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Preface

These are the course notes for the "Introduction to R" course given by the Monash Bioinformatics Platform. Some of this material is derived from work that is Copyright © Software Carpentry¹ with a CC BY 4.0 license 2 .

Data files we are working with are available from:

http://monashbioinformaticsplatform.github.io/r-intro/

 $^{^{1}} http://software-carpentry.org/ \\ ^{2} https://creativecommons.org/licenses/by/4.0/$

Chapter 1

Starting out in R

R is both a programming language and an interactive environment for statistics. Today we will be concentrating on R as an *interactive environment*.

Working with R is primarily text-based. The basic mode of use for R is that the user types in a command in the R language and presses enter, and then R computes and displays the result.

We will be working in RStudio¹. This surrounds the *console*, where one enters commands and views the results, with various conveniences. In addition to the console, RStudio provides panels containing:

- A text editor, where R commands can be recorded for future reference.
- A history of commands that have been typed on the console.
- A list of variables, which contain values that R has been told to save from previous commands.
- A file manager.
- Help on the functions available in R.
- A panel to show plots (graphs).

Open RStudio, click on the "Console" pane, type 1+1 and press enter. R displays the result of the calculation. In this document, we will be showing such an interaction with R as below.

```
1+1
## [1] 2
```

- + is called an operator. R has the operators you would expect for for basic mathematics: + * /. It also has operators that do more obscure things.
- * has higher precedence than +. We can use brackets if necessary (). Try 1+2*3 and (1+2)*3.

Spaces can be used to make code easier to read.

We can compare with == < > <= >=. This produces a "logical" value, TRUE or FALSE. Note the double equals, ==, for equality comparison.

There are also character strings such as "string".

¹https://www.rstudio.com/products/rstudio/download/

Variables

A variable is a name for a value, such as x, current_temperature, or subject.id. We can create a new variable by assigning a value to it using <-

```
weight_kg <- 55
```

RStudio helpfully shows us the variable in the "Environment" pane. We can also print it by typing the name of the variable and hitting enter. In general, R will print to the console any object returned by a function or operation *unless* we assign it to a variable.

```
weight_kg
## [1] 55
```

Examples of valid variables names: hello, hello_there, hello.there, value1. Spaces aren't ok inside variable names. Dots (.) are ok, unlike in many other languages.

We can do arithmetic with the variable:

```
# weight in pounds:
2.2 * weight_kg
## [1] 121
```

Tip

We can add comments to our code using the # character. It is useful to document our code in this way so that others (and us the next time we read it) have an easier time following what the code is doing.

We can also change a variable's value by assigning it a new value:

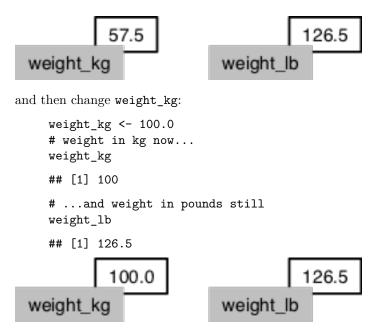
```
weight_kg <- 57.5
# weight in kilograms is now
weight_kg
## [1] 57.5</pre>
```

If we imagine the variable as a sticky note with a name written on it, assignment is like putting the sticky note on a particular value:



Assigning a new value to one variable does not change the values of other variables. For example, let's store the subject's weight in pounds in a variable:

```
weight_lb <- 2.2 * weight_kg
# weight in kg...
weight_kg
## [1] 57.5
# ...and in pounds
weight_lb
## [1] 126.5</pre>
```



Since weight_lb doesn't "remember" where its value came from, it isn't automatically updated when weight_kg changes. This is different from the way spreadsheets work.

Vectors

A "vector" of numbers is a collection of numbers. We call the individual numbers "elements" of the vector.

We can make vectors with c(), for example c(1,2,3), and do maths to them. c means "combine". Actually in R numbers are just vectors of length one. R is obsessed with vectors.

```
myvec <- c(10,20,30)
myvec + 1
## [1] 11 21 31
myvec + myvec
## [1] 20 40 60
length(myvec)
## [1] 3
c(40, myvec)
## [1] 40 10 20 30</pre>
```

When we talk about the length of a vector, we are talking about the number of numbers in the vector.

Access elements of a vector with [], for example myvec[1] to get the first element.

```
myvec[1]
## [1] 10
myvec[2]
## [1] 20
```

²We use the word vector here in the mathematical sense, as used in linear algebra, not in any biological sense, and not in the geometric sense.

```
myvec[2] <- 5
myvec
## [1] 10 5 30
```

We will also encounter vectors of character strings, for example "hello" or c("hello", "world"). Also we will encounter "logical" vectors, which contain TRUE and FALSE values. R also has "factors", which are categorical vectors, and behave very much like character vectors (think the factors in an experiment).

Challenge

Sometimes the best way to understand R is to try some examples and see what it does.

What happens when you try to make a vector containing different types, using c()? Make a vector with some numbers, and some words (eg. character strings like "test", or "hello").

Why does the output show the numbers surrounded by quotes " " like character strings are?

Because vectors can only contain one type of thing, R chooses a lowest common denominator type of vector, a type that can contain everything we are trying to put in it. A different language might stop with an error, but R tries to soldier on as best it can. A number can be represented as a character string, but a character string can not be represented as a number, so when we try to put both in the same vector R converts everything to a character string.

Functions

R has various functions, such as sum(). We can get help on a function with, eg ?sum.

```
?sum
sum(myvec)
## [1] 45
```

Here we have called the function sum with the argument myvec.

Because R is a language for statistics, it has many built in statistics-related functions. We will also be loading more specialized functions from "libraries" (also known as "packages").

Some functions take more than one argument. Let's look at the function rep, which means "repeat", and which can take a variety of different arguments. In the simplest case, it takes a value and the number of times to repeat that value.

```
rep(42, 10)
## [1] 42 42 42 42 42 42 42 42 42 42
```

As with many functions in R—which is obsessed with vectors—the thing to be repeated can be a vector with multiple elements.

```
rep(c(1,2,3), 10)
## [1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
```

So far we have used *positional* arguments, where R determines which argument is which by the order in which they are given. We can also give arguments by *name*. For example, the above is equivalent to

```
rep(c(1,2,3), times=10)
## [1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
```

```
rep(x=c(1,2,3), 10)

## [1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1
```

Arguments can have default values, and a function may have many different possible arguments that make it do obscure things. For example, rep can also take an argument each=. It's typical for a function to be invoked with some number of positional arguments, which are always given, plus some less commonly used arguments, typically given by name.

```
rep(c(1,2,3), each=3)
## [1] 1 1 1 2 2 2 3 3 3
rep(c(1,2,3), each=3, times=5)
## [1] 1 1 1 2 2 2 3 3 3 1 1 1 2 2 2 3 3 3 1 1 1 2 2 2 3 3
## [36] 3 1 1 1 2 2 2 3 3 3
```

Lists

Vectors contain all the same kind of thing. Lists can contain different kinds of thing. Lists can even contain vectors or other lists as elements.

We generally give the things in a list names. Try list(num=42, greeting="hello"). To access named elements we use \$.

```
mylist <- list(num=42, greeting="Hello, world")
mylist$greeting
## [1] "Hello, world"</pre>
```

Functions that need to return multiple outputs often do so as a list. We will be seeing examples of this today, and in the RNA-Seq class.

Overview of data types

We've seen several data types in this chapter, and will be seeing two more in the following chapters. This section serves as an overview of data types in R and their typical usage.

Each data type has various ways it can be created and various ways it can be accessed. If we have data in the wrong type, there are functions to "cast" it to the right type.

This will all make more sense once you have seen these data types in action.

Tip

If you're not sure what type of value you are dealing with you can use class, or for more detailed information str (structure).

vector

Vectors contain zero or more elements, all of the same basic type ("mode").

Elements can be named (names), but often aren't.

Access single elements: vec[5]

Take a subset of a vector: vec[c(1,3,5)] vec[c(TRUE,FALSE,TRUE,FALSE,TRUE)]

Vectors come in several different flavours.

numeric vector

Numbers. Internally stored as "floating point" so there is a limit to the number of digits accuracy, but this is usually entirely adequate.

Examples: 42 1e-3 c(1,2,0.7)
Casting: as.numeric("42")

character vector

Character strings.

Examples: "hello" c("Let", "the", "computer", "do", "the", "work")

Casting: as.character(42)

logical vector

TRUE or FALSE values.

Examples: TRUE FALSE T F c(TRUE, FALSE, TRUE)

factor vector

A categorical vector, where the elements can be one of several different "levels". There will be more on these in the chapter on data frames.

Creation/casting: factor(c("mutant","wildtype","mutant"), levels=c("wildtype","mutant"))

list

Lists contain zero or more elements, of any type. Elements of a list can even be vectors with their own multiple elements, or other lists. If your data can't be bundled up in any other type, bundle it up in a list.

List elements can and typically do have names (names).

Access an element: mylist[[5]] mylist[["elementname"]] mylist\$elementname

Creation: list(a=1, b="two", c=FALSE)

matrix

A matrix is a two dimensional tabular data structure in which all the elements are the same type. We will typically be dealing with numeric matrices, but it is also possible to have character or logical matrices, etc.

Matrix rows and columns may have names (rownames, colnames).

```
Access an element: mat[3,5] mat["arowname", "acolumnname"]

Get a whole row: mat[3,]

Get a whole column: mat[,5]

Creation: matrix()

Casting: as.matrix()
```

data.frame

A data frame is a two dimensional tabular data structure in which the columns may have different types, but all the elements in each column must have the same type.

Data frame rows and columns may have names (rownames, colnames). However in typical usage columns are named but rows are not.³

Accessing elements, rows, and columns is the same as for matrices, but we can also get a whole column using \$.

```
Creation: data.frame(colname1=values1,colname2=values2,...)
Casting: as.data.frame()
```

 $^{^3}$ For some reason, data frames use partial matching on row names, which can cause some very puzzling bugs.

Chapter 2

Working with data in a matrix

Loading data

Our example data is quality measurements (particle size) on PVC plastic production, using eight different resin batches, and three different machine operators.

The data set is stored in comma-separated value (CSV) format. Each row is a resin batch, and each column is an operator. In RStudio, open pvc.csv and have a look at what it contains.

```
read.csv("r-intro-files/pvc.csv", row.names=1)
```

Tip

The location of the file is given relative to your "working directory". You can see the location of your working directory in the title of the console pane in RStudio. It is most likely "~", indicating your personal home directory. You can change working directory with setwd.

The filename "r-intro-files/pvc.csv" means from the current working directory, in the sub-directory "r-intro-files", the file "pvc.csv".

You can check that the file is actually in this location using the "Files" pane in the bottom right corner of RStudio.

If you are working on your own machine rather than our training server, and downloaded and unarchived the r-intro-files.zip file, the file may be in a different location.

We have called <code>read.csv</code> with two arguments: the name of the file we want to read, and which column contains the row names. The filename needs to be a character string, so we put it in quotes. Assigning the second argument, <code>row.names</code>, to be 1 indicates that the data file has row names, and which column number they are stored in. If we don't specify <code>row.names</code> the result will not have row names.

Tip

read.csv actually has many more arguments that you may find useful when importing your own data in the future.

```
dat <- read.csv("r-intro-files/pvc.csv", row.names=1)
dat</pre>
```

```
##
          Alice
                  Bob Carl
## Resin1 36.25 35.40 35.30
## Resin2 35.15 35.35 33.35
## Resin3 30.70 29.65 29.20
## Resin4 29.70 30.05 28.65
## Resin5 31.85 31.40 29.30
## Resin6 30.20 30.65 29.75
## Resin7 32.90 32.50 32.80
## Resin8 36.80 36.45 33.15
class(dat)
## [1] "data.frame"
str(dat)
## 'data.frame':
                    8 obs. of 3 variables:
   $ Alice: num
                  36.2 35.1 30.7 29.7 31.9 ...
                  35.4 35.4 29.6 30.1 31.4 ...
   $ Bob : num
    $ Carl : num 35.3 33.4 29.2 28.6 29.3 ...
```

read.csv has loaded the data as a data frame. A data frame contains a collection of "things" (rows) each with a set of properties (columns) of different types.

Actually this data is better thought of as a matrix¹. In a data frame the columns contain different types of data, but in a matrix all the elements are the same type of data. A matrix in R is like a mathematical matrix, containing all the same type of thing (usually numbers).

R often but not always lets these be used interchangably. It's also helpful when thinking about data to distinguish between a data frame and a matrix. Different operations make sense for data frames and matrices.

Data frames are very central to R, and mastering R is very much about thinking in data frames. However when we get to RNA-Seq we will be using matrices of read counts, so it will be worth our time to learn to use matrices as well.

Let us insist to R that what we have is a matrix. as.matrix "casts" our data to have matrix type.

```
mat <- as.matrix(dat)
class(mat)
## [1] "matrix"
str(mat)
## num [1:8, 1:3] 36.2 35.1 30.7 29.7 31.9 ...
## - attr(*, "dimnames")=List of 2
## ..$ : chr [1:8] "Resin1" "Resin2" "Resin3" "Resin4" ...
## ..$ : chr [1:3] "Alice" "Bob" "Carl"</pre>
```

Much better.

Tip

Matrices can be created in various ways.

matrix converts a vector into a matrix with a specified number of rows and columns.

rbind stacks several vectors as rows one on top of another to form a matrix, or it can stack smaller matrices on top of each other to form a larger matrix.

¹We use matrix here in the mathematical sense, not the biological sense.

cbind similarly stacks several vectors as columns next to each other to form a matrix, or it can stack smaller matrices next to each other to form a larger matrix.

Indexing matrices

We can check the size of the matrix with the functions nrow and ncol:

```
nrow(mat)
## [1] 8
ncol(mat)
## [1] 3
```

This tells us that our matrix, mat, has 8 rows and 3 columns.

If we want to get a single value from the matrix, we can provide a row and column index in square brackets:

```
# first value in mat
mat[1, 1]
## [1] 36.25
# a middle value in mat
mat[4, 2]
## [1] 30.05
```

If our matrix has row names and column names, we can also refer to rows and columns by name.

```
mat["Resin4","Bob"]
## [1] 30.05
```

An index like [4, 2] selects a single element of a matrix, but we can select whole sections as well. For example, we can select the first two operators (columns) of values for the first four resins (rows) like this:

```
mat[1:4, 1:2]
## Alice Bob
## Resin1 36.25 35.40
## Resin2 35.15 35.35
## Resin3 30.70 29.65
## Resin4 29.70 30.05
```

The slice 1:4 means, the numbers from 1 to 4. It's the same as c(1,2,3,4), and doesn't need to be used inside [].

```
1:4
## [1] 1 2 3 4
```

The slice does not need to start at 1, e.g. the line below selects rows 5 through 8:

```
mat[5:8, 1:2]
## Alice Bob
## Resin5 31.85 31.40
## Resin6 30.20 30.65
## Resin7 32.90 32.50
## Resin8 36.80 36.45
```

We can use vectors created with c to select non-contiguous values:

```
mat[c(1,3,5), c(1,3)]
## Alice Carl
## Resin1 36.25 35.3
## Resin3 30.70 29.2
## Resin5 31.85 29.3
```

We also don't have to provide an index for either the rows or the columns. If we don't include an index for the rows, R returns all the rows; if we don't include an index for the columns, R returns all the columns. If we don't provide an index for either rows or columns, e.g. mat[,], R returns the full matrix.

```
# All columns from row 5
mat[5, ]
## Alice Bob Carl
## 31.85 31.40 29.30
# All rows from column 2
mat[, 2]
## Resin1 Resin2 Resin3 Resin4 Resin5 Resin6 Resin7 Resin8
## 35.40 35.35 29.65 30.05 31.40 30.65 32.50 36.45
```

Summary functions

Now let's perform some common mathematical operations to learn about our data. When analyzing data we often want to look at partial statistics, such as the maximum value per resin or the average value per operator. One way to do this is to select the data we want as a new temporary variable, and then perform the calculation on this subset:

```
# first row, all of the columns
resin_1 <- mat[1, ]
# max particle size for resin 1
max(resin_1)
## [1] 36.25</pre>
```

We don't actually need to store the row in a variable of its own. Instead, we can combine the selection and the function call:

```
# max particle size for resin 2
max(mat[2, ])
## [1] 35.35
```

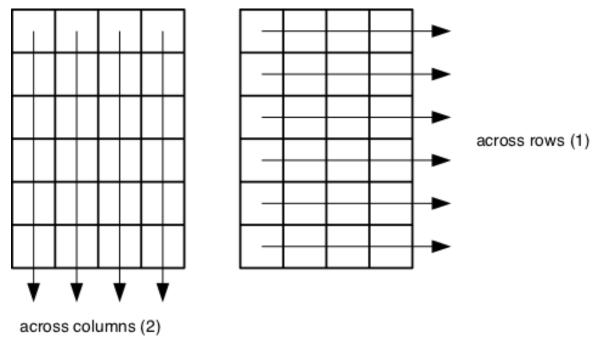
R has functions for other common calculations, e.g. finding the minimum, mean, median, and standard deviation of the data:

```
# minimum particle size for operator 3
min(mat[, 3])
## [1] 28.65
# mean for operator 3
mean(mat[, 3])
## [1] 31.4375
# median for operator 3
median(mat[, 3])
```

```
## [1] 31.275
# standard deviation for operator 3
sd(mat[, 3])
## [1] 2.49453
```

Summarizing matrices

What if we need the maximum particle size for all resins, or the average for each operator? As the diagram below shows, we want to perform the operation across a margin of the matrix:



To support this, we can use the apply function.

Tip

To learn about a function in R, e.g. apply, we can read its help documention by running help(apply) or ?apply.

apply allows us to repeat a function on all of the rows (MARGIN = 1) or columns (MARGIN = 2) of a matrix. We can think of apply as collapsing the matrix down to just the dimension specified by MARGIN, with rows being dimension 1 and columns dimension 2 (recall that when indexing the matrix we give the row first and the column second).

Thus, to obtain the average particle size of each resin we will need to calculate the mean of all of the rows (MARGIN = 1) of the matrix.

```
avg_resin <- apply(mat, 1, mean)</pre>
```

And to obtain the average particle size for each operator we will need to calculate the mean of all of the columns (MARGIN = 2) of the matrix.

```
avg_operator <- apply(mat, 2, mean)</pre>
```

Since the second argument to apply is MARGIN, the above command is equivalent to apply(dat, MARGIN = 2, mean).

Tip

Some common operations have more concise alternatives. For example, you can calculate the row-wise or column-wise means with rowMeans and colMeans, respectively.

Challenge - Slicing (subsetting) data

We can take slices of character vectors as well:

```
phrase <- c("I", "don't", "know", "I", "know")
# first three words
phrase[1:3]
## [1] "I"      "don't" "know"
# last three words
phrase[3:5]
## [1] "know" "I"      "know"</pre>
```

- 1. If the first four words are selected using the slice phrase[1:4], how can we obtain the first four words in reverse order?
- 2. What is phrase[-2]? What is phrase[-5]? Given those answers, explain what phrase[-1:-3] does.
- 3. Use a slice of phrase to create a new character vector that forms the phrase "I know I don't", i.e. c("I", "know", "I", "don't").

Challenge - Subsetting data 2

Suppose you want to determine the maximum particle size for resin 5 across operators 2 and 3. To do this you would extract the relevant slice from the matrix and calculate the maximum value. Which of the following lines of R code gives the correct answer?

```
(a) max(dat[5, ])
(b) max(dat[2:3, 5])
(c) max(dat[5, 2:3])
(d) max(dat[5, 2, 3])
```

t test

R has many statistical tests built in. One of the most commonly used tests is the t test. Do the means of two vectors differ significantly?

```
mat[1,]
## Alice Bob Carl
## 36.25 35.40 35.30
mat[2,]
```

```
## Alice Bob Carl
## 35.15 35.35 33.35

t.test(mat[1,], mat[2,])

##

## Welch Two Sample t-test
##

## data: mat[1,] and mat[2,]

## t = 1.4683, df = 2.8552, p-value = 0.2427

## alternative hypothesis: true difference in means is not equal to 0

## 95 percent confidence interval:
## -1.271985 3.338652

## sample estimates:
## mean of x mean of y

## 35.65000 34.61667
```

Actually, this can be considered a paired sample t-test, since the values can be paired up by operator. By default t.test performs an unpaired t test. We see in the documentation (?t.test) that we can give paired=TRUE as an argument in order to perform a paired t-test.

```
t.test(mat[1,], mat[2,], paired=TRUE)

##
## Paired t-test
##
## data: mat[1,] and mat[2,]
## t = 1.8805, df = 2, p-value = 0.2008
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.330952 3.397618
## sample estimates:
## mean of the differences
## 1.033333
```

Challenge - using t.test

Can you find a significant difference between any two resins?

When we call t.test it returns an object that behaves like a list. Recall that in R a list is a miscellaneous collection of values.

```
result <- t.test(mat[1,], mat[2,], paired=TRUE)
names(result)

## [1] "statistic" "parameter" "p.value" "conf.int" "estimate"

## [6] "null.value" "alternative" "method" "data.name"

result$p.value

## [1] 0.2007814</pre>
```

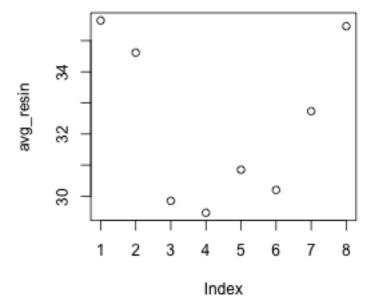
This means we can write software that uses the various results from t.test, for example performing a whole series of t tests and reporting the significant results.

Plotting

The mathematician Richard Hamming once said, "The purpose of computing is insight, not numbers," and the best way to develop insight is often to visualize data. Visualization deserves an entire lecture (or course) of its own, but we can explore a few of R's plotting features.

Let's take a look at the average particle size per resin. Recall that we already calculated these values above using apply(mat, 1, mean) and saved them in the variable avg_resin. Plotting the values is done with the function plot.

plot(avg_resin)

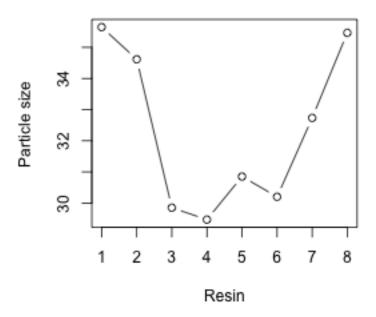


Above, we gave the function plot a vector of numbers corresponding to the average per resin across all operators. plot created a scatter plot where the y-axis is the average particle size and the x-axis is the order, or index, of the values in the vector, which in this case correspond to the 8 resins.

plot can take many different arguments to modify the appearance of the output. Here is a plot with some extra arguments:

```
plot(avg_resin,
    xlab="Resin",
    ylab="Particle size",
    main="Average particle size per resin",
    type="b")
```

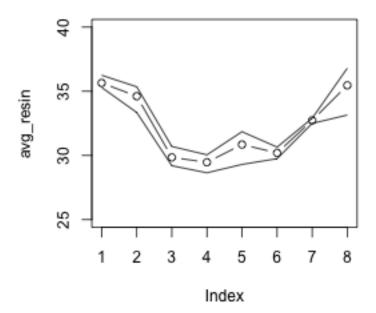
Average particle size per resin



Let's have a look at two other statistics: the maximum and minimum particle size per resin. Additional points or lines can be added to a plot with points or lines.

```
max_resin <- apply(mat, 1, max)
min_resin <- apply(mat, 1, min)

plot(avg_resin, type="b", ylim=c(25,40))
lines(max_resin)
lines(min_resin)</pre>
```



R doesn't know to adjust the y limits if we add new data outside the original limits, so we needed to specify ylim manually. This is R's base graphics system. If there is time today, we will look at a more advanced graphics package called "ggplot2" that handles this kind of issue more intelligently.

Challenge - Plotting data

Create a plot showing the standard deviation for each resin.

Advanced: Create a plot showing +/- two standard deviations about the mean.

Extension: Create similar plots for operator. Which dimension (resin or operator) is the major source of variation in this data?

Saving plots

It's possible to save a plot as a .PNG or .PDF from the RStudio interface with the "Export" button. However if we want to keep a complete record of exactly how we create each plot, we prefer to do this with R code.

Plotting in R is sent to a "device". By default, this device is RStudio. However we can temporarily send plots to a different device, such as a .PNG file (png("filename.png")) or .PDF file (pdf("filename.pdf")).

```
pdf("test.pdf")
plot(avg_resin)
dev.off()
```

dev.off() is very important. It tells R to stop outputting to the pdf device and return to using the default device. If you forget, your interactive plots will stop appearing as expected!

The file you created should appear in the file manager pane of RStudio, you can view it by clicking on it.

Chapter 3

Working with data in a data frame

As we saw earlier, read.csv loads tabular data from a CSV file into a data frame.

```
diabetes <- read.csv("r-intro-files/diabetes.csv")</pre>
class(diabetes)
## [1] "data.frame"
head(diabetes)
    subject glyhb location age gender height weight frame
      S1002 4.64 Buckingham 58 female
                                             61
                                                   256 large
      S1003 4.63 Buckingham 67
                                   male
                                             67
                                                   119 large
      S1005 7.72 Buckingham 64
                                  \mathtt{male}
                                             68
                                                   183 medium
      S1008 4.81 Buckingham
                              34
                                  \mathtt{male}
                                             71
                                                   190 large
## 5
      S1011 4.84 Buckingham 30
                                             69
                                                   191 medium
                                  male
      S1015 3.94 Buckingham 37
                                    male
                                             59
                                                   170 medium
colnames(diabetes)
## [1] "subject" "glyhb"
                             "location" "age"
                                                   "gender"
                                                              "height"
## [7] "weight"
                  "frame"
ncol(diabetes)
## [1] 8
nrow(diabetes)
## [1] 354
```

Tip

A data frame can also be created from vectors, with the data.frame function. For example

```
data.frame(foo=c(10,20,30), bar=c("a","b","c"))
```

```
## foo bar
## 1 10 a
## 2 20 b
## 3 30 c
```

Tip

A data frame can have both column names (colnames) and rownames (rownames). However, the modern convention is for a data frame to use column names but not row names. Typically a data frame contains a collection of items (rows), each having various properties (columns). If an item has an identifier such as a unique name, this would be given as just another column.

Indexing data frames

As with a matrix, a data frame can be accessed by row and column with [,].

One difference is that if we try to get a single row of the data frame, we get back a data frame with one row, rather than a vector. This is because the row may contain data of different types, and a vector can only hold elements of all the same type.

Internally, a data frame is a list of column vectors. We can use the \$ syntax we saw with lists to access columns by name.

Logical indexing

A method of indexing that we haven't discussed yet is logical indexing. Instead of specifying the row number or numbers that we want, we can give a logical vector which is TRUE for the rows we want and FALSE otherwise. This can also be used with vectors and matrices.

Suppose we want to look at all the subjects over 80 years of age. We first make a logical vector:

```
is_over_80 <- diabetes$age >= 80
head(is_over_80)
## [1] FALSE FALSE FALSE FALSE FALSE FALSE
sum(is_over_80)
## [1] 9
```

>= is a comparison operator meaning greater than or equal to. We can then grab just these rows of the data frame where is_over_80 is TRUE.

diabetes[is_over_80,]

```
##
       subject glyhb
                       location age gender height weight
                                                           frame
## 45
         S2770 4.98 Buckingham 92 female
                                                62
                                                      217
                                                           large
## 56
                8.40 Buckingham 91 female
         S2794
                                                61
                                                      127
                                                            <NA>
## 90
         S4803
                5.71
                         Louisa
                                 83 female
                                                59
                                                      125 medium
## 130 S13500 5.60
                         Louisa
                                 82
                                      male
                                                66
                                                      163
                                                            <NA>
        S15013 4.57
                                 81 female
                                                      158 medium
## 139
                         Louisa
                                                64
## 193
       S15815 4.92 Buckingham
                                 82 female
                                                63
                                                      170 medium
## 321
        S40784 10.07
                         Louisa
                                 84 female
                                                60
                                                      192
                                                           small
## 323
                                                71
        S40786 6.48
                         Louisa
                                 80
                                      male
                                                      212 medium
## 324
        S40789 11.18
                                                62
                         Louisa
                                 80 female
                                                           small
```

We might also want to know *which* rows our logical vector is TRUE for. This is achieved with the which function. The result of this can also be used to index the data frame.

```
which_over_80 <- which(is_over_80)
which_over_80</pre>
```

```
## [1] 45 56 90 130 139 193 321 323 324
diabetes[which_over_80,]
##
                       location age gender height weight
       subject glyhb
                                                           frame
## 45
         S2770 4.98 Buckingham
                                 92 female
                                                62
                                                      217
                                                           large
## 56
         S2794 8.40 Buckingham
                                 91 female
                                                61
                                                      127
                                                            <NA>
                                 83 female
## 90
                                                      125 medium
         S4803
                5.71
                         Louisa
                                                59
## 130
                                 82
       S13500
                5.60
                         Louisa
                                      male
                                                66
                                                      163
                                                            <NA>
## 139
        S15013
                4.57
                         Louisa
                                 81 female
                                                64
                                                      158 medium
## 193
       S15815
               4.92 Buckingham
                                 82 female
                                                63
                                                      170 medium
## 321
        S40784 10.07
                         Louisa
                                 84 female
                                                60
                                                      192
                                                           small
                                                71
## 323
        S40786 6.48
                                 80
                                                      212 medium
                         Louisa
                                       male
## 324
       S40789 11.18
                         Louisa
                                 80 female
                                                62
                                                      162
                                                           small
```

Comparison operators available are:

- x == y ``equal to''
- x != y "not equal to"
- x < y "less than"
- x > y "greater than"
- $x \le y -$ "less than or equal to"
- $x \ge y$ "greater than or equal to"

More complicated conditions can be constructed using logical operators:

- a & b "and", true only if both a and b are true.
- a | b "or", true if either a or b or both are true.
- ! a "not", true if a is false, and false if a is true.

```
is_over_80_and_female <- is_over_80 & diabetes$gender == "female"
is_not_from_buckingham <- !(diabetes$location == "Buckingham")
# or
is_not_from_buckingham <- diabetes$location != "Buckingham"</pre>
```

The data we are working with is derived from a dataset called **diabetes** in the **faraway** package. The rows are people interviewed as part of a study of diabetes prevalence. The column **glyhb** is a measurement of percent glycosylated haemoglobin, which gives information about long term glucose levels in blood. Values greater than 7% are usually taken as a positive diagnosis of diabetes. Let's add this as a column.

```
diabetes$diabetic <- diabetes$glyhb > 7.0
```

head(diabetes)

```
location age gender height weight
     subject glyhb
                                                         frame diabetic
## 1
       S1002 4.64 Buckingham
                                58 female
                                              61
                                                     256
                                                          large
                                                                   FALSE
## 2
       S1003 4.63 Buckingham
                                67
                                     male
                                              67
                                                     119
                                                          large
                                                                   FALSE
## 3
       S1005 7.72 Buckingham
                                64
                                              68
                                                     183 medium
                                                                    TRUE
                                     male
## 4
       S1008 4.81 Buckingham
                                34
                                     male
                                              71
                                                     190
                                                          large
                                                                   FALSE
## 5
             4.84 Buckingham
                                              69
                                                     191 medium
                                                                   FALSE
       S1011
                                30
                                     male
       S1015 3.94 Buckingham
                                37
                                     male
                                              59
                                                     170 medium
                                                                   FALSE
```

Challenge

Which female subjects from Buckingham are under the age of 25?

What is their average glybb?

Are any of them diabetic?

Missing data

summary gives an overview of a data frame.

summary(diabetes)

```
##
       subject
                       glyhb
                                            location
                                                             age
##
    S10000 : 1
                         : 2.680
                                     Buckingham: 178
                   Min.
                                                       Min.
                                                               :19.00
##
    S10001:
              1
                   1st Qu.: 4.385
                                     Louisa
                                                :176
                                                       1st Qu.:35.00
##
    S10016:
              1
                   Median : 4.840
                                                       Median :45.00
    S1002
                   Mean
                          : 5.580
                                                       Mean
                                                               :46.91
    S10020 :
                   3rd Qu.: 5.565
                                                       3rd Qu.:60.00
##
              1
##
    S1003
                   Max.
                           :16.110
                                                       Max.
                                                               :92.00
    (Other):348
                   NA's
##
                           :11
##
                                                                   diabetic
       gender
                      height
                                       weight
                                                       frame
##
    female:206
                  Min.
                          :52.00
                                          : 99.0
                                                    large: 91
                                                                  Mode :logical
##
    male :148
                  1st Qu.:63.00
                                   1st Qu.:150.0
                                                    medium:155
                                                                  FALSE:291
##
                  Median :66.00
                                   Median :171.0
                                                    small : 96
                                                                  TRUE :52
##
                  Mean
                          :65.93
                                   Mean
                                           :176.2
                                                    NA's : 12
                                                                  NA's :11
##
                  3rd Qu.:69.00
                                   3rd Qu.:198.0
                          :76.00
##
                  Max.
                                   Max.
                                           :325.0
##
                  NA's
                          :5
                                   NA's
                                           :1
```

We see that some columns contain NAs. NA is R's way of indicating missing data. Missing data is important in statistics, so R is careful with its treatment of this. If we try to calculate with an NA the result will be NA.

```
1 + NA

## [1] NA

mean(diabetes$glyhb)

## [1] NA
```

Many summary functions, such as mean, have a flag to say ignore NA values.

```
mean(diabetes$glyhb, na.rm=TRUE)
## [1] 5.580292
```

There is also an is.na function, allowing us to find which values are NA, and na.omit which removes NAs.

```
not_missing <- !is.na(diabetes$glyhb)
mean( diabetes$glyhb[not_missing] )
## [1] 5.580292
mean( na.omit(diabetes$glyhb) )
## [1] 5.580292</pre>
```

na.omit can also be used on a whole data frame, and removes rows with NA in any column.

Factors

When R loads a CSV file, it tries to give appropriate types to the columns. Let's examine what types R has given our data.

```
str(diabetes)
## 'data.frame': 354 obs. of 9 variables:
## $ subject : Factor w/ 354 levels "S10000", "S10001", ...: 4 6 7 8 9 10 11 12 13 14 ...
## $ glyhb : num    4.64 4.63 7.72 4.81 4.84 ...
## $ location: Factor w/ 2 levels "Buckingham", "Louisa": 1 1 1 1 1 1 1 1 1 2 2 ...
## $ age : int 58 67 64 34 30 37 45 55 60 38 ...
## $ gender : Factor w/ 2 levels "female", "male": 1 2 2 2 2 2 2 1 1 1 ...
## $ height : int 61 67 68 71 69 59 69 63 65 58 ...
## $ weight : int 256 119 183 190 191 170 166 202 156 195 ...
## $ frame : Factor w/ 3 levels "large", "medium", ..: 1 1 2 1 2 2 1 3 2 2 ...
## $ diabetic: logi FALSE FALSE TRUE FALSE FALSE FALSE ...
```

We might have expected the text columns to be the "character" data type, but they are instead "factor"s.

```
head( diabetes$frame )
## [1] large large medium large medium medium
## Levels: large medium small
```

R uses the factor data type to store a vector of *categorical* data. The different possible categories are called "levels".

Factors can be created from character vectors with factor. We sometimes care what order the levels are in, since this can affect how data is plotted or tabulated by various functions. If there is some sort of baseline level, such as "wildtype strain" or "no treatment", it is usually given first. factor has an argument levels= to specify the desired order of levels.

Factors can be converted back to a character vector with as.character.

When R loaded our data, it chose levels in alphabetical order. Let's adjust that for the column diabetes\$frame.

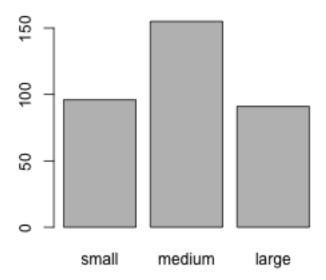
```
diabetes$frame <- factor(diabetes$frame, levels=c("small","medium","large"))
head( diabetes$frame )
## [1] large large medium large medium medium
## Levels: small medium large</pre>
```

Plotting factors

Some functions in R do different things if you give them different types of argument. summary and plot are examples of such functions.

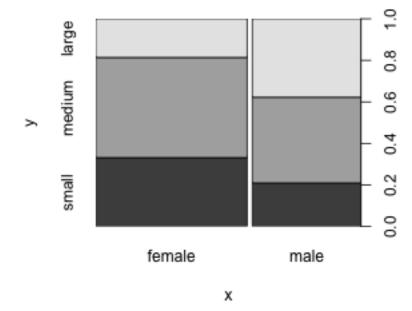
If we plot factors, R shows the proportions of each level in the factor. We can also see that R uses the order of levels we gave it in the plot.

```
plot( diabetes$frame )
```



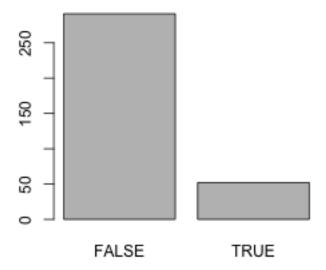
When we give R two factors to plot it produces a "mosaic plot" that helps us see if there is any relationship between the two factors.

plot(diabetes\$gender, diabetes\$frame)

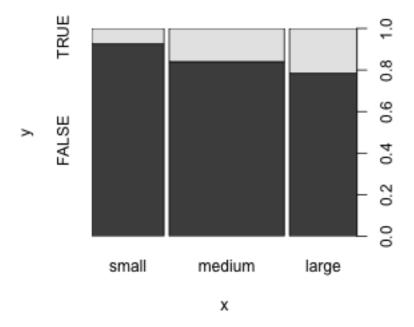


diabetes\$diabetic is logical, but we can tell R to turn it into a factor to produce this type of plot for this column as well.

plot(factor(diabetes\$diabetic))



plot(diabetes\$frame, factor(diabetes\$diabetic))



Summarizing factors

The table function gives us the actual numbers behind the graphical summaries we just plotted (a "contingency table").

```
table(diabetes$frame)
##
##
    small medium
                   large
##
       96
              155
                       91
table(diabetes$diabetic, diabetes$frame)
##
##
            small medium large
##
     {\tt FALSE}
               87
                      126
                             69
     TRUE
```

Fisher's Exact Test (fisher.test) or a chi-squared test (chiseq.test) can be used to show that two factors are not independent.

```
fisher.test( table(diabetes$diabetic, diabetes$frame) )
##
## Fisher's Exact Test for Count Data
##
## data: table(diabetes$diabetic, diabetes$frame)
## p-value = 0.02069
## alternative hypothesis: two.sided
```

Challenge - gender and diabetes

Do you think there is any association between gender and whether a person is diabetic shown by this data set?

Why, or why not?

Summarizing data frames

We were able to summarize the dimensions (rows or columns) of a matrix with apply. In a data frame instead of summarizing along different dimensions, we can summarize with respect to different factor columns.

We already saw how to count different levels in a factor with table.

We can use summary functions such as mean with a function called tapply, which works similarly to apply. The three arguments we need are very similar to the three arguments we used with apply:

- 1. The data to summarize.
- 2. What we want *not* to be collapsed away in the output.
- 3. The function to use to summarize the data.

However rather than specifying a dimension for argument 2 we specify a factor.

```
tapply(diabetes$glyhb, diabetes$frame, mean)
## small medium large
## NA NA NA
```

We obtain NAs because our data contains NAs. We need to tell mean to ignore these. Additional arguments to tapply are passed to the function given, here mean, so we can tell mean to ignore NA with

```
tapply(diabetes$glyhb, diabetes$frame, mean, na.rm=TRUE)
## small medium large
## 4.971064 5.721333 6.035795
```

The result is a vector, with names from the classifying factor. These means of a continuous measurement seem to be bearing out our earlier observation using a discrete form of the measurement, that this data show some link between body frame and diabetes prevalence.

We can summarize over several factors, in which case they must be given as a list. Two factors produces a matrix. More factors would produce a higher dimensional *array*.

tapply(diabetes\$glyhb, list(diabetes\$frame, diabetes\$gender), mean, na.rm=TRUE)

```
## small 5.042308 4.811379
## medium 5.490106 6.109464
## large 6.196286 5.929811
```

This is similar to a "pivot table", which you may have used in a spreadsheet.

Challenge

Find the age of the youngest and oldest subject, for each gender and in each location in the study.

Extension: How could we clean up the data frame so we never needed to use na.rm=TRUE when summarizing glyhb values?

Melting a matrix into a data frame

You may be starting to see that the idea of a matrix and the idea of a data frame with some factor columns are interchangeable. Depending on what we are doing, we may shift between these two representations of the same data.

Modern R usage emphasizes use of data frames over matrices, as data frames are the more flexible representation. Everything we can represent with a matrix we can represent with a data frame, but not vice versa.

tapply took us from a data frame to a matrix. We can go the other way, from a matrix to a data frame, with the melt function in the package reshape2.

```
library(reshape2)
averages <- tapply(diabetes$glyhb, list(diabetes$frame, diabetes$gender), mean, na.rm=TRUE)
melt(averages)
       Var1
              Var2
                      value
## 1 small female 5.042308
## 2 medium female 5.490106
## 3 large female 6.196286
## 4 small
              male 4.811379
## 5 medium
              male 6.109464
## 6 large
              male 5.929811
counts <- table(diabetes$frame, diabetes$gender)</pre>
melt(counts)
       Var1
              Var2 value
## 1 small female
## 2 medium female
## 3 large female
                      37
## 4 small
              male
                      30
## 5 medium
                      59
              male
## 6 large
                      54
              male
```

Tip

The aggregate function effectively combines these two steps for you. This can also be done with the popular dplyr library's summarise function. There are many variations on the basic idea behind apply!

Merging two data frames

One often wishes to merge data from two different sources. We want a new data frame with columns from both of the input data frames. This is also called a join operation.

Information about cholesterol levels for our diabetes study has been collected, and we have it in a second CSV file.

```
cholesterol <- read.csv("r-intro-files/chol.csv")
head(cholesterol)
## subject chol
## 1 S1000 203</pre>
```

```
## 2 S1001 165
## 3 S1002 228
## 4 S1005 249
## 5 S1008 248
## 6 S1011 195
```

diabetes2 <- diabetes

Great! We'll just add this new column of data to our data frame.

```
diabetes2$chol <- cholesterol$chol
## Error in `$<-.data.frame`(`*tmp*`, "chol", value = c(203L, 165L, 228L, : replacement has 362 row</pre>
```

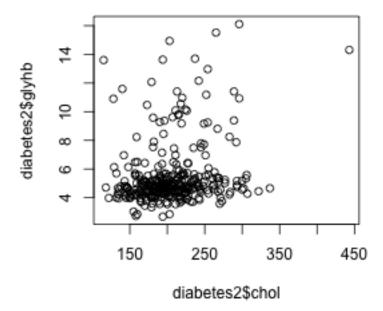
Oh. The two data frames don't have exactly the same set of subjects. We should also have checked if they were even in the same order before blithely combining them. R has shown an error this time, but there are ways to mess up like this that would not show an error. How embarassing.

```
nrow(diabetes)
## [1] 354
nrow(cholesterol)
## [1] 362
length( intersect(diabetes$subject, cholesterol$subject) )
## [1] 320
```

Inner join using the merge function

We will have to do the best we can with the subjects that are present in both data frames (an "inner join"). The merge function lets us merge the data frames.

```
diabetes2 <- merge(diabetes, cholesterol, by="subject")</pre>
nrow(diabetes2)
## [1] 320
head(diabetes2)
     subject glyhb
                     location age gender height weight frame diabetic chol
## 1 S10001 4.01 Buckingham 21 female
                                              65
                                                    169
                                                         large
                                                                  FALSE 132
     S10016 6.39 Buckingham
                               71 female
                                              63
                                                    244
                                                         large
                                                                  FALSE
                                                                         228
       S1002 4.64 Buckingham
                               58 female
                                              61
                                                    256
                                                         large
                                                                  FALSE
                                                                         228
     S10020 7.53 Buckingham
## 4
                               64
                                              71
                                                    225
                                                                         181
                                    male
                                                         large
                                                                   TRUE
## 5
       S1005 7.72 Buckingham
                                              68
                                                    183 medium
                                                                   TRUE
                                                                         249
                                    male
       S1008 4.81 Buckingham
                                                                  FALSE
## 6
                                              71
                                                    190
                                                                         248
                                    male
                                                         large
plot(diabetes2$chol, diabetes2$glyhb)
```



Note that the result is in a different order to the input. However it contains the correct rows.

Left join using the merge function

merge has various optional arguments that let us tweak how it operates. For example if we wanted to retain all rows from our first data frame we could specify all.x=TRUE. This is a "left join".

```
diabetes3 <- merge(diabetes, cholesterol, by="subject", all.x=TRUE)</pre>
nrow(diabetes3)
## [1] 354
head(diabetes3)
     subject glyhb
                      location age gender height weight frame diabetic chol
      S10000 4.83 Buckingham
                                                      164 small
                                                                    FALSE
                                      male
                                                76
                                                                            NA
      S10001 4.01 Buckingham
                                 21 female
                                                65
                                                      169 large
                                                                    FALSE
                                                                           132
## 3
      S10016
              6.39 Buckingham
                                 71 female
                                                63
                                                      244 large
                                                                    FALSE
                                                                           228
       S1002
              4.64 Buckingham
                                 58
                                    female
                                                61
                                                      256 large
                                                                    FALSE
                                                                           228
## 5
      S10020
              7.53 Buckingham
                                 64
                                      male
                                                71
                                                      225 large
                                                                     TRUE
                                                                           181
## 6
       S1003
              4.63 Buckingham
                                                      119 large
                                                                    FALSE
                                      male
                                                67
                                                                            NA
```

The data missing from the second data frame is indicated by NAs.

Tip

Besides merge, there are various ways to join two data frames in R.

• In the simplest case, if the data frames are the same length and in the same order, cbind ("column bind") can be used to put them next to each other in one larger data frame.

- The match function can be used to determine how a second data frame needs to be shuffled in order to match the first one. Its result can be used as a row index for the second data frame
- The dplyr package offers various join functions: left_join, inner_join, outer_join, etc. One advantage of these functions is that they preserve the order of the first data frame.

Chapter 4

For loops

We are not covering much about the programming side of R today. However for loops are useful even for interactive work.

If you intend to take your knowledge of R further, you should also investigate writing your own functions, and if statements.

For loops are the way we tell a computer to perform a repetitive task. Under the hood, many of the functions we have been using today use for loops.

If we can't find a ready made function to do what we want, we may need to write our own for loop.

Preliminary: blocks of code

Suppose we want to print each word in a sentence, and for some reason we want to do this all at once. One way is to use six calls to print:

```
sentence <- c("Let", "the", "computer", "do", "the", "work")
{
   print(sentence[1])
   print(sentence[2])
   print(sentence[3])
   print(sentence[4])
   print(sentence[6])
}

## [1] "Let"
## [1] "the"
## [1] "do"
## [1] "the"
## [1] "work"</pre>
```

R treats the code between the { and the } as a single "block". It reads it in as a single unit, and then executes each line in turn with no further interaction.

For loops

What we did above was quite repetitive. It's always better when the computer does repetitive work for us. Here's a better approach, using a for loop:

We can name the loop variable anything we like (with a few restrictions, e.g. the name of the variable cannot start with a digit). in is part of the for syntax. Note that the body of the loop is enclosed in curly braces { }. For a single-line loop body, as here, the braces aren't needed, but it is good practice to include them as we did.

Accumulating a result

Here's another loop that repeatedly updates a variable:

```
len <- 0
vowels <- c("a", "e", "i", "o", "u")
for(v in vowels) {
  len <- len + 1
}
# Number of vowels
len
## [1] 5</pre>
```

It's worth tracing the execution of this little program step by step. Since there are five elements in the vector vowels, the statement inside the loop will be executed five times. The first time around, len is zero (the value assigned to it on line 1) and v is "a". The statement adds 1 to the old value of len, producing 1, and updates len to refer to that new value. The next time around, v is "e" and len is 1, so len is updated to be 2. After three more updates, len is 5; since there is nothing left in the vector vowels for R to process, the loop finishes.

By inserting calls to print or cat in the code, we can see that this is exactly what has happened:

```
len <- 0
vowels <- c("a", "e", "i", "o", "u")
for(v in vowels) {
  len <- len + 1
  cat("v is", v ,"and len is now", len, "\n")
}</pre>
```

```
## v is a and len is now 1
## v is e and len is now 2
## v is i and len is now 3
## v is o and len is now 4
## v is u and len is now 5
```

Note that a loop variable is just a variable that's being used to record progress in a loop. It still exists after the loop is over, and we can re-use variables previously defined as loop variables as well:

```
letter <- "z"
for(letter in c("a", "b", "c")) {
    print(letter)
}
## [1] "a"
## [1] "b"
## [1] "c"
# after the loop, letter is
letter
## [1] "c"</pre>
```

Challenge - Using loops

1. Recall that we can use: to create a sequence of numbers.

```
1:5
## [1] 1 2 3 4 5
```

Suppose the variable n has been set with some value, and we want to print out the numbers up to that value, one per line.

```
n <- 7
```

Write a for loop to achieve this.

2. Suppose we have a vector called vec and we want to find the total of all the numbers in vec.

```
vec <- c(7, 30, 300, 1000)
```

Write a for loop to calculate this total.

(R has a built-in function called sum that does this for you. Please don't use it for this exercise.)

3. Multiplication.

Suppose variables a and b have been set to whole numbers:

```
a <- 6
b <- 7
```

Use a for loop to calculate a times b. Do not use *.

Hint: In challenge 1 you found a way to do something n times!

Try your loop with various different values in a and b.

Loading a set of files

for(year in years) {

Let's look at a more practical example of a for loop, following the pattern of accumulating a result that we've just seen. We have been given some demographic data from the Gapminder project, but unfortunately it is split into individual years r-intro-files/gapminder-NNNN.csv. We would like to load all of these CSV files into a single data frame.

read.csv can only read one file at a time, so we will need to call read.csv many times.

I will be using a couple of useful functions we haven't seen before, seq and paste0. As usual, you can look these up in the help system with ?seq and ?paste0.

```
years <- seq(1952, 2007, 5)
years
## [1] 1952 1957 1962 1967 1972 1977 1982 1987 1992 1997 2002 2007
# We could also have written
# years <- c(1952, 1957, <etc> )
```

We will make filenames with paste0 which pastes several values together as a character string.

```
paste0("r-intro-files/gapminder-", 1952, ".csv")
## [1] "r-intro-files/gapminder-1952.csv"
```

We will loop over all of the years, and build up a data frame. We start with NULL, which is a special value in R meaning nothing at all. We add to this with rbind, which concatenates the rows of data frames.

```
gap <- NULL
for(year in years) {
    filename <- paste0("r-intro-files/gapminder-", year, ".csv")
    gap_year <- read.csv(filename)
    gap <- rbind(gap, gap_year)
}</pre>
Again, print or cat can be used to check everything is working correctly.
```

gap <- NULL

```
filename <- pasteO("r-intro-files/gapminder-", year, ".csv")

cat("Loading", filename, "\n")

gap_year <- read.csv(filename)

cat("Read", nrow(gap_year), "rows\n")

gap <- rbind(gap, gap_year)

cat("Now have", nrow(gap), "rows in gap\n")
}

## Loading r-intro-files/gapminder-1952.csv

## Read 142 rows

## Now have 142 rows in gap

## Loading r-intro-files/gapminder-1957.csv

## Read 142 rows

## Now have 284 rows in gap

## Loading r-intro-files/gapminder-1962.csv</pre>
```

```
## Read 142 rows
## Now have 426 rows in gap
## Loading r-intro-files/gapminder-1967.csv
## Read 142 rows
## Now have 568 rows in gap
## Loading r-intro-files/gapminder-1972.csv
## Read 142 rows
## Now have 710 rows in gap
## Loading r-intro-files/gapminder-1977.csv
## Read 142 rows
## Now have 852 rows in gap
## Loading r-intro-files/gapminder-1982.csv
## Read 142 rows
## Now have 994 rows in gap
## Loading r-intro-files/gapminder-1987.csv
## Read 142 rows
## Now have 1136 rows in gap
## Loading r-intro-files/gapminder-1992.csv
## Read 142 rows
## Now have 1278 rows in gap
## Loading r-intro-files/gapminder-1997.csv
## Read 142 rows
## Now have 1420 rows in gap
## Loading r-intro-files/gapminder-2002.csv
## Read 142 rows
## Now have 1562 rows in gap
## Loading r-intro-files/gapminder-2007.csv
## Read 142 rows
## Now have 1704 rows in gap
nrow(gap)
## [1] 1704
head(gap)
##
         country continent year lifeExp
                                             pop
                                                  gdpPercap
## 1 Afghanistan
                      Asia 1952 28.801 8425333
                                                   779.4453
## 2
         Albania
                   Europe 1952 55.230
                                        1282697
                                                  1601.0561
## 3
         Algeria
                    Africa 1952 43.077 9279525
                                                  2449.0082
## 4
                    Africa 1952 30.015 4232095
                                                  3520.6103
          Angola
## 5
       Argentina Americas 1952 62.485 17876956 5911.3151
## 6
       Australia
                   Oceania 1952 69.120 8691212 10039.5956
```

When to use for loops

Many of the functions and operators we have been using are implemented using for loops, so often in R we are able to use these rather than directly writing a for loop. However when we need to do something complicated, for loops are there for us. Some real world reasons you might use a for loop:

- To create a collection of similar plots.
- To load and process a collection of files, all in the same way.
- To run a program outside of R, such as a read aligner, with each of a collection of files as input. Programs outside of R can be run using system.

• To perform resampling such as a permutation test or a bootstrap, to assure yourself that some region you have calculated is not simply due to chance.					

Chapter 5

Plotting with ggplot2

We already saw some of R's built in plotting facilities with the function plot. A more recent and much more powerful plotting library is ggplot2. This implements ideas from a book called "The Grammar of Graphics". The syntax is a little strange, but there are plenty of examples in the online documentation¹.

If ggplot2 isn't already installed, we need to install it.

```
install.packages("ggplot2")
```

We then need to load it.

```
library(ggplot2)
```

Producing a plot with ggplot2, we must give three things:

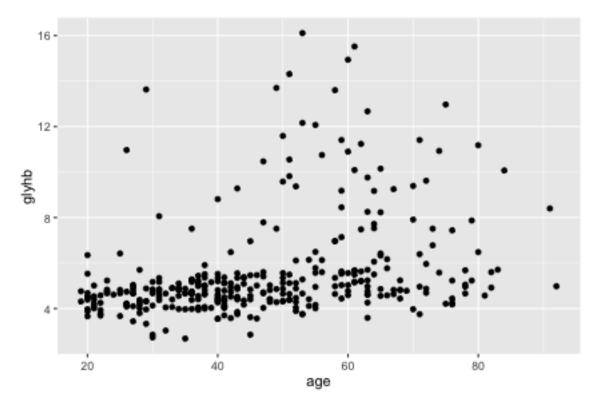
- 1. A data frame containing our data.
- 2. How the columns of the data frame can be translated into positions, colors, sizes, and shapes of graphical elements ("aesthetics").
- 3. The actual graphical elements to display ("geometric objects").

Using ggplot2 with a data frame

Let's load up our diabetes data frame again.

```
diabetes <- read.csv("r-intro-files/diabetes.csv")
ggplot(diabetes, aes(y=glyhb, x=age)) +
    geom_point()
## Warning: Removed 11 rows containing missing values (geom_point).</pre>
```

¹http://docs.ggplot2.org/current/



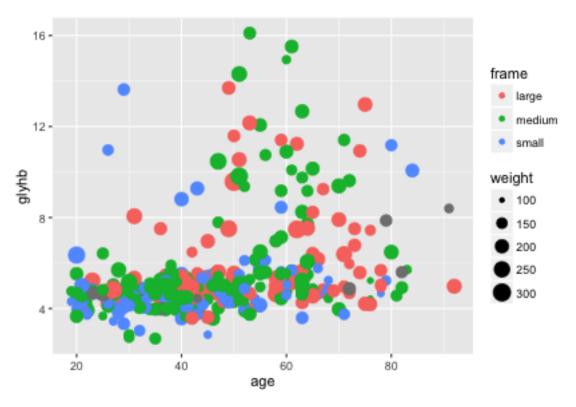
The call to ggplot and aes sets up the basics of how we are going to represent the various columns of the data frame. aes defines the "aesthetics", which is how columns of the data frame map to graphical attributes such as x and y position, color, size, etc. We then literally add layers of graphics to this.

Seasoned programmers may notice with some alarm that aes does something very odd, since its bare arguments refer to columns of the data frame as though they were variables. To those programmers we comment briefly that R uses lazy evaluation of function parameters to allow any function to potentially behave like a macro, and that this is exactly as dangerous and powerful as it sounds. It's used tastefully, for the most part.

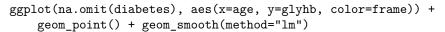
Further aesthetics can be used. Hans Rosling would be excited!

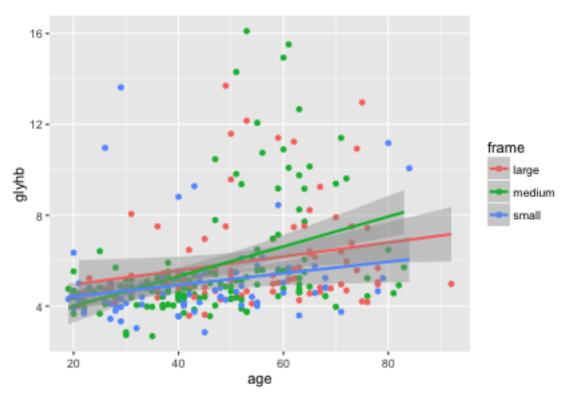
```
ggplot(diabetes, aes(x=age, y=glyhb, size=weight, color=frame)) +
    geom_point()
```

Warning: Removed 12 rows containing missing values (geom point).



We can see some trend for glyhb to increase with age, and we tend to see medium and large framed people at higher levels of glyhb. Let's try to show this trend.

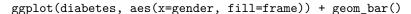


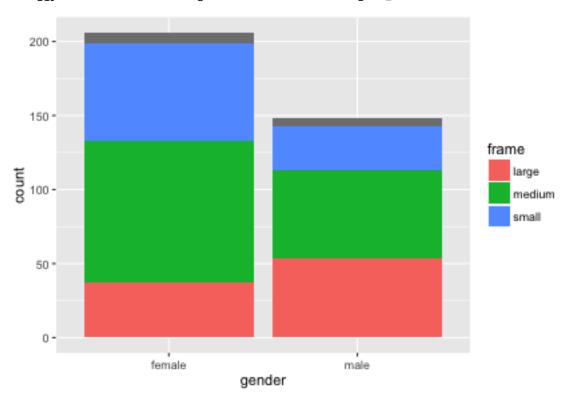


Hmm.

Some "geom"s have an associated "stat". For example <code>geom_smooth</code> overlays a curve fitted to the data. You can see in the help it has a default parameter of <code>stat="smooth"</code> which calculates this curve. If there is a grouping of the data, for example by color, then separate curves are shown for each group.

geom_bar by default uses a stat called "count", and so shows counts of rows in your data. (If you want to not use this stat, you could use geom_bar(stat="identity").)





Challenge - Gapminder data

Use the Gapminder data you loaded earlier, or cheat and load it all with:

Plot life expectancy vs GDP per capita, coloring points by continent and sizing by year.

Using ggplot2 with a matrix

Let's return to our first matrix example.

```
dat <- read.csv(file="r-intro-files/pvc.csv", row.names=1)
mat <- as.matrix(dat)</pre>
```

ggplot only works with data frames, so we need to convert this matrix into data frame form, with one measurement in each row. We can convert to this "long" form with the melt function in the library reshape2.

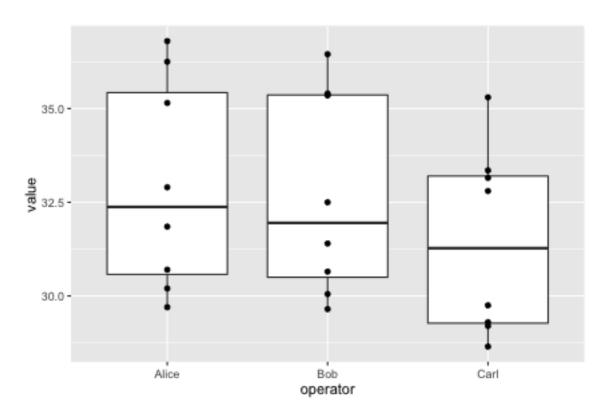
```
library(reshape2)
 long <- melt(mat)</pre>
 head(long)
 ##
        Var1 Var2 value
 ## 1 Resin1 Alice 36.25
 ## 2 Resin2 Alice 35.15
 ## 3 Resin3 Alice 30.70
 ## 4 Resin4 Alice 29.70
 ## 5 Resin5 Alice 31.85
 ## 6 Resin6 Alice 30.20
 colnames(long) <- c("resin", "operator", "value")</pre>
 head(long)
 ##
       resin operator value
 ## 1 Resin1
                Alice 36.25
 ## 2 Resin2
               Alice 35.15
 ## 3 Resin3
              Alice 30.70
 ## 4 Resin4
              Alice 29.70
 ## 5 Resin5
              Alice 31.85
 ## 6 Resin6
              Alice 30.20
 ggplot(long, aes(x=operator, y=value)) + geom_point()
35.0 -
30.0 -
                Alice
                                         Bob
                                                                 Carl
```

Notice how ggplot2 is able to use either numerical or categorical (factor) data as x and y coordinates.

operator

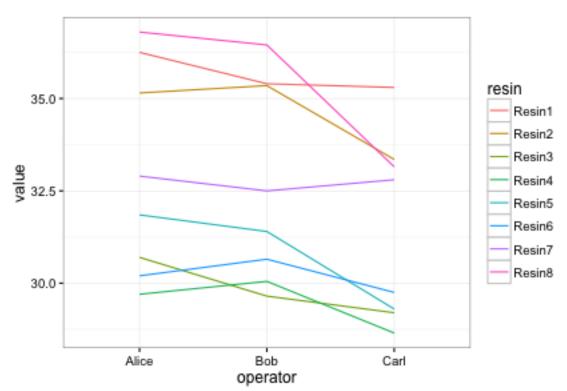
More complex plots

```
ggplot(long, aes(x=operator, y=value)) + geom_boxplot() + geom_point()
```



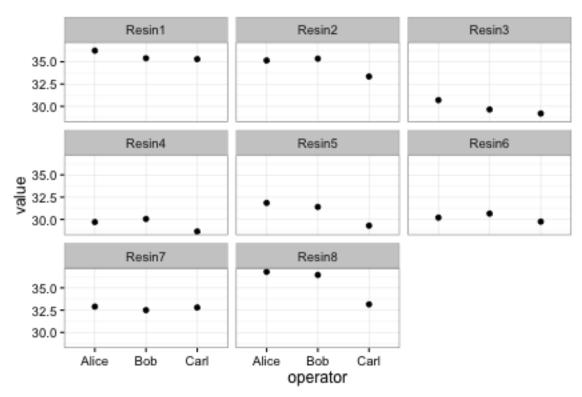
Lines need a group aesthetic.

ggplot(long, aes(x=operator, y=value, group=resin, color=resin)) +
 geom_line() + theme_bw()



Faceting lets us quickly produce a collection of small plots.

```
ggplot(long, aes(x=operator, y=value)) +
   facet_wrap(~ resin) + geom_point() + theme_bw()
```



Saving ggplots

ggplots can be saved as we talked about earlier, but with one small twist to keep in mind. The act of plotting a ggplot is actually triggered when it is printed. In an interactive session we are automatically printing each value we calculate, but if you are using a for loop, or other R programming constructs, you might need to explcitly print() the plot.

```
# Plot created but not shown.
p <- ggplot(long, aes(x=operator, y=value)) + geom_point()
# Only when we try to look at the value p is it shown
p
# Alternatively, we can explicitly print it
print(p)
# To save to a file
png("test.png")
print(p)
dev.off()</pre>
```

See also the function ggsave.

Challenge - Gapminder data

Apply what you have just learned to explore the Gapminder data further.

Think of a way to summarise the Gapminder data with tapply and visualize the summarised data.

Chapter 6

Next steps

We have barely touched the surface of what R has to offer. If you want to take your skills to the next level, here are some topics to investigate:

Cheat sheets

- RStudio's collection of cheat sheets¹ cover newer packages in R.
- An old-school cheat sheet² for dinosaurs and people wishing to go deeper.
- Bioconductor cheat sheet 3

More packages

- CRAN⁴ has hundreds of contributed packages which can be installed with install.packages.
- Bioconductor⁵ is another huge collection of packages with a biological focus.

Statistics

- Many statistical tests are built in to R.
- Linear models, and the linear model formula syntax ~, are core to much of what R has to offer statistically.
 - Many statistical techniques take linear models as their starting point, including limma which we will be using to test for differential gene expression, glm for logistic regression (etc), survival analysis, mixed models to characterize variation within populations.
 - Many R function repurpose the ~ formula syntax for other ways of relating response and explanatory variables.

See "The R Book" by Michael J. Crawley for general reference.

The books "Linear Models with R" and "Extending the Linear Model with R" by Julian J. Faraway cover linear models, with many practical examples.

¹https://www.rstudio.com/resources/cheatsheets/

²https://cran.r-project.org/doc/contrib/Short-refcard.pdf

³https://github.com/mikelove/bioc-refcard/blob/master/README.Rmd

⁴https://cran.rstudio.com/

⁵http://bioconductor.org/

Q&A sites

Stackoverflow-style sites are great for getting help:

- support.bioconductor.org⁶ for bioconductor related questions.
- biostars.org⁷ for general bioinformatics questions.
- stats.stackexchange.com⁸ for statistics questions.
- stackoverflow.com⁹ for general programming questions.

Community

The Monash Bioinformatics Platform offers:

- Weekly drop in sessions where you can get help with R, or general bioinformatics problems.
- Informal Wednesday afternoon talks, which often relate to R.
- Courses on various topics through the year.

Join the mailing list to hear about future events. 10

Also, the COMBINE¹¹ student and early career researcher organization runs Software Carpentry workshops.

⁶https://support.bioconductor.org

⁷https://biostars.org

⁸http://stats.stackexchange.com

⁹http://stackoverflow.com

¹⁰ http://bioinformatics.erc.monash.edu/ 11 https://combine.org.au/