretation easier.

```
flyls2 <- lm(longevity ~ type + thorax + sleep, data = fruitfly)
drop1(flyls2, test = "F")
```

Single term deletions

Model:

	longevity ~ type + thorax + sleep	Df	Sum of Sq	RSS	AIC	F value	Pr(>F)					
<none>			15125	609.47								
type	2	7576.9	22701	656.23	30.0578	2.617e-11 ***						
thorax	1	15282.8	30407	694.77	121.2556	< 2.2e-16 ***						
sleep	1	86.3	15211	608.18	0.6846	0.4097						

	Signif. codes:	0	'***'	0.001	'**'	0.01	'*'	0.05	'.'	0.1	' '	1

Question - Should we drop sleep from this model?

There is good reason to remove non-significant *interaction terms* from a model, they complicate estimates and make interpretations more difficult. For **main** effects things are a little more ambiguous.

When the main aim is prediction, it makes sense to be cautious and retain non-significant terms, as extra terms make no difference to the R² of a model.

When the focus is on hypothesis testing, then removal of non-significant terms can help produce a ‘true’ model, but this is optional. Generally speaking it

is often simpler to leave main effects in the model (you should have carefully considered the terms which were included in the first place).

In this example we can also see that AIC has not really changed - so the quality of the model is also not improved by dropping this term.

19.6 Posthoc

Using the emmeans package is a very easy way to produce the estimate mean values (rather than mean differences) for different categories `emmeans`. If the term `pairwise` is included then it will also include post-hoc pairwise comparisons between all levels with a tukey test `contrasts`.

```
emmeans::emmeans(flyls2, specs = pairwise ~ type + thorax + sleep)
```

```
$emmeans
  type      thorax sleep emmean   SE  df lower.CL upper.CL
Control    0.821  23.5   61.3 2.26 120     56.8    65.8
Inseminated 0.821  23.5   64.9 1.59 120     61.8    68.1
Virgin     0.821  23.5   48.0 1.59 120     44.9    51.2
```

Confidence level used: 0.95

```
$contrasts
  contrast                               estimate   SE  df t.ratio p.value
Control 0.82096 23.464 - Inseminated 0.82096 23.464     -3.63 2.77 120 -1.309  0.3929
Control 0.82096 23.464 - Virgin 0.82096 23.464       13.25 2.76 120  4.796 <.0001
Inseminated 0.82096 23.464 - Virgin 0.82096 23.464     16.87 2.25 120  7.508 <.0001
```

P value adjustment: tukey method for comparing a family of 3 estimates

Note - for continuous variables (sleep and thorax) - emmeans has set these to the mean value within the dataset, so comparisons are constant between categories at the average value of all continuous variables.

19.7 Write-up

Write-up- Can you write a Results section?

I tested the hypothesis that sexual activity is costly for male *Drosophila melanogaster* fruitflies. Previous research indicated that sleep deprived males are less attractive to females, this would indicate that levels of sexual activity might be affected by sleep and impact the effect on longevity, as such this was included as an interaction term in the full model. Body size is also known to affect lifespan, as such this was included as a covariate in the model.

Table 19.2: Linear model coefficients

Predictors	Estimates	Z-value	P	Lower 95% CI	Upper 95% CI
(Intercept)	-56.05	-5.01	0.00	-78.18	-33.91
typeInseminated	3.63	1.31	0.19	-1.86	9.11
typeVirgin	-13.25	-4.80	0.00	-18.71	-7.78
thorax	144.43	11.01	0.00	118.46	170.40
sleep	-0.05	-0.83	0.41	-0.18	0.07

There was a small interaction effect of decreased lifespan with increasing sleep in the treatment groups compared to control in our samples, but this was not significantly different from no effect ($F_{2,118} = 0.512$, $P = 0.6$), and was therefore dropped from the full model (Table 15.1).

There was a significant overall effect of treatment on male longevity (Linear model: $F_{2,120} = 30.1$, $P < 0.001$), with males paired to virgin females having the lowest mean longevity (48 days, [95%CI: 44.9 - 51.2]) (when holding body size and sleep constant), compared to control males (61.3 days [56.8 - 65.8]) and males paired with inseminated females (64.9 days [61.8 - 68.1 days]).

Post hoc analysis showed that these differences were statistically significant for males paired with control females compared to the inseminated (Tukey test: $t_{120} = 4.8$, $P < 0.001$) and virgin groups ($t_{120} = 7.5$, $P < 0.001$), but there was no overall evidence of a difference between inseminated and virgin groups ($t_{120} = -1.309$ $P < 0.3929$) (Figure 19.4).

Comparing the treatment effects against other predictors of longevity such as body size and sleep, I found that sleep had a very small effect on longevity (mean change -0.05 days [-0.18 - 0.07]) which was not significantly different from no effect (Linear model: $F_{1,120} = 0.68$, $P = 0.41$). Body size (taken from thorax length) was a significant predictor of longevity ($F_{1,120} = 121$, $P < 0.001$), with each 0.1 mm increase in body size adding 14.4 days to the individual lifespan [11.8 - 17]. It appears as though body size has a stronger effect on longevity than treatment, indicating that while there is a measurable cost of sexual activity to males, it may be less severe than in females (not compared here), and less severe than other measurable predictors.

Note - there are formal measures of partial effect sizes for complex linear models, but they are not often reported in papers, and become increasingly complicated to produce and ensure they are robust, so I have not tried to compute these here.⁷

⁷<https://www.frontiersin.org/articles/10.3389/fpsyg.2013.00863/full>

Note - for the effect of thorax, I could also have reported t from the summary - as it is a single **level**, continuous effect so here $F = t^2$.

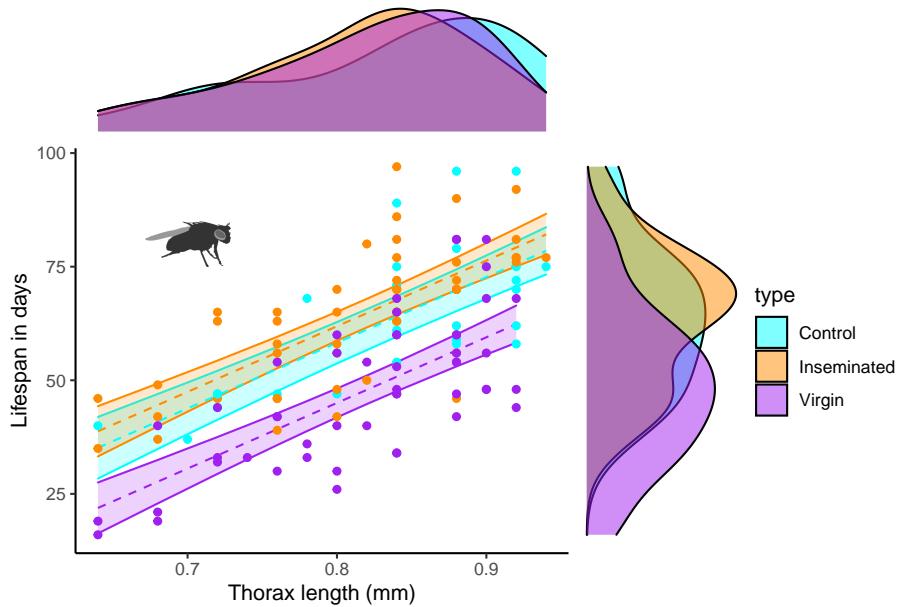


Figure 19.1: A scatter plot of longevity against body size across three treatments of differing male sexual activity. Fitted model slopes are from the reduced linear model (main effects only of thorax size, sleep and treatment group), with 95% confidence intervals, circles are individual data points. Marginal plots are density plot distributions for thorax length and longevity split by treatments.

19.8 Summary

In this chapter we have worked with our scientific knowledge to develop testable hypotheses and built statistical models to formally assess them. We now have a working pipeline for tackling complex datasets, developing insights and producing and explaining robust linear models.

19.8.1 Checklist

- Think carefully about the hypotheses to test, use your scientific knowledge and background reading to support this
- Import, clean and understand your dataset: use data visuals to investigate trends and determine if there is clear support for your hypotheses

- Fit a linear model, including interaction terms with caution
- Investigate the fit of your model, understand that parameters may never be perfect, but that classic patterns in residuals may indicate a poorly fitting model - sometimes this can be fixed with careful consideration of missing variables or through data transformation
- Test the removal of any interaction terms from a model, look at AIC and significance tests
- Make sure you understand the output of a model summary, sense check this against the graphs you have made
- The direction and size of any effects are the priority - produce estimates and uncertainties. Make sure the observations are clear.
- Write-up your significance test results, taking care to report not just significance (and all required parts of a significance test). Do you know *what* to report? Within a complex model - reporting *t* will indicate the slope of the line for that single term against the intercept, *F* is the overall effect of a predictor across all levels, *post-hoc* if you wish to compare across all levels.
- Well described tables and figures can enhance your results sections - take the time to make sure these are informative and attractive.

19.9 Supplementary code

Code to pipe the tidy model summary to kable to produce a table

```
library(kableExtra)
flyls2 %>% broom::tidy(conf.int = T) %>%
  select(-`std.error`) %>%
  mutate_if(is.numeric, round, 2) %>%
  kbl(col.names = c("Predictors",
                    "Estimates",
                    "Z-value",
                    "P",
                    "Lower 95% CI",
                    "Upper 95% CI"),
      caption = "Linear model coefficients",
      booktabs = TRUE) %>%
  kable_styling(full_width = FALSE, font_size=16)
```

sjPlot A really nice package that helps produce model summaries for you automatically

```
library(sjPlot)
tab_model(flyls2)
```

Produce phylopic images⁸

```
# optional extras for adding animal silhouette
library(rphylopic)
# search for the right image in the database
fruitfly_phylo <- name_search(text = "Drosophila melanogaster", options = "namebankID")

# name_images(uuid = fruitfly$uid[1])

# extract the ID of the right image
fruitfly_2 <- name_images(uuid = fruitfly_phylo$uid[1])$same[[2]]$ui

# get the actual image
fruitfly_pic <- image_data(fruitfly_2, size = 256)[[1]]
```

Manual calculation of regression lines

```
equation1=function(x){coef(flyls2)[4]*x+coef(flyls2)[5]*23.5+coef(flyls2)[1]}
equation2=function(x){coef(flyls2)[4]*x+coef(flyls2)[5]*23.5+coef(flyls2)[1]+coef(flyls2)[2]}
equation3=function(x){coef(flyls2)[4]*x+coef(flyls2)[5]*23.5+coef(flyls2)[1]+coef(flyls2)[2]}

flyls2 %>%
  ggplot(aes(x=thorax, y = longevity, colour = type))+
  stat_function(fun=equation1,geom="line",color="cyan")+
  stat_function(fun=equation2,geom="line",color="darkorange")+
  stat_function(fun=equation3,geom="line",color="purple")+
  geom_point()+
  scale_colour_manual(values = colours)+
  theme_minimal()+
  theme(legend.position = "none")+
  add_phylopic(fruitfly_pic2, alpha = 0.8, x = 0.7, y = 80, ysize = 10)
```

Code to produce a model summary plot that plots the exact slopes calculate by your model from using `augment()`

```
# density plot of thorax length by treatment
marginal1 <- fruitfly %>%
```

⁸<https://jacintak.github.io/post/2021-08-01-rphylopic/>

```

ggplot()+
  geom_density(aes(x = thorax, fill = type),
               alpha = 0.5)+
  scale_fill_manual(values = colours)+
  theme_void()+
  theme(legend.position = "none")

# density plot of longevity by treatment
marginal2 <- fruitfly %>%
  ggplot()+
  geom_density(aes(x = longevity, fill = type),
               alpha = 0.5)+
  scale_fill_manual(values = colours)+
  theme_void()+
  coord_flip()

# create a new dataset where all sleep values are set to a constant (mean) values - see the emmed

fruitfly_2 <- fruitfly %>%
  mutate(sleep = mean(sleep))

# use the final model to produce model predictions set to the new constant sleep dataframe
model_plot <- broom::augment(flyls2, newdata = fruitfly_2, interval = "confidence") %>%
  ggplot(aes(x=thorax, y = .fitted, colour = type, fill = type))+
  geom_ribbon(aes(ymin=.lower, ymax=.upper), alpha = 0.2)+
  geom_line(linetype = "dashed", show.legend = FALSE)+
  geom_point(data = fruitfly, aes(x = thorax, y = longevity),
             show.legend = FALSE)+
  scale_colour_manual(values = colours)+
  scale_fill_manual(values = colours)+
  labs(y = "Lifespan in days",
       x = "Thorax",
       fill = "Female exposure")+
  guides(colour = "none")+
  theme_classic()+
  theme(legend.position = "none")+
  add_phylopic(fruitfly_pic, alpha = 0.8, x = 0.7, y = 80, ysize = 10)

layout <- "
AAA#
BBC"

```

```
BBBC  
BBCB"
```

```
marginal1+model_plot+marginal2 +plot_layout(design = layout)
```

Chapter 20

Generalized Linear Models

20.1 Maximum Likelihood and Generalized Linear Models

20.1.1 BoxCox

20.1.2 Transformations

20.1.3 Distributions

20.2 Checklist

*R Cheat Sheets

Bibliography

- Bryan, J. (2012). *Happy Git and GitHub for the useR*. Chapman and Hall/CRC, Boca Raton, Florida.
- Csárdi, G. (2020). *gitcreds: Query git Credentials from R*. R package version 0.1.1.
- Darwin, C. (1876). *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. Public Domain.
- Firke, S. (2021). *janitor: Simple Tools for Examining and Cleaning Dirty Data*. R package version 2.1.0.
- Gorman, K., Williams, T., and Fraser, W. (2014). Ecological sexual dimorphism and environmental variability within a community of antarctic penguins (genus *pygoscelis*). *PLoS One*, 9(3):e90081.
- Hadley Wickham, D. N. and Pedersen, T. L. (2020). *ggplot2: Elegant Graphics for Data Analysis*. Chapman and Hall/CRC, Boca Raton, Florida.
- Hadley Wickham, G. G. (2020). *R for Data Science*. Chapman and Hall/CRC, Boca Raton, Florida.
- Henry, L. and Wickham, H. (2020). *purrr: Functional Programming Tools*. R package version 0.3.4.
- Horst, A., Hill, A., and Gorman, K. (2020). *palmerpenguins: Palmer Archipelago (Antarctica) Penguin Data*. R package version 0.1.0.
- Kassambara, A. (2020). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. R package version 0.6.0.
- Katherine Ralls, Paul Sunnucks, R. L. and Frankham, R. (2020). Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. *Biological Conservation*, 251:108784.
- Müller, K. (2020). *here: A Simpler Way to Find Your Files*. R package version 1.0.1.

- Ou, J. (2021). *colorBlindness: Safe Color Set for Color Blindness*. R package version 0.1.9.
- Pedersen, T. L. (2020). *patchwork: The Composer of Plots*. R package version 1.1.1.
- Ram, K. and Wickham, H. (2018). *wesanderson: A Wes Anderson Palette Generator*. R package version 0.3.6.
- Sievert, C., Parmer, C., Hocking, T., Chamberlain, S., Ram, K., Corvellec, M., and Despouy, P. (2021). *plotly: Create Interactive Web Graphics via plotly.js*. R package version 4.9.3.
- Spinu, V., Golemund, G., and Wickham, H. (2021). *lubridate: Make Dealing with Dates a Little Easier*. R package version 1.7.10.
- Wickham, H. (2019). *stringr: Simple, Consistent Wrappers for Common String Operations*. R package version 1.4.0.
- Wickham, H. (2021). *tidyverse: Tidy Messy Data*. R package version 1.1.3.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Golemud, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., and Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4(43):1686.
- Wickham, H., Bryan, J., and Barrett, M. (2021a). *usethis: Automate Package and Project Setup*. R package version 2.1.3.
- Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., and Dunnington, D. (2021b). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics*. R package version 3.3.5.
- Wickham, H., François, R., Henry, L., and Müller, K. (2021c). *dplyr: A Grammar of Data Manipulation*. R package version 1.0.6.
- Wickham, H., Hester, J., and Bryan, J. (2021d). *readr: Read Rectangular Text Data*. R package version 2.1.1.
- Wickham, H. and Seidel, D. (2020). *scales: Scale Functions for Visualization*. R package version 1.1.1.
- Wilke, C. O. (2020). *Fundamentals of Data Visualization*. Chapman and Hall/CRC, Boca Raton, Florida.
- Wilke, C. O. (2021). *ggridges: Ridgeline Plots in ggplot2*. R package version 0.5.3.
- Xiao, N. (2018). *ggsci: Scientific Journal and Sci-Fi Themed Color Palettes for ggplot2*. R package version 2.9.

Xie, Y. (2015). *Dynamic Documents with R and knitr*. Chapman and Hall/CRC, Boca Raton, Florida, 2nd edition. ISBN 978-1498716963.

Zhu, H. (2020). *kableExtra: Construct Complex Table with kable and Pipe Syntax*. R package version 1.3.1.