

Introduction to R

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Preface

These are the course notes for the “Introduction to R” course given by the Monash Bioinformatics Platform on the 30th of November 2015.

Some of this material is derived from work that is Copyright © Software Carpentry¹ with a CC BY 4.0 license².

Data files we are working with are available from:

<http://monashbioinformaticsplatform.github.io/2015-11-30-intro-r/>

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"`.

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
```

¹<https://www.rstudio.com/>

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** (or **return**). 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

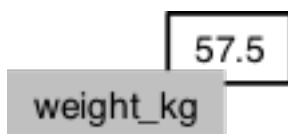
We can add comments to our code using the `#` character. It is useful to document our code in this way so that others (and us the next time we read it) have an easier time following what the code is doing.

We can also change an object’s value by assigning it a new value:

```
weight_kg <- 57.5  
# weight in kilograms is now  
weight_kg
```

```
## [1] 57.5
```

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:



This means that assigning a value to one object 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
```



and then change `weight_kg`:

```
weight_kg <- 100.0
# weight in kg now...
weight_kg

## [1] 100

# ...and weight in pounds still
weight_lb

## [1] 126.5
```



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 vector² 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, values are just vectors of length one. R is obsessed with vectors.

```
myvec <- c(1,2,3)
myvec + 1

## [1] 2 3 4

myvec + myvec

## [1] 2 4 6

length(myvec)

## [1] 3

c(10, myvec)

## [1] 10 1 2 3
```

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.

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).

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.

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()`? Try a few different combinations of types, and try to explain what R is doing.

Functions

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

```
?sum
```

```
sum( c(1,2,3) )
```

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”).

Functions take some number of *arguments*. 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
```

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
```

```
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
```

```
rep(x=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
```

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 more rarely 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 3 1 1 1 2 2 2 3 3 3
## [36] 3 1 1 1 2 2 2 3 3 3
```

Lists

Vectors contain all the same kind of thing. Try `c(42, "hello")`. Lists can contain different kinds of thing.

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"
```

This terminology is peculiar to R. Other languages make the same distinction but they may use different words for vectors and lists.

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

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 also functions to “cast” it to the right type.

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

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”. 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. 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()`

³For 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 sets are stored in comma-separated values (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(file="data/intro-r/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.

The filename means from the current working directory, in the sub-directory “data”, in the sub-directory “intro-r”, 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 `intro-r.zip` file, the files may be in a different location.

`read.csv` has 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, `row.names`, to be 1 indicates that the data file has row names, and which column number they are stored in.

Tip

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

```
dat <- read.csv(file="data/intro-r/pvc.csv", row.names=1)
```

```
dat
```

```
##      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 ...
## $ Bob  : num  35.4 35.4 29.6 30.1 31.4 ...
## $ 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 interchangeably. 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.

```
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"
```

Much better.

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

Tip

Matrices can also be created de novo 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 top of each other to form a matrix. Or it can stack smaller matrices on top of each other to form a larger matrix.

`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 see the dimensions, or “shape”, 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 data frame, we can provide an 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 data frame, 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:

```
1:4
```

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

```
1:2
```

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

```
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 `[]`.

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 to create a new temporary vector (or matrix, or data frame), 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
```

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 also 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])
```

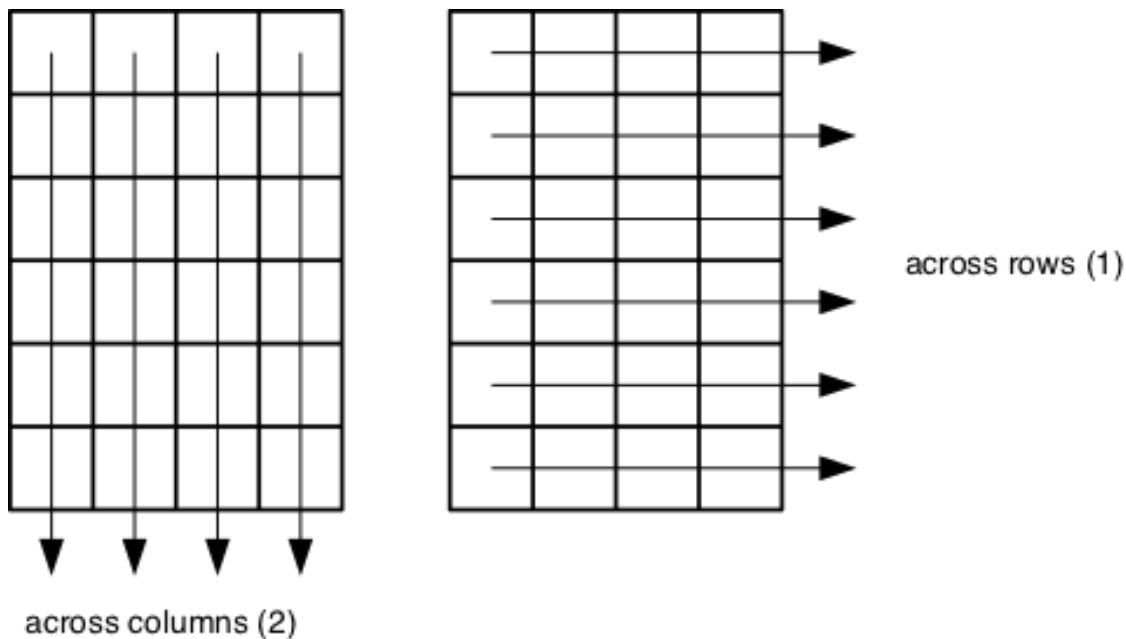
```
## [1] 31.275
```

```
# 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 documentation 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.

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)
```

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)
```

Since the second argument to `apply` is `MARGIN`, the above command is equivalent to `apply(dat, MARGIN = 2, mean)`. We'll learn why this is so in the next lesson.

Tip

Some common operations have more efficient 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:

```
animal <- c("m", "o", "n", "k", "e", "y")
# first three characters
animal[1:3]
```

```
## [1] "m" "o" "n"
```

```
# last three characters
```

```
animal[4:6]
```

```
## [1] "k" "e" "y"
```

1. If the first four characters are selected using the slice `animal[1:4]`, how can we obtain the first four characters in reverse order?
2. What is `animal[-1]`? What is `animal[-4]`? Given those answers, explain what `animal[-1:-4]` does.
3. Use a slice of `animal` to create a new character vector that spells the word “eon”, i.e. `c("e", "o", "n")`.

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 data frame 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. A classic test 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 data.

```
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
```

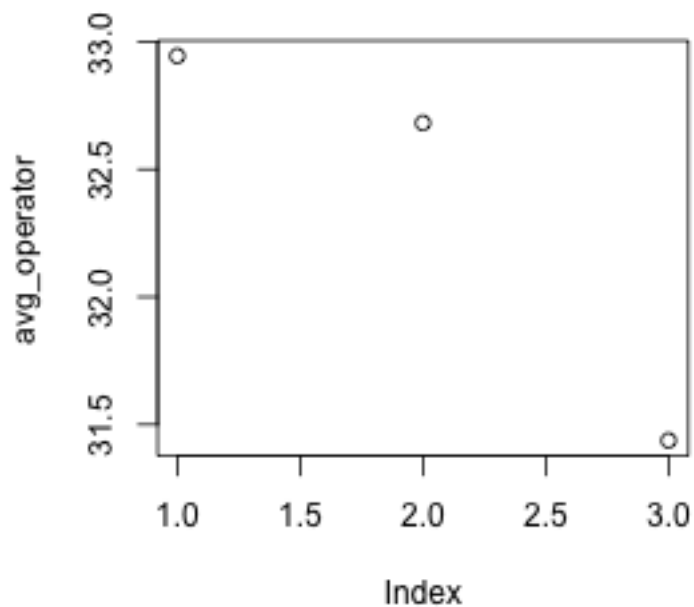
This means we can write software that uses the various results from t-test, for example performing a whole series of t-tests and reporting the significant results.

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.

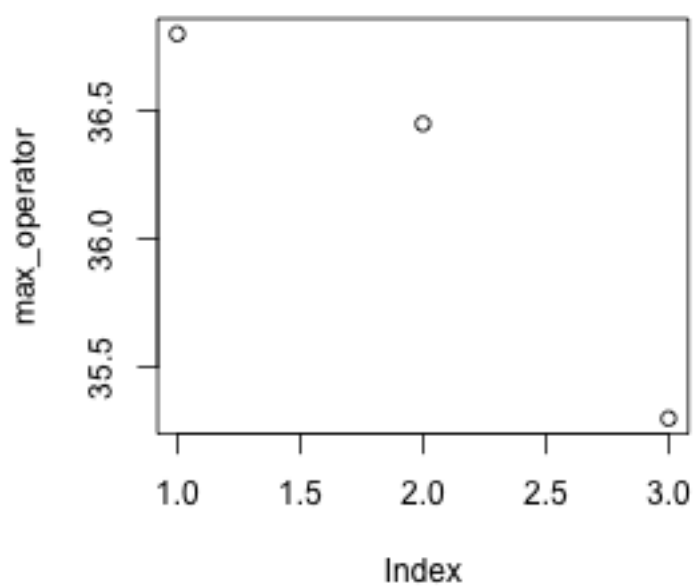
Let’s take a look at the average inflammation over time. Recall that we already calculated these values above using `apply(mat, 2, mean)` and saved them in the variable `avg_operator`. Plotting the values is done with the function `plot`.

```
plot(avg_operator)
```

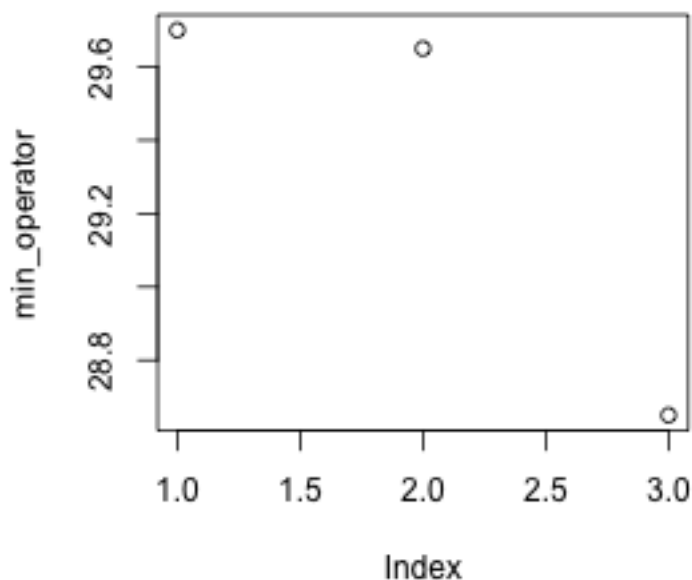


Above, we gave the function `plot` a vector of numbers corresponding to the average per operator across all resins. `plot` created a scatter plot where the y-axis is the average particle size and the x-axis is the order, or index, of the values in the vector, which in this case correspond to the 3 operators. Let's have a look at two other statistics: the maximum and minimum inflammation per operator.

```
max_operator <- apply(mat, 2, max)
plot(max_operator)
```



```
min_operator <- apply(dat, 2, min)
plot(min_operator)
```



Challenge - Plotting data

Create a plot showing the standard deviation of for each operator across all resins.

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 automate plot making, we need 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 it, your interactive plots will stop appearing as expected!

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("data/intro-r/diabetes.csv")

class(diabetes)

## [1] "data.frame"

head(diabetes)

##   subject glyhb   location age gender height weight  frame
## 1  S1002  4.64 Buckingham  58 female    61   256   large
## 2  S1003  4.63 Buckingham  67  male    67   119   large
## 3  S1005  7.72 Buckingham  64  male    68   183 medium
## 4  S1008  4.81 Buckingham  34  male    71   190   large
## 5  S1011  4.84 Buckingham  30  male    69   191 medium
## 6  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 de novo 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 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      S2794  8.40 Buckingham 91 female    61    127   <NA>
## 90      S4803  5.71      Louisa 83 female    59    125 medium
## 130     S13500  5.60      Louisa 82  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
## 323     S40786  6.48      Louisa 80  male    71    212 medium
## 324     S40789 11.18      Louisa 80 female    62    162  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
```

```
## [1] 45 56 90 130 139 193 321 323 324
```

```
diabetes[which_over_80,]
```

```
##      subject glyhb  location age gender height weight  frame
## 45      S2770  4.98 Buckingham 92 female    62    217  large
## 56      S2794  8.40 Buckingham 91 female    61    127  <NA>
## 90      S4803  5.71      Louisa 83 female    59    125 medium
## 130     S13500  5.60      Louisa 82  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
## 323     S40786  6.48      Louisa 80  male    71    212 medium
## 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 <= y` “less than or equal to”
- `x >= 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"
```

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 Glycosylated Haemoglobin. 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)
```

```
##      subject glyhb  location age gender height weight  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  male    68    183 medium     TRUE
## 4      S1008  4.81 Buckingham 34  male    71    190  large    FALSE
## 5      S1011  4.84 Buckingham 30  male    69    191 medium    FALSE
## 6      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 glyhb?

Are any of them diabetic?

Factors

When R loads a CSV file, it tries to give appropriate types to the columns. Lets 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 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 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 a parameter **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. Lets adjust that for the column **diabetes\$frame**.

```
diabetes$frame <- factor(diabetes$frame, levels=c("small","medium","large"))

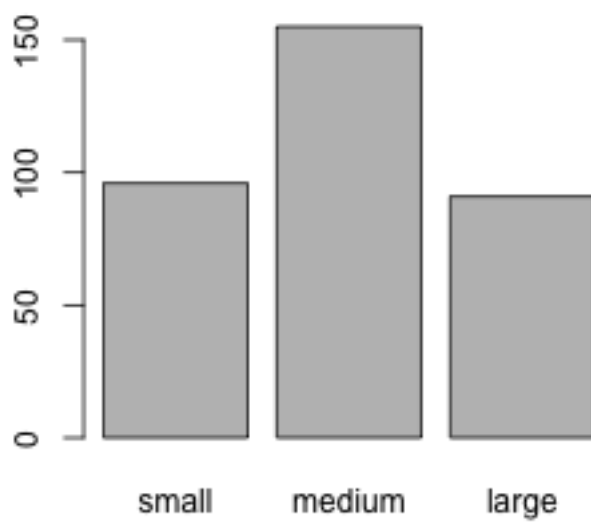
head( diabetes$frame )

## [1] large large medium large medium medium
## Levels: small medium large
```

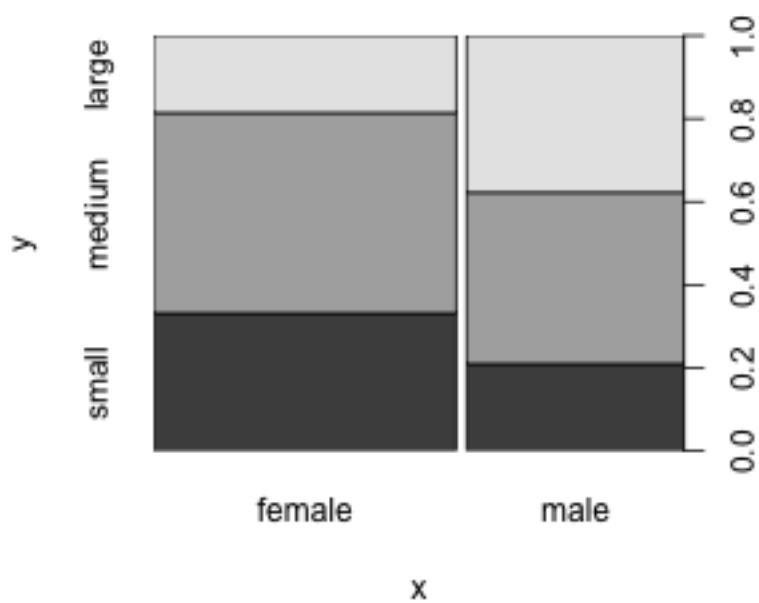
Plotting factors

If we plot factors, we can see that R uses the order of levels in the plot.

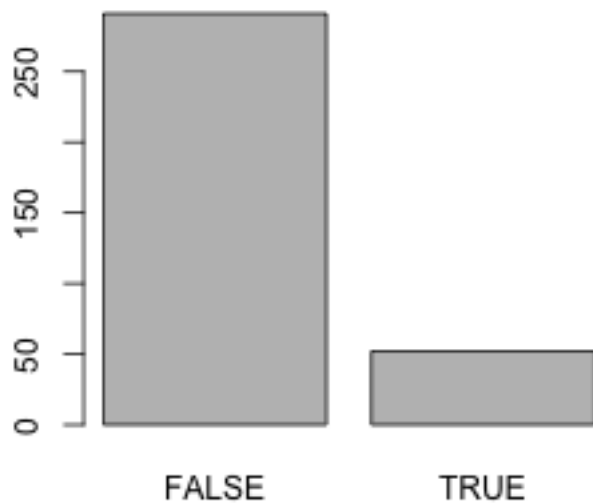
```
plot( diabetes$frame )
```



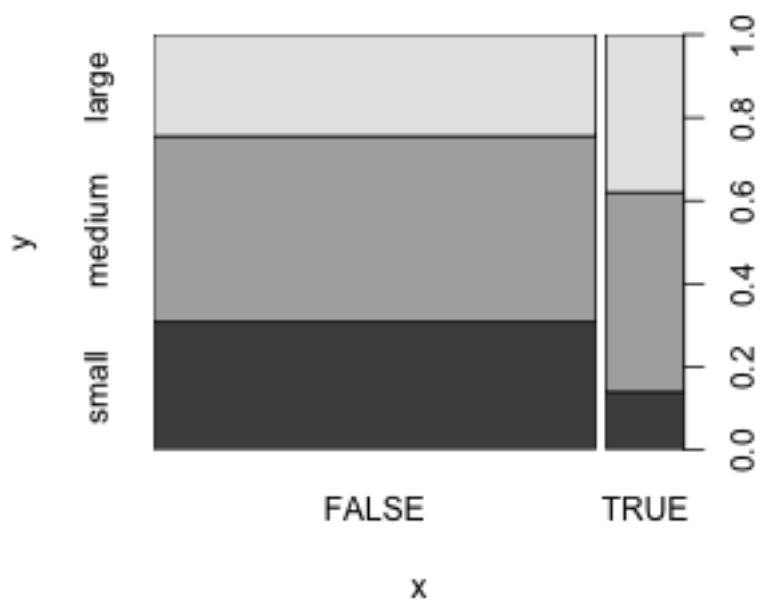
```
plot( diabetes$gender, diabetes$frame )
```




```
plot( factor(diabetes$diabetic) )
```



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



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
##  FALSE      87    126    69
##  TRUE       7     24    19
```

Fisher’s Exact Test (`fisher.test`) or a chi-squared test (`chisq.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 any association between gender and whether a person is diabetic is shown by this data set?

Why?

Missing data

`summary` gives an overview of a data frame.

```
summary(diabetes)

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

```
##           Median :66.00   Median :171.0   large : 91   TRUE :52
##           Mean   :65.93   Mean   :176.2   NA's  : 12   NA's :11
##           3rd Qu.:69.00   3rd Qu.:198.0
##           Max.   :76.00   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 very 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 perform this removal manually using logical indexing.

```
mean( diabetes$glyhb[ !is.na(diabetes$glyhb) ] )
```

```
## [1] 5.580292
```

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`.

```
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.

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)
```

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

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)
melt(counts)
```

```
##      Var1  Var2 value
## 1 small female    66
## 2 medium female    96
## 3 large  female    37
## 4 small   male     30
## 5 medium   male     59
## 6 large   male     54
```

Tip

The `aggregate` function effectively combines these two steps for you. See also the `ddply` function in package `plyr`, and the `dplyr` package. 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("data/intro-r/chol.csv")
head(cholesterol)
```

```
##   subject chol
## 1   S1000  203
## 2   S1001  165
## 3   S1002  228
## 4   S1005  249
## 5   S1008  248
## 6   S1011  195
```

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

```
diabetes2 <- diabetes
diabetes2$chol <- cholesterol$chol
```

```
## Error in `$<-.data.frame`(`*tmp*`, "chol", value = c(203L, 165L, 228L, : replacement has 362 rows
```

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

```
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")
```

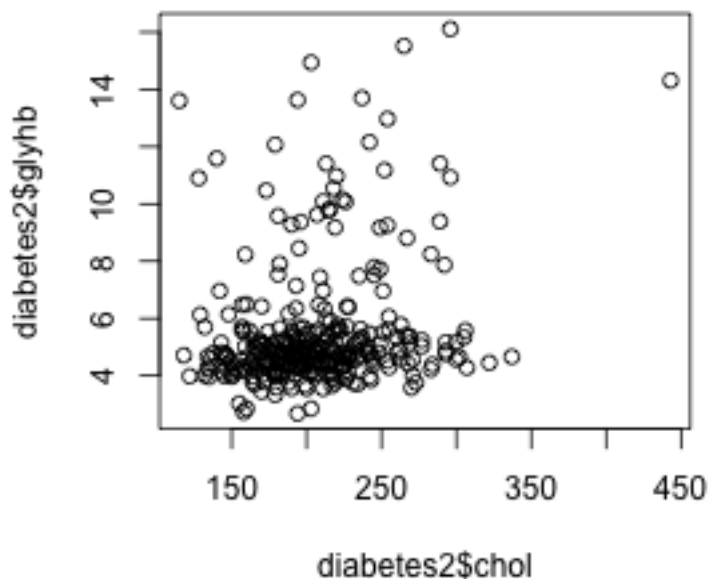
```
nrow(diabetes2)
```

```
## [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
## 2  S10016  6.39 Buckingham 71 female   63   244  large   FALSE  228
## 3   S1002  4.64 Buckingham 58 female   61   256  large   FALSE  228
## 4  S10020  7.53 Buckingham 64  male   71   225  large    TRUE  181
## 5   S1005  7.72 Buckingham 64  male   68   183 medium    TRUE  249
## 6   S1008  4.81 Buckingham 34  male   71   190  large   FALSE  248
```

```
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)
```

```
nrow(diabetes3)
```

```
## [1] 354
```

```
head(diabetes3)
```

```
##   subject glyhb   location age gender height weight frame diabetic chol
## 1  S10000  4.83 Buckingham 23  male   76   164 small   FALSE   NA
## 2  S10001  4.01 Buckingham 21 female   65   169 large   FALSE  132
## 3  S10016  6.39 Buckingham 71 female   63   244 large   FALSE  228
## 4   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 67  male   67   119 large   FALSE   NA
```

The missing data 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[5])
  print(sentence[6])
}

## [1] "Let"
## [1] "the"
## [1] "computer"
## [1] "do"
## [1] "the"
## [1] "work"
```

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:


```
for (word in sentence) {
  print(word)
}
```

```
## [1] "Let"
## [1] "the"
## [1] "computer"
## [1] "do"
## [1] "the"
## [1] "work"
```

The general form of a loop is:

```
for (variable in collection) {
  do things with variable
}
```

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
```

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.

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"
```

You can perhaps now start to see how functions like `sum` work internally.

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.

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`.

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. Exponentiation is built into R:

```
2^4

## [1] 16
```

Suppose variables `base` and `power` have been set.

Write a for loop to raise `base` to the power `power`.

Try it with various different values in `base` and `power`.

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)
```

```
## Loading required package: methods
```

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

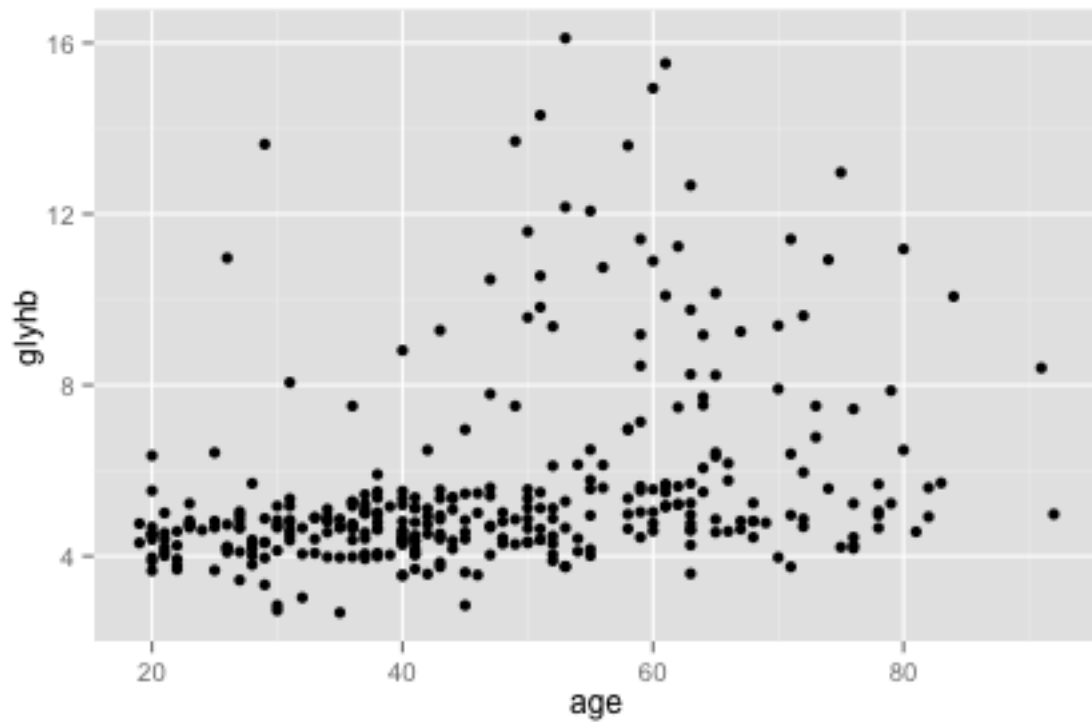
Let’s load up our diabetes data frame again.

```
diabetes <- read.csv("data/intro-r/diabetes.csv")
```

```
ggplot(diabetes, aes(y=glyhb, x=age)) +  
  geom_point()
```

```
## Warning: Removed 11 rows containing missing values (geom_point).
```

¹<http://docs.ggplot2.org/current/>

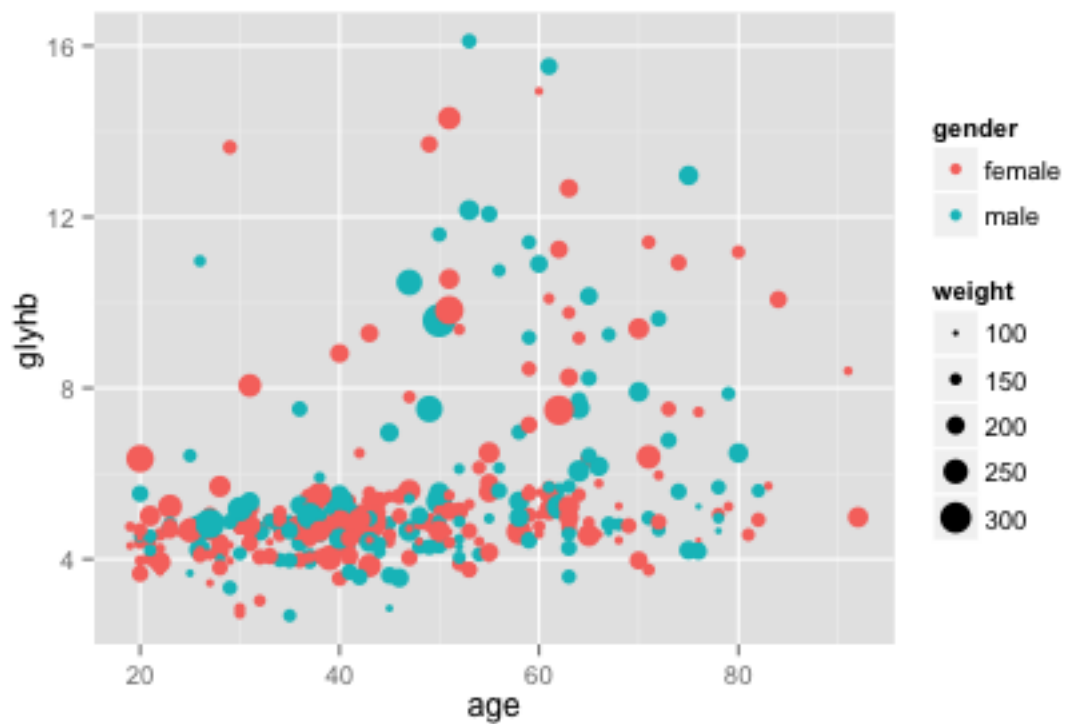


The call to `ggplot` sets up the basics of how we are going to represent the various columns of the data frame. We then literally add layers of graphics to this.

Further aesthetics can be added.

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

Warning: Removed 12 rows containing missing values (geom_point).



Using ggplot2 with a matrix

Let's return to our first matrix example.

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

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`.

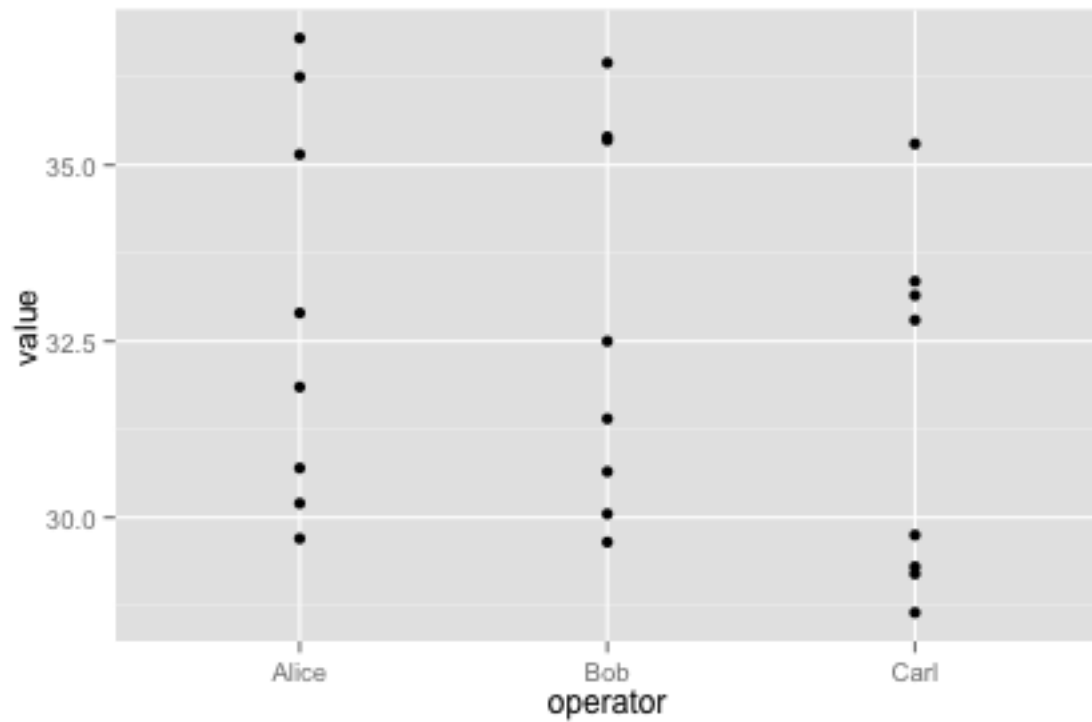
```
library(reshape2)
long <- melt(mat)
head(long)
```

```
##      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")
head(long)
```

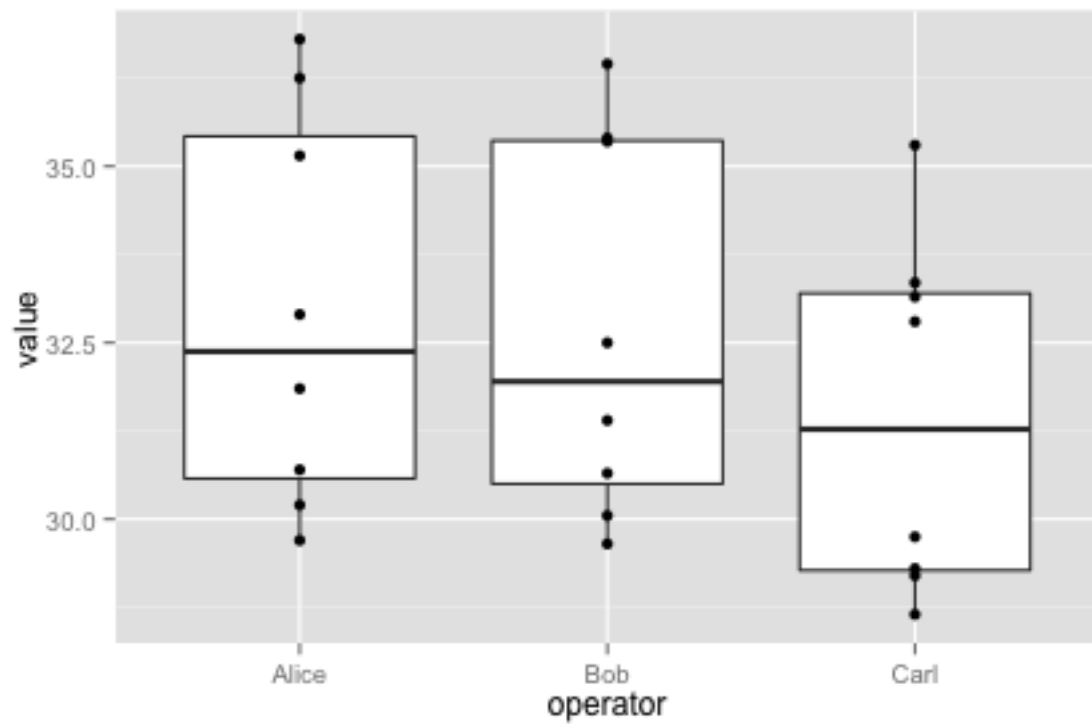
```
##      resin operator value
## 1 Resin1      Alice 36.25
## 2 Resin2      Alice 35.15
## 3 Resin3      Alice 30.70
## 4 Resin4      Alice 29.70
## 5 Resin5      Alice 31.85
## 6 Resin6      Alice 30.20
```

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

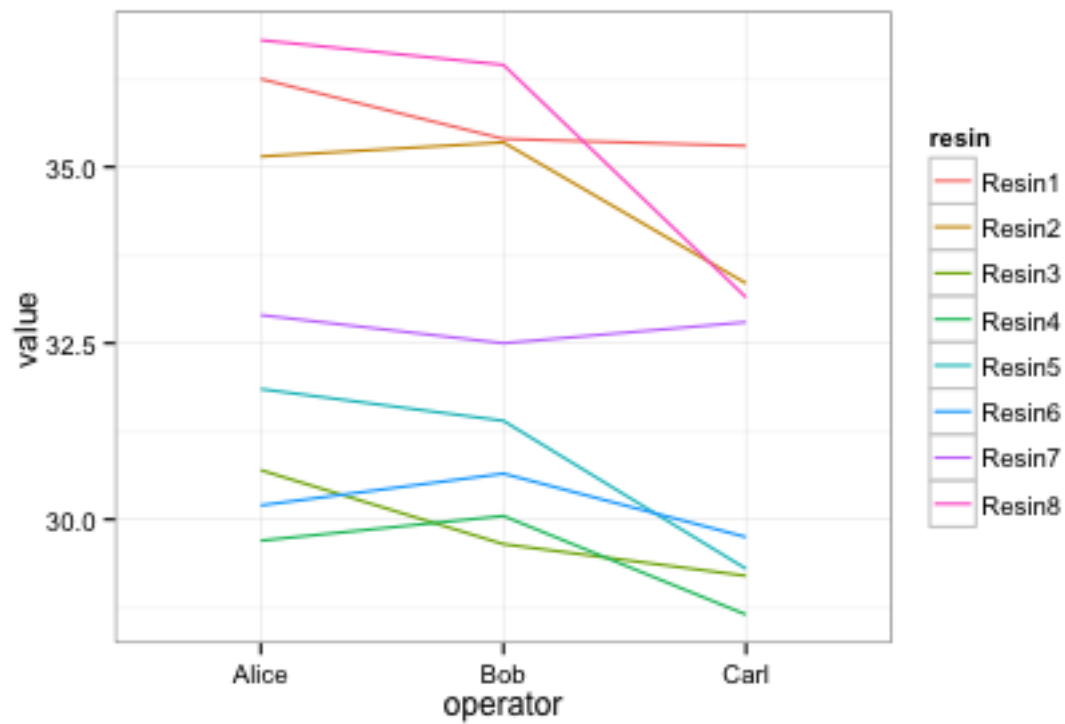


Notice how ggplot2 is able to use either numerical or categorical (factor) data as x and y coordinates.

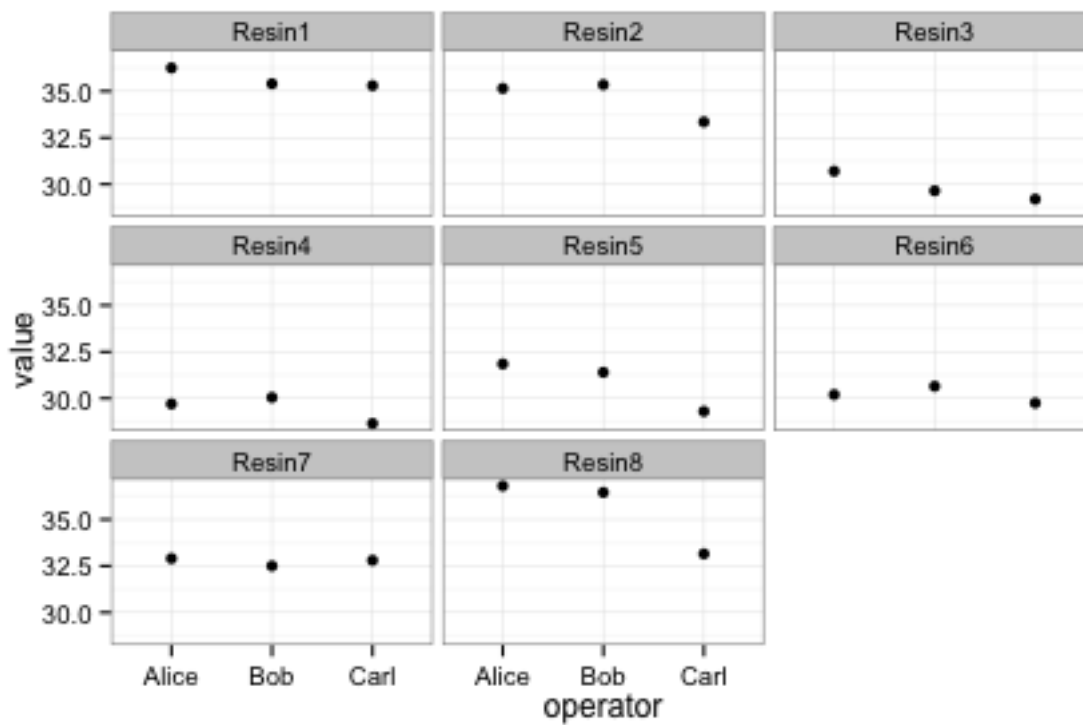
```
ggplot(long, aes(x=operator, y=value)) + geom_boxplot() + geom_point()
```



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



```
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 explicitly `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()
```

See also the function `ggsave`.

Chapter 6

Next steps

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

Programming

- Writing functions.
- Using if statements.

The Software Carpentry in R¹ course introduces R as a programming language.

Tidying and summarizing data

- `plyr`², `dplyr`³, and `tidyr`⁴ packages by Hadley Wickham.
- `magrittr`⁵'s `%>%` operator for chaining together data frame manipulations.

These tools play well with `ggplot2`, which we saw in the previous chapter.

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 `edgeR` which we will be using to test for differential gene expression.
 - 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.

¹<http://swcarpentry.github.io/r-novice-inflammation/>

²<http://plyr.had.co.nz/>

³<https://cran.rstudio.com/web/packages/dplyr/vignettes/introduction.html>

⁴<http://blog.rstudio.org/2014/07/22/introducing-tidyr/>

⁵<https://cran.r-project.org/web/packages/magrittr/vignettes/magrittr.html>

Bioinformatics

Bioconductor⁶ is a collection of bioinformatics related packages, including the popular `limma` and `edgeR` packages for RNA-Seq analysis developed at the Walter and Eliza Hall Institute.

Getting help

Stackoverflow-style sites are great for getting help:

- support.bioconductor.org⁷ for bioconductor related questions.
- biostars.org⁸ for general bioinformatics questions.
- stats.stackexchange.com⁹ for statistics questions.
- stackoverflow.com¹⁰ for general programming questions.

⁶<http://bioconductor.org>

⁷<https://support.bioconductor.org>

⁸<https://biostars.org>

⁹<http://stats.stackexchange.com>

¹⁰<http://stackoverflow.com>