

First steps with R in Life Sciences: Graphics

February, 2022 - Streamed

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-- with slides from Diana Marek, Wandrille Duchemin, Leonore Wigger

















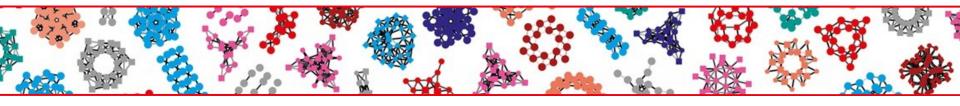
Exam – for 0.5 ECTS credit points

Take-home exam: data analysis tasks, available on course page.

Exam is graded as "pass" or "fail".

- Submit analysis by February 23, 2022, 23:59:59
- (e-mail in the chat + google doc).
- You will receive a certificate of achievement from the SIB Training Team, which you can submit to your educational institution.
- If you don't take the exam, you will receive a certificate of attendance.

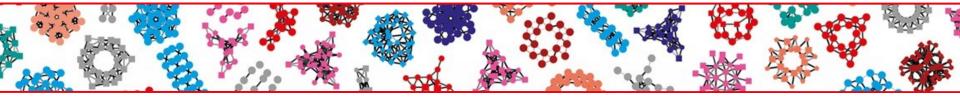
Outline



Day 2

- Building graphics in R (basic plotting)
- Starting with statistics in R (hypothesis testing, simple linear regression)

Examples and exercises are integrated in the chapters



Building graphics in R

R graphics

R is powerful for plotting graphs and figures. It provides several plotting systems:

- base (widely used, comes with basic R installation)
- ggplot2 (widely used)
- lattice (mainly used for specialized needs, e.g. 3D plots)

They have very different syntax, cannot be mixed, and need to be learned separately. This course gives an introduction to the R base plotting system

R base plotting system

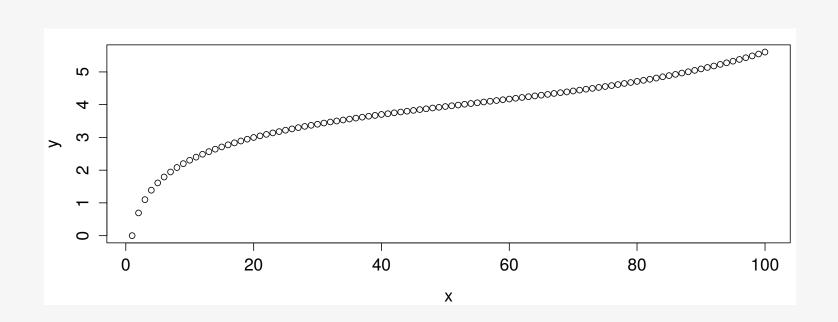
Plots are built up step-by-step with multiple function calls.

- High level graphics functions:
 - Draw a new plot. Tailor its appearance with optional arguments.
- Low level graphics functions:
 - Add graphical elements to an existing plot, piece by piece.

Plotting - the basics

- The generic function to use is plot(), which plots a variable y against a variable x.
- Takes the argument type to indicate the type of plot ("I" for lines, "p" for points and "b" for both). The default is points.

```
> x <- 1:100
> y <- log(x) + (x/100)^5
> plot(x,y) \# equivalent to plot(x, y, type)
```

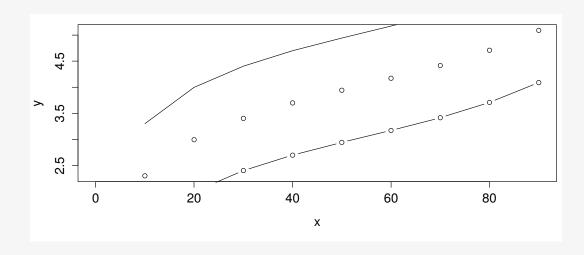


Adding elements to a plot

- Every time the plot function is called, a new plot is created.
- In order to add more graphical elements to an already existing plot, low-level plotting commands can be used, such as:
 - points() to add points to an existing plot
 - lines() to add a line to an existing plot

The **type** argument can also be provided to those functions ("**I**" for lines, "**p**" for points and "**b**" for both). Default for points(): "**p**", default for lines(): "**I**".

```
>x <- seq(0,100, by=10)
>y <- log(x) + (x/100)^5
>plot(x,y)
>lines(x,y+1)
>points(x,y-1, type
```

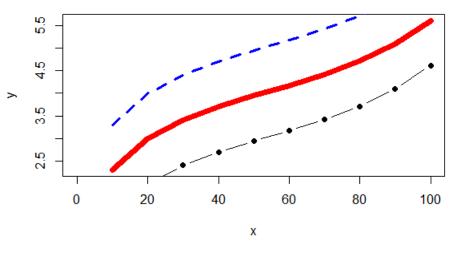


Customizing plots - Part 1

- plot(), points() and lines() all take customizing arguments, including:
 - col indicating the colour
 - **Iwd** indicating the line width
 - Ity indicating the line type
 - pch indicating the plotting character (symbol)

>points(x, y-1, type="b",

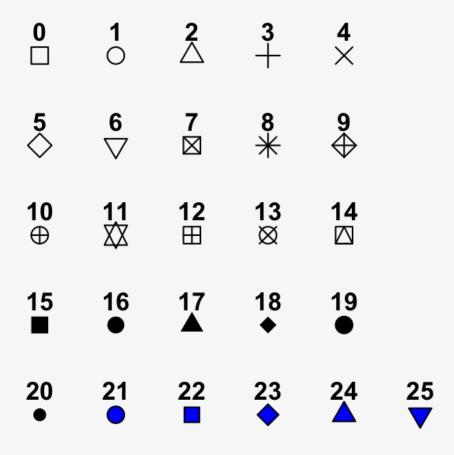
pch=19)



R line types, to use with Ity

 Ity=1 or 'solid'	
 Ity=2 or 'dashed'	
 Ity=3 or 'dotted'	
 Ity=4 or 'dotdash'	
 lty=5 or 'longdash'	
 Ity=6 or 'twodash'	

R plotting characters, to use with pch



R color

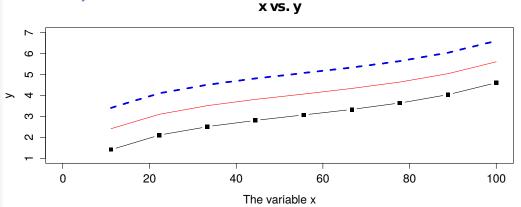
- default palette : 1 to 8 |
- Hexadecimal:
 - "#1492AD"
 - "#BC520088" ← transparency
- 657 built-in color names,
 - here is a subset:
- colors() will output a list of all color names

See R color cheat sheet for the full color chart and other ways to define colors

white	aliceblue	antiquewhite	antiquewhite1	antiquewhite2
antiquewhite3	antiquewhite4	aquamarine	aquamarine1	aquamarine2
aquamarine3	aquamarine4	azure	azure1	azure2
azure3	azure4	beige	bisque	bisque1
bisque2	bisque3	bisque4		blanchedalmond
blue	blue1	blue2	blue3	blue4
blueviolet	brown	brown1	brown2	brown3
brown4	burlywood	burlywood1	burlywood2	burlywood3
burlywood4	cadetblue	cadetblue1	cadetblue2	cadetblue3
cadetblue4	chartreuse	chartreuse1	chartreuse2	chartreuse3
chartreuse4	chocolate	chocolate1	chocolate2	chocolate3
chocolate4	coral	coral1	coral2	coral3
coral4	cornflowerblue	cornsilk	cornsilk1	cornsilk2
cornsilk3	cornsilk4	cyan	cyan1	cyan2
cyan3	cyan4	darkblue	darkcyan	darkgoldenrod
darkgoldenrod1	darkgoldenrod2	darkgoldenrod3	darkgoldenrod4	darkgray
darkgreen	darkgrey	darkkhaki	darkmagenta	darkolivegreen
darkolivegreen1	darkolivegreen2	darkolivegreen3	darkolivegreen4	darkorange
darkorange1	darkorange2	darkorange3	darkorange4	darkorchid
darkorchid1	darkorchid2	darkorchid3	darkorchid4	darkred
darksalmon	darkseagreen	darkseagreen1	darkseagreen2	darkseagreen3
darkseagreen4	darkslateblue	darkslategray	darkslategray1	darkslategray2
darkslategray3	darkslategray4	darkslategrey	darkturquoise	darkviolet
deeppink	deeppink1	deeppink2	deeppink3	deeppink4
deepskyblue	deepskyblue1	deepskyblue2	deepskyblue3	deepskyblue4

Customizing plots - Part 2

- The plot() command takes further arguments to customize the plotting area:
 - xlim and ylim to set the limits on the x- and y-axis, respectively
 - xlab and ylab to set the labels for the x- and y-axis, respectively
 - main to set a title

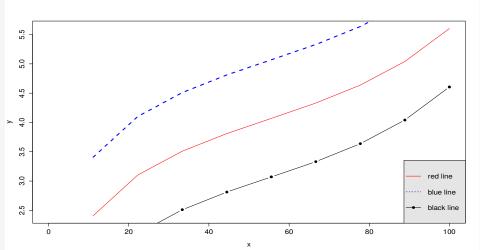


Customizing plots - Part 3

- The legend() command can be used to add legends to plots:
 - x , y to set the numeric coordinates for positioning the legend.
 - x can be used by itself with a keyword for legend position: "bottomright", "bottom", "bottomleft", "left", "topleft", "top", "topright", "right", "center"
 - legend to set the text to appear in the legend
 - col , lty , lwd , pch : set graphical elements in the legend
 - **bty** for box type around the legend ("o" for box, "n" for no box)
 - bg for background color
- > legend(x="bottomright",

legend=c("red line","blue line", "black line"),

```
lty=c(1,2,1),
pch=c(NA,NA,19),
col=c("red", "blue", "black"),
bg="gray90")
```



How to get data for practicing and playing

R can easily simulate data drawn from a given distribution. The function rnorm() generates normally distributed data.

Example:

How to get data for practicing and playing

R can easily simulate data drawn from a given distribution. The function rnorm() generates normally distributed data.

Example:

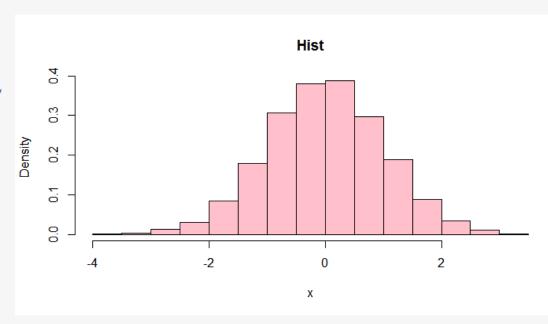
```
>rnorm(10, mean=10, sd=2) #customized mean and sd [1] 6.253392 9.527140 9.398857 11.932284 11.472909 [2] 10.714245 7.656026 11.302829 9.332930 10.264157
```

If you want data from other distributions than normal: rpois() for poisson, rbinom() for binomial (see R help)

The hist() function

- The function hist() produces a histogram, which counts the number of observations that fall into different ranges (bins)
- Rough visual representation of the distribution of the data.
 - x vector of data values for which the histogram will be constructed
 - **breaks** either a vector indicating breakpoints between histogram bins, or a single number for the number of bins (used as suggestion)
 - **freq** logical. If TRUE, cell height represents counts per bin. If FALSE, cell height is the fraction of values that fall into each bin (probability density).

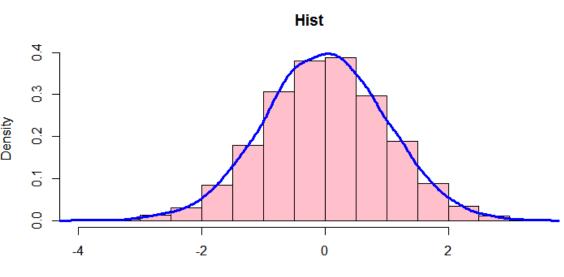
```
> x <- rnorm(10000)
> hist(x, breaks=20, freq=FALSE,
  main="Hist", col="pink")
```



The hist() and density() functions

- To add a smooth line to a histogram, use density(), which computes estimates of the probability density (kernel density estimates).
- This works as a complementary representation of the histogram only when freq = FALSE
- The line produced by density() often reflects the distribution better than a histogram.
- Use lines() to plot the result as a line on top of the

histogram.



Х

Draftsman's or Pairs Scatter Plots

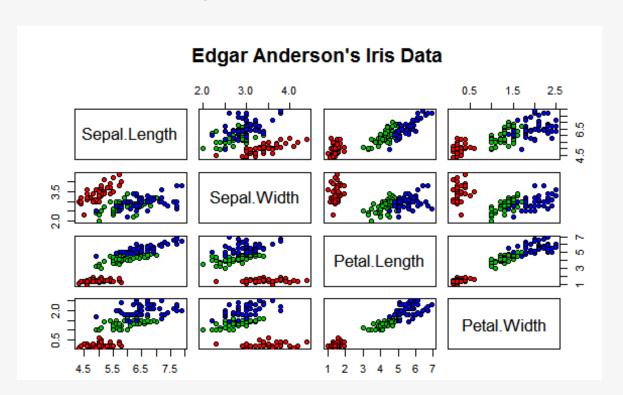
- **P5S**

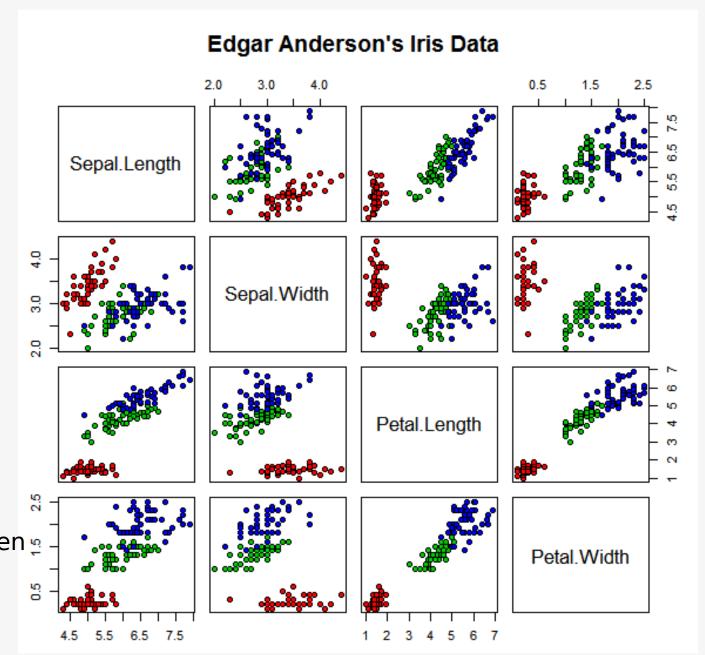
- If x is a matrix or a data frame, **pairs()** draws all possible bivariate plots between the columns of x.
- > data(iris) #contains 4 measurements for 150 flowers from 3 species of iris (Iris setosa, versicolor and virginica)
- > pairs(iris[,1:4], main="Edgar Anderson's Iris Data", pch=21, bg=c("red", "green3", "blue")[iris\$Species])

bg: color fill of circles

Colors:

setosa in red versicolor in green virginica in blue





Colors:
setosa in red
versicolor in green
virginica in blue

Excursus: Coloring data points in the iris data(I)

bg=c("red", "green3", "blue")[iris\$Species]) #color fill

How does this work?

iris\$Species #factor with 3 levels

```
setosa
      [1] setosa
                                                                                                                                           setosa
                                                                                                                                                                                                                                               setosa
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      [92] versicolor versic
 [105] virginica 
 [118] virginica 
[131] virginica 
[144] virginica virginica virginica virginica virginica virginica virginica
```

Levels: setosa versicolor virginica

as.numeric(iris\$Species) #factor coerced to numeric

Excursus: Coloring data points in the iris data (II)

The coloring strategy in the iris draftman's plot involves subsetting and coercion:

```
bg=c("red", "green3", "blue")[iris$Species]
     is equivalent to
bg=c("red", "green3", "blue")[as.numeric(iris$Species)]
      is equivalent to
bg=c("red","green3",blue")
```

Inside the square brackets [] , the factor is automatically coerced to a numeric

Excursus: Coloring data points in the iris data (III)

This results in a vector of color terms with 150 entries in the correct order.

```
> bg=c("red", "green3", "blue")[iris$Species] #color fill
```

> bg

```
[1] "red"
           "red"
                  "red"
                         "red"
                                "red" "red"
                                              "red" "red"
                                                              "red"
                                                                     "red"
                                                                            "red"
                                                                                   "red" "red"
                          "red"
                                                               "red"
                                                                      "red"
[14] "red" "red"
                   "red"
                                "red"
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[27] "red"
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[40] "red"
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                   "red"
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                                 "red"
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                                                                             "red"
                                                                                    "green3"
"green3"
[53] "green3" "green3" "green3" "green3" "green3" "green3" "green3" "green3" "green3" "green3"
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[92] "green3" "blue"
"blue" "blue"
[105] "blue" "blue"
"blue"
[118] "blue" "blue"
"blue"
[131] "blue" "blue"
"blue"
[144] "blue" "blue" "blue" "blue" "blue" "blue" "blue"
```

Let's practice - 7

Let's come back to the mice dataset stored in the "mice_data" data frame (Let's practice – 3). If mice_data is not currently in your workspace, either get it back by loading the .Rdata file or import the data again from the original .csv file.

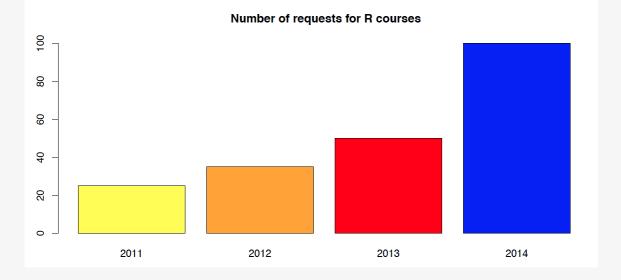
- 1) Check your data frame: did it load correctly? Make sure genotype and diet are factor variables.
- 2) Plot an histogram of mouse weight and customize it with colours, labels, title and represent the density line on top.
- 3) Make a scatter plot of mouse weights using the function plot(), with no additional arguments. (You do not need to define values for a second axis.) Inspect the plot what appears on the two axes? Then re-do the plot by adding function arguments: Use solid circles as plotting symbol, add a title, customise the y-axis label, and colour the points **by genotype.** Add a legend.

The barplot() function

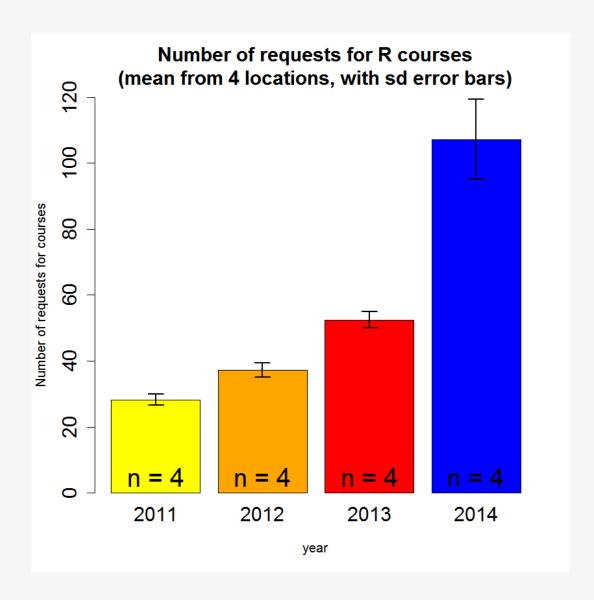
 Barplots allow simple visualization of counts or numeric data.

Optional arguments can be used to customize the

plot.



Customized barplot with sd error bars



Each year, R course requests were counted in 4 cities.

Bar heights: mean across all cities per year

Custom plotting using low level functions:

- add error bars (std dev.)
- add text to each bar (n=4)

Customized barplot with sd error bars (I)

Create a data frame

```
df <- data.frame(
    year = c("2011","2012","2013","2014",
         "2011", "2012", "2013", "2014",
         "2011","2012","2013","2014",
         "2011","2012","2013","2014"),
    city = c("A","A","A","A", "B","B","B","B",
         "C","C","C","C", "D","D","D","D"),
    nb requests courses = c(30,36,50,98,26,35,54,101,
                 28.38.51.105. 29.40.55.125))
# Check what is inside
> head(df)
        city nb_requests_courses
year
1 2011
           Α
                         30
2 2012
                         36
            Α
3 2013
                         50
            Α
4 2014
                         98
            Α
5 2011
            B
                         26
```

Customized barplots with sd error bars (II)

Compute summary statistics

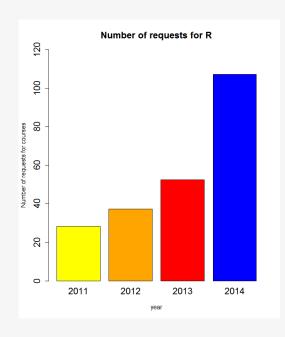
```
# Compute mean, sd, number of observations per year
mean_nb <- tapply(df$nb_requests_courses, df$year, mean)</pre>
sd_nb <- tapply(df$nb_requests_courses, df$year, sd)</pre>
n_values <- tapply(df$nb_requests_courses, df$year, length)</pre>
 > mean nb
  2011 2012 2013 2014
  28.25 37.25 52.50 107.25
 > sd nb
  2011
          2012 2013
                           2014
  1.707825 2.217356 2.380476 12.175796
 > n values
  2011 2012 2013 2014
```

Customized barplots with sd error bars (III)

High-level: Generate plot

mids contains the location of the middle of the bars on the x-axis

```
> mids
[,1]
[1,] 0.7
[2,] 1.9
[3,] 3.1
[4,] 4.3
```



Customized barplots with sd error bars (IV)

Low-level: Add elements (error bars and text)

```
# Use arrows() to put sd error bars on the plot
```

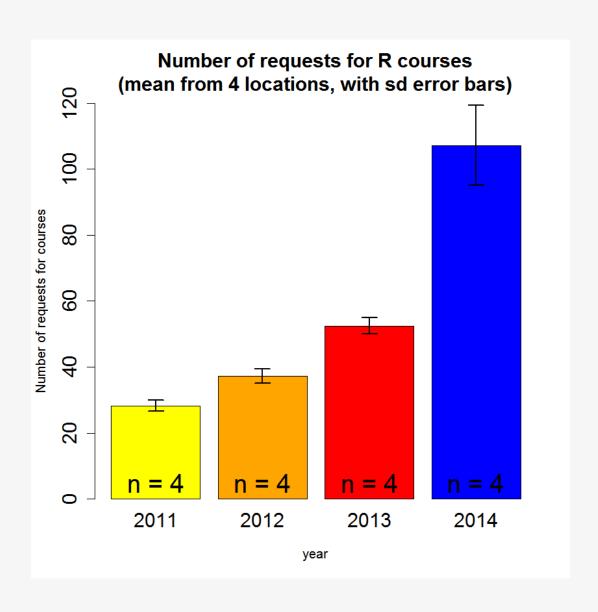
```
T— "arrow head"
— "arrow shaft"
```

```
arrows(mids, mean_nb-sd_nb, # coordinates of lower point
    mids, mean_nb+sd_nb, # coordinates of upper point
    code=3, # type of arrow: "head at both ends" angle=90, #
angle between shaft and head of arrow
    length=0.1, # length of edges of arrow head
    lwd=2) # line width
```

Add text at the midpoints and at height 5 on the y-axis: number of observations

```
text(x=mids, y=5, paste("n =",n_values), cex=2
```

Customized barplot with sd error bars



Barplot digression...

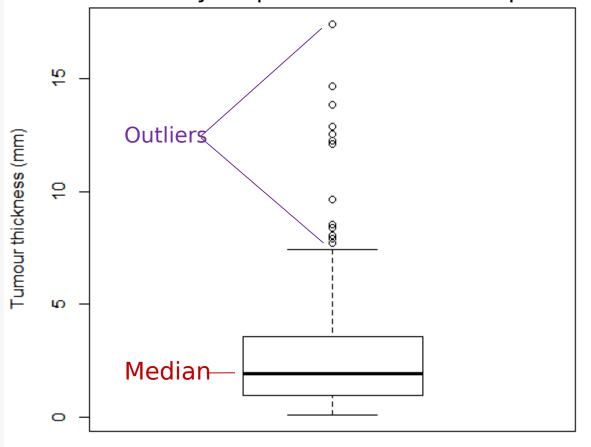
- Bar plots with error bars are widely used in biology
- Be aware: different ways of calculating error bars (sd or sem*)
 (standard deviation or standard error of the mean)
- Often not the most informative way to look at the data, combine them with other plots (box plot, violin plots, scatter plot)
- https://stekhoven.shinyapps.io/barplotNonsense/

*sd: standard deviation sem: standard error of the mean

The boxplot() function

Convenient way of depicting the spread of numerical data

- **Box**: Interquartile range (IQR), contains 50% of points
- Whiskers: Extend from box, indicate variability outside upper and lower quartiles
- Outliers: May be plotted as individual points



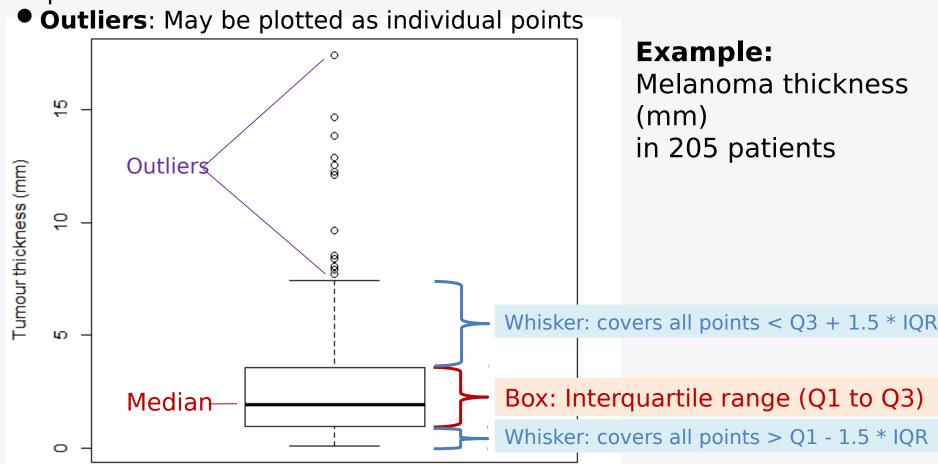
Example:

Melanoma thickness (mm) in 205 patients

The boxplot() function

Convenient way of depicting the spread of numerical data

- **Box**: Interquartile range (IQR), contains 50% of points
- Whiskers: Extend from box, indicate variability outside upper and lower quartiles



Boxplot: data and plotting code

>library(MASS)

>data(Melanoma) #Data from MASS package. 205 patients in Denmark

with malignant melanoma

>head(Melanoma) #look inside the data set time status sex age year thickness ulcer

```
      1
      10
      3
      1
      76
      1972
      6.76
      1

      2
      30
      3
      1
      56
      1968
      0.65
      0

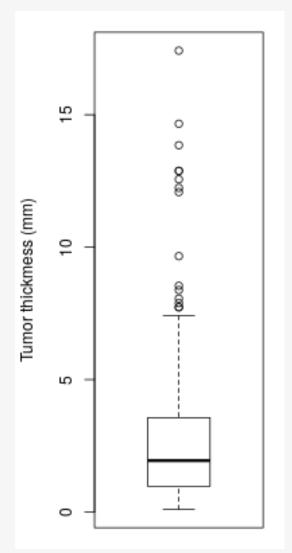
      3
      35
      2
      1
      41
      1977
      1.34
      0

      4
      99
      3
      0
      71
      1968
      2.90
      0

      5
      185
      1
      1
      52
      1965
      12.08
      1

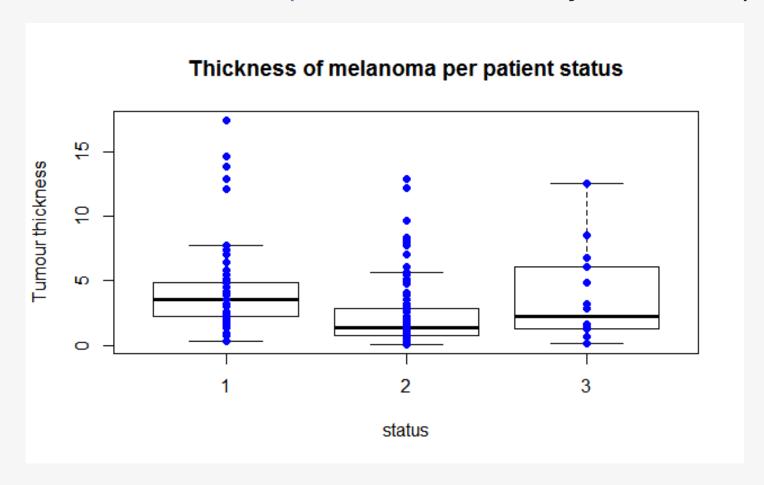
      6
      204
      1
      1
      28
      1971
      4.84
      1
```

>boxplot(Melanoma\$thickness, ylab="Tumour thickness (mm)")



More boxplots

- Make separate boxplots for subgroups of data
- Plot individual data points as an overlay of the boxplots.



status: 1 died from melanoma, 2 alive, 3 dead from other causes

More boxplots: data preparation

#coerce the grouping variable to factor

>Melanoma\$status <- factor(Melanoma\$status)

```
#check if the grouping variable is a factor (it is not!)
>str(Melanoma)
'data.frame': 205 obs. of 10 variables:
$ time : int 10 30 35 99 185 204 210 232 232 279 ...
$ status : int 3 2 3 1 1 1 3 1 1 ...
$ sex : int 1 1 1 0 1 1 1 0 1 0 ...
$ age : int 76 56 41 71 52 28 77 60 49 68 ...
$ year : int 1972 1968 1977 1968 1965 1971 1972 1974
$ thickness: num 6.76 0.65 1.34 2.9 12.08 ...
$ ulcer : int 1 0 0 0 1 1 1 1 1 1
```

More boxplots: plotting code

Method 1: Data subsets

>points(Melanoma\$status, Melanoma\$thickness, col="blue",pch=19) #adds the actual data points to the plot

Method 2: Formulas

```
>boxplot(thickness ~ status, data=Melanoma,
main="Thickness of melanoma per patient status",
xlab="status", ylab="Tumour thickness")
```

```
>points(thickness ~ status, data=Melanoma, col="blue", pch=19) #adds the actual data points to the plot
```

More boxplots: plotting code

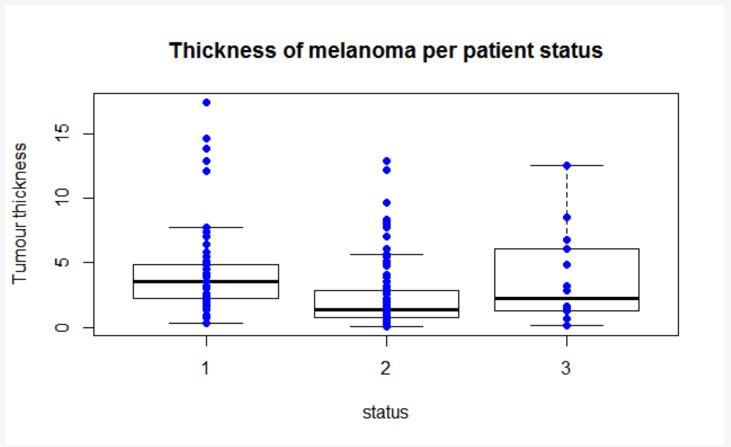
- **P5S**

Method 1: Data subsets - boxplot(df\$y[df\$x=='a'], df\$y[df\$x=='b'])

Method 2: Formulas

- boxplot(y ~ x , data=df)

→ give the same plot



The abline() function

abline() adds one or more straight lines through the current plot – vertical, horizontal or sloped.

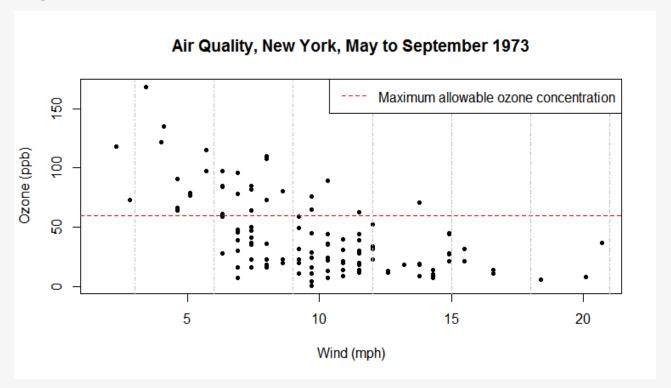
Useful for

- showing boundaries and cutoffs
- fitting straight trend lines through the data (cf. ?lm)

Arguments:

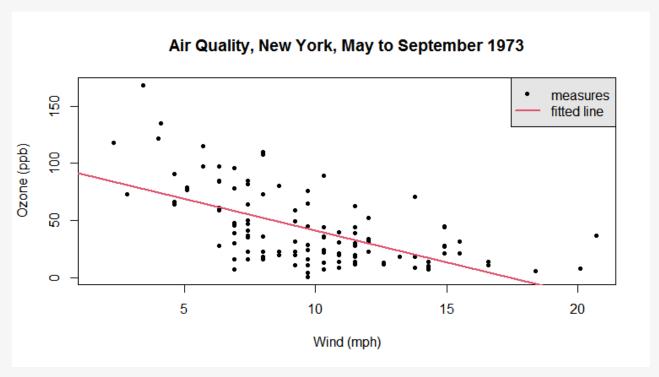
- abline(v=c(...)): add vertical line(s) at the given x value(s)
- abline(h=c(...)): add horizontal line(s) at the given y value(s)
- abline(a= ,b=): add an affine line with intercept a and slope b
- abline(reg=lm(...)): add a trend line from a linear regression equivalent to abline(lm(...))

Example 1: Horizontal and vertical lines



- > data(airquality) # Daily measurements, New York, May-Sept. 1973
- > abline(h=60, col="red", lty="dashed")
- > abline(v=seq(3,21,3), col="grey", lty="dotdash")

Example 2: Fitting a trend line



- > abline(lm(airquality\$Ozone ~ airquality\$Wind), col=2, lwd=2)
- > legend("topright", legend= c("measures","fitted line"), pch= c(20, NA), lty = c(0, 1), lwd=c(NA, 2), col = c(1, 2), bg = "gray90")

Let's practice - 8

Let's come back to the mice dataset stored in the "mice_data" data frame (Let's practice – 3). If mice_data is not currently in your workspace, either get it back by loading the .Rdata file or import the data again from the original .csv file.

- 1) Make boxplots of weights from WT and KO mice. Customise with title, labels, colours.
- 2) Make a barplot of the mean weights of WT and KO mice, using the means returned by tapply(). Customise the barplot with title, labels, colours.
 - Optional: Add number of observations to each bar.
 - Optional: add errors bars.

Permanent Graphic Changes (I)

- The function par() allows to change the default values of many plotting parameters. All future calls to graphics functions will be affected.
- Example 1: set plotting colors and symbols

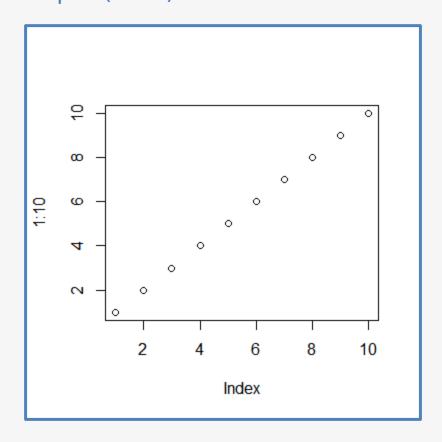
```
>par(col="red", pch=15)
```

- Example 2: set margin widths for subsequent plots
 - mar sets plot margins in number of lines
 - mai sets plot margins in inches
 - use vectors of 4 values (c(0,1,1,2)) for the bottom, left, top, and right margins

```
>par(mar=c(5.1,4.1,4.1,2.1)) #set margins in lines
>par(mai=c(1.02,0.82,0.82,0.42)) #set margins in inches
```

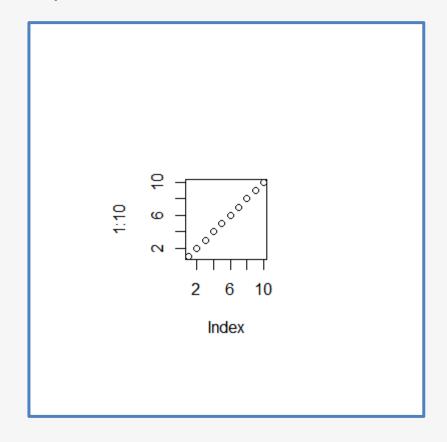
Normal Margins (bottom, left, top right):

```
>par(mar=c(5.1,4.1,4.1,2.1))
>plot(1:10)
```



Wide Margins (bottom, left, top, right):

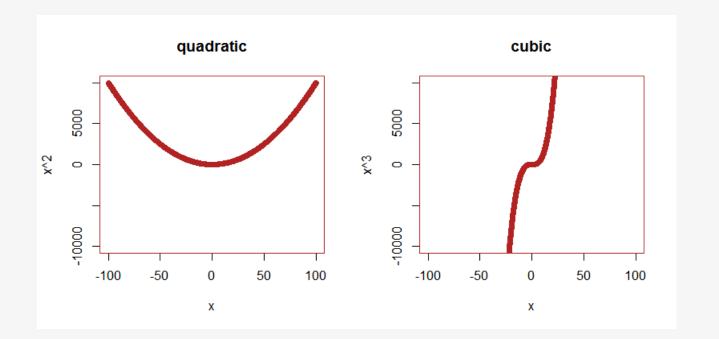
```
>par(mar=c(8.1,8.1,8.1,8.1))
>plot(1:10)
```



Permanent Graphic Changes (II)

• **Example 3:** Generate multi-panel figures using **par() mfrow (or mfcol)**: A vector of the form c(nr, nc). Subsequent figures will be drawn in an nr-by-nc array by rows (or columns, respectively).

```
> par(mfrow=c(1,2),col="firebrick", pch=19) #1x2 plot array
> x <- seq(-100, 100, 0.1)
> plot(x, y=x^2, ylim = c(-10000,10000), "quadratic")
> plot(x, y=x^3, ylim = c(-10000,10000), "cubic")
```



Current settings of par()

- P5S
- Calling par() without parameters displays current settings
- If you changed nothing, all parameters are at default values

Resetting par()

- par() is automatically reset to defaults when you:
 - Restart R or close/switch Rstudio projects
 - Run dev.off(), which closes the most recent plot/plotting device
 - Run graphics.off(), which closes plots/plotting devices
 - In RStudio, clear all plots using the broom icon

Saving figures to files

- By default, R plots all graphics to the screen.
- R offers functions to export graphics to many formats (pdf, postscript, bmp, jpeg, png, tiff). The basic concept is to redirect the graphics output to a different "device".
- Use pdf() to start redirection to a .pdf file, png() for a .png file, etc.
- Use dev.off() to close the redirection.

```
> pdf(file="quadratic_cubic.pdf", width=10, height=5,
    paper="a4")
> par(mfrow=c(1,2),col="firebrick", pch=19)
> x <- seq(-100, 100, 0.1)
> plot(x, y=x^2, ylim=c(-10000,10000), "quadratic")
> plot(x, y=x^3, ylim=c(-10000,10000), "cubic")
> dev.off()
```

- Alternatively you can use the RStudio interface:
 - Plots > Export > Save as Image (PNG,JPEG,TIFF,BMP,...)
 - Plots > Save as PDF.

Arguments to graphics export functions

Use correct file extension:

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

- Each graphics device has a specific set of arguments that dictate characteristics of the outputted file: height=, width=, horizontal=, res=, paper=, pointsize=
- For png, jpeg, tiff (raster formats), the width and height of the graphics are given in pixels.
- For pdf and postscript (vector formats), the width and height of the graphics region are given in inches. Default values are 7. (Tip: A4 = 8.3" x 11.7"; set the width and height a little smaller for printing to A4 size).
- Only pdf() and postscript have an argument "paper". This can be set to common paper formats (paper="a4" for A4 in portrait orientation, paper="

Multi-page vs single page output

- Files in png, jpeg, tiff (raster formats) can only have one page.
- Files in pdf and postscript formats (vector format) receive multiple pages by default. You can deactivate this behaviour using the onefile argument.

One Multi-page file

```
> pdf(file="my_file.pdf")
... #make a few plots, each will go on a separate page #in the same file
> dev.off()
```

Several one-page files

```
> pdf(file="my_file_%d.pdf", onefile= FALSE )
... #make a few plots, each will go into its own file
> dev.off()
```

The format specifier %d causes the resulting files to be numbered:

Choosing an image file format

Raster graphics (png, tiff, jpeg):

file sizes depend on the image size (number of pixels) once created, stretching the image leads to poor quality

Vector graphics (pdf, ps, eps, svg):

file sizes depend on the number of drawing actions (e.g. number of points, lines,...) all elements can be scaled as desired

Embedding image files in MS Office documents (Word, PowerPoint):

In Windows, png and tiff work best, pdf can get blurry. In macOS, pdf works well.

Publication-quality figures:

Vector graphics (pdf, eps) tend to be easier to adapt as they can be resized

File size tip: pdfs can become large in file size and slow to display when a large number of points is plotted. When this is an issue, consider png.

Let's practice - 9

- 1)Make a multi-panel figure with the **four graphics on one page**, exporting the figure to a **png** file. Set width and height arguments in the call to png() to make it look nice.
- **2)Optional:** Perform the steps of the 2 previous practice sessions, but for diet in place of genotype. (Step 2 will not change). This time, make a **pdf** with two pages and **two graphics on each page**. Again, set width and height arguments in the call to pdf() to make it look nice.
- **3)Optional:** Look at the multi-panel figures. Are your impressions about mouse weight from yesterday's exploration of data summaries confirmed by today's visualizations?

In a nutshell

- Introduction to high-level and low-level plotting functions in R
 - plot(), lines(), points(), hist(), barplot(), boxplot() ...
- Customization of plotting functions
 - Colours, line types, line widths, plotting characters...
 - Titles, labels, legend...
- Permanent graphic changes
- Exporting graphics