DAY 2. A beginners guide to solving biological problems in R

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Course material:
http://logic.sysbiol.cam.ac.uk/teaching/Rcourse/

Original slides by Ian Roberts and Robert Stojnić

Day 2
Schedule

1. Writing scripts

2. Writing functions

3. Data analysis examples

4. Graphics

Writing custom scripts for data analysis

1

The R scripting language
Scripting

- A script is a series of instructions that when executed sequentially automates a task
  - A script is a good solution to a repetitive problem
  - The art of good script writing is
    - understanding exactly what you want to do
    - expressing the steps as concisely as possible
    - making use of error checking
    - including descriptive comments
- R is a powerful scripting language, and embodies aspects found in most standard programming environments
  - procedural statements
  - loops
  - functions
  - conditional branching
- Scripts may be written in any standard text editor, e.g. notepad, gedit, kate
  - We will use RStudio

Colony forming experiment

- We have been asked by some collaborators to analyse some trial data to see if an experiment will work.
- We are interested in the behaviour of a gene, X, which is involved in a cell proliferation pathway.
- This pathway causes cells to proliferate in the presence of a compound, Z.
- Gene X turns the pathway off, reducing cell proliferation.

- Our collaborators want to test what happens when we knock down X in the presence of Z.
- To do this, they want to grow cell colonies in the presence of Z, with or without X, and count the number of colonies that result.

Initial trial

- Our collaborators have sent us a first batch of test data, growing colonies in different concentrations of compound Z.
- Does increasing concentration of Z have an effect on colony growth?
- We want to do the following:
  - Load the data into R
  - Plot the data to inspect it
  - Calculate an Analysis of Variance to see if growth is influenced by Z concentration
  - Calculate the mean growth for each level of Z concentration, to see the direction of change
  - (We will ignore full post hoc testing)

Initial trial exercise

- The initial trial data is in the file **2.1_colony_trial.xls**. This is an Excel file and the data is not in the right format for R. Enter the data into a plain text file in a data frame format, and load it into R.
- Plot the data using a formula. Recall how we did this yesterday with linear modelling:

```
plot(y~x)
```

- Calculate an analysis of variance for the data. The R function for ANOVA is aov(), which works like lm() for linear modelling – recall this from yesterday:

```
summary(lm(y~x))
```

- There are four concentrations of Z, and each concentration has been replicated three times. What is the mean colony count for each concentration? See if you can figure out a way to calculate this with what we learned yesterday. You will need to use logical indexing and you may want to use a for loop.

Importing data

- In the Excel file, the data has this format:
- But this is not a data frame format, where columns are variables and rows are observations of those variables.
- There are three variables, Z, Replicate, and Count. We need to reshape the data with these three columns.
- We can do this in Excel, and then save the file in CSV format, or we can just type up the results in a CSV file ourselves.
- Once we have a CSV file, we can load it with read.csv:

```
colony<-read.csv("2.1_colony_trial.csv")
```

Replicate	1	2	3
No Z	150	180	223
Low Z	87	40	53
Medium Z	5	1	9
High Z	0	0	0

Z	Replicate	Count
None	1	150
None	2	180
None	3	223
Low	1	87
Low	2	40
Low	3	53
Medium	1	5
Medium	2	1
Medium	3	9
High	1	0
High	2	0
High	3	0

Plotting

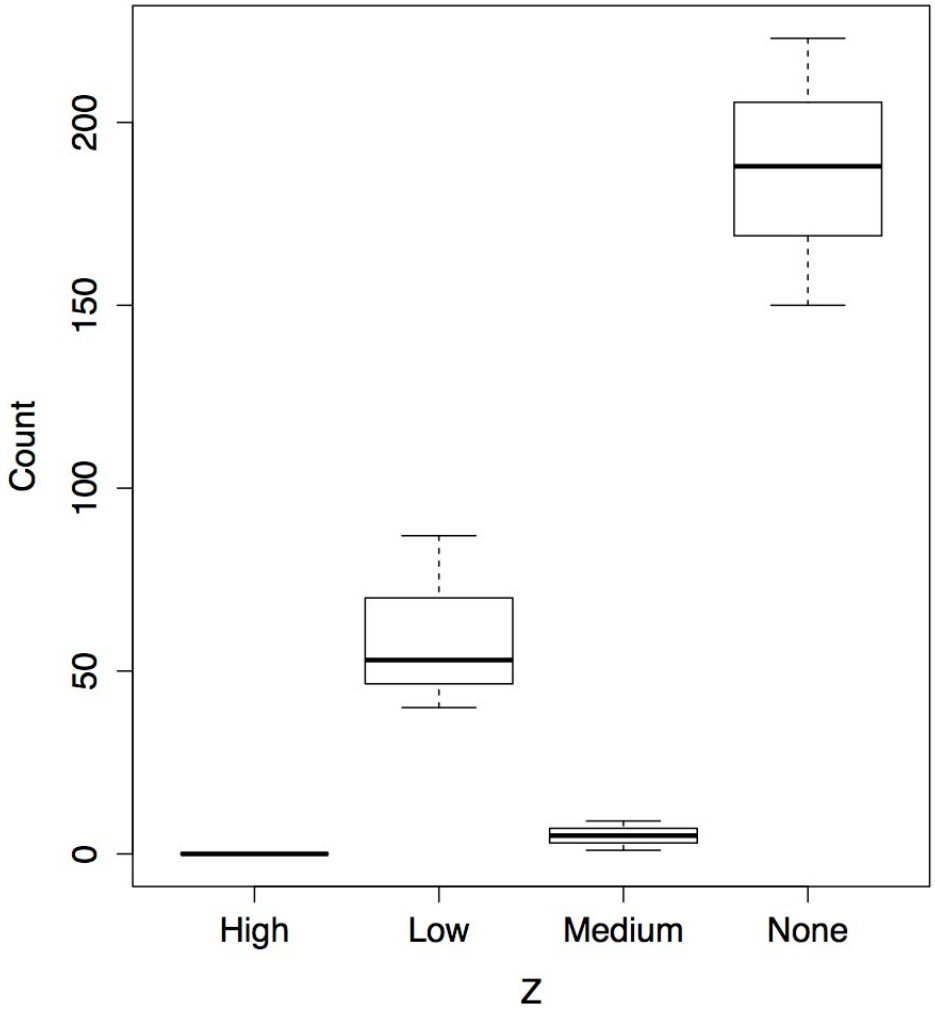
We want to plot the colony growth in response to changing Z concentration.

Z is the explanatory variable, and Count is the response variable.

We don't want to plot replicates separately here, but get R to summarise each Z concentration over all replicates.

We can call plot using the same formula syntax we learnt yesterday:

```
plot(colony$Count~colony$Z)
```



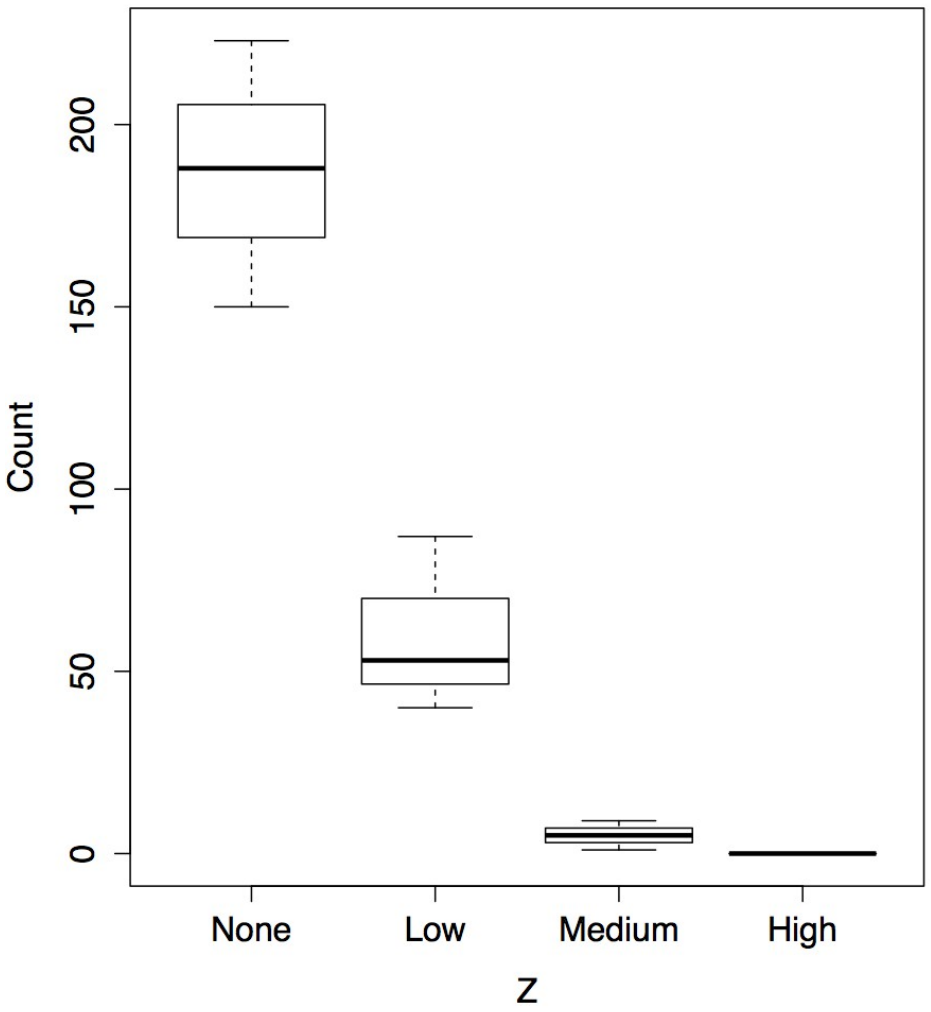
Plotting

We can improve on this. Firstly, we want to order the Z categories. Z is a factor, so we need to supply new levels to this factor in the colony data frame:

```
colony$Z<-factor(colony$Z,
   levels=c("None","Low","M
   edium","High"))
```

Second, we can tell the plot command which data frame to use, rather than using the dollar operator:

```
plot(Count~Z, data=colony)
```



Analysis of Variance

We can use the same formula syntax to calculate an analysis of variance:

```
colony.aov<-aov(Count~Z, colony)
summary(colony.aov)
            Df Sum Sq Mean Sq F value   Pr(>F)
Z            3  68154   22718   46.89 2.02e-05 ***
Residuals    8   3876     484
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

This tells us what we can already see from the plot, that there is a highly significant relationship between Z concentration and colony growth.

We would like to investigate this relationship. For example, we might want to calculate the mean colony count for each concentration of Z.

Calculating group means

We can calculate a mean for a particular group like this:

```
> mean(colony[colony$Z=="None",]$Count)
[1] 187
> mean(colony[colony$Z=="Low",]$Count)
[1] 60
> mean(colony[colony$Z=="Medium",]$Count)
[1] 5
> mean(colony[colony$Z=="High",]$Count)
[1] 0
```

We could generalise this with a for loop:

```
for (z in levels(colony$Z)) {
     print(mean(colony[colony$Z==z,]$Count))
}
[1] 187
[1] 60
[1] 5
[1] 0
```

But there is a better way.

The tapply function
a brief digression

- The apply family of functions allow us to group data by variable and calculate something for each group.
- Assume we have the following data for heights of 5 males and females:

```
data <- data.frame(gender=c("Male", "Male", "Female",
        "Female", "Female"), height=c(6, 6.1, 5.8, 6, 5.95))
```

```
gender height
1   Male   6.00
2   Male   6.10
3 Female   5.80
4 Female   6.00
5 Female   5.95
```

- How can we get mean height of males and females separately?

  `tapply()` lets us do exactly this:

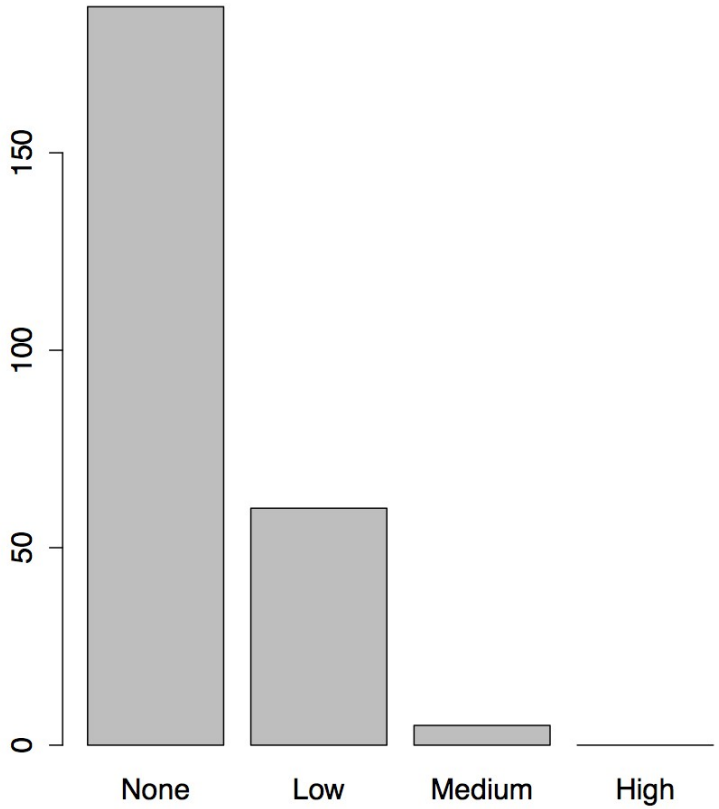
- **`tapply( data$height, data$gender, mean )`**

  data groups function

Using tapply on colony

- We can use tapply to calculate group means on colony like this:

```
> colony.means<-tapply( colony$Count, colony$Z, mean )
> colony.means
  None    Low Medium   High
   187     60      5      0
> barplot(colony.means)
```



A complete script

We now have a complete script to analyse this data:

```
# Load data, order Z and plot
colony<-read.csv("2.1_colony_trial.csv")
colony$Z<-factor(colony$Z,c("None","Low","Medium","High"))
plot(Count~Z,colony)

# Analysis of Variance
colony.aov<-aov(Count~Z,colony)
print(summary(colony.aov))

# Calculate group means
colony.means<-tapply(colony$Count,colony$Z,mean)
print(colony.means)
barplot(colony.means)
```

We need to print the results we want to see on screen, otherwise they will not be output.

Make sure you can source your commands (or the file 2.1_colony_1.R) from Rstudio and generate the results and plot.

Knocking down gene X: revising the script

As the trial worked, our collaborators have gone ahead with an experiment to knock down gene X in the same concentrations of Z.

On our request, they have delivered the data in a data frame format in a CSV file: 2.1_colony_Run1Counts.csv.

They want us to see if knocking down X affects colony growth.

Because we saved our analysis in a script, we can rerun the same script to analyse the data, just by changing the name of the file we are loading.

Run your script on this new data file and confirm that you can calculate an ANOVA and group means for this new data set.

Knocking down gene X: revising the script

Our current script only analyses Z, not X. We need to modify it to include X and see how both X and Z influence colony growth.

1. We need to include X and the interaction between Z and X in our formulae for plotting and for ANOVA. Look up the 'Modelling formulae' slide from Day 1 to see how to do this.

2. What does **plot** do with a formula including both X and Z? Try using **boxplot** instead. What difference does it make if you change the order of X and Z?

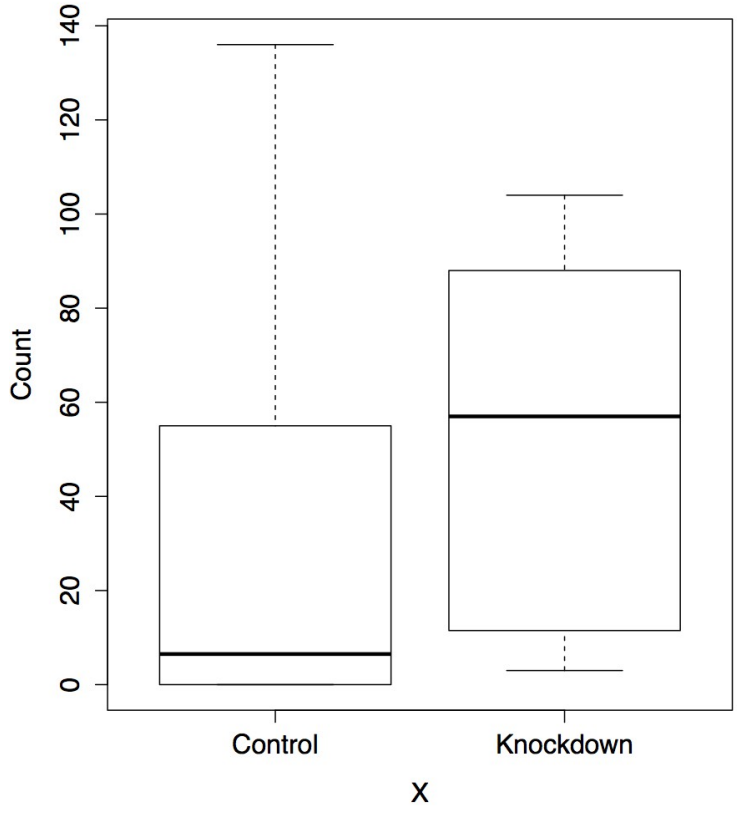
3. We need to include both X and Z in our call to **tapply**. Modify the call to **tapply** by changing the second argument, which should be a list containing the data for both X and Z.

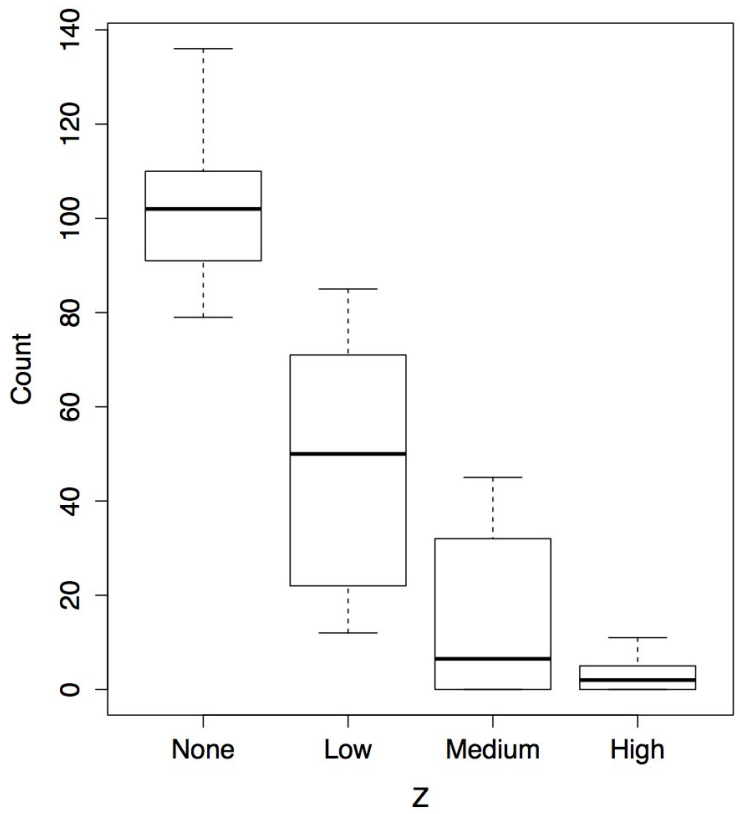
4. Plot the group means you calculated with **tapply** using **barplot**. Plot bars for different conditions *beside* each other, not on top of each other. Check the help page for an option to do this.

Plotting interactions

Including interactions in formulae is straightforward, but **plot** doesn't show us the interaction, only the main effects:

```
> plot(Count~X*Z,colony)
```

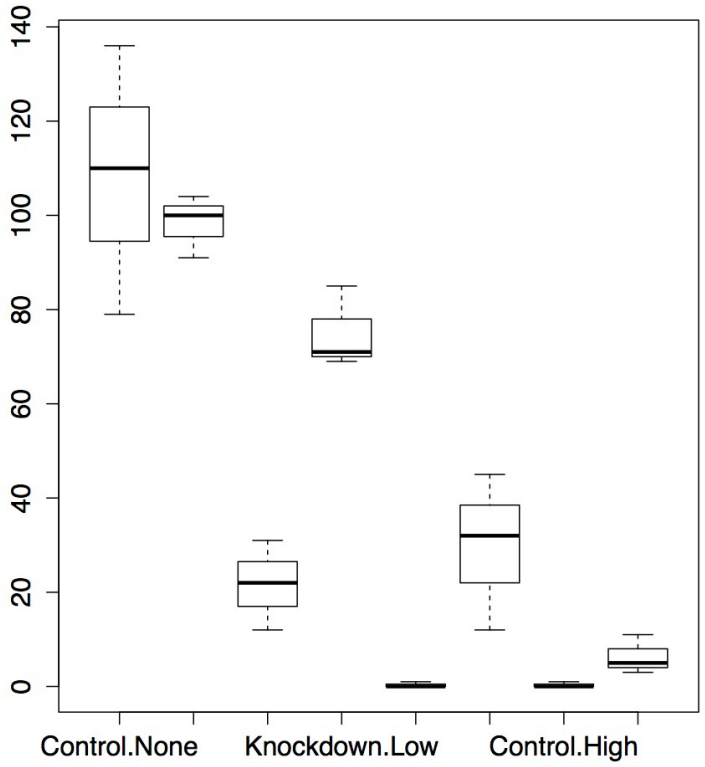




Plotting interactions

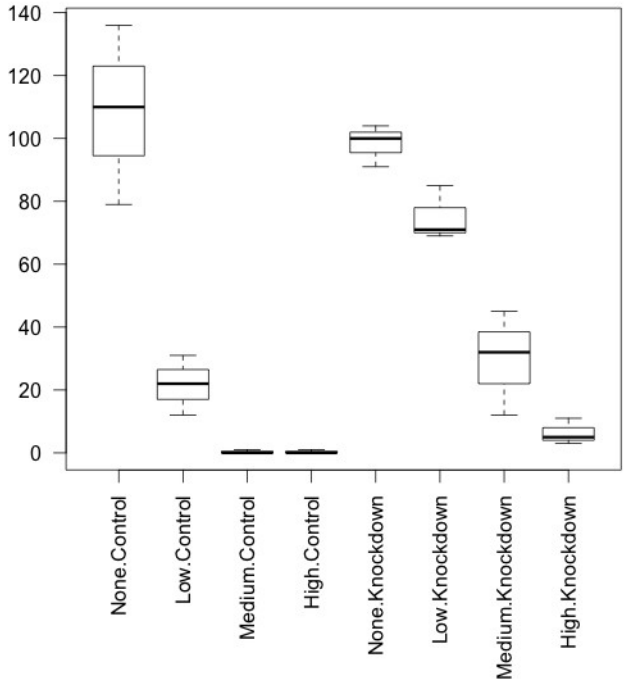
To get a sense of what's happening with the interactions, use **boxplot**:

```
> boxplot(Count~X*Z,colony)
```



To make the labels visible, we'll use some graphics commands to increase the size of the lower margin and make the x-axis labels vertical (full details on this this afternoon):

```
> par(oma=c(6,2,2,2))
> boxplot(Count~X*Z,colony,las=2)
```



It looks like knocking down X increases colony growth, except when Z is completely absent.

Analysis of variance with interactions

Including interactions in the analysis of variance is straightforward:

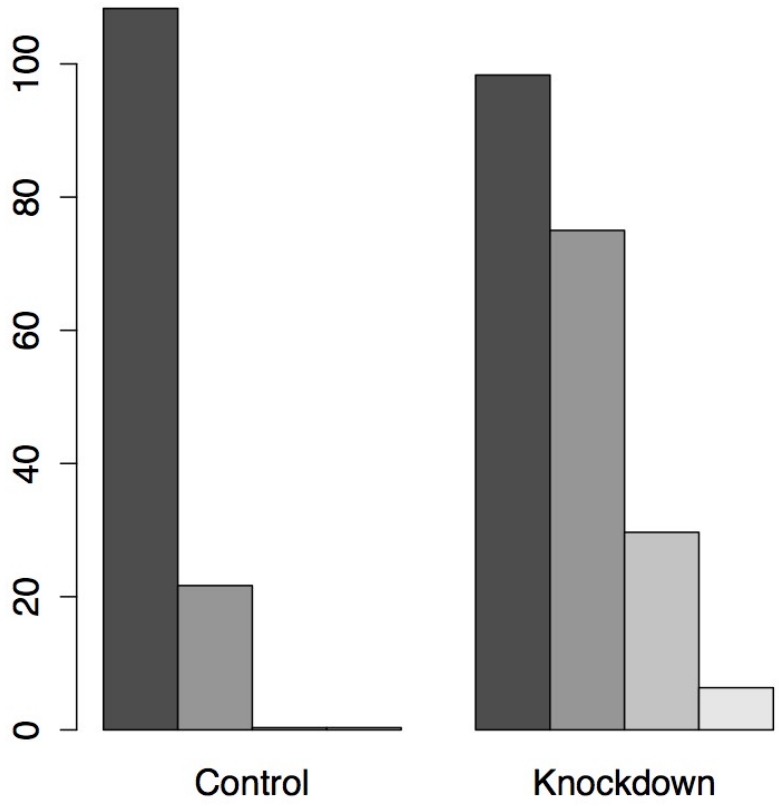
```
> colony.aov<-aov(Count~X*Z,colony)
> print(summary(colony.aov))
            Df Sum Sq Mean Sq F value   Pr(>F)
X            1   2321    2321  14.072  0.00174 **
Z            3  36150   12050  73.067 1.48e-09 ***
X:Z          3   3441    1147   6.954  0.00329 **
Residuals   16   2639     165
---
Signif. Codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Not only do X and Z have a significant effect on colony growth individually, but there is also a significant interaction between them.

tapply with multiple variables

Including Z in the call to tapply is a little fiddly, but easy when you know how. Use the **beside** option in the call to **barplot**. (What happens if you put X first in the list?)

```
> colony.means<-tapply(colony$Count,list(colony$Z,colony$X),mean)
> print(colony.means)
           Control Knockdown
None   108.3333333 98.333333
Low     21.6666667 75.000000
Medium   0.3333333 29.666667
High     0.3333333  6.333333
> barplot(colony.means,beside=TRUE)
```



Complete script: first revision

Our script now looks like this (see 2.1_colony_2.R):

```
# Load data, order Z and plot
colony<-read.csv("2.1_colony_Run1Counts.csv")
colony$Z<-factor(colony$Z,c("None","Low","Medium","High"))

par(oma=c(6,2,2,2))
boxplot(Count~Z*X,colony,las=2)

# Analysis of Variance
colony.aov<-aov(Count~X*Z,colony)
print(summary(colony.aov))

# Calculate group means
colony.means<-tapply(colony$Count,list(colony$Z,colony$X),mean)
print(colony.means)
barplot(colony.means,beside=TRUE)
```

Incorporating multiple runs: second revision

As it looks like there is an interaction between Z and X, our collaborators have repeated the experiment twice more, to increase the sample size. They have delivered two more data files, one for each extra run, in the files 2.1_colony_Run2Counts.csv and 2.1_colony_Run3Counts.csv.

1. Modify your script to load in the two new files. You may wish to use the looping code you wrote yesterday. Combine all three data sets into one **colony** data frame.

2. Now we have an additional variable, the **Run** each observation came from. Create a **Run** vector to record this, and add it to your colony data frame with **cbind**. You will need to use the **rep** function with its **each** argument – look it up to see what it does.

3. Modify your boxplot and analysis of variance to include **Run**. How does this data set look? Would you trust an analysis of it?

Loading multiple files

This should look familiar from yesterday:

```
# Load data
colony.files<-dir(pattern="Counts.csv")
colony<-data.frame()
for (cf in colony.files) {
  colony<-rbind(colony,read.csv(cf))
}
```

We *could* just call **read.csv** three times, but this would be very brittle. What if we were given fifty more files? What if the filenames changed? This code will handle these cases – we would only have to change the pattern in the first line, not every call to **read.csv**.

Creating the Run variable

If we inspect the data files, we can see there are 24 observations in each file. So we can hardcode a Run variable like this:

```
Run<-rep(1:3,each=24)
colony<-cbind(Run,colony)
```

But this is brittle in the same way as calling **read.csv** three times is brittle. It would be better to get R to calculate **Run** from what it knows about the data.

We can count the observations in **colony** with **nrow**, and the number of runs (number of files) by getting the **length** of **colony.files**.

```
nruns<-length(colony.files)
Run<-rep(1:nruns,each=nrow(colony)/nruns)
colony<-cbind(Run,colony)
```

Analysis of variance with Run variable

Adding the Run variable to our analysis of variance is easy:

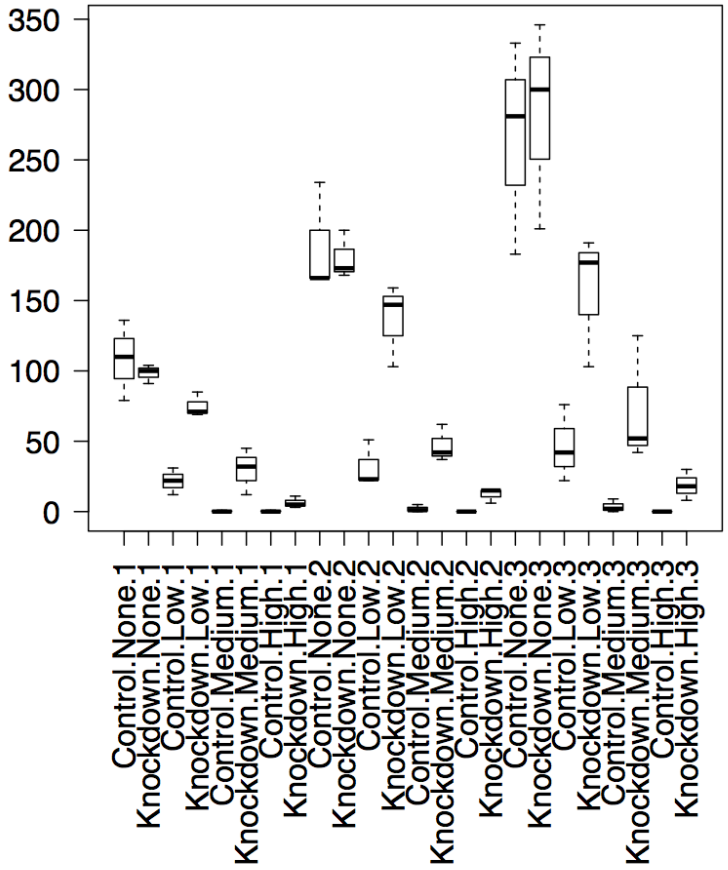
```
> colony.aov<-aov(Count~X*Z*Run,colony)
> print(summary(colony.aov))
             Df Sum Sq Mean Sq F value   Pr(>F)
X             1  24939   24939  32.454 4.71e-07 ***
Z             3 355524  118508 154.221  < 2e-16 ***
Run           1  48197   48197  62.721 1.05e-10 ***
X:Z           3  21967    7322   9.529 3.51e-05 ***
X:Run         1   3485    3485   4.535   0.0376 *
Z:Run         3  49513   16504  21.478 2.19e-09 ***
X:Z:Run       3    805     268   0.349   0.7897
Residuals    56  43032     768
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

But the result is troubling – why should Run be correlated with colony growth? Let's have a look at the plot.

Plotting the Run variable

It is also easy to add Run to our boxplot:

```
boxplot(Count~X*Z*Run,colony,las=2)
```



Unfortunately, this shows that something has gone wrong during the experiments. The later runs have much higher colony growth than the first.

We probably can't rely on this data to tell us something about X and Z. At the very least, we will have to control for Run during the analysis.

Scripting summary

You can find the final version of the script in 2.1_colony_3.R.

We have seen the value of writing a reusable script, and how we can modify scripts as new data comes in.

We have also seen how easy it is to incorporate new variables into R, and to repeat complicated analyses with single commands.

Finally, we have learnt a little about generalising our code to cope with variations in current and future input. Now we'll go on to learn how to define our own functions in R, to help us to generalise even further.

User functions

2

Introducing ...
User functions

- All R commands are functions.
- Functions perform calculations, possibly involving several arguments, then return a value to the calling statement.
- The calculation maybe any process, might or might not have return value
  - It need not be arithmetic
- User functions extend the capabilities of R by adapting or creating new tasks that are tailored to your specific requirements.
- User functions are a special kind of object

Defining a new function

- Parts of function definition: name, arguments, procedural steps, return value

```
sqXplusX <- function(x){
    x^2 + x
}
```

- **sqXplusX** is the function name
- **x** is the single argument to this function and it exists only within the function
- everything between brackets { } are procedural steps
- the **last** calculated value is the function return value
- after defining the function, we can use it:

```
> sqXplusX(10)
[1] 110
```

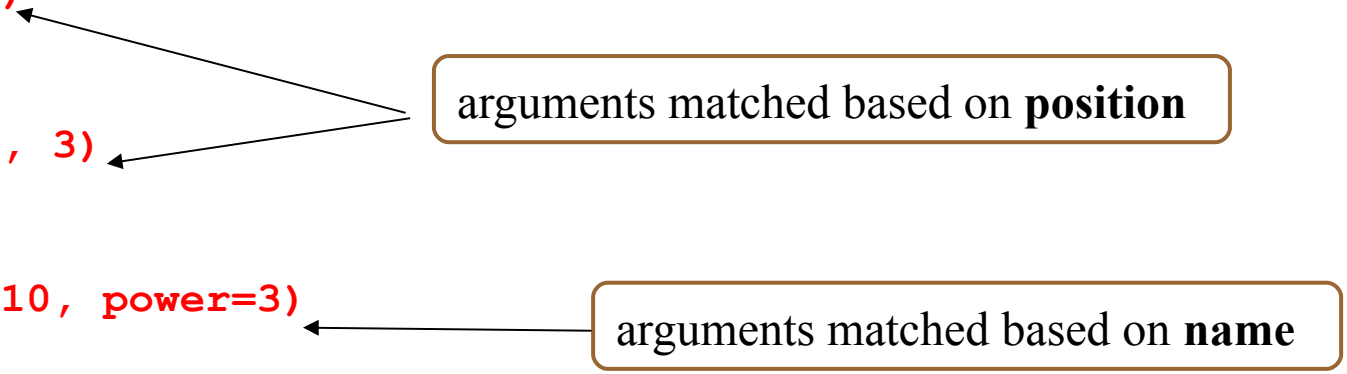
Named and default arguments

- Example of function with more than one named argument:

```
powXplusX <- function(x, power=2){
    x^power + x
}
```

- Now we have two arguments. The second argument has a default value of 2.
- Arguments without default value are required, those with default values are optional.

```
> powXplusX(10)
[1] 110
> powXplusX(10, 3)
[1] 1010
> powXplusX(x=10, power=3)
[1] 1010
```



Assignments with arguments
User functions

```
sqXplusX <- function(x){
   x^2 + x
}
```

You can use a blank document in gedit, nedit or other text editor to hold these commands for you, then copy / paste the instructions into R

- Now try this …

```
a <- matrix(1:100, ncol=10, byrow=T)  # make some dummy data
b <- sqXplusX(a)  # transform a by sqXplusX, assign result to b
b # to view the result
```

- sqXplusX user function is now an R object, check its arguments and list it in the current workspace

```
> args(sqXplusX)
> ls()
> sqXplusX
```

Don’t add brackets to see the definition of sqXplusX

Assigned or anonymous ...
User functions

- Functions may be assigned a name, or anonymously created within an operation
  - Anonymous functions are really useful in `apply()` style procedures

```
apply(object, margin, function)
```

  - E.g. I have a 10 x 10 matrix and want to square each item, and add the item to itself

```
a<-matrix(1:100, ncol=10, byrow=T)
a # to view new object
apply(a, c(1,2), function(x) x^2+x)
```

`x` is transiently assigned each item of `a`, and this is passed as an argument to the anonymous function

1 means by rows, 2 means by columns [1st or 2nd margin]
c(1,2) means do both rows and columns

Functions occupy their own space
User functions

- Objects created in functions are not available to the general environment unless returned.
  - they are said to be out of scope
    - Scope relates to the accessibility of an object.
- A function can only return one object.
- Custom functions disappear when R sessions end, unless the function object is saved in an Rdata file or sourced from a script.
  - A really useful function could be added to your .Rprofile file, and would always be ready for you at launch
- You could also make a package
  - Beyond the scope of the beginners course!!!!

Script / function tips
User functions

- If your script repeats the same style command more than twice, you should consider writing a function
- Writing functions makes your code more easily understandable because they encapsulate a procedure into a well-defined boundary with consistent input/output
- Functions should not be longer than one-to-two screens of code, keep functions clean and simple
- Look at other functions to get ideas for how to write your own …
  - Display function code by entering the function's name without brackets.

File commands for extending
scripts & user functions

Generic file commands

```
dir(...,pattern="txt")
```

Retrieve working directory file listing filtered by pattern. Note pattern is a regular expression, not a shell wildcard

```
glob2rx("*.txt")
```

Changes wildcards to regular expressions!

```
> glob2rx("*.txt")
[1] "^.*\\.txt$"
```

```
unlink(...)
```

Remove (permanently) a file from system

```
system(...)
```

Execute a shell command from within R

Result can not be coerced to an object, only available to linux R

Text manipulation for extending
scripts & user functions

- Text manipulation and file name mangling ... that's a technical term

```
grep( pattern, object )
```

  - If pattern is not found, grep returns a 0 length object.
    - Test for null with `is.null()`

```
sub( pattern, replacement, object )
```

```
gsub( pattern, replacement, object)
```

  - Sub replaces first occurrence only, gsub does them all.

```
cat( "...", file=...)
```

  - Outputs text to a file, or prints it on screen if file=""
    - cat requires "\n" to be given for new lines ... try ...

```
cat("Hello World!") ; cat("Hello World!",sep="\n") ; cat("Hello
   World!",sep="\n",file="world.txt")
```

  - cat is extremely useful for writing scripts or generating reports on-the-fly

Error reporting for extending scripts & user functions

- Your code should report errors if inconsistency is detected.

`stop(...)`

- Stops execution of a function and reports a custom error message

`is.family(...)`

- Functions that can be used to test for a variety of conditions place them inside `if` structures to check that all is well

```
if( !is.numeric(x) ){ stop ("Non numeric value entered.  Cannot
   continue.") }
```

If the object x is non numeric (e.g. Text has been entered when numbers were required), then stop execution and report message

The `is.family`

	is.language	is.primitive
.array	is.leaf	is.qr
s.atomic	is.list	is.R
isBaseNamespace	is.loaded	is.raw
is.call	is.logical	is.real
is.character	is.matrix	is.recursive
isClass	is.mts	is.relistable
isClassDef	is.na	isRestart
isClassUnion	is.na<-	isS4
is.complex	is.na.data.frame	isSealedClass
is.data.frame	is.na<-.default	isSealedMethod
isdebugged	is.na<-.factor	isSeekable
is.double	is.name	is.single
is.element	isNamespace	is.stepfun
is.empty.model	is.nan	is.symbol
is.environment	is.na.POSIXlt	isSymmetric
is.expression	is.null	isSymmetric.matrix
is.factor	is.numeric	is.table
is.finite	is.numeric.Date	isTRUE
is.function	is.numeric.POSIXt	is.ts
isGeneric	is.numeric_version	is.tskernel
isGrammarSymbol	is.object	is.unsorted
isGroup	isOpen	is.vector
isIncomplete	is.ordered	isVirtualClass
s.infinite	isoreg	isXS3Class
.integer	is.package_version	
nds	is.pairlist	

Temperature conversion exercise
User functions

- Centigrade to Fahrenheit conversion is given by
  - F = 9/5 C +32
    - Write a function that converts between temperatures.
      - The function will need two named arguments
        - *temperature* ***(t)*** *is numeric*
        - *units* ***(unit)*** *is character*
        - *They will need default values, e.g t=0, unit="c"*
      - The function should report an appropriate error if inappropriate values are given

```
if( !is.numeric(t) ) { .... }
if( !(unit %in% c("c","f")) ){...}
```

      - The function should print out the temperature in F if given in C, and vica versa

Functions with named arguments are defined with the following syntax

```
myFunc<-function(arg=defaultValue,…)
```

- Why not add a third scale?

  K=C+273.15

Example code:
12_convTemp.R

Building the solution

- It is difficult to write large chunks of code, instead start with something that works and build upon it
- E.g. to solve the temperature conversion exercise:
  - start with the function powXplusX (from some slides ago)
  - modify the argument names
  - delete the old code, for now just print out the input arguments
  - save the function file, load it into R and try it out
  - now add the two lines for input checking from the previous slide
  - try it out, see if passing a character for temperature gives the expected error
  - now try to convert C into F and print out the result
  - when that works, add the conversion from F to C
- If you get stuck, call us to help you !

Temperature conversion script

```
convTemp<-function(t=0,unit="c"){ # convTemp is defined as a new user function requiring two
arguments, t and unit, the default values are 0 and "c", respectively.

     if( !is.numeric(t) ){
          stop("Non numeric temparture entered") # Exception error if character given for
temperature
     }

     if(!(unit %in% c("c","f","k"))){
          stop("Unrecognized temperature unit. \n Enter either (c)entigrade, (f)ahreneinheit
or (k)elvin") # Exception error if unrecognized units entered
     }
# Conversion for centigrade
     if(unit=="c"){
          fTemp <- 9/5 * t + 32
          kTemp <- t + 273.15
          output <- paste(t,"C is: \n",fTemp,"F \n",kTemp,"K \n")
          cat(output)
     }
# Conversion for Fahrenheit
     if(unit=="f"){
          cTemp <- 5/9 * (t-32)
          kTemp <- cTemp + 273.15
          output <- paste(t,"F is: \n",cTemp,"C \n",kTemp,"K \n")
          cat(output)
     }
# Conversion for Kelvin
     if(unit=="k"){
          cTemp <- t - 273.15
          fTemp <- 9/5 * cTemp + 32
          output <-paste(t,"K is: \n",cTemp,"C \n",fTemp,"F \n")
          cat(output)
     }
}
```

"\n" -> puts text on a new line

Units must be entered in quotes, as it's a character object

```
> convTemp(t=-273,unit="c")
-273 C is:
 -459.4 F
 0.149999999999977 K
```

Example code: 12_convTemp.R

Advanced data processing

3

Combining data from multiple sources
Gene clustering example

- R has powerful functions to combine heterogeneous data into a single data set
- Gene clustering example data:
  - five sets of differentially expressed genes from various experimental conditions
  - file with names of experimentally verified genes
- Gene clustering exercise:
  1. combine this dataset into a single table and cluster to see which conditions are similar
  2. repeat the clustering but only on a subset of experimentally verified genes

Combining gene tables

- input files have two columns: gene names and fold change
- we want to combine all five tables into a single table, with 0 for missing values

LpR2	3.5795
fs(1)h	3.1376
CG6954	2.7492
Psa	2.7012
zfh2	2.6247
Fur1	2.4413
ct	2.3804
S	2.3674
rux	2.3574
RhoBTB	2.26
CG14889	2.1735
oc	2.1421
pros	2.0882
Kr-h1	-2.0447
CG5149	-2.1521
tna	-2.2102
CG14888	-2.4346
CG31368	-2.4793
Trim9	-2.616
Awd	-3.0595

+

Psa	3.8529
vnd	3.6457
ct	3.201
fs(1)h	3.1489
btd	3.1229
zfh2	2.8421
RhoBTB	2.6022
pros	2.5679
CG1124	2.5475
S	2.5424
oc	2.5111
Fur1	2.43
PHDP	2.304
CG31241	2.2802
rux	2.2232
CG14889	2.1752
CG31163	2.1606
HmgZ	2.0795
svp	-2.0404
TER94	-2.1807
corto	-2.3481
olf413	-2.4404
brat	-2.7256
CG31368	-2.7293
mub	-2.9555
Awd	-3.1413
lola	-3.8882

+

lola	3.0121
CG31368	2.8063
Kr-h1	2.7262
svp	2.7055
mub	2.6475
CG5149	2.5248
run	2.4759
tna	2.4302
CG6954	2.4235
CG11153	2.3045
Awd	2.2295
CG6919	2.1324
CG14888	2.067
Psa	-2.0276
rux	-2.093
fs(1)h	-2.141
CG1124	-2.155
Fur1	-2.1588
S	-2.2539
corto	-2.2618
oc	-2.3017
CG14889	-2.4393
zfh2	-2.5884
HmgZ	-3.6328
btd	-3.7627
brat	-3.7716

+

lola	3.3019
CG6919	2.9965
CG31368	2.817
CG5149	2.7675
Kr-h1	2.7647
TER94	2.6286
tna	2.5748
CG11153	2.4795
run	2.3831
CG14888	2.0938
S	-2.0243
rux	-2.0668
oc	-2.3437
corto	-2.5556
fs(1)h	-2.6211
brat	-2.9904
ct	-3.3404
zfh2	-4.4947
CG6954	-4.7244

+

brat	5.2812
ct	4.828
CG31163	4.3345
LpR2	3.6882
vnd	3.6866
zfh2	3.5314
pros	3.4307
Psa	3.3998
fs(1)h	3.3869
CG31241	2.9973
HmgZ	2.9226
Fur1	2.7469
RhoBTB	2.7189
oc	2.6543
Toll-7	2.6161
rux	2.5975
CG14889	2.3054
S	2.2324
CG1124	2.0216
Kr-h1	-2.1439
tna	-2.1793
CG5149	-2.1892
run	-2.2194
Trim9	-2.251
olf413	-2.3821
btd	-3.0293
CG6919	-3.3719

Gene clustering
Script walkthrough 1

- To make the big table we first need to find out all the genes present in at least one of the files
- Make sure not to use factors in read.delim()

```
# start with en empty collection of genes
genes <- c()
for( fileNum in 1:5 ){
   # load in files 13_DiffGenes1.tsv ...
   t <- read.delim(paste("13_DiffGenes", fileNum, ".tsv", sep=""),
                   as.is=TRUE, header=FALSE)
   # label the input columns to help code readability
   names(t) <- c("gene", "expression")
   genes <- union(genes, t$gene)
}

# for tidiness order our genes by name
genes <- sort(genes)

genes # show all genes
```

when loading in character data use **as.is=T** to prevent it being converted to factors!

union() is a set operation, combines two vectors by eliminating duplicates. There are also intersect() and setdiff()

Example code:
13_geneClustering.R

Gene clustering
Script walkthrough 2

- Using the complete list of genes, we can create the big table and fill in the values:

```
# make the destination table [rows = unique genes, cols = file numbers]
values <- matrix(0, nrow=length(genes), ncol=5)
rownames(values) <- genes # name the rows with the complete gene names

for(fileNum in 1:5){
  # read in the file again
     t <- read.delim(paste("13_DiffGenes", fileNum, ".tsv", sep=""),
            as.is=T, header=F)
     names(t) <- c("gene", "expression")

     # match the names of the genes to the rows in our big table
     index <- match(t$gene, rownames(values))
     # copy the expression levels
     values[index,fileNum] <- t$expression
}
```

match() returns the index of first argument in the second, i.e. index of input file genes in the big table

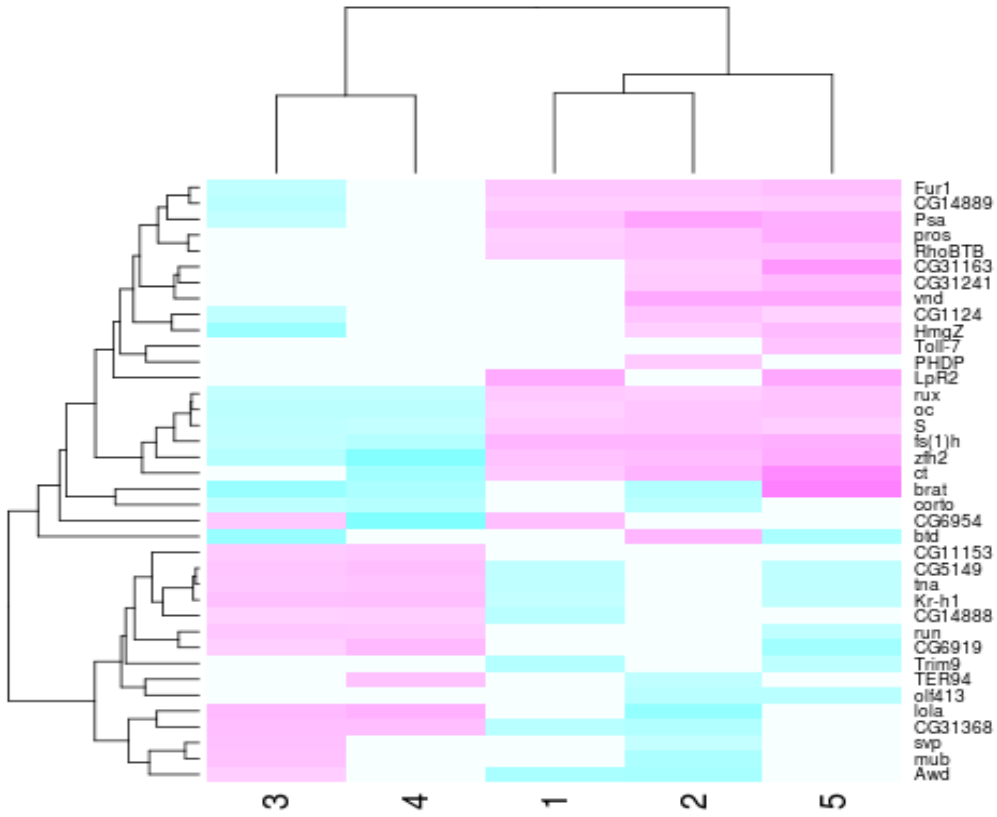
we use index to pick the rows in such way that they match the gene order in the input file

Gene clustering
Script walkthrough 3

- Now we can do hierarchical clustering:

```
heatmap(values, scale="none", col = cm.colors(256))
```

Values from the matrix are colour-coded. Rows and columns are re-arranged according to similarity



Gene clustering

Script walkthrough 4

- In a second part of our analysis, we want to produce the same heatmap but only based on a list of experimentally verified genes

- The problem is data is not formatted in the most convenient way:

genes	citation
oc,run,RhoBTB,CG5149,CG11153,S,Fur1	Segal et al, Development 2001
tna,Kr-h1,rux	Krejci et al, Development 2002

Gene clustering

Script walkthrough 5

- We load in this table, and only extract the gene names, then we use them to select a subset of values matrix

```
# load in the tab-delimited file with genes and citations
t.exp <- read.delim("13_ExperimentalGenes.tsv", as.is=T)
# split all gene names by "," and then flatten it out into a single vector
experim.genes <- unlist( strsplit(t.exp$genes, ",") )
```

unlist() flattens out a nested list into a single vector

strsplit() splits a vector of strings by a custom split character (","). The result is a list of split values for each element of the input vector

```
# redo the heatmap by using just the genes in the experimentally verified set
is.experimental <- rownames(values) %in% experim.genes
heatmap(values[ is.experimental, ], scale="none", col = cm.colors(256))
```

Gene clustering review

- We load in the five tables twice - first to collect gene names, then to load expression values
- Based on expression table (values) we construct a clustered heatmap first on the whole set of genes, then on a selected subset

- Go through the code, try it out it and understand it

- Try answering the following questions:
  - what is rownames(values) ?
  - why is rownames(values)[index] and t$gene giving the same output?
  - what is the difference between rownames(values) %in% experim.genes and experim.genes %in% rownames(values)

Example code:
13_geneClustering.R

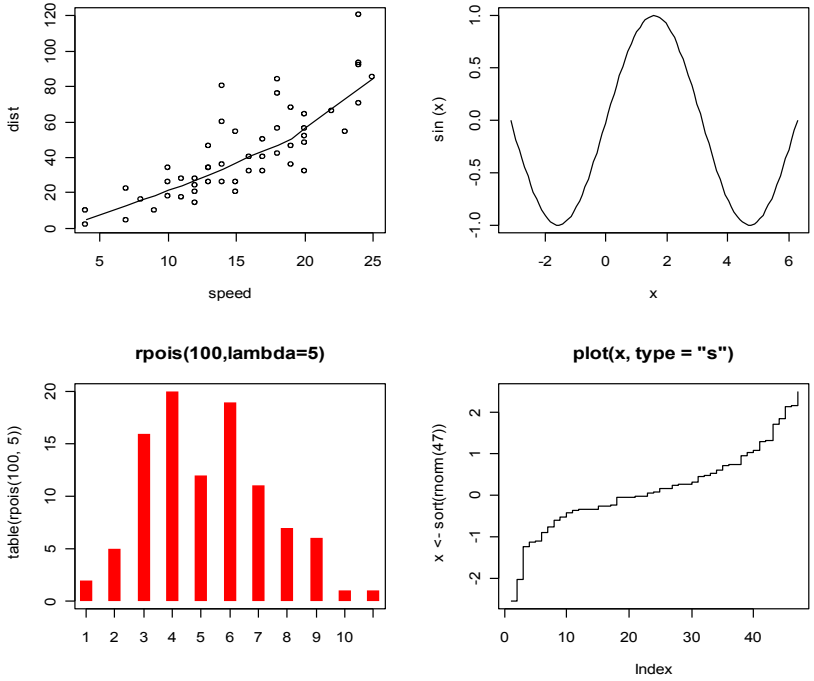
Graphics

4

Starting out with R graphics
Graphics

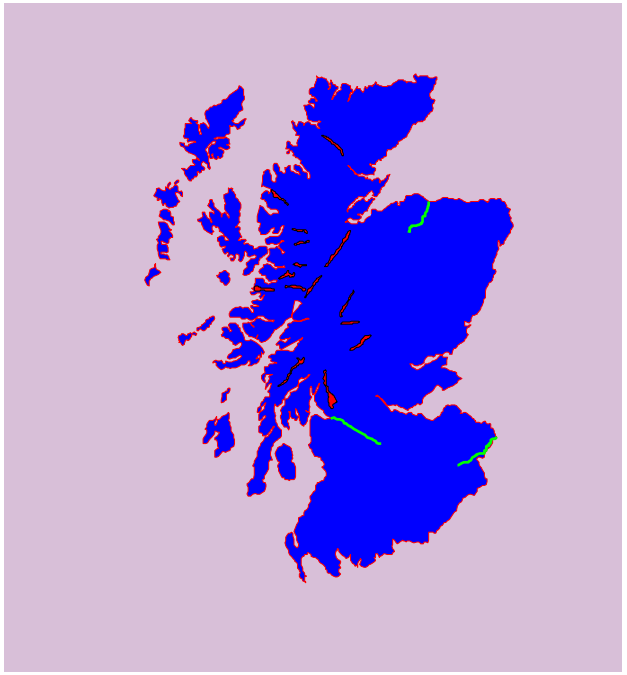
- R provides several mechanisms for producing graphical output
  - Functionality depends on the level at which the user seeks interaction with R
    - graphics systems, packages, devices & engines
- High level graphics
  - Functions compute an appropriate chart based up on the information provided. Optional arguments may tailor the chart as required
    - Interaction is at traditional graphics system level. The user isn't required to know much about anything
- Low level graphics
  - The user interacts with the drawing device to build up a picture of the chart piece by piece.
    - This fine granular control is only required if you seek to do something exceptional
- R graphics produces plots using a painter's model
  - Elements of the graph are added to the canvas one layer at a time, and the picture built up in levels. Lower levels are obscured by higher levels, allowing for blending, masking and overlaying of objects.

High level vs. Low level plotting
Graphics



High level plotting

`example(plot)`

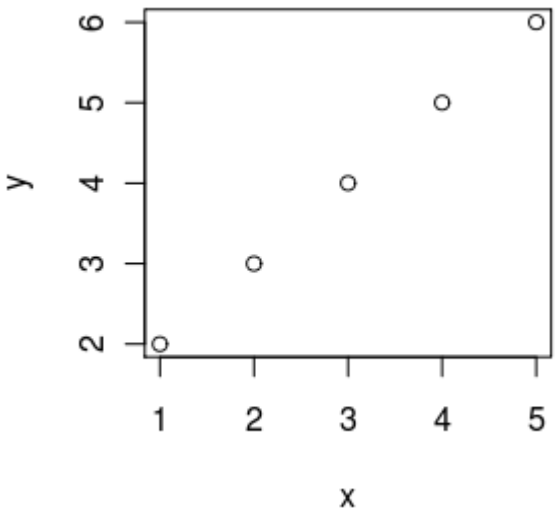


Low level plotting
(Scotland by blighty package)

Essential plotting - plot()

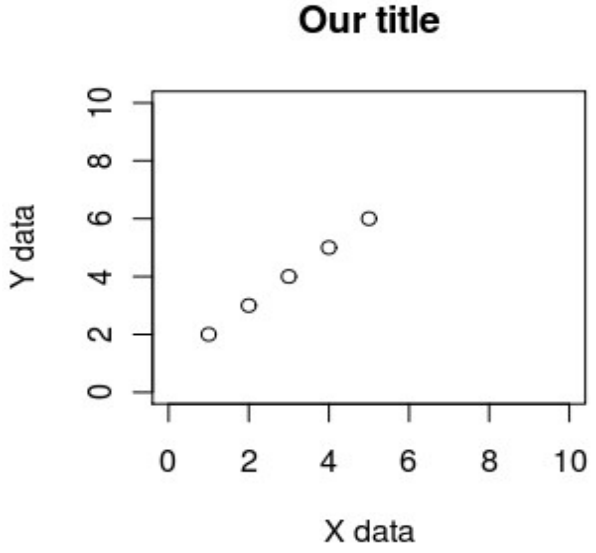
- plot() is the main function for plotting, it takes x,y values to plot and also lots of graphical parameters (see **?par** for all of them)

default plotting




```
x <- 1:5
y <- 2:6
plot(x,y)
```

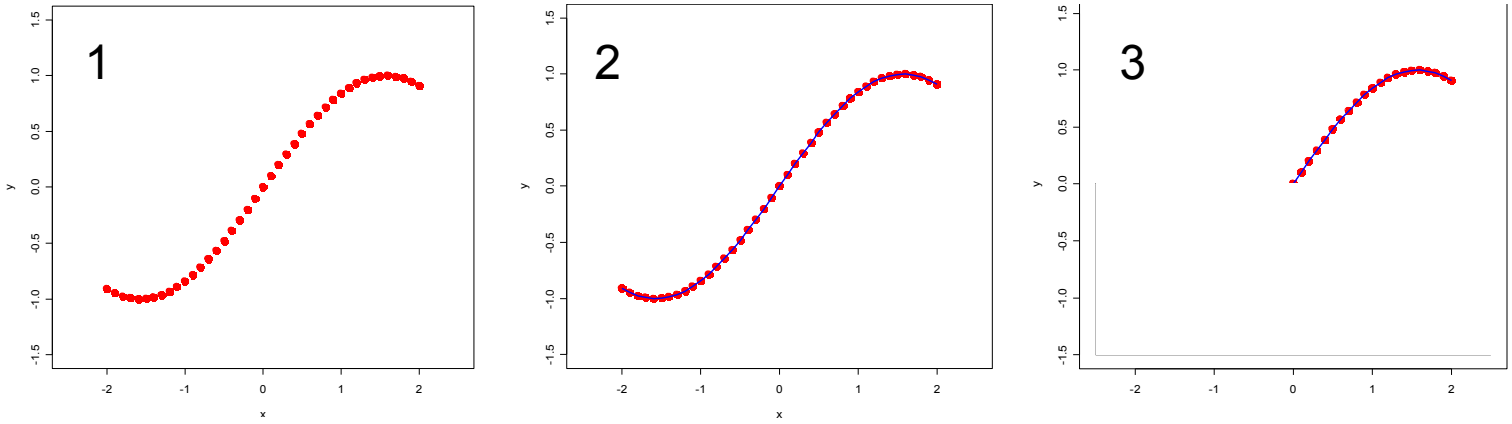
custom plotting




```
x <- 1:5
y <- 2:6
plot(x,y, xlab="X data", ylab="Y
data", xlim=c(0,10), ylim=c(0,10),
main="Our title")
```

R graphics uses a painter's model

```
x <- seq(-2, 2, 0.1)
y <- sin(x)
```




```
plot(y~x, ylim=c(-1.5,1.5),
xlim=c(-2.5,2.5),
col="red" ,pch=16, cex=1.4)
```

```
lines(y~x, ylim=c(-1.5,1.5),
xlim=c(-2.5,2.5), col="blue",
lty=1,lwd=2)
```

```
rect(-2.5,0,2.5,-1.5,
col="white", border="white")
```

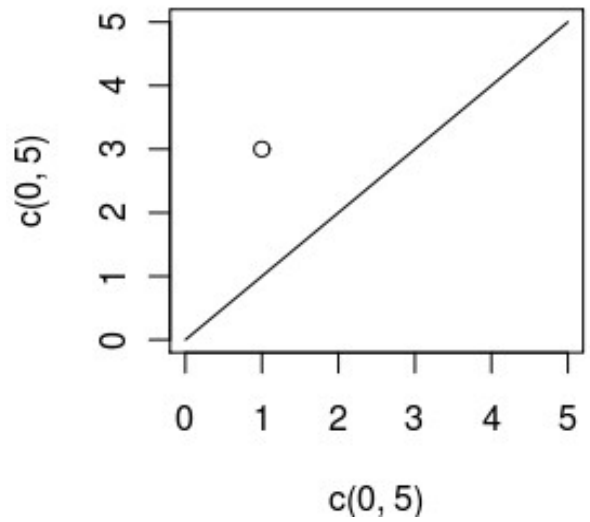
xlim, ylim = axis limits
col = line colour
pch = plotting character [`example(points)`]
cex = character expansion [scaling factor]

lty = line type
lwd = line width
rect = rectangle

Example code:
14_painterModel.R

Plotting x,y data - plot(), points(), lines()

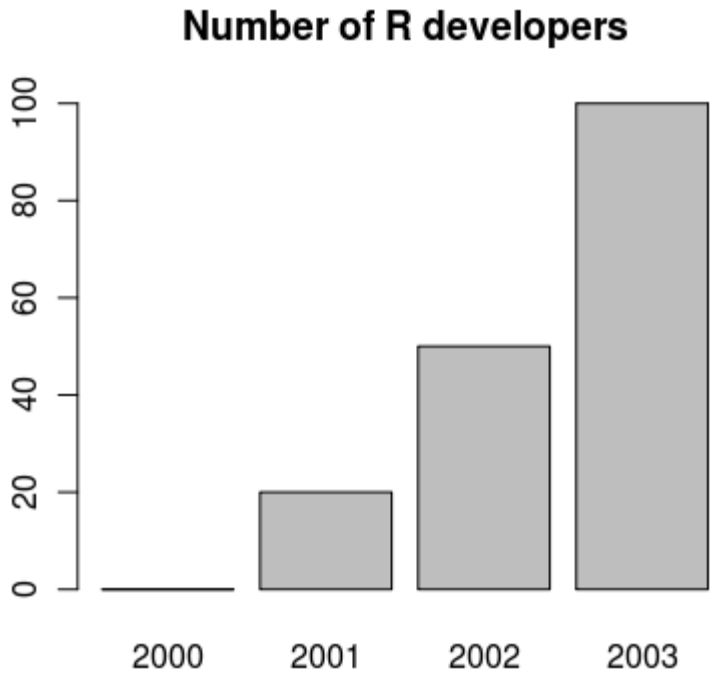
- **plot()** is used to start a new plot, accepts x,y data, but also data from some objects (like linear regression). Use the parameter **type** to draw points, lines, etc (see **?plot**)
- **points()** is used to add points to an existing plot
- **lines()** is used to add lines to an existing plot




```
plot(c(0, 5), c(0, 5), type="l") # draw as line from (0,0) to (5,5)
points(1, 3) # add a point at 1,3
```

Making bar plots - barplot()

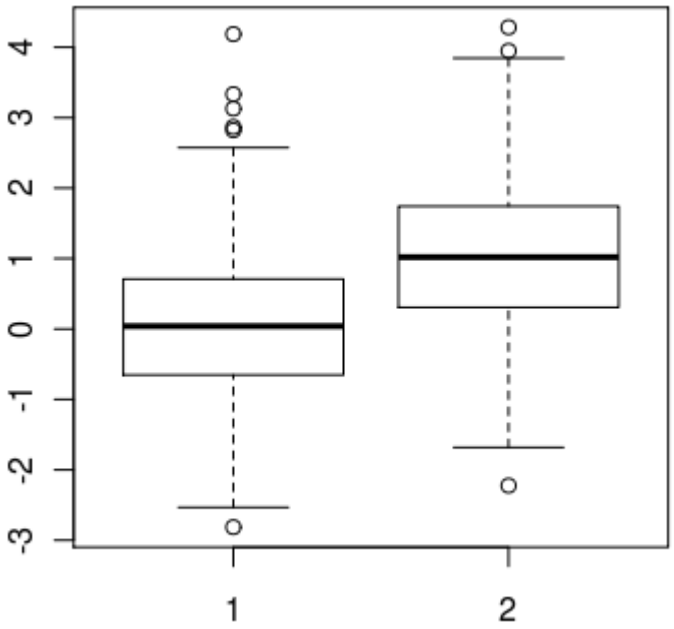
- visualizing a vector of data can be done with bar plots, using function **barplot()**




```
data <- c("2000"=0, "2001"=20, "2002"=50, "2003"=100)
barplot(data, main="Number of R developers")
```

Making box plots - boxplot()

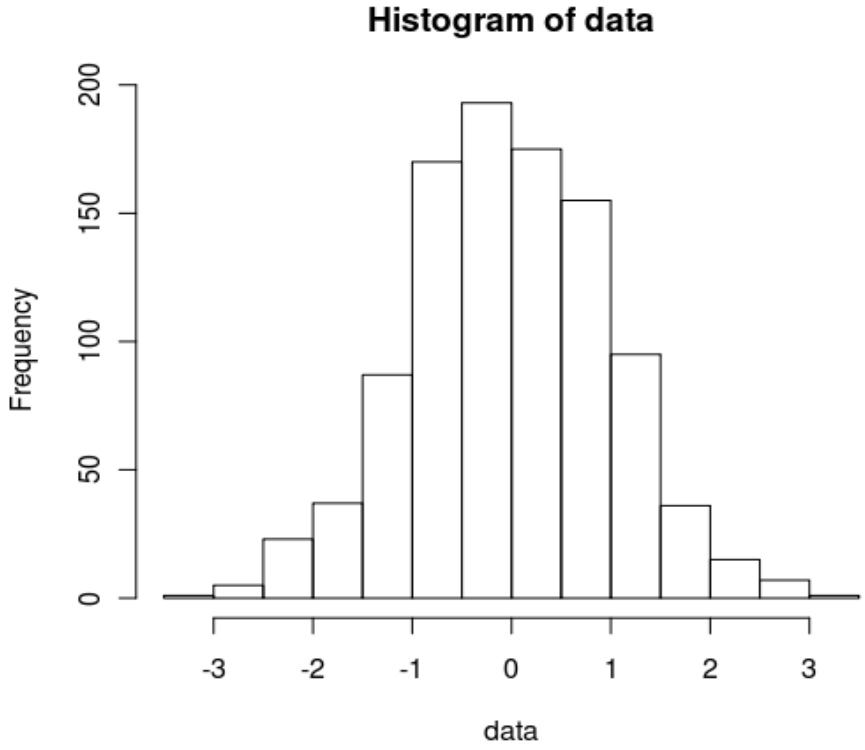
- when a spread of data needs to be visualised, we can use boxplots with function **boxplot()**




```
data1 <- rnorm(1000, mean=0)
data2 <- rnorm(1000, mean=1)
boxplot(data1, data2)
```

Making histograms - hist()

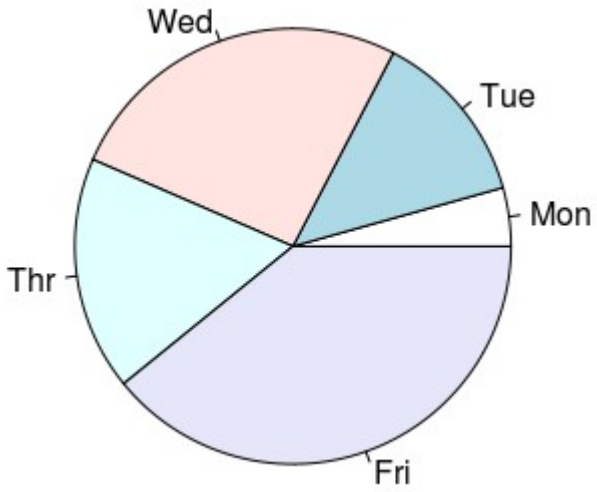
- when we need to look at the distribution of data, we can visualize it using histograms with function hist()




```
data <- rnorm(1000)
hist(data)
```

Pie charts - pie()

• to visualise percentages or parts of a whole we can use pie charts with function **pie()**




```
data <- c("Mon"=1, "Tue"=3, "Wed"=6, "Thr"=4, "Fri"=9)
pie(data)
```

Typical plotting workflow

- Set the plot layout and style - `par()`
  - Set the number of plots you want per page
  - Set the outer margins of the figure region
    - The distance between the edge of the page and the figure region, or between adjacent plots if there are multiple figures per page
  - Set the inner margins of the plot
    - The distance between the plot axes and the labels & titles
  - Set the styles for the plot
    - Colours, fonts, line styles and weights
- Draw the plot - `plot(x,y, …)`

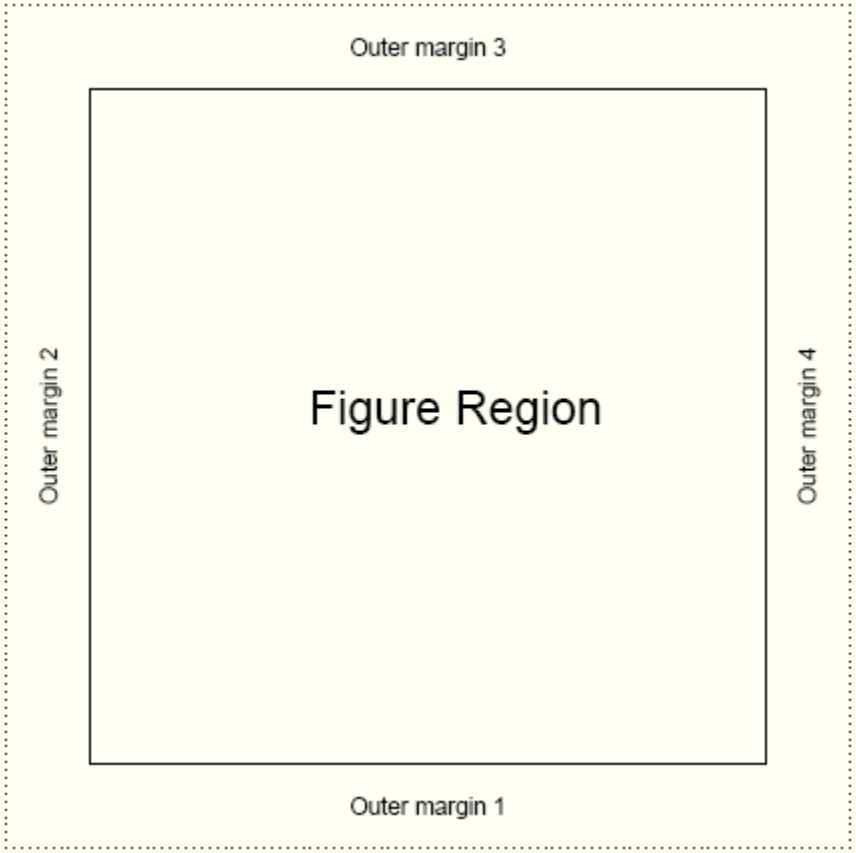
Setting graphics layout and style - par()

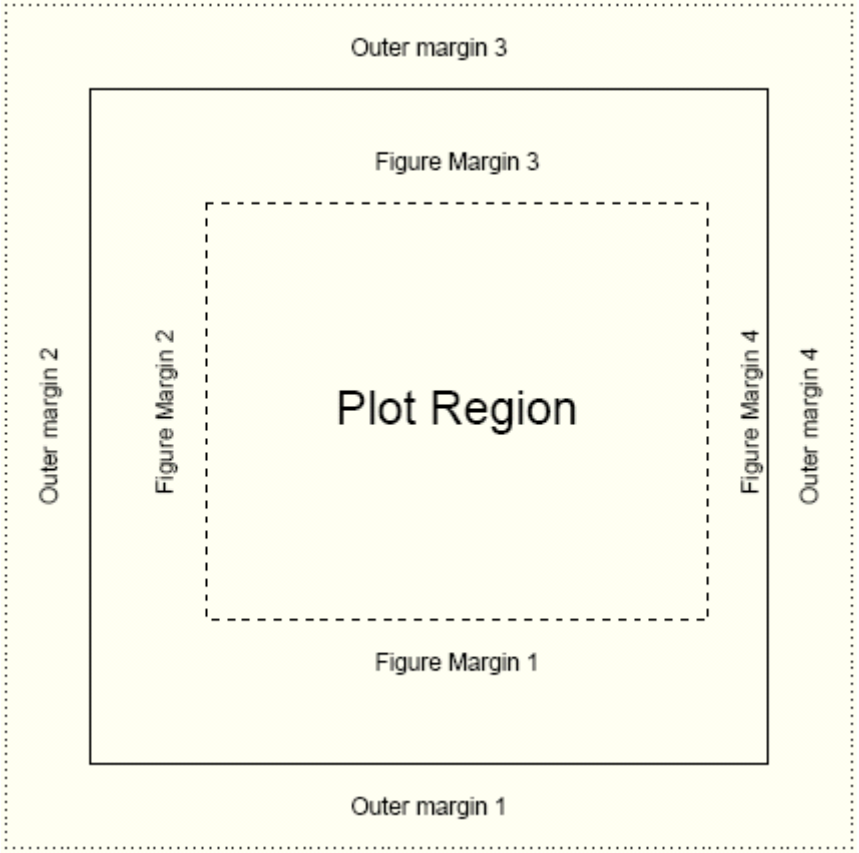
`par()` Top level graphics function

- parameter specifies various page settings. These are inherited by subordinate functions, if no other styles are set.
  - Specific colours and styles may be set globally with `par`, but changed ad hoc in plotting commands
  - The global setting will remain unchanged, and reused in future plotting calls.
- `par` sets the size of page and figure margins
  - Margin spacing is in 'lines'
- `par` is responsible for controlling the number of figures that are plotted on a page
- `par` may set global colouring of axes, text, background, foreground, line styles (solid/dashed), if figures should be boxed or open etc. etc.

type `par()` to get a list of top down settings which may be set globally

Page settings with `par`
Graphics





`par(mfrow=c(1,1))`
one figure on page
`par(oma=c(2,2,2,2))`
equal outer margins

`par(mar=c(5,4,4,2))`
Sets space for x & y labels, a main title, and a thin margin on the right

Numbering: bottom, left, top, right

Page layout plot exercise
Graphics

```
par(mfrow=c(2,2))
```
- 2 x 2 figures per page

```
par(oma=c(1,0,1,0)
```
- 1 line spacing top and bottom

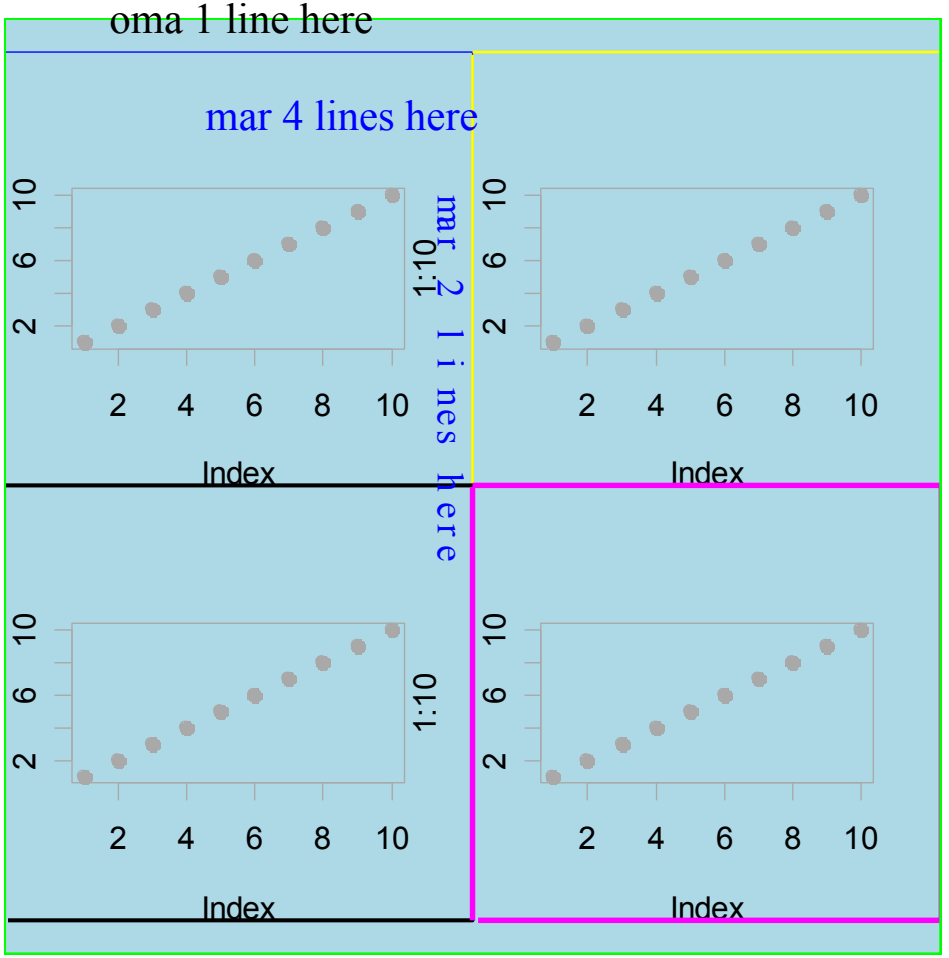
```
par(mar=c(4,2,4,2))
```
- 4 lines at bottom & top
- 2 lines left & right

```
par(bg="lightblue",fg="darkgrey")
```
- light blue background
- dark grey spots

```
par(pch=16,cex=1.4)
```
- Large circles for spots
- Execute 4 times with different colors:

```
plot(1:10)
box("figure",lty=3,col="blue")
```
- Draw a blue dashed line around plot

```
box("outer",lty=1,lwd=3,
col="green")
```
- Draw a green solid line around figure



See how the figure margins overlap
Using painter's model

15_parExample.R

Plotting characters for plot()
size and orientation

pch= ...

Sets one of the 26 standard plotting character used.

Can also use characters, such as "."

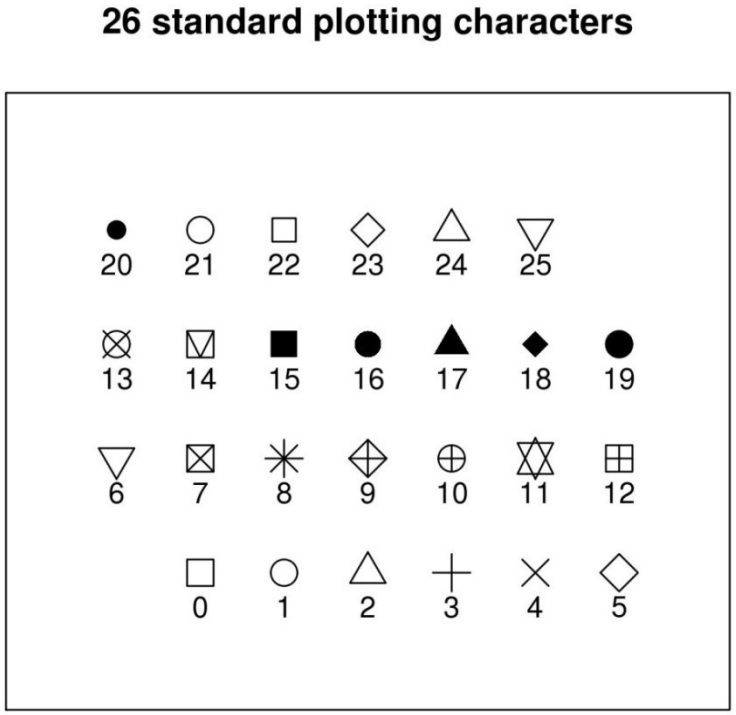
cex= ...

Character expansion. Sets the scaling factor of the printing character

las= ...

Axes label style. 1 normal, 2 rotated 90º

4 styles (0-3)



Plotting characters exercise
Graphics

16_plottingChars.R

```
xCounter<-1
yCounter<-1
plotChar<-0

plot(NULL, xlim=c(0,8),
ylim=c(0,5),xaxt="n",
     yaxt="n",ylab="",xlab=""
     ,main="26 standard plotting
characters")

while (plotChar < 26){
  if(xCounter < 7){
    xCounter <- xCounter+1
  } else {
    xCounter <- 1
    yCounter <- yCounter+1
  }

  points(xCounter,yCounter,pch=plotChar,
cex=2)
  text(xCounter,(yCounter-0.3),plotChar)
  plotChar <- plotChar+1
}
```

X-Y coordinates,
Plotting character index counter

Sets up an empty plotting area.
Axis scale limits, xlim, ylim
Don't draw axis ticks, xaxt, yaxt="n"
Don't annotate axis, xlab, ylab=""
Set a main title, main

We want to print the characters in a 7 x 4 grid. The if statement sets up the character plotting coordinates such that each time x =7, make it 1 again and increment the y axis by 1 at the same time

While loop counts up to 25
(0 to 25 = 26 iterations)
And cycles through each pch available

Annotating the plot

- plot accepts main title, subtitle, X label, Y label as standard arguments

```
plot(x, y, main="...", sub="...", xlab="...", ylab="...")
```

```
mtext(text="…", side= …)
```

- allows text to be written directly into the margin of a plot

```
text(x,y,labels="…")
```

- allows text to be written in the plot at x,y

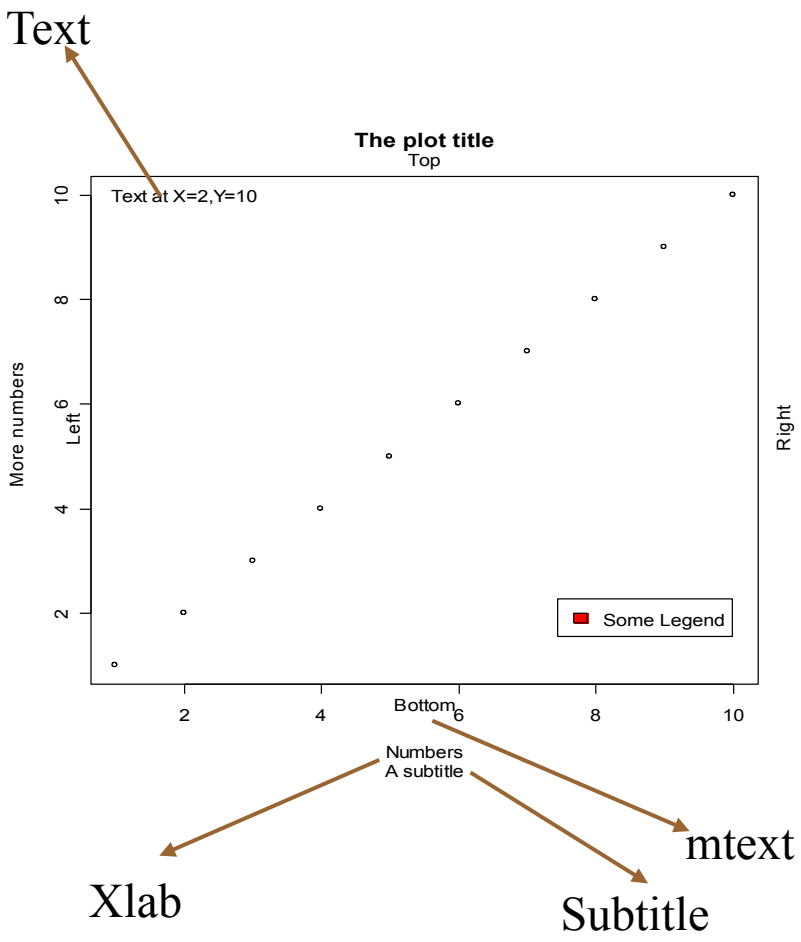
```
legend(x,y, legend=…)
```

- produces a legend for the plot

Appreciating drawing coordinates

- How do we know where to place items within the plot region when building up our customized graphs?
- Most of the time we can specify X,Y coordinates.
  - R calculates sensible pixel coordinates of plots from the data we provide. We don't need to worry about pixels, centimetre distances etc.
- `locator(…)`
  - Returns x,y coordinates from a mouse click within a plot
  - good for working out where to place legend items
- `identify(…)`
  - provides an id tag for the closest plotted point to a mouse click
  - useful if you want to label points on a chart
- `xy.coords(…)`
  - translates x,y coordinates into pixel coordinates
- Margin spacing is in lines
  - The exact distance is a factor of font family, style and size
    - Text may appear bunched or squashed if sufficient distance is not left between the axes and the caption

Building up a plot
Graphics



17_buildingAplot.R

R code

```
par(mfrow=c(1,1))
par(bg="white",fg="black",cex=1)
par(oma=c(1,1,1,1))
par(mar=c(5,4,4,2)+0.1)

plot(1:10,main="The plot title",
sub="A subtitle", xlab="Numbers",
        ylab="More numbers")

mtext(c("Bottom", "Left", "Top",
"Right"), c(1,2,3,4),     line=.5)

text(2,10,"Text at X=2,Y=10")
legend(locator(1),"Some
Legend",fill="red")
```

Adding legend ...
Don't forget to mouse click!

align text left, right & centre with adj=(i,j) i.e centre is adj=(0.5,0.5), left is adj=(1,0) and right is adj=(0,1)

Plots with custom axes
Graphics

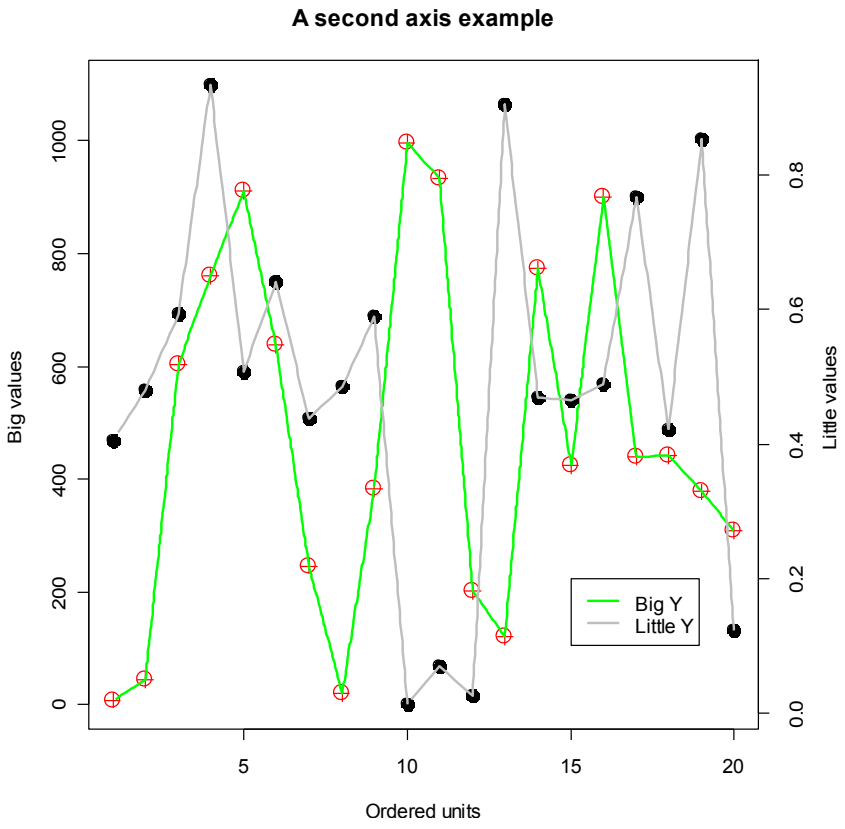
- R `plot` doesn't support multiple Y axis by default
  - You have to make additional axes yourself!
- Adding custom axis

```
axis(side=, at=, labels=, …)
```

- If you want to specify custom axes, make sure you turn off the automatic axes in the plot / points call

```
plot( …, axes=F)
```

Adding a second Y axis
Graphics



The trick

1. plot first Y series
2. use `par(new=T)` to overlay a second figure region
3. plot second series without axes
4. `axis(side=4, …)` to add second Y axis
5. `mtext(side=4, …)` to label second Y

Example: The second Y series
Graphics

18_secondYaxis.R

```
x1<-1:20
y1<-sample(1000,20)
y2<-runif(20)
y2axis<-seq(0,1,.2)

par(mar=c(4,4,4,4))

plot(x1,y1,type="p",pch=10,cex=2,col="red",
        main="A second axis example",
        ylab="Big values",ylim=c(0,1100),
        xlab="Ordered units")
points(x1,y1,type="l",lty=3,lwd=2,col="green")

par(new=T)

plot(x1,y2,type="p",pch=20,cex=2,col="black",axes=FALSE,bty="n",xlab="",ylab="")
points(x1,y2,type="l",lty=2,lwd=2,col="grey")

axis(side=4,at=pretty(y2axis))
mtext("Little values",side=4,line=2.5)

legend(15,0.2,c("Big Y","Little Y"),lty=1,lwd=2,col=c("green","grey"))
```

Demo data

Set up equivalent figure margins

Plot and label first Y series

Connect dots with a line

Overlay a second plot region

Plot second Y series, but suppress labels

Anotate second Y axis

Add legend, note X,Y is on second Y axis scale

Use of colour in R
Graphics

- Colour is usually expressed as a hexadecimal code of Red, Green, and Blue counterparts
  - No good for humans.
- R supports numerous colour palettes which are available through several "colour" functions.
  - `colours()` # get inbuilt names of known colours
    - RGB primaries may take on a decimal intensity value of 0 to 255
      - 255 is #FF in hexidecimal
        - White is #FF FF FF
        - Black is #00 00 00
  - `rgb()` # converts red green blue intensities to colour
    - Strangely, likes decimalized intensities (ie. 0 is black, 1 is white)

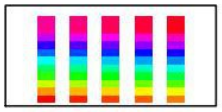
```
> rgb(1,1,1)
[1] "#FFFFFF"

> par(mfrow=c(2,2))
> plot(1:10,col="#FF00FF")
> plot(1:10,col=rgb(1,0,1))
> plot(1:10,col="magenta")
```

Colour Ramps & Palettes
Graphics

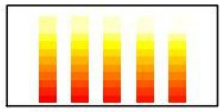
- Heatmaps use colour depth to convey data values. Cold colours are typically low values, and light colours are high state values. This is a colour ramp.
- R supports numerous graded colour charts. Specify *n*, to set the number of gradations required in the palette

```
rainbow(n)
heat.colors(n)
terrain.colors(n)
topo.colors(n)
cm.colors(n)
```

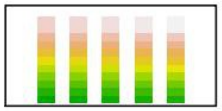
Rainbow Colours



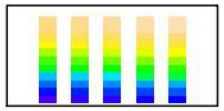
Heat Colours



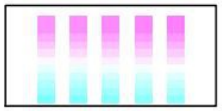
Terrain Colours



Topological Colours



Cyan Magenta Colours



19_colourCharts.R

You can specify a user defined palette of indexed colours:

```
palette(rainbow(7)) # creates 7 indexed colours (1:7) based on
                    # rainbow palette  R O Y G B I V !!!
```

Colour packages: RColorBrewer
Graphics

- This add on package provides a series of well defined colour palettes. The colours in these palettes are selected to permit maximum visual discrimination
- Access the RColorBrewer library functions ...

```
library("RColorBrewer")
```

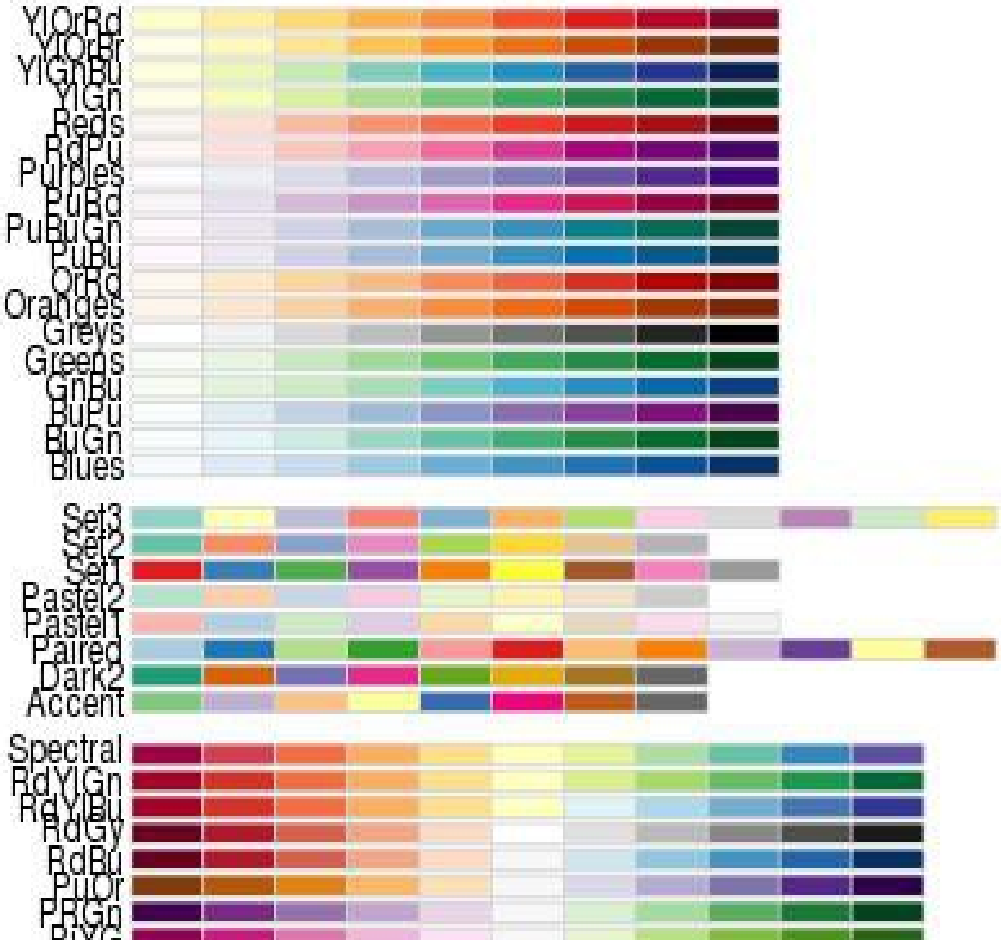
- Check out the available palettes

```
display.brewer.all(n=NULL, type="all", select=NULL, exact.n=TRUE)
```

- Define your own palette based on one of RColorBrewers'

```
myCol<-brewer.pal(n,"...") # n=number of colours, "..." is the palette name
```

RColorBrewer named palettes
Graphics



Saving plots to files

- Unless specified, R plots all graphics to the screen
- To send plots to a file, you need to set up an appropriate graphics device …

```
postscript(file="a_name.ps", …)
pdf(file="…pdf", …)
jpeg(file=" …jpg", …)
png(file=" ….png", …)
```

  - Each graphics device will have a specific set of arguments that dictate characteristics of the outputted file
    - `height=, width=, horizontal=, res=, paper=`
      - Top tip: jpg, A4 @ 300 dpi, portrait, size in pixels
      - `jpg(file="my_Figure.jpg", height=3510, width=2490, res=300)`
      - Postscript & pdf work in inches by default, A4 = 8.3" x 11.7"

- Graphics devices need closing when printing is finished

```
dev.off()
```

for example:

```
png("tenPoints.png", width=300, height=300)
plot(1:10)
dev.off()
```

Thoughts when plotting to a file
Graphics

- Its very tempting to send all graphical output to a pdf file. Caution!
  - For high resolution publication quality images you need postscript. Set up postscript file capture with the following function

```
postscript("a_file.ps",paper="a4")
```

    - postscript images can be converted to JPEG using ghostscript (free to download) for low resolution lab book photos and talks
  - PDF images will grow too large for acrobat to render if plots contain many data points (e.g. Affymetrix MA plots)
  - Automatically send multiple page outputs to separate image files using …`file="somename%02d.jpg"`
  - Don't forget to close graphics devices (i.e. the file) by using
    - `dev.off()`

Plotting exercise
Graphics

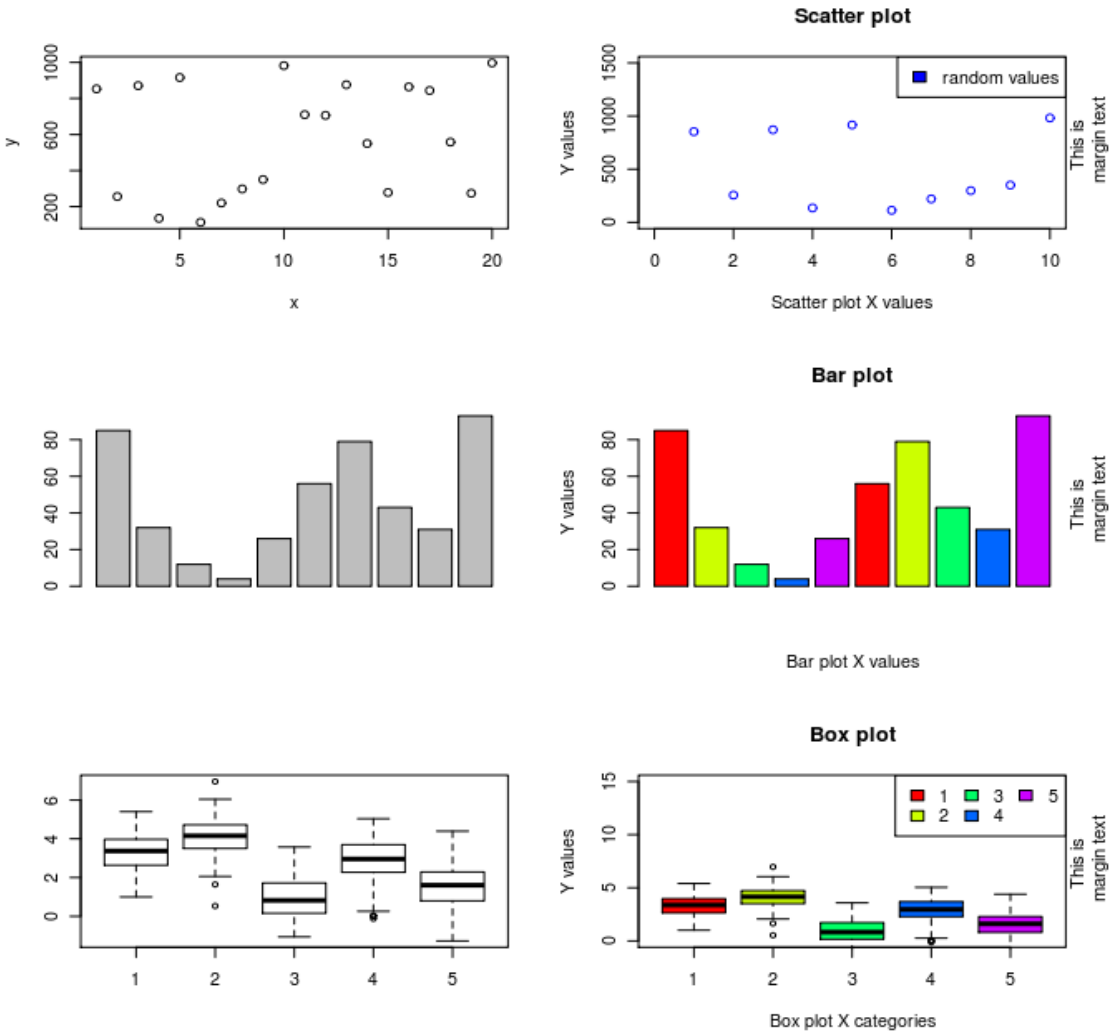
- Exercise:
  - Make a full A4 page figure comprising of 6 plots: 2 each of XY plot (`plot()`), barchart (`barplot()`) and box plots (`boxplot()`)
  - The two version of each plots should consistent of: the default plot and a customised plot (change for instance colours, range, captions...)
  - Output the completed 6-panel figure to: screen, jpeg, postscript and pdf file

- Suggested route to solution:

  1. Generate some plotting data appropriate for each type of plot
  2. Write the code to produce the six plots, once plotting the data by using default plotting, one with some customisations you want
  3. To output the plot to screen, jpeg, postscript and pdf you will need to redo the plot multiple times - create a function to do a plotting and call it by redirecting graphical output to screen, jpeg file, poscript file and pdf file

20_6PanelPlotScript.R

6 Panel plots exercise
Graphics



References

- Official documentation on:
  - http://cran.r-project.org/manuals.html
- A good repository of R recipes:
  - Quick-R: http://www.statmethods.net/
- Don't forget that many packages come with tutorials (vignettes)
- Website of this course:
  - http://logic.sysbiol.cam.ac.uk/teaching/Rcourse/
- R forums (stackoverflow & official):
  - http://stackoverflow.com/questions/tagged/r
  - http://news.gmane.org/gmane.comp.lang.r.general
- Plenty of textbooks to choose from, comprehensive list + reviews:
  - http://www.r-project.org/doc/bib/R-books.html

Thanks for your attention!

END OF COURSE