

## Introduction to R

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# Chapter 1

## Introduction

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

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

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## Chapter 2

# Starting out in R

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 RStudio<sup>1</sup>. 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
```

`*` 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
```

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.

---

<sup>1</sup><https://www.rstudio.com/>

```
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

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

c(10, myvec)

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

Access elements of a vector with `[ ]`, for example `myvec[1]`.

We will also encounter character strings, for example `"hello"`. R also has something called "factors", which are categorical vectors, and behave very much like character vectors (think the factors in an experiment). R likes turning character vectors into factors, which is usually fine.

## 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*. Lets 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
```

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
```

```
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
```

```
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
```

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 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"
```

I'm skipping a lot of details here.

This terminology is peculiar to R. Other languages make the same distinction 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).

## Chapter 3

# 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<sup>1</sup> (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/pvc.csv", row.names=1)
```

The expression `read.csv(...)` is a function call<sup>2</sup> that asks R to run the function `read.csv`.

`read.csv` has two arguments<sup>3</sup>: the name of the file we want to read, and which column contains the row names. The filename needs to be a character string (or string<sup>4</sup> for short), 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. You can learn more about these options in this supplementary lesson<sup>5</sup>.

The utility of a function is that it will perform its given action on whatever value is passed to the named argument(s). For example, in this case if we provided the name of a different file to the argument `file`, `read.csv` would read it instead. We'll learn more of the details about functions and their arguments shortly.

```
dat <- read.csv(file="data/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
```

---

<sup>1</sup>[reference.html#comma-separated-values-\(csv\)](#)

<sup>2</sup>[reference.html#function-call](#)

<sup>3</sup>[reference.html#argument](#)

<sup>4</sup>[reference.html#string](#)

<sup>5</sup>[01-supp-read-write-csv.html](#)

```
## 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 a matrix. 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"
```

Much better.

## 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.

## Manipulating Data

We can see the dimensions, or shape<sup>6</sup>, of the matrix with the functions `nrow` and `ncol`:

---

<sup>6</sup>[reference.html#shape-\(of-an-array\)](#)

```
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<sup>7</sup> in square brackets, just as we do in math:

```
# 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:

```
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<sup>8</sup> `1:4` means, “Start at index 1 and go to index 4.” It’s the same as `c(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
```

---

<sup>7</sup>[reference.html#index](#)

<sup>8</sup>[reference.html#slice](#)

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 a slice for either the rows or the columns. If we don't include a slice for the rows, R returns all the rows; if we don't include a slice for the columns, R returns all the columns. If we don't provide a slice for either rows or columns, e.g. `dat[, ]`, 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
```

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])

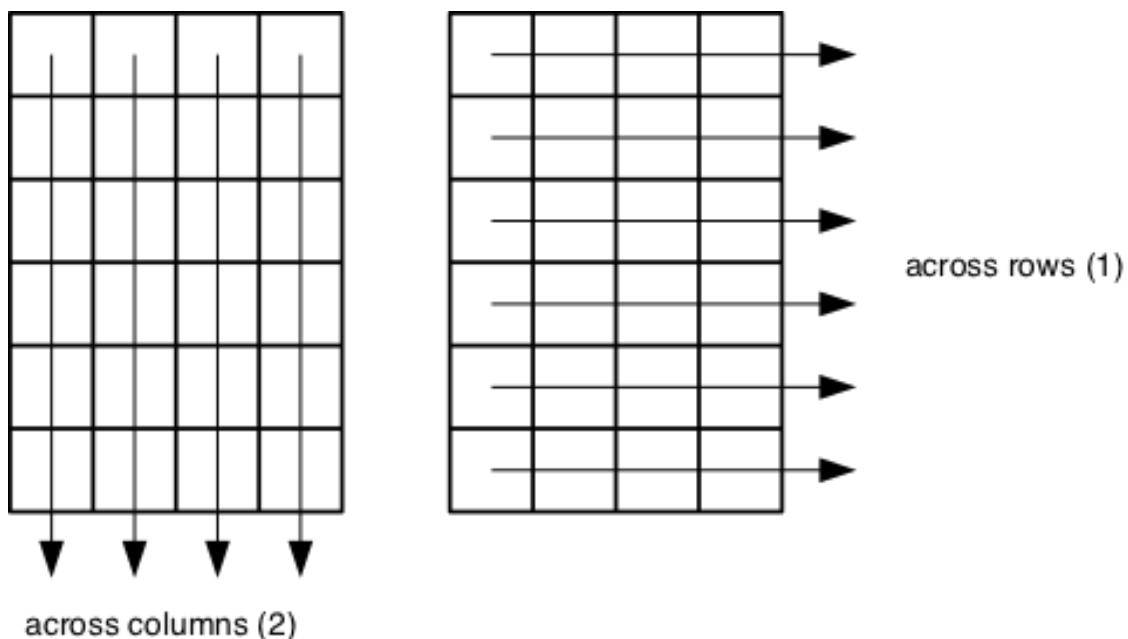
## [1] 31.275

# standard deviation for operator 3
sd(mat[, 3])

## [1] 2.49453

```

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])`

## A t-test or two

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.

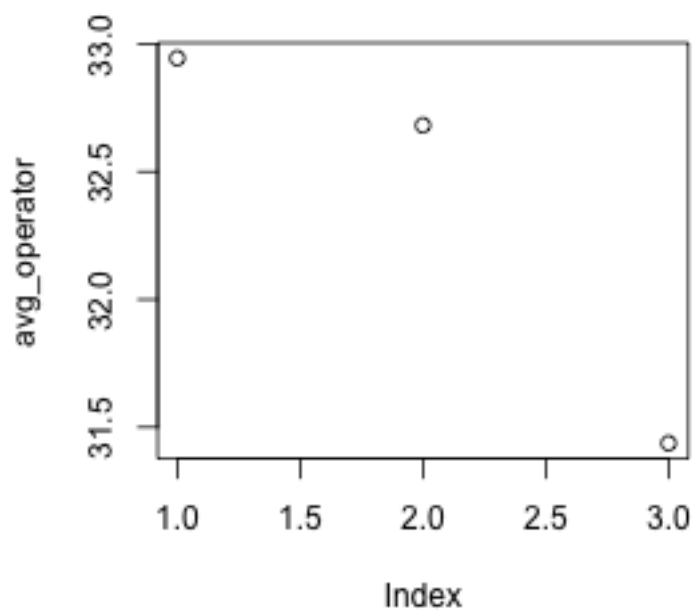


## Plotting

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.

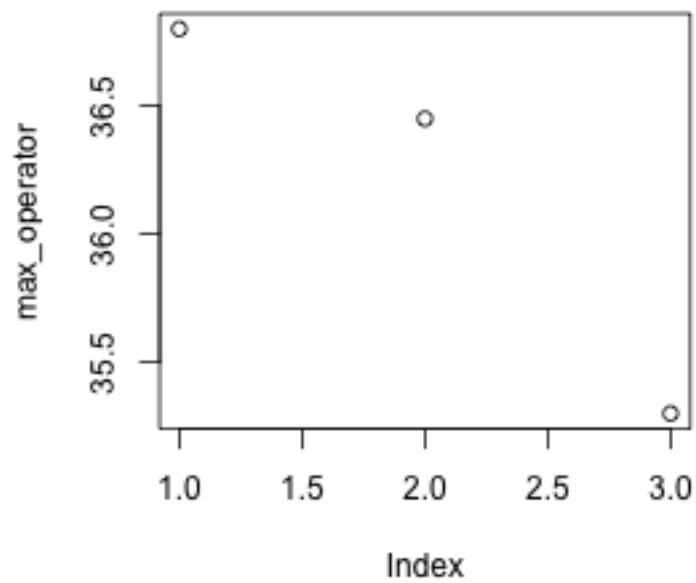
Let’s take a look at the average inflammation over time. Recall that we already calculated these values above using `apply(mat, 2, mean)` and saved them in the variable `avg_operator`. Plotting the values is done with the function `plot`.

```
plot(avg_operator)
```

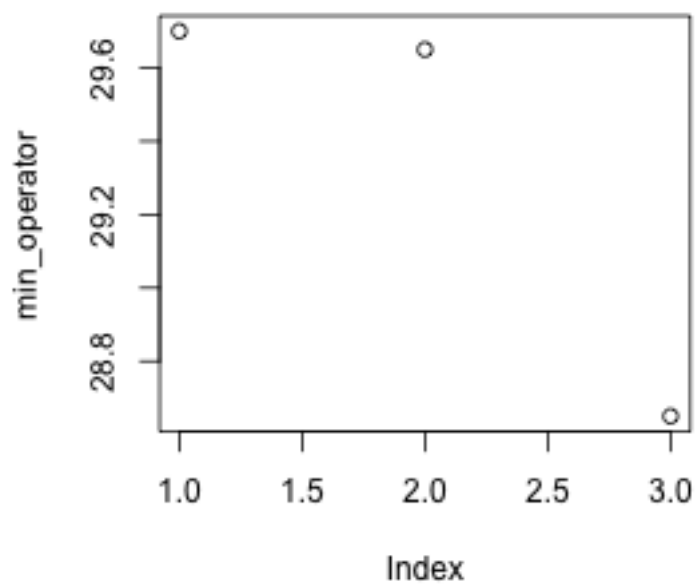


Above, we gave the function `plot` a vector of numbers corresponding to the average per operator across all resins. `plot` created a scatter plot where the y-axis is the average particle size and the x-axis is the order, or index, of the values in the vector, which in this case correspond to the 3 operators. 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 4

# 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/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 some column vectors with `data.frame`. 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 `[,]`.

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

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. Lets 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
```

## 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"))
```

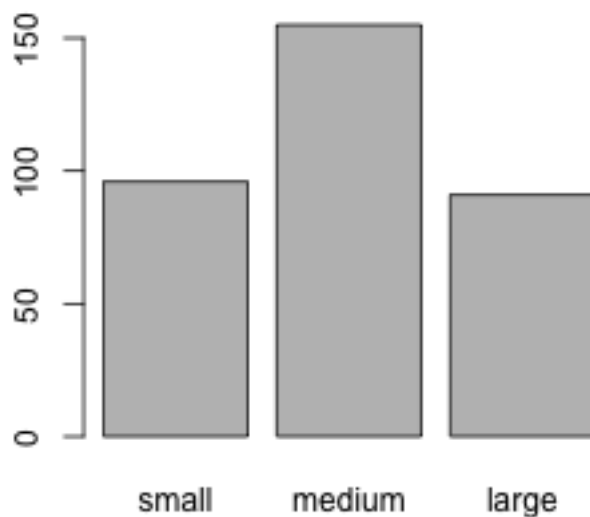
```
head( diabetes$frame )
```

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

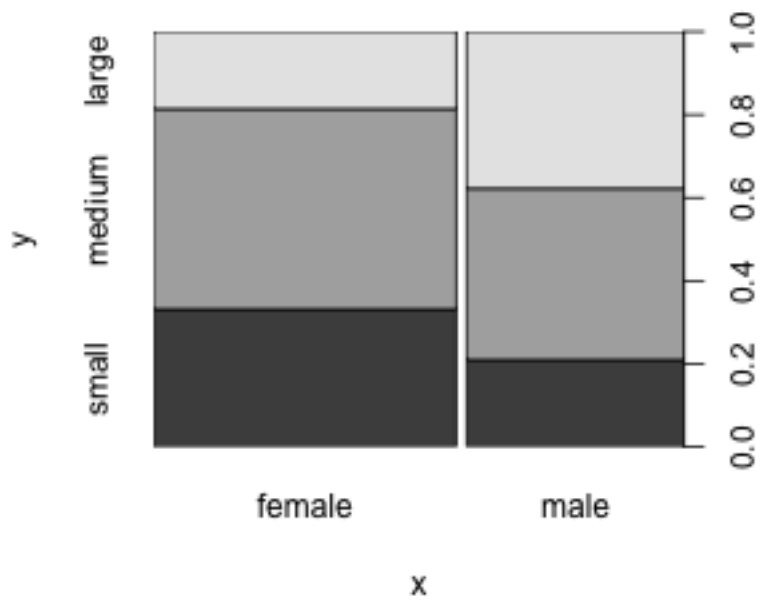
## Plotting factors

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

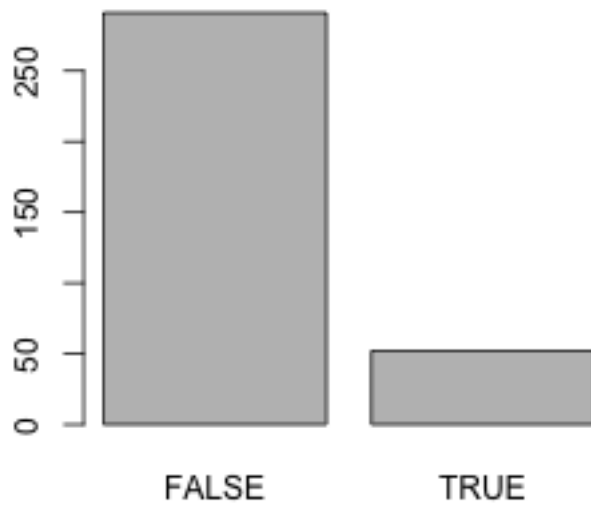
```
plot( diabetes$frame )
```



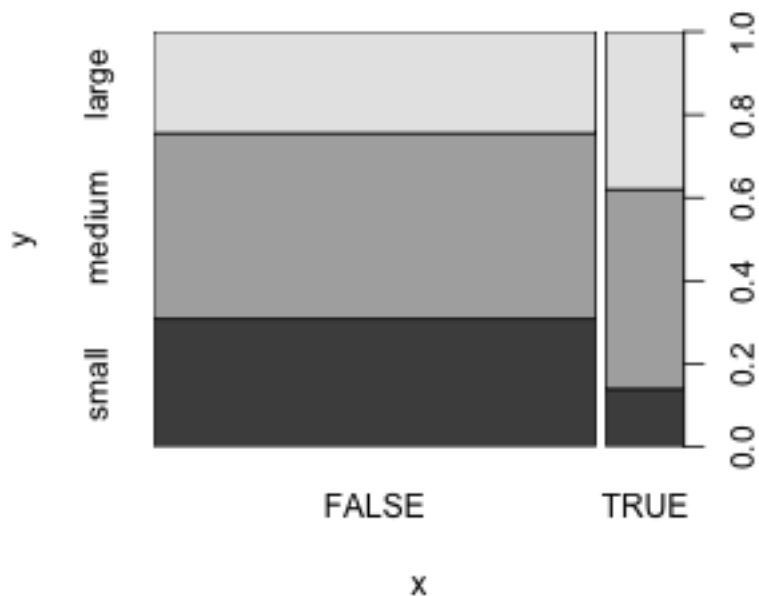
```
plot( diabetes$gender, diabetes$frame )
```



```
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
```

## 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/chol.csv")
head(cholesterol)
```

```
##
## 1 function (x, ...)
## 2 UseMethod("chol")
```

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")

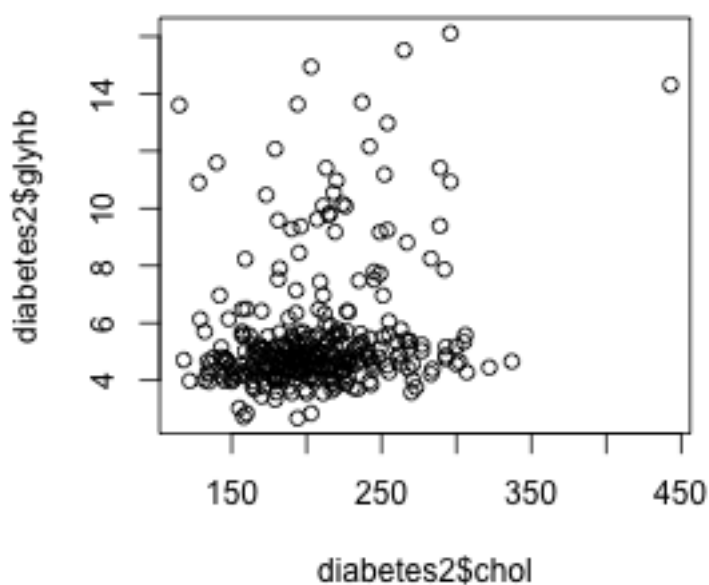
nrow(diabetes2)

## [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 5

# 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**:

```
best_practice <- 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])
}
```

```
## Error in print(sentence[1]): object 'sentence' not found
```

R treats that code between the { and the } as a single “block” of code. 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)
}
```

```
## Error in eval(expr, envir, enclos): object 'sentence' not found
```

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 6

# 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 documentation<sup>1</sup>.

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.

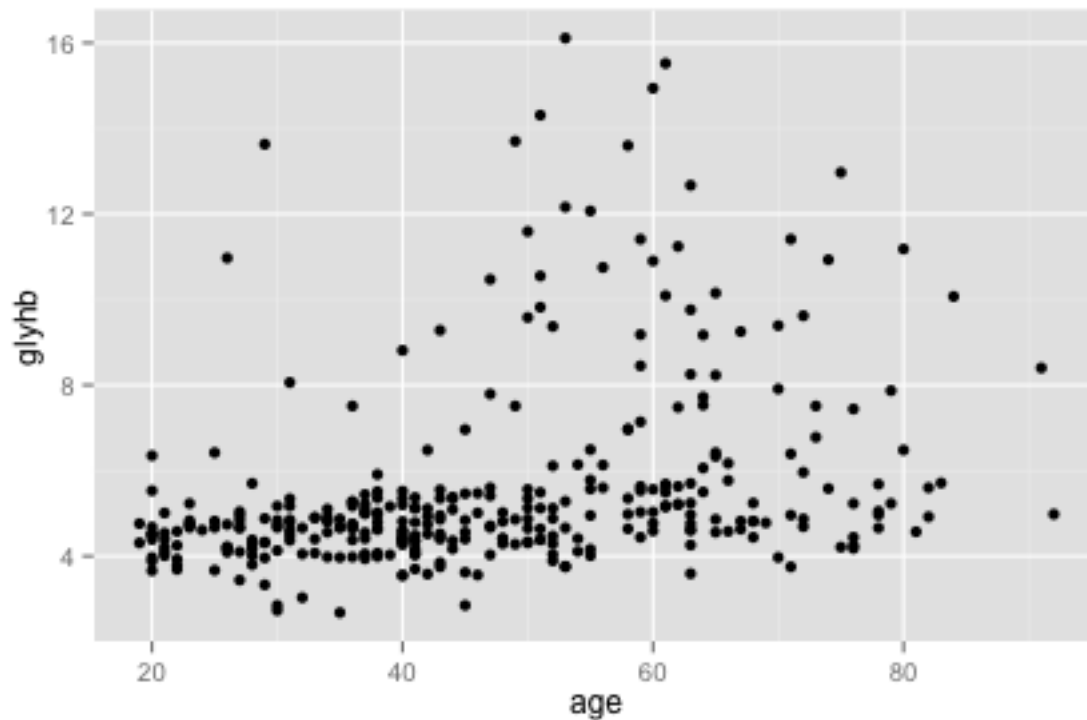
```
diabetes <- read.csv("data/diabetes.csv")
```

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

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

---

<sup>1</sup><http://docs.ggplot2.org/current/>

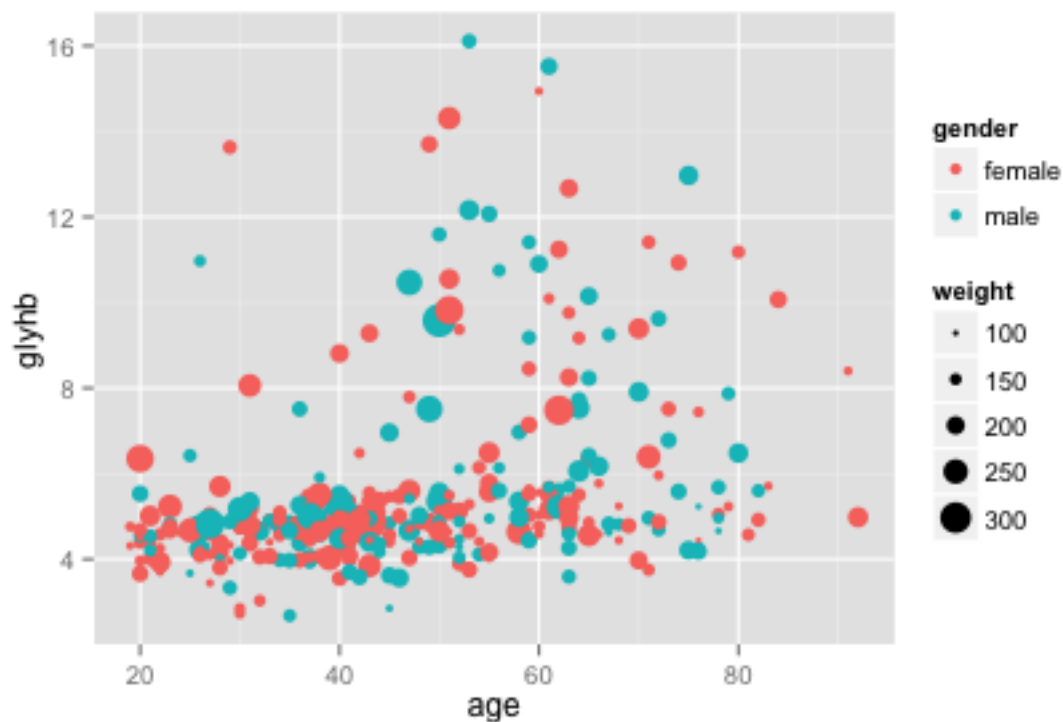


The call to `ggplot` sets up the basics of how we are going to represent the various columns of the data frame. We then literally add layers of graphics to this.

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/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`.

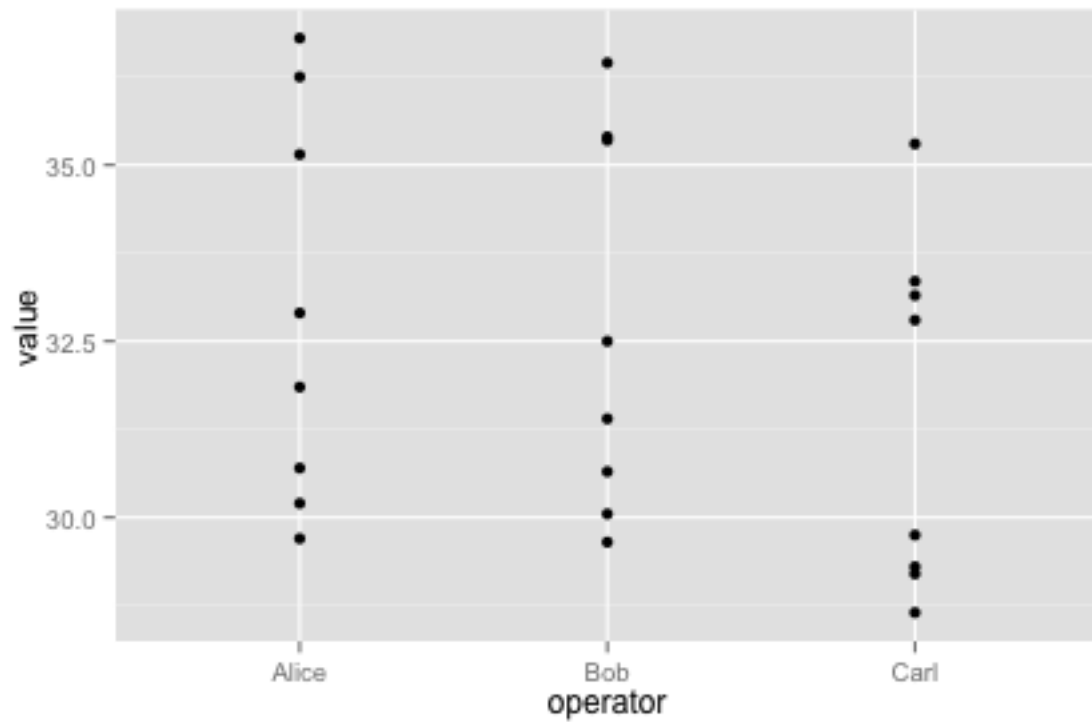
```
library(reshape2)
long <- melt(mat)
head(long)
```

```
##      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)
```

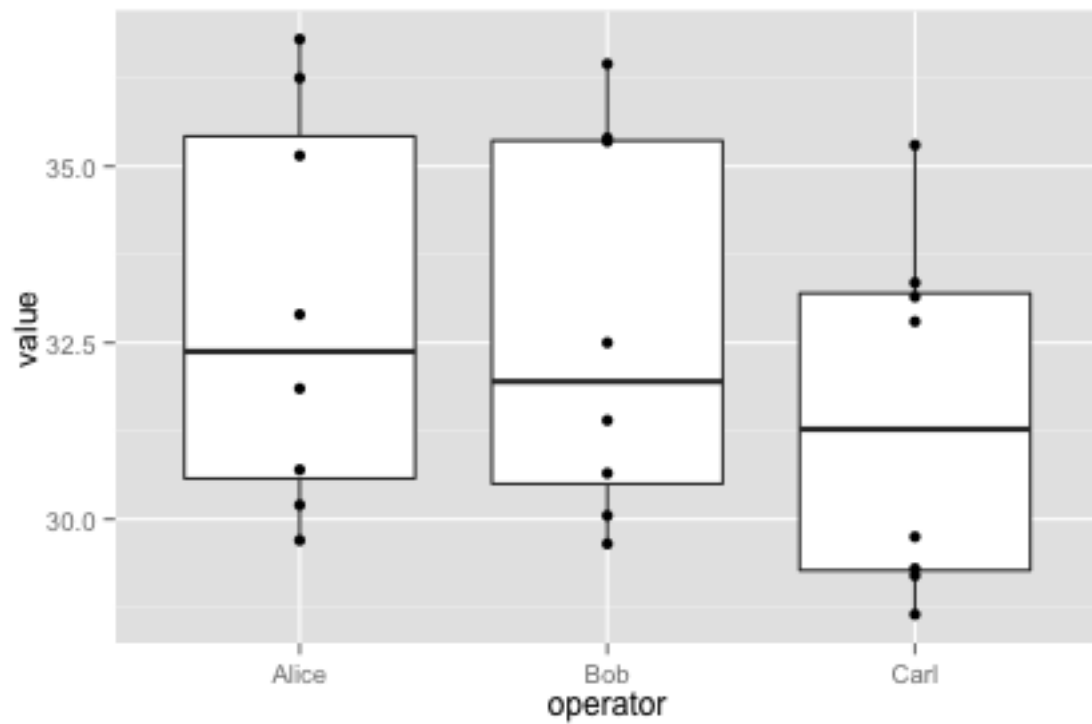
```
##      resin operator value
## 1 Resin1      Alice 36.25
## 2 Resin2      Alice 35.15
## 3 Resin3      Alice 30.70
## 4 Resin4      Alice 29.70
## 5 Resin5      Alice 31.85
## 6 Resin6      Alice 30.20
```

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

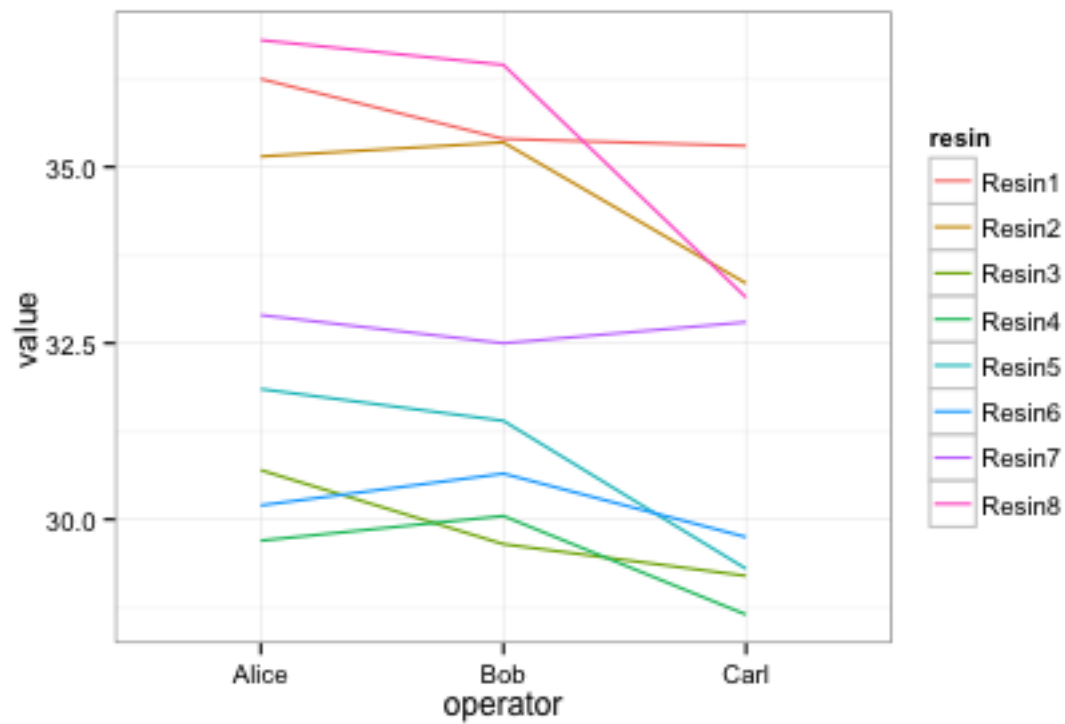


Notice how ggplot2 is able to use either numerical or categorical (factor) data as x and y coordinates.

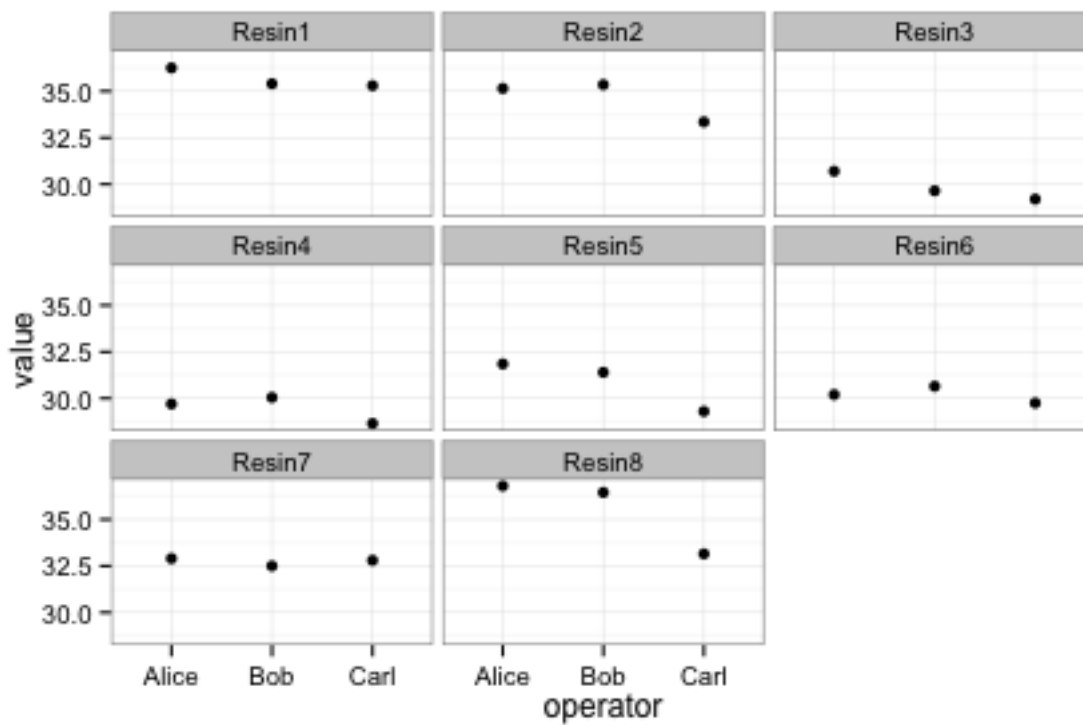
```
ggplot(long, aes(x=operator, y=value)) + geom_boxplot() + geom_point()
```



```
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 7

## Next steps

We can barely touch 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.

Tidying and summarizing data:

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

Data visualization:

- ggplot2 for general plotting.

Statistics:

- Many statistical tests are built in to R.
- Linear models and the linear model formula syntax ~.
- 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.

RNA-Seq:

- This will be covered in the Thursday class.