SISG 2014

SISG Module 9: Elements of R

19th Summer Institute in Statistical Genetics

UNIVERSITY of WASHINGTON

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Elements of R for Genetics and Bioinformatics

Thomas Lumley
Ken Rice

Universities of Washington and Auckland

Seattle, July 2014

Introduction: Course Aims

- Under the hood of R;
  - R essentials, and programming skills
  - Examples implement common genetic analyses, efficiently

- Using R for more sophisticated analyses;
  - Using tools, packages produced by others
  - Bioconductor-based bioinformatics

While no R knowledge is assumed, we will move quickly – and knowing *either* some programming or some genetics will help a lot – also trying our coding ideas on your data, later.

If you are new(er) to R and need help with e.g. reading in data, manipulating data, understanding help files, *please ask* Thomas, Ken, or someone else in class, during hands-on periods.

Introduction: About Prof Lumley

- Professor, Univ of Auckland
- R Core developer
- Genetic/Genomic research in Cardiovascular Epidemiology
- Sings bass

Introduction: About Prof Rice

- Associate Prof, UW Biostat
- Not an authoR, but a useR (and a teacheR)
- Genetic/Genomic research in Cardiovascular Epidemiology
- Sings bass!

... and you?

(who are you, what area of genetics, what are you looking for from the course)

Introduction: Course structure

10 sessions over 2.5 days

- Day 1; Review of `R`, graphics, data-wrangling
- Day 2; Programming (loops), big data, bioconductor
- Day 2.5; More bioconductor, interfacing with other code

Web page: `http://faculty.washington.edu/kenrice/sisg/`

Introduction: Session structure

- 45 mins teaching (questions welcome – please interrupt!)
- 30 mins hands-on
- 15 mins summary, discussion

Keys are posted after each session – though there is more than one correct answer

1. Review of R

Ken Rice
Thomas Lumley

Universities of Washington and Auckland

Seattle, July 2014

What is R?

R is a 'programming environment for statistics and graphics'

The base R has fewer prepackaged statistics procedure than SPSS or SAS, but it is much easier to extend with new procedures.

There are about 3500 published extension packages for R, many aimed at genetics and genomics research.

Using R

R is a free implemention of S, for which John Chambers won the ACM Software Systems award.

> For the S system, which has forever altered how people analyze, visualize, and manipulate data.

The downside is that using R effectively may require changing how *you* analyze, visualize, and manipulate data.

R is a command-line system, not a point-and-click system.

A calculator

```
> 2+2
[1] 4
> 1536/317000
[1] 0.004845426
> exp(pi)-pi
[1] 19.9991
> x <- 3
> y <- 2
> x+y
[1] 5
> ls()
[1] "x" "y"
> round(pi, 6)
[1] 3.141593
> round(pi,
+ 6)
[1] 3.141593
```

Scripts

For longer analyses (and for this course), it's better to type code into a script and then run it. In base R;

- Windows: *File* | *New script*, CTRL-R to run lines or regions
- Mac: *File* | *New Document*, command-return to run
- Some other text editors offer this: Emacs, Tinn, WinEDT, JGR, Eclipse. RStudio has a script editor *and* data viewer

Please note we *strongly* recommend doing the exercises in pairs

Scripts

Default interface (and how to use it);

Reading data

Ability to read in data is assumed. But to illustrate commands, and show some less-standard approaches, we'll review reading in;

- Text files
- Other statistics packages datasets
- Web pages

Much more information is in the Data Import/Export manual.

Reading text data

The easiest format has variable names in the first row

```
 case   id gender   deg yrdeg field startyr year   rank admin
    1    1      F Other    92 Other      95   95 Assist     0
    2    2      M Other    91 Other      94   94 Assist     0
    3    2      M Other    91 Other      94   95 Assist     0
    4    4      M   PhD    96 Other      95   95 Assist     0
```

and fields separated by spaces. In R, use

```
salary <- read.table("salary.txt", header=TRUE)
```

to read the data from the file `salary.txt` in the current working directory into the data frame `salary`.

Syntax notes

- Spaces in commands don't matter (except for readability), but Capitalisation Does Matter.
- `TRUE` (and `FALSE`) are logical constants
- Unlike many systems, R does not distinguish between commands that do something and commands that compute a value. Everything is a function: i.e. it returns a value.
- Arguments to functions can be named (`header=TRUE`) or unnamed (`"salary.txt"`)
- A whole data set (called a `data frame` is stored in a variable (`salary`), so more than one dataset can be available at the same time.

Did it work?

The `head()` function shows the first few lines of the data frame

```
> head(salary)
 case id gender   deg yrdeg field startyr year   rank admin salary
1    1  1      F Other    92 Other      95   95 Assist     0   6684
2    2  2      M Other    91 Other      94   94 Assist     0   4743
3    3  2      M Other    91 Other      94   95 Assist     0   4881
4    4  4      M   PhD    96 Other      95   95 Assist     0   4231
5    5  6      M   PhD    66 Other      91   91   Full     1  11182
6    6  6      M   PhD    66 Other      91   92   Full     1  11507
```

It *should* look like this!

The `View()` command similarly shows your data frame, in a manageable way.

Where's my file?

- Find out your current directory with `getwd()`
- Change directory with the menus
- or with `setwd("I:/like/this/directory/better")`
- `file.choose()` pops up a dialog for choosing a file and returns the name, so

  ```
  salary <- read.table(file.choose(), header=TRUE)
  ```


Reading text data

Sometimes columns are separated by commas (or tabs)

```
Ozone,Solar.R,Wind,Temp,Month,Day
41,190,7.4,67,5,1
36,118,8,72,5,2
12,149,12.6,74,5,3
18,313,11.5,62,5,4
NA,NA,14.3,56,5,5
```

Use

```
ozone <- read.table("ozone.csv", header=TRUE, sep=",")
```

or

```
ozone <- read.csv("ozone.csv")
```

Syntax notes

- Forgetting `header=TRUE` in `read.table()` is bad (try it!)

- Functions can have optional arguments (`sep` wasn't used the first time). Use `help("read.table")` or `?read.table` for a complete description of the function and all its arguments.

- There's more than one way to do it.

- `NA` is the code for missing data. Think of it as "Don't Know". R handles it sensibly in computations: eg 1+NA, NA & FALSE, NA & TRUE. You cannot test `temp==NA` (Is temperature equal to some number I don't know?), so there is a function `is.na()`.

Reading text data

Sometime the variable names aren't included

```
 1      0.2      115       90      1        3       68    42 yes
 2      0.7      193       90      3        1       61    48 yes
 3      0.2       58       90      1        3       63    40 yes
 4      0.2        5       80      2        3       65    75 yes
 5      0.2      8.5       90      1        2       64    30 yes
```

and you have to supply them

```
psa <- read.table("psa.txt", col.names=c("ptid","nadirpsa",
       "pretxpsa", "ps","bss","grade","age",
       "obstime","inrem"))
```

or

```
psa <- read.table("psa.txt")
names(psa) <- c("ptid","nadirpsa","pretxpsa", "ps",
           "bss","grade","age","obstime","inrem")
```

Syntax notes

- Assigning a single vector (or anything else) to a variable uses the same syntax as assigning a whole data frame.

- `c()` is a function that makes a single vector from its arguments.

- `names()` is a function that accesses the variable names of a data frame

- Some functions, such as `names()`, can be used on the LHS of an assignment.

Other statistical packages

```
library("foreign")
stata <- read.dta("salary.dta")
spss <- read.spss("salary.sav", to.data.frame=TRUE)
sasxport <- read.xport("salary.xpt")
epiinfo <- read.epiinfo("salary.rec")
```

Notes:

- Many functions in R live in optional packages. The `library()` function lists packages, shows help, or loads packages from the package library.

- The foreign package is in the standard distribution. It handles import and export of data. Thousands of extra packages are available at `http://cran.r-project.org`.

The web

Files for `read.table()` can live on the web

```
fl2000<-read.table("http://faculty.washington.edu/tlumley/
data/FLvote.dat", header=TRUE)
```

It's also possible to read from more complex web services (such as the genome databases)

Operating on data

We assume you know how to use the $ sign, to indicate columns of interest in a dataset, e.g. `antibiotics$duration` means the variable `duration` in the data frame `antibiotics`.

```
## This is a comment
## Convert temperature to real degrees
antibiotics$tempC <- (antibiotics$temp-32)*5/9
## display mean, quartiles of all variables
summary(antibiotics)
```

Subsets

Everything in R is a vector (but some have only one element). The re are several ways to use `[]` to extract subsets

```
## First element
antibiotics$temp[1]
## All but first element
antibiotics$temp[-1]
## Elements 5 through 10
antibiotics$temp[5:10]
## Elements 5 and 7
antibiotics$temp[c(5,7)]
## People who received antibiotics (note ==)
antibiotics$temp[ antibiotics$antib==1 ]
## or
with(antibiotics, temp[antib==1])
```


Notes

- Positive indices select elements, negative indices drop elements
- `5:10` is the sequence from 5 to 10
- You need `==` to test equality, not just `=`
- `with()` temporarily sets up a data frame as the default place to look up variables.

More subsets

For data frames you need two indices – naming rows and columns can also be useful;

```
## First row
antibiotics[1,]
## Second column
antibiotics[,2]
## Some rows and columns
antibiotics[3:7, 2:4]
## Columns by name
antibiotics[, c("id","temp","wbc")]
## People who received antibiotics
antibiotics[antibiotics$antib==1, ]
## Put this subset into a new data frame
yes <- antibiotics[antibiotics$antib==1,]
```


Computations

```
mean(antibiotics$temp)
median(antibiotics$temp)
var(antibiotics$temp)
sd(antibiotics$temp)
mean(yes$temp)
mean(antibiotics$temp[antibiotics$antib==1])
with(antibiotics, mean(temp[sex==2]))
toohot <- with(antibiotics, temp>99)
mean(toohot)
```

Factors

Factors represent categorical variables. You can't do mathematical operations on them (except for ==)

```
> table(salary$rank,salary$field)

        Arts Other Prof
  Assist  668 2626   754
  Assoc  1229 4229  1071
  Full    942 6285  1984
> antibiotics$antib<-factor(antibiotics$antib,
                            labels=c("Yes","No"))
> antibiotics$agegp<-cut(antibiotics$age, c(0,18,65,100))
> table(antibiotics$agegp)
  (0,18]  (18,65] (65,100]
       2       19        4
```


Help!

- `?fn` or `help(fn)` for help on fn
- `help.search("topic")` for help pages related to "topic"
- `apropos("tab")` for functions whose names contain "tab"
- `RSiteSearch("FDR")` to search the R Project website (requires internet access)

2. Graphics in R

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Seattle, July 2014

Graphics

R can produce graphics in many formats, including:

- on screen
- PDF files for LaTeX or emailing to people
- PNG or JPEG bitmap formats for web pages (or on non-Windows platforms to produce graphics for MS Office). PNG is also useful for graphs of large data sets.
- On Windows, metafiles for Word, Powerpoint, and similar programs

Setup

Graphs should usually be designed on the screen and then may be replotted on eg a PDF file (for Word/Powerpoint you can just copy and paste)

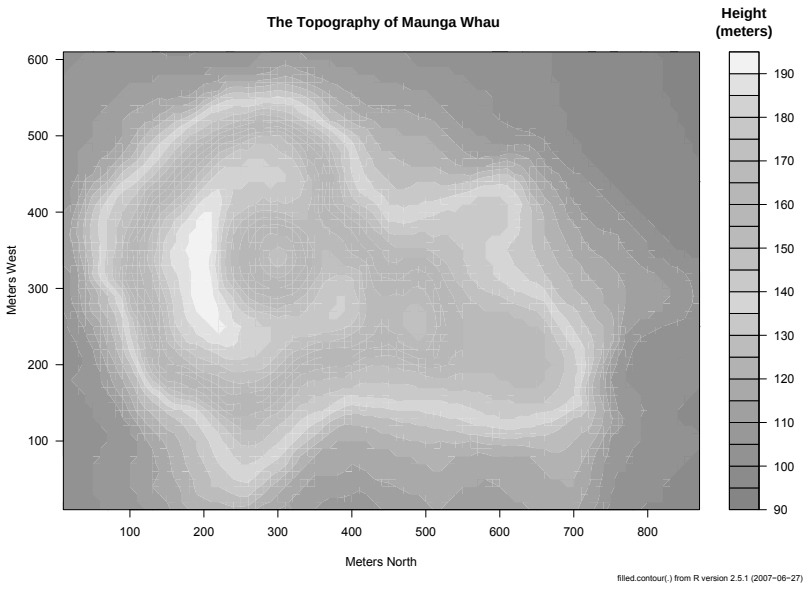
For printed graphs, you will get better results if you design the graph at the size it will end up, eg:

```
## on Windows
windows(height=4,width=6)
## on Unix
x11(height=4,width=6)
```

Word or LaTeX can rescale the graph, but when the graph gets smaller, so do the axis labels...

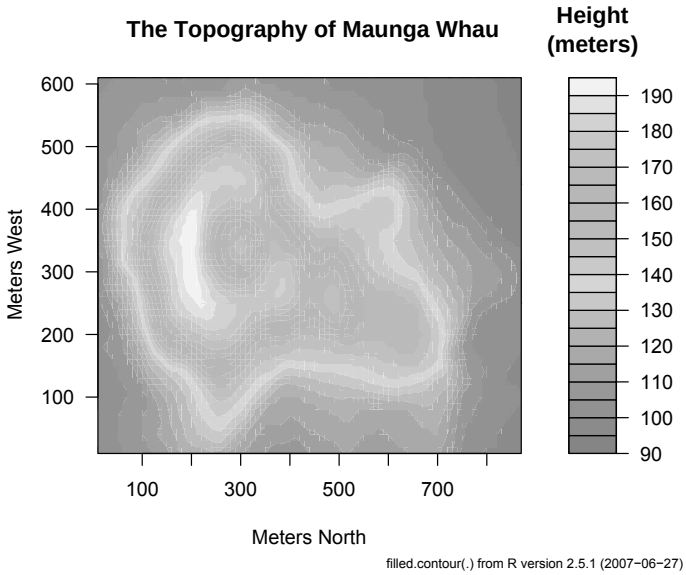
Setup

Created at full-page size (11×8.5 inches)



Better scaling

Created at 6×5 inches



The real thing

http://www.teara.govt.nz/en/photograph/3920/maungawhau-mt-eden

Finishing

After you have the right commands to draw the graph you can produce it in another format: eg

```
## start a PDF file
pdf("picture.pdf",height=4,width=6)
## your drawing commands here
...
### close the PDF file
dev.off()
```

You may find `dir()`, `getwd()` and `setwd()` helpful

Drawing

Usually, use `plot()` to create a graph and then `lines()`, `points()`, `legend()`, `text()`, `rect()`, `segments()`, `symbols()`, `arrows()` and other commands to annotate it. There is no 'erase' function, so keep your commands in a script.

`plot()` is a generic function: it does appropriate things for different types of input

```
## scatterplot
plot(salary$year, salary$salary)
## boxplot
plot(salary$rank, salary$salary)
## stacked barplot
plot(salary$field, salary$rank)
```

and others for other types of input.

Formula interface

The `plot()` command can also be used this way;

```
plot(salary~rank, data=salary)
```

where we introduce the formula system, that is also used for regression models. Here, think of `Y~X`.

The variables in the formula are automatically looked up in the data= argument.

Designing graphs

Two important aspects of designing a graph

- It should have something to say
- It should be legible

Having something to say is *your* problem; software can help with legibility.

Designing graphs

Important points

- Axes need labels (with units, large enough to read)
- Color can be very helpful (but not if the graph is going to be printed in black and white).
- Different line or point styles usually should be labelled.
- Points plotted on top of each other won't be seen

After these are satisfied, it can't hurt to have the graph look nice.

Options

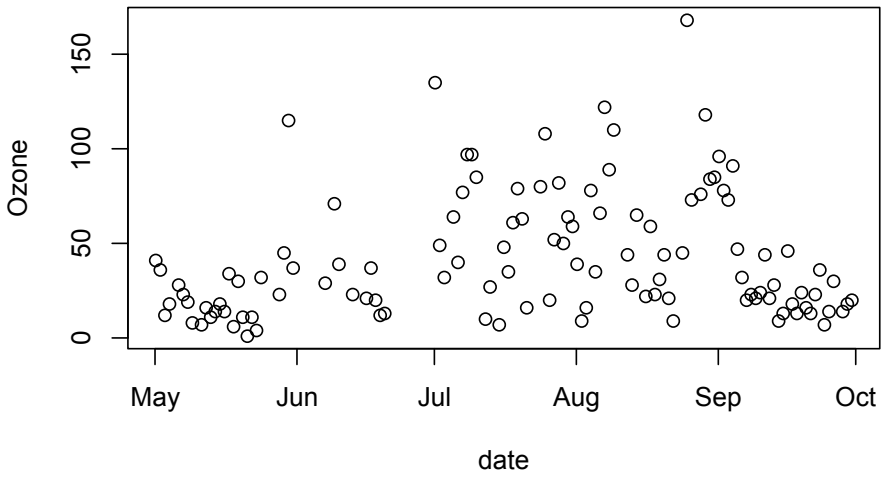
First we set up the data – in this case, a built-in dataset, containing daily ozone concentrations in New York, summer 1973

```
data(airquality)
names(airquality)
airquality$date<-with(airquality, ISOdate(1973,Month,Day))
```

All these graphs were designed at 4in×6in and stored as PDF files;

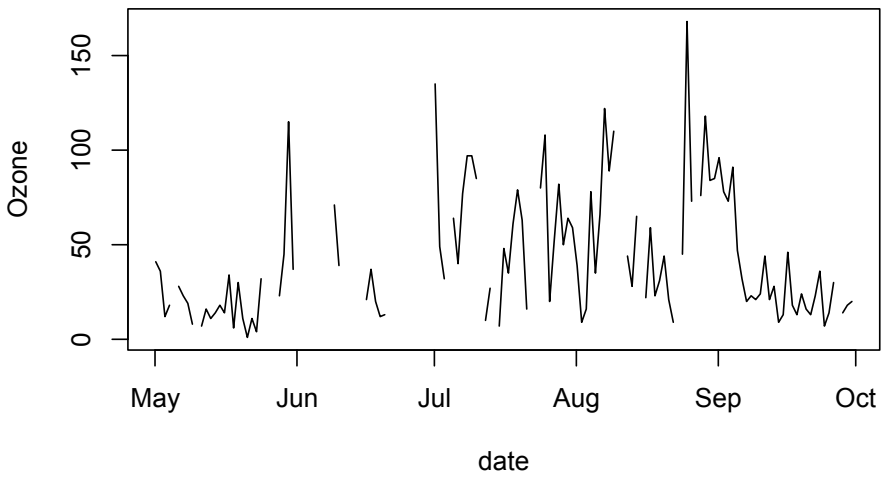
Options

```
plot(Ozone~date, data=airquality)
```



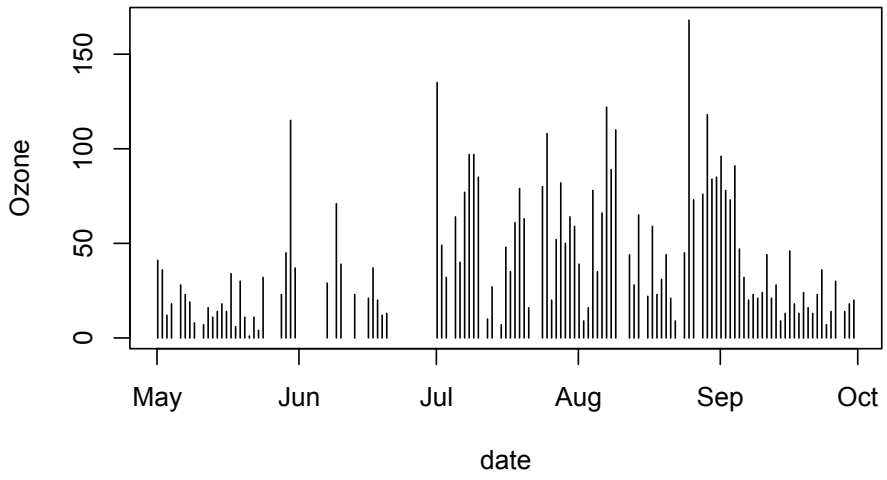
Options

```
plot(Ozone~date, data=airquality,type="l")
```



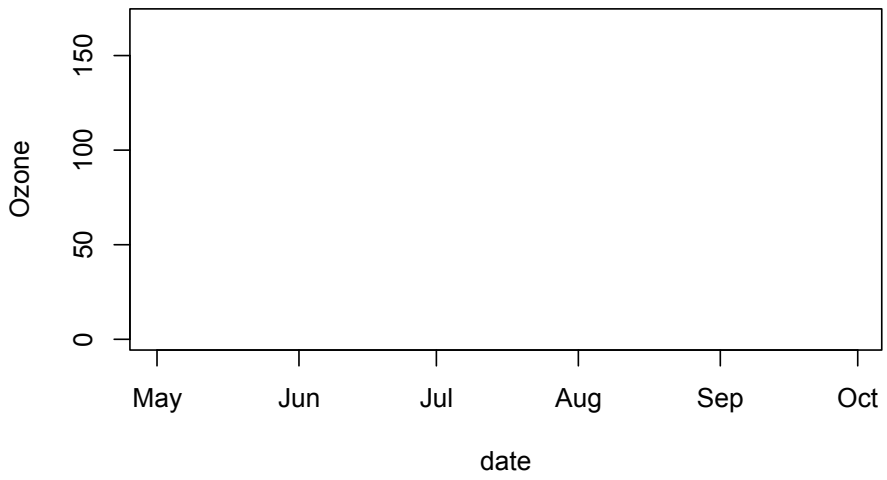
Options

```
plot(Ozone~date, data=airquality,type="h")
```



Options

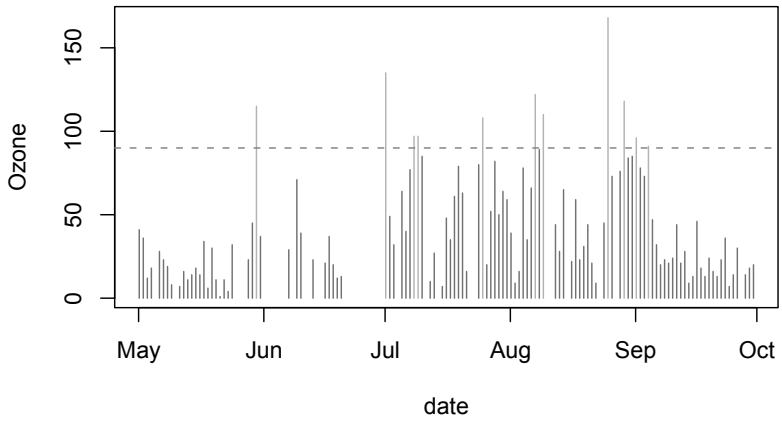
```
plot(Ozone~date, data=airquality,type="n")
```



Options

Commands to do a more complex plot;

```
bad <- ifelse(airquality$Ozone>=90, "orange", "forestgreen")
plot(Ozone~date, data=airquality, type="h", col=bad)
abline(h=90, lty=2, col="red")
```



Notes

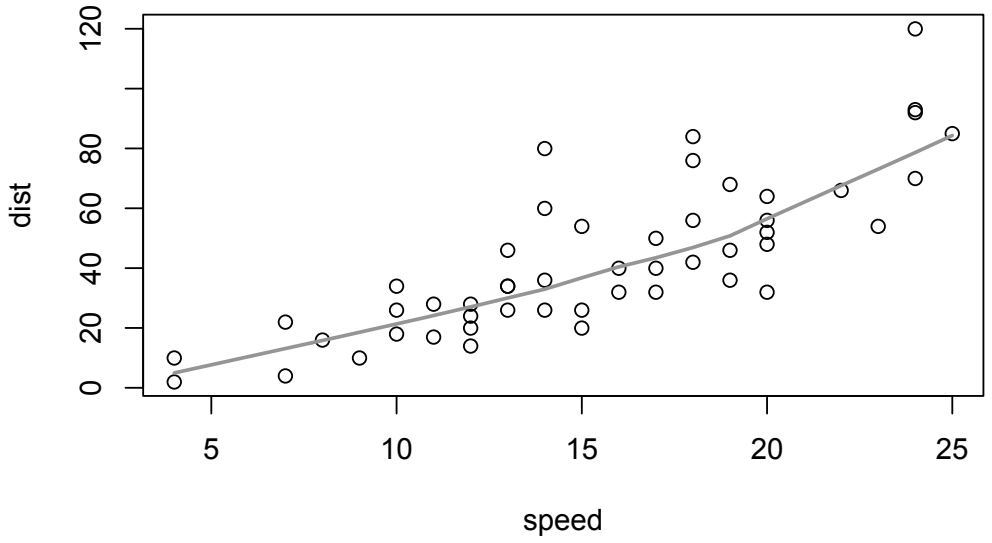
- `type=` controls how data are plotted. `type="n"` is not as useless as it looks: it can set up a plot for latter additions.
- Colors can be specified by name (the `colors()` function gives all the names), by red/green/blue values ("#rrggbb" with six base-sixteen digits) or by position (1:8) in the standard palette of 8 colors.
- `abline` draws a single straight line on a plot
- `ifelse()` selects between two vectors based on a logical variable.
- `lty` specifies the line type: 1 is solid, 2 is dashed, 3 is dotted, then it gets more complicated. See `?par`, then search for `lty`

Adding to a plot

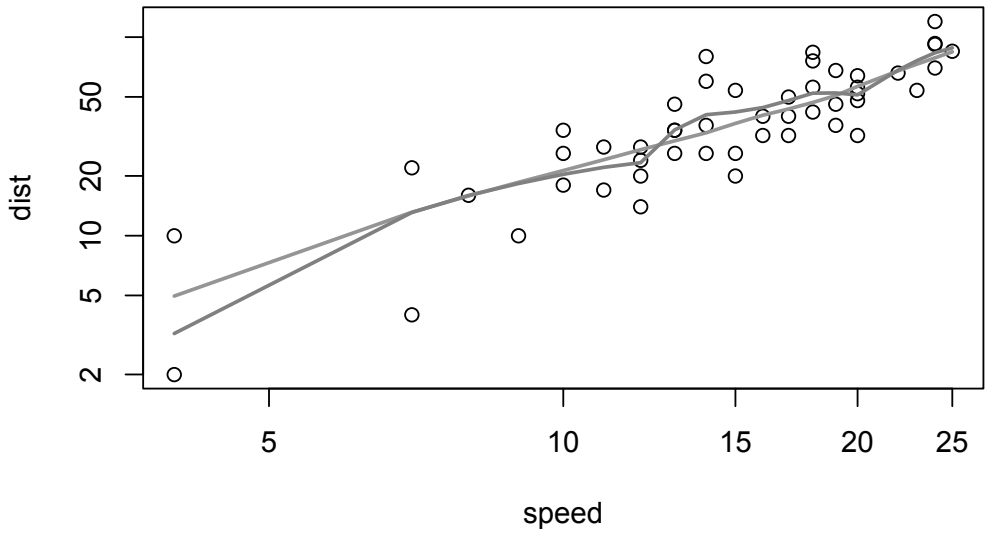
More example commands

```
data(cars)
plot(dist~speed,data=cars)
with(cars, lines(lowess(speed, dist), col="tomato", lwd=2))

plot(dist~speed,data=cars, log="xy")
with(cars, lines(lowess(speed, dist), col="tomato", lwd=2))
with(cars, lines(supsmu(speed, dist), col="purple", lwd=2))

legend("bottomright", legend=c("lowess","supersmoother"),bty="n",
  lwd=2, col=c("tomato","purple"))
```

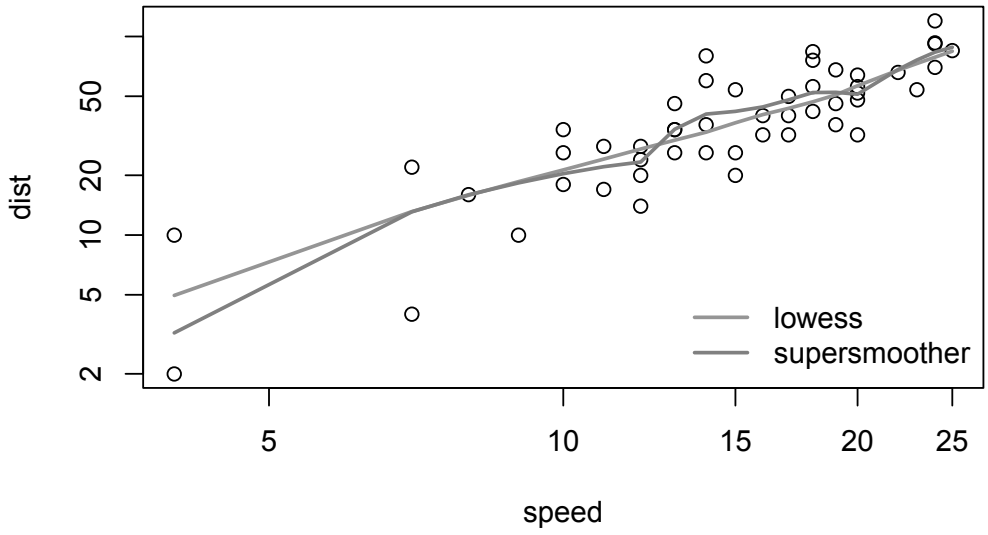

Adding to a plot



Adding to a plot



Adding to a plot



Notes

- `lines()` adds lines to an existing plot (and `points()` adds points)

- `lowess()` and `supsmu()` are scatterplot smoothers. They calculate smooth curves that fit the relationship between y and x locally. Their output has attributes `$x` and `$y`, that generic function `lines()` can cope with

- `log="xy"` asks for both axes to be logarithm (`log="x"` would just be the x-axis)

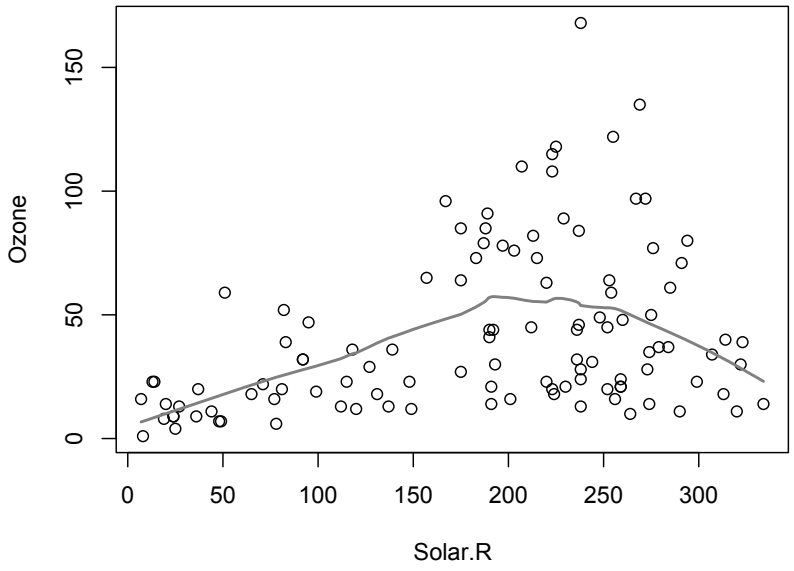
- `legend()` adds a legend

Conditioning plots

Ozone is a secondary pollutant, it is produced from organic compounds and atmostpheric oxygen in reactions catalyzed by nitrogen oxides and powered by su nlight.

However, looking at ozone concentrations in NY in summer we see a non-monotone relationship with sunlight

Conditioning plots

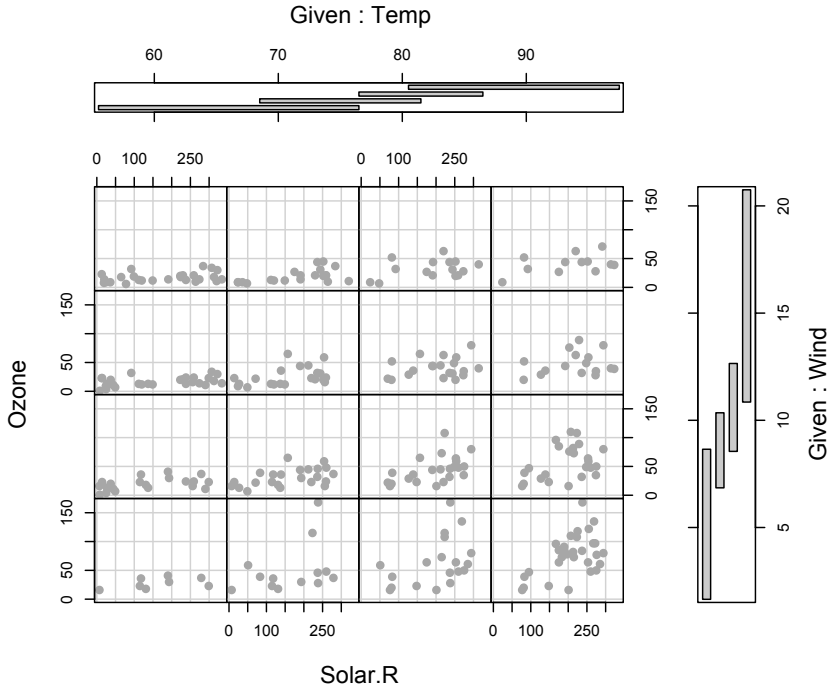


Conditioning plots

Here we draw a scatterplot of `Ozone` vs `Solar.R` for various subranges of `Temp` and `Wind`. For more examples like this, see the commands in the `lattice` package.

```
data(airquality)
coplot(Ozone ~ Solar.R | Temp * Wind, number = c(4, 4),
     data = airquality,
    pch = 21, col = "goldenrod", bg = "goldenrod")
```

Conditioning plots



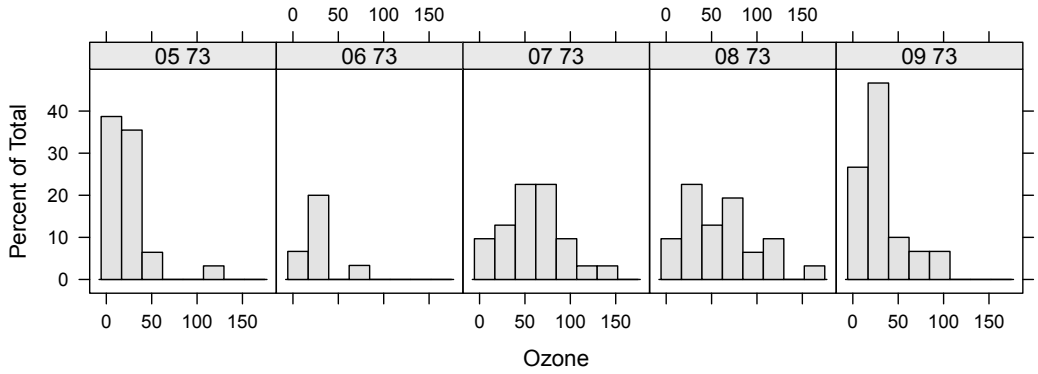
Conditioning plots

- A 4-D relationship is illustrated; the Ozone/sunlight relationship changes in strength depending on both the Temperature and Wind
- The vertical bar | is statistician-speak for 'conditioning on' (nb this is different to use of |'s meaning as Boolean 'OR')
- The horizontal/vertical 'shingles' tell you which data appear in which plot. The overlap can be set to zero, if preferred
- `coplot()`'s default layout is a bit odd; try setting `rows`, `columns` to different values
- For more plotting commands that support conditioning, see `library(help="lattice")`

Conditioning plots: general

Functions in the `lattice` package allow various forms of conditioning plot – here for histograms;

```
library("lattice")
airquality$date  <- with(airquality, ISOdate(1973,Month,Day))
airquality$month <- strftime(airquality$date, "%m %y")
histogram(~Ozone|month, data=airquality)
```



Conditioning plots: general

Some other plots that can be conditioned;

- `xyplot()` for scatterplots – more flexible version of `coplot()`
- `barchart()` for boxplots
- `dotplot()` and `stripchart()`
- `qq()` and `qqmath()`, e.g. comparing to $N(0, 1)$
- `levelplot()` – heatmaps, see also `image()`
- `contourplot()` – see also `contour()`
- `cloud()` and `wireframe()`, for fake 3D

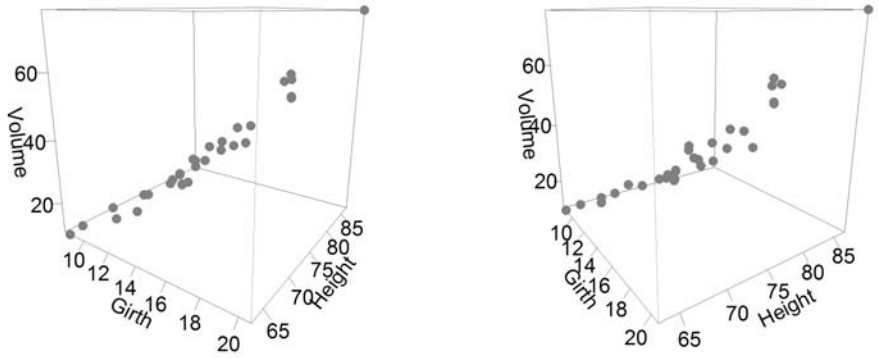
For still others see `?Lattice` after loading the `lattice` package ... or `demo(lattice)`.

For fine control, these functions all permit `panel` arguments, i.e. you can supply a function implemented in each subplot. `?panel.abline` describes some pre-written versions.

persp() and friends

Should you *ever* need them; (and you may not!)

```
with(trees,{
per <- persp(x=1:2, y=1:2, z=matrix(NA,2,2), theta=35,
     xlim=range(Girth), ylim=range(Height), zlim=range(Volume),
     xlab="Girth",ylab="Height", zlab="Volume", axes=TRUE, ticktype="detailed")
points(trans3d(x=Girth, y=Height, z=Volume, per), col="red", pch=19)
})
```



`persp()` is okay for wireframes, otherwise try `cloud()` first.

Toys: Mathematical annotation

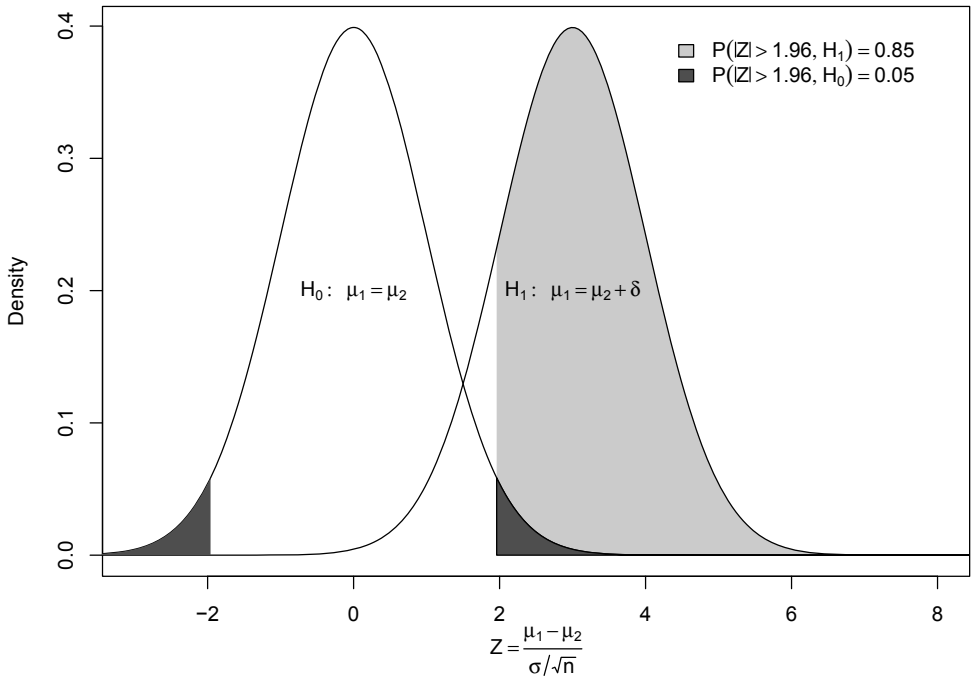
An expression can be specified in R for any text in a graph (`help(plotmath)` for details). Here we annotate a figure drawn with `polygon()`.

```
x<-seq(-10,10,length=400)
y1<-dnorm(x)
y2<-dnorm(x,m=3)
par(mar=c(5,4,2,1))
plot(x,y2,xlim=c(-3,8),type="n",
    xlab=quote(Z==frac(mu[1]-mu[2],sigma/sqrt(n))),
    ylab="Density")
polygon(c(1.96,1.96,x[240:400],10),
        c(0,dnorm(1.96,m=3),y2[240:400],0),
        col="grey80",lty=0)
lines(x,y2)
lines(x,y1)
polygon(c(-1.96,-1.96,x[161:1],-10),
        c(0,dnorm(-1.96,m=0),y1[161:1],0),
        col="grey30",lty=0)
polygon(c(1.96,1.96,x[240:400],10),
        c(0,dnorm(1.96,m=0),y1[240:400],0),
        col="grey30")
```

Toys: Mathematical annotation

```
legend(4.2,.4,fill=c("grey80","grey30"),
    legend=expression(P(abs(Z)>1.96,H[1])==0.85,
        P(abs(Z)>1.96,H[0])==0.05),bty="n")
text(0,.2,quote(H[0]:~~mu[1]==mu[2]))
text(3,.2,quote(H[1]:~~mu[1]==mu[2]+delta))
```

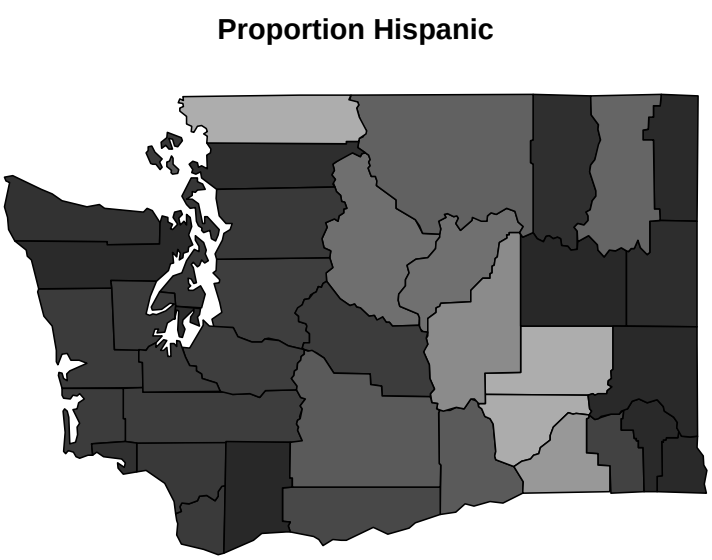

Toys: Mathematical annotation



Toys: Maps

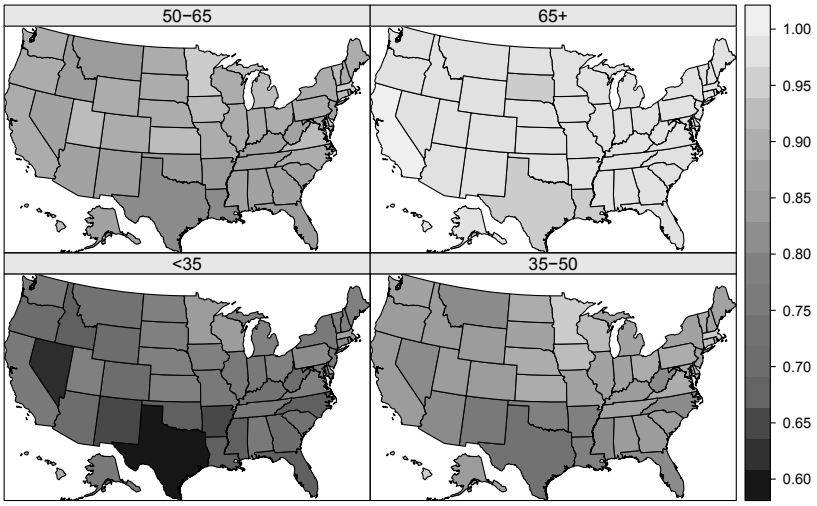
```
> library("maps")
> map('county', 'washington', fill = TRUE,
        col = grey(sqrt(wa[,10]/(wa[,1]))) )
> title(main="Proportion Hispanic")
```


Toys: Maps



Even conditioned maps!

Health insurance coverage by age and state



2.36

3. More Advanced Graphics

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Seattle, July 2014

Outline

- Colour and pre-attentive perception: facts about graphics
- Too many variables: parallel coordinates, transparency
- Too many dimensions: hexagonal binning, transparency

Colour coding

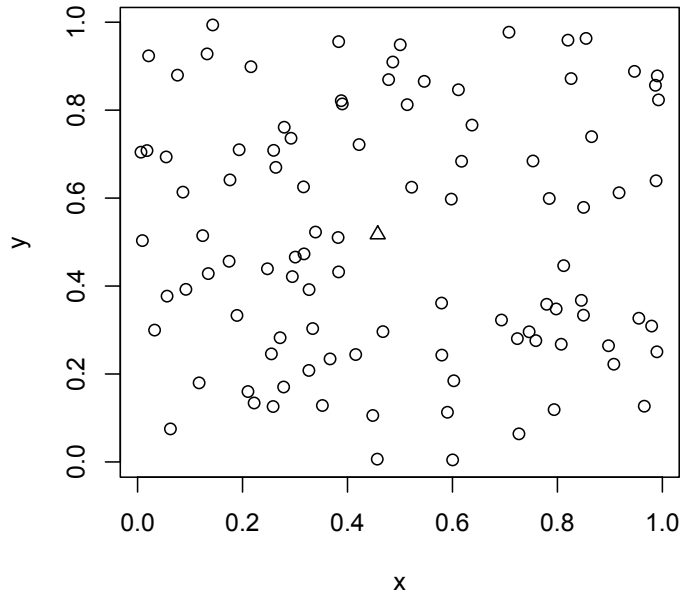
'Simple' plots involve two-dimensional data, which we measure on the x and y axes.

For higher-dimensions, some traditional approaches are;

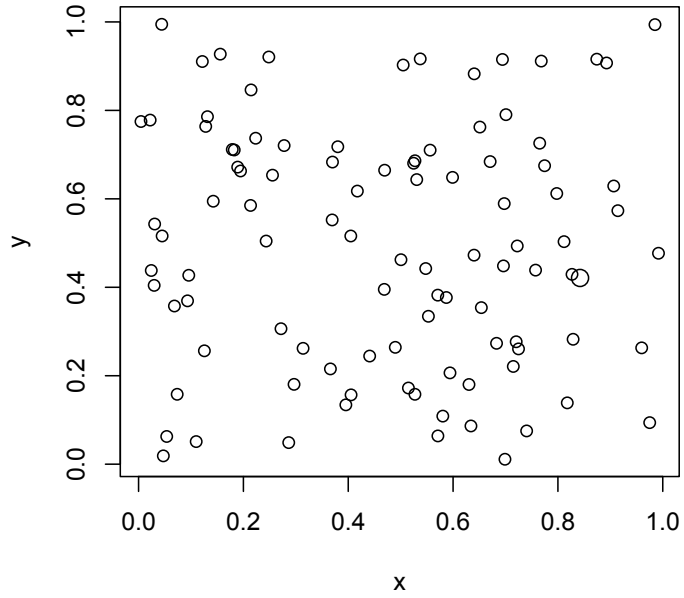
- Different colors for e.g. men, women (`col`)
- Different-shaped symbols (`pch`), or different sizes (`cex`)

For ≤ 100's of data points, modest use of these is fine. But your eye is not good at concentrating e.g. just on the purple points, in a fully Technicolor plot;

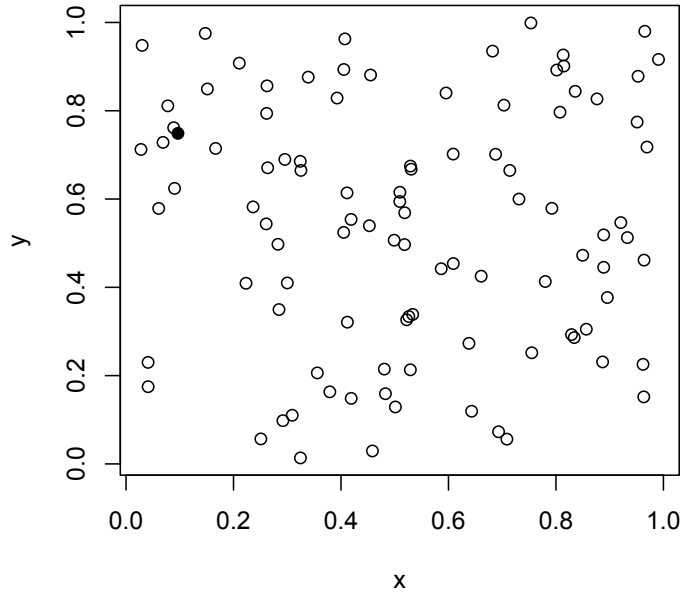
Which point is different?



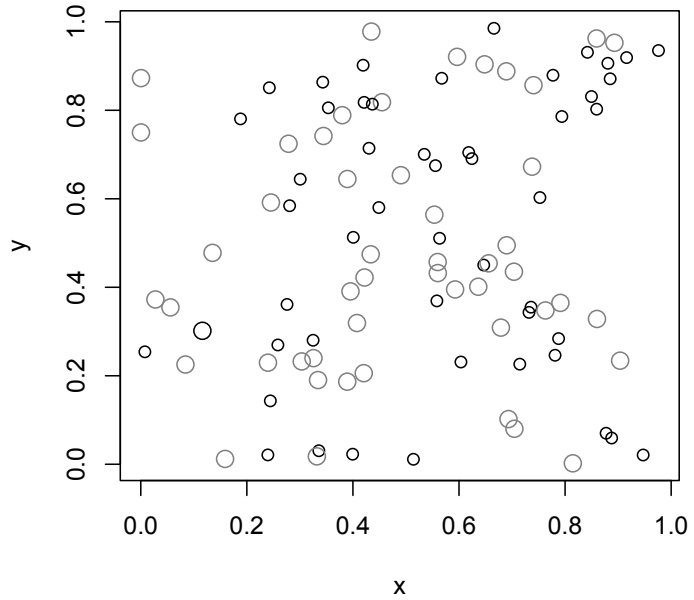
Which point is different?



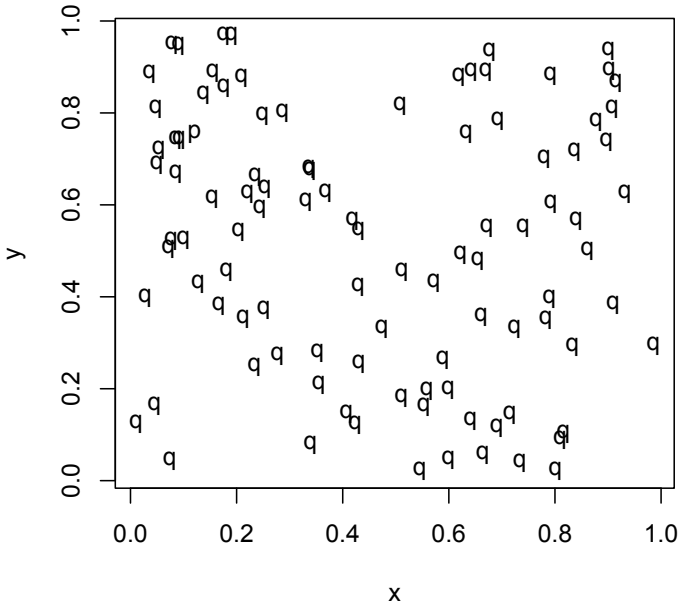
Which point is different?



Which point is different?



Which point is different?



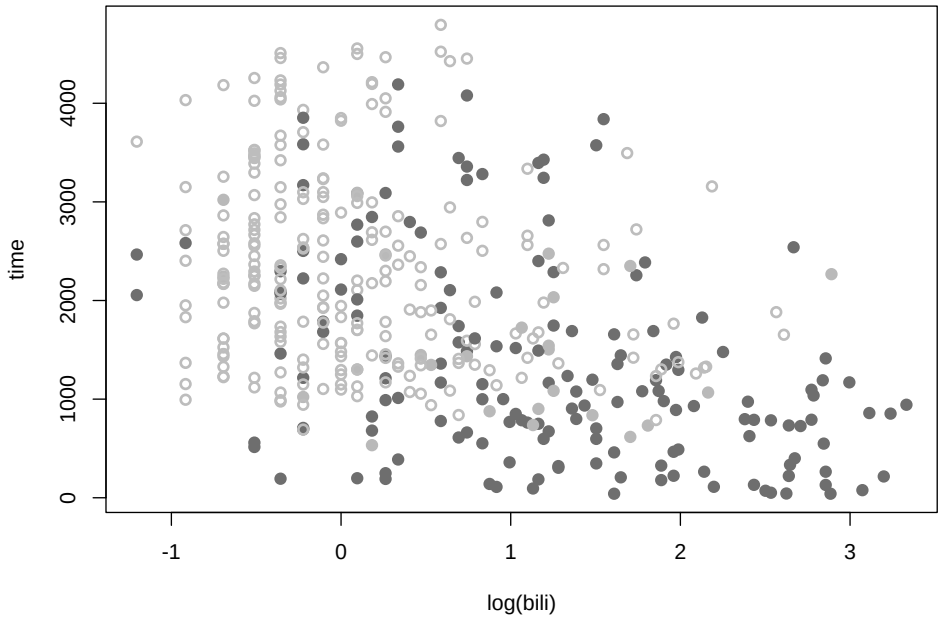
Preattentive perception

Some differences are processed by the brain before you get to see the image: pre-attentive perception.

Important because

- It's easier to see things
- You can look at just one subset of the points and see patterns
- Like colour-blindness, illustrates that there are **facts** about graphics, not just artistic taste

Preattentive perception



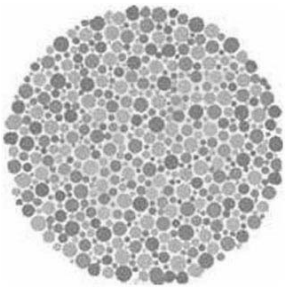
Color schemes

Color choice is best left to experts, or people with taste.

`http://www.colorbrewer.org` has color schemes designed for the National Cancer Atlas, also in package `RColorBrewer`

`colorspace` package has color schemes based on straight lines in a perceptually-based color space (rather than RGB).

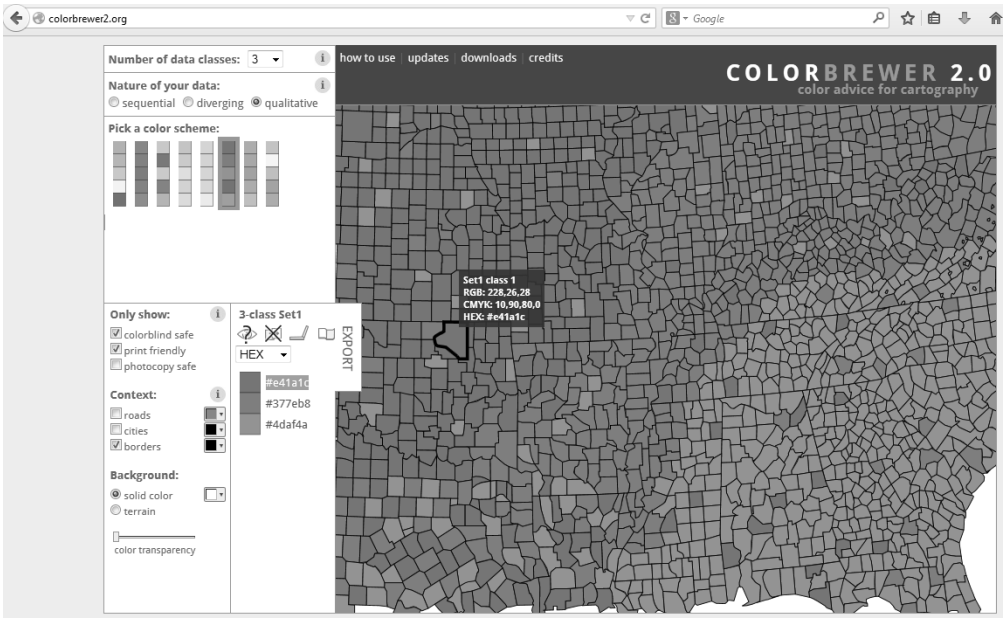
`dichromat` package attempts to show the impact of red:green color blindness on your R color schemes.



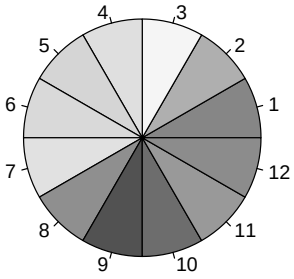
[Code for examples is in file `colorpalettes.R` on course website]

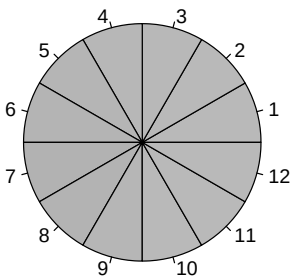
Color brewer

R accepts 'hex' colors, e.g. `col="#e41a1c"` here;



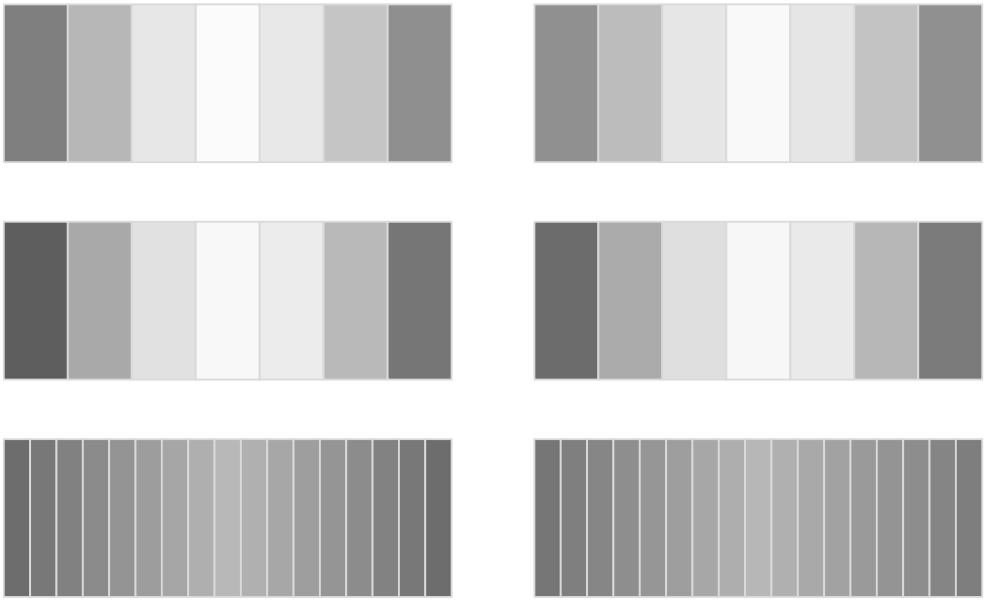
Color choice





(nb B&W printed copies of this slide may not be helpful!)

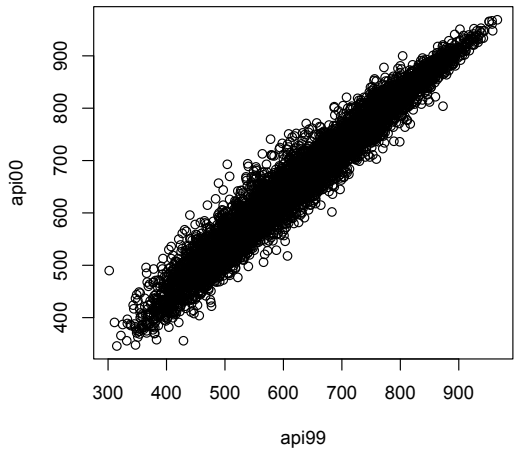
Color blindness



(nb B&W printed copies of this slide may not be helpful!)

Larger data

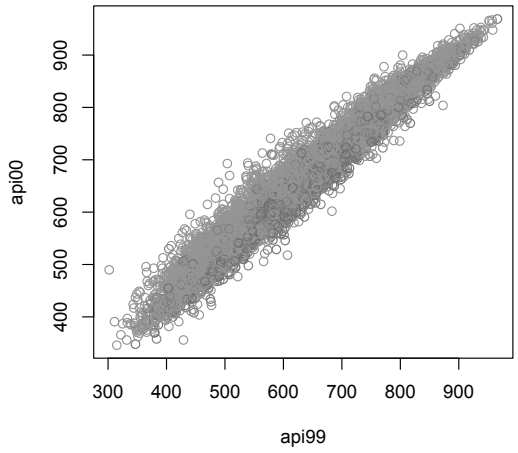
For large(ish) data, 'overlap' is a fundamental problem...



(California Academic Performance Index on 6194 schools)

Larger data

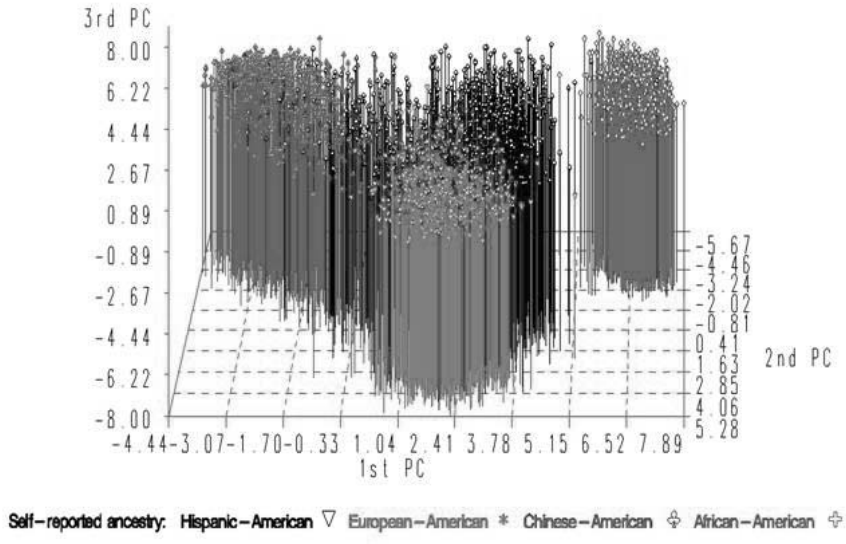
... which remains, when we color-code.



Colors denote Elementary, Middle & High Schools

Larger data

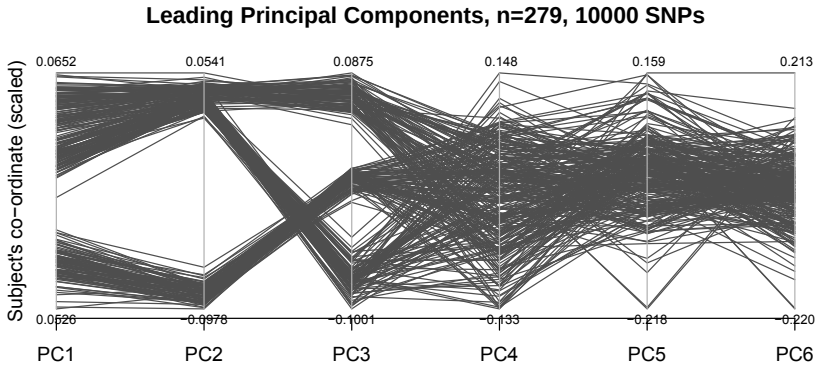
With three dimensions + color-codes, this can happen;



(R does have `persp()`, for occasional use)

Parallel Coordinate Plots

For even higher-dimensional data, scatterplots can not provide adequate summaries. For data where the dimensions can be ordered, the parallel co-ordinates plot is useful;

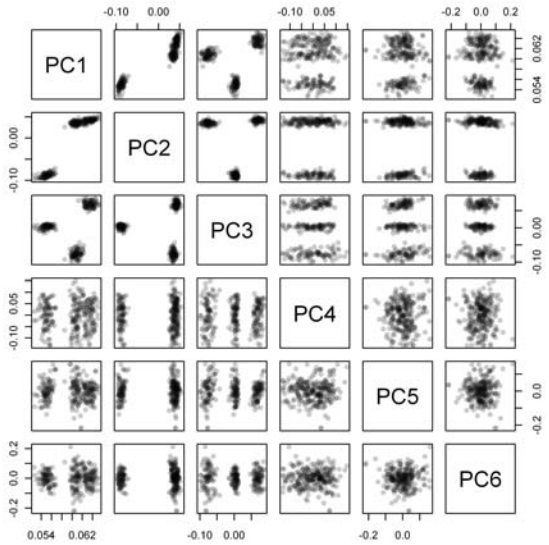


Parallel Coordinate Plots

- Each multi-dimensional data point (i.e. each person) is represented by a line – not a point
- `parcoord()` in the `MASS` package is one simple implementation – writing your own version is not a big job
- Coloring the lines also helps (example later)
- Scaling of axes, and their vertical positions are arbitrary
- Doing 'Principal Components Analysis' is just choosing axes for your data so that their variance is maximized on axis 1, then axis 2, ...

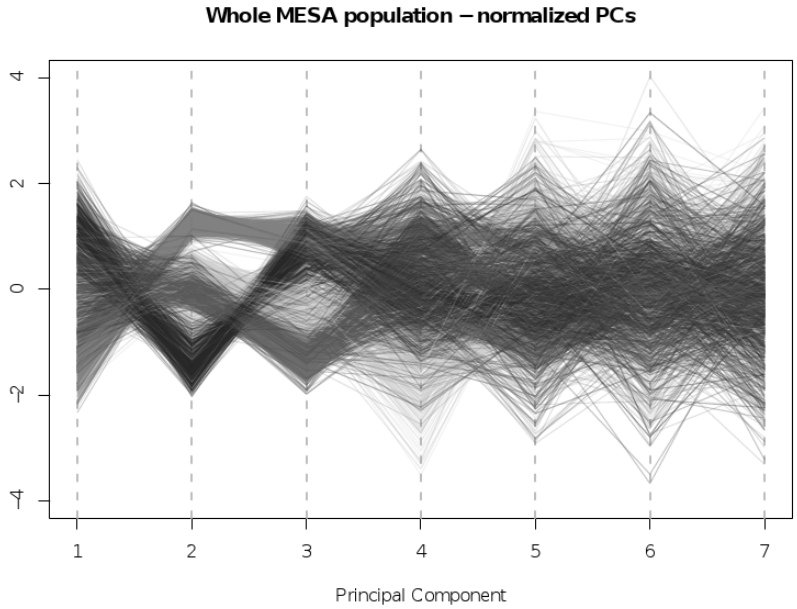
Parallel Coordinate Plots

A `pairs()` plot of the same thing; (nasty!)



Parallel Coordinate Plots

The pin cushion data++ : colors indicate self-report ancestry



Transparency

The colors in the last examples were transparent. As well as specifiying e.g. `col=2` or `col="red"`, you can also specify

```
col="#FF000033"
```

– coded as RRGGBB in hexadecimal, with transparency 33 (also hexadecimal). This is a 'pale' red – 33/FF $\approx$ 20%.

Get from color names to RGB with `col2rgb()`, and from base 10 to base 16 using `format(as.hexmode(11), width=2)`

(Or, go to colorbrewer or a similar site and take the hex from there!)

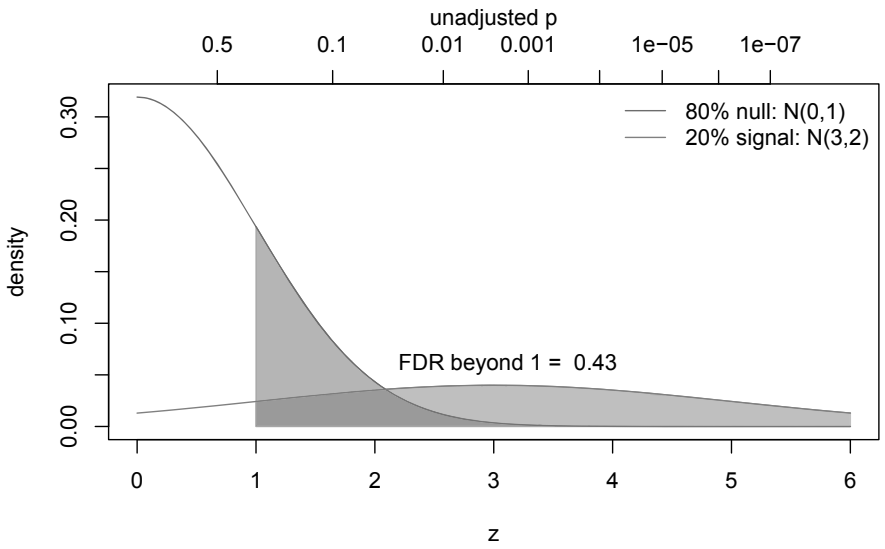
Transparency

An example; (also shows other graphics commands)

```
curve(0.8*dnorm(x), 0, 6, col="blue", ylab="density", xlab="z")
curve(0.2*dnorm(x,3,2), 0, 6, col="red", add=T)

xvals <- seq(1, 6, l=101)
polygon(
c(xvals,6,1), c(0.8*dnorm(xvals), 0,0),
density=NA, col="#0000FF80" ) # tranparent blue
polygon(
c(xvals,6,1), c(0.2*dnorm(xvals,3,2), 0,0),
density=NA, col="#FF000080" ) # tranparent red

legend("topright", bty="n", lty=1, col=c("blue","red"),
c("80% null: N(0,1)", "20% signal: N(3,2)"))
axis(3, at=qnorm(c(0.25, 0.5*10^(-1:-7)), lower=F), c(0.5, 10^(-1:-7)) )
mtext(side=3, line=2, "unadjusted p")

text(2.2, 0.07, adj=c(0,1), paste("FDR beyond 1 = ",
round(0.8*pnorm(1,lower=F)/(0.8*pnorm(1,lower=F) + 0.2*pnorm(1,3,2,lower=F)),3)))
```

Transparency

Here's the output;



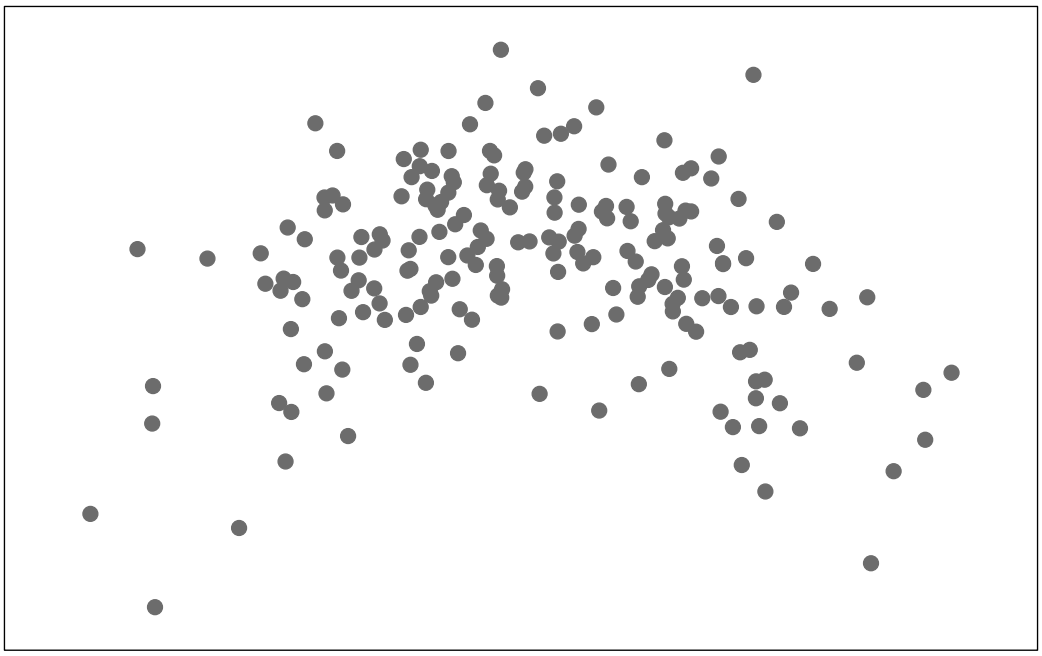
Hexagonal binning

Using transparent plotting symbols is a quick-and-dirty way to adapt scatterplots for use with large datasets.

A better method is 'hexagonal binning'; this is a 2D analog of a histogram – where you would count the number of data in one area, and then draw a bar with height proportional to that count.

Hexagonal binning

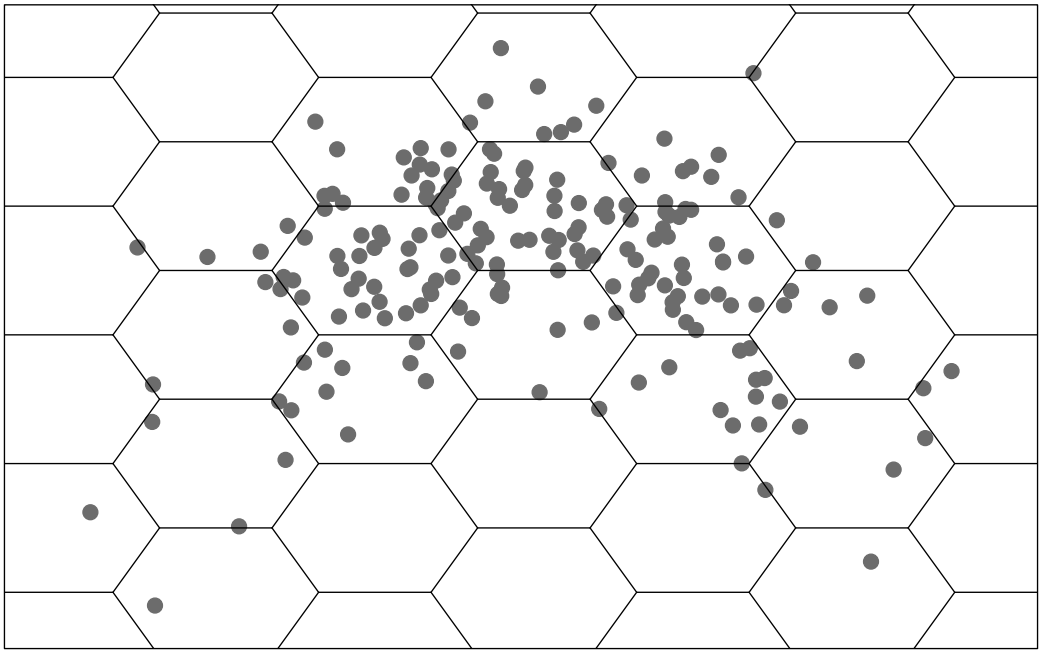
Binning in two dimensions;



3.25

Hexagonal binning

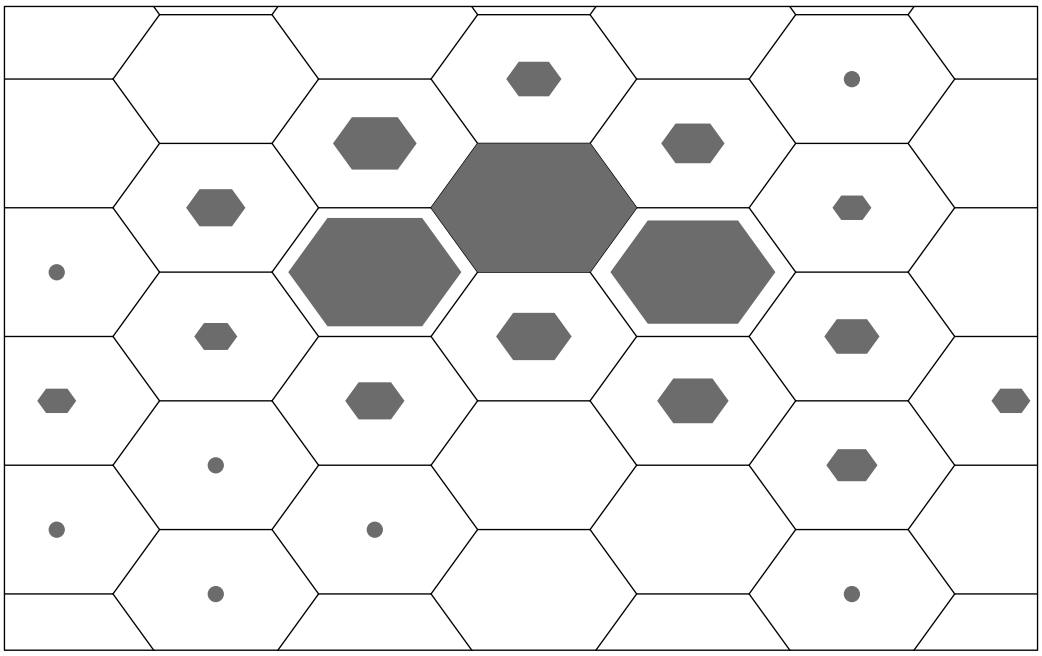
Binning in two dimensions;



3.26

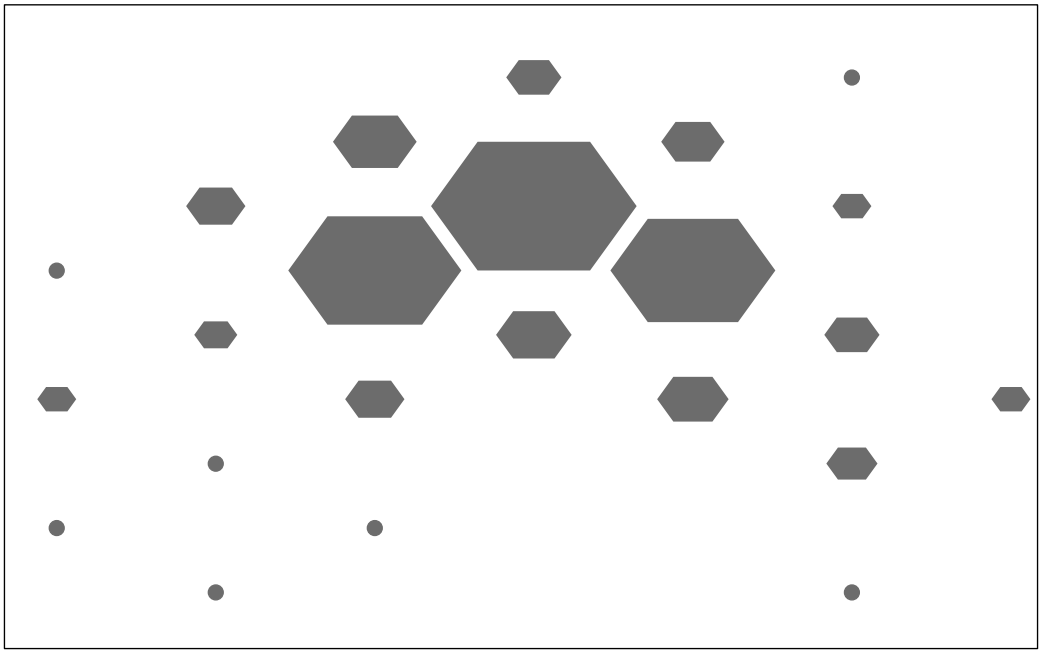
Hexagonal binning

Binning in two dimensions;



Hexagonal binning

Binning in two dimensions;

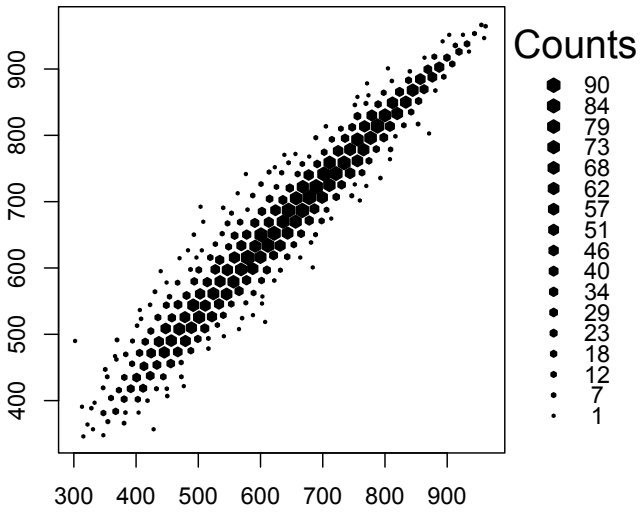


Hexagonal binning

The `hexbin()` package does all the bin construction, and counting. It has a `plot` method for its `hexbin` objects;

```
install.packages(c("hexbin","survey"))
library("hexbin")
library("survey")# for apipop data frame

with(apipop, plot(hexbin(api99,api00), style="centroids"))
```


Hexagonal binning



Hexagonal binning

Hexbin is used when you don't *really* care about the exact location of every single point

- Singleton points are plotted 'as usual'; you do (perhaps) care about them
- `hexbin` centers the 'ink' at the cell data's 'center of gravity'
- `style="centroids"` gives the center-of-gravity version; the default `style` is `colorscale` – usually grayscale. See `?gplot.hexagons` for more options

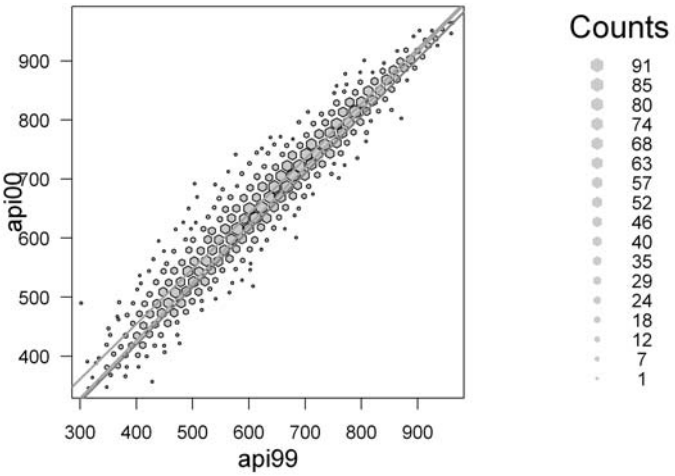
Hexagonal binning

For keen people: the `hexbin` package doesn't use the standard R graphics plotting devices; instead, it operates through the `Grid` system (in the `grid` package) which defines rectangular regions on a graphics device; these `viewport` regions can have a number of coordinate systems. To add lines to a hexbin plot, the options are;

- Use `hexVP.abline()` to add these directly
- Move everything into 'standard' graphics – not `Grid` graphics (see `?Grid`). The `Grid` system lets you alter graphics *after* plotting them
- Write your own plot method for hexbin objects, with standard R graphics commands
- Make do with `hexBinning()` in the `fMultivar` package

Hexagonal binning

An example; color-coded lines of best fit, by school type;




```
lm.e <- coef(lm(api00~api99, data=apipop, subset=stype=="E"))
lm.m <- coef(lm(api00~api99, data=apipop, subset=stype=="M"))
lm.h <- coef(lm(api00~api99, data=apipop, subset=stype=="H"))

hexVP.abline(vp1$plot.vp, lm.e[1], lm.e[2], col="coral")
```


Large data: multiple groups

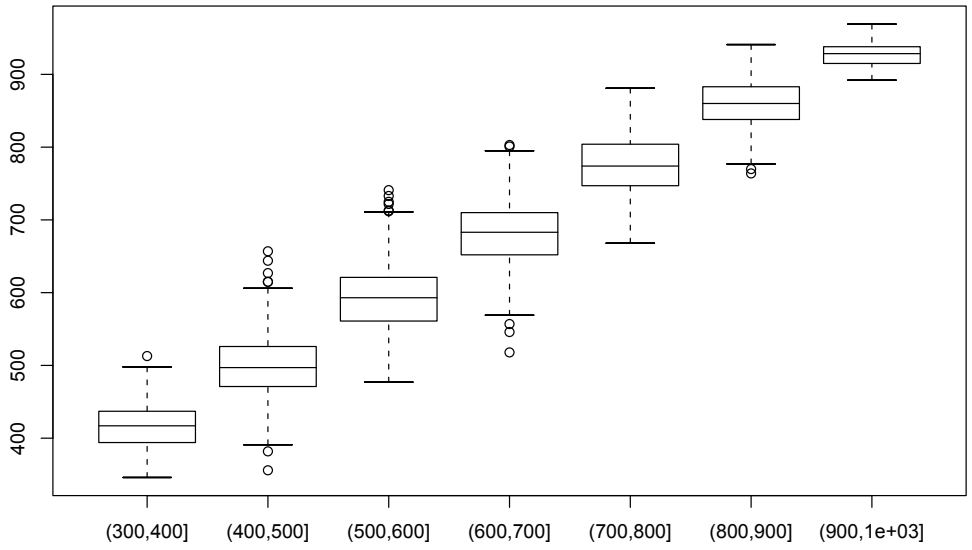
For showing multiple groups, a scatterplot smoother or perhaps boxplots or conditioning may be better.

```
boxplot(api00~cut(api99,(3:10)*100), data=apipop)
par(las=1)
par(mar=c(5.1,10.1,2.1,2.1))
boxplot(api00~interaction(stype,
                          cut(api99,(3:10)*100)),
        data=apipop, horizontal=TRUE,col=1:3)
```

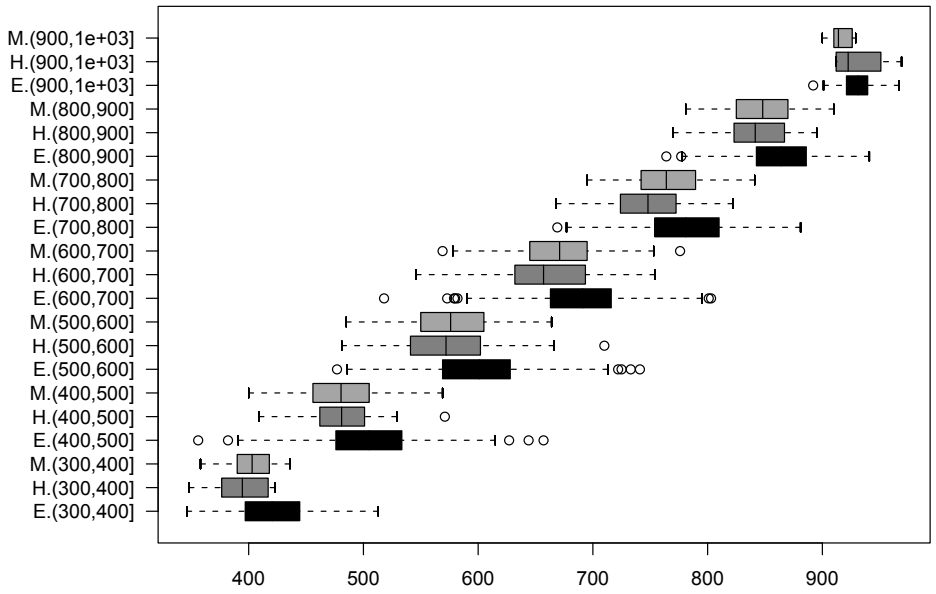
- `cut()` turns a variable into a factor by cutting it at the specified points.

- `par(mar=)` sets the margins around the plot. We need a large left margin for the labels.

Large data: multiple groups



Large data: multiple groups



Smoothers

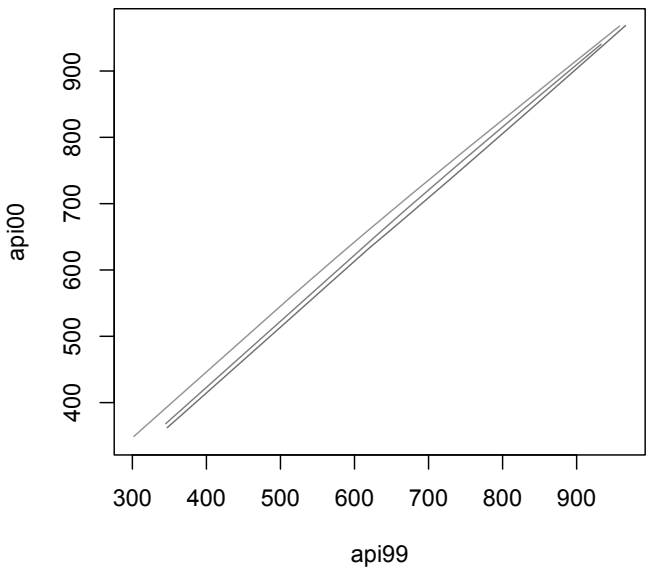
We don't plot the data at all, just means (could also plot quantiles with `quantreg` package)

```
plot(api00~api99,data=apipop,type="n")
with(subset(apipop, stype=="E"),
     lines(lowess(api99, api00), col="tomato"))
with(subset(apipop, stype=="H"),
     lines(lowess(api99, api00), col="forestgreen"))
with(subset(apipop, stype=="M"),
     lines(lowess(api99, api00), col="purple"))
```

Note the use of `type="n"`

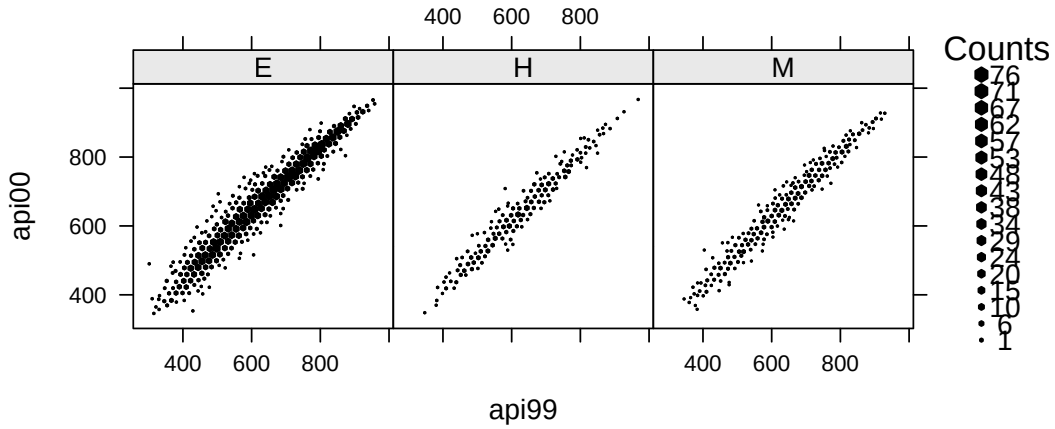
`subset()` returns a subset of a data frame.

Smoothers



Conditioning

```
hexbinplot(api00~api99|stype,data=apipop,style="centroid")
```



3.39

4. Data manipulation

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Universities of Washington and Auckland

Seattle, July 2014

Merging and matching

The data for an analysis often do not come in a single file. Combining multiple files is necessary.

If two data sets have the same individuals in the same order, they can simply be pasted together side by side.

```
## CHS baseline data
baseline <- read.spss("I:/DISTRIB/BASEBOTH.SAV", to.data.frame=TRUE)
## Events data (eg death, heart attack, ...)
events <- read.spss("I:/SAVEFILES/EVSUM04.SAV", to.data.frame=TRUE)

if (!all(baseline$IDNO==events$IDNO)) {
   stop("PANIC: They don't match!")
} else {
   alldata <- cbind(baseline, events[,c("TTODTH","DEATH",
                    "TTOMI","INCMI")])
}
```


Merging: order

The data might need to be sorted first

```
index1 <- order(baseline$IDNO)
baseline <- baseline[index1,]
index2 <- order(events$IDNO)
events <- events[index2,]
if (!all(baseline$IDNO==events$IDNO)) {
   stop("PANIC: They still don't match!")
} else {
   alldata <- cbind(baseline, events[,c("TTODTH","DEATH",
                    "TTOMI","INCMI")])
}
```

Note that `order(baseline$IDNO)` gives a subset of row numbers containing all the rows but in a different (increasing) order.

Merging: merge

Or there might be different rows in the two data sets

- Some people are missing from one or other data set (eg baseline and year 5 visits)
- Some people have multiple records in one data set (eg baseline data and all hospitalisations

The `merge()` function can do an database outer join, giving a data set that has all the possible matches between a row in one and a row in the other

Merging: merge

```
combined <- merge(baseline, hospvisits, by="IDNO", all=TRUE)
```

- `by=IDNO` says that the IDNO variable indicates individuals who should be matched.
- `all=TRUE` says that even people with no records in the `hospvisits` data set should be kept in the merged version.

How does it work: match

You could imagine a dumb algorithm for merging

```
for(row in firstdataset){
    for(otherrow in seconddataset){
     if (row$IDNO==otherrow$IDNO)
       ##add the row to the result
 }
}
```

More efficiently, the match function gives indices to match one variable to another

```
> match(c("B","I","O","S","T","A","T"),LETTERS)
[1]  2  9 15 19 20  1 20
> letters[match(c("B","I","O","S","T","A","T"),LETTERS)]
[1] "b" "i" "o" "s" "t" "a" "t"
```


Reshaping

Sometimes data sets are the wrong shape. Data with multiple observations of similar quantities can be in long form (multiple records per person) or wide form (multiple variables per person).

Example: The SeattleSNPs genetic variation discovery resource supplies data in a format

```
 SNP   sample al1 al2
000095 D001 C T
000095 D002 T T
000095 D003 T T
```

so that data for a single person is broken across many lines. To convert this to one line per person

```
> data<-read.table("http://pga.gs.washington.edu/data/il6
                 /ilkn6.prettybase.txt",
                 col.names=c("SNP","sample","allele1","allele2"))
> dim(data)
[1] 2303    4
> wideData<-reshape(data, direction="wide", idvar="sample",
                          timevar="SNP")
> dim(wideData)
[1] 47 99
> names(wideData)
 [1] "sample"       "allele1.95"   "allele2.95"   "allele1.205"
 [5] "allele2.205"  "allele1.276"  "allele2.276"  "allele1.321"
 [9] "allele2.321"  "allele1.657"  "allele2.657"  "allele1.1086"
...
```


- `direction="wide"` says we are going from long to wide format
- `idvar="sample"` says that sample identifies the rows in wide format
- `timevar="SNP"` says that SNP identifies which rows go into the same column in wide form (for repeated measurements over time it would be the time variable)

Broken down `by()` age and sex

A common request for Table 1 or Table 2 in a medical paper is to compute means and standard deviations, percentages, or frequency tables of many variables broken down by groups (eg case/control status, age and sex, exposure,...).

That is, we need to apply a simple computation to subsets of the data, and apply it to many variables. One useful function is `by()`, another is `tapply()`, which is very similar (but harder to remember).

```
> by(airquality$Ozone, list(month=airquality$Month),
       mean, na.rm=TRUE)
month: 5
[1] 23.61538
------------------------------------------------------
month: 6
[1] 29.44444
------------------------------------------------------
month: 7
[1] 59.11538
------------------------------------------------------
month: 8
[1] 59.96154
------------------------------------------------------
month: 9
[1] 31.44828
```

Notes

- The first argument is the variable to be analyzed.
- The second argument is a list of variable defining subsets. In this case, a single variable, but we could do `list(month=airquality$Month, toohot=airquality$Temp>85)` to get a breakdown by month and temperature
- The third argument is the analysis function to use on each subset
- Any other arguments (na.rm=TRUE) are also given to the analysis function
- The result is really a vector (with a single grouping variable) or array (with multiple grouping variables). It prints differently.

Confusing digression: str()

How do we know it is an array? Because `str()` summarises the internal structure of a variable.

```
> a<- by(airquality$Ozone, list(month=airquality$Month,
                           toohot=airquality$Temp>85),
                           mean, na.rm=TRUE)
> str(a)
 by [1:5, 1:2] 23.6 22.1 49.3 40.9 22.0 ...
 - attr(*, "dimnames")=List of 2
  ..$ month : chr [1:5] "5" "6" "7" "8" ...
  ..$ toohot: chr [1:2] "FALSE" "TRUE"
 - attr(*, "call")= language by.data.frame(data =
     as.data.frame(data), INDICES = INDICES,
     FUN = FUN, na.rm = TRUE)
 - attr(*, "class")= chr "by"
```

One function, many variables

There is a general function, `apply()` for doing something to rows or columns of a matrix (or slices of a higher-dimensional array).

```
> apply(psa[,1:8],2,mean,na.rm=TRUE)
        id      nadir      pretx         ps        bss
 25.500000  16.360000 670.751163  80.833333   2.520833
      grade       age    obstime
   2.146341 67.440000  28.460000
```

In this case there is a special, faster, function `colMeans`, but `apply()` can be used with other functions such as `sd()`, `IQR()`, `min()`,...

apply

- the first argument is an array or matrix or dataframe
- the second argument says which margins to keep (1=rows, 2=columns, ...), so 2 means that the result should keep the columns: apply the function to each column.
- the third argument is the analysis function
- any other arguments are given to the analysis function

There is a widespread belief that `apply()` is faster than a `for()` loop over the columns. This is a useful belief, since it encourages people to use `apply()`, but it is not true. (We'll see `for()` loops later)

New functions

Suppose you want the mean and standard deviation for each variable. One solution is to apply a new function;

```
> apply(psa[,1:8], 2,
   function(x){c(mean=mean(x,na.rm=TRUE), stddev=sd(x,na.rm=TRUE))}
  )
             id   nadir     pretx       ps       bss     grade
mean   25.50000 16.3600  670.7512 80.83333 2.5208333 2.1463415
stddev 14.57738 39.2462 1287.6384 11.07678 0.6838434 0.7924953
             age  obstime
mean   67.440000 28.46000
stddev  5.771711 18.39056
```


New functions

```
function(x){ c(mean=mean(x,na.rm=TRUE), stddev=sd(x,na.rm=TRUE)) }
```

translates as: "If you give me a vector, which I will call `x`, I will mean it and sd it and give you the results"

We could give this function a name and then refer to it by name

```
mean.and.sd <- function(x){ c(mean=mean(x,na.rm=TRUE),
                              stddev=sd(x,na.rm=TRUE))
                          }
apply(psa[,1:8], 2, mean.and.sd)
```

which would save typing if we used the function many times. The {curly brackets} are optional for a function with just one expression, but necessary for longer functions.

by() revisited

With our own functions, we can use `by()` more generally

```
> by(psa[,1:8], list(remission=psa$inrem),
     function(subset){round( apply(subset, 2, mean.and.sd), 2)} )
remission: no
          id nadir   pretx    ps  bss grade   age obstime
mean   31.03 22.52  725.99 79.71 2.71  2.11 67.17   21.75
stddev 11.34 44.91 1362.34 10.29 0.52  0.83  5.62   15.45
------------------------------------------------------
remission: yes
          id nadir   pretx    ps  bss grade   age obstime
mean   11.29  0.53  488.45 83.57 2.07  2.23 68.14   45.71
stddev 12.36  0.74 1044.14 12.77 0.83  0.73  6.30   13.67
```


Notes

`function(subset){ round(apply(subset, 2, mean.and.sd), 2) }`

translates as "If you give me a data frame, which I will call `subset`, I will apply the `mean.and.sd` function to each variable, round to 2 decimal places, and give you the results"

5. Replication: simulation and permutation

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Seattle, July 2014

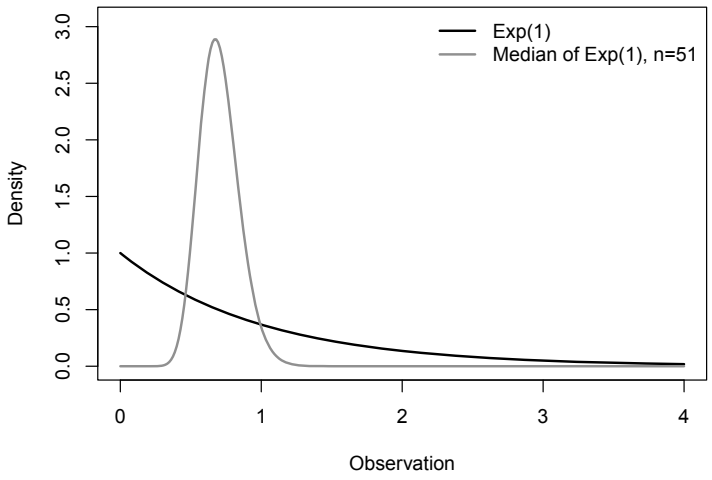
Overview

- Simulation
- Permutation tests
- Power (briefly)
- Smallest p-value across multiple models
- Cautionary notes, which we won't get to

Simulation: easier than doing maths

A question from analysis of survival traits – and its answer!

What is the expected value of the median of a sample, size $n = 51$, of independent data from $Exp(1)$? What is its variance?



Simulation: easier than doing maths

The picture didn't make it obvious? Here are the *exact* answers;

$$\mathbb{E}[\,\text{Median}_{51}\,] = \frac{2178178936539108674153}{3099044504245996706400}$$

$$\mathbb{E}[\,\text{Median}_{51}^2\,] = \frac{24672823160636679674592332321392579768019 59}{4802038419648657749001278815379823900480000}$$

These are 0.70286 and 0.51380 to 5 d.p. – so the variance is $0.51380 - 0.70286^2 = 0.01978$.

- Yes, there are 'pretty' answers here
- In general there aren't – but the 'expectation' ($\mathbb{E}[\ldots]$) terms just mean averaging over lots of datasets – which is easy, with a computer
- We can get a good-enough answer very quickly

Simulation: easier than doing maths

We'll write code that;

1. Generates samples of size $n = 51$ from $Exp(1)$
2. Calculates their median and returns this number
3. Replicate steps 1 and 2 many times, then work out the mean and variance of the stored numbers

Steps 1 and 2 are inside the curly brackets;

```
set.seed(4)
many.medians <- replicate(10000, {
   mysample <- rexp(n=51, rate=1)   # take a sample, size 51
   median(mysample)                 # calcuate & output its median
   })
```

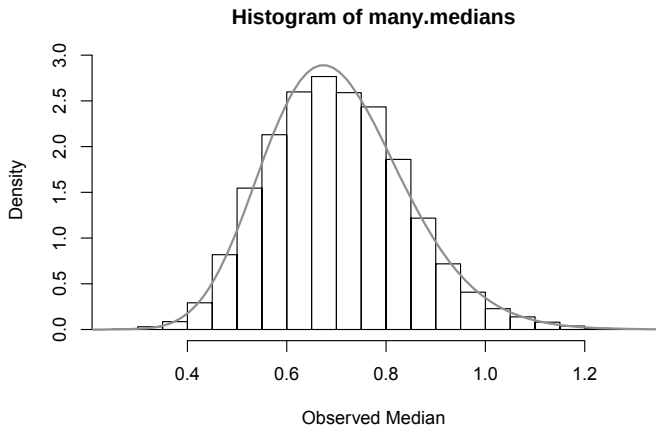
The function `set.seed()` tells R where to start its random-number generator – this is important, as it means we can repeat the code and get the same answers. Choose any 'seed' you like.

Simulation: easier than doing maths

To finish, take mean & variance of our 10000 sample medians;

```
> mean(many.medians)
[1] 0.702171         # exact answer is 0.70286
> var(many.medians)
[1] 0.01955728       # exact answer is 0.01978
```

NB: for large-enough values of 10000, we could work basically *anything* about the sample median, with little extra work;



Simulation: easier than doing maths

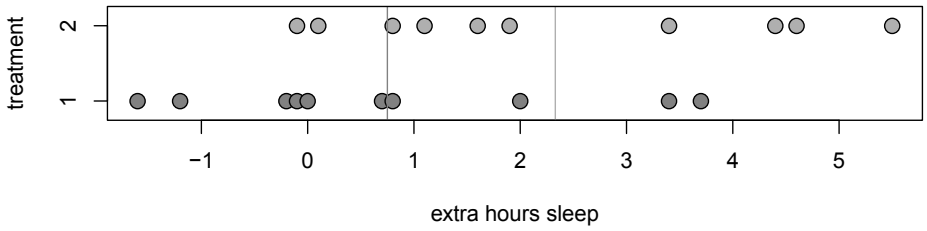
Notes on the coding;

- Yes, you could write a `for()` loop. (See Session 6, or `?Control`) But this approach helps break down the job into manageable pieces – then *finally* deal with the looping
- This approach is also easier to setup – just create `many.medians` and easier to edit afterwards, e.g. changing the value of 10000. Using `for()` loops, it's surprisingly easy to mess this up (more in Session 6)
- By default, the last object evaluated in a function is what it returns. Can also use `return()`
- We used `rexp()`, see also `rnorm()`, `rgamma()`, `rbinom`, `rpois()` etc etc

Permutation test

A classical statistical question: are the data we've observed *unexpected*, if there's nothing going on?

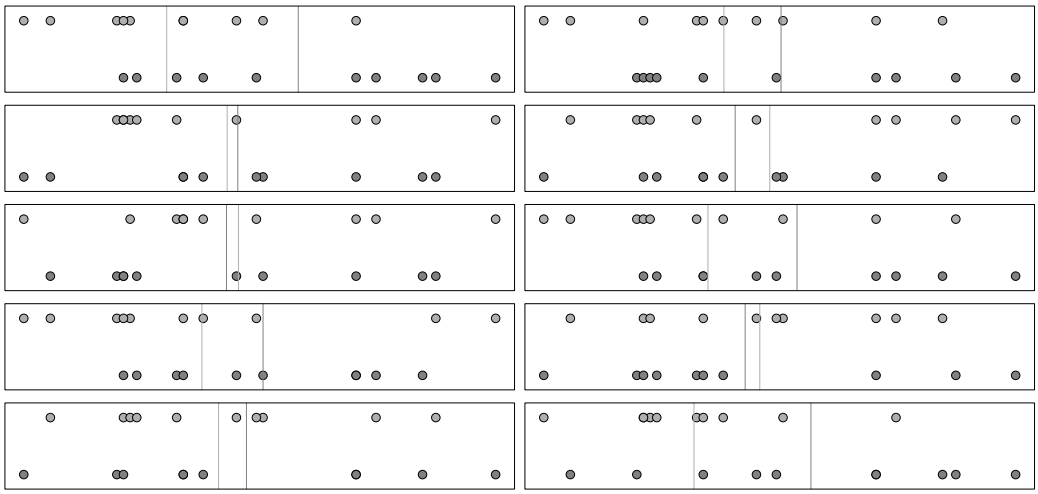
An example where we can answer this is the `sleep` data, from a small clinical trial;



- 10 subjects per group
- Groups receive different treatments, we record how many hours sleep they get, compared to baseline
- Mean extra hours sleep is higher in group 2 (2.33 hrs vs 0.75 hrs, so difference is 1.58 hrs)

Permutation test

What if there were nothing going on*, i.e. what if any differences in mean were just chance? If so, the data we saw would be just as likely as that obtained assigning the group labels *at random*;



* Formally, what if the *null hypothesis* of equal means held, in the population from which this data has been sampled?

Permutation test

To measure how unexpected our data is, we compute the red/green difference in means for many of these *permutations*, and see how the *observed* data compares.

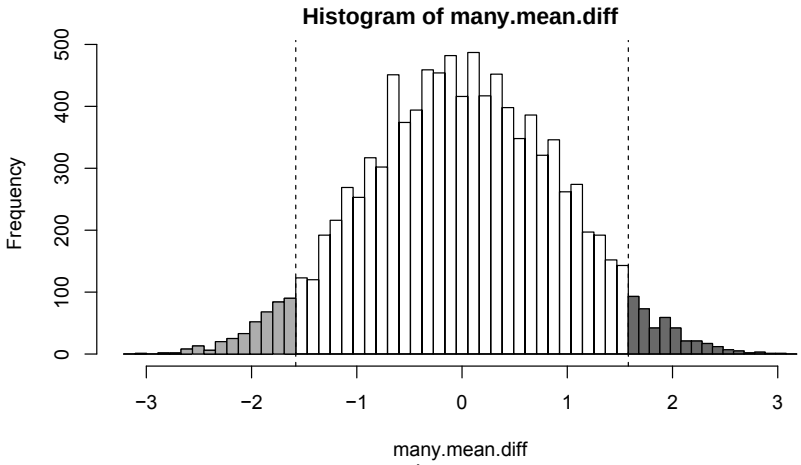
```
orig.mean.diff <-
   mean(sleep$extra[group==2])        - mean(sleep$extra[group==1])
orig.mean.diff

set.seed(4)
many.mean.diff <- replicate(10000,{
  group.shuffle <- sample(sleep$group)
  mean(sleep$extra[group.shuffle==2]) - mean(sleep$extra[group.shuffle==1])
  })
```

- `sample()` returns a random shuffle of a vector
- The same calculation is made, for the original data and the shuffled versions; the difference in means is called the *test statistic*

Permutation test

How does original data (w/ mean diff=1.58) compare to these?




```
> table(many.mean.diff>orig.mean.diff)
FALSE  TRUE
 9601   399
> mean(many.mean.diff>orig.mean.diff)
[1] 0.0399
> mean(abs(many.mean.diff)>abs(orig.mean.diff))
[1] 0.0789
```


Permutation test

- The proportion of sample in the RH tail is a (valid) p-value for a one-tailed test, where the alternative is that green $>$ red. $p = 0.04$, here
- The proportion in both tails is the p-value for a two-tail test; $p = 0.079$
- There is some 'Monte Carlo' error in these p-values; roughly ± 0.004 here, i.e. 2 decimal places in p. If that's not good enough, use more permutations. (Here, could use all 184,756 – but in larger samples it's not possible)

For a quicker but *somewhat* approximate version of this test;

```
> t.test(extra~group, data=sleep)
Welch Two Sample t-test
data:  extra by group
t = -1.8608, df = 17.776, p-value = 0.07939
alternative hypothesis: true difference in means is not equal to 0
```

The t-test makes fewer assumptions than many people think!

How many permutations?

With 10000 permutations the smallest possible p-value is 0.0001, and the uncertainty near $p = 0.05$ is about $\pm 0.4\%$

If we have multiple testing we may need much more precision.

Using 100,000 permutations reduces the uncertainty near $p = 0.05$ to $\pm 0.1\%$ and allows accurate p-values as small as 0.00001.

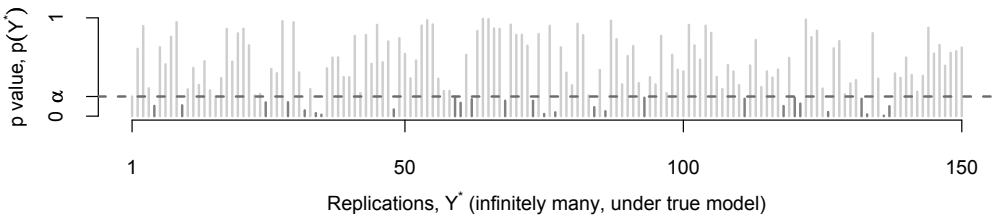
A useful strategy is to start with 1000 permutations and continue to larger numbers only if p is small enough to be interesting, eg $p < 0.1$.

Parallel computing of permutations is easy: just run `R` on multiple computers.

Example: power calculation

A reminder: statistical tests **reject** H_0 whenever $p(Y) < \alpha$;
α is known as the **size** or **level** of the test; it is the *probability* of declaring a signal when none is present. (By convention, we almost always use $\alpha = 0.05$.)

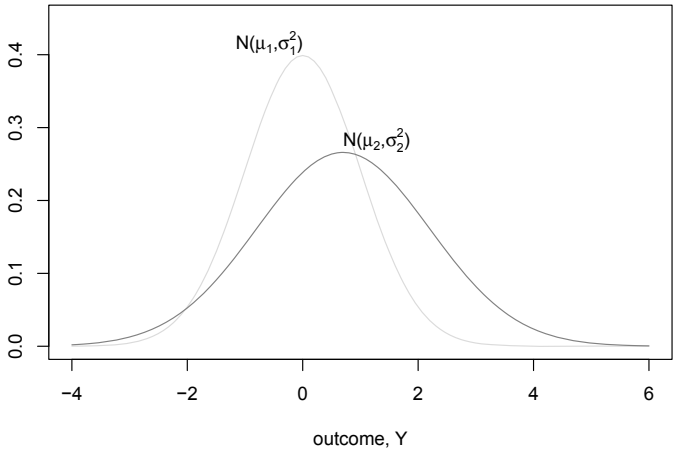
The **power** of the test is the *probability* you reject H_0, (i.e. get $p(Y) < \alpha$) when a signal is truly present.



Note: *probabilities* are means of binary (0/1) variables.

Example: power calculation

To evaluate power, for a comparison of two groups – like the sleep study;



- Assume outcomes are Normal, means μ_1, μ_2, SDs σ_1, σ_2
- Sample sizes n_1, n_2 in each group
- Use unequal-variance two-sided t-test for analysis

Example: power calculation

Using `replicate()` to do the work;

```
do.one <- function(n1, n2, mu1, mu2, s1, s2, alpha){
   y1 <- rnorm(n1, mu1, s1)
   y2 <- rnorm(n2, mu2, s2)
   t.test(y1, y2)$p.value < alpha # default is unequal variance
}

set.seed(4)
bigB <- 10000
mean(replicate(bigB, do.one(20, 20, 0, 0.7, 1, 1.5, 0.05)))
```

- Mean here is 3895/10000 = 0.3895, i.e. about 40% power. Precision to multiple decimal places matters much less than earlier example, calculating p
- For equal variances, can use `power.t.test()`, i.e. built-in maths-only version
- This version is slower to compute – but much more flexible, e.g. regression-based analyses, multi-step analyses, any distribution/design you like

Debugging

R error messages are sometimes hard to follow, when the error occurs in a low-level function. To see what happened after an error, `traceback()` reports the entire call stack, which is useful for seeing where things went wrong.

For example, in the permutation test example, suppose our outcome variable were actually a data frame with one column rather than a vector:

```
> wrong.extra <- as.data.frame(sleep$extra) # easy mistake!
> many.mean.diff <- replicate(10000,{
+   group.shuffle <- sample(sleep$group)
+   mean(wrong.extra[group.shuffle==2]) - mean(wrong.extra[group.shuffle==1])
+   })
Error in `[.data.frame`(wrong.extra, group.shuffle == 2) :
  undefined columns selected
```

We didn't know we were calling `` `[.data.frame` ``, so the message appears opaque and unhelpful.

Debugging

```
> traceback()
8: stop("undefined columns selected")
7: `[.data.frame`(wrong.extra, group.shuffle == 2)
6: wrong.extra[group.shuffle == 2]
5: mean(wrong.extra[group.shuffle == 2]) at #3
4: FUN(c(0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L, 0L,
3: lapply(X = X, FUN = FUN, ...)
2: sapply(integer(n), eval.parent(substitute(function(...) expr)),
       simplify = simplify)
1: replicate(10000, {
       group.shuffle <- sample(sleep$group)
       mean(wrong.extra[group.shuffle == 2]) - mean(wrong.extra[group.shuffle == 1
   })
```

This mean the problem happens in our code `mean(wrong.extra[group.shuffle == 2])` and is a problem with computing `wrong.extra[group.shuffle == 2]`.

We want to have a look at `wrong.extra` and `group.shuffle`...

Debugging

The post-mortem debugger lets you look inside the code where the error occurred;

```
> options(error=recover)
> many.mean.diff <- replicate(10000,{
+   group.shuffle <- sample(sleep$group)
+   mean(wrong.extra[group.shuffle==2]) - mean(wrong.extra[group.shuffle==1])
+   })
Error in `[.data.frame`(wrong.extra, group.shuffle == 2) :
  undefined columns selected

Enter a frame number, or 0 to exit

1: replicate(10000, {
    group.shuffle <- sample(sleep$group)
    mean(wrong.ex
2: sapply(integer(n), eval.parent(substitute(function(...) expr)), simplify =
3: lapply(X = X, FUN = FUN, ...)
4: FUN(c(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
5: #3: mean(wrong.extra[group.shuffle == 2])
6: wrong.extra[group.shuffle == 2]
7: `[.data.frame`(wrong.extra, group.shuffle == 2)
```

We want 6, the last step of 'our' code

Debugging

```
Called from: eval(substitute(browser(skipCalls = skip), list(skip = 7 - which)),
    envir = sys.frame(which))
Browse[1]> ls()
character(0)
Browse[1]> str(wrong.extra)
'data.frame':   20 obs. of  1 variable:
 $ sleep$extra: num  0.7 -1.6 -0.2 -1.2 -0.1 3.4 3.7 0.8 0 2 ...
Browse[1]> is.vector(wrong.extra)
[1] FALSE
Browse[1]> c
```

As well as data in unexpected formats, watch out for 'weird' data that might lead to e.g. missing values in regression output.

Finally; turn the post-mortem debugger off with

```
options(error=NULL) # turn it off! turn it off!!!
```

Minimum p-value

Little point in permutation test for the mean: same result as t-test

Permutation test is useful when we do not know how to compute the distribution of a test statistic.

Suppose we test additive effects of 8 SNPs, one at a time, and we want to know if the most significant association is real.

For any one SNP the z-statistic from a logistic regression model has a Normal distribution.

We need to know the distribution of the most extreme of eight z-statistics. This is not a standard distribution, but a permutation test is still straightforward.

Minimum p-value

```
dat <- data.frame(y=rep(0:1,each=100),
SNP1=rbinom(200,size=2,prob=.1), SNP2=rbinom(200,size=2,prob=.2),
SNP3=rbinom(200,size=2,prob=.2), SNP4=rbinom(200,size=2,prob=.4),
SNP5=rbinom(200,size=2,prob=.1), SNP6=rbinom(200,size=2,prob=.2),
SNP7=rbinom(200,size=2, prob=.2), SNP8=rbinom(200,size=2,prob=.4))
```

```
> head(dat)
  y SNP1 SNP2 SNP3 SNP4 SNP5 SNP6 SNP7 SNP8
1 0    0    0    0    0    0    1    0    0
2 0    0    1    0    1    0    1    0    2
3 0    0    1    0    1    1    0    0    0
4 0    0    0    1    1    0    0    0    0
5 0    0    1    0    1    1    0    0    0
6 0    0    0    0    1    0    1    0    1
```

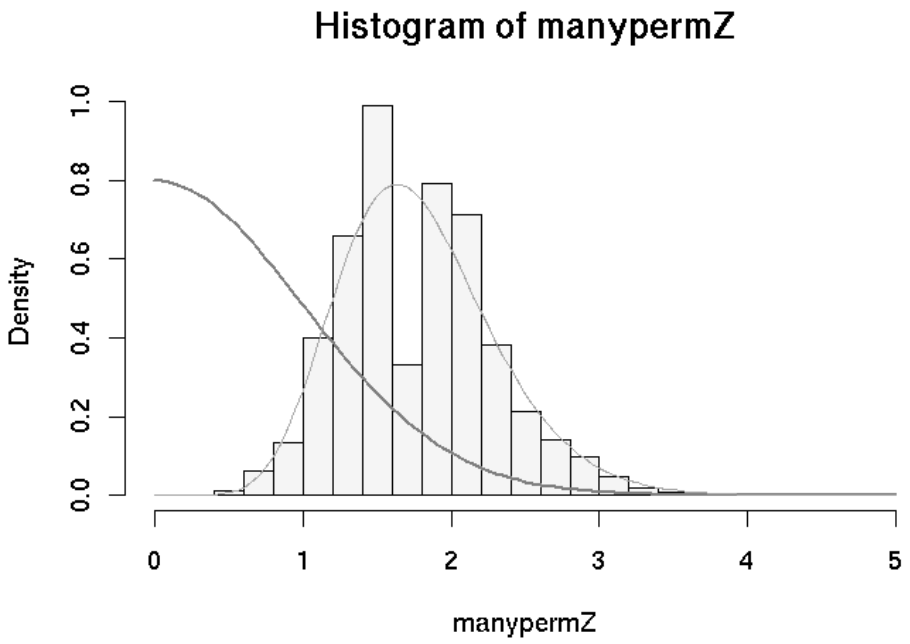
Minimum p-value

```
oneZ<-function(outcome, snp){
    model <- glm(outcome~snp, family=binomial)
    coef(summary(model))["snp","z value"]
}
maxZ<-function(outcome, snps){
    allZs <- sapply(snps,
         function(this.snp){ oneZ(outcome, snp=this.snp) })
    max(abs(allZs))
}


true.maxZ<-maxZ(outcome=dat$y, snps=dat[,-1])

manypermZ<-replicate(10000,
       maxZ(outcome=sample(dat$y), snps=dat[,-1]))
```


Minimum p-value



Minimum p-value

The histogram shows the permutation distribution for the maximum Z-statistic.

The blue curve is the theoretical distribution for one Z-statistic

The yellow curve is the theoretical distribution for the maximum of eight independent Z-statistics.

Clearly the multiple testing is important: a Z of 2.5 gives $p = 0.012$ for a single test but $p = 0.075$ for the permutation test.

The theoretical distribution for the maximum has the right range but the permutation distribution is quite discrete. The discreteness is more serious with small sample size and rare SNPs.

[The theoretical distribution is not easy to compute except when the tests are independent.]

More debugging

Permutation tests on other people's code might reveal a lack of robustness.

For example, a permutation might result in all controls being homozygous for one of the SNPs and this might give an error message

We can work around this with `tryCatch()`

```
oneZ<-function(outcome, snp){
    tryCatch({model <- glm(outcome~snp, family=binomial())
              coef(summary(model))["snp","z value"]},
       error=function(e) NA
       )
}
```

Now `oneZ()` will return `NA` if there is an error in the model fitting.

Caution: wrong null

Permutation tests cannot solve all problems: they are valid only when the null hypothesis is 'no association'

Suppose we are studying a set of SNPs that each have some effect on outcome and we want to test for interactions (epistasis).

Permuting the genotype data would break the links between genotype and outcome and created shuffled data with no main effects of SNPs.

Even if there are no interactions the shuffled data will look different from the real data.

Caution: weak null hypothesis

A polymorphism could increase the variability of an outcome but not change the mean.

In this case the strong null hypothesis is false, but the hypothesis of equal means is still true.

- If we want to detect this difference the permutation test is unsuitable because it has low power
- If we do not want to detect this difference the permutation test is invalid, because it does not have the correct Type I error rate.

When groups are the same size the Type I error rate is typically close to the nominal level, otherwise it can be too high or too low.

To illustrate this we need many replications of a permutation test. We will do 1000 permutation tests for a mean, each with 1000 permutations.

```
meandiff<-function(x,trt){
mean(x[trt==1])-mean(x[trt==2])
}
meanpermtest<-function(x,trt,n=1000){
observed<-meandiff(x,trt)
perms<-replicate(n, meandiff(x, sample(trt)))
mean(abs(observed)>abs(perms))
}

trt1<-rep(c(1,2),c(10,90))

perm.p<-replicate(1000, {
          x1<-rnorm(100, 0, s=trt1)
          meanpermtest(x1,trt1)})

table(cut(perm.p,c(0,.05,.1,.5,.9,.95,1)))/1000
```

```
  (0,0.05] (0.05,0.1]  (0.1,0.5]  (0.5,0.9] (0.9,0.95]   (0.95,1]
        86         99        564        244           6           0
```

The p-values are too small, relative to a uniform distribution. If we reverse the standard errors we get

```
  (0,0.05] (0.05,0.1]  (0.1,0.5]  (0.5,0.9] (0.9,0.95]   (0.95,1]
        27         28        275        354          67         249
```

If the two groups each have 50 observations we get

```
  (0,0.05] (0.05,0.1]  (0.1,0.5]  (0.5,0.9] (0.9,0.95]   (0.95,1]
        50         45        403        407          52          43
```

which is much better.

6. Writing Big Loops

Ken Rice
Thomas Lumley

Universities of Washington and Auckland

Seattle, July 2014

Writing loops in R

We saw that `replicate()`, and `apply(), sapply()` are R's preferred way of looping (doing the same thing many times).

Even for expert useRs, their use requires some careful thought; debugging code may be complex. Also, there are some jobs (e.g. iteration) that these loops cannot do.

In this session we'll talk about some alternatives, and their application to genome-wide studies.

`for()` loops

Your first computer program?

```
for(i in 1:100){
   print("Hello world!")
   print(i*i)
}
```

- Everything inside the curly brackets {...} is done 100 times
- Looped commands can depend on `i` (or whatever you called the counter)
- `R` creates a vector `i` with `1:100` in it. You could use **any vector that's convenient**

`for()` loops

`for()` loops are very intuitive, but have some drawbacks;

- They can be **slow**;
  - 'growing' a large dataset is a bad idea;
    `mydata <- cbind(mydata, rnorm(1000, mean=i))`
  - so set up blank output **first**, then 'fill it in'
- `apply()` is interpreted slightly faster than `for()` – but typically this will not matter, contrary to some urban myths
- `for()` loops require more typing than `apply()`! For tasks which will be repeated, writing a function really is the Right Thing to do, in the long run.

Using `for(i in 1:N)` sets up a vector (`i`) of length `N`. Do you really need this?

`for()` loops

Two alternatives; (see `?Control` for details)

```
i <- 1; my.mat <- matrix(NA, N, 3)
while(i <= N){
  z <- work.on.gene(i)
  my.mat[i,] <- summary(z)
  i <- i+1
  }
```

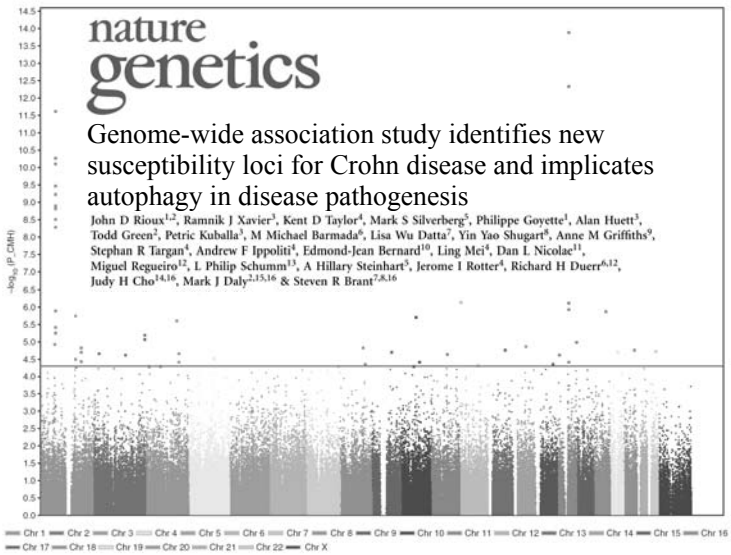
– note that we avoided 'growing' the output

```
i <- 1; my.mat <- matrix(NA, N, 3)
repeat{
  z <- work.on.gene(i)
  my.mat[i,] <- summary(z)
  i <- i+1
  if(i>=N) break
  }
```

Use `apply()`, `sapply()` to avoid the 'setup' stage

Application to whole-genome study

Whole genome studies look **very intimidating** ...



Application to whole-genome study

... however, each p-value on that picture comes from a single logistic regression.

There may be 2,500,000 tests in total; if each one takes 1/10 sec, the analysis is done in under an hour;

Time per test	Total time	
0.01 sec	7 hrs	
0.1 sec	2 days 22 hrs	
1 sec	28 days	(!)
5 sec	5 months	(!!)
5 mins	24 yrs	(!!!)

Cutting time per test from 1 sec $\rightarrow$ 0.1 sec is clearly worthwhile – but *proposing* analyses where each test takes $>$ 5 secs may be silly.

Making code run faster, part 1

Some easy 'streamlining' ideas;

- Write a function to do just the analysis you want
  `> my.output <- apply(my.data, 1, my.function)`
- Make a compiled version of it – use `cmpfun()` in the (standard) `compiler` package
- Pre-process/'clean' your data before analysis; e.g. `sum(x)/length(x)` doesn't error-check like `mean(x)`
- Similarly, you can streamline `glm()` to just `glm.fit()` [as we'll see, in some examples]
- Use vectorized operations, where possible
- Store data as matrices, not data.frames

Making code run faster, part 2

Streamlining, for experts;

- Write small but *important* pieces of code in `C`, and call these from `R`
- **Batch mode** processing lets you break down e.g. the whole genome into 23 chromosomes – great if you have 23 processors to use.
  - Save your analysis in 23 output files
  - read in the answers
  - finally produce e.g. multi-color pictures

  Various packages help implement this, but use is platform-specific

Timing

"Premature optimization is the root of all evil"

Donald Knuth

Do you need to optimize your code? Running 2 or 3 times faster may **not be worth the time spent coding/debugging!**

But going an **order of magnitude** faster is A Good Thing.

After you have code that works, you may need to speed it up. Experienced useRs may be able to 'eyeball' the problem; measurement is an **easier and more reliable** approach

Timing

- `proc.time()` returns the current time. Save it before a task and subtract from the value after a task.

- `system.time()` times the evaluation of expression

- `R` has a **profiler**; this records which functions are being run, many times per second. `Rprof(filename)` turns on the profiler, `Rprof(NULL)` turns it off. `summaryRprof(filename)` reports how much time was spent in each function.

Remember that a 1000-fold speedup in a function used 10% of the time is **less helpful** than a 30% speedup in a function used 50% of the time.

High-throughput code – caveats

We saw yesterday that 'weird' datasets can crash your code. These **will appear** in genome-wide studies, and a crash at SNP 299,999 will be **very frustrating**.

- Some 'weirdness' is easy to spot;
  - Everyone is homozygous
  - All cases missing
  - No variation in outcome ...

- In more complex models, it's easier to 'try it and see'. Use `tryCatch()`

- When 'weirdness' is found, high-throughput code should;
  - Produce sensible output (`NA`, `-999` etc)
  - Handle these appropriately in summary output

7. Working with Big Data

Thomas Lumley
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Universities of Washington and Auckland

Seattle, July 2014

Large data

"R is well known to be unable to handle large data sets."

Solutions:

- Get a bigger computer: 8-core Linux computer with 32Gb memory for $< \$3000$
- Don't load all the data at once (methods from the mainframe days).

Large data

Data won't fit in memory on current computers: R can comfortably handle data up to

- About 1/3 of physical RAM
- About 10% of address space (ie, no more than 400MB for 32-bit R, no real constraint for 64-bit R)

R can't (currently) handle a single matrix with more than $2^{31} - 1 \approx 2$ billion entries even if your computer has memory for it.

Storing data on disk means extra programming work, but has the benefit of making you aware of data reads/writes in algorithm design.

Storage formats

R has two convenient data formats for large data sets

- For ordinary large data sets, direct interfaces to relational databases allow the problem to be delegated to the experts.
- For very large 'array-structured' data sets the `ncdf` package provides storage using the netCDF data format.

SQL-based interfaces

Relational databases are the natural habitat of large datasets.

- Optimized for loading subsets of data from disk
- Fast at merging, selecting
- Standardized language (SQL) and protocols (ODBC, JDBC)

Elementary SQL

Basic statement: `SELECT var1, var2 FROM table`

- `WHERE condition` to choose rows where condition is true
- `table1 INNER JOIN table2 USING(id)` to merge two tables on a identifier
- Nesting: `table` can be a complete `SELECT` statement

R database interfaces

- RODBC package for ODBC connections (mostly Windows)
- DBI: standardized wrapper classes for other interfaces
  - RSQLite: small, zero-configuration database for embedded storage
  - RJDBC: Java interface
  - also for Oracle, MySQL, PostgreSQL.

Setup: DBI

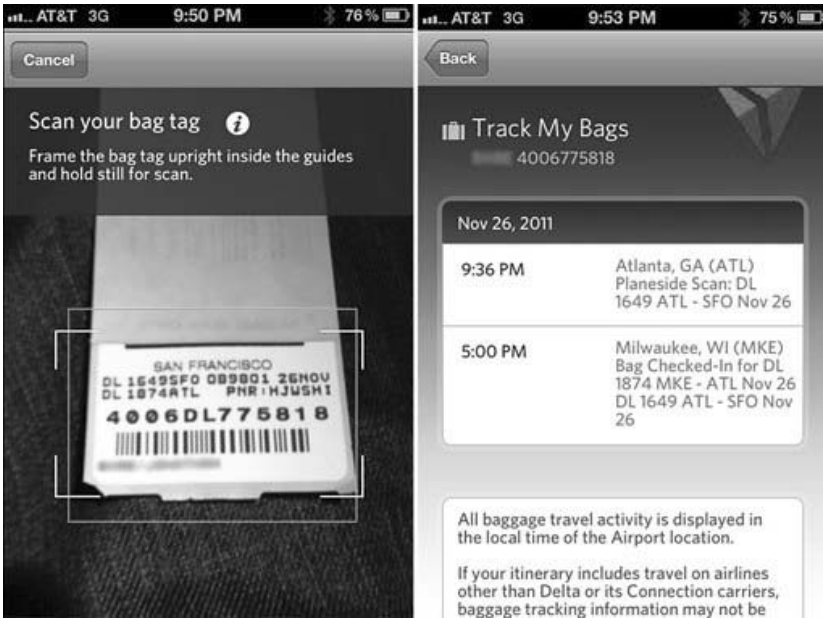
Needs DBI package + specific interface package

```
library("RSQLite") ## also loads DBI package

sqlite <- dbDriver("SQLite")
conn <- dbConnect(sqlite, "example.db")

dbListTables(conn) ## what tables are available?
                   ## see also dbListFields()

dbDisconnect(conn) ## when you are done
```

Now use `conn` to identify this database connection in future requests

Setup: DBI

How to think about `conn`, and what it does;



Queries: DBI

- `dbGetQuery(conn, "select var1, var2, var22 from smalltable")`: runs the SQL query and returns the results (if any)
- `dbSendQuery(conn, "select var1, var2, var22 from hugetable")` runs the SQL and returns a result set object
- `fetch(resultset, n=1000)` asks the database for the next 1000 records from the result set
- `dbClearResult(resultset)` releases the result set

Whole tables: DBI

- `dbWriteTable(conn, "tablename", dataframe)` writes the whole data frame to a new database table (use `append=TRUE` to append to existing table)
- `dbReadTable(conn, "tablename")` reads a whole table
- `dbDropTable(conn, "tablename")` deletes a table.

Setup: ODBC

Just needs RODBC package. Database must be given a "Data Source Name" (DSN) using the ODBC adminstrator on the Control Panel

```
library("RODBC")

conn <- odbcConnect(dsn)

close(conn) ## when you are done
```

Now use `conn` to identify this database connection in future requests

Queries: ODBC

- `sqlQuery(conn, "select var1, var2, var22 from smalltable")`: runs the SQL query and returns the results (if any)

- `odbcQuery(conn, "select var1, var2, var22 from hugetable")` runs the SQL and returns a result set object

- `sqlGetResults(con, max=1000)` asks the database for the next 1000 records from the result set

Whole tables: ODBC

- `sqlSave(conn, dataframe, "tablename")` writes the whole data frame to a new database table (use `append=TRUE` to append to existing table)

- `sqlFetch(conn, "tablename")` reads a whole table

Example: SQLite and Bioconductor

Bioconductor's AnnotationDBI system maps from one system of identifiers (eg probe ID) to another (eg GO categories).

Each annotation package contains a set of two-column SQLite tables describing one mapping.

'Chains' of tables allow mappings to be composed so, eg, only gene ids need to be mapped directly to GO categories.

Original annotation system kept all tables in memory; they are now getting too large.

AnnotationDBI

```
> library("hgu95av") # a Bioconductor package: see Session 8
> hgu95av2CHR[["1001_at"]]
[1] "1"
> hgu95av2OMIM[["1001_at"]]
[1] "600222"
> hgu95av2SYMBOL[["1001_at"]]
[1] "TIE1"
> length(hgu95av2GO[["1001_at"]])
[1] 16
```

Under the hood

```
> ls("package:hgu95av2.db")
 [1] "hgu95av2"              "hgu95av2_dbconn"       "hgu95av2_dbfile"
 [4] "hgu95av2_dbInfo"       "hgu95av2_dbschema"     "hgu95av2ACCNUM"
 [7] "hgu95av2ALIAS2PROBE"   "hgu95av2CHR"           "hgu95av2CHRLENGTHS"
[10] "hgu95av2CHRLOC"        "hgu95av2CHRLOCEND"     "hgu95av2ENSEMBL"
> hgu95av2_dbconn()
<SQLiteConnection: DBI CON (7458, 1)>
> dbGetQuery(hgu95av2_dbconn(), "select * from probes limit 5")
    probe_id gene_id is_multiple
1    1000_at    5595           0
2    1001_at    7075           0
3  1002_f_at    1557           0
4  1003_s_at     643           0
5    1004_at     643           0
```


Under the hood

The `[[` method calls the `mget` method, which also handles multiple queries.

These (eventually) produce SQLite `SELECT` statements with `INNER JOIN`s across the tables needed for the mapping.

netCDF

netCDF was designed by the NSF-funded UCAR consortium, who also manage the National Center for Atmospheric Research.

Atmospheric data are often array-oriented: eg temperature, humidity, wind speed on a regular grid of (x, y, z, t).

Need to be able to select 'rectangles' of data – eg range of (x, y, z) on a particular day t.

Because the data are on a regular grid, the software can work out where to look on disk without reading the whole file: efficient data access.

Many processes can read the same netCDF file at once: efficient parallel computing.

Current uses in biology

- Whole-genome genetic data (us and people we talk to)
  - Two dimensions: genomic location $\times$ sample, for multiple variables
  - Data sizes in tens to thousands of gigabytes.
- Flow cytometry data (proposed new FCS standard)
  - 5–20 (to 100, soon) fluorescence channels $\times$ 10,000–10,000,000 cells $\times$ 5–5000 samples
  - Data sizes in gigabytes to thousands of gigabytes.

Using netCDF data

Interact with netCDF files via the `ncdf` package, in much the same way as databases;

`open.ncdf()` opens a netCDF file and returns a connection to the file (rather than loading the data)

`get.var.ncdf()` retrieves all or part of a variable.

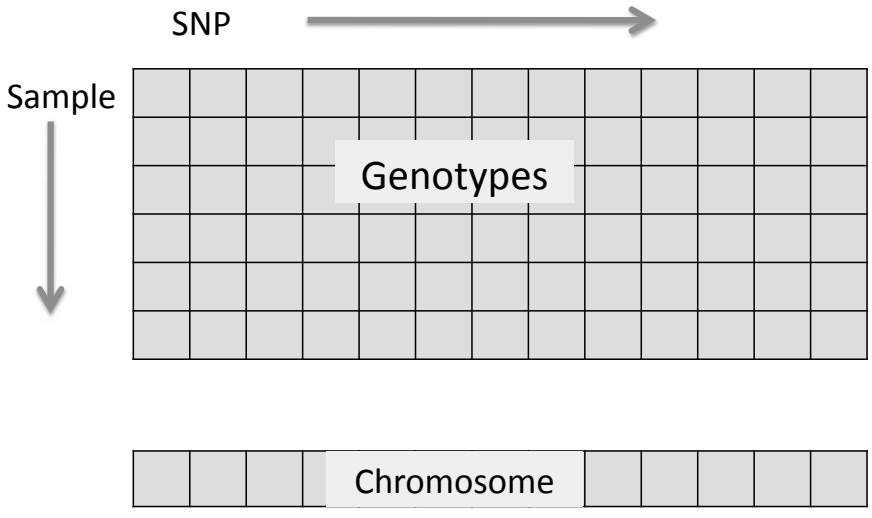
`close.ncdf()` closes the connection to the file.

To see what variables a file contains, and their dimensions, e.g.

```
conn <- open.ncdf("mynetCDFfile.nc")
conn
```


Dimensions

Variables can use one or more array dimensions of a file



Dimensions

To read data that has given dimensions, netCDF's `get.var.ncdf()` function uses an unusual (but logical) system, with these arguments;

- `nc` – the netCDF connector object, returned by `create.ncdf`
- `varid` – the name of the variable you want to read, e.g. `"genotype"` or `"chromosome"`
- `start` – a vector containing the location at which you start reading, e.g. `c(1,1)` or just `c(10)`
- `count` – *how far* you want to read, in each dimension, e.g. `c(50,100)` for a 50×100 rectangle, just `99` for 99 elements in a 1D object

For `count`, `-1` is a special 'code', that means 'all the entries in that dimension until the end'. It's very useful!

Example

Finding long homozygous runs (possible deletions)

```
library("ncdf")
nc <- open.ncdf("hapmap.nc")

## read all of chromosome variable
chromosome <- get.var.ncdf(nc, "chr", start=1, count=-1)
## set up list for results
runs<-vector("list", nsamples)

for(i in 1:nsamples}{
   ## read all genotypes for one person
   genotypes <- get.var.ncdf(nc, "geno", start=c(1,i),count=c(-1,1))
   ## zero for htzygous, chrm number for hmzygous
   hmzygous <- genotypes != 1
   hmzygous <- as.vector(hmzygous*chromosome)
```

Example

```
   ## consecutive runs of same value
   r <- rle(hmzygous)
   begin <- cumsum(c(1, r$lengths))
   end   <- cumsum(r$lengths)
   long  <- which ( r$lengths > 250 & r$values !=0)
   runs[[i]] <- cbind(begin[long], end[long], r$lengths[long])
}

close.ncdf(nc)
```

Notes

- `chr` uses only the 'SNP' dimension, so `start` and `count` are single numbers
- `geno` uses both SNP and sample dimensions, so `start` and `count` have two entries.
- `rle` compresses runs of the same value to a single entry.

Advanced: creating netCDF files

Creating files is more complicated

- Define dimensions
- Define variables and specify which dimensions they use
- Create an empty file
- Write data to the file.

Advanced: defining dimensions

Specify the name of the dimension, the units, and the allowed values in the `dim.def.ncdf()` function.

One dimension can be 'unlimited', allowing expansion of the file in the future. An unlimited dimension is important, otherwise the maximum variable size is 2Gb.

```
snpdim    <-dim.def.ncdf("position","bases", positions)
sampledim <-dim.def.ncdf("seqnum","count",1:10, unlim=TRUE)
```


Advanced: defining variables

Variables are defined by name, units, and dimensions

```
varChrm <- var.def.ncdf("chr","count",dim=snpdim,
         missval=-1, prec="byte")
varSNP <- var.def.ncdf("SNP","rs",dim=snpdim,
        missval=-1, prec="integer")
vargeno <- var.def.ncdf("geno","base",dim=list(snpdim, sampledim),
        missval=-1, prec="byte")
vartheta <- var.def.ncdf("theta","deg",dim=list(snpdim, sampledim),
        missval=-1, prec="double")
varr <- var.def.ncdf("r","copies",dim=list(snpdim, sampledim),
       missval=-1, prec="double")
```

Advanced: creating the file

The file is created by specifying the file name ad a list of variables.

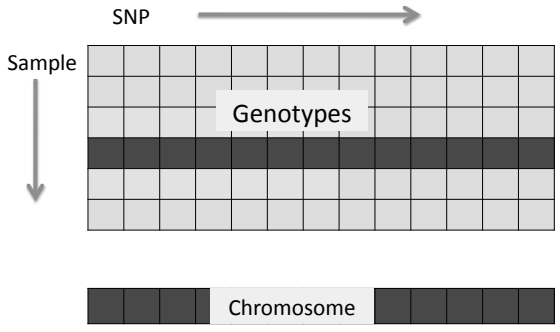
```
genofile<-create.ncdf("hapmap.nc", list(varChrm, varSNP, vargeno,
                 vartheta, varr))
```

The file is empty when it is created. Data can be written using `put.var.ncdf()`. Because the whole data set is too large to read, we might read raw data and save to netCDF for one person at a time;

```
for(i in 1:4000){
    temp.geno.data <-readRawData(i) ## somehow
    put.var.ncdf(genofile, "geno", temp.geno.data,
           start=c(1,i), count=c(-1,1))
}
```

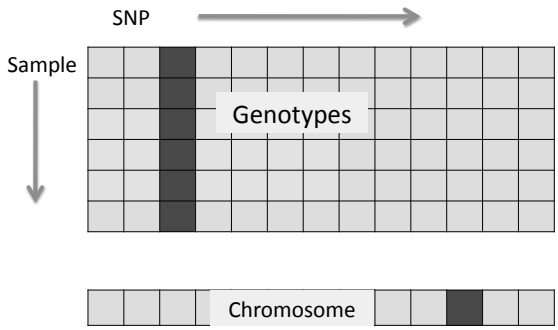

Efficient use of netCDF

Read all SNPs, one sample



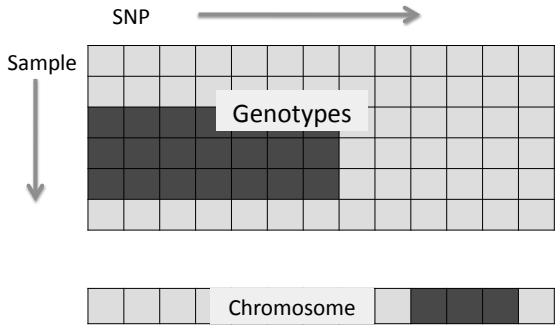
Efficient use of netCDF

Read all samples, one SNP



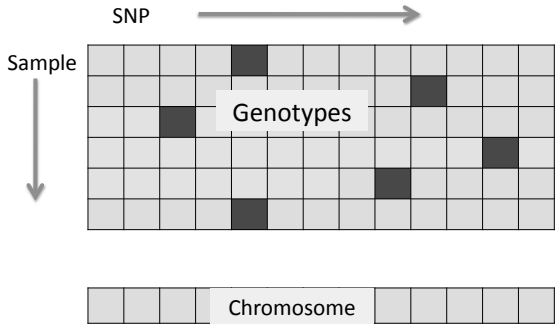
Efficient use of netCDF

Read some samples, some SNPs.



Efficient use of netCDF

Random access is not efficient: eg read probe intensities for all missing genotype calls.



Efficient use of netCDF

- Association testing: read all data for one SNP at a time
- Computing linkage disequilibrium near a SNP: read all data for a contiguous range of SNPs
- QC for aneuploidy: read all data for one individual at a time (and parents or offspring if relevant)
- Population structure and relatedness: read all SNPs for two individuals at a time.

8. Bioconductor Intro and Annotation

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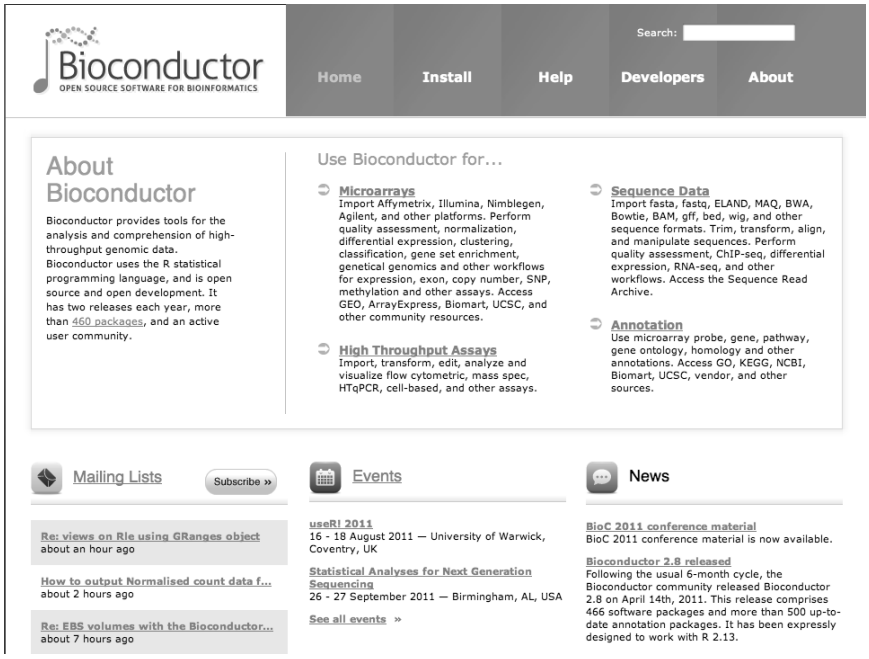
Universities of Washington and Auckland

Seattle, July 2014

What is Bioconductor?

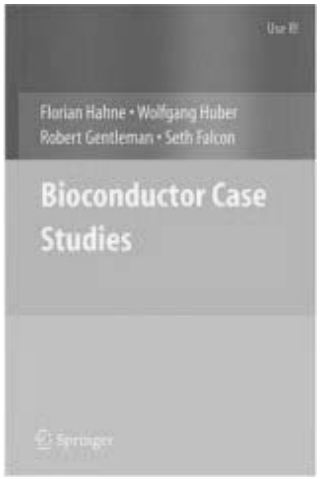


What is Bioconductor?

- `www.bioconductor.org`
- Software project for analysis of genomic data – and related tools, resources/datasets
- **Open source** and **Open development**
- **Free**

You could use commercial software; but experts typically write `R` code first. Also, the help manuals are not a sales pitch and encourage appropriate use.

Bioconductor basics

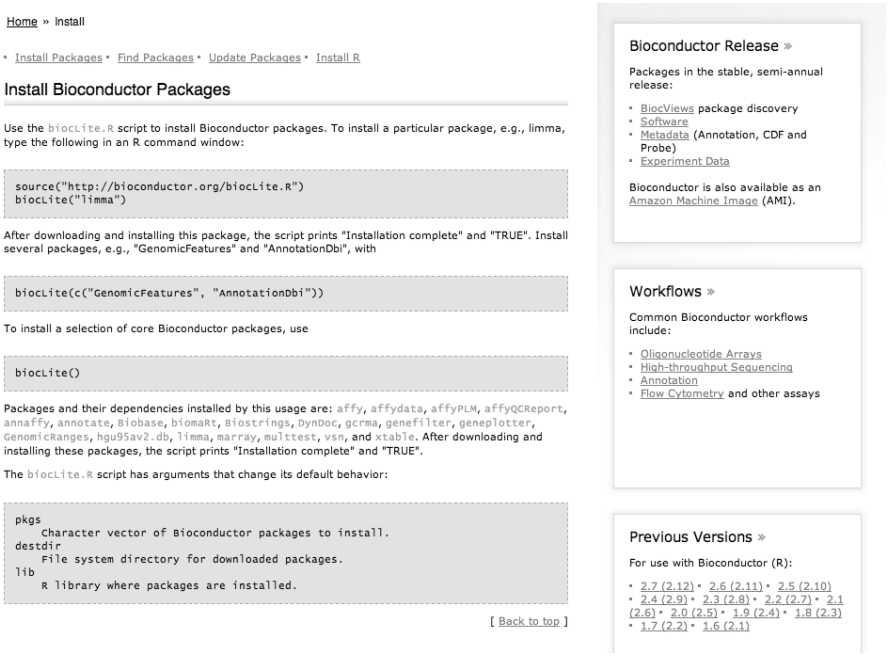


- Begun in 2001, based at Harvard and now FHCRC (Seattle)
- A large collection of `R` packages (they also convert good software to `R`)
- Far too much for our little course!

We'll give examples of what Bioconductor can do, and how to learn more. Hahne et al (above) is a helpful reference text

Bioconductor basics

Getting started...



Home » Install

• Install Packages • Find Packages • Update Packages • Install R

Install Bioconductor Packages

Use the biocLite.R script to install Bioconductor packages. To install a particular package, e.g., limma, type the following in an R command window:

```
source("http://bioconductor.org/biocLite.R")
biocLite("limma")
```

After downloading and installing this package, the script prints "Installation complete" and "TRUE". Install several packages, e.g., "GenomicFeatures" and "AnnotationDbi", with

```
biocLite(c("GenomicFeatures", "AnnotationDbi"))
```

To install a selection of core Bioconductor packages, use

```
biocLite()
```

Packages and their dependencies installed by this usage are: affy, affydata, affyPLM, affyQCReport, annaffy, annotate, Biobase, biomaRt, Biostrings, DynDoc, gcrma, genefilter, geneplotter, GenomicRanges, hgu95av2.db, limma, marray, multtest, vsn, and xtable. After downloading and installing these packages, the script prints "Installation complete" and "TRUE".

The biocLite.R script has arguments that change its default behavior:

```
pkgs
    Character vector of Bioconductor packages to install.
destdir
    File system directory for downloaded packages.
lib
    R library where packages are installed.
```

[Back to top]

Bioconductor Release »

Packages in the stable, semi-annual release:

- BiocViews package discovery
- Software
- Metadata (Annotation, CDF and Probe)
- Experiment Data

Bioconductor is also available as an Amazon Machine Image (AMI).

Workflows »

Common Bioconductor workflows include:

- Oligonucleotide Arrays
- High-throughput Sequencing
- Annotation
- Flow Cytometry and other assays

Previous Versions »

For use with Bioconductor (R):

• 2.7 (2.12) • 2.6 (2.11) • 2.5 (2.10) • 2.4 (2.9) • 2.3 (2.8) • 2.2 (2.7) • 2.1 (2.6) • 2.0 (2.5) • 1.9 (2.4) • 1.8 (2.3) • 1.7 (2.2) • 1.6 (2.1)

Bioconductor basics

```
> source("http://bioconductor.org/biocLite.R")
> biocLite()
```

installs the following general-purpose libraries;

`Biobase, IRanges, AnnotationDbi`

... then you use e.g. `library("Biobase")` as before. (NB older versions used to download much more than this)

`vignette(package="Biobase")` tells you to look at `vignette("esApply")` for a worked example – a very helpful introduction. (Or use e.g. `openVignette()`, which is in the `Biobase` package itself)

Bioconductor basics

To get other packages, use the `source()` command as before, then use e.g.

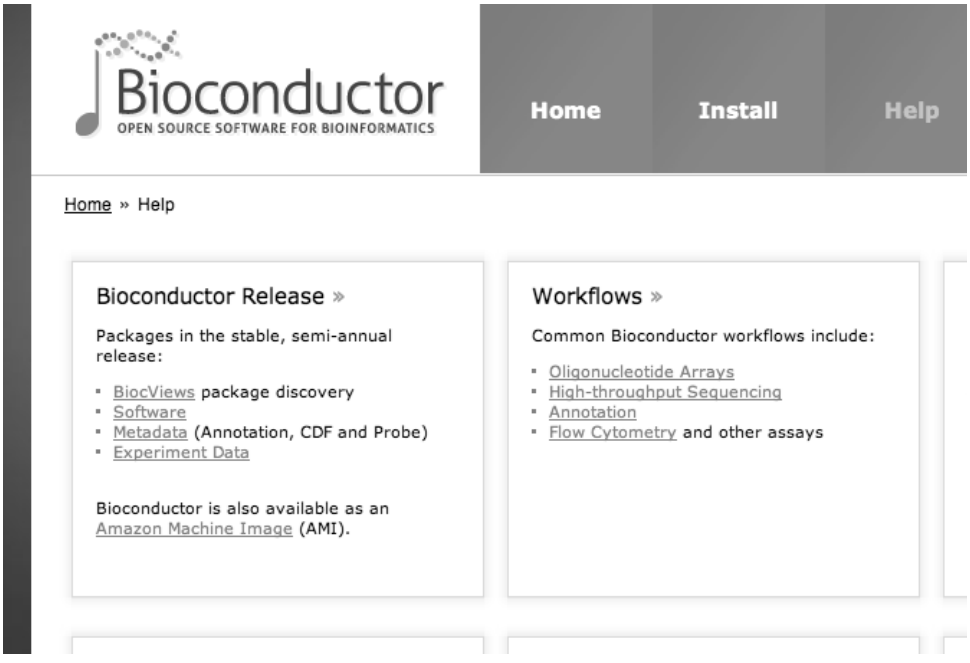
```
biocLite("SNPchip")
biocLite(c("limma", "siggenes"))
```

You do not need to type `biocLite()` again (even in a new R session). This would install the general-purpose packages again – which is harmless, but a waste of time.

Note; if, due to access privileges, you need to write to non-default directories, follow the onscreen commands and then start again. On Windows, 'Run as Administrator' may cut out this step.

What to install?

Back to the front page – click 'Help'



What to install?

- **Software** – probably what you want
- **Metadata** – e.g. annotation data, probe sequence data for microarrays of different types
- **Experiment data** – e.g. datasets from `hapmap.org`, some expression datasets

Simple QC graphics

The "splots" package plots values from 96 or 384-well plates, for QC purposes

First, install it

```
biocLite("splots")
```

Then load into R

```
library("splots")
```

There is a single function: `plotScreen()` for displaying the results

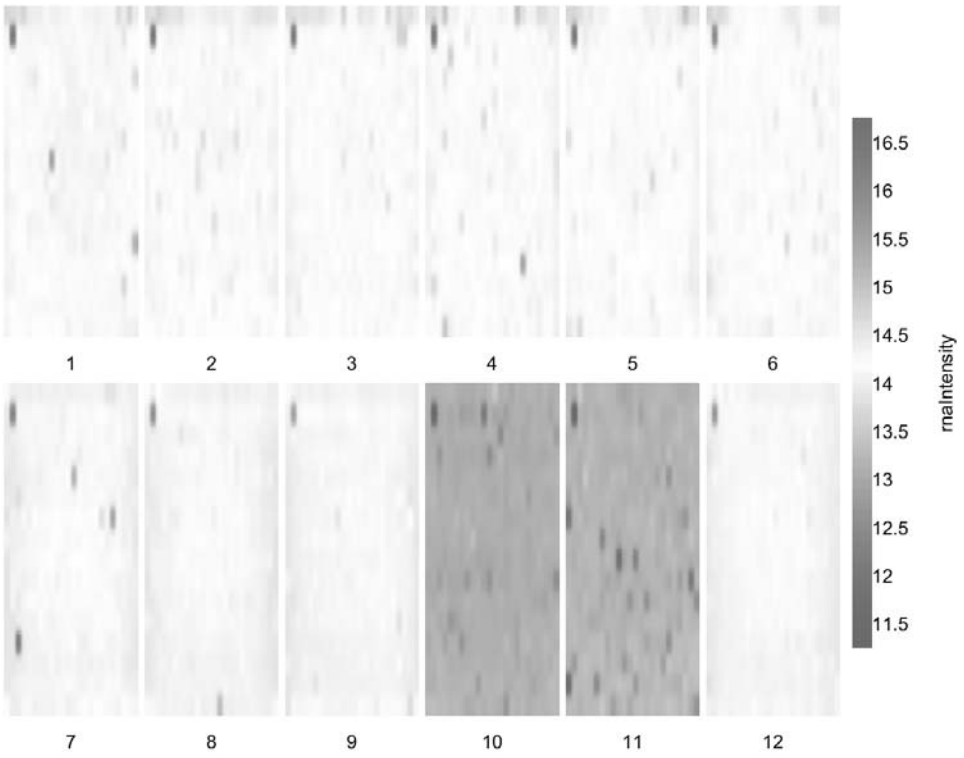
Example

The file "drosophila.rda" contains 12 of 114 plates from a RNAi gene-knockout study in fruit flies. Each spot represents a gene, and the intensity is low if knockout of that gene is lethal (data from the "RNAither" package)

```
load("drosophila.rda")
plotScreen(rnai)
```

The positive controls in the same position each plate are clear, and there are obvious plate effects that you might need to correct by normalization.

Example



Outline

One goal of Bioconductor is to provide efficient access inside R to the genome databases that are vital to interpreting associations.

We will look at a few of these

- annotate
- biomaRt
- genomeGraphs

The reason to have an R interface to these databases is to be able to analyze annotation data for many SNPs or RNA transcripts.

Online or stored data

Annotation data can be downloaded in a single file or retrieved for each query from an online database.

Local storage is faster, but may require too much space (eg Ensembl) or become obsolete too quickly.

Local storage is ideal for fixed annotation data such as gene names for a microarray or SNP chip.

Types of database

Translations of names: Affy probe 32972_at is the gene NADPH oxidase 1 with symbol NOX1 and Ensembl gene id ENSG00000007952

Location: NOX1 is on Xq22.1, from 99984969 to 100015990, coded on the negative strand. There are 120 known polymorphisms (SNPs or indels) in this range.

Homology: The mouse version of NOX1 is also on the X chromosome, starting at 130621066 (and called Nox1)

Structure and function: NOX1 is a membrane protein (location), involved in voltage-gated ion channel activity (molecular function), and involved in signal transduction (biological process).

Annotate

Bioconductor distributes annotation packages for a wide range of gene expression microarrays. The annotate package is one way to use this annotation information.

```
> library("annotate")
> library("hgu95av2.db")
> library("GO.db")
```

loads the annotate package and the databases for the Gene Ontology and one of the Affymetrix human microarray chips.

Lookups

The databases are queried with `get()` or `mget()` for multiple queries

```
> mget(c("738_at", "40840_at", "32972_at"),  envir=hgu95av2GENENAME)
$‘738_at‘
[1] "5’-nucleotidase, cytosolic II"

$‘40840_at‘
[1] "peptidylprolyl isomerase F (cyclophilin F)"

$‘32972_at‘
[1] "NADPH oxidase 1"

> go<-get("738_at", envir=hgu95av2GO)
> names(go)
[1] "GO:0009117" "GO:0005829" "GO:0005737" "GO:0000166" "GO:0000287"
[6] "GO:0008253" "GO:0008253" "GO:0016787"
```


Lookups

```
> get("GO:0009117",  envir=GOTERM)
GOID: GO:0009117
Term: nucleotide metabolic process
Ontology: BP
Definition: The chemical reactions and pathways involving a
   nucleotide, a nucleoside that is esterified with (ortho)phosphate
   or an oligophosphate at any hydroxyl group on the glycose
   moiety; may be mono-, di- or triphosphate; this definition
   includes cyclic nucleotides (nucleoside cyclic phosphates).
Synonym: nucleotide metabolism
```

What lookups are there?

```
> library(help="hgu95av2.db")

hgu95av2ALIAS2PROBE  Map between Common Gene Symbol Identifiers and
                     Manufacturer Identifiers

> get("NOX1", envir=hgu95av2ALIAS2PROBE)
[1] "32972_at"   "32973_s_at"
```

You can also reverse a lookup table with `revmap()`

```
> get("NOX1", envir=revmap(hgu95av2SYMBOL))
[1] "32972_at"   "32973_s_at"
> get("X",revmap(hgu95av2CHR))
 [1] "1016_s_at"  "107_at"     "1100_at"    "112_g_at"   "1155_at"
 .... and lots more
```


BioMart

BioMart (www.biomart.org)is a query-oriented data management system developed jointly by the European Bioinformatics Institute (EBI) and Cold Spring Harbor Laboratory (CSHL).

`biomaRt` is an R interface to BioMart systems, in particular to Ensembl (www.ensembl.org). Ensembl is a joint project between EMBL - European Bioinformatics Institute (EBI) and the Wellcome Trust Sanger Institute (WTSI) to develop a software system which produces and maintains automatic annotation on selected eukaryotic genomes.

BioMart

We begin by choosing which BioMart to use

```
> library(biomaRt)
Loading required package: RCurl
> listMarts()
                              biomart
1                            ensembl                       ENSEMBL GENES 63 (SANGER U
2                                snp                    ENSEMBL  VARIATION 63 (SANGER U
3              functional_genomics                    ENSEMBL REGULATION 63 (SANGER U
4                               vega                                VEGA 43  (SANGER U
5                  bacteria_mart_10                       ENSEMBL BACTERIA 10 (EBI U
6                     fungi_mart_10                          ENSEMBL FUNGI 10 (EBI U
7              fungi_variations_10                 ENSEMBL FUNGI VARIATION 10 (EBI U
8                  metazoa_mart_10                         ENSEMBL METAZOA 10 (EBI U
9            metazoa_variations_10               ENSEMBL METAZOA VARIATION 10 (EBI U
...
60               ENSEMBL_MART_PLANT          GRAMENE 30 ENSEMBL GENES (CSHL/CORNELL U
61           ENSEMBL_MART_PLANT_SNP              GRAMENE 30 VARIATION (CSHL/CORNELL U
62                GRAMENE_MARKER_30                GRAMENE 30 MARKERS (CSHL/CORNELL U
63                   GRAMENE_MAP_30                GRAMENE 30 MAPPINGS (CSHL/CORNELL U
64                         QTL_MART                  GRAMENE 32 QTL DB (CSHL/CORNELL U
65                 salmosalar2_mart             UNIGENE SALMO SALAR DATABASE (CMM CHIL
66                      trucha_mart    UNIGENE ONCORHYNCHUS MYKISS DATABASE (CMM CHIL
> ens <- useMart("ensembl")
```


BioMart

We then choose a database to use

```
> listDatasets(ens)
                           dataset                                     description
                           dataset                                   description
1            oanatinus_gene_ensembl      Ornithorhynchus anatinus genes (OANA5)
2             tguttata_gene_ensembl     Taeniopygia guttata genes (taeGut3.2.4)
3           cporcellus_gene_ensembl             Cavia porcellus genes (cavPor3)
4           gaculeatus_gene_ensembl      Gasterosteus aculeatus genes (BROADS1)
5            lafricana_gene_ensembl          Loxodonta africana genes (loxAfr3)
...
30           pvampyrus_gene_ensembl           Pteropus vampyrus genes (pteVam1)
...
58             btaurus_gene_ensembl                   Bos taurus genes (UMD3.1)
59            meugenii_gene_ensembl           Macropus eugenii genes (Meug_1.0)
60           sharrisii_gene_ensembl       Sarcophilus harrisii genes (DEVIL7.0)
61         cfamiliaris_gene_ensembl          Canis familiaris genes (CanFam3.1)
> hsap <- useDataset("hsapiens_gene_ensembl",mart=ens)
```

BioMart

The `getGene` function queries the database for gene information. It accepts many forms of gene identifier, eg Entrez, HUGO, Affy transcript

```
> getGene(id=1440, type="entrezgene", mart=hsap)
  entrezgene hgnc_symbol
1       1440        CSF3
                                                              description
1 colony stimulating factor 3 (granulocyte) [Source:HGNC Symbol;Acc:2438]
  chromosome_name  band strand start_position end_position ensembl_gene_id
1              17 q21.1      1       38171614     38174066 ENSG00000108342

>  getGene(id=c("AGT","AGTR1"), type="hgnc_symbol", mart=hsap)
  hgnc_symbol hgnc_symbol
1         AGT         AGT
2       AGTR1       AGTR1

1 angiotensinogen (serpin peptidase inhibitor, clade A, member 8) [Source:HGNC Sym
2                                angiotensin II receptor, type 1 [Source:HGNC Sym
  chromosome_name  band strand start_position end_position ensembl_gene_id
1               1 q42.2     -1      230838269    230850043 ENSG00000135744
2               3   q24      1      148415571    148460795 ENSG00000144891
```


BioMart

`getBM` is more general than `getGene`. It specifies a list of **filters** for selecting genes or SNPs and **attributes** to return from the database.

```
> affyids <- c("202763_at", "209310_s_at", "207500_at")
>  getBM(attributes = c("affy_hg_u133_plus_2", "hgnc_symbol", "chromosome_name",
    "start_position", "end_position", "band"), filters = "affy_hg_u133_plus_2",
     values = affyids, mart = hsap)
     affy_hg_u133        hgnc   chromosome_name start_position end_position  band
1           202763_at       CASP3          4      185785844    185807623 q35.1
2           207500_at       CASP5         11      104370180    104384957 q22.3
3         209310_s_at       CASP4         11      104318804    104344535 q22.3
```

`listAttributes(hsap)` and `listFilters(hsap)` list the avilable attributes and filters (hundreds)

BioMart

```
> getBM(mart=hsap, attributes=c("band","hgnc_symbol"),
         filters=c("band_start","band_end","chromosome_name"),
         values=list("p21.33","p21.33",6))
      band hgnc_symbol
1   p21.33
2   p21.33    SNORD117
3   p21.33     SNORA38
4   p21.33     SNORD48
5   p21.33     SNORD52
6   p21.33      MIR877
7   p21.33     MIR1236
8   p21.33      GTF2H4
9   p21.33       VARS2
10  p21.33       SFTA2
11  p21.33       DPCR1
12  p21.33       MUC21
...
121 p21.33      HSPA1A
122 p21.33        TNXB
123 p21.33       STK19
124 p21.33         C4A
125 p21.33         C4B
```


Homology

`getLDS()` combines two data marts, for example to homologous genes in other species. We can look up the mouse equivalents of a particular Affy transcript, or of the NOX1 gene.

```
> human = useMart("ensembl", dataset = "hsapiens_gene_ensembl")
> mouse = useMart("ensembl", dataset = "mmusculus_gene_ensembl")
> getLDS(attributes = c("hgnc_symbol","chromosome_name", "start_position"),
+ filters = "hgnc_symbol", values = "NOX1", mart = human,
+ attributesL = c("chromosome_name","start_position","external_gene_id"),
+ martL = mouse)
    V1 V2        V3 V4        V5   V6
1 NOX1  X 100098313  X 134086421 Nox1
```

The mouse gene name is the same as the human one apart from capitalisation.

Homology

The `getSequence` function looks up DNA or protein sequences by chromosome position or gene identifiers

```
> agt<-getSequence(id="AGT",type="hgnc_symbol", seqType="peptide",mart=hsap)
> agt

1 MRKRAPQSEMAPAGVSLRATILCLLAWAGLAAGDRVYIHPFHLVIHNESTCEQLAKANAGKPKDPTFIPAPIQAKTS
PVDEKALQDQLVLVAAKLDTEDKLRAAMVGMLANFLGFRIYGMHSELWGVVHGATVLSPTAVFGTLASLYLGALDHTAD
RLQAILGVPWKDKNCTSRLDAHKVLSALQAVQGLLVAQGRADSQAQLLLSTVVGVFTAPGLHLKQPFVQGLALYTPVVL
PRSLDFTELDVAAEKIDRFMQAVTGWKTGCSLMGASVDSTLAFNTYVHFQGKMKGFSLLAEPQEFWVDNSTSVSVPMLS
GMGTFQHWSDIQDNFSVTQVPFTESACLLLIQPHYASDLDKVEGLTFQQNSLNWMKKLSPRTIHLTMPQLVLQGSYDLQ
DLLAQAELPAILHTELNLQKLSNDRIRVGEVLNSIFFELEADEREPTESTQQLNKPEVLEVTLNRPFLFAVYDQSATAL
HFLGRVANPLSTA*
```


Example: finding chromosomes

We had a set 1524 SNPs, of which 409 did not have their chromosome listed.

I needed to know which SNPs were on the X chromosome, to estimate sex from DNA intensity and heterozygous X-chromosome loci, for QC.

```
> head(unknown)
[1] "UGT1A3-001449-0_B_R_1538822" "LIPC-002761-0_B_R_1538453"
[3] "CETP-001265-0_B_R_1538254"   "F8-165293-0_T_F_1538626"
[5] "CPB2-051208-0_B_F_1539402"   "VDRDIL-1355-0_T_F_1539404"
```

A hand-search would be easy but tedious, so we want an automated approach

Example: finding chromosomes

First extract the gene names

```
genes <- sapply(unknown, function(snp) strsplit(snp,"-")[[1]][1])
ugenes <- unique(genes)
```

Now call out to Ensembl

```
getBM(attributes="chromosome_name", filters="hgnc_symbol",values=ugenes,
  mart=hsap)
```

works for all except VRDIL, which isn't recognized.

Finding SNPs

Human SNPs (and short indels) are in a separate database from gene information. We can look up known SNPs and other polymorphisms for the NOX1 gene

```
> snpmart = useMart("snp", dataset = "hsapiens_snp")
Checking attributes ... ok
Checking filters ... ok
> getBM(c("refsnp_id", "allele", "chrom_start", "chrom_strand"),
       filters = c("chr_name", "chrom_start","chrom_end"),
       values = list("X",99984969,100015990), mart = snpmart)
         refsnp_id             allele chrom_start chrom_strand
1     rs7054049              T/A    99985184            1
2    rs60975472              G/T    99985304            1
3    rs58902780              A/G    99985571            1
4   rs182188185              G/A    99985618            1
5   rs186748080              A/G    99985798            1
```

More metadata

The `citation()` function prints out information about how to cite a package

```
> citation("biomaRt")

To cite the biomaRt package in publications use:

  Mapping identifiers for the integration of genomic datasets with the
  R/Bioconductor package biomaRt. Steffen Durinck, Paul T. Spellman,
  Ewan Birney and Wolfgang Huber, Nature Protocols 4, 1184-1191 (2009).

  BioMart and Bioconductor: a powerful link between biological
  databases and microarray data analysis. Steffen Durinck, Yves Moreau,
  Arek Kasprzyk, Sean Davis, Bart De Moor, Alvis Brazma and Wolfgang
  Huber, Bioinformatics 21, 3439-3440 (2005).
```

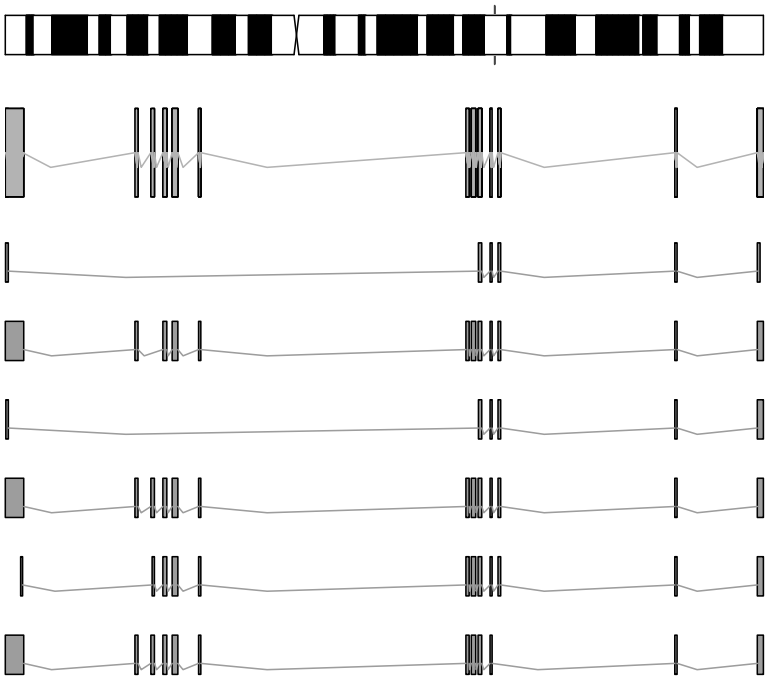
Citations are one way academic software authors can prove to funders and promotion committees that software is worthwhile.

GenomeGraphs

This package makes pretty pictures from the annotation data.

For example, a pictures showing the standard and alternative splices for the NOX1 gene and the location of the gene on the X chromosome

```
> library(GenomeGraphs)
> gene <- makeGene(id = "NOX1", type = "hgnc_symbol",
     biomart = hsap)
> transcript<-makeTranscript(id="NOX1",type="hgnc_symbol",
     biomart=hsap)
> ideogram <- makeIdeogram(chromosome ="X")
> gdPlot(list(ideogram,gene,transcript))
```



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9. More Bioconductor packages

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Seattle, July 2014

GWAS analysis

Genome-Wide Association Studies (GWAS) are currently popular – typically, these genotype e.g. 1M SNPs on several thousand subjects in (large) established studies

- Usually on 1000's of subjects
- 'Simple' t-tests, regressions, for each SNP (like microarrays)
- 1M *anything* takes a long time! (up to 72 hours)
- Just **loading** big datasets is non-trivial – but some tools are available

GWAS analysis

`snpStats` is a Bioconductor package for GWAS analysis – maintained by David Clayton (analysis lead on Wellcome Trust)

```
> biocLite("snpStats") #in a new session
> library(snpStats)
> data(for.exercise)
> ls()
[1] "snp.support" "snps.10" "subject.support"
```

A 'little' case-control dataset (Chr 10) based on HapMap – three objects; `snp.support`, `subject.support` and `snps.10`

GWAS analysis

```
> summary(snp.support)
   chromosome    position         A1        A2
 Min.   :10   Min.   :   101955   A:14019   C: 2349
 1st Qu.:10   1st Qu.: 28981867   C:12166   G:12254
 Median :10   Median : 67409719   G: 2316   T:13898
 Mean   :10   Mean   : 66874497
 3rd Qu.:10   3rd Qu.:101966491
 Max.   :10   Max.   :135323432
> summary(subject.support)
       cc         stratum
 Min.   :0.0   CEU    :494
 1st Qu.:0.0   JPT+CHB:506
 Median :0.5
 Mean   :0.5
 3rd Qu.:1.0
 Max.   :1.0
```


GWAS analysis

```
> show(snps.10) # show() is generic
A SnpMatrix with  1000 rows and  28501 columns
Row names:  jpt.869 ... ceu.464
Col names:  rs7909677 ... rs12218790
> summary(snps.10)
$rows
   Call.rate      Heterozygosity
 Min.   :0.9879   Min.   :0.0000
 Median :0.9900   Median :0.3078
 Mean   :0.9900   Mean   :0.3074
 Max.   :0.9919   Max.   :0.3386
$cols
     Calls        Call.rate          MAF              P.AA
 Min.   : 975   Min.   :0.975   Min.   :0.0000   Min.   :0.00000
 Median : 990   Median :0.990   Median :0.2315   Median :0.26876
 Mean   : 990   Mean   :0.990   Mean   :0.2424   Mean   :0.34617
 Max.   :1000   Max.   :1.000   Max.   :0.5000   Max.   :1.00000
      P.AB             P.BB             z.HWE
 Min.   :0.0000   Min.   :0.00000   Min.   :-21.9725
 Median :0.3198   Median :0.27492   Median : -1.1910
 Mean   :0.3074   Mean   :0.34647   Mean   : -1.8610
 Max.   :0.5504   Max.   :1.00000   Max.   :  3.7085
                                    NA's   :  4.0000
```

GWAS analysis

- 28501 SNPs, all with Allele 1, Allele 2
- 1000 subjects, 500 controls (`cc=0`) and 500 cases (`cc=1`)
- **Far too much** data for a regular `summary()` of `snps.10` – even in this small example

GWAS analysis

We'll use just the column summaries, and a (mildly) 'clean' subset;

```
> snpsum <- col.summary(snps.10)
> use <- with(snpsum, MAF > 0.01 & z.HWE^2 < 200)

> table(use)
use
FALSE  TRUE
  317 28184
```

GWAS analysis

Now do single-SNP tests for each SNP, and extract the p-value for each SNP, along with its location;

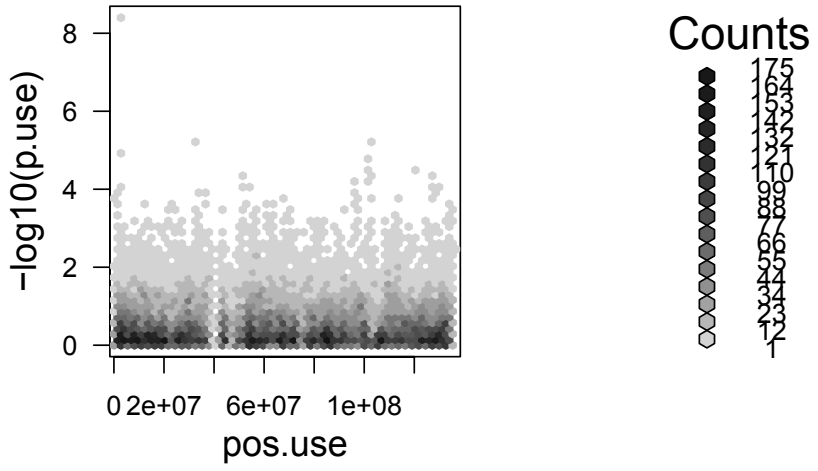
```
tests <- single.snp.tests(cc, data = subject.support,
+ snp.data = snps.10)

pos.use <- snp.support$position[use]
p.use   <- p.value(tests, df=1)[use]
```

We'd usually give a table of 'top hits,' but...

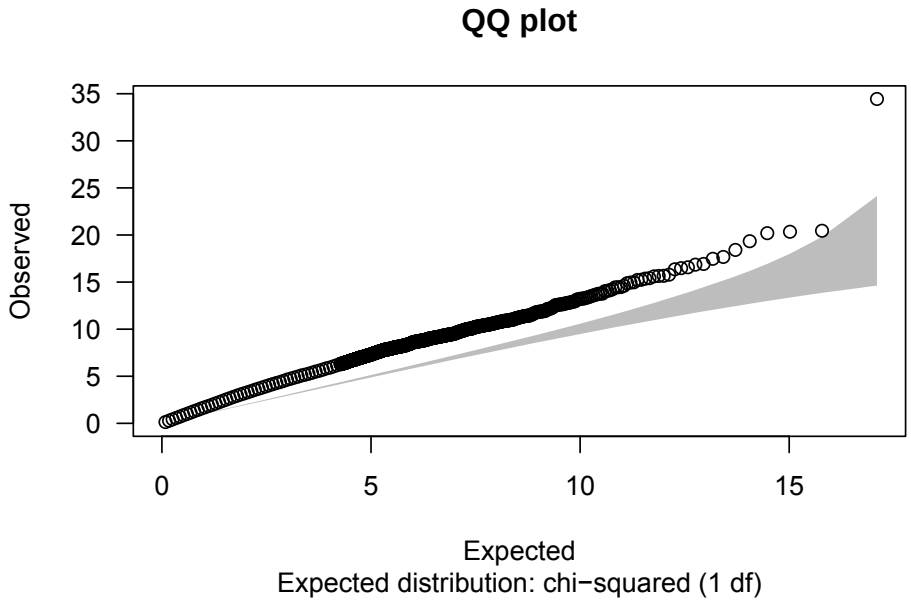
GWAS analysis

```
plot(hexbin(pos.use, -log10(p.use), xbin = 50))
```



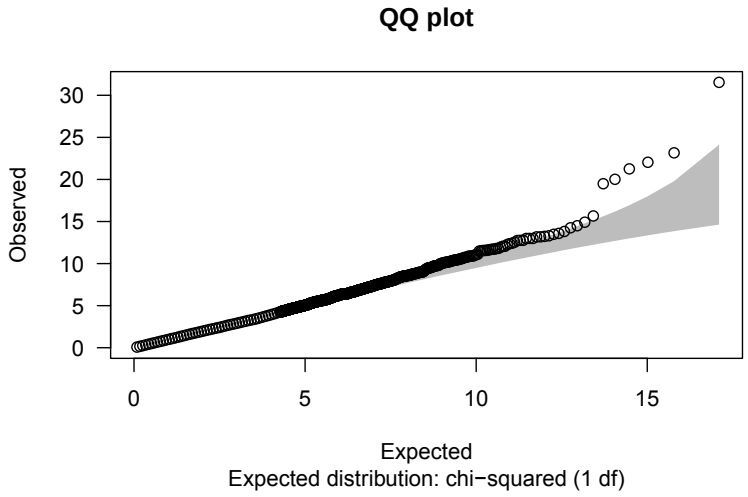
GWAS analysis

```
qq.chisq(chi.squared(tests, df=1)[use], df=1)
```



GWAS analysis

```
tests2 <- single.snp.tests(cc, stratum, data = subject.support,
+ snp.data = snps.10)
qq.chisq(chi.squared(tests2, 1)[use], 1)
```



Handling short-read sequences

The `ShortRead` package handles short-read (aka 'next-generation') sequencing reads, mostly for QC and preprocessing.

It comes with a (small) subset of a Solexa sequencing run: we will look at this example.

The data are in a structured set of folders, so the first step is to specify where they can be found.

```
exptPath <- "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata"
```

and then use the `SolexaPath()` function to create a simple summary of the structure, which we will pass to other functions instead of a filename

Handling short-read sequences

```
> sp <- SolexaPath(exptPath)
> sp
class: SolexaPath
experimentPath: /Users/tlumley/Library/R/2.15/library/ShortRead/extdata
dataPath: Data
scanPath: NA
imageAnalysisPath: C1-36Firecrest
baseCallPath: Bustard
analysisPath: GERALD
> imageAnalysisPath(sp)
[1] "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata/
      Data/C1-36Firecrest"
> analysisPath(sp)
[1] "/Users/tlumley/Library/R/2.15/library/ShortRead/extdata/
     Data/ C1-36Firecrest/Bustard/GERALD"
```

Reading data

The function `readAligned()` reads in the aligned sequence fragments,

```
> aln <- readAligned(sp, "s_2_export.txt")
> aln
class: AlignedRead
length: 1000 reads; width: 35 cycles
chromosome: NM NM ... chr5.fa 29:255:255
position: NA NA ... 71805980 NA
strand: NA NA ... + NA
alignQuality: NumericQuality
alignData varLabels: run lane ... filtering contig
```


Examining data

The `sread()` function returns the bases read

```
> sread(aln)
  A DNAStringSet instance of length 1000
      width seq
   [1]    35 CCAGAGCCCCCCGCTCACTCCTGAACCAGTCTCTC
   [2]    35 AGCCTCCCTCTTTCTGAATATACGGCAGAGCTGTT
   [3]    35 ACCAAAAACACCACATACACGAGCAACACACGTAC
   [4]    35 AATCGGAAGAGCTCGTATGCCGGCTTCTGCTTGGA
   [5]    35 AAAGATAAACTCTAGGCCACCTCCTCCTTCTTCTA
...
```

and the `quality()` function gives the read quality, coded with Z as the best

Examining data

```
> quality(aln)
class: SFastqQuality
quality:
  A BStringSet instance of length 1000
      width seq
   [1]    35 YQMIMIMMLMMIGIGMFICMFFFIMMHIIHAAGAH
   [2]    35 ZXZUYXZQYYXUZXYZYYZZXXZZIMFHXQSUPPO
   [3]    35 LGDHLILLLLLLLIGFLLALDIFDILLHFIAECAE
   [4]    35 JJYYIYVSYYYYYYYYSDYYWVUYYNNVSVQQELQ
   [5]    35 LLLILIIIDLLHLLLLLLLLLLALLLLHLLLLEL
   [6]    35 YYYYYYYWVVMGGUHQHQMUFMICDMCDHQHEDDD
   [7]    35 ZZZZZZZZZZYZZZZZYZZZZYYZZZZZZZUUUUU
   [8]    35 ZZZZZZZZUZZUZZZZZZZZZZZZYZYZZZUUHUH
   [9]    35 ZZZZZZZYZZYZZZZYZZZZZZZZZZZZXUNUUU
```

Note how the quality goes down later in the read.

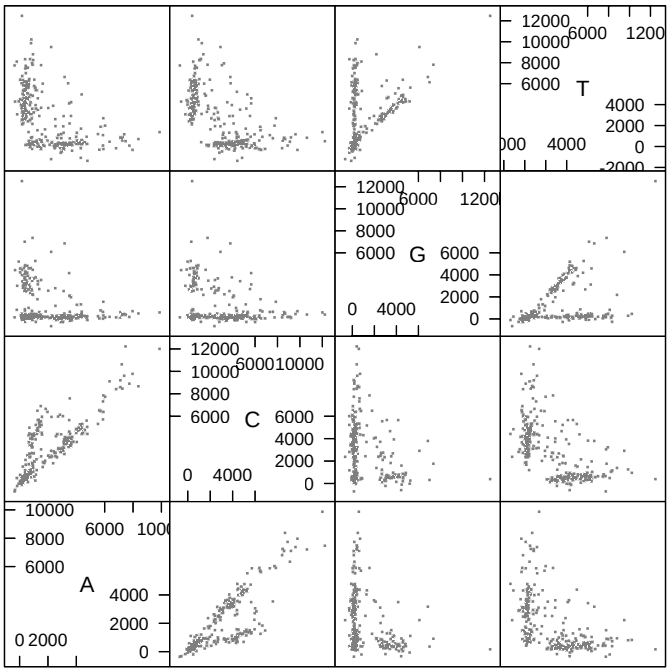
Examining data

We can read other information, such as the fluorescence intensities for each base

```
> int <- readIntensities(sp)
> int
class: SolexaIntensity
dim: 256 4 36
readInfo: SolexaIntensityInfo
intensity: ArrayIntensity
measurementError: ArrayIntensity
> splom(intensity(int)[[,,2]], pch=".", cex=3)
> splom(intensity(int)[[,,35]],pch=".",cex=3)
```

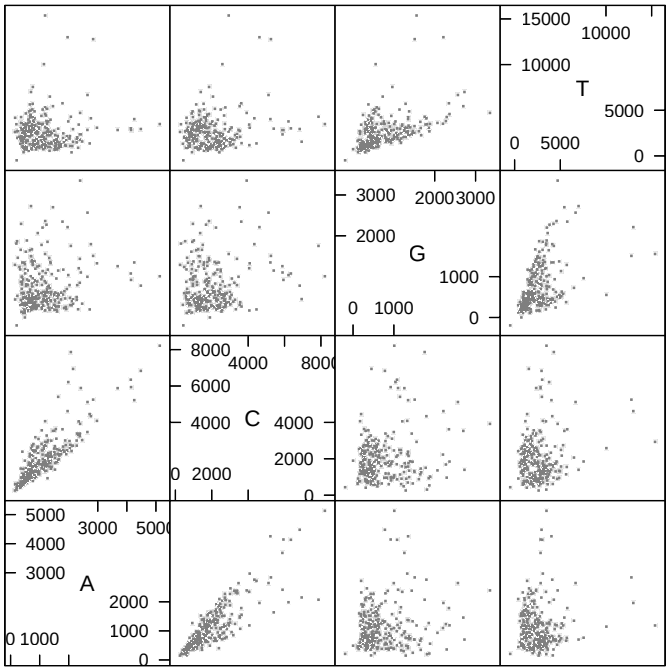
and then create a scatterplot matrix of the intensities for the 2nd and 35th bases. The plots show the correlation between A-T and C-G channels, and the deterioration towards the end of the read.

Intensities: 2nd position



Scatter Plot Matrix

Intensities: 35th position



Scatter Plot Matrix

DIfferential expression by RNAseq

The `edgeR` package (among others) estimates differential RNA expression from RNAseq experiments. It's a sequel to the `limma` package for microarray gene expression data.

RNAseq produces a count for each transcript, rather than the continuous measure produced by microarray experiments, and the statistical analysis relies on models for variation in counts.

We will look at an RNAseq experiment comparing gene expression in prostate cancer cells with and without treatment with an androgen (a testosterone analogue).

The experiment had three treated samples and four control samples, sequenced in seven of the lanes of a single flow cell on an Illumina 1G sequencer.

The data have been mapped to the human genome and turned into counts of transcripts in a simple text file

RNAseq of prostate cancer

```
> x <- read.delim("pnas_expression.txt", row.names=1,
       stringsAsFactors=FALSE)
> head(x)
                lane1 lane2 lane3 lane4 lane5 lane6 lane8  len
ENSG00000215696     0     0     0     0     0     0     0  330
ENSG00000215700     0     0     0     0     0     0     0 2370
ENSG00000215699     0     0     0     0     0     0     0 1842
ENSG00000215784     0     0     0     0     0     0     0 2393
ENSG00000212914     0     0     0     0     0     0     0  384
ENSG00000212042     0     0     0     0     0     0     0   92
```

The `stringsAsFactors` argument is needed because we want to keep the transcript names as strings, not turn them into factors.

RNAseq of prostate cancer

We also need to specify which 'lanes' are treated and which are control.

```
> targets
     Lane Treatment Label
Con1    1   Control  Con1
Con2    2   Control  Con2
Con3    2   Control  Con3
Con4    4   Control  Con4
DHT1    5       DHT  DHT1
DHT2    6       DHT  DHT2
DHT3    8       DHT  DHT3
```


RNAseq of prostate cancer

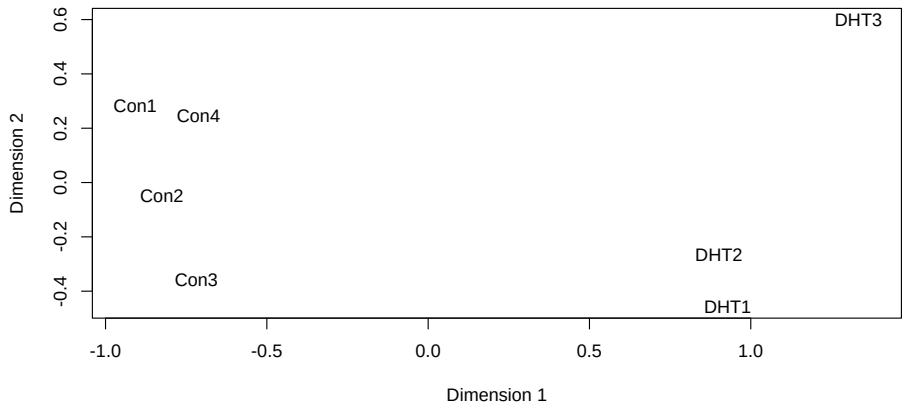
Putting the two together and filtering out low-expression transcripts gives data for analysis

```
y <- DGEList(counts=x[,1:7], group=targets$Treatment, genes=data.fra
colnames(y) <- targets$Label
keep <- rowSums(cpm(y)>1) >= 3
y <- y[keep,]
y$samples$lib.size <- colSums(y$counts)
```

Here, `cpm()` means counts per million

Overall differences?

`plotMDS(y)` does multidimensional scaling, projecting the data into two dimensions to see how well separated the samples are. In this case there are big treated/control differences and smaller between-replicate differences.



Differential expression (at last)

The statistical analysis has two steps. First, the variability is estimated, then the treatment and control groups are compared for each gene, using a test related to Fisher's exact test.

```
y <- estimateCommonDisp(y, verbose=TRUE)
y <- estimateTagwiseDisp(y)

et <- exactTest(y)
top <- topTags(et)
```

Differential expression (at last)

The output gives the gene name the log fold-change, the overall abundance, the p-value and FDR.

```
Comparison of groups:  DHT-Control
                Length    logFC    logCPM        PValue           FDR
ENSG00000151503   5605 5.704455  9.651026  0.000000e+00  0.000000e+00
ENSG00000096060   4093 4.881110  9.886097 1.065531e-315 8.787436e-312
ENSG00000166451   1556 4.539306  8.781549 1.168385e-217 6.423783e-214
ENSG00000127954   3919 8.054706  7.152971 3.838688e-200 1.582883e-196
ENSG00000162772   1377 3.204621  9.689874 8.819449e-170 2.909360e-166
ENSG00000113594  10078 3.960785  7.986306 1.108187e-144 3.046406e-141
ENSG00000116133   4286 3.143633  8.740383 1.291088e-138 3.042172e-135
ENSG00000115648   2920 2.513583 11.429414 6.775660e-130 1.396972e-126
ENSG00000123983   4305 3.473311  8.534717 9.250437e-129 1.695297e-125
ENSG00000116285   3076 4.104133  7.306976 1.758623e-128 2.900673e-125
```


Differential expression (at last)

We could use `biomaRt` or one of the other annotation packages to convert to other gene names

```
> getBM(attributes="hgnc_symbol",filters="ensembl_gene_id",
    values=rownames(top$table),mart=human)
   hgnc_symbol
1        FKBP5
2         LIFR
3         MLPH
4       DHCR24
5       ERRFI1
6        ACSL3
7       STEAP4
8       NCAPD3
9         ATF3
10       CENPN
```

10. Interfacing R

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Seattle, July 2014

Interfacing R

With Bioconductor, `R` can do a **huge** proportion of the analyses you'll want – but not everything

- Intensive (or anachronistic) `C++`, `FORTRAN` work, e.g. for pedigrees
- 'Speciality' analyses; some need different computing architecture
- Fancy interactive graphics

`R` can be used to 'manage' other software. Today we'll illustrate some favorite examples

Starting other software

NB these commands are for Windows only; see help files for e.g. Unix versions

- `shell()` does the equivalent of a DOS-style command
- `shell("notepad")` starts the Notepad editor
- **If** the command takes arguments, put them in the same string;
  `shell("notepad myfile.txt")`

The `system()` and `shell.exec()` commands do much the same thing.

Starting other software

Some more options for `shell()`;

- `wait`; `R` 'hangs' until completion
- `translate`; makes forward and backslashes work properly
- `intern`; return the output as an R object

For other options see the `system()` help page, for example `minimized=TRUE`.

Paths for files can be a little messy; `shell()` starts in your working directory (find it using `getwd()`). For files outside of this, give the full pathway.

`paste()` is useful, if you need to do a lot of this sort of thing.

Examples

Code for a really mundane job;

```
for(i in 1:100){
     infile <- paste("gene",i,"data.txt", sep="")
     outfile <-  paste("gene",i,"phase.out", sep="")
     shell(paste("PHASE",infile,outfile))
}
```

... this will churn away for hours, although with no error-control.

Why did we use `wait=TRUE` here? (the default)

Examples

- WinBUGS implements Bayesian analyse; it's not super-fast but is very flexible
- It needs special (& clever) architecture to achieve this
- WinBUGS' input, output, graphics are all rather clunky
- `R` is better; so `R2WinBUGS` calls WinBUGS for the difficult bits, and does all the 'translation' itself
- This is done with (repeated) use of `system()`

Outline

Many programs already exist to do useful analyses. It is more convenient to call them from R than to rewrite them in R.

Sometimes this involves calling the C code directly, sometimes just involves using R to write input files for another program

Examples:

- Graphviz: drawing networks
- PMF: input files for ancient Fortran software
- Google Earth: displaying outliers in context.

Drawing networks

GraphViz (http://www.graphviz.org) is a free program for drawing networks, written by AT&T researchers.

Its input format looks like

```
"15" [shape= box,regular=1 ,height= 0.5 ,width= 0.75 ,style=filled,color= grey ] ;
"16" [shape= circle ,height= 0.5 ,width= 0.75 ,style=filled,color= grey ] ;
"2x3" [shape=diamond,style=filled,label="",height=.1,width=.1] ;
"2"  ->  "2x3"  [dir=none,weight=1]  ;
"3"  ->  "2x3"  [dir=none,weight=1]  ;
"2x3"  ->  "1" [dir=none,weight=2]  ;
"2x3"  ->  "4" [dir=none,weight=2]  ;
"2x3"  ->  "5" [dir=none,weight=2]  ;
"2x3"  ->  "6" [dir=none,weight=2]  ;
```

The `sem` package uses GraphViz to display path diagrams for structural equation models and the `gap` package uses it to draw pedigrees.

Drawing networks

In gap the `pedtodot()` function writes a GraphViz input file from a pedigree in GAS or LINKAGE format.

```
     pid id fid mid sex aff GABRB1 D4S1645
1  10081  1   2   3   2   2    7/7    7/10
2  10081  2   0   0   1   1    -/-     -/-
3  10081  3   0   0   2   2    7/9    3/10
4  10081  4   2   3   2   2    7/9     3/7
5  10081  5   2   3   2   1    7/7    7/10
6  10081  6   2   3   1   1    7/7    7/10
7  10081  7   2   3   2   1    7/7    7/10
8  10081  8   0   0   1   1    -/-     -/-
9  10081  9   8   4   1   1    7/9    3/10
10 10081 10   0   0   2   1    -/-     -/-
11 10081 11   2  10   2   1    7/7     7/7
12 10081 12   2  10   2   2    6/7     7/7
13 10081 13   0   0   1   1    -/-     -/-
14 10081 14  13  11   1   1    7/8     7/8
15 10081 15   0   0   1   1    -/-     -/-
16 10081 16  15  12   2   1    6/6     7/7
```


Drawing networks

First the code prints nodes for each individual, with sex and affectedness information

```
for (s in 1:n) cat(paste("\"", id.j[s], "\" [shape=",
    sep = ""), shape.j[s], ",height=", height, ",width=",
    width, ",style=filled,color=", shade.j[s], "] ;\n")
```

giving output like

```
"16" [shape= circle ,height= 0.5 ,width= 0.75 ,style=filled,color= grey ] ;
```

It then works out all the matings and creates small nodes for each mating and lines connecting the parents to these nodes

```
mating <- paste("\"", s1, "x", s2, "\"", sep = "")
cat(mating, "[shape=diamond,style=filled,label=\"\",height=.1,width=.1] ;\n")
cat(paste("\"", s1, "\"", sep = ""), " -> ", mating,
    paste(" [dir=", dir, ",weight=1]", sep = ""),
     " ;\n")
cat(paste("\"", s2, "\"", sep = ""), " -> ", mating,
    paste(" [dir=", dir, ",weight=1]", sep = ""),
    " ;\n")
```

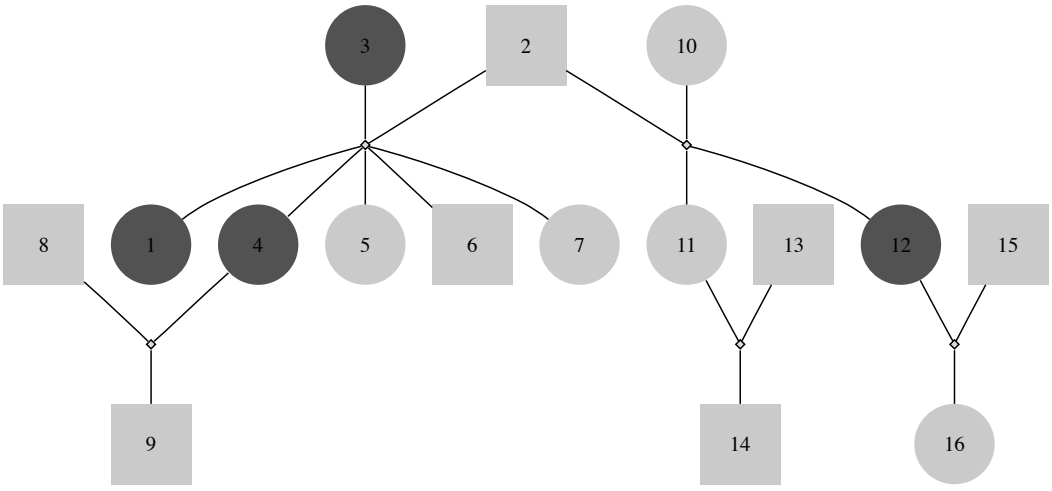
Drawing networks

giving output like

```
"2x3" [shape=diamond,style=filled,label="",height=.1,width=.1] ;
"2"  ->  "2x3"  [dir=none,weight=1]  ;
"3"  ->  "2x3"  [dir=none,weight=1]  ;
```

and then connects children to parents.

Drawing networks



pedigree 10081

[Bioconductor also has GraphViz more integrated with R in the `RGraphViz` package]

Chromosome simulation

MaCS, the Markov Coalescent Simulation (Chen et al, 2008) simulates realistic genotypes using an approximation to the coalescent.

It's a command-line program written in C++, with output:

```
/Users/tlumley/macs/macs 2000 15000 -t .001 -r .001 .001
1390319964

//
segsites: 134
positions: 0.000421536259 0.0100671644 0.0111485623 0.0230004096 0.0332452159...
10000000000000000000000000000010010001000100000000000000000001010000...
00000000000000000000000000000000000000000000000000000000000000000000...
00000000000000000000000000000000000000000000000000000000000000000000...
```

We want the same sort of simulation functions as in earlier sessions, so we need an R function that calls `MaCS` and returns a data matrix.

Chromosome simulation

Tasks

- Call MaCS
- Read in the lines of haploid genotypes as character strings
- Split into numbers
- Recode so 1 is the minor allele
- Combine pairs of haploids into a diploid

Chromosome simulation

```
makemacsdata<-function(N,length=15000,filter=0.05){
  f<-tempfile()
  system(paste("~/macs/macs",2*N,length,
      " -t .001 -r .001 2>/dev/null | ~/macs/msformatter >", f))
  input<-readLines(f)[-(1:6)]
  unlink(f)
  haplo<-do.call(rbind,lapply(strsplit(input,""),as.integer))
  diplo<-haplo[1:N,]+haplo[(N+1):(2*N),]
  af<-colMeans(diplo)/2
  diplo[,af>0.5]<- 2-diplo[,af>0.5,drop=FALSE]
  maf<-colMeans(diplo)/2
  diplo[,af<=filter,drop=FALSE]
}
```


Chromosome simulation

From the user's viewpoint it looks as though everything was done in R.

```
> d<-makemacsdata(1000)
> str(d)
 num [1:1000, 1:80] 0 0 0 0 0 0 0 0 0 0 ...
> summary(colMeans(d)/2) #maf
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
0.00050 0.00100 0.00450 0.01142 0.01512 0.05000
```

Chromosome simulation

Now simulate a new version of the SKaT rare-variant test

```
one.sim<-function(thresholds=c(Inf,1/2,1/3,1/4),
          sqrtweights=wuweights,
          N=4000, n=200, length=15000, filter=0.02)
{
  G <- makemacsdata(N,length,filter=filter)
  y <- sample(rep(0:1,c(N-n,n)))
  sapply(thresholds,
      function(c) winskat(G,y,threshold=c,sqrtweights))
}
```


SVG+tooltips

SVG (Scalable Vector Graphics) is a non-bitmap graphics format for the web.

The RSvgDevice and RSVGTipsDevice packages allow R output to SVG format.

We can use this to create graphs with links and tooltips. For example, a funnelplot showing associations between a large number of SNPs and VTE.

Point at a dot to see the SNP it represents, and click to go to information about the gene.

SVG+tooltips

```
for(i in 1:length(or)) {
     setSVGShapeToolTip(title=gene[i],
          desc1=snp[i],
          desc2=if(abs(lor[i]/se[i])>qnorm(0.5/n,lower.tail=FALSE))
                                qvals[i] else NULL
          )

      setSVGShapeURL(paste("http://pga.gs.washington.edu/data",
                                              tolower(gene[i]),
                                              sep="/")
           )
      points(prec[i],lor[i], cex=1, pch=19, col='grey')
  }
```


Google Earth

Google Earth is controlled by KML files specifying locations. KML is another plain text format.

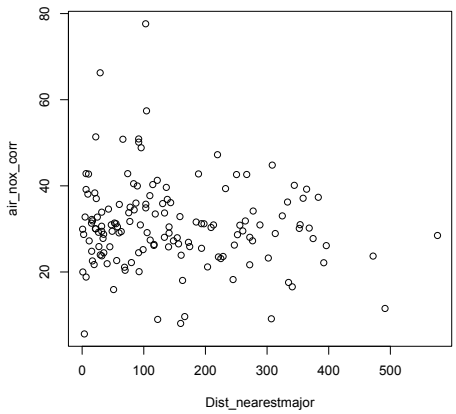
We can write a KML file

```
<?xml version="1.0" encoding="UTF-8"?>
        <kml xmlns="http://earth.google.com/kml/2.1">
        <Placemark>
        <name> 1 </name>
  <Point> <coordinates>-118.0256,34.11619,400</coordinates>
  </Point>
 </Placemark>
</kml>
```

and then send it to Google Earth with the `shell.exec(filename)` function, which opens a file using whatever is the appropriate program.

Google Earth

The `identify()` function lets the user select a point on a scatterplot.



In this example the points are locations where air pollution was measured, and we can call Google Earth to look at the location.