

Bio 723

Scientific Computing for Biologists

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1. Getting your feet wet with R

1.1. Getting Acquainted with R

1.1.1. Installing R

The R website is at <http://www.r-project.org/>. I recommend that you spend a few minutes checking out the resources, documentation, and links on this page. Download the appropriate R installer for your computer from the Comprehensive R Archive Network (CRAN). A direct link can be found at: <http://cran.stat.ucla.edu/>. As of mid August 2013 the latest R release is verison 3.0.1.

1.1.2. Starting and R Interactive Session

The OSX and Windows version of R provide a simple GUI interface for using R in interactive mode. When you start up the R GUI you'll be presented with a single window, the R console. See the your textbook, The Art of R Programming (AoRP) for a discussion of the difference between R's interactive and batch modes.

1.1.3. R Studio

R Studio <http://www.rstudio.com/> is an open source integrated development environment (IDE) that provides a nicer graphical interface to R than does the default GUI. R Studio also has built in support for various literate programming tools like knitr and Sweave.

1.1.4. Accessing the Help System on R

R comes with fairly extensive documentation and a simple help system. You can access HTML versions of R documentation under the Help menu in the GUI. The HTML documentation also includes information on any packages you've installed. Take a few minutes to browse through the R HTML documentation.

The help system can be invoked from the console itself using the `help` function or the `?operator`.

```
> help(length)
> ?length
> ?log
```

What if you don't know the name of the function you want? You can use the `help.search()` function.

```
> help.search("log")
```

In this case `help.search("log")` returns all the functions with the string 'log' in them. For more on `help.search` type `?help.search`. Other useful help related functions include `apropos()` and `example()`.

1.1.5. Navigating Directories in R

When you start the R environment your 'working directory' (i.e. the directory on your computer's file system that R currently 'sees') defaults to a specific directory. On Windows this is usually the same directory that R is installed in, on OS X it is typically your home directory. Here are examples showing how you can get information about your working directory and change your working directory.

```
> getwd()
[1] "/Users/pmagwene"
> setwd("/Users")
> getwd()
[1] "/Users"
```

Note that on Windows you can change your working directory by using the **Set Working Directory** item under the **Session** menu in R Studio.

To get a list of the files in your current working directory use the `list.files()` function.

```
> list.files()
[1] "Shared" "pmagwene"
```

1.1.6. Using R as a Calculator

The simplest way to use R is as a fancy calculator.

```
> 10 + 2 # addition
[1] 12
> 10 - 2 # subtraction
[1] 8
> 10 * 2 # multiplication
[1] 20
> 10 / 2 # division
[1] 5
> 10 ^ 2 # exponentiation
[1] 100
> 10 ** 2 # alternate exponentiation
[1] 100
> sqrt(10) # square root
[1] 3.162278
> 10 ^ 0.5 # same as square root
[1] 3.162278
> exp(1) # exponential function
```

```
[1] 2.718282
> 3.14 * 2.5^2
[1] 19.625
> pi * 2.5^2 # R knows about some constants such as Pi
[1] 19.63495
> cos(pi/3)
[1] 0.5
> sin(pi/3)
[1] 0.8660254
> log(10)
[1] 2.302585
> log10(10) # log base 10
[1] 1
> log2(10) # log base 2
[1] 3.321928
> (10 + 2)/(4-5)
[1] -12
> (10 + 2)/4-5 # compare the answer to the above
[1] -2
```

Be aware that certain operators have precedence over others. For example multiplication and division have higher precedence than addition and subtraction. Use parentheses to disambiguate potentially confusing statements.

```
> sqrt(pi)
[1] 1.772454
> sqrt(-1)
[1] NaN
Warning message:
NaNs produced in: sqrt(-1)
> sqrt(-1+0i)
[1] 0+1i
```

What happened when you tried to calculate `sqrt(-1)`? -1 is treated as a real number and since square roots are undefined for the negative reals, R produced a warning message and returned a special value called `NaN` (Not a Number). Note that square roots of negative complex numbers are well defined so `sqrt(-1+0i)` works fine.

```
> 1/0
[1] Inf
```

Division by zero produces an object that represents infinite numbers.

1.1.7. Comparison Operators

You've already been introduced to the most commonly used arithmetic operators. Also useful are the comparison operators:

```
> 10 < 9 # less than
[1] FALSE
> 10 > 9 # greater than
```

```
[1] TRUE  
> 10 <= (5 * 2) # less than or equal to  
[1] TRUE  
> 10 >= pi # greater than or equal to  
[1] TRUE  
> 10 == 10 # equals  
[1] TRUE  
> 10 != 10 # does not equal  
[1] FALSE  
> 10 == (sqrt(10)^2) # Surprised by the result? See below.  
[1] FALSE  
> 4 == (sqrt(4)^2) # Even more confused?  
[1] TRUE
```

Comparisons return boolean values. Be careful to distinguish between `==` (tests equality) and `=` (the alternative assignment operator equivalent to `<-`).

How about the last two statement comparing two values to the square of their square roots? Mathematically we know that both $(\sqrt{10})^2 = 10$ and $(\sqrt{4})^2 = 4$ are true statements. Why does R tell us the first statement is false? What we're running into here are the limits of computer precision. A computer can't represent $\sqrt{10}$ exactly, whereas $\sqrt{4}$ can be exactly represented. Precision in numerical computing is a complex subject and beyond the scope of this course. Later in the course we'll discuss some ways of implementing sanity checks to avoid situations like that illustrated above.

1.1.8. Working with Vectors in R

Vectors are the core data structure in R. Vectors store an ordered list of items all of the same type. Learning to compute effectively with vectors and one of the keys to efficient R programming. Vectors in R always have a length (accessed with the `length()` function) and a type (accessed with the `typeof()` function).

The simplest way to create a vector at the interactive prompt is to use the `c()` function, which is short hand for 'combine' or 'concatenate'.

```
> x <- c(2,4,6,8)  
[1] "double"  
> length(x)  
[1] 4  
> y <- c('joe','bob','fred')  
> typeof(y)  
[1] "character"  
> length(y)  
[1] 3  
> z <- c() # empty vector  
> length(z)  
[1] 0  
> typeof(z)  
[1] "NULL"
```

You can also use `c()` to concatenate two or more vectors together.

```
> v <- c(1, 3, 5, 7)
> w <- c(-1, -2, -3)
> vwx <- c(v, w, x)
> vwx
[1] 1 3 5 7 -1 -2 -3 2 4 6 8
```

Vector Arithmetic and Comparison

The basic R arithmetic operations work on vectors as well as on single numbers (in fact single numbers *are* vectors).

```
> x <- c(2, 4, 6, 8, 10)
> x * 2
[1] 4 8 12 16 20
> x * pi
[1] 6.283185 12.566371 18.849556 25.132741 31.415927
> y <- c(0, 1, 3, 5, 9)
> x + y
[1] 2 5 9 13 19
> x * y
[1] 0 4 18 40 90
> x/y
[1] Inf 4.000000 2.000000 1.600000 1.111111
> z <- c(1, 4, 7, 11)
> x + z
[1] 3 8 13 19 11
Warning message:
longer object length
      is not a multiple of shorter object length in: x + z
```

When vectors are not of the same length R ‘recycles’ the elements of the shorter vector to make the lengths conform. In the example above `z` was treated as if it was the vector `(1, 4, 7, 11, 1)`.

The comparison operators also work on vectors as shown below. Comparisons involving vectors return vectors of booleans.

```
> x > 5
[1] FALSE FALSE TRUE TRUE TRUE
> x != 4
[1] TRUE FALSE TRUE TRUE TRUE
```

If you try and apply arithmetic operations to non-numeric vectors, R will warn you of the error of your ways:

```
> w <- c('foo', 'bar', 'baz', 'qux')
> w**2
Error in w^2 : non-numeric argument to binary operator
```

Note, however that the comparison operators can work with non-numeric vectors. The results you get will depend on the type of the elements in the vector.

```
> w == 'bar'
[1] FALSE TRUE FALSE FALSE
> w < 'cat'
[1] FALSE TRUE TRUE FALSE
```

Indexing Vectors

For a vector of length n , we can access the elements by the indices $1 \dots n$. We say that R vectors (and other data structures like lists) are ‘one-indexed’. Many other programming languages, such as Python, C, and Java, use zero-indexing where the elements of a data structure are accessed by the indices $0 \dots n - 1$. Indexing errors are a common source of bugs. When moving back and forth between different programming languages keep the appropriate indexing straight!

Trying to access an element beyond these limits returns a special constant called NA (Not Available) that indicates missing or non-existent values.

```
> x <- c(2, 4, 6, 8, 10)
> length(x)
[1] 5
> x[1]
[1] 2
> x[4]
[1] 8
> x[6]
[1] NA
> x[-1]
[1] 4 6 8 10
> x[c(3,5)]
[1] 6 10
```

Negative indices are used to exclude particular elements. $x[-1]$ returns all elements of x except the first. You can get multiple elements of a vector by indexing by another vector. In the example above $x[c(3,5)]$ returns the third and fifth element of x .

Combining Indexing and Comparison

A very powerful feature of R is the ability to combine the comparison operators with indexing. This facilitates data filtering and subsetting. Some examples:

```
> x <- c(2, 4, 6, 8, 10)
> x[x > 5]
[1] 6 8 10
> x[x < 4 | x > 6]
[1] 2 8 10
```

In the first example we retrieved all the elements of x that are larger than 5 (read as ‘ x where x is greater than 5’). In the second example we retrieved those elements of x that were smaller than four *or* greater than six. The symbol $|$ is the ‘logical or’ operator. Other logical operators include $\&$ (‘logical and’ or ‘intersection’) and $!$

(negation). Combining indexing and comparison is a powerful concept and one you'll probably find useful for analyzing your own data.

Generating Regular Sequences

Creating sequences of numbers that are separated by a specified value or that follow a particular patterns turns out to be a common task in programming. R has some built-in operators and functions to simplify this task.

```
> s <- 1:10
> s
[1] 1 2 3 4 5 6 7 8 9 10
> s <- 10:1
> s
[1] 10 9 8 7 6 5 4 3 2 1
> s <- seq(0.5,1.5,by=0.1)
> s
[1] 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5
# 'by' is the 3rd argument so you don't have to specify it
> s <- seq(0.5, 1.5, 0.33)
> s
[1] 0.50 0.83 1.16 1.49
```

`rep()` is another way to generate patterned data.

```
> rep(c("Male","Female"),3)
[1] "Male"    "Female"   "Male"    "Female"   "Male"    "Female"
> rep(c(T,F),2)
[1] TRUE  TRUE FALSE  TRUE  TRUE FALSE
```

1.1.9. Some Useful Functions

You've already seem a number of functions (`c()`, `length()`, `sin()`, `log()`, `length()`, etc.). Functions are called by invoking the function name followed by parentheses containing zero or more *arguments* to the function. Arguments can include the data the function operates on as well as settings for function parameter values. We'll discuss function arguments in greater detail below.

Creating longer vectors

For vectors of more than 10 or so elements it gets tiresome and error prone to create vectors using `c()`. For medium length vectors the `scan()` function is very useful.

```
> test.scores <- scan()
1: 98 92 78 65 52 59 75 77 84 31 83 72 59 69 71 66
17:
Read 16 items
> test.scores
[1] 98 92 78 65 52 59 75 77 84 31 83 72 59 69 71 66
```

When you invoke `scan()` without any arguments the function will read in a list of values separated by white space (usually spaces or tabs). Values are read until `scan()` encounters a blank line or the end of file (EOF) signal (platform dependent). We'll see how to read in data from files below.

Note that we created a variable with the name `test.scores`. If you have previous programming experience you might be surprised that this works. Unlike most languages, R allows you to use periods in variable names. Descriptive variable names generally improve readability but they can also become cumbersome (e.g. `my.long.and.obnoxious.variable.name`). As a general rule of thumb use short variable names when working at the interpreter and more descriptive variable names in functions.

Useful Numerical Functions

Let's introduce some additional numerical functions that are useful for operating on vectors.

```
> sum(test.scores)
[1] 1131
> min(test.scores)
[1] 31
> max(test.scores)
[1] 98
> range(test.scores) # min,max returned as a vec of len 2
[1] 31 98
> sorted.scores <- sort(test.scores)
> sorted.scores
[1] 31 52 59 59 65 66 69 71 72 75 77 78 83 84 92 98
> w <- c(-1, 2, -3, 3)
> abs(w) # absolute value function
```

1.1.10. Function Arguments in R

Function arguments can specify the data that a function operates on or parameters that the function uses. Some arguments are required, while others are optional and are assigned default values if not specified.

Take for example the `log()` function. If you examine the help file for the `log()` function (type `?log` now) you'll see that it takes two arguments, referred to as '`x`' and '`base`'. The argument `x` represents the numeric vector you pass to the function and is a required argument (see what happens when you type `log()` without giving an argument). The argument `base` is optional. By default the value of `base` is $e = 2.71828 \dots$. Therefore by default the `log()` function returns natural logarithms. If you want logarithms to a different base you can change the `base` argument as in the following examples:

```
> log(2) # log of 2, base e
[1] 0.6931472
> log(2,2) # log of 2, base 2
```

```
[1] 1  
> log(2, 4) # log of 2, base 4  
[1] 0.5
```

Because base 2 and base 10 logarithms are fairly commonly used, there are convenient aliases for calling `log` with these bases.

```
> log2(8)  
[1] 3  
> log10(100)  
[1] 2
```

1.1.11. Lists in R

R lists are like vectors, but unlike a vector where all the elements are of the same type, the elements of a list can have arbitrary types (even other lists).

```
> l <- list('Bob', pi, 10, c(2,4,6,8))
```

Indexing of lists is different than indexing of vectors. Double brackets (`x[[i]]`) return the element at index i , single bracket return a list containing the element at index i .

```
> l[1] # single brackets  
[[1]]  
[1] "Bob"  
  
> l[[1]] # double brackets  
[1] "Bob"  
> typeof(l[1])  
[1] "list"  
> typeof(l[[1]])  
[1] "character"
```

The elements of a list can be given names, and those names objects can be accessed using the `$` operator. You can retrieve the names associated with a list using the `names()` function.

```
> l <- list(name='Bob', age=27, years.in.school=10)  
> l  
$name  
[1] "Bob"  
  
$age  
[1] 27  
  
$years.in.school  
[1] 10  
  
> l$years.in.school  
[1] 10  
> l$name
```

```
[1] "Bob"  
> names(1)  
[1] "name"           "age"                "years.in.school"
```

1.1.12. Simple Input in R

The `c()` and `scan()` functions are fine for creating small to medium vectors at the interpreter, but eventually you'll want to start manipulating larger collections of data. There are a variety of functions in R for retrieving data from files.

The most convenient file format to work with are tab delimited text files. Text files have the advantage that they are human readable and are easily shared across different platforms. If you get in the habit of archiving data as text files you'll never find yourself in a situation where you're unable to retrieve important data because the binary data format has changed between versions of a program.

1.1.13. Using `scan()` to input data

`scan()` itself can be used to read data out of a file. Download the file `algae.txt` from the class website and try the following (after changing your working directory):

```
> algae <- scan('algae.txt')  
Read 12 items  
> algae  
[1] 0.530 0.183 0.603 0.994 0.708 0.006 0.867 0.059 0.349 0.699 0.983  
    0.100
```

One of the things to be aware of when using `scan()` is that if the data type contained in the file can not be coerced to doubles than you must specify the data type using the `what` argument. The `what` argument is also used to enable the use of `scan()` with columnar data. Download `algae2.txt` and try the following:

```
> algae.table <- scan('algae2.txt', what=list('',double(0)))  
# note use of list argument to what  
> algae.table  
  
> algae.table  
[[1]]  
[1] "Jan" "Feb" "Mar" "Apr" "May" "Jun" "Jul" "Aug" "Sep" "Oct" "Nov"  
[12] "Dec"  
  
[[2]]  
[1] 0.530 0.183 0.603 0.994 0.708 0.006 0.867 0.059 0.349 0.699 0.983  
[12] 0.100  
  
> algae.table[[1]]  
[1] "Jan" "Feb" "Mar" "Apr" "May" "Jun" "Jul" "Aug" "Sep" "Oct" "Nov"  
[12] "Dec"  
> algae.table[[2]]
```

```
[1] 0.530 0.183 0.603 0.994 0.708 0.006 0.867 0.059 0.349 0.699 0.983  
[12] 0.100
```

Use help to learn more about `scan()`.

1.1.14. Using `read.table()` to input data

`read.table()` (and it's derivates - see the help file) provides a more convenient interface for reading tabular data. Download the `turtles.txt` data set from the class wiki. The data in `turtles.txt` are a set of linear measurements representing dimensions of the carapace (upper shell) of painted turtles (*Chrysemys picta*), as reported in Jolicoeur and Mosimann, 1960; Growth 24: 339-354.

Using the file `turtles.txt`:

```
> turtles <- read.table('turtles.txt', header=T)  
> turtles  
  sex length width height  
1   f     98     81     38  
2   f    103     84     38  
3   f    103     86     42  
# output truncated  
> names(turtles)  
[1] "sex"      "length"    "width"     "height"  
> length(turtles)  
[1] 4  
> length(turtles$sex)  
[1] 48
```

What kind of data structure is `turtles`? What happens when you call the `read.table()` function without specifying the argument `header=T`?

You'll be using the `read.table()` function frequently. Spend some time reading the documentation and playing around with different argument values (for example, try and figure out how to specify different column names on input).

Note: `read.table()` is more convenient but `scan()` is more efficient for large files. See the R documentation for more info.

1.1.15. Basic Statistical Functions in R

There are a wealth of statistical functions built into R. Let's start to put these to use.

If you wanted to know the mean carapace width of turtles in your sample you could calculate this simply as follows:

```
> sum(turtles$width)/length(turtles$width)  
[1] 95.4375
```

Of course R has a built in `mean()` function.

```
mean(turtles$width) [1] 95.4375
```

What if you wanted to calculate the mean of each variables in the data set? R has a set of ‘apply’ functions (lapply, sapply, mapply, etc) that facilitate applying a function repeatedly to different variables in a list or data frame. `sapply` is the one you’ll probably use most often. Here’s how to use `sapply` to calculate means for the turtle data set:

```
> sapply(turtles, mean)
  sex   length   width   height
NA 124.68750  95.43750  46.33333
Warning message:
In mean.default(X[[1L]], ...) :
  argument is not numeric or logical: returning NA
```

Can you figure out why the above produced a warning message? Spend some time reading the documentation for `lapply` and `sapply`, as they will become increasingly handy as you get into writing your own R functions.

Let’s take a look at some more standard statistical functions:

```
> min(turtles$width)
[1] 74
> max(turtles$width)
[1] 132
> range(turtles$width)
[1] 74 132
> median(turtles$width)
[1] 93
> summary(turtles$width)
  Min. 1st Qu. Median Mean 3rd Qu. Max.
 74.00  86.00  93.00  95.44 102.00 132.00
> var(turtles$width) # variance
[1] 160.6769
> sd(turtles$width) # standard deviation
[1] 12.67584
```

1.2. Exploring Univariate Distributions in R

1.2.1. Histograms

One of the most common ways to examine a the distribution of observations for a single variable is to use a histogram. The `hist()` function creates simple histograms in R.

```
> hist(turtles$length) # create histogram with fxn defaults
> ?hist # check out the documentation on hist
```

Note that by default the `hist()` function plots the frequencies in each bin. If you want the probability densities instead set the argument `freq=FALSE`.

```
> hist(turtles$length, freq=F) # y-axis gives probability density
```

Here's some other ways to fine tune a histogram in R.

```
> hist(turtles$length, breaks=12) # use 12 bins  
> mybreaks = seq(85,185,8)  
> hist(turtles$length, breaks=mybreaks) # specify bin boundaries  
> hist(turtles$length, breaks=mybreaks, col='red') # fill the bins with red
```

1.2.2. Density Plots

One of the problems with histograms is that they can be very sensitive to the size of the bins and the break points used. You probably noticed that in the example above as we changes the number of bins and the breakpoints to generate the histograms for the `turtles$length` variable. This is due to the discretization inherent in a histogram. A 'density plot' or 'density trace' is a continuous estimate of a probability distribution from a set of observations. Because it is continuous it doesn't suffer from the same sensitivity to bin sizes and break points. One way to think about a density plot is as the histogram you'd get if you averaged many individual histograms each with slightly different breakpoints.

```
> d <- density(turtles$length)  
> plot(d)
```

A density plot isn't entirely parameter free – the parameter you should be most aware of is the 'smoothing bandwidth'.

```
> d <- density(turtles$length) # let R pick the bandwidth  
> plot(d, ylim=c(0,0.020)) # gives ourselves some extra headroom on y-axis  
> d2 <- density(turtles$length, bw=5) # specify bandwidth  
> lines(d2, col='red') # use lines to draw over previous plot
```

The bandwidth determines the standard deviation of the 'kernel' that is used to calculate the density plot. There are a number of different types of kernels you can use; a Gaussian kernel is the R default and is the most common choice. In the example above, R picked a bandwidth of 8.5 (the black line in our plot). When we specified a smaller bandwith of 5, the resulting density plot (red) is less smooth. There exists a statistical literature on picking 'optimum' kernel sizes. In general, larger data sets support the use of smaller kernels. See the R documentation for more info on the `density()` function and references to the literature on density estimators.

The `lattice` package is an R library that makes it easier to create graphics that show conditional distributions. Here's how to create a simple density plot using the `lattice` package.

```
> library(lattice)  
> densityplot(turtles$length) # densityplot defined in lattice
```

Notice how by default the `lattice` package also drew points representing the observations along the x-axis. These points have been 'jittered' meaning they've been randomly shifted by a small amount so that overlapping points don't completely hide each other. We could have produced a similar plot, without the `lattice` package, as so:

```
> d <- density(turtles$length)
> plot(d)
> nobs <- length(turtles$length)
> points(jitter(turtles$length), rep(0,nobs))
```

Notice that in our version we only jittered the points along the x-axis. You can also combine a histogram and density trace, like so:

```
> hist(turtles$length, 10, xlab='Carapace Length (mm)', freq=F)
> d <- density(turtles$length)
> lines(d, col='red', lwd=2) # red lines, with pixel width 2
```

Notice the use of the `freq=F` argument to scale the histogram bars in terms of probability density.

Finally, let's see some of the features of `lattice` to produce density plots for the 'length' variable of the turtle data set, conditional on sex of the specimen.

```
> densityplot(~length | sex, data = turtles)
```

There are a number of new concepts here. The first is that we used what is called a 'formula' to specify what to plot. In this case the formula can be read as 'length conditional on sex'. We'll be using formulas in several other contexts and we discuss them at greater length below. The `data` argument allows us to specify a data frame or list so that we don't always have to write arguments like `turtles$length` or `turtles$sex` which can get a bit tedious.

1.2.3. Box Plots

Another common tool for depicting a univariate distribution is a 'box plot' (sometimes called a box-and-whisker plot). A standard box plot depicts five useful features of a set of observations: the median (center most line), the upper and lower quartiles (top and bottom of the box), and the minimum and maximum observations (ends of the whiskers).

There are many variants on box plots, particularly with respect to the 'whiskers'. It's always a good idea to be explicit about what a box plot you've created depicts.

Here's how to create box plots using the standard R functions as well as the `lattice` package:

```
> boxplot(turtles$length)
> boxplot(turtles$length, col='darkred', horizontal=T) # horizontal version
> title(main = 'Box plot: Carapace Length', ylab = 'Carapace Length (mm)')
> bwplot(~length,data=turtles) # using the bwplot function from lattice
```

Note how we used the `title()` function to change the axis labels and add a plot title.

Historical note – The box plot is one of many inventions of the statistician John W. Tukey. Tukey made many contributions to the field of statistics and computer science, particularly in the areas of graphical representations of data and exploratory data analysis.

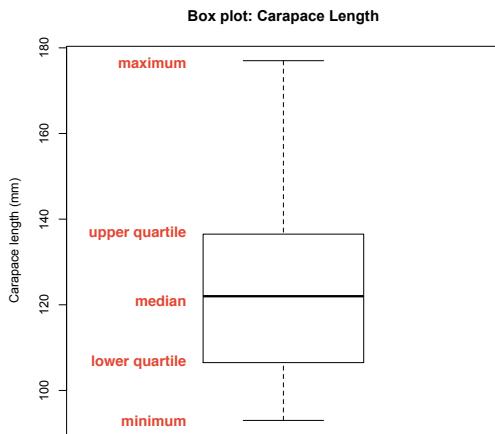


Figure 1.1.: A box plot represents a five number summary of a set of observations.

1.2.4. Bean Plots

My personal favorite way to depict univariate distributions is called a 'beanplot'. Beanplots combine features of density plots and boxplots and provide information rich graphical summaries of single variables. The standard features in a beanplot include the individual observations (depicted as lines), the density trace estimated from the observations, the mean of the observations, and in the case of multiple beanplots an overall mean.

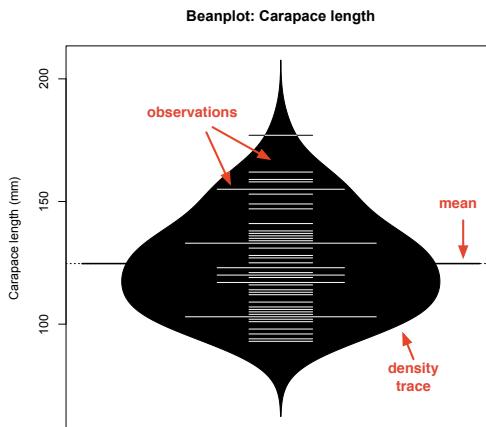


Figure 1.2.: Beanplots combine features of density and box plots.

The `beanplot` package is not installed by default. To download it and install it use the R package installer under the Packages & Data menu. If this is the first time

you use the package installer you'll have to choose a CRAN repository from which to download package info (I recommend you pick one in the US). Once you've done so you can search for 'beanplot' from the Package Installer window. You should also check the 'install dependencies' check box.

Once the beanplot package has been installed check out the examples to see some of the capabilities:

```
> library(beanplot)
```

Note the use of the `library()` function to make the functions in the `beanplot` library available for use. Here's some examples of using the `beanplot` function with the turtle data set:

```
> beanplot(turtles$length) # note the message about log='y'  
> beanplot(turtles$length, log='') # DON'T do the automatic log transform  
> beanplot(turtles$length, log='', col=c('white','blue','blue','red'))
```

In the final version we specified colors for the parts of the beanplot. See the explanation of the `col` argument int he `beanplot` function for details.

We can also compare the carapace length variable for male and female turtles.

```
> beanplot(length ~ sex, data = turtles, col=list(c('red'),c('black')),  
names = c('females','males'),xlab='Sex', ylab='Caparace length (mm)')
```

Note the use of the formula notation to compare the carapace length variable for males and females. Note the use of the list argument to `col`, and the use of vectors within the list to specify the colors for female and male beanplots.

There is also a asymmetrical version of the `beanplot` which can be used to more directly compare distributions between two groups. This can be specified by using the argument `side='both'` to the `beanplot` function.

```
> beanplot(length~sex, data=turtles, col=list(c('red'),c('black')),names=c(  
'females','males'),xlab='Sex', ylab='Carapace length (mm)',side='both')
```

Plots like this one are very convenient for comparing distributions between samples grouped by treatment, sex, species, etc.

We can also create a beanplot with multiple variables in the same plot if the variables are measured on the same scale.

```
> beanplot(turtles$length, turtles$width, turtles$height, log='',  
names=c('length','width','height'), ylab='carapace dimensions (mm)')
```

1.2.5. Demo Plots in R

To get a sense of some of the graphical power of R try the `demo()` function:

```
> demo(graphics)
```

1.3. Getting started with literate programming in R

1.3.1. knitr for R

knitr documents weave together documentation/discussion and code into a single document. The pieces of code and documentation are referred to as ‘chunks’. Knitr comes with a set of tools that allow you to extract just the code, or to turn the entire document into a nicely formatted report.

You can install knitr using the ‘Packages’ tab in the R studio IDE or at the command line as follows:

```
install.packages('knitr', dependencies = TRUE)
```

Restart R Studio after installing knitr.

Once knitr is installed, you can create your first knitr document. knitr documents are just plain text files, but R Studio includes some convenient tools to compile such documents in HTML. In R Studio select New > R Markdown to create a new knitr document, delete the template text, and enter the text shown below:

```
My First knitr Document
```

```
=====
```

```
This is very simple knitr document. It includes some *emphasized* and **bold** text, and a single code chunk.
```

```
```{r}
z <- rnorm(30, mean=0, sd=1)
summary(z)
```
```

Save this as a markdown file `knit1.Rmd` and ‘knit’ the document using the Knit HTML button in the R Studio IDE. If you entered everything correctly, R Studio will pop up a preview window showing the HTML document that was created from your knitr source code.

As you can see, knitr uses a simple way to markup text (using a formatting convention called ‘Markdown’), and code chunks are delineated from text using three backticks. In the HTML output notice that your text blocks includes some formatted italic and bold text, and that the code chunks are shown in grey boxes. Note that there’s also a table below the code chunk. This shows the result of evaluating the code chunk.

If you knit the document a second time you’ll find that the table output changes slightly. Figure out why this is so by reading the documentation for the `rnorm` function.

A fancier knitr document

Let’s get a little bit fancier and show how we can create graphics and use some knitr’s formatting features to produce a nicer document.

My Second knitr Document

This is a still a simple knitr file. However, now it includes several code chunks, graphics, and mathematical symbols.

```
## Sampling from the random normal distribution
```

```
```{r}
z <- rnorm(30, mean=0, sd=1)
summary(z)
```
```

That code chunk generated a random sample of 30 observations drawn from a normal distribution with mean zero ($\mu = 0$) and standard deviation one ($\sigma = 1$).

Note the use of the hashmarks to indicate section headings.

```
### Mathematical notation
```

knitr uses standard LaTeX conventions for writing mathematical formulas in text blocks.

```
## Generating figures
```

We can automatically embed graphics in our report. For example, the following will generate a histogram.

```
```{r}
hist(z)
```
```

For a full overview of knitr's capabilities see the documentation and examples at the knitr website <http://yihui.name/knitr/>.

Assignment 1.1

Find an example univariate data set of your choice from the literature. Read it into R using either `scan` or `read.table`. Plot the data using either a histogram or a density plot. Try several different bin widths or kernel sizes. Discuss why you think the plot and bin width/kernel you chose is the best way to represent your data. Also provide an example of a misspecification of the kernel or the bin width. Submit your assignment as a knitr literate programming document. Be sure to include a line of code to read in your data based on a relative path name and submit the accompanying data file.

Note that the `scan` and `read.table` functions can also take a url as input as illustrated below:

```
> algae <- scan(url("http://biology.duke.edu/magwelenlab/algae.txt", "r"))
```

Unfortunately, that only works with normal HTTP connections, *not* HTTPS, so you can't open files directly from the Bio 723 Github site.

2. Bivariate Data

2.1. Plotting Bivariate Data in R

Let's use a dataset called `iris` (included in the standard R distribution) to explore bivariate relationships between variables. This data set was made famous by R. A. Fisher who used it to illustrate many of the fundamental statistical methods he developed. The data set consists of four morphometric measurements on specimens of three different iris species. Use the R help to read about the `iris` data set (`?iris`). We'll be using this data set repeatedly in future weeks so familiarize yourself with it.

```
> ?iris
> names(iris)
[1] "Sepal.Length" "Sepal.Width"   "Petal.Length" "Petal.Width"
[5] "Species"
> unique(iris$Species)
[1] setosa      versicolor  virginica
Levels: setosa versicolor virginica
> dim(iris)
[1] 150    5
```

2.1.1. Bivariate scatter plots

We'll start with the conventional 'variable space' representation of bivariate relationships – the scatter plot.

```
> plot(iris$Sepal.Length, iris$Sepal.Width)
```

This plots Sepal Length on the x-axis and Petal Length on the y-axis. Here's an alternate way to generate the same plot:

```
> plot(Petal.Length ~ Sepal.Length, data = iris)
```

Did you notice what is different between the two versions above? In the second version, you can think of the tilde ('~') as short-hand for 'function of'. So the plotting call above can be translated roughly as "Plot Petal.Length as a function of Sepal.Length, where these variables can be found in the `iris` data set".

From these plot it is immediately obvious that these two variables are positively associated (i.e. when one increases the other tends to increase). You will also notice there seem to be distinct clusters of points in the plot. Recall that the `iris` data set consists of three different species. Let's regenerate the plot, this time coloring the points according to the species names. First, let's note that the `Species` column is a categorical variable, which in R we refer to as a 'factor'.

```
> iris$Species
[1] setosa    setosa    setosa    setosa ...
[51] versicolor versicolor versicolor versicolor ...
[101] virginica virginica virginica virginica ...
...
Levels: setosa versicolor virginica
> is.factor(iris$Species)
[1] TRUE
> levels(iris$Species)
[1] "setosa"      "versicolor" "virginica"
> nlevels(iris$Species)
[1] 3
> typeof(iris$Species)
[1] "integer"
```

The `is.factor()` function tests whether a vector is a factor, the `levels()` function returns the categorical labels associated with the factor, and `nlevels()` gives the total number of levels. Factor levels are represented internally as integers, as the `typeof()` function call illustrates. You can use the function `unclass()` to show the corresponding integer representations for a vector of factors:

```
> unclass(iris$Species)
[1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 ...
[59] 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 ...
[117] 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 ...
attr("levels")
[1] "setosa"      "versicolor" "virginica"
```

As you can see, the ‘setosa’ specimens have the value 1, ‘versicolor’ have the value 2, and ‘virginica’ the value 3.

Because of the mapping between factor levels and integers, we can use a variable of factors as indices into another vector, effectively creating a mapping between the factor levels, and the elements of the vector that is being indexed. This is shown below:

```
> clrs <- c('red', 'green', 'blue')
> clrs[iris$Species]
[1] "red"     "red"     "red"     "red"     "red"     "red"     ...
[57] "green"   "green"   "green"   "green"   "green"   "green"   ...
[99] "green"   "green"   "blue"    "blue"    "blue"    "blue"    ...
```

With that mapping in mind, let’s reconstruct our scatter plot:

```
> plot(Petal.Length ~ Sepal.Length, data = iris, col = clrs[iris$Species],
       main="Petal Length vs. Sepal Length")
> legend("topleft", pch = 1, col = clrs, legend = levels(iris$Species ))
```

In addition to plotting and coloring the bivariate scatter, we added a title to the plot using the `main` argument and created a legend, using the `legend()` function. Your output should look like Figure 2.1.

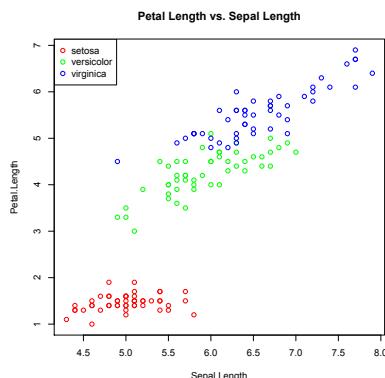


Figure 2.1.: Scatter plot created from the iris data set using the `plot` function.

2.2. Introducing ggplot2

Pretty much any statistical plot can be thought of as a mapping between data and one or more visual representations. For example, in a bivariate scatter plot we map two ordered sets of numbers (the variables of interest) to points in the Cartesian plane (x,y-coordinates). In our example above, we further embellished our plot with another mapping in which we mapped the Species labels to different colors.

This notion of representing plots in terms of their mappings is a powerful idea which is central to an approach for plotting that is represented in the R package `ggplot2`.

2.2.1. Installing ggplot2

Like all R packages, `ggplot2` can be installed either from the command line or via the GUI. Here's a reminder of how to do so from the command line:

```
> install.packages("ggplot2", dependencies=T)
```

2.2.2. Aesthetic and Geometric mappings in ggplot2

`ggplot2` considers two types of mappings from data to visual representations: 1) ‘aesthetic mappings’, which determine the way that data are represented in a plot (e.g. symbols, colors) and 2) ‘geometry’ or ‘geom’ mappings which determine the type of geometric representation that a plot uses.

The primary plotting function in `ggplot2` is `ggplot()`. The first argument to `ggplot` is always a data frame. The data frame is the one that `ggplot` will use to look for all the mappings that you define in the subsequent pieces of the plot. The nice thing about this is that there is no need to use the dollar sign notation. As you've seen, you can get similar behavior in base plots by specifying the ‘`data`’ argument.

The second argument to `ggplot()` is always a function called `aes()`. `aes()` takes named arguments. Each argument name is the ‘aesthetic’ that you want mapped to a particular variable (column) in the data.

The final piece of information that we need to draw our plot is the ‘geom’. All geoms are encoded as R functions. The syntax used to add them to a plot is simply a ‘+’ sign. There are many different ggplot geoms for different plot types. We’ll explore a few of the built-in geoms in this chapter; additional geoms will come up in later weeks.

2.2.3. Scatter plots using ggplot2

Let’s recreate our iris scatter plot using the function `ggplot` from the `ggplot2` library:

```
> library(ggplot2)
> ggplot(iris, aes(x = Sepal.Length, y = Petal.Length,
+ col = Species)) + geom_point()
```

Following the requirement outline above, `iris` is our data frame, the call to `aes` set’s up our aesthetic mapping, and we’re specifying the use of the point geom (`geom_point()`) to map the x- and y-values in the aesthetic mapping to points in the Cartesian plane. In the function call above, we told `ggplot` that we wanted the sepal length on the x axis, the petal length on the y axis, and the colors to be encoded by the species. However, we could choose any number of other aesthetic mappings. For example, could use shape instead of color to represent the Species labels:

```
> ggplot(iris, aes(x = Sepal.Length, y = Petal.Length,
+ shape = Species)) + geom_point()
```

or alternately, size:

```
> ggplot(iris, aes(x = Sepal.Length, y = Petal.Length,
+ size = Species)) + geom_point()
```

We can even combine multiple aesthetics in a single plot:

```
> ggplot(iris, aes(x = Sepal.Length, y = Petal.Length,
+ col = Species, shape = Species)) + geom_point()
```

The resulting plot is shown in Figure 2.2.

There’s a number of advantages to using `ggplot` rather than trying to replicate this plot with base graphics functions in R:

1. The legend is automatically drawn for you.
2. The code is very easy to change. Rather than having to figure out how to manually map a point size onto a variable using some difficult R code, it’s just as simple as saying to set the ‘size’ equal to a ‘variable’.
3. It’s easy to swap around variables from one aesthetic mapping to another.

Having a good understanding of both the base plotting functions and a powerful package like `ggplot2` allows you maximum flexibility in terms of the statistical graphics you are able to produce.

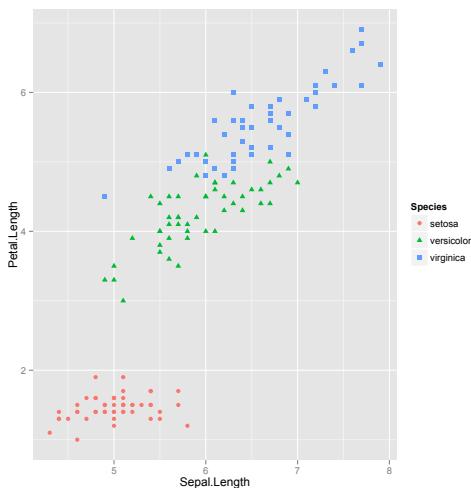


Figure 2.2.: Scatter plot created from the iris data set using the `ggplot` function.

2.2.4. Some additional ggplot geoms

So far we've only looked at a single geom (`geom_point()`). Let's revisiting some of the univariate plots from last week using `ggplot`.

Boxplots `geom_boxplot()` constructs boxplots in `ggplot`.

```
> ggplot(iris, aes(x = Species, y = Sepal.Length, col=Species)) +
  geom_boxplot()
```

Histograms `geom_histogram()` is used to construct histogram plots in `ggplot`.

```
> ggplot(iris, aes(x = Sepal.Length)) + geom_histogram()
```

Here we let `ggplot` pick the default bin widths. Below we show how to change the bin width:

```
> ggplot(iris, aes(x = Sepal.Length)) + geom_histogram(binwidth=0.25)
```

If we want to color histogram by species identity you need to set the `position = 'identity'` in the call to `geom_histogram`:

```
> ggplot(iris, aes(x = Sepal.Length, fill=Species)) +
  geom_histogram(binwidth=0.25, position='identity', alpha=0.65)
```

The above code also set the transparency of the bar fills using the `alpha` argument. As an alternative to overlaying the histogram bins for each species, you can show the bins side-by-side using the argument `position = 'dodge'`.

```
> ggplot(iris, aes(x = Sepal.Length, fill=Species)) +
  geom_histogram(binwidth=0.25, position='dodge')
```

Density plots `geom_density()` creates density plots in ggplot.

```
> ggplot(iris, aes(x = Sepal.Length, fill=Species)) +
  geom_density(alpha=0.65)
```

There's also a 2D version of the density plot, created using `geom_density2d()`. This can be usefully combined with `geom_points()` to create a bivariate scatter plot with density contours.

```
> ggplot(iris, aes(x = Sepal.Length, y = Petal.Length, col = Species)) +
  geom_point() + geom_density2d(alpha=0.25)
```

Scatter plots with marginal density plots The file `scatterWithMargins.R` from the course wiki contains a function that uses multiple calls to `ggplot()` to combine two marginal density plots with a scatter plot. To use this function you'll need to install a package called "gridExtra":

```
> install.packages("gridExtra", dependencies=T)
```

Then import the new function from `scatterWithMargins.R` and use it as so:

```
> source('scatterWithMargins.R')
> scatterWithMargins(iris, "Sepal.Length", "Petal.Length", "Species")
```

This produces the plot shown in Figure 2.3.

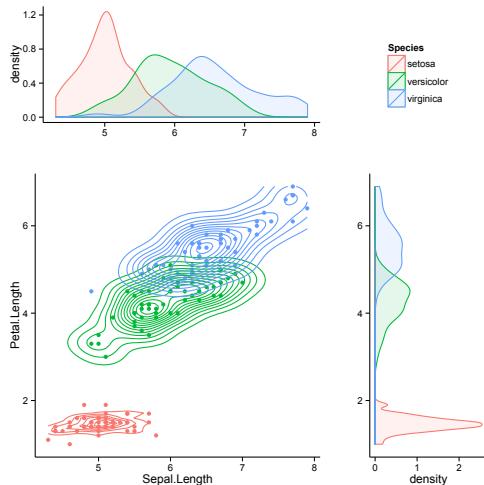


Figure 2.3.: Figure produced by the `scatterWithMargins` function from the course wiki.

2.3. Vector Mathematics in R

As you saw last week R vectors support basic arithmetic operations that correspond to the same operations on geometric vectors. For example:

```
> x <- 1:15
> y <- 10:24
> x
[1] 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
> y
[1] 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

> x + y          # vector addition
[1] 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
> x - y          # vector subtraction
[1] -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9
> x * 3          # multiplication by a scalar
[1] 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45
```

R also has an operator for the dot product, denoted `%*%`. This operator also designates matrix multiplication, which we will discuss next week. By default this operator returns an object of the R matrix class. If you want a scalar (or the R equivalent of a scalar, i.e. a vector of length 1) you need to use the `drop()` function.

```
> z <- x %*% x
> class(z)      # note use of class() function
[1] "matrix"
> z
[,1]
[1,] 1240
> drop(z)
[1] 1240
```

In lecture we saw that many useful geometric properties of vectors could be expressed in the form of dot products. Let's start with some two-dimensional vectors where the geometry is easy to visualize:

```
> a <- c(1, 0) # the point (1,0)
> b <- c(0, 1) # the point (0,1)
```

Now let's draw our vectors:

```
# create empty plot w/specify x- and y- limits
# the 'asp=1' argument maintains the scaling of the x- and y-axes
# so that units are equivalent for both axes (i.e. squares remain squares)
> plot(c(-2,2),c(-1,2),type='n', asp=1)

# draw an arrow from origin (0,0) to x,y coordinates of vector "a"
# the length argument changes the size of the arrowhead
# use the R help to read more about the arrows function
> arrows(0, 0, a[1], a[2], length=0.1)
```

```
# and now for the vector "b"
> arrows(0, 0, b[1], b[2], length=0.1)
```

You should now have a figure that looks like the one below: Let's see what the dot

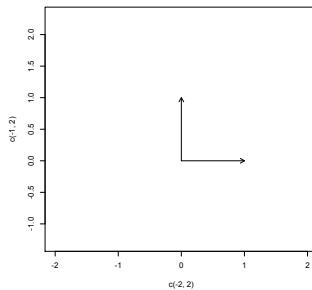


Figure 2.4.: A simple vector figure.

product can tell us about these vectors. First recall that we can calculate the length of a vector as the square-root of the dot product of the vector with itself ($|\vec{a}|^2 = \vec{a} \cdot \vec{a}$)

```
> len.a <- drop(sqrt(a %*% a))
> len.a
[1] 1
> len.b <- drop(sqrt(b %*% b))
```

How about the angle between a and b ?

```
> dot.ab <- a %*% b
> dot.ab
[,1]
[1,]    0
> cos.ab <- (a %*% b)/(len.a * len.b)
> cos.ab
[,1]
[1,]    0
```

A key point to remember dot product of two vectors is zero if, and only if, they are orthogonal to each other (regardless of their dimension).

2.4. Writing Functions in R

So far we've been mostly using R's built in functions. However the power of a true programming language is the ability to write your own functions.

The general form of an R function is as follows:

```
funcname <- function(arg1, arg2) {
  # one or more expressions
  # last expression is the object returned
```

```
# or you can explicitly return an object
}
```

To make this concrete, here's an example where we define a function in the interpreter and then put it to use:

```
> my.dot <- function(x,y){
+ # don't type the '+' symbols, these show continuation lines
+   return(sum(x*y))
+ }

> a <- 1:5
> b <- 6:10
> a
[1] 1 2 3 4 5
> b
[1] 6 7 8 9 10
> my.dot(a,b)
[1] 130
> my.dot
function(x,y){
  return(sum(x*y))
}
```

If you type a function name without parentheses R shows you the function's definition. This works for built-in functions as well (though sometimes these functions are defined in C code in which case R will tell you that the function is a 'Primitive').

2.4.1. Putting R functions in Scripts

When you define a function at the interactive prompt and then close the interpreter your function definition will be lost. The simple way around this is to define your R functions in a script that you can than access at any time.

In R Studio choose **File > New > R Script**. This will bring up a blank editor window. Enter your function into the editor and save the source file in your R working directory with a name like `vecgeom.R`.

```
# functions defined in vecgeom.R

veclength <- function(x) {
  # Given a numeric vector, returns length of that vector
  sqrt(drop(x %*% x))
}

unitvector <- function(x) {
  # Return a unit vector in the same direction as x
  x/veclength(x)
}
```

There are two functions defined above, one of which calls the other. Both take single vector arguments. These functions have no error checking to insure that the arguments passed to the functions are reasonable but R's built in error handling will do just fine for most cases.

Once your functions are in a script file you can make them accessible by using the `source()` function (See also the Source tab button in the R Studio GUI):

```
> source("vecgeom.R")
> x <- c(1,0.4)
> veclength(x)
[1] 1.077033
> ux <- unitvector(x)
> ux
[1] 0.9284767 0.3713907
> veclength(ux)
[1] 1
> a
[1] 1 2 3 4 5
> veclength(a)
[1] 7.416198
> ua <- unitvector(a)
> ua
[1] 0.1348400 0.2696799 0.4045199 0.5393599 0.6741999
> veclength(ua)
[1] 1
```

Note that our functions work with vectors of arbitrary dimension.

Assignment 2.1

Write a function that uses the dot product and the `acos()` function to calculate the angle (in radians) between two vectors of arbitrary dimension. By default, your function should return the angle in radians. Also include a logical (Boolean) argument that will return the answer in degrees. Test your function with the following two vectors: $x = [-3, -3, -1, -1, 0, 0, 1, 2, 2, 3]$ and $y = [-8, -5, -3, 0, -1, 0, 5, 1, 6, 5]$. The expected angle for these test vectors is 0.441 radians (25.3 degrees).

Let's also add the following function to `vecgeom.R` to aid in visualizing 2D vectors:

```
draw.vectors <- function(a, b, colors=c('red', 'blue'), clear.plot=TRUE){

  # figure out the limits such that the origin and the vector
  # end points are all included in the plot
  xhi <- max(0, a[1], b[1])
  xlo <- min(0, a[1], b[1])
  yhi <- max(0, a[2], b[2])
  ylo <- min(0, a[2], b[2])

  xlims <- c(xlo, xhi)*1.10 # give a little breathing space around
  # vectors
  ylims <- c(ylo, yhi)*1.10
```

```

if (clear.plot){
  plot(xlims, ylims, type='n', asp=1, xlab="x-coord", ylab="y-coord")
}
arrows(0, 0, a[1], a[2], length=0.1, col=colors[1])
arrows(0, 0, b[1], b[2], length=0.1, col=colors[2])
}

```

You can use this new function as follows:

```

# you need to source the file everytime you change it
> source("~/Users/pmagwene/Downloads/vecgeom.R")
> x <- c(1,0.4)
> y <- c(0.2, 0.8)
> draw.vectors(x,y) # draw the original vectors

```

The resulting figure should resemble the one below.

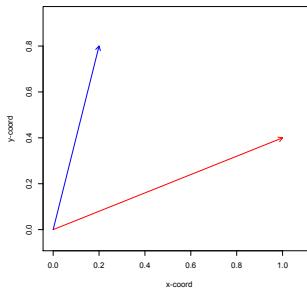


Figure 2.5.: Another vector figure.

Notice that we included a `clear.plot` argument in our `draw.vectors` function. I included this so we could add additional vectors to our plot, without overwriting the old vectors, as demonstrated below:

```

# draw the unit vectors that point in the same directors as the original
# vectors
> ux <- unitvector(x)
> uy <- unitvector(y)
> draw.vectors(ux, uy, colors=c('black', 'green'), clear.plot=F)

```

Unlike the other functions we wrote, `draw.vectors` only works properly with 2D vectors. Since any pair of vectors defines a plane, it is possible to generalize this function to work with arbitrary pairs of vectors.

Assignment 2.2

Write a function, `vproj()`, that takes two vectors, \vec{x} and \vec{y} , and returns a list containing the projection of \vec{y} on \vec{x} and the component of \vec{y} in \vec{x} :

$$P_{\vec{x}}(\vec{y}) = \left(\frac{\vec{x} \cdot \vec{y}}{|\vec{x}|} \right) \frac{\vec{x}}{|\vec{x}|}$$

and

$$C_{\vec{x}}(\vec{y}) = \frac{\vec{x} \cdot \vec{y}}{|\vec{x}|}$$

Use the test vectors from Assignment 2.1 to test your function. The list returned by your function for these test vectors should resemble that shown below:

```
> vproj(x, y)

$proj
[1] -6 -6 -2 -2  0  0  2  4  4  6

$comp
[1] 12.32883
```

2.5. Vector Geometry of Correlation and Regression

Let's return to our use of the dot product to explore the relationship between variables. First let's add a function to our module, `vecgeom.R`, to calculate the cosine of the angle between two vectors.

```
# add to vecgeom.R

vec.cos <- function(x,y) {
  # Calculate the cos of the angle between vectors x and y
  len.x <- veclength(x)
  len.y <- veclength(y)
  return( (x %*% y)/(len.x * len.y) )
}
```

We can then use this function to examine the relationships between the variables in the `iris` dataset. For now let's just work with the *I. setosa* specimens. Read the help file for `subset()`.

```
> setosa <- subset(iris, Species == 'setosa', select = -Species)
> dim(setosa)
[1] 50  4
> names(setosa)
[1] "Sepal.Length" "Sepal.Width"  "Petal.Length" "Petal.Width"
```

Often times it's useful to look at many bivariate relationships simultaneously. The `pairs()` function allows you to do this:

```
> pairs(setosa)
```

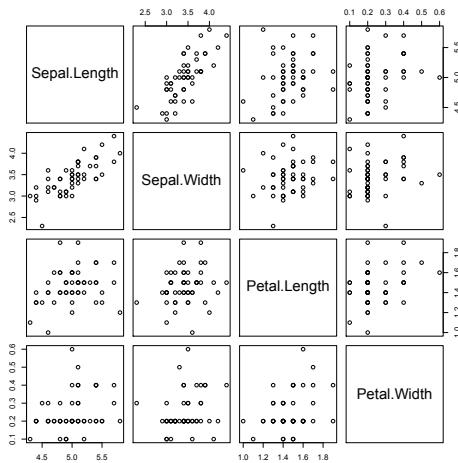


Figure 2.6.: Output of the `pairs()` function for the *I. setosa* specimens in the `iris` dataset.

First we'll center the setosa dataset using the `scale()` function. `scale()` has two logical arguments `center` and `scale`. By default both are `TRUE` which will center *and* scale the variables. But for now we just want to center the data. `scale()` returns a matrix object so we use the `data.frame` function to cast the object back to a data frame.

```
> source("/Users/pmagwene/Downloads/vecgeom.R")
> ctrd <- scale(setosa, center=T, scale=F)
> class(ctrd)
[1] "matrix"
> names(ctrd)
NULL
> ctrd <- data.frame(scale(setosa, center=T, scale=F))
> class(ctrd)
[1] "data.frame"
> names(ctrd)
[1] "Sepal.Length" "Sepal.Width" "Petal.Length" "Petal.Width"
> vec.cos(ctrd$Sepal.Length, ctrd$Sepal.Width)
[,1]
[1,] 0.7425467
> vec.cos(ctrd$Sepal.Length, ctrd$Petal.Length)
[,1]
[1,] 0.2671758
> vec.cos(ctrd$Sepal.Length, ctrd$Petal.Width)
[,1]
[1,] 0.2780984
```

Consider the values above in the context of the scatter plots you generated with the `pairs()` function; and then recall that for mean-centered variables, $\text{cor}(X, Y) = r_{XY} = \cos \theta = \frac{\vec{x} \cdot \vec{y}}{|\vec{x}| |\vec{y}|}$. So our `vec.cos()` function, when applied to centered data, is equivalent to calculating the correlation between x and y . Let's confirm this using the built in `cor()` function in R:

```
> cor(setosa$Sepal.Length, setosa$Sepal.Width)
[1] 0.7425467
> cor(setosa) # called like this will calculate all pairwise correlations
   Sepal.Length Sepal.Width Petal.Length Petal.Width
Sepal.Length     1.0000000  0.7425467  0.2671758  0.2780984
Sepal.Width      0.7425467  1.0000000  0.1777000  0.2327520
Petal.Length     0.2671758  0.1777000  1.0000000  0.3316300
Petal.Width      0.2780984  0.2327520  0.3316300  1.0000000
```

2.5.1. Bivariate Regression in R

R has a flexible built in function, `lm()` for fitting linear models. Bivariate regression is the simplest case of a linear model.

```
> setosa.lm <- lm(Sepal.Width ~ Sepal.Length, data=setosa)
> class(setosa.lm)
[1] "lm"
> names(setosa.lm)
[1] "coefficients"    "residuals"        "effects"          "rank"
[5] "fitted.values"   "assign"           "qr"              "df.residual"
[9] "xlevels"          "call"             "terms"           "model"
> coef(setosa.lm)
(Intercept) Sepal.Length
-0.5694327  0.7985283
```

The function `coef()` will return the intercept and slope of the line representing the bivariate regression. For a more complete summary of the linear model you've fit use the `summary()` function:

```
> summary(setosa.lm)

Call:
lm(formula = Sepal.Width ~ Sepal.Length, data = setosa)

Residuals:
    Min      1Q  Median      3Q      Max 
-0.72394 -0.18273 -0.00306  0.15738  0.51709 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) -0.5694     0.5217  -1.091   0.281    
Sepal.Length  0.7985     0.1040   7.681 6.71e-10 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Residual standard error: 0.2565 on 48 degrees of freedom  
Multiple R-squared: 0.5514, Adjusted R-squared: 0.542  
F-statistic: 58.99 on 1 and 48 DF, p-value: 6.71e-10
```

As demonstrated above, the `summary()` function spits out key diagnostic information about the model we fit. Now let's create a plot illustrating the fit of the model.

```
> plot(Sepal.Width ~ Sepal.Length, data=setosa, xlab="Sepal Length (cm)",  
       ylab="Sepal Width (cm)", main="Iris setosa")  
> abline(setosa.lm, col='red', lwd=2, lty=2) # see ?par for info about lwd  
and lty
```

Your output should resemble the figure below. Note the use of the function `abline()` to plot the regression line. Calling `plot()` with an object of class `lm` shows a series of diagnostic plots. Try this yourself.

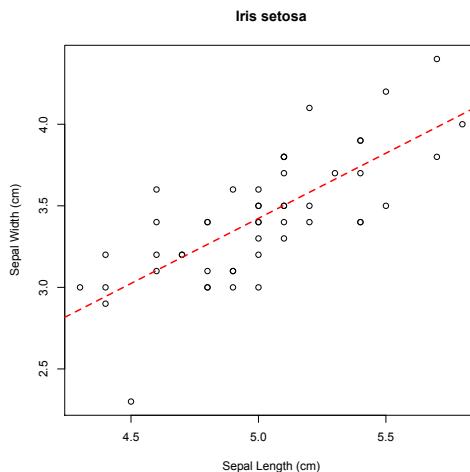


Figure 2.7.: Linear regression of Sepal Width on Sepal Length for *I. setosa*.

Assignment 2.3

Write your own regression function (i.e. your code shouldn't refer to the built in regression functions) for mean centered vectors in R. The function will take as its input two vectors, \vec{x} and \vec{y} . The function should return:

1. a list containing the mean-centered versions of these vectors
2. the regression coefficient b in the mean centered regression equation $\hat{y} = b\vec{x}$
3. the coefficient of determination, R^2

Demonstrate your regression function by using it to carry out regressions of Sepal.Length on Sepal.Width separately for the 'versicolor' and 'virginica' specimens from the iris data set. Include ggplot created plots in which you use the `geom_point()` and `geom_abline()` functions to illustrate your calculated regression line. To test your function, compare your regression coefficients and coefficient of determination to the same values returned by the built in `lm()` function.

3. Matrices and matrix operations in R

3.1. Matrices in R

In R matrices are two-dimensional collections of elements all of which have the same mode or type. This is different than a data frame in which the columns of the frame can hold elements of different type (but all of the same length), or from a list which can hold objects of arbitrary type and length. Matrices are more efficient for carrying out most numerical operations, so if you're working with a very large data set that is amenable to representation by a matrix you should consider using this data structure.

3.1.1. Creating matrices in R

There are a number of different ways to create matrices in R. For creating small matrices at the command line you can use the `matrix()` function.

```
> X <- matrix(1:5)
> X
     [,1]
[1,]    1
[2,]    2
[3,]    3
[4,]    4
[5,]    5
> X <- matrix(1:12, nrow=4)
> X
     [,1] [,2] [,3]
[1,]    1    5    9
[2,]    2    6   10
[3,]    3    7   11
[4,]    4    8   12
> dim(X) # give the shape of the matrix
[1] 4 3
```

`matrix()` takes a data vector as input and the shape of the matrix to be created is specified by using the `nrow` and `ncol` arguments. If the number of elements in the input data vector is less than `nrows × ncols` the elements will be 'recycled' as discussed in previous chapters. Without any shape arguments the `matrix()` function will create a column vector as shown above. By default the `matrix()` function fills in the matrix in a column-wise fashion. To fill in the matrix in a row-wise fashion use the argument `byrow=T`.

If you have a pre-existing data set in a list or data frame you can use the `as.matrix()` function to convert it to a matrix.

```
> turtles <- read.table('turtles.txt', header=T)
> tmtx <- as.matrix(turtles)
> head(tmtx) # see ?head and ?tail
  sex length width height
[1,] "f"   "98"  "81"  "38"
[2,] "f"   "103" "84"  "38"
[3,] "f"   "103" "86"  "42"
[4,] "f"   "105" "86"  "40"
[5,] "f"   "109" "88"  "44"
[6,] "f"   "123" "92"  "50"
# NOTE: the elements were all converted to character

> tmtx <- as.matrix(subset(turtles, select=-sex))
> head(tmtx)
  length width height
1      98    81     38
2     103    84     38
3     103    86     42
4     105    86     40
5     109    88     44
6     123    92     50
# This is probably more along the lines of what you want
```

You can use the various indexing operations to get particular rows, columns, or elements. Here are some examples:

```
> X <- matrix(1:12, nrow=4)
> X
  [,1] [,2] [,3]
[1,]    1    5    9
[2,]    2    6   10
[3,]    3    7   11
[4,]    4    8   12
> X[1,] # get the first row
[1] 1 5 9
> X[,1] # get the first column
[1] 1 2 3 4
> X[1:2,] # get the first two rows
  [,1] [,2] [,3]
[1,]    1    5    9
[2,]    2    6   10
> X[,2:3] # get the second and third columns
  [,1] [,2]
[1,]    5    9
[2,]    6   10
[3,]    7   11
[4,]    8   12
```

```

> Y <- matrix(1:12, byrow=T, nrow=4)
> Y
     [,1] [,2] [,3]
[1,]    1    2    3
[2,]    4    5    6
[3,]    7    8    9
[4,]   10   11   12
> Y[4] # see explanation below
[1] 10
> Y[5]
[1] 2
> dim(Y) <- c(2,6)
> Y
     [,1] [,2] [,3] [,4] [,5] [,6]
[1,]    1    7    2    8    3    9
[2,]    4   10    5   11    6   12
> Y[5]
[1] 2

```

The example above where we create a matrix `Y` is meant to show that matrices are stored internally in a column wise fashion (think of the columns stacked one atop the other), regardless of whether we use the `byrow=T` argument. Therefore using single indices returns the elements with respect to this arrangement. Note also the use of assignment operator in conjunction with the `dim()` function to reshape the matrix. Despite the reshaping, the internal representation in memory hasn't changed so `Y[5]` still gives the same element.

You can use the `diag()` function to get the diagonal of a matrix or to create a diagonal matrix as shown below:

```

> Z <- matrix(rnorm(16), ncol=4)
> Z
     [,1]          [,2]          [,3]          [,4]
[1,] -1.7666373  2.1353032 -0.903786375 -0.70527447
[2,] -0.9129580  1.1873620  0.002903752  0.51174408
[3,] -1.5694273 -0.5670293 -0.883259848  0.05694691
[4,]  0.9903785 -1.6138958  0.408543336  2.39152400
> diag(Z)
[1] -1.7666373  1.1873620 -0.8832598  2.3915240
> diag(5) # create the 5 x 5 identity matrix
     [,1] [,2] [,3] [,4] [,5]
[1,]    1    0    0    0    0
[2,]    0    1    0    0    0
[3,]    0    0    1    0    0
[4,]    0    0    0    1    0
[5,]    0    0    0    0    1
> s <- sqrt(10:13)
> diag(s)
     [,1]      [,2]      [,3]      [,4]
[1,] 3.162278 0.000000 0.000000 0.000000

```

```
[2,] 0.000000 3.316625 0.000000 0.000000  
[3,] 0.000000 0.000000 3.464102 0.000000  
[4,] 0.000000 0.000000 0.000000 3.605551
```

Matrix operations in R

The standard mathematical operations of addition and subtraction and scalar multiplication work element-wise for matrices in the same way as they did for vectors. Matrix multiplication uses the operator `%*%` which you saw last week for the dot product. To get the transpose of a matrix use the function `t()`. The `solve()` function can be used to get the inverse of a matrix (assuming it's non-singular) or to solve a set of linear equations.

```
> A <- matrix(1:12, nrow=4)  
> A <- matrix(1:12, nrow=4)  
> A  
     [,1] [,2] [,3]  
[1,]    1    5    9  
[2,]    2    6   10  
[3,]    3    7   11  
[4,]    4    8   12  
> t(A)  
     [,1] [,2] [,3] [,4]  
[1,]    1    2    3    4  
[2,]    5    6    7    8  
[3,]    9   10   11   12  
> B <- matrix(rnorm(12), nrow=4)  
> B  
     [,1]          [,2]          [,3]  
[1,] -2.9143953  0.38204730 -1.33207235  
[2,]  0.1778266 -0.44563686  0.76143612  
[3,]  1.7226235  0.03320553 -0.06652767  
[4,]  0.5291281 -0.13145408  0.14108766  
> A + B  
     [,1]          [,2]          [,3]  
[1,] -1.914395  5.382047  7.667928  
[2,]  2.177827  5.554363 10.761436  
[3,]  4.722623  7.033206 10.933472  
[4,]  4.529128  7.868546 12.141088  
> A - B  
     [,1]          [,2]          [,3]  
[1,] 3.914395  4.617953 10.332072  
[2,] 1.822173  6.445637  9.238564  
[3,] 1.277377  6.966794 11.066528  
[4,] 3.470872  8.131454 11.858912  
> 5 * A  
     [,1] [,2] [,3]  
[1,]    5    25   45
```

```
[2,]   10   30   50
[3,]   15   35   55
[4,]   20   40   60
> A %*% B # do you understand why this generated an error?
Error in A %*% B : non-conformable arguments
> A %*% t(B)
      [,1]      [,2]      [,3]      [,4]
[1,] -12.99281 4.802567 1.289902 1.141647
[2,] -16.85723 5.296193 2.979203 1.680408
[3,] -20.72165 5.789819 4.668505 2.219170
[4,] -24.58607 6.283445 6.357806 2.757932
> C <- matrix(1:16, nrow=4)
> solve(C) # not all square matrices are invertible!
Error in solve.default(C) : Lapack routine dgesv: system is exactly
    singular
> C <- matrix(rnorm(16), nrow=4) # you'll get a different matrix than I
    did
> C
      [,1]      [,2]      [,3]      [,4]
[1,] -1.6920758 -0.8104245 0.9940420 0.3592050
[2,]  1.5949448 -0.9508142 -0.1960434 -0.5678855
[3,] -1.2443831  0.6400100  0.2645679 -0.8733987
[4,]  0.2129116  0.6719323  0.7494698 -0.3856085
> Cinv <- solve(C) # this should return something that looks like an
    identity matrix
> C %*% Cinv
      [,1]      [,2]      [,3]      [,4]
[1,] 1.000000e+00 -2.360850e-17 6.193505e-17 4.189425e-18
[2,] 2.710844e-17  1.000000e+00 3.577867e-18 -7.264493e-17
[3,] 4.944640e-17  7.643625e-17 1.000000e+00 5.134714e-17
[4,] 1.978161e-17 -1.187201e-17 -4.022390e-17 1.000000e+00
> all.equal(C %*% Cinv, diag(4)) # test approximately equality
[1] TRUE
```

We expect that CC^{-1} should return the above should return the 4×4 identity matrix. As shown above this is true up to the approximate floating point precision of the machine you're operating on.

3.2. Descriptive statistics as matrix functions

Assume you have a data set represented as a $n \times p$ matrix, X , with observations in rows and variables in columns. Below I give formulae for calculating some descriptive statistics as matrix functions.

3.2.1. Mean vector and matrix

You can calculate a row vector of means, \mathbf{m} , as:

$$\mathbf{m} = \frac{1}{n} \mathbf{1}^T X$$

where $\mathbf{1}$ is a $n \times 1$ vector of ones.

A $n \times p$ matrix M where each column is filled with the mean value for that column is:

$$M = \mathbf{1}\mathbf{m}$$

3.2.2. Deviation matrix

To re-express each value as the deviation from the variable means (i.e. each columns is a mean centered vector) we calculate a deviation matrix:

$$D = X - M$$

3.2.3. Covariance matrix

The $p \times p$ covariance matrix can be expressed as a matrix product of the deviation matrix:

$$S = \frac{1}{n-1} D^T D$$

3.2.4. Correlation matrix

The correlation matrix, R , can be calculated from the covariance matrix by:

$$R = VSV$$

where V is a $p \times p$ diagonal matrix where $V_{ii} = 1/\sqrt{S_{ii}}$.

3.2.5. Concentration matrix and Partial Correlations

If the covariance matrix, S is invertible, than inverse of the covariance matrix, S^{-1} , is called the ‘concentration matrix’ or ‘precision matrix’. We can relate the concentration matrix to partial correlations as follow. Let

$$P = S^{-1}$$

Then:

$$\text{corr}(x_i, x_j | X \setminus \{x_i, x_j\}) = -\frac{p_{ij}}{\sqrt{p_{ii}p_{jj}}}$$

where $X \setminus \{x_i, x_j\}$ indicates all variables other than x_j and x_i . You can read this as ‘the correlation between x and y conditional on all other variables.’

Assignment 3.1

Create an R library that includes functions that use matrix operations to calculate each of the descriptive statistics discussed above (except the concentration matrix / partial correlations). Calculate these statistics for `iris` data set and check the results of your functions against the built-in R functions.

3.3. Visualizing Multivariate data in R

Plotting and visualizing multivariate data sets can be challenge and a variety of representations are possible. We cover some of the basic ones here.

Get the file `yeast-subset-clean.txt` from the class website. This data set consists of gene expression measurements on 15 genes from 173 two-color microarray experiments (see Gasch et al. 2000). These genes are members of a gene regulatory network that determines how yeast cells respond to nitrogen starvation. The values in the data set are expression ratios (treatment:control) that have been transformed by applying the \log_2 function (so that a ratio of 1:1 has the value 0, a ratio of 2:1 has the value 1, and a ratio of 1:2 has the value 0.5).

3.3.1. Scatter plot matrix

We already been introduced to the `pairs()` function which creates a set of scatter plots, arranged like a matrix, showing the bivariate relationships for every pair of variables. The size of this plot is p^2 where p is the number of variables so you should only use it for relatively small subsets of variables (maybe up to 7 or 8 variables at a time).

```
> yeast.clean <- read.delim("yeast-subnetwork-clean.txt")
> names(yeast.clean)
[1] "FL08" "RAS2" "TEC1" "PHD1" "ACE2" "SWI5" "SOK2" "RME1" "IME1" "GPA2"
   "MEP2" "IME2" "CLN2"
[14] "ASH1" "MUC1"
> pairs(yeast.clean[1:4]) # create a scatter plot matrix of the first 4
   variables
```

The `pairs` function can be extended in various ways. The package `PerformanceAnalytics`, which is mostly geared for econometrics analyses, has a very nice extended `pairs` function. As discussed in a previous class session you can install packages from the `Packages & Data` menu in the GUI or from the command line as shown below:

```
> install.packages('PerformanceAnalytics', dependencies=T)
> library(PerformanceAnalytics)
> chart.Correlation(yeast.clean[5:8])
```

The output of the `chart.Correlation()` function for this subset of the yeast data is shown in Fig. 3.1. The diagonal of this scatterplot matrix shows the univariate distributions. The lower triangle shows the bivariate relationships, over which has been

superimposed curves representing the ‘LOESS’ regressions for each variable (we’ll discuss LOESS in a later lecture). The upper triangle gives the absolute value of the correlations, with stars indicating significance of the p-value associated with each correlation. So for example, you can see from the figure that the genes SOK2 and RME1 are negatively correlated, and this correlation is significantly different from zero (under the assumption of bivariate normality). Note that there is no correction for multiple comparisons.

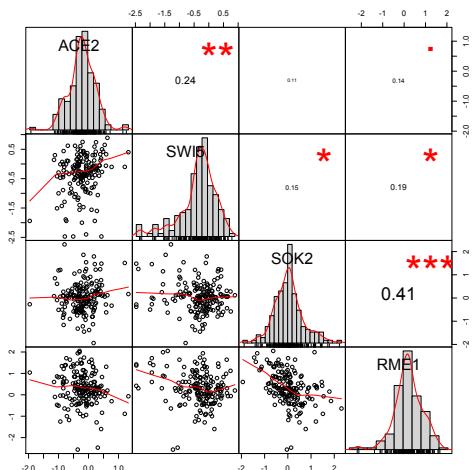


Figure 3.1.: Output of the `chart.Correlation()` function in the `PerformanceAnalytics` package, applied to the yeast expression data set.

3.3.2. 3D Scatter Plots

A three-dimensional scatter plot can come in handy. The R library `lattice` has a function called `cloud()` that allows you to make such plots.

```
> library(lattice)
> cloud(ACE2 ~ ASH1 * RAS2, data=yeast.clean)
> cloud(ACE2 ~ ASH1 * RAS2, data=yeast.clean, screen=list(x=-90, y=70)) # same plot from different angle
```

See the help file for `cloud()` and `panel.cloud()` for information on setting parameters.

3.3.3. Scatterplot3D

There is also a package available on CRAN called `scatterplot3d` with similar functionality.

```
> attach(yeast.clean) # so we can access the variables directly
> install.packages('scatterplot3d', dependencies=T) # installs scatterplot3d
```

```
> library(scatterplot3d) # assumes package is properly installed  
> scatterplot3d(ASH1, RAS2, ACE2)  
> scatterplot3d(ASH1, RAS2, ACE2, highlight.3d=T, pch=20,angle=25)
```

The `highlight.3d` argument colors points to help the viewer determine near and far points. Points that are closer to the viewer are lighter colors (more red in the default color scheme).

Using Package Vignettes

The Scatterplot3D package is quite flexible but this flexibility is hard to grok from the standard R help files (try `?scatterplot3d` to see for yourself). Luckily the Scatterplot3D package includes a ‘vignette’ – a PDF document that discusses the design of the package and illustrates its use. Many packages include such vignettes. To see the list of vignettes available for your installed packages do the following:

```
> vignette(all=T)
```

You should see that the vignette for the Scatterplot3D package is called `s3d`. You can access this vignette as follows, which should open the document in your default PDF viewer.

```
> vignette("s3d")
```

In this case, the ‘good stuff’ (i.e. the examples) starts on page 9 of the vignette.

3.3.4. The `rgl` Package

The 3D plots in `lattice` and `scatterplot3d` are fairly nice, but they don’t allow the user to interact with the figures. For example, wouldn’t it be nice to be able to rotate a 3D scatter of points around to understand the relationships? The `rgl` package allows you to do this, and can produce figures like that shown in Fig. 3.2. Most R figures can be saved using the Save option under the file menu. That’s not the case for `rgl` plots. Instead we need to use the `rgl.postscript()` (creates a postscript or PDF version of the figure) or `snapshot3d()` (creates a screenshot) functions.

```
> install.packages('rgl', dependencies=T)  
> library(rgl)  
> plot3d(ASH1, RAS2, ACE2, col='red', size=1, type='s')  
> rgl.postscript('rgl3d-example.pdf', fmt='pdf')
```

3.3.5. Colored grid plots

A colored grid (or ‘heatmap’) is another way of representing 3D data. It most often is used to represent a variable of interest as a function of two parameters. Grid plots can be created using the `image()` function in R.

```
> x <- seq(0, 2*pi, pi/20)  
> y <- seq(0, 2*pi, pi/20)
```

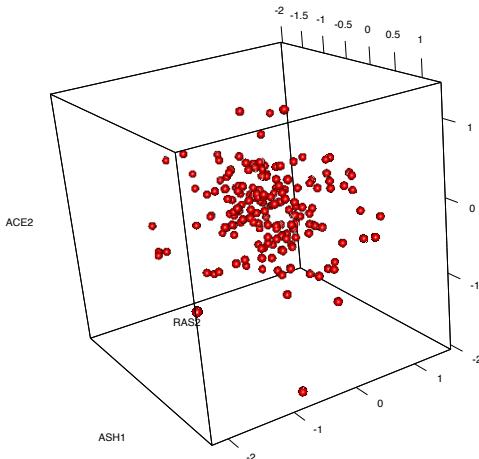


Figure 3.2.: Output of the `plot3d()` function in the `rgl` package.

```
> coolfxn <- function(x,y){
+   cos(x) * cos(y)}
> z <- outer(x,y,coolfxn) # the outer product of two matrices or vectors,
  see docs
> dim(z)
[1] 41 41
> image(x,y,z)
```

The `x` and `y` arguments to `image()` are vectors, the `z` argument is a matrix (in this case created using the outer product operator in conjunction with our function of interest).

A somewhat more flexible function called `levelplot()` is found in the `lattice` package. For example, we can create a similar heatmap using `levelplot()` as follows:

```
> library(lattice)
> levelplot(z) # just the colors
> levelplot(z, contour=T) # colors plus contour lines
```

We can also apply the `levelplot` function to create a representation of a correlation matrix, as shown here:

```
> levelplot(cor(yeast.clean))
```

The default `levelplot()` colors are decent, but let's see how we can change the colors used to our liking. The `colorRampPalette()` function returns a function that interpolates between the values given as arguments to `colorRampPalette()`. So in the example below, it will create a series of colors from blue to white to red.

```
> lvs <- seq(-1,1,0.1) # set thresholds for our colors
> colors <- colorRampPalette(c('blue', 'white', 'red'))(length(lvs))
> levelplot(cor(yeast.clean), col.regions=colors, at=lvs)
```

The `colorRampPalette()` function can also take hexadecimal colors, as is commonly used in HTML. For a list of R colors see <http://research.stowers-institute.org/efg/R/Color/Chart/>. For a list of color schemes, developed by a geographer for effective cartographic representations, see the [ColorBrewer web page](#). For example, here's how to create the representation of the yeast data set correlation matrix shown in Fig. 3.3:

```
# this generates a color ramp from green to black to purple
> colors <- colorRampPalette(c('#1B7837', 'black', '#762A83'))(length(lvls))
  )
> levelplot(cor(yeast.clean), col.regions=colors, at=lvls, scales=list(cex
  =0.6), xlab="", ylab="",main="Correlation Matrix\nYeast Expression Data
  ")
```

The `scales` argument to `levelplot` changes the scaling of the tick marks and labels on the axes.

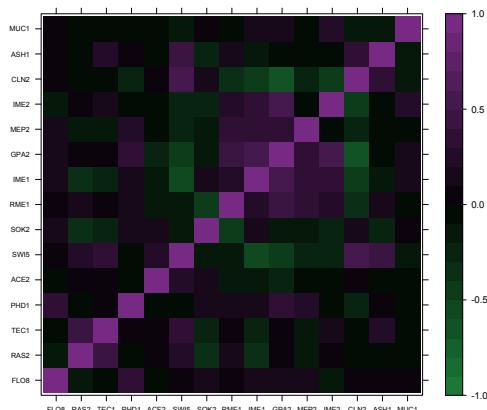


Figure 3.3.: A heatmap, representing the correlation matrix for the yeast expression data set, generated by the `levelplot()` function in the lattice package.

3.4. The Reshape package

`reshape2` is an R package for restructuring, transforming, and summarizing multivariate data sets. `reshape2` was written by Hadley Wickham, a statistician at Rice University, who is also the author of `ggplot`. In this section we'll give a brief overview of the `reshape2` package; for a more detailed discussion see the documentation available on `reshape` web page – [reshape](#). Install the `reshape2` package before proceeding.

The `reshape2` package allows us to restructure and aggregate data more easily than the built-in R functions. There are two primary functions associated with the package

- `melt()` and `cast()`. We use `melt()` to restructure a data frame or list into a generic structure that can then be `cast()` into the form we want.

`melt`

Import the `reshape2` package with `library(reshape2)` and read the docs for the `melt()` function. `melt()` needs at least three arguments: 1) a data frame or list, 2) a vector specifying which columns to treat as ‘identification variables’ (`id.vars`), and 3) a vector specifying which columns to use as ‘measured variables’ (`measured.vars`). ID variables are typically the fixed variables that represent aspects of the experimental design, while measured variables represent the variables that were measured on each unit of interest. If `id.vars` and `measured.vars` aren’t specified, the `melt()` function will try and infer the `id.vars` based on those columns that are factors, and treat the remaining variables as `measured.vars`. If only `id.vars` is specified, the remaining variables will be treated as `measured.vars`.

Let’s create a simple data set that we can use to explore `melt()` and `cast()` functions.

```
> group1 <- c(rep("A", 9), rep("B", 9))
> group2 <- rep(c("1","2","3"),6)
> data1 <- c(rnorm(9,mean=0), rnorm(9,mean=1))
> data2 <- as.vector(t(mapply(rnorm, n = c(3,3,3,3,3,3), mean = c(0,1,3))))
> test.data <- data.frame(species=as.factor(group1),
                           treatment=as.factor(group2),
                           v1=data1, v2=data2)

> test.data
   species treatment        v1        v2
1       A         1  0.75357665 -1.83527194
2       A         2  0.40335481  1.22663973
3       A         3  1.18084161  4.47310654
4       A         1 -0.18393749 -1.61953719
5       A         2 -0.85328571  2.75230914
6       A         3  0.99141392  3.39575430
7       A         1 -1.12026845 -0.61442409
8       A         2 -0.01716075  2.08970130
9       A         3 -1.84389967  2.08822132
10      B         1 -0.30507545 -0.01171179
11      B         2  0.54634457 -0.31179921
12      B         3  2.38310469  4.02453740
13      B         1  0.95729799  0.14273026
14      B         2  3.14992630  1.01718329
15      B         3  1.28400301  2.16849192
16      B         1  0.94616082 -0.26436515
17      B         2  1.19047574  0.58964302
18      B         3  0.35085358  2.46990925
```

Let’s apply `melt()`, specifying the ‘species’ and ‘treatment’ columns as the `id.vars`.

```
> melt.test <- melt(test.data, id.vars = c("species","treatment"))
```

```
> melt.test
  species treatment variable      value
1       A          1     v1  0.75357665
2       A          2     v1  0.40335481
3       A          3     v1  1.18084161
.....
13      B          1     v1  0.95729799
14      B          2     v1  3.14992630
15      B          3     v1  1.28400301
.....
19      A          1     v2  1.91999253
20      A          2     v2  1.76509214
21      A          3     v2  3.33803728
.....
28      B          1     v2  3.63924057
29      B          2     v2  1.81988816
30      B          3     v2  2.00163369
```

Examining the melted data set, you'll see that the columns representing the measured variables have been collapsed into a single new column called 'value'. There is also another column called 'variable' which specifies which of the measured variables the items in 'value' came from.

cast

Having melted our data set, we can then use the `cast()` function to reshape and aggregate the data into the form we desire. Read the docs for `cast()`. Note that the `cast()` function is actually called as `dcast()` or `acast()` depending on whether you want the function to return a data frame or a vector/array. Minimally, `cast()` takes: 1) a melted data set, 2) a formula specifying how to shape the melted data; and 3) a function to apply to any aggregates that are specified for the `cast` formula. These are most easily illustrated by example, as shown below.

In the first example we're going to aggregate the measurements of each variable for each species and calculate the species mean. Notice the form of the formula - `species ~ variable`.

```
> recast.test <- dcast(melt.test, species ~ variable, mean)
> recast.test
  species      v1      v2
1       A -0.07659612 1.328500
2       B  1.16701014 1.091624
```

In the second example, we want to aggregate across species *and* experimental treatments. The resulting values in the table show the per-species-per-treatment means.

```
> recast.test <- dcast(melt.test, species + treatment ~ variable, mean)
> recast.test
  species treatment      v1      v2
1       A          1 -0.1835431 -1.35641107
2       A          2 -0.1556972  2.02288339
```

```

3      A      3  0.1094520  3.31902738
4      B      1  0.5327945 -0.04444889
5      B      2  1.6289155  0.43167570
6      B      3  1.3393204  2.88764619

```

Yeast NanoString Dataset

To illustrate the use of the `reshape2` package in conjunction with `ggplot2` we will use a gene expression data set my lab has generated. This data set includes time series expression measurements on 192 genes, collected on each of four different yeast strains grown under two different media conditions. Each combination of treatments (time point, media condition, strain) was replicated three times. The expression platform used for this study is a technology called [NanoString](#).

Download the data set `yeast-timeseries.csv` from the course wiki. The data file is a plain text file that uses the "comma separated values" format. Use the `read.csv` function to read this data into R.

```

> yeast.time <- read.csv('yeast-timeseries.csv')
> dim(yeast.time)
[1] 108 196
> names(yeast.time)
 [1] "sample.id"    "media"       "strain"      "time.pt"     "replicate"
 [7] "ACE2"         "ACT1"        "ADR1"        "AGA2"        "AMN1"        "ASG7"        "ASH1"
...

```

We want to treat `sample.id`, `media`, `strain`, and `replicate` as factors. Of these, `strain` is the only variable that is not automatically treated as a factor, because the strain names are numbers. Let's change that as follows:

```
> yeast.time$strain <- as.factor(yeast.time$strain)
```

Now let's melt the data set:

```

> yeast.melt <- melt(yeast.time,
+                      id.vars = c("sample.id","strain","media","replicate","time.pt"))
> dim(yeast.melt)
[1] 20628      7

```

For our example we'll aggregate across species, media conditions, and time points and calculate the respective means. Below is the appropriate `dcast()` call and the first few rows and columns of the reshaped matrix.

```

> yeast.cast <- dcast(yeast.melt, strain + media + time.pt ~ variable, mean
+ )
> dim(yeast.cast)
[1] 36 194
> yeast.cast[1:10,1:5]
   strain media time.pt      ACE2      ACT1
1      144 YEPLD      24 479.99997 95666.580
2      144 YEPLD      48 198.66663 50803.660
3      144 YEPLD      72 119.83327 25328.243

```

| | | | | | |
|----|-----|-------|----|-----------|-----------|
| 4 | 144 | YEPLD | 96 | 53.19444 | 18782.137 |
| 5 | 144 | YPD | 0 | 470.88887 | 92196.660 |
| 6 | 144 | YPD | 24 | 481.80550 | 95400.580 |
| 7 | 144 | YPD | 48 | 122.55554 | 40365.830 |
| 8 | 144 | YPD | 72 | 53.55555 | 18454.160 |
| 9 | 144 | YPD | 96 | 53.33333 | 8317.555 |
| 10 | 497 | YEPLD | 24 | 510.72220 | 95666.580 |

Now let's generate a time series plot for the first gene in the data set, ACE2:

```
> ggplot(subset(yeast.cast, media == 'YPD'),
aes(x=time.pt, y=ACE2, col=strain)) + geom_line() + geom_point()
```

Notice the use of the `subset()` function to focus specifically on the YPD media treatment. However, it would be much more useful to be able to compare the two media treatments, YPD and YEPLD, side by side. To do so we can use what ggplot calls a 'facet'. A facet specifies one or more variables to condition against. So if we treat the variable `media` as a facet, we will generate a set of plots that differ only by media type. Here's how to do this with the `facet_wrap()` function from ggplot:

```
> ggplot(yeast.cast, aes(x=time.pt, y=ACE2, col=strain)) + geom_line() +
geom_point() + facet_wrap(facets=c('media'))
```

Your plot should resemble Fig. 3.4. You can examine the times series for different genes by changing the y variable in the ggplot aesthetic (do `name(yeast.cast)` to see all the variable names).

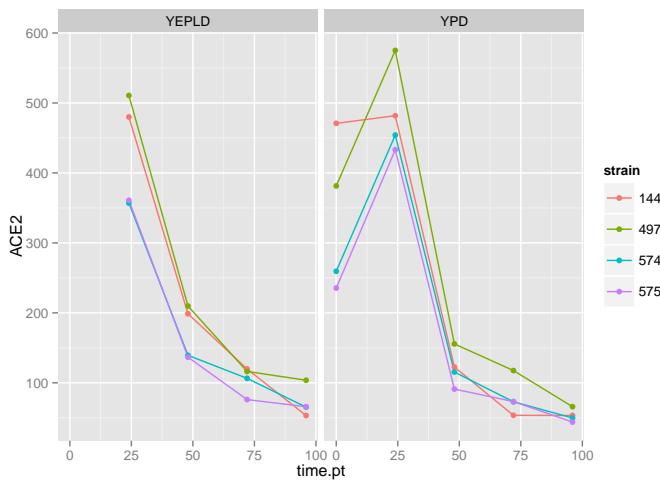


Figure 3.4.: Gene expression time series for four yeast strains, grown in two different media conditions.

Assignment 3.2

Rather than plotting the time series for the two media conditions side-by-side, as in Fig. 3.4, you can place them on the same plot and use different line type (e.g. solid vs. dashed) to distinguish between them (see Fig. 3.5).

Write a function the encapsulates the steps needed to produce a figure shown like that shown in Fig. 3.5. This function should take as input: 1) a dataset with column names as in `yeast.cast` used above; and 2) a string giving the name of the gene you want to plot, e.g. "ACE2" or "MUC1". The function `aes_string()` will be useful for writing this function. For an example of the use of `aes_string()` see the `scatterWithMargins()` function from week 2.

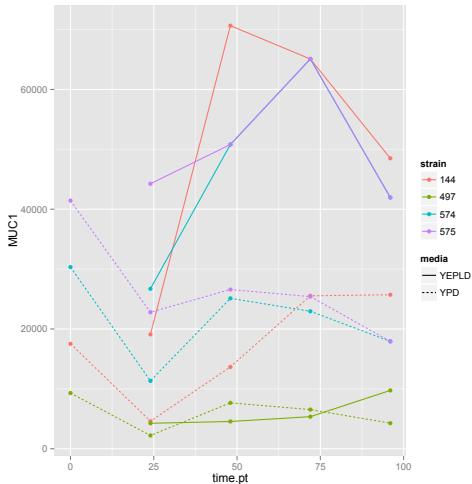


Figure 3.5.: An alternate representation of the expression time series data set.

4. Multiple Regression in R

4.1. Multiple Regression in R

To illustrate multiple regression in R we'll use a built in dataset called `trees`. `trees` consists of measurements of the girth, height, and volume of 31 black cherry trees (`?trees` for more info). We'll start with some summary tables and diagnostic plots to familiarize ourselves with the data:

```
> names(trees)
[1] "Girth" "Height" "Volume"
> dim(trees)
[1] 31  3
> summary(trees)
   Girth        Height       Volume
Min. : 8.30  Min. :63   Min. :10.20
1st Qu.:11.05 1st Qu.:72   1st Qu.:19.40
Median :12.90 Median :76   Median :24.20
Mean   :13.25 Mean   :76   Mean   :30.17
3rd Qu.:15.25 3rd Qu.:80  3rd Qu.:37.30
Max.  :20.60  Max.  :87   Max.  :77.00

# we'll use the chart.Correlation fnx that we introduced last week
> library(PerformanceAnalytics)
> chart.Correlation(trees)
```

As one might expect, the scatterplot matrix shows that all the variables are positively correlated, and girth and volume have a particularly strong correlation.

Let's assume we're lumberjacks, but our permit only allows us to harvest a fixed number of trees. We get paid by the total volume of wood we harvest, so we're interested in predicting a tree's volume (hard to measure directly) as a function of its girth and height (relatively easy to measure), so we can pick the best trees to harvest. We'll therefore calculate a multiple regression of volume on height and width. Let's start by taking a look at the 3D scatter of the data using the `plot3d` function from the `rgl` package.

```
> library(rgl)
> plot3d(trees, col='red', size=1, type='s') # use your mouse to rotate the
   plot
```

From the 3D scatter plot it looks like we ought to be able to find a plane through the data that fits the scatter fairly well. Let's use the `lm()` function to calculate the multiple regression:

```
> l1 <- lm(Volume ~ Girth + Height, data=trees)
```

To visualize the multiple regression, let's use the `scatterplot3d` package to draw the 3D scatter of plots and the plane that corresponds to the regression model:

```
> library(scatterplot3d) # install this package first if needed
> p <- scatterplot3d(trees, angle=55, type='h')
> title('Tree Volume as\na function of Girth and Height')
> p$plane3d(l1, col='orangered')
> dev.copy(pdf, 'trees-regrfit.pdf') # copy plot to a pdf file
> dev.off() # write the file
```

Notice the use of `dev.copy()` and `dev.off()` to save the plot from the console. The output this generates should look similar to Fig. 4.1.

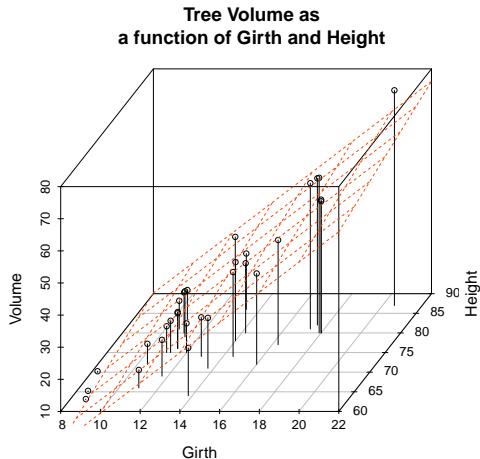


Figure 4.1.: Multiple regression plot of cherry tree volume on girth and height, generated using the `scatterplot3d` library

From the figure it looks like the regression model fits pretty well, as we anticipated from the pairwise relationships. Let's use the `summary()` function to obtain details of the model:

```
> summary(l1)

Call:
lm(formula = Volume ~ Girth + Height, data = trees)
```

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|---------|---------|--------|--------|
| -6.4065 | -2.6493 | -0.2876 | 2.2003 | 8.4847 |

Coefficients:

```

Estimate Std. Error t value Pr(>|t|)
(Intercept) -57.9877    8.6382 -6.713 2.75e-07 ***
Girth        4.7082    0.2643 17.816 < 2e-16 ***
Height       0.3393    0.1302  2.607  0.0145 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 3.882 on 28 degrees of freedom
Multiple R-squared: 0.948, Adjusted R-squared: 0.9442
F-statistic: 255 on 2 and 28 DF, p-value: < 2.2e-16

```

The regression equation is: $\hat{y} = 4.71x_1 + 0.34x_2$, where y is Volume, and x_1 and x_2 are Girth and Height respectively. Since they're on different scales the coefficients for Girth and Height aren't directly comparable. Both coefficients are significant at the $p < 0.05$ level, but note that Girth is the much stronger predictor. In fact the addition of height explains only a minor additional fraction of variation in tree volume, so from the lumberjack's perspective the additional trouble of measuring height probably isn't worth it.

4.1.1. Exploring the Vector Geometry of a Regression Model

The object returned by the `lm()` function hold lots of useful information:

```

> names(l)
[1] "coefficients"   "residuals"      "effects"
      "rank"           "fitted.values" "assign"
[7] "qr"              "df.residual"   "xlevels"
      "call"           "terms"         "model"

```

The `fitted.values` correspond to the predicted values of the outcome variable (\hat{y}). Let's use our knowledge of vector geometry to further explore the relationship between the predicted Volume and the predictor variables. By definition the vector representing the predicted values lies in the plane defined by Height and Girth, so let's do some simple calculations to understand their length and angular relationships:

```

# proportional to length of vectors
> sd(l$fitted.values)
[1] 16.00434
> sd(trees$Height)
[1] 6.371813
> sd(trees$Girth)
[1] 3.138139

# cosines of angles btw vectors
> cor(trees$Height, trees$Girth)
[1] 0.5192801
> cor(trees$Height, l$fitted.values)
[1] 0.6144545
> cor(trees$Girth, l$fitted.values)
[1] 0.9933158

```

```
# angles btw vectors in degrees
> acos(cor(trees$Height, l$fitted.values)) * (180/pi)
[1] 52.08771
> acos(cor(trees$Girth, l$fitted.values)) * (180/pi)
[1] 6.628322
> acos(cor(trees$Girth, trees$Height)) * (180/pi)
[1] 58.71603
```

In class assignment

Using the calculations above you should now be able to sketch out by hand, a diagram depicting the vector relationships between Height, Girth, and the predicted Volume . Once you've finished with your sketch, discuss it with your fellow classmates. Did you get similar answers? If not, discuss it and try to come up with an agreed upon representation.

4.1.2. Exploring the Residuals from the Model Fit

Now let's look at the residuals from the regression. The residuals represent the 'unexplained' variance:

```
> plot(trees$Volume, l$residuals, xlab='Volume', ylab='Regression Residuals')
> abline(h=0, lty='dashed', col='red')
```

Ideally the residuals should be evenly scattered around zero, with no trends as we go from high to low values of the dependent variable. As you can see in Fig. 4.2 it looks like that the residuals on the left tend to be below zero, while those on the far right of the plot are consistently above zero, suggesting that there may be a non-linear aspect of the relationship that our model isn't capturing.

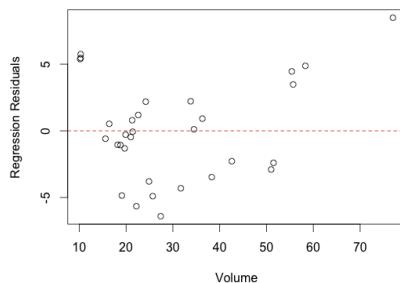


Figure 4.2.: Residual plot based on the multiple regression plot of cherry tree volume on girth and height,

Let's think about the relationships we're actually modeling for a few minutes. For the sake of simplicity let's consider the trunk of a tree to be a cylinder. How do

the dimensions of this cylinder relate to its volume? You can look up the formula for the volume of a cylinder, but the key thing you'll want to note is that volume of the cylinder should be proportional to a characteristic length of the cylinder cubed ($V \propto L^3$). This suggests that if we want to fit a linear model we should relate Girth to $\sqrt[3]{\text{Volume}}$. Let's explore this a little. Since our initial multiple regression suggested that height had relatively little predictive power, we'll simplify our model down to a single predictor:

```
> cuberoot.V <- trees$Volume^0.33
> cor(trees$Volume, trees$Girth)
[1] 0.9671194
> cor(cuberoot.V, trees$Girth)
[1] 0.9777078
> l.orig <- lm(trees$Volume ~ trees$Girth)
> l.transf <- lm(cuberoot.V ~ trees$Girth)
> summary(l.orig)
```

Call:

```
lm(formula = trees$Volume ~ trees$Girth)
```

Residuals:

| Min | 1Q | Median | 3Q | Max |
|--------|--------|--------|-------|-------|
| -8.065 | -3.107 | 0.152 | 3.495 | 9.587 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|--------------|----------|------------|---------|--------------|
| (Intercept) | -36.9435 | 3.3651 | -10.98 | 7.62e-12 *** |
| trees\$Girth | 5.0659 | 0.2474 | 20.48 | < 2e-16 *** |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.252 on 29 degrees of freedom

Multiple R-squared: 0.9353, Adjusted R-squared: 0.9331

F-statistic: 419.4 on 1 and 29 DF, p-value: < 2.2e-16

```
> summary(l.transf)
```

Call:

```
lm(formula = cuberoot.V ~ trees$Girth)
```

Residuals:

| Min | 1Q | Median | 3Q | Max |
|----------|----------|----------|---------|---------|
| -0.18919 | -0.09775 | -0.01488 | 0.07855 | 0.26427 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|--------------|----------|------------|---------|--------------|
| (Intercept) | 0.82543 | 0.08856 | 9.321 | 3.18e-10 *** |
| trees\$Girth | 0.16324 | 0.00651 | 25.076 | < 2e-16 *** |

```
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Residual standard error: 0.1119 on 29 degrees of freedom

Multiple R-squared: 0.9559, Adjusted R-squared: 0.9544

F-statistic: 628.8 on 1 and 29 DF, p-value: < 2.2e-16

Comparing the summary tables, we see indeed that using the cube root of Volume improves the fit of our model some. Let's examine the residuals.

```
> layout(c(1,2), widths=c(3,3), heights=c(2,2))
> plot(trees$Volume, l.orig$residuals, xlab='Volume', ylab="Residuals")
> abline(h = 0, col='red', lty='dashed')
> plot(cuberoot.V, l.transf$residuals,
      xlab='Volume^0.33', ylab='Residuals')

> abline(h = 0, col='red', lty='dashed')
> dev.copy(pdf, 'compare-residuals.pdf')
> dev.off()
> layout(c(1,1)) # reset the layout
```

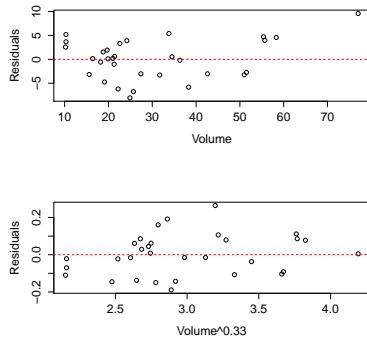


Figure 4.3.: Residual plot based on the bivariate regression of tree volume on girth, or $\sqrt[3]{V}$ on girth

As we can see the transformation we applied to the data did seem to make our residuals more uniform across the range of observations. Note the use of the `layout()` function to put multiple plots in the same figure.

4.1.3. Fitting a curvilinear model using `lm()`

Above we transformed the volume data in order to fit a straight line relationship between $\sqrt[3]{V}$ and Girth. However, we could just as easily have applied a cubic regression to the original variables as shown below (remember this is still linear in the coefficients):

```
> lm.3 <- lm(Volume ~ I(Girth^3), data=trees)
```

```
> summary(lm.3)

Call:
lm(formula = Volume ~ I(Girth^3), data = trees)

Residuals:
    Min      1Q  Median      3Q     Max 
-4.526 -3.036  0.215  2.419  8.291 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept) 8.0426960  1.0426698  7.714 1.66e-08 ***
I(Girth^3)  0.0081365  0.0003118 26.098 < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Residual standard error: 3.379 on 29 degrees of freedom
 Multiple R-squared: 0.9592, Adjusted R-squared: 0.9578
 F-statistic: 681.1 on 1 and 29 DF, p-value: < 2.2e-16

```
> lm.3$coefficients
(Intercept) I(Girth^3)
8.042696007 0.008136533
> a0 = lm.3$coefficients[[1]]
> B1 = lm.3$coefficients[[2]]
> x <- seq(8,25,0.25) # range of values to evaluate model over
> fit <- a0 + B1*x^3
> plot(Volume ~ Girth, data=trees)
> lines(x,fit,col='red')
> figtext <- paste(c("Volume = ", round(a0,2), "+", round(B1,4), "*Girth^3"),
  collapse='')
> text(12, 60, figtext)
```

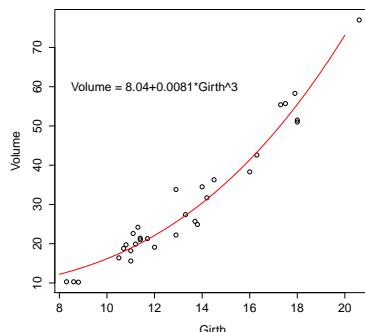


Figure 4.4.: Cubic regression of tree volume on girth

The `I()` function used above requires a little explanation. Normally, the R formula syntax (see `?formula`) treats the carat symbol, '`^`', as short-hand for factor crossing to the specified degree. For example, the formula `(a+b+c)^2` would be interpreted as the model with main effects and all second order interaction terms, i.e. $a + b + c + a:b + a:c + b:c$ where the colons indicate interactions. The `I()` function 'protects' the object in its argument; in this case telling the regression function to treat this as Girth raised to the third power as opposed to trying to construct interaction terms for Girth.

Assignment 4.1

Write a function, `mult.regr(X, y)` that calculates the multiple regression of y on multiple predictors, x_1, x_2, \dots, x_k using matrix operations. Your function should take two arguments, X and y , where X is a matrix representing the predictor variables and y is a vector for the outcome variable. Your function should return a list containing the vector of regression coefficients, B , the coefficient of determination (R^2), and a vector, \hat{y} , representing the fitted values. Refer to the slides from lecture 4 (and possibly lecture 2 if you need a refresher) to review the matrix solution to the regression problem.

4.2. Exploring the impact of nearly collinear predictors on regression

In lecture we discussed the problems that can arise in regression when your predictor variables are nearly collinear. In this section we'll illustrate some of these issues.

Consider again the `trees` data set. Recall that two of the variables – Girth and Volume – are highly correlated and thus nearly collinear.

```
> cor(trees)
      Girth     Height     Volume
Girth  1.0000000  0.5192801  0.9671194
Height  0.5192801  1.0000000  0.5982497
Volume  0.9671194  0.5982497  1.0000000
```

Let's explore what happens when we treat Height as the dependent variable, and Girth and Volume as the predictor variables.

```
> lm.H <- lm(Height ~ Girth + Volume, data = trees)
> summary(lm.H)
```

Call:

```
lm(formula = Height ~ Girth + Volume, data = trees)
```

Residuals:

| | Min | 1Q | Median | 3Q | Max |
|--|---------|---------|--------|--------|---------|
| | -9.7855 | -3.3649 | 0.5683 | 2.3747 | 11.6910 |

Coefficients:

```

Estimate Std. Error t value Pr(>|t|)
(Intercept) 83.2958     9.0866   9.167 6.33e-10 ***
Girth       -1.8615     1.1567  -1.609   0.1188
Volume      0.5756     0.2208   2.607   0.0145 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 5.056 on 28 degrees of freedom
Multiple R-squared:  0.4123,    Adjusted R-squared:  0.3703
F-statistic: 9.82 on 2 and 28 DF,  p-value: 0.0005868

```

We can, of course, fit the linear model despite the collinearity, and we find that the model does have some predictive power, with $R^2 = 0.41$, and with Volume being the more significant predictor.

Now, let's create a slightly different version of the trees data set by add some noise to the three variables. Our goal here is to simulate a data set we might have created had we measured a slightly different set of trees during our sampling. We'll use the jitter function to add uniform noise to the data set.

```

> jitter.Girth <- jitter(trees$Girth, amount= 0.25 * sd(trees$Girth))
> jitter.Height <- jitter(trees$Height, amount= 0.25 * sd(trees$Height))
> jitter.Volume <- jitter(trees$Volume, amount= 0.25 * sd(trees$Volume))
> jitter.trees <- data.frame(Girth = jitter.Girth,
                                Height = jitter.Height,
                                Volume = jitter.Volume)

```

Here we added uniform noise proportional to the one-quarter the standard deviation of each variable. Let's take a moment to convince ourselves that our new data set, `jitter.trees`, is not too different from the `trees` data set from which it was derived.

```

# compare this to summary(trees)
# You will get slightly different answers because jitter adds random noise

> summary(jitter.trees)
   Girth          Height          Volume      
Min.   : 7.913  Min.   :62.31  Min.   :10.75 
1st Qu.:10.971  1st Qu.:72.37  1st Qu.:18.99 
Median :12.606  Median :76.54  Median :22.38 
Mean   :13.170  Mean   :75.84  Mean   :29.77 
3rd Qu.:15.183  3rd Qu.:80.63  3rd Qu.:37.71 
Max.   :20.722  Max.   :85.91  Max.   :77.69 

# correlations among jittered variables are
# similar to those of the original variables

> cor(jitter.trees)
            Girth   Height   Volume
Girth  1.0000000 0.4924240 0.9433214
Height 0.4924240 1.0000000 0.5531763
Volume 0.9433214 0.5531763 1.0000000

```

```
## jittered variables are highly correlated with original variables

> cor(trees$Height, jitter.trees$Height)
[1] 0.9861006
> cor(trees$Girth, jitter.trees$Girth)
[1] 0.9928097
> cor(trees$Volume, jitter.trees$Volume)
[1] 0.9883385

> plot(trees$Height, jitter.trees$Height)
> plot(trees$Girth, jitter.trees$Girth)
> plot(trees$Volume, jitter.trees$Volume)
```

Now that we've convinced ourselves that our jittered data set is a decent approximation to our original data set, let's re-calculate the linear regression, and compare the coefficients of the jittered model to the original model:

```
> lm.H.jitter <- lm(Height ~ Girth + Volume, data = jitter.trees)
> coefficients(lm.H.jitter)
(Intercept)      Girth      Volume
73.3492169   -0.5437115   0.3241854
> coefficients(lm.H)
(Intercept)      Girth      Volume
83.2957705   -1.8615109   0.5755946
```

We see that the coefficients of the linear model have changed quite a bit between the original data and the jittered data. Our model is unstable to relatively modest changes to the data!

Let's draw some plots to illustrate how different the models fit to the original and jittered data are:

```
# draw 3d scatter plots with small points so as not to obscure regression
# planes
> p <- scatterplot3d(x=trees$Girth, y=trees$Volume, z=trees$Height,
angle=15, type='p', pch='.')

# original model
> p$plane3d(lm.H, col='orangered')

# jittered model
> p$plane3d(lm.H.jitter, col='blue')
```

The figure you generated should look something like Fig. 4.5.

Let's do the same comparison for the multiple regression of Volume on Height and Girth. In this case the predictor variables are *not* nearly collinear.

```
> lm.V <- lm(Volume ~ Girth + Height, data = trees)
> lm.V.jitter <- lm(Volume ~ Girth + Height, data = jitter.trees)
> coefficients(lm.V)
(Intercept)      Girth      Height
```

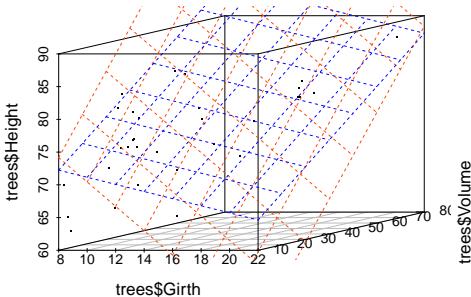


Figure 4.5.: Multiple regression plot of cherry tree height on girth and volume, for the original data (red) and the jittered data (blue).

```
-57.9876589  4.7081605  0.3392512
> coefficients(lm.V.jitter)
(Intercept)      Girth      Height
-51.2670818   4.4798268   0.2906203
```

For this model, we see that the coefficients have changed only a small amount. The underlying data, `jitter.trees`, is the same in both cases, but now our model is stable because the predictor variables are only modestly correlated with each other.

Let's generate another plot to illustrate the similarity of the models fit to the original and jittered data when Girth and Height are used to predict Volume. The corresponding output is shown in Fig. 4.6.

```
> p <- scatterplot3d(x=trees$Girth, y=trees$Height, z=trees$Volume,
                      angle=55, type='p', pch='.')
> p$plane3d(lm.V, col='orangered')
> p$plane3d(lm.V.jitter, col='blue')
```

Finally, let's do some vector calculations to quantify how the angular deviation between the fit data and the predictor variables changes between the original and jittered data set for the two different multiple regressions:

```
# write a quickie fxn to express angle between vectors in degrees
> vec.angle <- function(x,y) { acos(cor(x,y)) * (180/pi) }

# vector angles for fit of Height ~ Girth + Volume (orig)
> vec.angle(lm.H$fitted.values, trees$Girth)
[1] 36.02644
> vec.angle(lm.H$fitted.values, trees$Volume)
[1] 21.29297

# vector angles for fit of Height ~ Girth + Volume (jittered)
```

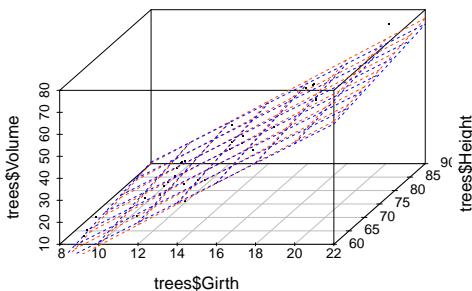


Figure 4.6.: Multiple regression plot of cherry tree volume on girth and height, for the original data (red) and the jittered data (blue).

```

> vec.angle(lm.H.jitter$fitted.values, jitter.trees$Girth)
[1] 28.48079
> vec.angle(lm.H.jitter$fitted.values, jitter.trees$Volume)
[1] 9.097828

# CONCLUSION -- angular changes of about 8 and 12 degrees

# vector angles for fit of Volume ~ Girth + Height (orig)
> vec.angle(lm.V$fitted.values, trees$Girth)
[1] 6.628322
> vec.angle(lm.V$fitted.values, trees$Height)
[1] 52.08771

# vector angles for fit of Volume ~ Girth + Height (jittered)
> vec.angle(lm.V.jitter$fitted.values, jitter.trees$Girth)
[1] 6.163463
> vec.angle(lm.V.jitter$fitted.values, jitter.trees$Height)
[1] 54.33651

# CONCLUSION -- angular changes of about 0.5 and 2 degrees

```

4.3. Manipulating data using split

Last week we introduced the `reshape()` function from the `reshape2` package. `reshape()` is good for computing simple statistics across multiple ‘facets’ of data. However, more complicated statistics are made possible by using the `split()` function, which is defined in the R base package.

`split()` takes two arguments: 1) a vector or data frame to split and 2) a character vector defining what to split the first argument by. For example, we can split the iris data set by species in order to get a list containing three data frames; one for each species.

```
> iris.split <- split(iris, iris$Species)
> names(iris.split)
[1] "setosa"      "versicolor"   "virginica"
> str(iris.split) # see the documentation for the str() function
List of 3
 $ setosa      :'data.frame': 50 obs. of  5 variables:
 ..$ Sepal.Length: num [1:50] 5.1 4.9 4.7 4.6 5 5.4 4.6 5 4.4 4.9 ...
 ..$ Sepal.Width : num [1:50] 3.5 3 3.2 3.1 3.6 3.9 3.4 3.4 2.9 3.1 ...
 ..$ Petal.Length: num [1:50] 1.4 1.4 1.3 1.5 1.4 1.7 1.4 1.5 1.4 1.5 ...
 ..$ Petal.Width : num [1:50] 0.2 0.2 0.2 0.2 0.2 0.4 0.3 0.2 0.2 0.1 ...
 ..$ Species     : Factor w/ 3 levels "setosa","versicolor",...: 1 1 1 1 1
    1 1 1 1 1 ...
$ versicolor:'data.frame': 50 obs. of  5 variables:
 ..$ Sepal.Length: num [1:50] 7 6.4 6.9 5.5 6.5 5.7 6.3 4.9 6.6 5.2 ...
 ..$ Sepal.Width : num [1:50] 3.2 3.2 3.1 2.3 2.8 2.8 3.3 2.4 2.9 2.7 ...
 ..$ Petal.Length: num [1:50] 4.7 4.5 4.9 4 4.6 4.5 4.7 3.3 4.6 3.9 ...
 ..$ Petal.Width : num [1:50] 1.4 1.5 1.5 1.3 1.5 1.3 1.6 1 1.3 1.4 ...
 ..$ Species     : Factor w/ 3 levels "setosa","versicolor",...: 2 2 2 2 2
    2 2 2 2 2 ...
$ virginica  :'data.frame': 50 obs. of  5 variables:
 ..$ Sepal.Length: num [1:50] 6.3 5.8 7.1 6.3 6.5 7.6 4.9 7.3 6.7 7.2 ...
 ..$ Sepal.Width : num [1:50] 3.3 2.7 3 2.9 3 3 2.5 2.9 2.5 3.6 ...
 ..$ Petal.Length: num [1:50] 6 5.1 5.9 5.6 5.8 6.6 4.5 6.3 5.8 6.1 ...
 ..$ Petal.Width : num [1:50] 2.5 1.9 2.1 1.8 2.2 2.1 1.7 1.8 1.8 2.5 ...
 ..$ Species     : Factor w/ 3 levels "setosa","versicolor",...: 3 3 3 3 3
    3 3 3 3 3 ...
```

Now that we have a split data frame, it's easy to use `lapply` or `sapply` to calculate complicated summary statistics. For example, this function calculates the mean ratio of `Sepal.Length` to `Petal.Length`:

```
> ratio.sepall2petal <- function(x) {
+   mean( x$Sepal.Length / x$Petal.Length)
+ }
> sapply(iris.split, ratio.sepall2petal)
  setosa versicolor  virginica
 3.464906   1.400896   1.188350
```

We could also write a function to return the coefficients of fitting a linear model to each facet of the data:

```
> sepal.on.petal.coeff <- function(x){
+   model <- lm(Sepal.Length ~ Petal.Length, data=x)
+   return(model$coeff)
+ }
> sapply(iris.split, sepal.on.petal.coeff)
```

```
      setosa versicolor virginica
(Intercept) 4.2131682  2.407523 1.0596591
Petal.Length 0.5422926  0.828281 0.9957386
```

Of course, no analysis would be complete without examining the fit of the linear models. In order to visualize whether the linear model is a good representation of the data, we'll write another function to return a data frame containing the fitted values, residuals, and species names for each element of the list.

```
> sepal.on.petal.lm.fit <- function(x){
+   model <- lm(Sepal.Length ~ Petal.Length, data=x)
+   data.frame(fitted = fitted(model),
+             residuals = residuals(model),
+             species = x$Species)
+ }
> iris.fit <- lapply(iris.split, sepal.on.petal.lm.fit)
> str(iris.fit)
List of 3
 $ setosa    :'data.frame': 50 obs. of  3 variables:
 ..$ fitted   : num [1:50] 4.97 4.97 4.92 5.03 4.97 ...
 ..$ residuals: num [1:50] 0.1276 -0.0724 -0.2181 -0.4266 0.0276 ...
 ..$ species  : Factor w/ 3 levels "setosa","versicolor",...: 1 1 1 1 1 1 1
               1 1 1 ...
 $ versicolor:'data.frame': 50 obs. of  3 variables:
 ..$ fitted   : num [1:50] 6.3 6.13 6.47 5.72 6.22 ...
 ..$ residuals: num [1:50] 0.7 0.265 0.434 -0.221 0.282 ...
 ..$ species  : Factor w/ 3 levels "setosa","versicolor",...: 2 2 2 2 2 2 2
               2 2 2 ...
 $ virginica  :'data.frame': 50 obs. of  3 variables:
 ..$ fitted   : num [1:50] 7.03 6.14 6.93 6.64 6.83 ...
 ..$ residuals: num [1:50] -0.734 -0.338 0.165 -0.336 -0.335 ...
 ..$ species  : Factor w/ 3 levels "setosa","versicolor",...: 3 3 3 3 3 3 3
               3 3 3 ...
>
```

Next, we'll join the data back into a data frame using the `do.call` and `rbind` functions. Read the documentation to figure out what they do.

```
> iris.joined <- do.call('rbind', iris.fit)
> str(iris.joined)
'data.frame': 150 obs. of  3 variables:
 $ fitted   : num  4.97 4.97 4.92 5.03 4.97 ...
 $ residuals: num  0.1276 -0.0724 -0.2181 -0.4266 0.0276 ...
 $ species  : Factor w/ 3 levels "setosa","versicolor",...: 1 1 1 1 1 1 1 1
               1 1 ...
```

Finally, we'll visualize our model fits by plotting our data using `ggplot`:

```
> library(ggplot2)
> ggplot(iris.joined, aes(x=fitted, y=residuals))+
+   geom_point()+
+   facet_wrap(~species, scale='free') +
```

```
+ ggtitle("Residuals from Regression of \nSepal Length on Petal Length\nfor 3 Iris Species")
```

Examining the residuals (Fig. 4.7), we see they look fairly uniform across the range of fit values. The term that statisticians use for this is ‘homoscedastic’; when the residuals are non uniform we say they are ‘heteroscedastic’.

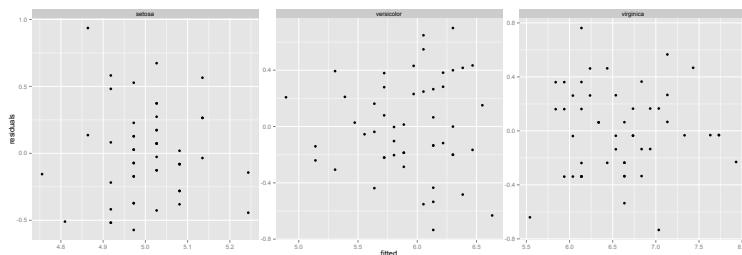


Figure 4.7.: Residuals from regressions of Sepal Length on Petal Length, for the Iris data set split by species.

An alternate way to visualize the fits, without the benefit of getting the info on the model fits back for further examination, is to use the `stat_smooth()` to plot a linear fit of our data for each facet (Fig. 4.8). Read the `stat_smooth` documentation to how this works.

```
> ggplot(iris, aes(x=Petal.Length, y=Sepal.Length)) +
  geom_point() +
  stat_smooth(method="lm") +
  facet_wrap(~Species, scale='free') +
  ggtitle("Regressions of Sepal Length on Petal Length")
```

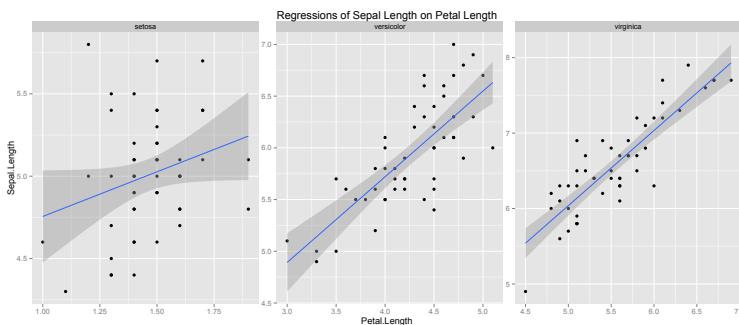


Figure 4.8.: Regressions of Sepal Length on Petal Length, for the Iris data, produced using the `stat_smooth()` function in `ggplot2`.

5. Eigenanalysis and PCA in R

5.1. Eigenanalysis in R

The `eigen()` function computes the eigenvalues and eigenvectors of a square matrix.

```
> A <- matrix(c(2,1,2,3), nrow=2)
> A
[,1] [,2]
[1,]    2    2
[2,]    1    3
> eigen.A <- eigen(A)
> eigen.A
$values
[1] 4 1
$vectors
[,1]      [,2]
[1,] -0.7071068 -0.8944272
[2,] -0.7071068  0.4472136
> V <- eigen.A$vectors
> D <- diag(eigen.A$values) # diagonal matrix of eigenvalues
> Vinv <- solve(V)
> V %*% D %*% Vinv # reconstruct our original matrix (see lecture slides)
[,1] [,2]
[1,]    2    2
[2,]    1    3
> Vinv %*% A %*% V
[,1] [,2]
[1,] 4.000000e+00    0
[2,] 2.220446e-16    1
> all.equal(Vinv %*% A %*% V, D) # test 'near equality'
[1] TRUE
> V[,1] %*% V[,2] # note that the eigenvectors are NOT orthogonal. Why?
[,1]
[1,] 0.3162278
> B <- matrix(c(2,2,2,3), nrow=2) # define another transformation
> B
[,1] [,2]
[1,]    2    2
[2,]    2    3
> eigen.B$values
[1] 4.5615528 0.4384472
> eigen.B$vectors
```

```
[,1]      [,2]
[1,] 0.6154122 -0.7882054
[2,] 0.7882054  0.6154122
> Vb <- eigen.B$vectors
> Vb[,1] %*% Vb[,2] # these eigenvectors ARE orthogonal.
[,1]
[1,]    0
```

As we discussed in lecture, the eigenvectors of a square matrix, A, point in the directions that are unchanged by the transformation specified by A. The following relationships relate A to its eigenvectors and eigenvalues:

$$\mathbf{V}^{-1}\mathbf{A}\mathbf{V} = \mathbf{D}$$

$$\mathbf{A} = \mathbf{V}\mathbf{D}\mathbf{V}^{-1}$$

where **V** is a matrix where the columns represent the eigenvectors, and **D** is a diagonal matrix of eigenvalues.

Since *A* and *B* represent 2D transformations we can visualize the effect of these transformations using points in the plane. We'll show how they distort a set of points that make up a square.

```
# define the corners of a square
> pts <- matrix(c(1,1, 1,-1, -1,-1, -1,1),4,2,byrow=T)
> pts
[,1] [,2]
[1,]    1    1
[2,]    1   -1
[3,]   -1   -1
[4,]   -1    1
> plot(pts,xlim=c(-6,6),ylim=c(-6,6),asp=1) # plot the corners
> polygon(pts) # draw edges of square
> transA <- A %*% t(pts)
> transA
[,1] [,2] [,3] [,4]
[1,]    4    0   -4    0
[2,]    4   -2   -4    2
> newA <- t(transA)
> newA
[,1] [,2]
[1,]    4    4
[2,]    0   -2
[3,]   -4   -4
[4,]    0    2
> points(newA, col='red') # plot the A transformation
> polygon(newA, lty='dashed', border='red')
> newB <- t(B %*% t(pts)) # do the same for the B transformation
> polygon(newB, lty='dashed', border='blue')
> points(newB, col='blue')
```

```
> legend("topleft", c("transformation A","transformation B"),
  lty=c("dashed","dashed"),col=c("red","blue"))
```

The code given above will produce the plot shown in the figure below.

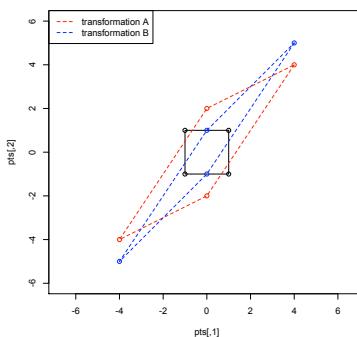


Figure 5.1.: Transformation of a square represented by two matrices, A and B

Assignment 5.1

Fig. 5.2 illustrates the geometry of the eigenvectors for matrices A and B as defined above. Note that the lengths of the eigenvector depictions are scaled to be proportional to their eigenvalues. Write R code to reconstruct this figure.

Extra Credit: For extra credit, write a function called `draw_eigenvector()` that will create a similar figure for any arbitrary matrix that represents a 2D transformation. Your function should take as input a matrix A, and a set of points in the plane. Make sure to include code to handle cases where A is singular.

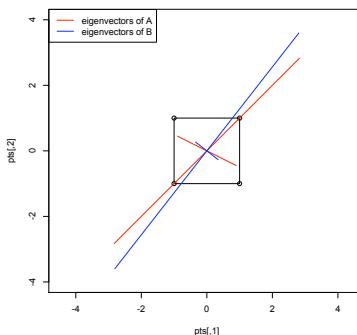


Figure 5.2.: Eigenvectors of matrices A and B

5.2. Principal Components Analysis in R

There are two functions in R for carrying out PCA - `princomp()` and `prcomp()`. The `princomp()` function uses the `eigen()` function to carry out the analysis on the covariance matrix or correlation matrix, while `prcomp()` carries out an equivalent analysis, starting from a data matrix, using a technique called singular value decomposition (SVD). The SVD routine has greater numerical accuracy, so the `prcomp()` function should generally be preferred. The `princomp()` function is also useful when you don't have access to the original data, but you do have a covariance or correlation matrix (a frequent situation when re-analyzing data from the literature). We'll concentrate on using the `prcomp()` function.

5.2.1. Bioenv dataset

To demonstrate PCA we'll use a dataset called 'bioenv.txt' (see class wiki), obtained from a book called "Biplots in Practice" (M. Greenacre, 2010). Here is Greenacre's description of the dataset:

The context is in marine biology and the data consist of two sets of variables observed at the same locations on the sea-bed: the first is a set of biological variables, the counts of five groups of species, and the second is a set of four environmental variables. The data set, called "bioenv", is shown in Exhibit 2.1. The species groups are abbreviated as "a" to "e". The environmental variables are "pollution", a composite index of pollution combining measurements of heavy metal concentrations and hydrocarbons; depth, the depth in metres of the sea-bed where the sample was taken; "temperature", the temperature of the water at the sampling point; and "sediment", a classification of the substrate of the sample into one of three sediment categories.

The first column has no header, and corresponds to the site labels.

```
> b <- read.delim('bioenv.txt', row.names=1) # note use of row.names argument
[1] "a"          "b"          "c"          "d"          "e"
[6] "Pollution"  "Depth"      "Temperature" "Sediment"
```

The columns labeled 'a' to 'e' contain the counts of the five species at each site. We'll work with this abundance data for now.

```
> abund <- subset(b, select=c(a,b,c,d,e))
> boxplot(abund, xlab="Species", ylab="Counts", main="Distribution of\
nSpecies Counts per Site")
```

From the boxplot it looks like the counts for species 'e' are smaller on average, and less variable. The mean and variance functions confirm that.

```
> apply(abund, 2, mean)
    a      b      c      d      e
  1.0  10.0  10.0  10.0  1.0
```

```
13.466667 8.733333 8.400000 10.900000 2.966667
> apply(abund, 2, var)
      a          b          c          d          e
157.63678 83.44368 73.62759 44.43793 15.68851
```

A correlation matrix suggests weak to moderate associations between the variables, but the scatterplot matrix generated by the `chart.Correlation()` function suggests that many of the relationships have a strong non-linear element.

```
> cor(abund)
      a          b          c          d          e
a 1.0000000 0.67339954 -0.23992888 0.358192050 0.273522301
b 0.6733995 1.00000000 -0.08041947 0.501834036 0.036914702
c -0.2399289 -0.08041947 1.00000000 0.081504483 -0.343540453
d 0.3581921 0.50183404 0.08150448 1.000000000 -0.004048517
e 0.2735223 0.03691470 -0.34354045 -0.004048517 1.000000000

> library(PerformanceAnalytics)
> chart.Correlation(abund)
```

5.2.2. PCA of the Bioenv dataset

Linearity is not a requirement for PCA, as it's simply a rigid rotation of the original data. So we'll continue with our analysis after taking a moment to read the help on the `prcomp()` function.

```
> ?prcomp
> a.pca <- prcomp(abund, center=T, retx=T)
  # center=T mean centers the data
  # retx=T returns the PC scores
  # if you want to do PCA on correlation matrix set scale.=T
  #   -- notice the period after scale!

> summary(a.pca)
Importance of components:
                    PC1        PC2        PC3        PC4        PC5 
Standard deviation     14.8653   8.8149   6.2193   5.03477  3.48231 
Proportion of Variance 0.5895   0.2073   0.1032   0.06763  0.03235 
Cumulative Proportion  0.5895   0.7968   0.9000   0.96765  1.00000
```

We see that approximately 59% of the variance in the data is captured by the first PC, and approximately 90% by the first three PCs.

Let's compare the values returned by PCA to what we would get if we carried out eigenanalysis of the covariance matrix that corresponds to our data.

```
> a.pca
Standard deviations:
[1] 14.865306 8.814912 6.219250 5.034774 3.482308

Rotation:
```

```

          PC1        PC2        PC3        PC4        PC5
a  0.81064462  0.07052882 -0.53108427  0.18442140 -0.14771336
b  0.51264394 -0.27799671  0.47711910 -0.63418946  0.17342177
c -0.16235135 -0.88665551 -0.40897655 -0.01149647  0.14173943
d  0.22207108 -0.31665237  0.56250980  0.72941223 -0.04422938
e  0.06616623  0.17696554 -0.08141111  0.17781482  0.96231977
> eigen(cov(abund))
$values
[1] 220.97732  77.70266  38.67908  25.34895  12.12647

$vectors
      [,1]      [,2]      [,3]      [,4]      [,5]
[1,] 0.81064462 -0.07052882  0.53108427  0.18442140 -0.14771336
[2,] 0.51264394  0.27799671 -0.47711910 -0.63418946  0.17342177
[3,] -0.16235135  0.88665551  0.40897655 -0.01149647  0.14173943
[4,]  0.22207108  0.31665237 -0.56250980  0.72941223 -0.04422938
[5,]  0.06616623 -0.17696554  0.08141111  0.17781482  0.96231977

```

Notice that the ‘rotation’ object returned by the `prcomp` function are the scaled eigenvectors (scaled to have length 1). The standard deviations of the PCA are the square roots of the eigenvalues of the covariance matrix.

5.2.3. Calculating Factor Loadings

Let’s calculate the ‘factor loadings’ associated with the PCs:

```

> V <- a.pca$rotation # eigenvectors
> L <- diag(a.pca$sdev) # diag mtx w/sqrt of eigenvalues on diag.

> a.loadings <- V %*% L
> a.loadings
      [,1]      [,2]      [,3]      [,4]      [,5]
a 12.0504801  0.6217053 -3.3029460  0.92852016 -0.5143835
b  7.6206090 -2.4505164  2.9673232 -3.19300085  0.6039081
c -2.4134024 -7.8157898 -2.5435276 -0.05788214  0.4935804
d  3.3011545 -2.7912626  3.4983893  3.67242602 -0.1540203
e  0.9835813  1.5599356 -0.5063161  0.89525751  3.3510942

```

The magnitude of the loadings is what you want to focus on. For example, species ‘a’ and ‘b’ contribute most to the first PC, while species ‘c’ has the largest influence on PC2.

You can think of the loadings, as defined above, as the components (i.e lengths of the projected vectors) of the original variables with respect to the PC basis vectors. Since vector length is proportional to the standard deviation of the variables they represent, you can think of the loadings as giving the standard deviation of the original variables with respect the PC axes. This implies that the loadings squared sum to the total variance in the original data, as illustrated below.

```

> apply(a.loadings**2, 1, sum)
     a       b       c       d       e

```

```
157.63678 83.44368 73.62759 44.43793 15.68851
> apply(abund, 2, var)
      a         b         c         d         e
157.63678 83.44368 73.62759 44.43793 15.68851
```

5.2.4. Drawing Figures to Represent PCA

PC Score Plots

The simplest PCA figure is to depict the PC scores, i.e. the projection of the observations into the space defined by the PC axes. Let's make a figure with three subplots, depicting PC1 vs PC2, PC1 vs PC3, and PC2 vs. PC3.

```
> par(mfrow=c(1,3))
> plot(a.pca$x[,1], a.pca$x[,2],asp=1,pch=16, xlab='PC1', ylab='PC2',xlim=c
(-30,30),ylim=c(-30,30))
> plot(a.pca$x[,1], a.pca$x[,3],asp=1,pch=16, xlab='PC1', ylab='PC3',xlim=c
(-30,30),ylim=c(-30,30))
> plot(a.pca$x[,2], a.pca$x[,3],asp=1,pch=16, xlab='PC2', ylab='PC3',xlim=c
(-30,30),ylim=c(-30,30))
```

Note that you should always set `asp=1` when plotting PC scores, so that the distances between points are accurate representations. Note too that I used the `xlim` and `ylim` arguments to keep the axis limits the same in all plots; comparable scaling of axes is important when comparing plots. Also note the use of the `mfrow` argument to `par()` in order to setup a multicolumn plot.

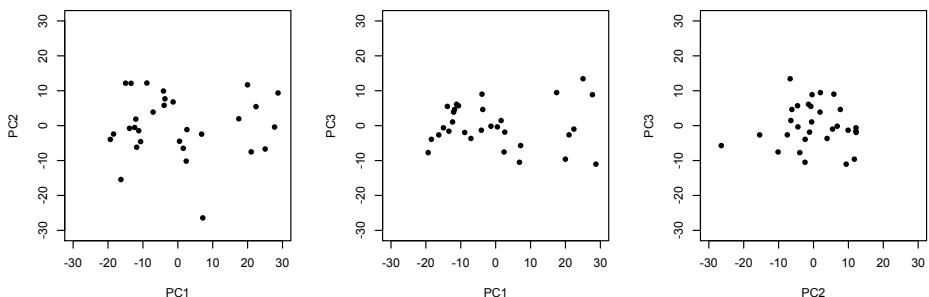


Figure 5.3.: Projection of the bioenv dataset into the basis defined by the first three PCs.

As we did in previous weeks we can also use one of the 3D plotting functions to make a 3D scatterplot of the scores.

```
> library(rgl)
> plot3d(a.pca$x[,1:3], asp=1, type='s', xlim=c(-30,30), ylim=c(-30,30),
zlim=c(-30,30),col='red',size=2)
```

Simultaneous Depiction of Observations and Variables in the PC Space

Let's return to our simple PC score plot. As we discussed above, the loadings are components of the original variables in the space of the PCs. This implies we can depict those loadings in the same PC basis that we use to depict the scores.

```
> plot(a.pca$x[,1], a.pca$x[,2],asp=1,pch=16, xlab='PC1', ylab='PC2',xlim=c(-30,30),ylim=c(-30,30))

# get the loadings for each variable w/respect to PCs 1 and 2
> load2d.a <- a.loadings[1,1:2]
> load2d.b <- a.loadings[2,1:2]
> load2d.c <- a.loadings[3,1:2]
> load2d.d <- a.loadings[4,1:2]
> load2d.e <- a.loadings[5,1:2]

# draw arrows depicting Loadings
> arrows(0, 0, load2d.a[1], load2d.a[2], length=0.1, col='red')
> text(load2d.a[1], load2d.a[2], 'a', col='red')
> arrows(0, 0, load2d.b[1], load2d.b[2], length=0.1, col='red')
> text(load2d.b[1], load2d.b[2], 'b', col='red')
> arrows(0, 0, load2d.c[1], load2d.c[2], length=0.1, col='red')
> text(load2d.c[1], load2d.c[2], 'c', col='red')
> arrows(0, 0, load2d.d[1], load2d.d[2], length=0.1, col='red')
> text(load2d.d[1], load2d.d[2], 'd', col='red')
> arrows(0, 0, load2d.e[1], load2d.e[2], length=0.1, col='red')
> text(load2d.e[1], load2d.e[2], 'e', col='red')
```

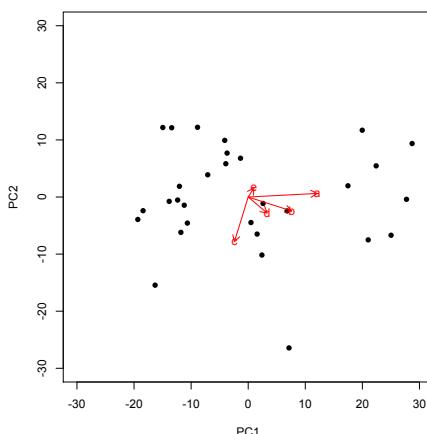


Figure 5.4.: PCA of the bioenv dataset. This biplot represents both the observations (black points) and variables (red vectors) in the space of PCs 1 and 2.

The output of the code above should look like Fig. 5.4. Fig. 5.4 is called a ‘biplot’, as it simultaneously depicts both the observations and variables in the same space. From this biplot we can immediately see that variable ‘a’ is highly correlated with PC1, but only weakly associated with PC2. Conversely, variable ‘c’ is strongly correlated with PC2 but only weakly so with PC1. We can also approximate the correlations among the variables themselves – for example ‘b’ and ‘d’ are fairly strongly correlated, but weakly correlated with ‘c’. Keep in mind however that with respect to the relationships among the variables, this visualization is a 2D projection of a 5D space so the geometry is approximate.

The biplot is a generally useful tool for multivariate analyses and there are a number of different ways to define biplots. We’ll study biplots more formally in a few weeks after we’ve covered singular value decomposition.

Assignment 5.2

Do a PCA analysis on the iris data set with all three species pooled together. Generate a plot showing the projection of the specimens on the first two PC axes as shown in Fig. 5.5. Represent the specimens from a given species with different colors. Make sure you include a legend for your plot.

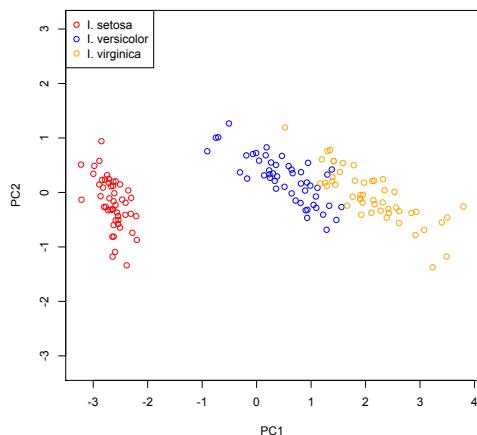


Figure 5.5.: PCA of the iris data set. One of your assignments is to reconstruct this figure on your own.

6. Singular value decomposition

6.1. SVD in R

If A is an $n \times p$ matrix, and the singular value decomposition of A is given by $A = USV^T$, the columns of the matrix V^T are the eigenvectors of the square matrix $A^T A$ (sometimes referred to as the minor product of A). The singular values of A are equal to the square roots of the eigenvalues of $A^T A$.

The `svd()` function computes the singular value decomposition of an arbitrary rectangular matrix. Below I demonstrate the use of the `svd()` function and confirm the relationships described above:

```
> A <- matrix(c(2,1,2,3), nrow=2)
> A
[,1] [,2]
[1,]    2    2
[2,]    1    3
> a.svd <- svd(A)
> a.svd$u
[,1]      [,2]
[1,] -0.6618026 -0.7496782
[2,] -0.7496782  0.6618026
# R uses the notation  $A = u d v'$  rather than  $A = u s v'$ 
> a.svd$d
[1] 4.1306486 0.9683709
> all.equal(A, a.svd$u %*% diag(a.svd$d) %*% t(a.svd$v))
[1] TRUE
> AtA <- t(A) %*% A
> eigen.AtA <- eigen(AtA)
> eigen.AtA
$values
[1] 17.0622577  0.9377423
$vectors
[,1]      [,2]
[1,] 0.5019268 -0.8649101
[2,] 0.8649101  0.5019268
> all.equal(a.svd$d, sqrt(eigen.AtA$values))
[1] TRUE
```

As we discussed in lecture, the eigenvectors of square matrix, A , point in the directions that are unchanged by the transformation specified by A .

6.1.1. Writing our own PCA function

In lecture we discussed the relationship between SVD and PCA. Let's walk through some code that carries out PCA via SVD, and then we'll implement our own PCA function.

```
> i.sub <- subset(iris, select=-Species)
> i.ctr <- scale(i.sub, center=T, scale=F)
> i.svd <- svd(i.ctr)

> U <- i.svd$u
> S <- diag(i.svd$d)
> V <- i.svd$v

> pc.scores <- U %*% S
# compare to fig 5.5 in your workbook
> plot(pc.scores, asp=1, col=c('red', 'darkolivegreen', 'blue')[iris$Species], pch=16)

> n <- nrow(i.ctr)
> pc.sdev <- sqrt((S**2/(n-1)))
> pc.sdev
      [,1]      [,2]      [,3]      [,4]
[1,] 2.056269 0.0000000 0.0000000 0.0000000
[2,] 0.000000 0.4926162 0.0000000 0.0000000
[3,] 0.000000 0.0000000 0.2796596 0.0000000
[4,] 0.000000 0.0000000 0.0000000 0.1543862

> V
      [,1]      [,2]      [,3]      [,4]
[1,] 0.36138659 -0.65658877 0.58202985 0.3154872
[2,] -0.08452251 -0.73016143 -0.59791083 -0.3197231
[3,] 0.85667061 0.17337266 -0.07623608 -0.4798390
[4,] 0.35828920 0.07548102 -0.54583143 0.7536574
```

For comparison, here's what the built-in `prcomp` function gives us:

```
> i.pca <- prcomp(i.ctr)
> i.pca$sdev
[1] 2.0562689 0.4926162 0.2796596 0.1543862
> i.pca$rotation
          PC1        PC2        PC3        PC4
Sepal.Length 0.36138659 -0.65658877 0.58202985 0.3154872
Sepal.Width -0.08452251 -0.73016143 -0.59791083 -0.3197231
Petal.Length 0.85667061 0.17337266 -0.07623608 -0.4798390
Petal.Width  0.35828920 0.07548102 -0.54583143 0.7536574
```

Now that we have a sense of the key calculations, let's turn this into a function. Save the following code in file named `mypca.R`.

```
# a user defined version of principal components analysis
PCA <- function(X, center=T, scale=F){
  x <- scale(X, center=center, scale=scale)
  n <- nrow(x)
  p <- ncol(x)

  x.svd <- svd(x)
  U <- x.svd$u
  S <- diag(x.svd$d)
  V <- x.svd$v

  # check for zero eigenvalues
  tolerance = .Machine$double.eps^0.5
  has.zero.singval <- any(x.svd$d <= tolerance)
  if(has.zero.singval)
    print("WARNING: Zero singular values detected")

  pc.scores <- U %*% S
  pc.sdev <- diag(sqrt((S**2/(n-1))))
  return(list(vectors = V, scores=pc.scores, sdev = pc.sdev))
}
```

Note I also included some code to warn the user when the covariance matrix is singular. Use the help to read about variables defined in ‘.Machine’.

Let's put our function through it's paces:

```
> source('mypca.R')
> iris.pca <- PCA(i.sub)
> plot(iris.pca$scores, asp=1)

> sing.pca <- PCA(t(i.sub)) # should have singular values equal to zero
[1] "WARNING: Zero singular values detected"

> tree.pca <- PCA(trees)
> tree.pca$sdev
[1] 17.1834214 4.9820035 0.7485858
> prcomp(trees)$sdev # compare to prcomp
[1] 17.1834214 4.9820035 0.7485858
```

To bring things full circle, let's make sure that the covariance matrix we reconstruct from our PCA analysis is equal to the covariance matrix calculated directly from the data set:

```
> n <- nrow(i.sub)
> V <- iris.pca$vectors
> S <- diag(sqrt(iris.pca$sdev**2 * (n-1))) # turn sdev's back into
   singular values
> reconstructed.cov <- (1/(n-1)) * V %*% S %*% S %*% t(V) # see pg. 11 of
   slides
> all.equal(reconstructed.cov, cov(i.sub), check.attributes=F)
```

```
[1] TRUE
```

Great! It seems like things are working as expected.

6.2. Creating Biplots in R

To illustrate the construction of biplots we'll use the iris data set. The built-in R function is `biplot()`.

```
# leave out the Species variable
> iris.vars <- subset(iris, select=-Species)
# read the prcomp docs and note differences from princomp
> iris.pca <- prcomp(iris.vars)
> summary(iris.pca)

Importance of components:
              PC1       PC2       PC3       PC4
Standard deviation   2.0563  0.49262  0.2797  0.15439
Proportion of Variance 0.9246  0.05307  0.0171  0.00521
Cumulative Proportion  0.9246  0.97769  0.9948  1.00000

> ?biplot # read the help for biplot
> ?biplot.prcomp # more detailed info on how biplot works with objects
     return by prcomp
> biplot(iris.pca, scale=1) # scale = 1 - alpha
# change the biplot scaling - how does this differ?
> biplot(iris.pca, scale=0)
```

Note that the `scale` argument to `biplot` sets the α value we discussed during lecture, however `scale = 1 - α` (i.e. if `scale = 1`, $\alpha = 0$, and if `scale = 0`, $\alpha = 1$).

Assignment 6.1

1. Apply PCA to the `yeast-subnetwork-clean.txt` data set.
2. Create biplots in the first two principal components using both $\alpha = 0$ and $\alpha = 1$ (i.e. the `scale` argument to `biplot`).
3. In your biplots change the labels for the observations to integers using the `xlab` argument to `biplot()`. To make the plot more readable use the `cex` argument to `biplot` to make the font size for the observations half the size of the variable labels.
4. An obvious pattern emerges in the biplot with respect to the gene MEP2. What is this pattern? What subset of conditions (rownames) is most closely related to the vector representing MEP2?

6.3. Data compression and noise filtering using SVD

Two common uses for singular value decomposition are for data compression and noise filtering. Will illustrate these with two examples involving matrices which represent image data. This example is drawn from an article by David Austin, found on a tutorial about SVD at the American Mathematical Society Website ([link](#)).

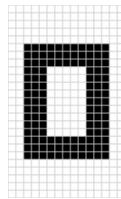
6.3.1. Data compression

Download the file `zeros.dat` from the course wiki. This is a 25×15 binary matrix that represents pixel values in a simple binary (black-and-white) image.

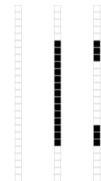
```
> z <- read.delim('zero.dat', header=F)
> z
   V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15
1  1  1  1  1  1  1  1  1  1  1  1  1  1  1  1
2  1  1  1  1  1  1  1  1  1  1  1  1  1  1  1
...
... output truncated ...

# we'll use the image() function to visualize z
> image(1:15, 1:25, t(z), col=c('black', 'white'), asp=1)
```

This matrix data is shown below in a slightly different form that emphasizes the individual elements of the matrix. As you can see, this matrix can be thought of as being composed of just three types of vectors.



(a) The 'zero' matrix.



(b) The three vector types in the 'zero' matrix.

If SVD is working like expected it should capture that feature of our input matrix, and we should be able to represent the entire image using just three singular values and their associated left- and right-singular vectors.

```
> zsvd <- svd(z)
> round(zsvd$d, 2)
[1] 14.72 5.22 3.31 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
     0.00 0.00 0.00
[15] 0.00
> D <- diag(zsvd$d[1:3])
> D
      [,1]      [,2]      [,3]
```

```
[1,] 14.72425 0.000000 0.000000
[2,] 0.00000 5.216623 0.000000
[3,] 0.00000 0.000000 3.314094
> U <- zsvd$u[,1:3]
> V <- zsvd$v[,1:3]
> newZ <- U %*% D %*% t(V)
> all.equal(newZ, z, check.attributes=F)
[1] TRUE

# and let's double check using the image() function
> image(1:15,1:25,t(newZ),col=c('black','white'),asp=1)
```

Our original matrix required 25×15 ($= 375$) storage elements. Using the SVD we can represent the same data using only $15 \times 3 + 25 \times 3 + 3 = 123$ units of storage (corresponding to the truncated U, V, and D in the example above). Thus our SVD allows us to represent the same data with at less than 1/3 the size of the original matrix. In this case, because all the singular values after the 3rd were zero this is a lossless data compression procedure.

6.3.2. Noise filtering using SVD

The file `noisy-zero.dat` is the same 'zero' image, but now sprinkled with Gaussian noise draw from a normal distribution ($N(0, 0.1)$). As in the data compression case we can use SVD to approximate the input matrix with a lower-dimensional approximation. Here the SVD is 'lossy' as our approximation throws away information. In this case we hope to choose the approximating dimension such that the information we lose corresponds to the noise which is 'polluting' our data.

```
> nz <- as.matrix(read.delim('noisy-zero.dat',header=F))
> dim(nz)
[1] 25 15
> x <- 1:15
> y <- 1:25
# create a gray-scale representation of the matrix
> image(x,y,t(nz),asp=1,xlim=c(1,15),ylim=c(1,25),col=gray(seq(0,1,0.05)))
> round(nz.svd$d,2)
[1] 13.63 4.87 3.07 0.40 0.36 0.31 0.27 0.26 0.21 0.19 0.13
     0.11 0.09 0.06
[15] 0.04
# as before the first three singular values dominate
> nD <- diag(nz.svd$d[1:3])
> nU <- nz.svd$u[,1:3]
> nV <- nz.svd$v[,1:3]
> approx.nz <- nU %*% nD %*% t(nV)

# now plot the original and approximating matrix side-by-side
> par(mfrow=c(1,2))
> image(x,y,t(nz),asp=1,xlim=c(1,15),ylim=c(1,25),col=gray(seq(0,1,0.05)))
```

```
> image(x,y,t(approx.nz),asp=1,xlim=c(1,15),ylim=c(1,25),col=gray(seq(0,1,0.05)))
```

As you can see from the images you created the approximation based on the approximation based on the SVD manages to capture the major features of the matrix and filters out much of (but not all) the noise.

6.4. Image Approximation Using SVD in R

R doesn't have native support for common image files like JPEG and PNG. However, there are a couple of packages we can install that will allow us to read in such files and treat them as matrices:

```
> install.packages("png", dependencies=T)
> install.packages("jpeg", dependencies=T)
```

The png and jpeg libraries provide simple functions for reading and writing image files. The following code shows how to read in the chesterbw.jpg image which can be found in the course datasets.

The function grid.raster in the grid library can be used to draw the matrix of image data returned from the readJPEG. There is also a lower-level rasterImage() function that can be used to draw images, as shown below. The image() function included in R base will also draw images, but to do so conveniently we'll write a simple wrapper function called GreyscaleImage().

```
> library(jpeg)
> img <- readJPEG("chesterbw.jpg")
> dim(img)
[1] 556 605
> typeof(img)
[1] "double"
> class(img)
[1] "matrix"
> ny <- dim(img)[1] # rasterImage will draw rows along vertical axis
> nx <- dim(img)[2]
> max.pixels <- max(nx,ny)
> plot(0:max.pixels, 0:max.pixels, type='n', xlab='', ylab='', asp=1)
> ?rasterImage
> rasterImage(img, 0, 0, nx, ny)
> library(grid) # provides grid.raster function
> ?grid.raster
> grid.raster(img) # more convenient but less flexible than rasterImage
> GreyscaleImage <- function(im){
+   rotated <- t(im[rev(1:nrow(im)), 1:ncol(im)])
+   image( rotated, col= grey(seq(0,1, length=256)), useRaster=TRUE )
+ }
> GreyscaleImage(img)
```

The output of the code above is shown in Fig 6.2.



Figure 6.2.: My ever-faithful companion Chester.

Now we'll use SVD to create a low-dimensional approximation of this image.

```
> img.svd <- svd(img)
> U <- img.svd$u
> S <- diag(img.svd$d)
> Vt <- t(img.svd$v)

> U15 <- U[,1:15] # first 15 left singular vectors
> S15 <- S[1:15,1:15] # first 15 singular values
> Vt15 <- Vt[1:15,] # first 15 right singular values, NOTE: we're getting
  rows rather than columns here

> approx15 <- U15 %*% S15 %*% Vt15
> GreyScaleImage(approx15)
```

The output of our approximate image is shown in Fig 6.3.



Figure 6.3.: A low-dimensional approximation of Chester.

Above we created a rank 15 approximation to the rank 556 original image matrix. This approximation is crude (as judged by the visual quality of the approximating image) but it does represent a very large savings in space. Our original image required the storage of $605 \times 556 = 336380$ integer values. Our approximation requires the storage of only $15 \times 556 + 15 \times 605 + 15 = 17430$ integers. This is a saving of roughly 95%. Of course, as with any lossy compression algorithm, you need to decide

what is the appropriate tradeoff between compression and data loss for your given application.

Finally, let's look at the 'error term' associated with our approximation, i.e. what we *did not* capture in the 15 singular vectors.

```
> img.diff <- img - approx15  
> GreyScaleImage(img.diff)
```

An image representing the information our approximation didn't capture is shown in Fig 6.4.

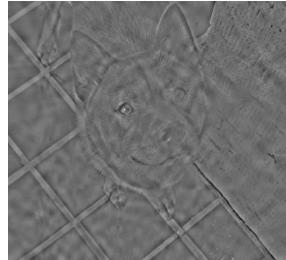


Figure 6.4.: A representation of the information *not* captured by our approximation.

Assignment 6.2

Write a function, `svd_img()`, that automates the creation of a lower dimensional approximation of a grayscale image using SVD.

1. Your function should take as input a matrix representing the original image and an integer specifying the approximating dimension - i.e. function will be called as `svd_img(imgmtx, dim)`.
2. Your function should return a list of two objects: 1) an array representing the approximated image; and 2) an array representing the difference between the original and approximating images (i.e. original - approximation).
3. Test your function on various images using a variety of approximating dimensions (e.g. 5, 10, 25, 50, 100, 250) on the `chesterbw.jpg` image.

In addition to your code consider the following questions:

- When analyzing `chesterbw.jpg`, at some approximating dimensions you'll notice interesting artifacts. How do these relate to the original image?
- What is the lowest approximating dimension where you would consider the image to be recognizable as a dog?
- At what approximating dimension would you judge the image to be "close enough" to the original by the casual observer? What is the storage saving of this approximation relative to the original image?
- How does the difference array change as the approximating dimension changes? Is there a particular type of image information that seems most prominent in the difference array?

7. ANOVA and Discriminant Analysis

7.1. ANOVA in R

We'll start our introduction to ANOVA in R by reconstructing the example used in the lectures slides:

```
> y <- c(20, 17, 17, 21, 16, 14, 17, 16, 15, 8, 11, 8)
> groups <- c(1,1,1, 2,2,2, 3,3,3, 4,4,4)
> group.factor <- as.factor(groups)
```

Since we're doing this example by hand, let's check that our entries were correct by comparing the grand and group means to the example in the slides:

```
> mean(y) # grand mean
[1] 15

# means of each group
> mean(y[group.factor == 1])
[1] 18
> mean(y[group.factor == 2])
[1] 17
> mean(y[group.factor == 3])
[1] 16
> mean(y[group.factor == 4])
[1] 9
```

The `aov()` function in R is suitable for doing ANOVA with balanced designs.

```
> ex.anova <- aov(y ~ group.factor)
> ex.anova
Call:
aov(formula = y ~ group.factor)
```

Terms:

| | group.factor | Residuals |
|-----------------|--------------|-----------|
| Sum of Squares | 150 | 40 |
| Deg. of Freedom | 3 | 8 |

Residual standard error: 2.236068

Estimated effects may be unbalanced

```
> summary(ex.anova)
      Df Sum Sq Mean Sq F value    Pr(>F)
group.factor  3     150      50      10 0.00441 ***
Residuals     8      40       5
```

```
---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> coefficients(ex.anova)
(Intercept) group.factor2 group.factor3 group.factor4
18           -1            -2            -9
```

The ANOVA table shown by `summary()` looks the same as what I presented in the slides, but the model coefficients don't look the same because by default `aov()` uses dummy coding. To see how R creates contrasts from our grouping variable use the `contrasts()` function:

```
> contrasts(group.factor)
 2 3 4
1 0 0 0
2 1 0 0
3 0 1 0
4 0 0 1
```

We can interpret the above as saying that samples from group 1 get coded as '0 0 0', those from group 2 as '1 0 0', etc.

We can use `contrasts()` in combination with `contr.sum()` to change this to effect coding. The argument to `contr.sum()` should be the total number of groups:

```
> contrasts(group.factor) <- contr.sum(4)
> contrasts(group.factor)
 [,1] [,2] [,3]
1     1     0     0
2     0     1     0
3     0     0     1
4    -1    -1    -1
```

Now that we've changed the matrix of contrasts, let's refit the model:

```
> anova.2 <- aov(y ~ group.factor)
> summary(anova.2)
   Df Sum Sq Mean Sq F value    Pr(>F)
group.factor  3    150      50     10 0.00441 **
Residuals    8     40       5
---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> coefficients(anova.2)
(Intercept) group.factor1 group.factor2 group.factor3
15           3             2             1
```

7.1.1. ANOVA via Multiple Regression

In lecture we discussed how ANOVA can be fit as a multiple regression, using grouping variables as the predictor variables. Let's confirm that, first by hand and then using the `lm()` function:

```
> Y <- matrix(y)
```

```
> X <- model.matrix(~group.factor) # note the leading tilde
>      # X will be effect coding if you used the contr.sum function above
>      # otherwise will be dummy coding
> X
  (Intercept) group.factor1 group.factor2 group.factor3
1             1            1            0            0
2             1            1            0            0
3             1            1            0            0
4             1            0            1            0
5             1            0            1            0
6             1            0            1            0
7             1            0            0            1
8             1            0            0            1
9             1            0            0            1
10            1           -1           -1           -1
11            1           -1           -1           -1
12            1           -1           -1           -1
attr(",assign")
[1] 0 1 1 1
attr(",contrasts")
attr(",contrasts")$group.factor
 [,1] [,2] [,3]
1    1    0    0
2    0    1    0
3    0    0    1
4   -1   -1   -1

>
> b <- solve(t(X) %*% X) %*% t(X) %*% Y
> b
[,1]
(Intercept)    15
group.factor1     3
group.factor2     2
group.factor3     1
>
> yhat <- X %*% b
> yhat.ctr <- yhat - mean(yhat)
> len.yhat <- t(yhat.ctr) %*% yhat.ctr
> dim.yhat <- 3
>
> e <- Y - yhat
> len.e <- t(e) %*% e
> dim.e <- 8
>
> F.stat <- (dim.e * len.yhat)/(dim.yhat * len.e)
> F.stat
[,1]
[1,]  10
```

```
> ?FDist # read the docs on the F distribution functions
# includes information on the pf() fxn used below
# probability of observing the F, with given degrees of freedom
> pf(F.stat, 3, 8, lower.tail = FALSE)
[1] 0.004407445
```

And now, more compactly with the `lm()` function:

```
> a.lm <- lm(y ~ group.factor)
```

```
> anova(a.lm)
```

Analysis of Variance Table

Response: y

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|--------------|----|--------|---------|---------|-------------|
| group.factor | 3 | 150 | 50 | 10 | 0.004407 ** |
| Residuals | 8 | 40 | 5 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

```
> summary(a.lm)
```

Call:

```
lm(formula = y ~ group.factor)
```

Residuals:

| Min | 1Q | Median | 3Q | Max |
|-------|-------|--------|------|------|
| -3.00 | -1.00 | -1.00 | 1.25 | 4.00 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|---------------|----------|------------|---------|--------------|
| (Intercept) | 15.0000 | 0.6455 | 23.238 | 1.25e-08 *** |
| group.factor1 | 3.0000 | 1.1180 | 2.683 | 0.0278 * |
| group.factor2 | 2.0000 | 1.1180 | 1.789 | 0.1114 |
| group.factor3 | 1.0000 | 1.1180 | 0.894 | 0.3972 |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.236 on 8 degrees of freedom

Multiple R-squared: 0.7895, Adjusted R-squared: 0.7105

F-statistic: 10 on 3 and 8 DF, p-value: 0.004407

7.1.2. Graphical Depictions of ANOVA

The `granova` package provides nice graphical representations of ANOVA. We'll apply this to a dataset called `genotypes` available in the `MASS` package (part of the basic R installation). As described in the R help, rats with four different genotypes (A, B, I, and J) were separated from their natural mothers at birth, and give to foster mothers to rear. There are two grouping variables we can explore here, the genotype of the foster mother and that of the litter.

```

> install.packages("granova", dependencies=T)
> library(granova)
> library(MASS) # for the genotype dataset
> attach(genotype) # read about attach/detach in the docs
> aov.litter <- aov(Wt ~ Litter)
> summary(aov.litter)
      Df Sum Sq Mean Sq F value Pr(>F)
Litter      3     60   20.05   0.283  0.838
Residuals  57  4040    70.88
> g.litter <- granova.1w(Wt, Litter)

> aov.mother <- aov(Wt ~ Mother)
> summary(aov.mother)
      Df Sum Sq Mean Sq F value Pr(>F)
Mother      3     772   257.2   4.405 0.000743 ***
Residuals  57   3329    58.4
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> g.mother <- granova.1w(Wt, Mother)

```

The output of the `granova.1w()` function is shown below. Use your common sense and the `granova.1w` docs to understand what the different elements of the plot mean. For more details about the `granova` plots check out the paper the authors have made available on the web.

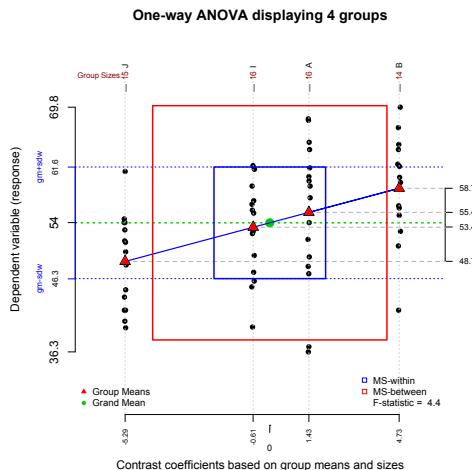


Figure 7.1.: A graphical representation of a one-way ANOVA, created using the `granova` package.

7.2. Discriminant Analysis in R

The function `lda()`, found in the R library MASS, carries out linear discriminant analysis (i.e. canonical variates analysis).

```
> library(MASS) #load the MASS package
> z <- lda(Species ~ Sepal.Length + Sepal.Width + Petal.Length + Petal.Width
   , iris, prior=c(1,1,1)/3)
> z
Call:
lda(Species ~ Sepal.Length + Sepal.Width + Petal.Length + Petal.Width,
     data = iris, prior = c(1, 1, 1)/3)
```

Prior probabilities of groups:

| | | |
|-----------|------------|-----------|
| setosa | versicolor | virginica |
| 0.3333333 | 0.3333333 | 0.3333333 |

Group means:

| | Sepal.Length | Sepal.Width | Petal.Length | Petal.Width |
|------------|--------------|-------------|--------------|-------------|
| setosa | 5.006 | 3.428 | 1.462 | 0.246 |
| versicolor | 5.936 | 2.770 | 4.260 | 1.326 |
| virginica | 6.588 | 2.974 | 5.552 | 2.026 |

Coefficients of linear discriminants:

| | LD1 | LD2 |
|--------------|------------|-------------|
| Sepal.Length | 0.8293776 | 0.02410215 |
| Sepal.Width | 1.5344731 | 2.16452123 |
| Petal.Length | -2.2012117 | -0.93192121 |
| Petal.Width | -2.8104603 | 2.83918785 |

Proportion of trace:

| | |
|--------|--------|
| LD1 | LD2 |
| 0.9912 | 0.0088 |

The prior argument given in the `lda()` function call isn't strictly necessary because by default the `lda` function will assign equal probabilities among the groups. However I included this argument call to illustrate how to change the prior if you wanted. The output give some simple summary statistics for the group means for each of the variables and then gives the coefficients of the canonical variates. The 'Proportion of trace' output above tells us that 99.12% of the between-group variance is captured along the first discriminant axis.

7.2.1. Shorthand Formulae in R

You've encountered the use of model formulae in R several times, such as in the call to `lda()` above and when carrying out various regressions. The document "An Introduction to R" (distributed with R and available at the R project website) gives a concise summary and a number of examples of how to construct formulae in R (see [Defining statistical models: formulae](#)).

Relevant to our current example is a shorthand way for specifying multiple variables in a formula. In the example above we called the `lda()` function with a formula of the form:

```
Species ~ Sepal.Length + Sepal.Width + ....
```

Writing the names of all those variables is tedious and error prone and would be unmanageable if we were analyzing a data set with tens or hundreds of variables. Luckily we can use the shorthand name ‘.’ to specify all other variables in the data frame except the variable on the left. For example, we can rewrite the `lda()` call above as:

```
> z <- lda(Species ~ ., data = iris, prior = c(1,1,1)/3)
```

7.2.2. Fine Tuning Your Plot

To get a graphical representation of the specimens in the space of the canonical variates you can use the `plot()` function on the object returned by the call to `lda()`.

```
> plot(z) # 2D scatter plot of specimens in CVs 1 and 2
> plot(z, abbrev=T) # use abbreviated group names
```

You can also create a plot to look at group variation along just the first canonical variate:

```
> plot(z, dimen=1, type='both') # plot histograms and density plots for each
   group along 1st CV
```

The plot call on the object returned by `lda()` allows some additional customization of the plot, but the extent of graphical tuning is limited:

```
> plot(z, abbrev=T, xlab='CV1', ylab='CV2') # change the x- and y- labels
```

If you want to do any more fine tuning of the plot you'll have to calculate the CV scores from the coefficients and reconstruct the plot to your liking. Below I give an example of how to do that:

```
> iris.data <- subset(iris, select=-Species)
> iris.mtx <- as.matrix(iris.data)
> dim(iris.mtx)
[1] 150   4
> iris.cv <- iris.mtx %*% z$scaling # gives scores in the CV space
> dim(iris.cv)
[1] 150   2
> group.symbols <- (1:3)[iris$Species] # specify the symbols for each group
> plot(iris.cv, pch=group.symbols, asp=1, xlab="CV1", ylab="CV2")
```

The definition of `group.symbols` and the use of the `pch` argument require a little explanation. `pch` is short-hand for ‘plotting character’ and specifies the symbols used to represent each observation in the plot. These symbols can either be letters or integers in the range 0-25. The integers refer to a standard set of symbols shapes defined in R. Figure 1 gives those symbols.

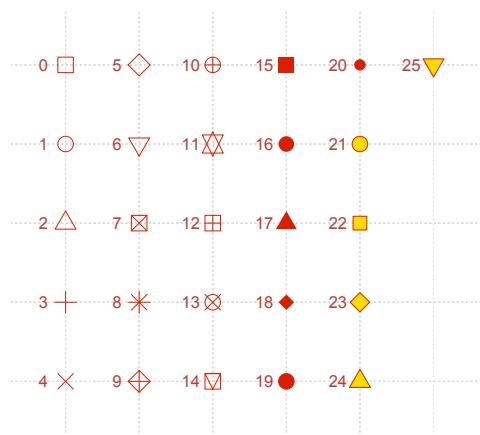


Figure 7.2.: Standard R symbols, and their corresponding integer values, accessible via the pch argument to plot.

If you'd like to see a function that prints out all the standard symbols type `?points` and check out the `pchShow` function defined in the example at the bottom of the documentation page. To see this example in action type `example(points)`. After typing `example(points)` you can call the `pchShow` function directly (that's how I generated the figure).

The `group.symbols <- ...` line constructs a vector of length n (where n is the length of the `iris$Species` vector) where each element of the `group.symbols` vector has the value 1, 2 or 3 according to which species the corresponding specimen represents. A simpler example might make this clearer:

```
> sexes = as.factor(c('M', 'F', 'F', 'M', 'F'))
> sexes
[1] M F F M F
Levels: F M
> c("a", "b")[sexes]
[1] "b" "a" "a" "b" "a"
```

Here I created a simple example involving five specimens where each specimen was categorized by sex. The `as.factor` function tells R to treat the characters in the vector as factor levels. I then assigned each specimen a label, either "a" or "b" depending on its sex. If I wanted to extend that example to our three species iris data set I could do something like:

```
> group.symbols = c("a", "b", "c")[iris$Species]
> plot(iris.cv, pch=group.symbols, cex=0.75, asp=1, xlab="CV1", ylab="CV2")
```

This draws each specimen with the label "a", "b", or "c" depending on which species it is assigned to. Notice that in the last example I used the `cex` argument to make the symbols smaller than normal.

What if I wanted to also plot the group means in the canonical variate space? The following example shows how to do that:

```
> group.symbols = c(0,2,4)[iris$Species] # I switched back to symbols
> group.colors = c('red','darkorange','blue')[iris$Species] # I also want
   to use colors
> cv1.means <- tapply(iris.cv[,1], iris$Species, mean)
> cv1.means
  setosa versicolor virginica
  5.502493 -3.930156 -7.887657
> cv2.means <- tapply(iris.cv[,2], iris$Species, mean)
> cv2.means
  setosa versicolor virginica
  6.876606  5.933573  7.174239
> plot(iris.cv, pch=group.symbols, cex=0.75, asp=1,
+       xlab="CV1", ylab="CV2", col=group.colors)
> points(cv1.means, cv2.means, pch=16, cex=1.5, col='black')
```

Note the use of the `points()` function. This function draws on top of rather than erasing the previous plot. Note too the use of the `col` argument in the `plot()` call to specify different colors. If you'd like to see a chart of all the colors in R check out this web page: [A Chart of R Colors](#).

I stated in lecture that for the canonical variate diagram we can estimate the $100(1 - \alpha)$ confidence region for a group mean as a circle centered at the mean having a radius $(\chi^2_{\alpha,r}/n_i)^{1/2}$ where r is the number of canonical variate dimensions considered. Using similar reasoning the $100(1 - \alpha)$ confidence region for the whole population is given by a hypersphere centered at the mean with radius $(\chi^2_{\alpha,r})^{1/2}$. To calculate these confidence regions you could look up the appropriate value of the the χ^2 distribution in a book of statistical tables, or we can use the `qchisq()` function which gives the inverse cumulative probability distribution for the χ^2 function:

```
> chi2 = qchisq(0.05,2, lower.tail=F)
> chi2
[1] 5.991465
> group.lengths = tapply(iris$Species, iris$Species, length)
> group.lengths
  setosa versicolor virginica
      50          50          50
> mean.radii = sqrt(chi2/group.lengths)
> pop.radii = rep(sqrt(chi2),3)
> help.search("circle") # I don't remember off hand how to draw circles so
   let's look it up
> library(tripack) # Let's use the circles function in the 'tripack'
   package
> circles(cv1.means, cv2.means, pop.radii, lty='dashed')
> circles(cv1.means, cv2.means, mean.radii, lty='dotted')
```

Let's put the finishing touch on our plots by adding some color coded rug plots to the first CV axis. For completeness I'll include all the previous steps used to generate the plot:

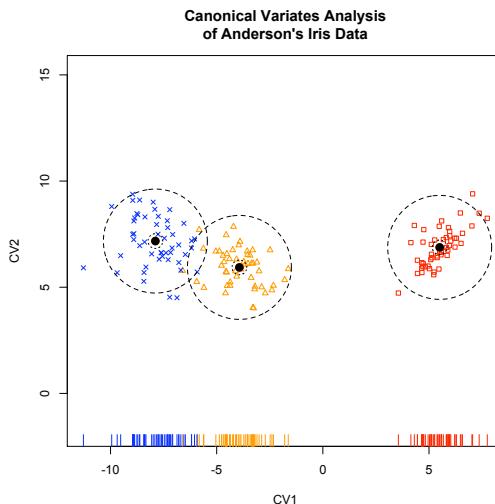


Figure 7.3.: Ordination of iris specimens in the space of the first two canonical variates. The dashed circles surrounding each species distribution give the approximate 95% tolerance regions for the population distributions. See text for details on the construction of this plot.

```
> plot(iris.cv, pch=group.symbols, cex = 0.75, asp=1, xlab="CV1", ylab="CV2"
      , col=group.colors)
> points(cv1.means, cv2.means, pch=16, cex=1.5,col='red')
> circles(cv1.means, cv2.means, pop.radii,lty='dashed')
> circles(cv1.means, cv2.means, mean.radii, lty='dotted')
> rug(iris.cv[,1][iris$Species=="setosa"],col="red")
> rug(iris.cv[,1][iris$Species=="versicolor"],col="darkorange")
> rug(iris.cv[,1][iris$Species=="virginica"],col="blue")
> title("Canonical Variates Analysis\nof Anderson's Iris Data")
```

If you did everything right (and I cut and pasted correctly!) you should get a plot that looks like Fig. 7.3. If I was going to be repeatedly generate these types of plots I would wrap up the key steps discussed above into a convenient function.

7.2.3. Calculating the Within and Between Group Covariance Matrices

The `lda()` function conveniently carries out the key steps of a canonical variates analysis for you. However, what if we wanted some of the intermediate matrices relevant to the analysis such as the within- and between group covariances matrices? The code below shows you how to calculate these:

```
> g = iris$Species
```

```

> group.means <- rowsum(iris.mtx, g)/as.vector(table(g))
> group.means
      Sepal.Length Sepal.Width Petal.Length Petal.Width
setosa          5.006       3.428      1.462      0.246
versicolor      5.936       2.770      4.260      1.326
virginica       6.588       2.974      5.552      2.026
> Dwin <- iris.mtx - group.means[g,]
> nobs <- dim(iris.mtx)[1]
> ngroups <- length(levels(g))
> win.cov <- 1/(nobs-ngroups) * t(Dwin) %*% Dwin
> btw.cov.unweighted <- cov(group.means)

```

Having now calculated the within group covariance matrix we can calculate the Mahalanobis distance between the means of each group as follows:

```

> mahalanobis(group.means, group.means[1,], win.cov)
    setosa versicolor virginica
  0.00000  89.86419 179.38471
> mahalanobis(group.means, group.means[2,], win.cov)
    setosa versicolor virginica
  89.86419  0.00000 17.20107
> mahalanobis(group.means, group.means[3,], win.cov)
    setosa versicolor virginica
179.38471 17.20107  0.00000

```

Assignment 7.1

Identify a paper from the literature relevant to your research interests that employs one or more of the following multivariate statistical techniques:

1. Multivariate regression
2. Principal Component Analysis
3. Singular Value Decomposition
4. Canonical Variate Analysis (or an alternate discriminant function)

Write a short report discussing the use of these techniques in the paper and how the application of these methods contributed to the author's conclusions or understanding of the data. Your report should touch on any assumptions (explicit or implicit) that are relevant to the statistical analysis and discuss whether you feel the author's conclusions are justified or well supported (again based on the statistical analysis). Did the author(s) provide sufficient detail for you repeat the analysis if you had the data? Have the authors(s) made their multivariate data set available?

Include in your report a brief outline (bullet points) that lays out the key steps (e.g. handling of missing data, normalization) and the primary R functions that you would use to repeat the analysis yourself. You don't actually have to carry out the analysis, but rather give a 'road map' for doing so.

8. Introduction to Python

8.1. About Python

Python is a programming language invented in the early 1980's by a Dutch programmer named Guido van Rossum who was working at the Dutch National Research Institute for Mathematics and Computer Science. Python is a high-level programming language with a simple syntax that is easy to learn. The language supports a variety of programming paradigms including procedural programming, object-oriented programming, as well as some functional programming idioms. The name of the language is a whimsical nod toward Monty Python's Flying Circus.

Python has a very active development community. There is a stable core to the language, but new language features are also being developed. Python has an extensive standard library that includes facilities for a wide range of programming tasks. There is a very large user community that provides support and helps to develop an extensive set of third-party libraries. Python is also highly portable - it is available on pretty much any computing platform you're likely to use. Python is also open-source and free!

8.2. Python Resources

There are many resources available online and in bookstores for learning Python. A few handy resources are listed here:

- [Python Website](#) - the official website for the programming language.
- [The Python Tutorial](#) - the 'official' Python tutorial.
- [Python Library Reference](#) - a reference guide to the many modules that come included with Python.
- [Think Python: How to Think Like a Computer Scientist](#) - a free book that provides an introduction to programming using Python.

8.3. Starting the Python interpreter

The Python interpreter can be started in a number of ways. The simplest way is to open a shell (terminal) and type `python`. You can open a terminal as follows:

- On a Mac (OS X) run the terminal program available under Applications > Utilities
- On Windows open up a command prompt, available from Start Menu > Accessories

Once you're at the command prompt type the following command:

```
python
```

If everything is working correctly you should see something like:

```
Enthought Python Distribution -- www.enthought.com
```

```
Version: 7.2-2 (32-bit)
```

```
Python 2.7.2 |EPD 7.2-2 (32-bit)| (default, Sep 7 2011, 09:16:50)
```

```
[GCC 4.0.1 (Apple Inc. build 5493)] on darwin
```

```
Type "packages", "demo" or "enthought" for more information.
```

```
>>>
```

If that command didn't work, please see me for further help configuring your Python installation. From within the default interpreter you can type `Ctrl-d` (Unix, OS X), `Ctrl-z` (Windows) or type `quit()` to stop the interpreter and return to the command line.

For interactive use, the default interpreter isn't very feature rich, so the Python community has developed a number of GUIs or shell interfaces that provide more functionality. For this class we will be using a shell interface called [IPython](#). IPython is one of the tools that was included when you installed the Enthought Python Distribution.

Recent versions of IPython provides both terminal and GUI-based shells. The EPD installer will place a number of shortcuts on your Start Menu or in Launchpad on OS X 10.7, including ones that read PyLab and QtConsole. These are a terminal based and GUI based versions of IPython respectively, both of which automatically load key numerical and plotting libraries. Click on both of these icons to compare their interfaces.

To get the functionality of PyLab from the terminal, run the following command from your shell:

```
ipython --pylab
```

Again, `Ctrl-d` or `Ctrl-z` will quit the terminal.

To get the equivalent of QtConsole you can run ipython with the following arguments:

```
ipython qtconsole --pylab
```

QtConsole is a recent addition to IPython and there may still be bugs to be sorted out, but it provides some very nice features like ' tooltips' (shows you useful information about functions as you type) and the ability to embed figures and plots directly into the console, and the ability to save a console session as a web page (with figures embedded!).

8.3.1. Quick IPython tips

IPython has a wealth of features, many of which are detailed in its [documentation](#). There are also a number of videos available on the IPython page which demonstrate some of it's power. Here are a few key features to get you started and save you time:

- *Don't retype that long command!* — You can scroll back and forth through your previous inputs using the up and down arrow keys (or `Ctrl-p` and `Ctrl-n`); once you find what you were looking forward you can edit or change it. For even faster searching, start to type the beginning of the input and then hit the up arrow.
- *Navigate using standard Unix commands* — IPython lets you use standard Unix commands like `ls` and `cd` and `pwd` to navigate around your file system (even on Windows!)
- *Use <Tab> for command completion* — when you are navigating paths or typing function names in you can hit the `<Tab>` key and IPython will show you matching functions or filenames (depending on context). For example, type `cd ./<Tab>` and IPython will show you all the files and subdirectories of your current working directory. Type a few of the letters of the names of one of the subdirectories and hit `<Tab>` again and IPython will complete the name if it finds a unique match. Tab completion allows you to very quickly navigate around the file system or enter function names so get the hang of using it.

8.3.2. IP[y] Notebooks

For most of this class we'll be using a web-browser based 'notebook' to interface with Python. This notebook tool, called IP[y], is included with IPython. IP[y] notebooks are similar to Mathematica notebooks, or Sweave/Knitr documents, in that you can weave together code and text.

To start an IP[y] notebook first open up a terminal or command prompt and type the following command:

```
ipython notebook --pylab=inline
```

If IPython was installed correctly this will open up a new tab or window in your webbrowser, as shown in Fig. 8.1. Click the "New Notebook" button in the upper right and you'll be presented with an interface like the one shown in Fig. 8.2.

8.3.3. Entering commands in IP[y] Notebooks

Unlike the normal Python interpreter, when you hit `Enter` in an IP[y] notebook, the commands you enter in a notebook cell are not immediately evaluated. You have to use `Shift-Enter` (hold the `Shift` key while you hit the `Enter` key) when you want a cell to be evaluated.

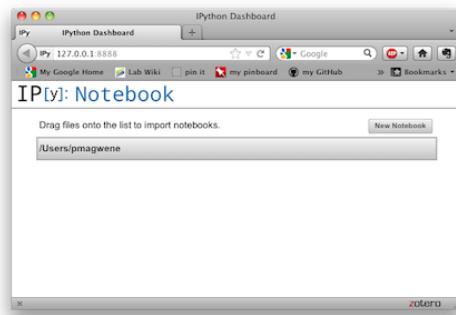


Figure 8.1.: The web-browser based IP[y] Notebook is a new feature of IPython, available in version 0.12.



Figure 8.2.: The IP[y] Notebook interface.

In the examples that follow, lines that begin with >>> indicate lines that you should enter in the IP[y] notebook. Lines that follow will indicate the output produced by that command (sometimes the output will be omitted).

8.3.4. Exploring some of the power Python

Let's start off by demonstrating some of the cool things you can do with Python. This will also serve to demonstrate some of the powerful features of the IP[y] notebook format. The examples should be fairly self-explanatory; I will defer explanation of specific function calls and the various libraries until later.

```
>>> x = array([1,2,3,4,5,6,7,8,9,10])  
>>> plot(x, x**2)
```

Alert!

When entering these lines from inside IP[y], remember to hit Shift-Enter to evaluate the commands!

Now click on the notebook cell with the `plot` command, change it to the following, and hit Shift-Enter to re-evaluate the cell.

```
plot(x, x**2, color='red', marker='o')  
xlabel("Length")  
ylabel("Area")  
title("Length vs. Area for Squares")
```

One of the coolest features of IP[y] notebooks is that they allow you to interactively enter some code, evaluate the results, and then go back and fix, edit or change the code and re-evaluate it without having to reload or compile anything. This is particularly useful for interactively creating complex graphics.

Let's create a more complicated figure, illustrating a histogram of random draws from a normal distribution, compared to the expected probability distribution function (PDF) for a normal distribution with the same parameters:

```
mean = 100  
sd = 15  
  
# draw 1000 random samples from a normal distn  
normaldraw = normal(mean, sd, size=1000)  
  
# draw a histogram  
# "normed" means normalize the counts  
n, bins, patches = hist(normaldraw, bins=50, normed=True)  
xlabel("x")  
ylabel("density")  
  
# draw the normal PDF for the same parameters  
# evaluated at the bins we used to construct the histogram  
y = normpdf(bins, mean, sd)  
l = plot(bins, y, "r--", linewidth=2)
```

This produces the plot shown in Fig. 8.3.

In this final set of examples we create several representations of the function $z = \cos(x) \sin(y)$.

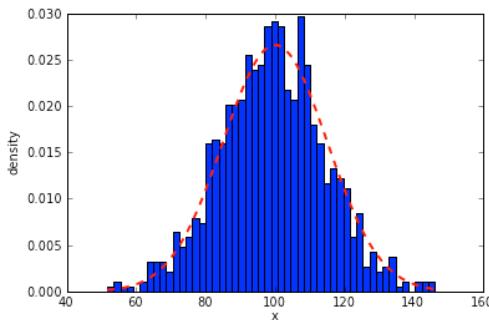


Figure 8.3.: A histogram created using IP[y] and the `matplotlib` library.

```

def f(x,y):
    # multiply by pi/180 to convert degrees to radians
    return cos(x*pi/180) * sin(y*pi/180)

# note we set the upper boundary as 361
# so that 360 get's included
x,y = ogrid[0:361:10, 0:361:10]
z = f(x,y)

# ravel insures the x and y are 1d arrays
# try with 'contour' rather than 'contourf'
p = contourf(ravel(x), ravel(y),z)

lx = xlabel("x (degrees)")
tx = xticks(arange(0,361,45))
ly = ylabel("y (degrees)")
ty = yticks(arange(0,361,45))

```

And the same function represented in 3D, that produces Fig. 8.4.

```

from mpl_toolkits.mplot3d import Axes3D
fig = figure()
ax = Axes3D(fig)

# create x,y grid
x,y = meshgrid(arange(0,361,10), arange(0,361,10))
z = f(x,y) # uses fxn f from previous cell
ax.plot_surface(x,y,z,rstride=2,cstride=2,cmap="jet")

# setup axes labels
ax.set_xlabel("x (degrees)")
ax.set_ylabel("y (degrees)")
ax.set_zlabel("z")

# set elevation and azimuth for viewing

```

```
ax.view_init(68,-11)
fig.show()
```

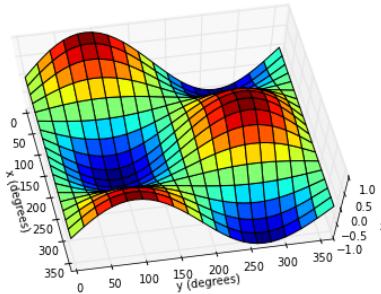


Figure 8.4.: A 3D representation of $z = \cos(x) \sin(y)$

8.3.5. Accessing the Documentation

Python comes with extensive [HTML documentation](#). If you have a network connection, you can access the online documentation for Python (and several other packages) by clicking the appropriate button under “Help” in the left-hand frame of IP[y]. Alternately, you can use the `help()` function (a built-in function in Python), or the `?` command (specific to IPython):

```
>>> help(len)
>>> ?len
```

8.4. Using Python as a Calculator

As with R, the simplest way to use Python is as a fancy calculator. Let’s explore some simple arithmetic operations:

```
>>> 2 + 10    # this is a comment
12
>>> 2 + 10.3
12.30000000000001 # 0.3 can't be represented exactly in floating point
precision
>>> 2 - 10
-8
>>> 1/2    # integer division
0
>>> 1/2.0   # floating point division
0.5
>>> 2 * 10.0
```

```
20.0
>>> 10**2 # raised to the power 2
100
>>> 10**0.5 # raised to a fractional power
3.1622776601683795
>>> (10+2)/(4-5)
-12
>>> (10+2)/4-5 # compare this answer to the one above
-2
```

In addition to integers and reals (represented as floating points numbers), Python knows about complex numbers:

```
>>> 1+2j # Engineers use 'j' to represent imaginary numbers
(1+2j)
>>> (1 + 2j) + (0 + 3j)
(1+5j)
```

Some things to remember about mathematical operations in Python:

- Integer and floating point division are not the same in Python. Generally you'll want to use floating point numbers.
- The exponentiation operator in Python is `**`
- Be aware that certain operators have precedence over others. For example multiplication and division have higher precedence than addition and subtraction. Use parentheses to disambiguate potentially confusing statements.
- The standard math functions like `cos()` and `log()` are not available to the Python interpreter by default. To use these functions you'll need to `import` the math library as shown below. This doesn't apply when you're using an interpreter environment like '`ipython -pylab`' which automatically imports the commonly used math functions.

For example:

```
>>> cos(0.5)
Traceback (most recent call last):
  File "<pyshell#2>", line 1, in -toplevel-
    cos(0.5)
NameError: name 'cos' is not defined
>>> import math # make the math module available
>>> math.cos(0.5) # cos() function in the math module
0.87758256189037276
>>> pi # pi isn't defined in the default namespace
Traceback (most recent call last):
  File "<pyshell#5>", line 1, in -toplevel-
    pi
NameError: name 'pi' is not defined
>>> math.pi # however pi is defined in math
```

```
3.1415926535897931
>>> from math import * # bring everything in the math module into the
      current namespace
>>> pi
3.1415926535897931
>>> cos(pi)
-1.0
```

8.4.1. Comparison Operators in Python

The comparison operators in Python work the same was as they do in R (except they don't work on lists default). Repeat the comparison excercises given above.

8.5. More Data Types in Python

You've already seen the three basic numeric data types in Python - integers, floating point numbers, and complex numbers. There are two other basic data types - Booleans and strings.

Here's some examples of using the Boolean data type:

```
>>> x = True
>>> type(x)
<type 'bool'>
>>> y = False
>>> x == y
False
>>> if x is True:
...     print 'Oh yeah!'
...
Oh yeah!
>>> if y is True:
...     print 'You betcha!'
... else:
...     print 'Sorry, Charlie'
...
Sorry, Charlie
>>>
```

And some examples of using the string data type:

```
>>> s1 = 'It was the best of times'
>>> type(s1)
<type 'str'>
>>> s1 # show representation of string
'It was the best of times'
>>> print s1 # print the string, notice difference from above
It was the best of times
>>> s2 = 'it was the worst of times'
```

```
>>> s1 + s2
'It was the best of timesit was the worst of times'
>>> s1 + ', ' + s2
'It was the best of times, it was the worst of times'
>>> 'times' in s1 # is 'times' a substring of s1?
True
>>> s1.count('t') # count the occurrences of 't' in s1
4
>>> s3 = "You can nest 'single quotes' in double quotes"
>>> s4 = 'or "double quotes" in single quotes'
>>> s5 = "but you can't nest "double quotes" in double quotes"
      File "<stdin>", line 1
          s5 = "but you can't nest "double quotes" in double quotes"
                           ^
SyntaxError: invalid syntax
```

Note that you can use either single or double quotes to specify strings.

8.6. Elementary data structures in Python

8.6.1. Python Lists

Lists are the simplest ‘built-in’ data structure in Python. List represent ordered collections of arbitrary objects.

```
>>> l = [2, 4, 6, 8, 'fred']
>>> l
[2, 4, 6, 8, 'fred']
>>> len(l)
5
```

Python lists are zero-indexed. This means you can access lists elements in the range 0 to `len(x)-1`.

```
>>> l[0]
2
>>> l[3]
8
>>> l[5]
Traceback (most recent call last):
  File "<stdin>", line 1, in <module>
IndexError: list index out of range
```

You can use negative indexing to get elements from the end of the list:

```
>>> l[-1] # the last element
'fred'
>>> l[-2] # the 2nd to last element
8
>>> l[-3] # ... etc ...
6
```

Indexing can be used to both get and set items in a list.

```
>>> l = [2, 4, 6, 8, 'hike!']
>>> l[-1]
'hike!'
>>> l[-1] = "learning python is so great!"
>>> l
[2, 4, 6, 8, 'learning python is so great!']
```

You can append and delete list elements as well as concatenate two lists:

```
>>> l1 = [1,2,3]
>>> l2 = ['a','b','c','d']
>>> l1.append(4)
>>> l1
[1, 2, 3, 4]
>>> del l2[2]
>>> l2
['a', 'b', 'd']
>>> l3 = l1 + l2
>>> l3
[1, 2, 3, 4, 'a', 'b', 'd']
```

Python lists support the notion of ‘slices’ - a continuous sublist of a larger list. The following code illustrates this concept:

```
>>> y = range(10) # use help(range) to read about this fnx
>>> y
[0, 1, 2, 3, 4, 5, 6, 7, 8, 9]
>>> y[:3] # get first 3 elements of y
[0, 1, 2]
>>> y[2:8] # get elements from 2 to 8
[2, 3, 4, 5, 6, 7]
>>> y[2:-1] # get elements from 2 to the last element
[2, 3, 4, 5, 6, 7, 8]
>>> y[-1:0] # how come this didn't work?
[]
# slice from last to first, stepping backwards by 2
>>> y[-1:0:-2]
[9, 7, 5, 3, 1]
```

As with single indexing, the slice notation can be used to set elements of a list as well:

```
>>> s = ['a', 'b', 'c', 'd', 'e']
>>> s[2:4] = ['C', 'D']
>>> s
['a', 'b', 'C', 'D', 'e']
```

Finally, there are a number of useful methods associated with list objects, such as `reverse()` and `sort()`:

```
>>> l4 = [1, 5, 3, 4, 10, 11, 3]
>>> l4.sort() # sort in-place
```

```
>>> l4  
[1, 3, 3, 4, 5, 10, 11]  
>>> l4.reverse() # reverse in-place  
>>> l4  
[11, 10, 5, 4, 3, 3, 1]
```

Read, ‘Think Python’ [Chapter 10](#) for more about lists. See the [Python library documentation](#) for a summary of methods associated with lists.

8.6.2. Python Tuples

As shown above, the elements of a Python list are mutable and the length of lists can be changed. Sometimes it’s useful for reasons of both programming logic and computational efficiency to have immutable collections of items. The data structure that Python uses to represent such immutable collection is called a ‘Tuple’.

Parentheses rather than square brackets are used to create tuples. A common use of tuples is to represent a pair, i.e. a tuple of length two. For example, you might use a pair to represent a point in the Cartesian plane:

```
>>> pt = (1, 5)  
>>> pt  
(1, 5)  
>>> pt[0]  
1  
>>> pt[1]  
5  
>>> pt[0] = 2  
Traceback (most recent call last):  
  File "<ipython-input-11-648d2cfb62f4>", line 1, in <module>  
    pt[0] = 2  
TypeError: 'tuple' object does not support item assignment
```

The above illustrates that tuples can be indexed like lists, however you can’t assign to or extend a tuple after it’s created. As we will illustrate later, tuples are often used to return multiple objects from functions. Read, ‘Think Python’ [Chapter 12](#) for more about tuples.

8.6.3. Python Dictionaries

Python dictionaries (sometimes called ‘hash maps’ in other programming languages) map a set of keys (labels) to values. Dictionaries provide efficient access to non-ordered data.

```
## create a dictionary  
>>> d = {'fred':26, 'jim':22, 'mary':34, 'jill': 12}  
>>> d  
{'jim': 22, 'mary': 34, 'jill': 12, 'fred': 26}  
>>> d['fred'] ## get value associated with the key 'fred'  
26
```

```
>>> d.keys() # return list of keys in dict
['jim', 'mary', 'jill', 'fred']
>>> d.values() # return list of values in dict
[22, 34, 12, 26]
>>> for key in d.keys():    # iterate over the keys in the dict
...     print key, "is", d[key], "years old."
...
jim is 22 years old.
mary is 34 years old.
jill is 12 years old.
fred is 26 years old.
>>> d['joe'] = 99 # add a new key,value pair to dict
>>> d
{'joe': 99, 'jim': 22, 'mary': 34, 'jill': 12, 'fred': 26}
>>> del d['mary'] # remove a key,value pair from dict
>>> d
{'joe': 99, 'jim': 22, 'jill': 12, 'fred': 26}
```

Dictionary keys have to be immutable objects, but associated values can be arbitrary python objects, even other dictionaries.

```
>>> d2 = {} # create an empty dict
>>> d2[[1,2,3]] = 'b' # a list can't be a key
Traceback (most recent call last):
  File "<ipython-input-52-497f3cfcc4ef>", line 1, in <module>
    d2[[1,2,3]] = 'b'
TypeError: unhashable type: 'list'

>>> d2[(1,2,3)] = 'b' # but a tuple works
>>> d2
{(1, 2, 3): 'b'}
>>> d3 = dict() # alternate way to create empty dict
# create a dictionary of dictionaries
>>> d3['fred'] = {'age': 26, 'sex': 'male', 'occupation':'nurse'}
>>> d3['mary'] = {'age': 34, 'sex': 'female', 'occupation':'engineer'}
>>> d3['fred']['age']
26
>>> d3['mary']['occupation']
'engineer'
```

Read, ‘Think Python’ [Chapter 11](#) for more about dictionaries.

8.7. Introduction to NumPy

Python does not have a built-in data structure that behaves in quite the same way as do vectors in R. However, we can get very similar behavior using a library called NumPy.

NumPy does not come with the standard Python distribution, but it does come as an included package if you use the Enthought Python distribution. Alternately you

can download NumPy from the SciPy project page at: <http://scipy.org>. The NumPy package comes with documentation and a tutorial. You can access the documentation at <http://docs.scipy.org/doc/>.

Here's some examples illustrating using of NumPy:

```
>>> from numpy import array # a third form of import
>>> x = array([2,4,6,8,10])
>>> x
array([ 2,  4,  6,  8, 10])
>>> -x
array([-2, -4, -6, -8, -10])
>>> x**2
array([ 4, 16, 36, 64, 100])
>>> pi * x # assumes pi is in the current namespace
array([ 6.28318531, 12.56637061, 18.84955592, 25.13274123,
       31.41592654])
>>> y = array([0, 1, 3, 5, 9])
>>> x + y
array([ 2,  5,  9, 13, 19])
>>> x * y
array([ 0,  4, 18, 40, 90])
>>> z = array([1, 4, 7, 11])
>>> x+z
Traceback (most recent call last):
  File "<stdin>", line 1, in <module>
ValueError: shape mismatch: objects cannot be broadcast to a single shape
```

The last example above shows that, unlike R, NumPy arrays in Python are not 'recycled' if lengths do not match.

Remember that lists and arrays in Python are zero-indexed rather than one-indexed.

```
>>> x
array([ 2,  4,  6,  8, 10])
>>> len(x)
5
>>> x[0]
2
>>> x[1]
4
>>> x[4]
10
>>> x[5]
Traceback (most recent call last):
  File "<pyshell#52>", line 1, in -toplevel-
    x[5]
IndexError: index out of bounds
>>> x[-1]
10
```

NumPy arrays support the comparison operators and return arrays of booleans.

```
>>> x < 5  
array([ True, True, False, False, False], dtype=bool)  
>>> x >= 6  
array([0, 0, 1, 1, 1])
```

NumPy also supports the combination of comparison and indexing that R vectors can do. There are also a variety of more complicated indexing functions available for NumPy; see the [Indexing Routines](#) in the NumPy docs.

```
>>> x[x < 5]  
array([2, 4])  
>>> x[x >= 6]  
array([ 6,  8, 10])  
>>> x[(x<4)+(x>6)] # 'or'  
array([ 2,  8, 10])
```

Note that Boolean addition is equivalent to ‘or’ and Boolean multiplication is equivalent to ‘and’.

Most of the standard mathematical functions can be applied to NumPy arrays however you must use the functions defined in the NumPy module.

```
>>> x  
array([ 2,  4,  6,  8, 10])  
>>> import math  
>>> math.cos(x)  
  
Traceback (most recent call last):  
  File "<pyshell#67>", line 1, in -toplevel-  
    math.cos(x)  
TypeError: only length-1 arrays can be converted to Python scalars.  
>>> import numpy  
>>> numpy.cos(x)  
array([-0.41614684, -0.65364362,  0.96017029, -0.14550003, -0.83907153])
```

8.7.1. Vector Operations in NumPy

Like R vectors, NumPy arrays support element-wise arithmetic, so we can treat 1-dimensional NumPy arrays like vectors.

```
>>> import numpy  
>>> x = numpy.arange(start=1, stop=16, step=1)  
>>> y = numpy.arange(10,25) # default step = 1  
>>> x  
array([ 1,  2,  3,  4,  5,  6,  7,  8,  9, 10, 11, 12, 13, 14, 15])  
>>> y  
array([10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24])  
>>> x+y  
array([11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39])  
>>> x-y  
array([-9, -9, -9, -9, -9, -9, -9, -9, -9, -9, -9, -9, -9, -9])
```

```
>>> 3*x
array([ 3,  6,  9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45])
>>> z = numpy.dot(x,x) # no built-in dot operator, but a dot fn in numpy
>>> z
1240
```

Note the use of the `numpy.arange()` function. `numpy.arange()` works like R's `seq()` function and it returns a Numpy array. However, notice that the values go up to but don't include the specified stop value. Use `help()` to lookup the documentation for `numpy.arange()`. Python also includes a `range()` function that generates a regular sequence as a Python list object. The `range()` function has `start`, `stop`, and `step` arguments but these can only be integers. Here are some additional examples of the use of `arange()` and `range()`:

```
>>> z = numpy.arange(1,5,0.5)
>>> z
array([ 1. ,  1.5,  2. ,  2.5,  3. ,  3.5,  4. ,  4.5])
>>> range(1,20,2)
[1, 3, 5, 7, 9, 11, 13, 15, 17, 19]
>>> range(1,5,0.5)
Traceback (most recent call last):
  File "<stdin>", line 1, in <module>
TypeError: range() integer step argument expected, got float.
```

8.7.2. Matrices in NumPy

Matrices in Python are created are created using the `Numeric.array()` function. In Python you need to be a little more aware of the type of the arrays that you create. If the argument you pass to the `array()` function is composed only of integers than Numeric will assume you want an integer matrix which has consequences in terms of operations like those illustrated below. To make sure you're matrix has floating type values you can use the argument `typecode=Numeric.Float`.

```
>>> import numpy as np # I'm 'aliasing' the name so I can type 'np' instead
       of 'numpy'
>>> array = np.array # setup another alias
>>> X = array(range(1,13))
>>> X
array([ 1,  2,  3,  4,  5,  6,  7,  8,  9, 10, 11, 12])
>>> X.shape = (4,3) # rows, columns
>>> X
array([[ 1,  2,  3],
       [ 4,  5,  6],
       [ 7,  8,  9],
       [10, 11, 12]])
>>> 1/X # probably not what you expected
array([[1, 0, 0],
       [0, 0, 0],
       [0, 0, 0],
```

```
[0, 0, 0]])  
>>> X = array(range(1,13), dtype=np.float)  
>>> X.shape = 4,3  
>>> X  
array([[ 1.,  2.,  3.],  
       [ 4.,  5.,  6.],  
       [ 7.,  8.,  9.],  
       [10., 11., 12.]])  
>>> 1/X # that's more like it  
array([[ 1.          ,  0.5         ,  0.33333333],  
       [ 0.25        ,  0.2         ,  0.16666667],  
       [ 0.14285714,  0.125        ,  0.11111111],  
       [ 0.1          ,  0.09090909,  0.08333333]])  
>>> X + X  
array([[ 2.,  4.,  6.],  
       [ 8., 10., 12.],  
       [14., 16., 18.],  
       [20., 22., 24.]])  
>>> X - X  
array([[ 0.,  0.,  0.],  
       [ 0.,  0.,  0.],  
       [ 0.,  0.,  0.],  
       [ 0.,  0.,  0.]])  
>>> np.dot(X,np.transpose(X)) # dot fxn in numpy gives matrix  
multiplication for arrays  
array([[ 14.,  32.,  50.,  68.],  
       [ 32.,  77., 122., 167.],  
       [ 50., 122., 194., 266.],  
       [ 68., 167., 266., 365.]])  
>>> np.dot(X, X.transpose()) # transpose() is also a method associated  
with the array object  
array([[ 14.,  32.,  50.,  68.],  
       [ 32.,  77., 122., 167.],  
       [ 50., 122., 194., 266.],  
       [ 68., 167., 266., 365.]])  
>>> np.sqrt(X)  
array([[ 1.          ,  1.41421356,  1.73205081],  
       [ 2.          ,  2.23606798,  2.44948974],  
       [ 2.64575131,  2.82842712,  3.          ],  
       [ 3.16227766,  3.31662479,  3.46410162]])  
>>> np.cos(X)  
array([[ 0.54030231, -0.41614684, -0.9899925 ],  
       [-0.65364362,  0.28366219,  0.96017029],  
       [ 0.75390225, -0.14550003, -0.91113026],  
       [-0.83907153,  0.0044257 ,  0.84385396]])  
>>> np.identity(4)  
array([[1, 0, 0, 0],  
       [0, 1, 0, 0],  
       [0, 0, 1, 0],  
       [0, 0, 0, 1]])
```

```
[0, 0, 0, 1]])  
>>> np.ones((4,4))  
array([[ 1.,  1.,  1.,  1.],  
       [ 1.,  1.,  1.,  1.],  
       [ 1.,  1.,  1.,  1.],  
       [ 1.,  1.,  1.,  1.]])
```

The code above also demonstrated the Numpy functions `dot`, `transpose`, `identity` and `ones`. Note too that Numpy has a variety of functions such as `sqrt()` and `cos()` that work on an element-wise basis.

Indexing of arrays in Numpy is demonstrated below. You'll see that Python arrays support 'slicing' operations. For more on slicing and other array basics see the Numpy documentation at <http://docs.scipy.org/doc/>.

```
>>> X  
array([[ 1.,  2.,  3.],  
       [ 4.,  5.,  6.],  
       [ 7.,  8.,  9.],  
       [10., 11., 12.]])  
>>> X[0,0] # get the 0th row, 0th column (remember that Python sequences  
# are zero-indexed!)  
1.0  
>>> X[3,0] # get the fourth row, 1st column  
10.0  
>>> X[2:,:2] # slicing, first two columns and rows (i.e. indices 0 and 1)  
array([[ 1.,  2.],  
       [ 4.,  5.]])  
>>> X[1:,:2] # get everything after the 0th row and the first two columns  
array([[ 4.,  5.],  
       [ 7.,  8.],  
       [10., 11.]])
```

To calculate matrix inverses in Python you need to import the `numpy.linalg` package.

```
>>> import numpy.linalg as la  
>>> import numpy.random as ra # for matrices with elements from random  
# distributions  
>>> C = ra.normal(loc=0,scale=1,size=(4,4)) # do help(ra.normal) for  
# explanation of arguments  
>>> C  
array([[ 0.79525679,  1.11730719, -2.19257712, -0.06289276],  
       [ 0.7087366 ,  0.70574975, -1.51599336, -0.90360945],  
       [-0.33845153, -0.20109722, -0.75245988, -0.56027025],  
       [-0.51692665,  0.59972543,  1.55562234,  1.88639367]])  
>>> Cinv = la.inv(C)  
>>> np.dot(C, Cinv) # again result is approx the identity matrix due to  
# floating point precision  
array([[ 1.0000000e+000, -5.55111512e-017, -6.93889390e-017,  2.94902991e  
-017],  
       [ 1.11022302e-016,  1.0000000e+000, -1.11022302e-016, -5.55111512e  
-017],
```

```
[ 1.11022302e-016, -2.22044605e-016,  1.00000000e+000,  2.77555756e
   -017],
[ 0.00000000e+000, -4.44089210e-016,  0.00000000e+000,  1.00000000e
   +000]])
>>> CCinv = np.dot(C, Cinv)
>>> np.allclose(CCinv, np.identity(4)) # test for approx. equality
True
>>> print np.array2string(CCinv,precision=2, suppress_small=True)
[[ 1. -0.  0.  0.]
 [-0.  1.  0.  0.]
 [ 0.  0.  1.  0.]
 [-0. -0. -0.  1.]]
```

8.8. Writing Functions in Python

The general form of a Python function is as follows:

```
def funcname(arg1,arg2):
    # one or more expressions
    return someresult # arbitrary python object
                      # (could even be another function)
```

An important thing to remember when writing functions is that Python is white space sensitive. In Python code indentation indicates scoping rather than braces. Therefore you need to maintain consistent indentation. This may surprise those of you who have extensive programming experience in another language. However, white space sensitivity contributes significantly to the readability of Python code. Use a Python aware programmer's editor and it will become second nature to you after a short while. I recommend you set your editor to substitute spaces for tabs (4 spaces per tab), as this is the default convention within the python community.

Here's an example of defining and using a function in the Python interpreter:

```
>>> def mypyfunc(x,y):
...     return x**2 + y**2 + 3*x*y
...
>>> mypyfunc(10,12)
604
>>> a = numpy.arange(1,5,0.5)
>>> b = numpy.arange(2,6,0.5)
>>> mypyfunc(a,b)
array([ 11.   ,  19.75,  31.   ,  44.75,  61.   ,  79.75,  101.   ,
       124.75])
>>> a = range(1,5)
>>> b = range(1,5)
>>> mypyfunc(a,b)

Traceback (most recent call last):
  File "<pyshell#52>", line 1, in <module>
    mypyfunc(a,b)
```

```
mypyfunc(a,b)
File "<pyshell#45>", line 2, in mypyfunc
    return x**2 + y**2 + 3*x*y
TypeError: unsupported operand type(s) for ** or pow(): 'list' and 'int'
>>>
```

Note that this function works for numeric types (`ints` and `floats`) as well as `numpy`.
arrays but not for simple Python lists. If you wanted to make this function work for
lists as well you could define the function as follows:

```
>>> def mypyfunc(x,y):
...     x = numpy.array(x)
...     y = numpy.array(y)
...     return x**2 + y**2 + 3*x*y
...
>>> a
[1, 2, 3, 4]
>>> b
[1, 2, 3, 4]
>>> mypyfunc(a,b)
array([ 5, 20, 45, 80])
```

8.8.1. Putting Python functions in Modules

As with R, you can define your own Python modules that contain user defined functions. Using a programmer's text editor, write your function(s) and save it to a file with a `.py` extension in a directory in your `PYTHONPATH` (see below).

```
# functions defined in vecgeom.py
import numpy

def veclength(x):
    """Calculate length of a vector x."""
    x = numpy.array(x)
    return numpy.sqrt(numpy.dot(x,x))

def unitvector(x):
    """Return a unit vector in the same direction as x."""
    x = numpy.array(x)
    return x/veclength(x)
```

To access your function use an `import` statement:

```
>>> import vecgeom
>>> x = [-3,-3,-1,-1,0,0,1,2,2,3]
>>> help(vecgeom.veclength)
Help on function veclength in module vecgeom:

veclength(x)
    Calculate length of a vector x.
```

```
>>> vecgeom.veclength(x)
6.164414002968976
# import all fxns from the vecgeom module
>>> from vecgeom import *
>>> print vecgeom.unitvector(x)
[-0.48666426 -0.48666426 -0.16222142 -0.16222142  0.
 0.16222142  0.32444284  0.32444284  0.48666426]
```

8.9. Setting the PYTHONPATH

Like the operating system, Python searches a set of default directories whenever you ask it to load a specific module. Python knows where to find all of its base modules, and a well written package will install its files into one of the standard locations.

To see the directories that your Python installation searches by default try the following commands in the Python interpreter (your output will be different):

```
>>> import sys
>>> import sys
>>> sys.path
['', '/Library/Frameworks/Python.framework/Versions/7.1/bin', '/Users/
 pmagwene',
'/Users/pmagwene/synchronized/pyth', '/Users/pmagwene/pytest',
... output truncated ...]
```

For your own code it's useful to setup a separate directory. Create a directory called pycode in your home directory. In order for Python to "see" the code in this directory you must add it to your PYTHONPATH. To temporarily add a new directory to sys.path:

```
>>> sys.path.append('/Users/pmagwene/pycode') # substitute the path to the
directory you used
>>> print sys.path
['', '/Library/Frameworks/Python.framework/Versions/7.1/bin', '/Users/
pmagwene',
'/Users/pmagwene/synchronized/pyth', '/Users/pmagwene/pytest',
... output truncated...
'/Users/pmagwene/pycode']
```

This change applies only to your current interpreter and lasts until you close the interactive prompt. To make persistent changes to the Python search path you need to create an environment variable called PYTHONPATH and add the desired directories. You do this the same way you set your system PATH, by modifying your shell initialization file (Unix or OS X) or using the System Properties tool in the control panel to create a new environment variable (Windows). For example, on OS X add the following line to your .bash_profile (found in your home directory, create it if doesn't already exist):

```
export PYTHONPATH=$PYTHONPATH:$HOME/pycode
```

If you are keeping your Bio723 code somewhere other than `~/pycode` than change the location as needed. To have this change take effect, start a new Terminal window or re-start IP[y].

For more info on setting PATH variables see: <https://github.com/pmagwene/Bio313/wiki/setting-paths>.

8.10. Plotting in Python

Python doesn't have any 'native' data plotting tools but there are a variety of packages that provide tools for visualizing data. The Matplotlib package is the de facto standard for producing publication quality scientific graphics in Python. Matplotlib is included with the EPD and was automatically pulled into the interpreter namespace if you're using the IPython --pylab option. If you want to explore the full power of Matplotlib check out the example gallery and the documentation at <http://matplotlib.sourceforge.net/>.

8.10.1. Basic plots using matplotlib

If you invoked the Ipython shell using the pylab option than most of the basic matplotlib functions are already available to you. If not, import them as so:

```
>>> from pylab import * # only necessary if not using ipython --pylab
>>> import numpy as np # go ahead and import numpy as well, using an alias

# load the turtle data using the numpy.loadtxt function
# skipping the first row (header) and the first column
>>> turt = np.loadtxt('turtles.txt', skiprows=1,
                     usecols=(1,2,3))

>>> turt.shape
(48, 3)

# draw bivariate scatter plot
>>> scatter(turt[:,0], turt[:,1])
# give the axes some labels and a title for the plot
>>> xlabel('Length')
>>> ylabel('Width')
>>> title('Turtle morphometry')
```

Here's another example using the yeast expression data set:

```
>>> data = np.loadtxt('yeast-subnetwork-clean.txt',
                     skiprows=1, usecols=range(1,16))
>>> data.shape # check the dimensions of the resulting matrix
(173, 15)
```

The `skiprows` argument tells the function how many rows in the data file you want to skip. In this case we skipped only the first row which gives the variable names. The `usecols` arguments specifies which columns from the data file to use. Here we skipped the first (zeroth) column which had the names of the conditions. The

usecols loadtxt works when there is no missing data. Use numpy.genfromtxt instead when there are missing values. For a full tutorial on how to use the numpy.genfromtxt function see <http://docs.scipy.org/doc/numpy/user/basics.io.genfromtxt.html>.

Histograms in Matplotlib

Matplotlib has a histogram drawing function. Here's how to use it:

```
>>> hist? # in Ipython calls the help function
>>> h = hist(data[:,0]) # plot a histogram of the first variable (column)
   in our data set
>>> clf() # clear the plot window, don't need this if you closed the plot
   window
>>> h = hist(data[:,0], bins=20) # plot histogram w/20 bins
>>> clf()
>>> h = hist(data[:,0:2]) # histograms of the first two variables
```

There's no built in density plot function, but we can create a function that will do the necessary calculations for us to create our own density plot. This uses a kernel density estimator function in the scipy library (included with EPD). Put the following code in a file called myplots.py somewhere on your PYTHONPATH:

```
# myplots.py

import numpy as np
from scipy import stats

def density_trace(x):
    kde = stats.gaussian_kde(x)
    xmin,xmax = min(x), max(x)
    xspan = xmax - xmin
    xpts = np.arange(xmin, xmax, xspan/1000.)
    ypts = kde.evaluate(xpts) # evaluate the estimate at the xpts
    return xpts,ypts
```

You can then use the density_trace function as follows:

```
>>> import myplots
>>> h = hist(data[:,0], normed=True) # use normed=True so histogram
   # is normalized to form a prob. density
>>> x,y = myplots.density_trace(data[:,0])
>>> plot(x,y, 'red')
```

Boxplots in Matplotlib

Box-and-whisker plots are straightforward in Matplotlib:

```
>>> b = boxplot(data[:,0])
>>> clf()
>>> b = boxplot(data[:,0:5]) # boxplots of first 5 variables
```

The `boxplot` function has quite a few facilities for customizing your boxplots. For example, here's how we can create a notched box-plot using 1000 bootstrap replicates (we'll discuss the bootstrap in more detail in a later lecture) to calculate confidence intervals for the median.

```
>>> boxplot(data[:,0], notch=1, bootstrap=True)
```

See the Matplotlib docs for more info.

3D Scatter Plots in Matplotlib

Recent version of Matplotlib include facilities for creating 3D plots. Here's an example of a 3D scatter plot:

```
>>> from mpl_toolkits.mplot3d import Axes3D
>>> fig = figure()
>>> ax = fig.add_subplot(111, projection = '3d')
>>> ax.scatter(data[:,0],data[:,1],data[:,2])
<mpl_toolkits.mplot3d.art3d.Patch3DCollection object at 0x1a0bbd70>
>>> ax.set_xlabel('Gene 1')
<matplotlib.text.Text object at 0x1a0ae7d0>
>>> ax.set_ylabel('Gene 2')
<matplotlib.text.Text object at 0x1a0bb2b0>
>>> ax.set_zlabel('Gene 3')
<matplotlib.text.Text object at 0x1a0bbcd0>
>>> show()
```

Retying all those commands is tedious and error prone so let's turn it into a function. Add the following code to `myplots.py`:

```
from matplotlib import pyplot
from mpl_toolkits.mplot3d import Axes3D

def scatter3d(x,y,z, labels=None):
    fig = pyplot.figure()
    ax = fig.add_subplot(111, projection='3d')
    ax.scatter(x,y,z)

    if labels is not None:
        try:
            ax.set_xlabel(labels[0])
            ax.set_ylabel(labels[1])
            ax.set_zlabel(labels[2])
        except IndexError:
            print "You specified less than 3 labels."
    return fig
```

Now reload myplots and call the `scatter3d` function as so:

```
>>> reload(myplots)
>>> myplots.scatter3d(data[:,0], data[:,1], data[:,2])
>>> myplots.scatter3d(data[:,0], data[:,1], data[:,2], lab)
```

```
>>> myplots.scatter3d(data[:,0], data[:,1], data[:,2], labels=('X','Y','Z'))
```

9. Clustering Methods

9.1. Hierarchical Clustering in R

The function `hclust()` provides a simple mechanism for carrying out standard hierarchical clustering in R. The `method` argument determines the group distance function used (single linkage, complete linkage, average, etc.).

The input to `hclust()` is a dissimilarity matrix. The function `dist()` provides some of the basic dissimilarity measures (e.g. Euclidean, Manhattan, Canberra; see `method` argument of `dist()`) but you can convert an arbitrary square matrix to a distance object by applying the `as.dist()` function to the matrix.

```
> iris.data <- subset(iris, select=-Species)
> iris.cl <- hclust(dist(iris.data), method='single')
> plot(iris.cl) # plot a dendrogram
# let's improve the look a little bit
> plot(iris.cl, labels=iris$Species, cex=0.7)
> # use neg. values of hang to make labels on leaves line up
> plot(iris.cl, labels=iris$Species, hang=-0.1, cex=0.7)
```

Other functions of interest related to dendograms include `cuttree()` for cutting the tree at a specified height (or number of groups) and `identify()` for graphically highlighting a cluster of interest in a dendrogram.

```
> plot(iris.cl, labels=iris$Species, cex=0.7)
# identify fxn doesn't work in R
-Studio
> interesting.cluster <- identify(iris.cl) # use left-mouse to choose,
right-mouse to stop choosing
> interesting.cluster
# [output omitted]
```

Fancy formatting of dendrogram plots in R is awkward. You need to use the `plot()` function in combination with the `as.dendrogram()` function to access many options. See the help for 'dendrogram' in R for a discussion of options and type `example(dendrogram)` to see some possibilities. A few of them are illustrated here:

```
> plot(as.dendrogram(iris.cl)) # contrast this with plot(iris.cl)
> plot(as.dendrogram(iris.cl), horiz=T) # draw horizontally
> # here's one way to change the labels
> iris.cl$labels <- iris$Species
> levels(iris.cl$labels) <- factor(c("S", "Ve", "Vi"))
> iris.dend <- as.dendrogram(iris.cl)
> plot(iris.dend)
```

The `heatmap()` function combines a false color image of a matrix with a dendrogram. Here's we apply it to the yeast-subnetwork data set from previous weeks.

```
> yeast <- read.delim('yeast-subnetwork-clean.txt')
> ymap <- heatmap(as.matrix(yeast), labRow=NA) # suppress the numerous row
   labels
> ymap <- heatmap(as.matrix(yeast), labRow=rownames(yeast)) # w/ row labels,
   kinda messy
```

The R package `cluster` provides some slightly fancier clustering routines. The basic agglomerative clustering methods in `cluster` are accessed via the function `agnes()`

Compare the results of different hierarchical clustering methods (single linkage, complete linkage, etc.) as applied to the `iris` data set using the `hclust()` or `agnes()` functions. For single and average linkage use both Euclidean and Manhattan distance as the dissimilarity measures.

9.2. K-means Clustering in R

The `kmeans()` function calculates standard k-means clusters in R. The input is a data matrix (perhaps transformed before hand) and k , the number of clusters. Alternatively you can specify starting cluster centers. You can also run the algorithm multiple times with different random starting positions by using the `nstart` argument.

```
# generate data set w/two groups (one of size 50, the other of size 75)
# note the different means and std dev between the two groups
> test.data <- rbind(matrix(rnorm(100, mean=0, sd=0.2), ncol=2),
   matrix(rnorm(150, mean=1, sd=0.5), ncol=2))
> colnames(test.data) <- c("x", "y")
> plot(test.data)
> cl <- kmeans(test.data, 2)
> names(cl)
[1] "cluster" "centers" "withinss" "size"
> cl$cluster # which cluster each object is assigned to
... output deleted ...
> plot(test.data, col = cl$cluster)
> cl$centers # compare to the "true" means for the groups
      x         y
1 0.009479636 0.1182016
2 1.109641398 1.0427396
> points(cl$centers, col = 1:2, pch = 8, cex=2)

> # what if we pick the wrong number of clusters?
> cl <- kmeans(test.data, 5)
> plot(test.data, col = cl$cluster)
> points(cl$centers, col = 1:5, pch = 8, cex=2)

> # as above but using nstart argument
> cl <- kmeans(test.data, 5, nstart=25)
> plot(test.data, col = cl$cluster)
```

```
> points(cl$centers, col = 1:5, pch = 8, cex=2)
```

9.2.1. Applying K-means to the iris data set

Now that we've seen how to apply k-mean clustering to a synthetic data set, let's go ahead and apply it to our old friend the iris data set. Note that this is a four dimensional data set so we'll need to pick a projection in which to depict the cluster. The space of the first two principal components is a natural choice (but note that the fact that we're using the PCA space doesn't impact the k-means clustering in this context).

```
# drop the fifth column (species names)
# we'll assume we know how many groups there are
> k.iris <- kmeans(as.matrix(iris[,-5]), 3)
> iris.pca <- prcomp(iris[,-5])
# the following plot colors the specimens by the group
# they were assigned to by the k-means clustering
> plot(iris.pca$x,col=k.iris$cluster)

# this plot colors specimens by k-means grouping
# and chooses plot symbol by real species grouping.
# This can help us quickly pick out the misclassified
# specimens
> plot(iris.pca$x, col=k.iris$cluster, pch=c(1,2,16)[iris[,5]])
```

9.3. Hierarchical Clustering in Python

The `scipy` library provide a variety of hierarchical clustering routines for Python. These are found in the module `scipy.cluster.hierarchy`. The clustering routines take as input an array giving the pairwise distances between the objects you want to cluster. Functions for calculating various dissimilarity measures are found in `scipy.spatial.distance`.

In our first example we will carry out single-linkage clustering using Euclidean distance as our dissimilarity measures.

```
In [6]: import numpy as np
In [7]: iris = np.loadtxt('iris.txt', skiprows=1, usecols=range(4))
In [8]: iris.shape
Out[8]: (150, 3)

In [9]: import scipy.spatial.distance as dist
In [10]: d = dist.pdist(iris, 'euclidean')

In [11]: d.shape
Out[11]: (11175,)
```

```
In [12]: (150*149)/2 # check number of pairs of specimens
Out[12]: 11175

In [13]: import scipy.cluster.hierarchy as hier
In [14]: ilink = hier.linkage(d)
In [15]: dendro = hier.dendrogram(ilink)
In [16]: dendro = hier.dendrogram(ilink, color_threshold=0.5) # colors the
         subtrees at a different threshold

# get species names from data file
In [17]: species = np.loadtxt('iris.txt', dtype=str, skiprows=1, usecols
   =[4])

# redraw dendrogram w/species names as labels
# root of tree to the left
In [18]: dendro = hier.dendrogram(ilink, color_threshold=0.5, labels=species
   , orientation='right', leaf_font_size=10)
```

And here's the equivalent version using city block (i.e. Manhattan) distance and UPGMA.

```
In [26]: d2 = dist.pdist(iris, 'cityblock')
In [27]: iupgma = hier.average(d2)
In [30]: dendro2 = hier.dendrogram(iupgma, labels=species, orientation='right',
   leaf_font_size=10)
```

See the SciPy documentation for the full details on [scipy.cluster.hierarchy](#) and [scipy.spatial.distance](#).

9.4. K-means clustering in Python

The module `scipy.cluster.vq` in the SciPy package implements k-means clustering. Using this module, there are three key steps you need to carry out: 1) normalizing (whitening) the input data set using the `whiten()` function; 2) running the `kmeans()` algorithm to calculate the cluster centroids; and 3) assigning each observation to the respective cluster using the `vq()` function ("vq" is short for vector quantization).

```
>>> from scipy.cluster import vq
>>> normiris = vq.whiten(iris)

# std deviation of variables before normalization
>>> np.std(iris, axis=0)
array([ 0.82530129,  0.43441097,  1.75940407,  0.75969263])

# std deviation of variables after normalization
>>> np.std(normiris, axis=0)
array([ 1.,  1.,  1.,  1.])

# calculate kmeans, using 3 groups
```

```
>>> centroids, distortion = vq.kmeans(normiris, 3)
>>> centroids
array([[ 7.00300835,  6.10726115,  2.45908867,  1.81598687],
       [ 8.14913325,  7.0954768 ,  3.10488375,  2.58102322],
       [ 6.06566359,  7.89114515,  0.83096318,  0.32381517]])

# Distortion is the sum of the squared diffs. btw. obs and corresponding
# centroids
>>> distortion
0.85998478065872275

# Assign each observation to it's nearest centroid
>>> assign, distortion = vq.vq(normiris, centroids)

# first ten items are assigned to group 2
>>> assign[:10]
array([2, 2, 2, 2, 2, 2, 2, 2, 2, 2])

# some more assignments
>>> assign[40:55]
array([2, 2, 2, 2, 2, 2, 2, 2, 1, 1, 1, 0, 0])
```

9.4.1. Creating a PCA plot in Python

Now that we've carried out the k-means clustering, let's generate a plot to illustrate the results. As we did before, we'll project the specimens into the space of the first two principal components and then color the points using the centroid labels assigned by the k-means algorithm. T

here is no built-in PCA function in SciPy, but a number of packages that were included in the Enthought Python Distribution include PCA functions. These include the packages scikit-learn (a powerful machine learning library for Python; <http://scikit-learn.org/>) and MDP ('Modular toolkit for Data Processing', <http://mdp-toolkit.sourceforge.net/>). The scikit-learn package is more powerful, but the MDP pca() function is simpler, so for now we'll use MDP.

```
>>> import mdp
>>> irispca = mdp.pca(iris)

# mdp.pca returns a matrix of PC scores. The scores for each PC
# are in the columns
>>> irispca.shape
(150, 4)

# we'll draw each of the labeled groups separately
>>> group0 = irispca[assign == 0]
>>> group1 = irispca[assign == 1]
>>> group2 = irispca[assign == 2]
```

```
>>> plot(group0[:,0], group0[:,1], color='blue', marker='o', linestyle='none')
>>> plot(group1[:,0], group1[:,1], 'ro') # shorthand way of plotting with
   red
                                         # circular markers; see help(plot)
                                         ) for info
>>> plot(group2[:,0], group2[:,1], 'go')

>>> axes = gca() # get the python object that represents the plot axes
>>> axes.set_aspect('equal') # set equal aspect ratio for x- and y-axes
>>> draw() # call draw() to refresh the plot
```

For the iris data set we know the true clustering. The first 50 specimens are *I. setosa*, the next 50 *I. versicolor*, and the last 50 are *I. virginica*. Let's create a fancier plot with two subfigures. The left plot will be the k-means assignments again; the right plot will highlight the mis-assignments.

```
# let's examine the centroid assignments
>>> assign
array([2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 1, 1, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0,
       0, 1, 0, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 0,
       0, 0, 0, 0, 0, 0, 1, 0, 1, 1, 1, 0, 1, 1, 1, 1, 1, 1, 0, 0, 1, 1,
       1, 1, 1, 1, 0, 1, 0, 1, 1, 0, 1, 1, 1, 1, 1, 1, 1, 1, 0, 0, 1, 1,
       1, 1, 1, 1, 0, 1, 1, 1, 0, 1, 1, 1])

# it looks like setosa specimens were given the label 2, versicolor the
# label 0
# and virginica the label 1

# let's use nested numpy.where calls to assign true labels
# use help(where) to read about how this function works
>>> truelabels = where(species == 'setosa', 2, where(species == 'versicolor',
   ', 0, 1))
array([2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
       0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
       0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
       1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
       1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1])

# find the objects that are mismatched
>>> mismatch = irispca[assign != truelabels]
>>> mismatch.shape
(23, 4)

# we're going to create a figure with two subplot, arranged in a 1-by-2
grid
```

```
# create first subplot
>>> subplot2grid((1,2), (0,0))
>>> plot(group0[:,0], group0[:,1], 'bo')
>>> plot(group1[:,0], group1[:,1], 'ro')
>>> plot(group2[:,0], group2[:,1], 'go')

# create 2nd subplot
>>> subplot2grid((1,2), (0,1))
>>> plot(irispc[:,:] irispc[:,1], 'ko', alpha=0.1)
>>> plot(mismatch[:,0], mismatch[:,1], 'mo') # highlight mismatches in magenta

# add a title that spans both subplots
>>> fig = gcf()
>>> fig.suptitle('Left: K-means clustering of iris data set\nRight: Misclassified observations from k-means clustering')
```

The final output of your plot should look like Figure 9.1.

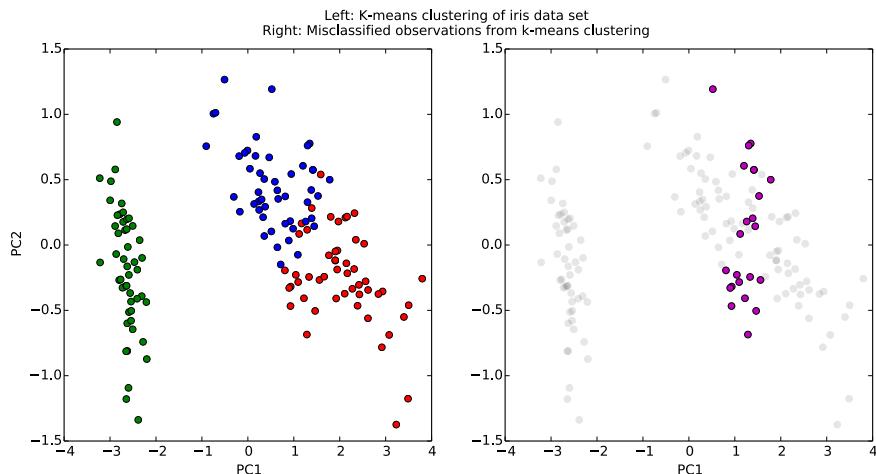


Figure 9.1.: Results of applying k-means clustering to the iris data set, using the k-means algorithm implemented in SciPy.

Assignment 9.1

Using the ‘naive’ k-means clustering algorithm described in lecture, implement your own k-means clustering function in either Python or R.

Your function should take as input:

1. A data matrix, X
2. an integer, k , giving the number of clusters
3. an integer, maxiter , giving the maximum number of iterations of the algorithm to perform. NOTE: maxiter is an upper limit; you might choose other criteria for the algorithm to converge, but maxiter will guarantee that it completes.

Illustrate your function with application to a data set of your choice.

10. Mixture Modeling and Multidimensional Scaling

10.1. Gaussian Mixture Models in R

There are multiple packages for fitting mixture models in R. We'll look at two - `mixtools` and `MCLUST`.

10.1.1. Installing mixtools

The package `mixtools` can be installed via the GUI or the `install.packages` command. A [mixtools vignette](#) can be downloaded from the CRAN website.

10.1.2. Using mixtools

We'll look at how to use `mixtools` using a data set on eruption times for the Old Faithful geyser in Yellowstone National Park (`?faithful` for details). We'll fit a univariate Gaussian mixture model to the time between eruptions data (`faithful$waiting`).

```
# allows us to refer to the variables within waiting time
# without using the standard list "$" syntax
> attach(faithful)

# create a nice histogram
> hist(waiting, main = "Time between Old Faithful eruptions",
xlab = "Minutes", ylab = "", cex.main = 1.5, cex.lab = 1.5, cex.axis = 1.4)

> library(mixtools)
> ?normalmixEM # read the docs!
> wait.mix <- normalmixEM(waiting)

> names(wait.mix)
[1] "x"          "lambda"      "mu"          "sigma"       "loglik"      "
 posterior"
[7] "all.loglik" "restarts"    "ft"

# Lambda is what we called "pi" in the lecture notes
> wait.mix[c("lambda","mu","sigma")]

> class(wait.mix)
[1] "mixEM"
```

```
> ?plot.mixEM # read about the plotting options for the mixEM object
> plot(wait.mix, density=TRUE)
> plot(wait.mix, loglik=FALSE, density = TRUE, cex.axis = 1.4, cex.lab =
  1.4, cex.main = 1.8, main2 = "Time between Old Faithful eruptions",
  xlab2 = "Minutes")
```

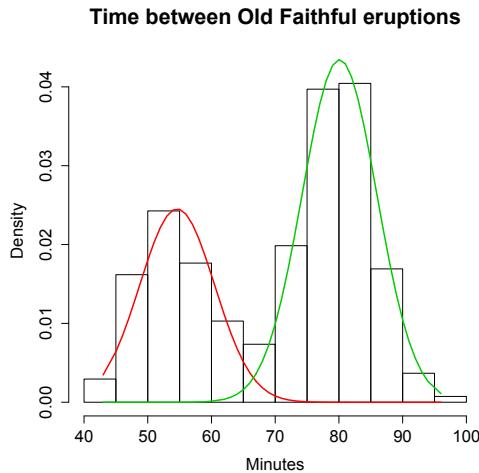


Figure 10.1.: A Gaussian mixture model for the Old Faithful dataset, estimated using the `mixtools` module in R.

10.1.3. Installing MCLUST

The package `MCLUST` is one of another package that provides maximum likelihood based estimation of mixture models. You will need to install the package (and it's dependencies) from the R GUI or using the `install.packages` command.

10.1.4. Using MCLUST

We're going to use two data set to illustrate some of `MCLUST`'s capabilities – the `iris` data set we've worked with before, and the bivariate version of the Old Faithful data set. We'll start off with the old faithful data set.

```
> plot(faithful$eruptions, faithful$waiting)
```

From visual inspection of the bivariate scatter plot, it looks like there two clusters. Let's apply the `Mclust` function and see what it suggests:

```
> library(mclust)
> fclust <- Mclust(faithful)
> fclust
```

```
best model: elliposidal, equal variance with 3 components
```

Now that we've run the mixture model, let's look at the results graphically. The following call to `plot` will produce a series of plots.

```
> plot(fclust)
```

The first plot gives is a diagnostic plot that shows the likelihood of the model as a function of the number of groups (see BIC below). In general, when considering many possible models you want to pick the simplest model that has a highest likelihood. The second graphically represents the classification. The third plots highlights those objects for which the cluster assignment is most uncertain. The fourth plot gives a graphical representation of the Gaussian densities.

The `Mclust` function used a likelihood criterion called the “Bayesian Information Criterion” (BIC) to estimate the number of components (clusters) in the mixture model. By this criterion it suggested 3 components. BIC, like other information criteria (the Akaike Information Criterion is another popular one), is designed to help choose among parametric models with different numbers of parameters. It tries to choose the simplest model that provides a good fit to the data.

```
> names(fclust)
[1] "modelName"      "n"           "d"           "G"          "
[BIC"
[6] "bic"            "loglik"       "parameters"  "z"          "
classification"
[11] "uncertainty"
> ?mclust # check out the docs to read about all the returned parameters
```

Of course you don't have to accept the number of clusters that the `Mclust` function estimated. Here's how you'd calculate the mixture model with a user determined number of clusters:

```
> fclust2 <- Mclust(faithful, G=2)
> plot(fclust2)
```

If you wanted to generate some of those plots individually you can do the following:

```
# generate a plot showing the classifications predicted by mixture model
> mclust2Dplot(data = faithful, what = "classification", identify = TRUE,
   parameters = fclust$parameters, z = fclust$z)
```

See the docs for the `mclust2Dplot` function for other options.

10.1.5. Mixture Models for the Iris data set

The `MCLUST` package includes the function `c1Pairs`, a very nice extension of the `pairs` function, for creating scatter plot matrices with group information. The following code illustrates this:

```
> names(iris) # remind yourself of the variables in the iris data set
[1] "Sepal.Length" "Sepal.Width"  "Petal.Length" "Petal.Width"  "Species"
```

```
# 5th variable is the Species classification
> clPairs(data=iris[,-5], classification=iris[,5])
```

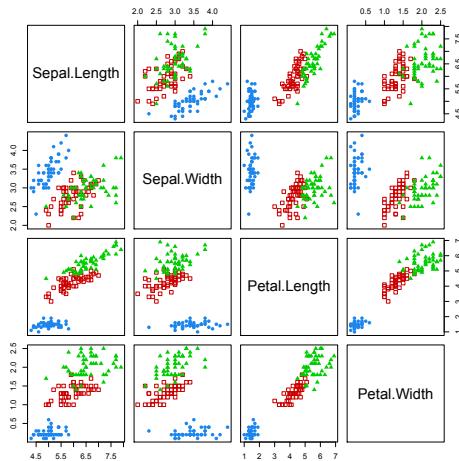


Figure 10.2.: Output of the `clpairs` function for the iris data set.

Let's see what `Mclust` makes of the iris data set:

```
> iclust <- Mclust(iris[,-5])
> iclust

best model: ellipsoidal, equal shape with 2 components
> plot(iclust)
```

Blind to the actual group structure the BIC suggests just two components, whereas we know there are three groups (though *I. versicolor* is thought to be an allopolyploid hybrid; see Kim et al. (2007) Ann Bot, 100: 219-224.). Examine the first graph produced by the `plot` call above to see how the 2 and 3 component models compare with respect to the BIC.

Now let's see how the mixture model does when we give it the true number of clusters:

```
> iclust3 <- Mclust(iris[,-5], G=3)
> plot(iclust3)
```

To calculate the classification error rate we can compare the estimated clustering to the "true" (known) classification with the `classError` function (`?classError` for details):

```
> classError(iclust3$classification, iris[,5])
$misclassified
[1] 69 71 73 78 84
```

```
$errorRate  
[1] 0.03333333
```

The uncerPlot command allows us to visualize the uncertainty implied by the mixture model to see how uncertain the model was about the misclassified samples.

```
> uncerPlot(iclust3$z, iris[,5])
```

In the uncertainty plot the vertical lines indicate the misclassified samples. As you can see those tend to be among the observations that the mixture model was most uncertain about with respect to which component they belonged to.

10.1.6. More details on MCLUST

See the [MCLUST docs](#) for in depth discussion of the use of MCLUST. The examples illustrated above were drawn from this documentation.

10.2. Mixture Modeling in Python

The package [scikit-learn](#) extends the SciPy library with a number of common machine learning algorithms, including an implementation of Gaussian mixture modeling. [scikit-learn](#) is included with the EPD distribution you have already installed. The code below demonstrates how to use [scikit-learn](#) to carry out mixture modeling.

Before you get started use the `write.table()` function in R to create a tab-delimited version of the `faithful` dataset (hint: use "\t" to specify tabs as the separator character, and dont include row names in the file). If you've properly formatted the file you should be able to open it as follows, and create a histogram:

```
In [1]: f = np.loadtxt('faithful.txt', skiprows=1)  
In [2]: f.shape  
Out[2]: (272, 2)
```

Assuming that worked, let's import [scikit-learn](#) and fit a mixture model:

```
In [4]: from sklearn import mixture  
In [6]: classifier = mixture.GMM(n_components = 2)  
In [7]: fit = classifier.fit(f[:,1])  
  
In [22]: fit.means_ # means of the estimated subdistributions  
Out[22]:  
array([[ 80.12056513],  
       [ 54.6623402 ]])  
  
In [23]: fit.covars_ # (co)variances of the estimated substritutions  
Out[23]:  
array([[ 34.08969904],  
       [ 34.95662279]])
```

```
In [66]: fit.weights_ # weighting factors (pi in slides)
Out[66]: array([ 0.63770034,  0.36229966])

In [32]: x = linspace(40, 100, 200) # points at which to evaluate the model

# plot histogram using prob density rather than straight up frequency
In [33]: hist(f[:,1], normed=True, alpha=0.2, color='gray')

# draw the PDFs for the two estimated subdistributions
# notice how we multiply each normal PDF by it's weight

In [63]: plot(x, fit.weights_[0] * normpdf(x, fit.means_[0], sqrt(fit.
    covars_[0])),color='red')
In [64]: plot(x, fit.weights_[1] * normpdf(x, fit.means_[1], sqrt(fit.
    covars_[1])),color='blue')
In [73]: xlabel("Waiting Time")
In [74]: ylabel("Density")
In [75]: title("Time between Old Faithful eruptions")
```

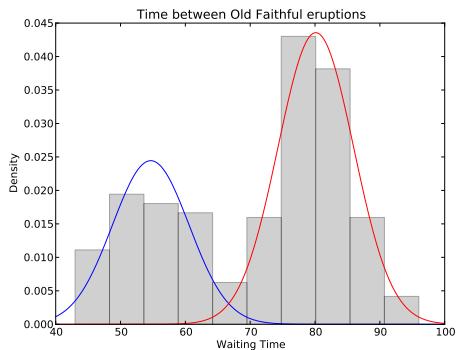


Figure 10.3.: A Gaussian mixture model for the waiting time variable in the Old Faithful dataset, estimated using the scikit-learn module in Python.

Now let's look at the binary data:

```
In [4]: plot(f[:,0], f[:,1], 'k.') # 'k.' gives small black dots as plot
    points
In [8]: xlabel("Eruption time (mins)")
In [9]: ylabel("Waiting time to next eruption (mins)")

# specify covariance_type = 'full' to estimate complete covariance matrices
In [10]: classifier2 = mixture.GMM(n_components=2, covariance_type='full')
In [11]: fit2 = classifier2.fit(f)

# setup grid to evaluate mixture model over
```

```
In [12]: x = linspace(1.5,5,200)
In [13]: y = linspace(40,100,100)
In [14]: X,Y = meshgrid(x, y)
In [23]: XY = np.c_[X.ravel(), Y.ravel()] # like R's c()

# evaluate the mixture model over the grid
In [24]: Z = log(-fit2.eval(XY)[0])
In [25]: Z = Z.reshape(X.shape)
In [26]: cntr = contour(X, Y, Z)
```

Check out the scikit-learn docs for more details and examples.

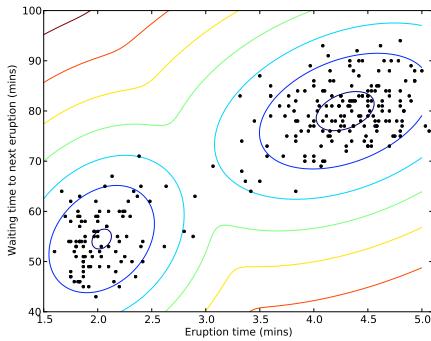


Figure 10.4.: A Gaussian mixture model for 2D Old Faithful dataset, estimated using the scikit-learn module in Python.

Assignment 10.1

Download the dataset `ddata.txt` from the course wiki. This data set consists of 64 variables measured on 720 specimens. Use the various clustering and ordination techniques you've learned over the course of the semester to explore this data set and estimate the number of clusters in the data. Use code and figures to support your conclusion. Hint: you might consider using a lower dimensional approximation of the data to facilitate your analyses.

10.3. Multidimensional scaling and Minimum Spanning Trees in R

10.3.1. Metric MDS

The implementation of classic metric scaling in R is carried out using the `cmdscale()` function. Read the documentation for `cmdscale` and then work through the exam-

ple showing the application of MDS to analysis of road distances between US cities available at the following link (but see notes below first):

<http://personality-project.org/r/mds.html>.

As you work through your example note the following:

- You can use the `source()` function not only with a local file but also with a URL. This is convenient but potentially a security issue so don't run code willy nilly without checking out what it does.
- You can download the code at <http://personality-project.org/r/useful.r> and check out the functions that it includes. I thought the `read.clipboard()` function was particularly nice.

10.3.2. Minimum Spanning Tree in R

The package `ape` has an `mst()` function. Several others packages, including `vegan` also have minimum spanning tree functions. Install `ape` using the standard `install.packages()` mechanism.

The `mst()` function takes a dissimilarity matrix as its input and returns a square adjacency matrix, A , where $A_{ij} = 1$ if (i, j) is an edge in the MST or 0 otherwise. Here's an application of the `MST` function to the `cities` example you completed above.

```
> library(ape) # install ape first if necessary
> city.mst <- mst(as.dist(cities))
> city.mst # see the adjacency matrix return by mst
```

If you want to create a nice looking plot you can use the `mat2listw()` function in the package `spdep`. `mat2listw` converts the adjacency matrix into a form that you can extract the neighbor information from:

```
> library(spdep) # install spdep first if necessary
> plot(city.location, type='n', xlab='PCoord1', ylab='PCoord2')
> text(city.location, labels=names(cities))

# note British spelling of 'neighbours'
> plot(mat2listw(city.mst)$neighbours, city.location, add=T)
```

10.3.3. Non-metric MDS

The `isoMDS()` function in the `MASS` package implements the Shepard-Kruskal version of non-metric scaling, while the `sammon()` function in the same package use the criterion proposed by Sammon (1969). You will need to utilize these functions, along with `cmdscale` and the hierarchical clustering functions covered last week for the following assignment.

Assignment 10.2

Harding and Sokal (1998; PNAS 85:9370-9372; see course wiki) used cluster analysis and non-metric MDS to explore the relationship between European language families as measured by genetic distances among the people who speak those languages. The classification they derived at large reflects geographic proximity but there are some language families that have distant genetic relationships to their geographic neighbors.

Harding and Sokal provide a table of genetic distances that they used in their analyses. Use R to reconstruct the cluster analysis they report (Fig. 1) and repeat this analysis using neighbor joining. In a similar manner use both metric scaling and the Shepard-Kruskal and Sammon criteria for non-metric scaling to do an MDS analysis (similar to Harding and Sokal's fig. 2). Try to also recreate the MST shown in their figure 2.

Submit your code as an R markdown document, and include a brief paragraph describing what differences, if any, you found in your re-analysis of Harding and Sokal data. Are these differences significant (i.e. do they change your interpretation of the data)?

10.4. Multidimensional scaling in Python

The scikit-learn package also includes facilities for metric and non-metric multidimensional scaling. The functions for carrying out MDS are included in the `sklearn.manifold` module which also includes a variety of other techniques for so-called ‘manifold learning’. Manifold learning is an area of the machine learning literature concerned with approaches to non-linear dimensionality reduction.

We'll apply metric MDS to the `iris` data set, using Euclidean distance as our dissimilarity measure. Recall that metric MDS on Euclidean distances is equivalent to PCA.

```
>>> import sklearn.datasets  
>>> iris = sklearn.datasets.load_iris()  
>>> print iris['DESCR']  
Iris Plants Database
```

Notes

Data Set Characteristics:

```
:Number of Instances: 150 (50 in each of three classes)  
:Number of Attributes: 4 numeric, predictive attributes and the class  
:Attribute Information:  
    - sepal length in cm  
... Output Truncated ...
```

```
>>> iris.data  
array([[ 5.1,  3.5,  1.4,  0.2],  
       [ 4.9,  3. ,  1.4,  0.2],  
       [ 4.7,  3.2,  1.3,  0.2],  
       [ 4.6,  3.1,  1.5,  0.2],
```

```
... Output Truncated ...

>>> import sklearn.manifold

# we setup the model first, asking for a two dimensional embedding (
    n_components)
>>> mds = sklearn.manifold.MDS(dissimilarity='euclidean', n_components=2)
>>> pos = mds.fit_transform(iris.data) # and then we fit the model to the
    data

# fti_transforms returns an array that
# holds the information about coordinates in the MDS space
>>> pos.shape
(150, 2)

# plot the ordination
>>> plot(pos[:,0], pos[:,1], 'ko')
[<matplotlib.lines.Line2D object at 0x10d683b50>]
>>> gca().set_aspect('equal')
>>> draw()
```

The iris data set, as represented in sklearn, has a variety of accessory attributes. For example, the `target_names` attribute gives the set of species labels, and the `'target'` attribute gives the corresponding classification for each sample. We will use this to create a fancier MDS ordination plot.

```
>>> iris.target_names
array(['setosa', 'versicolor', 'virginica'],
      dtype='|S10')
>>> iris.tar
iris.target      iris.target_names
>>> iris.target
array([0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
       0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
       0, 0, 0, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
       1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1,
       1, 1, 1, 1, 1, 1, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,
       2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2])]

# setup Boolean vectors to represent the different species
>>> setosa = iris.target == 0
>>> versicolor = iris.target == 1
>>> virginica = iris.target == 2
>>> plot(pos[setosa,0], pos[setosa,1], 'ro', label='I. setosa')
>>> plot(pos[versicolor,0], pos[versicolor,1], 'g*', label='I. versicolor')
>>> plot(pos[virginica,0], pos[virginica,1], 'b^', label='I. virginica')
>>> gca().set_aspect('equal')
```

11. Randomization, Bootstrap, and LOESS

11.1. Randomization Tests in R

There are a number of packages (e.g. `coin`) that include functions for carrying out randomization/permuation tests in R. However, it's often just as easy to write a quick set of functions to carry out such tests yourself. We'll illustrate a simple example of this using the "jackal" example from Manly (2006).

Consider the following data set composed of measures of mandible lengths (in mm) for male and female golden jackals. This set of measurements was taken from a set of skeletons in the collections of the British Museum of Natural History.

| | | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Males | 120 | 107 | 110 | 116 | 114 | 111 | 113 | 117 | 114 | 112 |
| Females | 110 | 111 | 107 | 108 | 110 | 105 | 107 | 106 | 111 | 111 |

Let's first create two vectors to represent this set of measurements and create a quick beanplot to visually compare the distributions.

```
> males <- c(120,107,110,116,114,111,113,117,114,112)
> females <- c(110,111,107,108,110,105,107,106,111,111)
> library(beanplot)
> beanplot(males,females,side='b',col=list('blue','pink'),names=c("Male",
  Female"))
> mean(males)
[1] 113.4
> mean(females)
[1] 108.6
> mean(males) - mean(females)
[1] 4.8
```

The hypothesis we want to test is that male jackals have, on average, larger mandibles than female jackals. The beanplot we constructed and difference in the means would seem to suggest so but let's carry out some more formal tests. The obvious way to compare this set of measurements would be to carry out a t-test, which is appropriate if the samples are normally distributed with approximately equal variance. We have small samples here, so it's hard to know if the normal distribution holds. Instead we'll use a randomization test to compare the observed difference in the means (4.8) to the distribution of differences we would expect to observe if the labels 'male' and 'female' were randomly applied to samples of equal size from the data at hand.

Let's create a function that takes a sample and randomly assigns the observations to two groups of a specified size. The function takes as input a vector of values (size

N) and two integers representing the sample sizes (n_1 and n_2 where $n_1 + n_2 = N$) of the two groups to be compared.

```
two.group <- function(x,n1,n2){
  # sample w/out replacement
  reordered <- sample(x, length(x)) # see help(sample) for more info
  g1 <- reordered[seq(1,n1)]
  g2 <- reordered[seq(n1+1,n1+n2)]
  list(g1,g2)
}
```

Test out this function by calling it repeatedly as shown below. You'll see that it returns a random reordering of the original data, split into two groups:

```
> jackals <- c(males,females)
> two.group(jackals,10,10)
# ... output not shown ...
> two.group(jackals,10,10) # call it again to get a different sample
# ... output not shown ...
```

Now let's write a simple function that returns the mean difference between two samples:

```
mean.diff <- function(x1,x2) {
  mean(x1) - mean(x2)
}
```

Now let's write a generic randomization function:

```
randomization <- function(x1,x2,fxn,nsamples=100){
  stats <- c()
  orig <- c(x1,x2)
  for (i in 1:nsamples){
    g <- two.group(orig, length(x1), length(x2))
    stats <- c(stats, fxn(g[[1]],g[[2]])))
  }
  return (stats)
}
```

We can then use the `randomization` function we wrote as follows to evaluate the significance of the observed difference in means in the original sample:

```
# generate 1000 samples of the mean.diff for randomized data
> rsample <- randomization(males,females,mean.diff,1000)
> hist(rsample) # examine the distribution

# in how many of the random samples is mean difference between the two
# groups
# as great or larger than the observed difference in our original samples?
# you might get a slightly different answer
> sum(rsample >= 4.8)
[1] 2
```

So our conclusion is that the probability of getting a mean difference between samples of this size is about $2/1000 = 0.002$. Note that we can't generalize this to golden jackals as a whole because we know nothing about whether these samples actually represent random samples of the golden jackal population or biases that might have been imposed on the collection (e.g. maybe the collectors liked to single out particularly large males). However, if we saw a similar trend (males larger than females) in multiple museum collections we might see this as supporting evidence that the trend held true in general.

Note that we wrote our `randomization` function to take an arbitrary function that takes as its input two vectors of data. That means we can use it to estimate the randomized distribution of arbitrary statistics of interest. Here we illustrate that with a function that calculates the ratio of variances.

```
ratio.var <- function(x1,x2){
  var(x1)/var(x2)
}

> ratio.var(males,females) # ratio of variances for the original samples
[1] 2.681034
> vsample <- randomization(males,females,ratio.var, 1000)
> hist(vsample)
> mean(vsample)
[1] 1.266088
> sum(vsample >= 2.68)
[1] 74
> 74/1000.
[1] 0.074
```

In this case the observed ratio of variances isn't particularly unusual. Let's make one more comparison. We know (or at least we should know!) that ratios of variances have an F -distribution so let's compare the distribution of ratios of variances from our randomized sample to that of a sample of the same size drawn from the F -distribution with the same degrees of freedom.

```
> randomF <- rf(1000, 9, 9) # see help(rf)
> plot(density(vsample),type='l',xlab="Ratio of variances", main="Ratio of
  Variances\n Theoretical (red) vs Randomization Estimate (black)")
> lines(density(randomF),col='red')
```

11.2. Jackknifing in R

Jackknife estimates of simple statistics are also relatively straightforward to calculate in R. Here's an example of a simple jackknife function:

```
jknife <- function(x, fxn, ci=0.95) {
  theta <- fxn(x)
  n <- length(x)
  partials <- rep(0,n)
```

```

for (i in 1:n){
    partials[i] <- fxn(x[-i])
}
pseudos <- (n*theta) - (n-1)*partials
jack.est <- mean(pseudos)
jack.se <- sqrt(var(pseudos)/n)
alpha = 1-ci
CI <- qt(alpha/2,n-1,lower.tail=FALSE)*jack.se
jack.ci <- c(jack.est - CI, jack.est + CI)
list(est=jack.est, se=jack.se, ci=jack.ci)
}

```

The bootstrap package (install if necessary) contains a very similar implementation of a jackknife function (`jackknife()`).

Let's illustrate our jackknife function using samples drawn from a Poisson distribution. The Poisson is a discrete probability distribution that is often used to describe the probability of a number of events occurring in a fixed period of time, where the events are independent and occur with an average rate λ . The Poisson distribution is used to model processes like mutations in DNA sequences or atomic decay. Both the mean and variance of a Poisson distribution are equal to λ . Let's see how well the jackknife does at estimating confidence intervals for the mean and variance of a modest number of samples drawn from a Poisson.

```

> psample <- rpois(25,4) # 25 obsevations from poisson with Lambda = 4
> psample # your sample will be different
[1] 3 1 1 3 3 3 5 4 1 4 6 5 6 3 4 5 6 1 1 2 3 7 3 4 5
> mean(psample)
[1] 3.56
> var(psample)
[1] 3.173333
> jknife(psample, mean)$ci
[1] 2.824680 4.295320
> jknife(psample, var)$ci
[1] 1.716397 4.630270

```

In both cases above, the true mean and variance were contained within the 95% confidence intervals estimated by the jackknife. Let's do a little experiment to see how often that's true for samples of this size:

```

# create 500 samples of size 25 drawn from Poisson w/lambda=4
> psamples <- matrix(rpois(25*500,4),ncol=25,byrow=T)
> dim(psamples)
[1] 500 25

# create a convenience function
> get.ci <- function(x) { return(x$ci) } #x$ci gives confidence interval

# generate jackknife estimates for mean
> j.mean <- apply(psamples, 1, jknife, mean)

```

```
# make matrix that holds 95% confidence intervals of mean
> mean.ci <- t(sapply(j.mean, get.ci))
> mean.ci[1,]
[1] 2.796265 4.323735
> mean.ci[2,]
[1] 3.562991 4.917009

# check how often true mean is w/in CI
> sum(mean.ci[,1] <=4 & mean.ci[,2] >= 4)
[1] 463
> 463/500
[1] 0.926
# true mean is w/in estimated 95% CI about 93% of the time.

# now the same for variances
> j.var <- apply(psamples, 1, jknife, var)
> var.ci <- t(sapply(j.var, get.ci))
> sum(var.ci[,1] <=4 & var.ci[,2] >= 4)
[1] 449
> 449/500.
[1] 0.898
# true variance is w/in 95% CI only 90% of time
```

In the case of the confidence intervals for the mean, the jackknife estimator did a decent job - the true mean is with the 95% confidence interval about 93% of the time. In the case of the variance it did less well. The jackknife confidence intervals work well when the estimator is normally distributed. This suggests that one way we might improve the jackknife CIs is by using a normalizing transformation, like the logarithm function:

```
> log.var <- function(x){log(var(x))}
> j.log.var <- apply(psamples, 1, jknife, log.var)
> log.var.ci <- t(sapply(j.log.var, get.ci))
> sum(log.var.ci[,1] <=log(4) & log.var.ci[,2] >= log(4))
[1] 472
> 472/500.
[1] 0.944
# a substantial improvement in the performance of the 95% CIs
```

This illustrates the type of simulation study you might do to check the robustness of the jackknife for a statistic of interest for a given class of distributions.

11.3. Bootstrapping in R

There are several packages that provide functions for doing bootstrapping in R. These include `bootstrap` and `boot`. We'll take a quick look at the functions in `bootstrap`. Install `bootstrap` using the standard package installation mechanism.

We'll use the same set of samples from the Poisson that we used before to illustrate the jackknife.

```

> library(bootstrap)
> ?bootstrap # as always, check out the docs
# generate 1000 bootstrap sample estiamte of var
> b <- bootstrap(psample, 1000, var)

# standard bootstrap confidence limits
# based on assumption of normality
> bstar <- b$thetastar
> c(mean(bstar)-1.96*sd(bstar), mean(bstar)+1.96*sd(bstar))
[1] 1.774923 4.348151

# estimate the bootstrap percentile confidence limits
> quantile(b$thetastar,c(0.025,0.975))
  2.5%   97.5%
1.839583 4.373333

# for comparison remind ourself of what the jackknife CI was
> jknife(psample,var)$ci
[1] 1.716397 4.630270

```

Now let's use the bootstrap to look at the distribution of a more complicated statistic – the fraction of the variance explained by the first principal component. To do this, first we need to define a function that return the statistic we're interested in.

```

> pca.1stpc.ratio <- function(x){
+   x.pca <- prcomp(x, center=T, scale.=T)
+   pca.var <- x.pca$sdev**2
+   return (pca.var[1]/sum(pca.var))
+ }

```

The `bootstrap()` function expects the data to be in the form of a vector, not a data frame or matrix, so we will write a second function that takes as it's first argument the set of observations to use to construct each bootstrap sample, and as it's second argument the full data set. We can then pass this function to `bootstrap()` with a vector of indices $1, 2, \dots, n$.

```

> pca.on.subset <- function(the.subset, orig.data){
+   data.subset <- orig.data[the.subset,]
+   pca.1stpc.ratio(data.subset)
+ }

```

Having defined this function we can generate bootstrap samples to estimate the distribution of this statistic:

```

> iris.sub <- subset(iris, select=-Species) # drop the species column
> iris.boot <- bootstrap(1:150, 1000, pca.on.subset, iris.sub)
> hist(iris.boot$thetastar)
> mean(iris.boot$thetastar)
[1] 0.7306235
> pca.1stpc.ratio(iris.sub)
[1] 0.7296245

```

For this particular statistic, the fraction of variation explained by the 1st PC, the bootstrap distribution looks fairly normal with a mean fairly close to our observed value.

11.4. LOESS Models

LOESS (aka LOWESS; ‘Locally weighted scatterplot smoothing’) is a modeling technique that fits a curve (or surface) to a set of data using a large number of local regressions. Local weighted regressions are fit at numerous regions across the data range, using a weighting function that drops off as you move away from the center of the fitting region (hence the ‘local’ aspect). LOESS combines the simplicity of least squares fitting with the flexibility of non-linear techniques and doesn’t require the user to specify a functional form ahead of time in order to fit the model. It does however require relatively dense sampling in order to produce robust fits.

Formally, at each point x_i we estimate the regression coefficients $\hat{\beta}_j(x)$ as the values that minimize:

$$\sum_{k=1}^n w_k(x_i)(y_k - \beta_0 - \beta_1 x_k - \dots - \beta_d x_k^d)^2$$

where d is the degree of the polynomial (usually 1 or 2) and w_k is a weight function. The most common choice of weighting function is called the “tri-cube” function which is defined as:

$$\begin{aligned} w(x) &= (1 - |x|^3)^3, \text{ for } |x| < 1 \\ &= 0, \text{ for } |x| \geq 1 \end{aligned}$$

The primary parameter that a user must decide on when using LOESS is the size of the neighborhood function to apply (i.e. over what distance should the weight function drop to zero). This is referred to as the “span” in the R documentation, or as the parameter α in many of the papers that discuss LOESS. The appropriate span can be determined by experimentation or, more rigorously by cross-validation.

We’ll illustrate fitting a LOESS model using data on Barack Obama’s approval ratings over the period from Jan 2007 to November 2012, using data downloaded from [pollster.com](#). This data is available as `obama-favorable-rating.csv` on the class wiki.

```
> polls <- read.csv('obama-favorable-rating.csv')
> names(polls)
[1] "Pollster"                 "Start.Date"                "End.Date"
[4] "Release.Date.Time..ET.."  "Number.of.Observations" "Population"
[7] "Mode"                     "Favorable"                 "Unfavorable"
[10] "Undecided"               "Pollster.URL"              "Source.URL"
[13] "Source.URL.1"
> dim(polls)
```

```
[1] 666 13
```

```
# polls are in reverse chronological order so let's reverse them
# so we can look at trend from earliest to most recent dates
> fav <- rev(polls$Favorable)
> pollnum <- 1:length(fav)
> plot(pollnum, fav,pch=16, cex=0.5,col='grey')
> loess.fav <- loess(fav ~ pollnum)
> pred.fav <- predict(loess.fav, pollnum)
> lines(pollnum, pred.fav, lwd=2, col='red')

# now with a smaller neighborhood span
> loess.fav2 <- loess(fav ~ pollnum, span=0.25)
> pred.fav2 <- predict(loess.fav2, pollnum)
> lines(pollnum, pred.fav2, lwd=2, col='blue')
```

Take note of how the LOESS curve changed when we made the span smaller. By decreasing the span we've increased the sensitivity of the model (perhaps overfitting in this case).

Assignment 11.1

Write an R function that generates a plot that simultaneously illustrates trends in both approval and disapproval ratings for Barack Obama, showing both the raw data and corresponding LOESS fits. Use colors and/or shapes to distinguish the two trends. Make sure both your x- and y-axes are scaled to show the full range of the data. Label your axes and create a title in the plot. Aim for a 'publication quality' figure.

12. Bioinformatics Pipelines I

12.1. Overview

Many types of analyses, especially those involving genomic data, require the investigator to carry out a large number of sequential steps. For example, given a set of uncharacterized genes in your organism of interest you might want to find out as much as you can about the structure and function of the proteins they encode, search for related proteins in other organisms, and try to identify pathways that they might be involved in. If you had only a single gene of interest you might apply each of the appropriate software tools by hand to carry out such an analysis. However, when the number of genes of interest grows beyond a small number (say 10-15) doing such an analysis by hand starts to become tedious and error prone. A bioinformatics pipeline can help to automate this process, will make the analysis easier to replicate or apply to new sets of genes, and can be modified to include additional tasks. Writing out a series of analysis steps as a pipeline also helps us to achieve the goal of ‘reproducible research’ in the same way that knitr helps you to do so in R.

During the next two class sessions we’re going to build a series of analysis pipelines, starting with simple shell scripts and eventually building up to a complex series of analyses integrating Python code, several command line programs, and web queries to NCBI.

12.2. Awk revisited

One of the tools that was introduced in the appendix on Unix command line tools was Awk, a programming language for processing structured text files. We’ll start by revisiting Awk to build some simple but useful programs that we’ll then incorporate into our first pipeline.

If you haven’t already done so, download the *Saccharomyces cerevisiae* GFF file from the yeast genome database as so:

```
$ curl -O http://downloads.yeastgenome.org/curation/chromosomal_feature/  
       saccharomyces_cerevisiae.gff
```

Recall that Awk works on the fields of a structured data file. By default fields are delimited by spaces or tabs, though this can be changed. Conveniently, GFF files consist of 9 columns separated by tabs, so they are well suited to manipulation by awk. The information that is found in each of the columns of the GFF format is described here: <http://www.sequenceontology.org/gff3.shtml>.

12.2.1. The pattern {action} syntax of Awk

The basic syntax of awk is often depicted in the form `pattern {action}`. In the example below the pattern can be read as – “if the 3rd field is ‘chromosome’”. For all lines that match that pattern the corresponding action is applied; in this case “print fields 1 and 5” (the chromosome name and its length):

```
awk '$3=="chromosome" {print $1, $5}' saccharomyces_cerevisiae.gff
```

Here’s another pattern {action} pair that shows how we could find all gene features with length less than 300:

```
$ awk '$3 == "gene" && ($5 - $4) < 300 {print $0 }'  
saccharomyces_cerevisiae.gff | wc -l
```

`&&` is the AND operator. The pattern for this example translates as “if the 3rd fields is ‘gene’ AND the the 5th field minus the 4th field is less than 300”; the corresponding action is “print the whole line.” Notice how we piped the output of awk to the utility `wc` to count the number of lines returned.

Let’s add one more condition to the previous example – we’ll look for the word ‘Dubious’ in the 9th field.

```
$ awk '$3 == "gene" && ($5 - $4) < 300 && match($9, "Dubious") {print $0 }'  
saccharomyces_cerevisiae.gff | wc -l
```

Comparing the output of the previous example to this one you’ll see that a significant proportion of small genes are classified as ‘Dubious’.

12.2.2. Writing an Awk script

There’s lots of powerful things you can do with awk one-liners, but writing short scripts often makes things easier to understand. You can think of an awk scripts as a series of pattern {action} statements. Our script will create a table giving both the length of each chromosome and the number of genes on that chromosome. Save the following script in a file called `gcount.awk`.

```
# gcount.awk  
# Length of each chromosome  
$3 == "chromosome" {  
    clen[$1] = $5  
}  
  
# increment the gene count for the given chromosome  
$3 == "gene" {  
    ngenes[$1] += 1  
}  
  
# END only gets carried out once  
# we've processed all the records  
END {  
    print "Chrom\tLength\t# Genes"
```

```
for (chr in clen) {  
    print chr "\t" clen[chr] "\t" ngenes[chr]  
}  
}
```

In this example we create two arrays – `clen` and `ngenes` – to keep track of the chromosome lengths and number of genes on each chromosome. Arrays can be indexed by either integers or strings; when they are indexed by strings we can think of them like Python dictionaries. We have two patterns – whether the 3rd field equals 1) “chromosome” or 2) “gene”. The final pattern, labeled `END`, says what to do once we’ve processed all the lines in the file. Run this script as follows:

```
$ awk -f gcount.awk saccharomyces_cerevisiae.gff
```

The `-f` option says to use the pattern/action pairs contained in the specified file. One possible shortcoming (at least on my system) is that the output wasn’t sorted. That’s easy to solve by piping the results to the `sort` command:

```
$ awk -f gcount.awk saccharomyces_cerevisiae.gff | sort
```

12.2.3. A more flexible Awk script

Our `gcount.awk` script works pretty well, but what if we wanted to count pseudogenes rather than genes, or tRNA features? In its current form the feature type is hardcoded into the script. Let’s see how we can get rid of that constraint. Save the following awk script as `fcount.awk`.

```
# fcount.awk  
BEGIN {  
# if var ftype has NOT been defined, assign it a default value  
if (!ftype)  
    ftype = "gene"  
}  
  
# length of each chromosome  
$3 == "chromosome" {  
    clen[$1] = $5  
}  
  
# increment the feature count for the given chromosome  
$3 == ftype {  
    ngenes[$1] += 1  
}  
  
END {  
for (chr in clen) {  
    print chr "\t" clen[chr] "\t" ngenes[chr]  
}
```

Here we introduced the BEGIN pattern. This pattern is carried out before any lines of the file are processed. By default, this new script will count genes like our previous script did, but if you specify the variable ftype using the -v option on the command line it will count the specified feature type:

```
# count pseudogenes
$ awk -f fcount.awk -v ftype="pseudogene" saccharomyces_cerevisiae.gff

# counts ARS sequences (origins of replication)
$ awk -f fcount.awk -v ftype="ARS" saccharomyces_cerevisiae.gff

# count tRNA genes
$ awk -f fcount.awk -v ftype="tRNA" saccharomyces_cerevisiae.gff
```

12.2.4. String substitution in Awk

As a final example of using awk, let's see how we can use string substitution to create more human friendly output when we output our GFF file in awk.

Here's a now familiar example of using the pattern {action} syntax to find all the pseudogenes in a GFF file:

```
$ awk '$3 == "pseudogene" { print $0 }' saccharomyces_cerevisiae.gff | less
```

This works, but the output is a bit ugly because of how the attribute field is specified in GFF format. Let's write a simple awk function that nicely formats the output. Save the following script as attribs.awk.

```
# attribs.awk
# parse the attributes field of a GFF file

NF >= 9 {
    # print some useful fields
    print "Chromosome =", $1
    print "Type =", $3
    print "Start =", $4
    print "End =", $5
    print "Strand =", $7
    #print $1, $2, $3, $4, $5, $6, $7, $8

    # break the attributes field up into individual attributes
    n = split($9, attributes, ";")
    for (i = 1; i <= n; i++) {
        tstr = attributes[i]
        gsub(/%20/, " ", tstr) # spaces
        gsub(/%2C/, ",", tstr) # commas
        gsub(/%3B/, ";", tstr) # semi-colons
        gsub(/%2F/, "/", tstr) # forward slash
        gsub("=", " = ", tstr) # add spaces around equal signs
```

```
    print tstr
}
print "\n"
}
```

The awk function `gsub()` globally substitutes one string for another. In this case it's replacing HTML type encoding of spaces, commas, semi-colons, etc. with more human friendly versions of the same. We can use our `attribs.awk` script as follows:

```
$ awk '$3 == "pseudogene" { print $0 }' saccharomyces_cerevisiae.gff | awk
-f attribs.awk | less
```

This produces output that is much nicer for a human reader to interpret, though perhaps less easy to parse computationally.

The GFF3 format is used by many organism specific genome projects besides yeast. If we take care to write our scripts to operate on GFF3 files generically then we can apply scripts we write for one organism easily to another organism. Let's test this out by downloading the X-chromosome GFF3 file for *Drosophila melanogaster* from FlyBase:

```
$ curl -O ftp://ftp.flybase.net/genomes/dmel/current/gff/dmel-X-r5.54.gff.gz
$ gunzip dmel-X-r5.54.gff.gz # unzip the compressed file
```

Now let's test our `attribs.awk` script with this new GFF file by generating a report on pseudogenes on the *Drosophila* X-chromosome:

```
$ awk '$3 == "pseudogene" {print $0}' dmel-X-r5.54.gff | awk -f attribs.awk > fly-X-pseudogenes.txt
```

Use `less` or a text editor to view your report.

12.3. Shell Scripting

To this point all of our examples have involved single command lines or scripts, occasionally tied together with pipes. This works well for quick analyses, but what if you wanted to run an analysis over and over again, say on a monthly basis as a genome project was updated, or as you generated new data as part of your research? In that context a shell script might be useful. A shell script is a small program written for the command line interpreter of an operating system. A shell script is convenient for tying together a series of commands that you might otherwise type by hand into a repeatable and documented set of operations. For these exercises we will assume that you use the “bash shell”, which is the default command line interface on OSX, Cygwin, and most Linux based systems. You can confirm that your default shell is bash by doing something like:

```
$ sh --version
GNU bash, version 3.2.48(1)-release (x86_64-apple-darwin10.0)
Copyright (C) 2007 Free Software Foundation, Inc.
```

Assuming, that you've got bash working on your system, enter the following code into your text-editor and save it with the filename `genome_reporter.sh` in the same directory where you've saved `fcount.awk` that we created earlier. Be careful that you enter the text as shown as bash is particularly picky about extra spaces around the equal sign (=) in variable assignment so if you get error messages when you try and run this script (see below), that's the first thing to check.

```
#!/bin/bash

URL='http://downloads.yeastgenome.org/curation/chromosomal_feature/
      saccharomyces_cerevisiae.gff'
BASEFILE='saccharomyces_cerevisiae.gff'

# get today's date
TODAY=$(date -u +%Y-%m-%d)

# create filename, prepended w/today's date
FILENAME="$TODAY-$BASEFILE"
REPORT="report-$FILENAME"

# if the GFF file does not already exist then
# use curl to download the file and save it with the name above
if [ ! -e $FILENAME ]
then
    curl -o $FILENAME $URL
fi

# create report with a series of awk calls
echo -e "Genome Report\nPrepared: $TODAY\n" > $REPORT

echo "Total genes: " >> $REPORT
awk '$3 == "gene" {print $0}' $FILENAME | wc -l >> $REPORT

echo -e "\nDubious ORFs: " >> $REPORT
awk '$3 == "gene" && match($9, "Dubious") {print $0 }' $FILENAME | wc -l
>> $REPORT

echo -e "\nPseudogenes: " >> $REPORT
awk -f fcount.awk -v ftype="pseudogene" $FILENAME | wc -l >> $REPORT

echo -e "\nChromosome, length, genes per chromosome: " >> $REPORT
awk -f fcount.awk $FILENAME | sort >> $REPORT

echo "Report written to: $REPORT"
```

Note that the line `#!/bin/bash` needs to be the first line in the file. This tells the operating system to run this script using the bash shell. This line is sometimes referred to as the 'she-bang' line by Unix programmers. We'll see next week how to set this for a Python program.

Having entered and saved that script, make the script executable by typing:

```
$ chmod +x genome_reporter.sh
```

from the command line. Once you've done that you can run the script, from the same directory, by typing:

```
$ ./genome_reporter.sh
```

Assuming you don't have any errors the script will download the GFF file from the Saccharomyces Genome Database, save it with the date prefixed to the file name, and then generate a short report listing some useful summaries generated from the file.

Most of the bottom half of the script should be easy to understand; it simply shows a bunch of echo and awk commands that you might have typed at the command line. In the top portion of the script we create a set of variables to hold the names of the files we'll be using. One new feature we haven't seen before is the use of the dollar sign (\$) to dereference variable names. For example, the variable FILENAME is constructed by creating a string by joining together the strings held in the variables TODAY and BASEFILE (and separated by a dash -). Depending on the date on which the script is run it generates a different set of file names, as specified by the variables TODAY, BASEFILE, and REPORT. The bottom half of the script is setup to generate the appropriate output given those changing variables. One other feature to take note of is the if-then-fi conditional statement. The portion in the square brackets ([! -e \$FILENAME]) asks whether the file for that date already exists. If so it doesn't bother downloading the file again, for efficiency reasons.

12.4. Beyond Shell Scripting

As we saw above, built-in Unix tools like Awk can be extremely useful for manipulating and processing data sources, especially in combination with shell scripts. Now we're going to scale up our pipeline building to include additional bioinformatics tools and a small Biopython script.

Our next pipeline will accomplish the following tasks:

1. Translate a nucleotide FASTA file to amino acid sequences
2. Perform a multiple alignment of the amino acid sequences using the aligner MAFFT
3. Place the translated output and multiple sequence alignments in separate files
4. Run HMMER on the the translated output and produce a report on recognize protein domains.

12.4.1. A First Brush with Biopython

We'll start with a small Python script to take care of step 1, translating the nucleotide sequence to a protein sequence. Save the following code as `aatranslate.py`. This small script uses a number of classes and functions from the Biopython library that we'll discuss in greater detail in the next class session.

```
#!/usr/bin/env python

import sys
from Bio import Seq, SeqIO
from Bio.SeqRecord import SeqRecord
from Bio.Data.CodonTable import TranslationError

recs = SeqIO.parse(sys.stdin, "fasta")
for rec in recs:
    try:
        newrec = SeqRecord(rec.seq.translate(), id=rec.id+"_translated",
                           description=rec.description + ' (translated)')
        print newrec.format("fasta")
    except TranslationError:
        print rec.format("fasta")
```

After saving the above code as `aatranslate.py`, make it executable by using `chmod +x`. This script takes a set of FASTA nucleotide sequences from `stdin` and translates them, sending the output to `stdout`. Test your function as shown below using the `unknown2.fas` data set from the class wiki. Notice how we use `cat` to pipe the fasta file to `aatranslate.py`.

```
$ less unknown2.fas # confirm that the unknowns are nuc. seqs, q to quit
$ cat unknown2.fas | ./aatranslate.py | less # pipe output to less
$ cat unknown2.fas | ./aatranslate.py > unknown2-AA.fas # write output to file
```

12.4.2. MAFFT

MAFFT is a multiple sequence alignment program. It's relatively fast and a number of studies have shown that it is amongst the best performing multiple sequence aligners. MAFFT is usually the sequence aligner I reach for first. Clustalw is the 'classic' alignment tool, so it's useful to have on your system, but MAFFT usually gives better alignments (though Clustalw2 is supposed to address some of the short-comings of the older versions of Clustalw). See the [MAFFT website](#) for additional references and information. There are pre-compiled MAFFT binaries available on the MAFFT website.

Once you've installed MAFFT check the installation location and confirm that the binary is working (on Windows, add the MAFFT install directory to your PATH):

```
$ which mafft
/usr/local/bin/mafft
$ mafft # type ctrl-c to exit from the interactive prompt
```

MAFFT v6.864b (2011/11/10)

NAR 30:3059–3066, NAR 33:511–518
<http://mafft.cbrc.jp/alignment/software/>

12.4.3. Testing MAFFT

Once you've confirmed that MAFFT is properly installed, let's test it with the amino acids translation of our `unknown2.fas` file. We'll use this data to do some quick tests to confirm that our software tools are working correctly. Of course, when putting together an analysis pipeline for your own purposes you'll want to spend a fair amount of time reading the documentation (and related papers) for each tool and make sure you understand the various options and settings.

```
$ mafft --auto unknown2-AA.fas > unknown2-AA-mafft.fas
```

The commands above should produce the following file: `unknown2-AA-mafft.fas` (MAFFT). To visualize the alignments there are a variety of different multiple alignment viewers. One such program is [SeaView](#), a free cross platform, alignment viewer. Take a look at the MAFFT alignment using SeaView.

12.4.4. HMMER

HMMER is an implementation of a profile Hidden Markov Model (HMM) for protein sequence analysis. You can read up on HMMER at the [HMMER website](#). We will use it here for finding protein domains in sequences in conjunction with the PFAM database. Download the appropriate version of HMMER from <http://hmmer.janelia.org/software>. See the bottom of the page for Cygwin binaries.

Get the PFAM HMM library

We will be using the Pfam database (Release 27) in conjunction with HMMER to search for known protein domains in our sequences of interest. Since the the Pfam HMM libraries are large I'll try and provide a couple of thumb drives with the necessary library. If you're using this document outside of class you can download the necessary library as follows:

```
$ curl -O ftp://ftp.sanger.ac.uk/pub/databases/Pfam/releases/Pfam27.0/Pfam-A.hmm.gz
```

This is a large file (202MB) and decompresses to an even larger file (approx. 1GB). Make sure you have adequate disk space. On OS X or Windows using Cygwin you can unzip it as follows:

```
$ gunzip Pfam-A.hmm.gz
```

If you aren't using Cygwin on Windows you can download the open source program [7-zip](#) which can unzip gzip'd files (and many other common compression formats).

12.4.5. Testing HMMER

To test out HMMER and Pfam download the `Rme1.fas` file from the class wiki. Rme1 is a transcription factor that regulates sporulation and meiosis in budding yeast, *Saccharomyces cerevisiae*. We'll illustrate how we can use HMMER to analyze the domain structure of Rme1.

The first thing you'll need to do is run `Pfam-A.hmm` through the `hmmpress` program which prepares the HMM database for fast scanning by creating binary files. This might take a few minutes depending on the speed of your machine.

```
$ hmmpress Pfam-A.hmm
```

This will create a number of additional files in the same directory as `Pfam-A.hmm`. We can now use `hmmscan` to search for known protein domains included in the Pfam database.

```
$ hmmscan Pfam-A.hmm Rme1.fas > Rme1-Pfam-out.txt
```

The default output from `hmmscan` is designed to be human readable. Open `Rme1-Pfam-out.txt` in a text editor to see the output. For outputs that are easier to parse computationally use the `--tblout` or `--domtblout` options to save output in a tabular format.

```
$ hmmscan --domtblout Rme1-output.txt -o /dev/null Pfam-A.hmm Rme1.fas
```

This call produces a file `Rme1-output.txt` that contains a space delimited text file summarizing the per-domain output. The `-o` option redirects the main human-readable output (in this case to the 'bit-bucket', `/dev/null`). You can then manipulate the tabular output in `Rme1-output.txt` using standard Unix tools like `awk`.

See pp. 24-26 of the [HMMER user guide](#) for more info on the `hmmscan` program and settings. E-values and bit-scores are the criteria you want to look at when trying to judge which domains your sequence of interest has good matches to. HMMER bit scores reflect the extent to which a sequence is a good match to a profile model (higher bit scores are better matches). See p. 43 of the HMMER 2.3 user guide (use Google to find a copy of the older version of the HMMER manual) for a discussion of E-values and bit scores. If you examine the output file `Rme1-output.txt` you'll see that the model with the lowest E-values and highest bit-scores is a "zinc finger" domain. There are three such domains in the Rme1 protein (see the column labeled "N" in the output), though two of them are weaker matches (larger E-values). Rme1 is a zinc finger transcriptional factor. The two weaker zinc finger domains are weak matches to the HMM model for zinc fingers but are nonetheless functional domains.

Combining Python, MAFFT, HMMER

Now that we know how to use each of the tools individually we can write a bash script to chain them together. Save the following code as `pipeline1.sh`:

```
#!/bin/bash
```

```
# change these if these executables and files are located elsewhere
```

```
TRANSLATE=$HOME/tmp/aatranslate.py
MAFFT=/usr/local/bin/mafft
HMMSCAN=/usr/local/bin/hmmSCAN
PFAMDB=$HOME/tmp/Pfam-A.hmm

scriptargs="fastafile aafile alignfile reportfile"
E_WRONG_ARGS=85
nexpargs=4
args=$#

# did we get the expected number of args to the script?
if [[ $args -ne $nexpargs ]]
then
    echo
    echo "Usage: `basename $0` $scriptargs"
    echo
    exit $E_WRONG_ARGS
fi

programname="$0" # the name of the program
fastafile="$1" # the input fasta file
aafile="$2" # the output file for the AA translation
alignfile="$3" # the output file for the aligned sequences
reportfile="$4" # output file for the HMMER report

echo "Translating sequences"
cat "$fastafile" | "$TRANSLATE" > "$aafile"

echo "Aligning sequences"
"$MAFFT" --auto --quiet "$aafile" > "$alignfile"

echo "Running hmmSCAN"
"$HMMSCAN" "$PFAMDB" "$aafile" > "$reportfile"
```

After setting the script to be executable (`chmod +x pipeline1.sh`) you can run it like so:

```
$ ./pipeline1.sh unknown2.fas unknown2-trans.fas unknown2-align.fas
unknown2-report.txt
```

This will produce three files, one containing the translated amino acid sequences, the second giving the multiple alignment of those amino acid sequences, and the third with the PFAM output. Use less or a text editor to take a look at the generated files. At the beginning of this script we added a little bit of error checking code to insure that the correct number of arguments were provided on the command line. If no arguments or the wrong number of arguments are provided the script gives the user a little usage information and exits gracefully. Try running the script as:

```
$ ./pipeline2.sh
```

13. Bioinformatics Pipelines II

13.1. Overview

Building on our initial forays into building bioinformatics pipelines last week, we now turn to a more complicated example that integrates BioPython along with several command line programs. This pipeline will incorporate such features as web based queries and conversion of information between different file formats.

13.2. The Pipeline

The tasks carried out by the pipeline will be as follows:

- Read in a nucleotide sequence from a FASTA file
- Translate the nucleotide sequence to an amino acid sequence
- Do a blastp search against human and fly proteins in the Swiss-Prot database using an interface to the NCBI web version of BLAST
- Download protein sequences for the best blast hits from Swiss-Prot
- Use MAFFT to do a multiple alignment of the original amino acid sequence and the presumed orthologs generated via the blast search
- Analyze the query protein for known protein domains using HMMER and Pfam

You will need a working installation of Python (2.7+), IPython, and the BioPython library (1.59+) as well as the command line tools we installed last week (MAFFT, HMMER).

13.3. Biopython

Now we turn our attention to Biopython. As we build our pipeline I will first demonstrate the use of various modules, classes, and functions in the interactive shell and then I will give a set of functions that consolidate the commands to make them convenient to use.

13.3.1. Test files

Download the file `unknown1.fas` and `unknown2.fas` from the class website. I recommend you place these in `~/tmp`.

13.3.2. Reading in a single sequence from a FASTA file

Fire up and ipython interpreter, either a text based command line (`ipython --pylab`) or an ipython notebook (`ipython notebook --pylab=inline`).

We'll start by showing how to read sequence data out of a FASTA file:

```
>>> cd ~/tmp
>>> from Bio import SeqIO
>>> u1 = SeqIO.read('unknown1.fas', 'fasta')
>>> type(u1)
<class 'Bio.SeqRecord.SeqRecord'>
>>> u1
SeqRecord(seq=Seq('ATGATGAATTTTTTACATCAAATCGTCGAAT
CAGGATACTGGATTAGCTCT...TGA', SingleLetterAlphabet()),
id='YHR205W', name='YHR205W', description='YHR205W  Chr 8', dbxrefs=[])
>>> u1.name
'YHR205W'
>>> u1.description
'YHR205W  Chr 8'
>>> u1.seq
Seq('ATGATGAATTTTTTACATCAAATCGTCGAATCAGGATACTGG
ATTAGCTCT...TGA', SingleLetterAlphabet())
>>> u1.seq[:10]
Seq('ATGATGAATT', SingleLetterAlphabet())
>>> u1.seq[0]
'A'
>>> u1.seq[9]
'T'
>>> u1.seq[:10].tostring()
'ATGATGAATT'
>>> u1.seq.translate()[:10]
Seq('MMNFFTSKSS', HasStopCodon(ExtendedIUPACProtein(), '*'))
```

`SeqIO` is a sub-module of the top-level module Biopython module `Bio`. The function `SeqIO.read()` reads a single sequence object from a file and returns an instance of a `SeqRecord` class (defined in the Biopython package). A *class* is a programming concept that groups data and functions that operate on that data into a single object. For example, in the code above we used the `.name` and `.description` attributes to examine information about the sequence (this information was retrieved from the FASTA file itself). A `SeqRecord` holds a `Seq` object (yet another class!) as well as accessory information like the name of the sequence, a description, etc. `Seq` objects act very much like strings in terms of slicing and element access but they also have specialized function like `.translate()` that can be used to translate a nucleotide sequence into a peptide sequence.

Reading in multiple sequences from a FASTA file

In the code above we demonstrated how to read a single sequence from a FASTA file. Here we demonstrate how to read multiple sequences. The key difference is the use of the SeqIO.parse() function rather than SeqIO.read().

```
>>> u2 = SeqIO.parse('unknown2.fas', 'fasta')
>>> type(u2)
<type 'generator'>
>>> s1 = u2.next()
>>> type(s1)
<class 'Bio.SeqRecord.SeqRecord'>
>>> s1
SeqRecord(seq=Seq('ATGTCATCAAAACCTGATACTGGTCGGA
AATTCTGGCCCTCAGCGACAGGAA...TGA', SingleLetterAlphabet()),
id='YJL005W', name='YJL005W', description='YJL005W', dbxrefs=[])
>>> s1.seq
Seq('ATGTCATCAAAACCTGATACTGGTCGGAATTCTGGCC
CTCAGCGACAGGAA...TGA', SingleLetterAlphabet())
>>> s2 = u2.next()
>>> s2
SeqRecord(seq=Seq('ATGTCATCAAATCATGCTATTAGTCCAGAA
ACTTCTGGCTCTCATGAGCAACAA...TGA', SingleLetterAlphabet()),
id='MIT_Sbay_c342_13338', name='MIT_Sbay_c342_13338',
description='MIT_Sbay_c342_13338', dbxrefs=[])
>>> s3 = u2.next()
>>> s4 = u2.next()
>>> s5 = u2.next()
-----
StopIteration                                     Traceback (most recent call last)
/Users/pmagwene/Desktop/tmp/<ipython console> in <module>()
StopIteration:
```

In this case the SeqIO.parse function returns an object that has *iterator* semantics (technically it's a 'generator' but this is a technical difference that you can ignore for now). An iterator is an object that 'acts like' a sequence (e.g. a list or tuple), but there are some major differences. The most important one is that an iterator does not have to compute the entire sequence at once. In the case of the SeqIO.parse() function that means that if you have a FASTA file with thousands of sequence entries it wouldn't try to suck them all into memory. The .next() method is used to call successive sequence entries in the FASTA file. When you call .next() on the iterator(generator) instance you get back SeqRecords, one at a time. However, as the last call demonstrates if there is no 'next' item in the iterator it raises a StopIteration exception. For more info about iterators and generators see Norman Matloff's [Tutorial on Python Iterators and Generators](#).

The steps for reading a FASTA sequence file can be wrapped up in the following function. We'll place each of the functions we develop in a module called pipeline.py (place this in your working directory or your PYTHONPATH). As you progress through the pipeline design you will add additional functions to this module.

```
# pipeline.py -- a simple bioinformatics pipeline
from Bio import SeqIO

def read_fasta(infile):
    """Read a single sequence from a FASTA file"""
    rec = SeqIO.read(infile, 'fasta')
    return rec

def parse_fasta(infile):
    """Read multiple sequences from a FASTA file"""
    recs = SeqIO.parse(infile, 'fasta')
    return [i for i in recs]
```

List comprehensions

The `parse_fasta()` function above introduces another new concept called *list comprehensions*. A list comprehension is a compact way of applying a function to each element in a sequence. In this case the list comprehension implicitly called `.next()` to get all the `SeqRecords` from the generator returned by `SeqIO.parse()`. You'll recall that most functions in R works in a vector-wise manner. List comprehensions provide similar semantics for Python. Below are some simpler examples of list comprehensions. Try and predict the output of each of these before typing them in:

```
In [1]: x = [2,4,6,8,10]
In [2]: [i**2 for i in x]
Out[2]: ???
In [3]: y = ['bob', 'tab', 'rob', 'snob']
In [4]: def juvenilize(s):
...:     return str(s) + "by"
...:
In [5]: [juvenilize(i) for i in y]
Out[5]: ???
```

You can use the `read_fast()` function as follows:

```
>>> import pipeline
>>> recs = pipeline.parse_fasta('unknown2.fas')
>>> len(recs)
4
>>> [i.name for i in recs]
['YJL005W', 'MIT_Sbay_c342_13338', 'MIT_Smik_c333_12160', '
    MIT_Spar_c300_12282']
```

Note that the `parse_fasta()` function will return a list of `SeqRecords` even when there is only a single sequence in the file. In contrast, if you use the function `read_fasta()` on a FASTA file with more than one sequence it will raise an error.

13.3.3. Translating nucleotide sequence to a protein sequence

The next step is to translate each DNA sequence into a corresponding protein sequence. This is very easy using the `.translate()` method associated with the `Seq` class.

```
>>> recs[0].seq.translate()
Seq('MSSKPDTGSEISGPQRQQEQQIEQQIEQSSPTEANDRSIHD
PKVKKRHEQNSGH...ST*', HasStopCodon(ExtendedIUPACProtein(), '*'))
```

Note that the above code returns an object of type `Seq`. That's usually what we want if we're manipulating nucleotide or protein sequences but if we want to write our translated sequences back out into a file we need to create new `SeqRecords`. I illustrate this in the function below (add this to `pipeline.py`).

```
from Bio import Seq
from Bio import SeqRecord

def translate_recs(seqrecs):
    """ nucleotide SeqRecords -> translated protein SeqRecords """
    proteins = []
    for rec in seqrecs:
        aaseq = rec.seq.translate()
        protrec = SeqRecord.SeqRecord(aaseq, id=rec.id, name=rec.name,
                                      description=rec.description)
        proteins.append(protrec)
    return proteins
```

We can then encapsulate the whole process of converting a nucleotide FASTA file to a peptide sequence FASTA file as so (add these to `pipeline.py`):

```
def write_fasta(recs, outfile):
    ofile = open(outfile, 'w')
    SeqIO.write(recs, ofile, 'fasta')

def translate_fasta(infile, outfile):
    """ nucleotide fasta file -> protein fasta file """
    nrecs = parse_fasta(infile)
    precs = translate_recs(nrecs)
    write_fasta(precs, outfile)
```

`open()` is a built-in Python function that when called with the '`w`' argument opens a file for writing. When called with '`r`' as its second argument it opens a file for reading.

We can use our `translate_fasta` function from the Python interpreter like so:

```
>>> reload(pipeline)
<module 'pipeline' from '/Users/pmagwene/synchronized/pyth/pipeline.py'>
>>> pipeline.translate_fasta('unknown2.fas', 'unknown2-protein.fasta')
```

Take a moment to open the file `unknown2-protein.fasta` in a text editor to confirm that the file now hold amino acid sequences rather than nucleotide sequences.

Globbing to get multiple files of a given type

As an aside, what if we wanted to repeat this for a whole directory full of DNA sequences in separate FASTA files? Here's a function to help accomplish that task:

```
import glob

def inout_pairs(insuffix, outsuffix):
    """ Files in directory with given suffix -> list of tuples w/ (infile,
    outfile) """
    infiles = glob.glob('*'+insuffix)
    pairs = []
    for infile in infiles:
        inprefix = infile[:-len(insuffix)]
        outfile = inprefix + outsuffix
        pairs.append((infile,outfile))
    return pairs
```

The `glob` module gives you filename 'globbing' functionality. Globbing is a means of matching specified file or pathnames; you can think about this as a simplified class of regular expressions. For example, you're probably familiar with command line searches like:

```
$ ls *.fas  # list all files with the extension .fas
$ ls unk*   # list all files that begin with 'unk'
```

The `inout_pairs()` function we defined above allows us to glob file files with the given `insuffix` and create a corresponding set of names for output files. The following illustrates this:

```
>>> pairs = pipeline.inout_pairs('.fas', '-protein.fasta')
>>> pairs
[('unknown1.fas', 'unknown1-protein.fasta'),
 ('unknown2.fas', 'unknown2-protein.fasta')]
>>> from Bio.Data.CodonTable import TranslationError
>>> for (i,o) in pairs:
...     try:
...         pipeline.translate_fasta(i,o)
...     except TranslationError:
...         continue
...
...
>>> ls *.fas*  # only works in ipython
unknown1-protein.fasta  unknown1.fas  unknown2-protein.fasta  unknown2.fas
```

Note that I changed the file suffix from `.fas` to `.fasta` on the output files. This isn't necessary but I find that doing so makes it easy to sort through large directories to distinguish generated files from the original files. The `inout_pairs()` function will come in handy when we combine our functions to generate a multi-sequence pipeline.

Another new concept I introduced in the for loop above is `try-except` block for exception handling. The Python starts by executing the code in the `try` clause. If

there are no problems the `except` clause is ignored. However, if an exception (error) is raised than it evaluates the `except` clause. In this case, our `except` clause says if the error is an exception of type `TranslationError` (defined in `Bio.Data.CodonTable`) then ignore it and just keep working. However, any other exception will stop program execution, as we haven't included any general error handling code. See Downey, Chap 14 for more discussion of exception handling.

13.3.4. BLAST searches via the NCBI server

We can use Biopython do network based BLAST searches. Here we will use `blastp` to search against protein sequences in the Swiss-Prot database.

```
>>> from Bio.Blast import NCBIWWW, NCBIXML
>>> prot1 = pipeline.read_fasta('unknown1-protein.fasta')
>>> results_handle = NCBIWWW.qblast('blastp','swissprot',prot1.seq.tostring()
() , entrez_query='(Homo sapiens[ORGN])')
>>> results = results_handle.read()
>>> sfile = open('prot1_blast.out','w')
>>> sfile.write(results)
>>> sfile.close()
>>> blast_out = open('prot1_blast.out','r')
>>> brec = NCBIXML.read(blast_out)
>>> brec
<Bio.Blast.Record.Blast instance at 0x2ec22d8>
>>> len(brec.alignments) # we got 50 blast hits in the query
50
>>> brec.alignments[0]
<Bio.Blast.Record.Alignment instance at 0x2ec23a0>
>>> brec.alignments[0].accession
u'P31749'
```

This code introduces another concept we'll call the *Producer-Consumer* pattern. The Producer-Consumer pattern is a general programming concept, but the key here is that the pattern generalizes the problem of parsing complex biological data types. The producer does the work of getting the information from a file (or from the web in this case). The consumer process the information into a form we can use. In the code above the function `NCBIWWW.qblast()` is the producer and `NCBIXML.read()` plays the role of the consumer. This pattern is used over and over again in Biopython so you should spend some time trying to understand the general idea. See the Biopython tutorial for a more complete discussion.

Our BLAST query returned the information in the form of XML data. XML stands for 'Extensible Markup Language', and is a generic way to encode documents in machine-readable form. XML data is usually plain text - go ahead and open up the file `prot1_blast.out` in a text editor to see the output. Since XML is a generic format, specific types of XML documents need a 'schema' or 'grammar' that specifies how the document is to be read and interpreted. In the example above, the module `NCBIXML` knows how to handle XML data returned from NCBI, hence our use of the function `NCBIXML.read()`.

In the example given, we limited our query to sequences from humans. If we wanted to include all metazoan sequences we could pass '(Metazoa[ORGN])' as the argument to `entrez_query`. If we didn't want to limit our search at all we would simply not include that argument (i.e. accept the default). The BLAST output is fairly complicated. See the BioPython tutorial section 7.5 for a complete breakdown of all the fields in the BLAST output.

Again, the commands above are rather involved so let's wrap them up in a function:

```
from Bio.Blast import NCBIWWW, NCBIXML

def blastp(seqrec, outfile, database='nr', entrez_query='(none)'):
    handle = NCBIWWW.qblast('blastp', database, seqrec.seq.tostring(),
                            entrez_query=entrez_query)
    results = handle.read()
    sfile = open(outfile, 'w')
    sfile.write(results)
    sfile.close()
    bout = open(outfile, 'r')
    brecord = NCBIXML.read(bout)
    return brecord

def summarize_blastoutput(brecord):
    hits = []
    for alignment in brecord.alignments:
        expect = alignment.hsps[0].expect
        accession = alignment.accession
        hits.append((expect, accession))
    hits.sort() # will sort tuples by their first value (i.e. expect)
    return hits
```

We can use this code as follows:

```
>>> humanblast = pipeline.blastp(prot1, 'prot1-hum-blast.out', database='swissprot', entrez_query='(Homo sapiens[ORGN])')
>>> flyblast = pipeline.blastp(prot1, 'prot1-fly-blast.out', database='swissprot', entrez_query='(Drosophila melanogaster[ORGN])')
>>> humanhits = pipeline.summarize_blastoutput(humanblast)
>>> flyhits = pipeline.summarize_blastoutput(flyblast)
>>> humanhits[0] # the first number is the E-value for the BLAST search
(4.98013e-95, u'P31749')
>>> print humanhits[0][1] # prints the swissprot accession number
P31749
>>> flyhits[0]
(4.09304e-96, u'Q8INB9')
```

Go to the UniProt [website](#) and use the search box to lookup those accession numbers.

13.3.5. Getting records from Swiss-Prot

For a small number of accession numbers it's easy to use the web interface to UniProt (Swiss-Prot). For hundred of blast hits that's just not an option. Conveniently, we can use Biopython to query the Swiss-Prot database to retrieve information about these presumed orthologs. You can access the Swiss-Prot database as follows:

```
>>> from Bio import ExPASy
>>> from Bio import SwissProt
>>> handle1 = ExPASy.get_sprot_raw(humanhits[0][1]) # access with the
   accession number
>>> rec1 = SwissProt.read(handle1)
>>> print rec1.description
RecName: Full=RAC-alpha serine/threonine-protein kinase; EC=2.7.11.1;
   AltName:
Full=RAC-PK-alpha; AltName: Full=Protein kinase B; Short=PKB; AltName: Full
   =C-
AKT;
>>> rec1.comments[0]
"FUNCTION: AKT1 is one of 3 closely related serine/threonine- protein
   kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate
   many processes including metabolism, proliferation, cell survival,
   growth and angiogenesis.
...
   output truncated ..."
>>> print dir(rec1) # lets see what other attributes the record has
['__doc__', '__init__', '__module__', 'accessions', 'annotation_update',
 'comments', 'created', 'cross_references', 'data_class', 'description',
 'entry_name', 'features', 'gene_name', 'host_organism', 'host_taxonomy_id',
 'keywords', 'molecule_type', 'organelle', 'organism',
 'organism_classification', 'references', 'seqinfo', 'sequence',
 'sequence_length', 'sequence_update', 'taxonomy_id']
>>> print rec1.gene_name
Name=AKT1; Synonyms=PKB, RAC;
>>> print rec1.sequence[:25] # first 25 amino acids
MSDVAIVKEGWLHKRGEYIKTWRPR
```

Here's some functions to make this more convenient:

```
from Bio import ExPASy
from Bio import SwissProt

def get_swissrec(accession):
    handle = ExPASy.get_sprot_raw(accession)
    record = SwissProt.read(handle)
    return record

def swissrec2seqrec(record):
    seq = Seq.Seq(record.sequence, Seq.IUPAC.protein)
    s = SeqRecord.SeqRecord(seq, description=record.description,
                           id=record.accessions[0], name=record.entry_name)
    return s
```

And here is an example of how we can apply these functions:

```
>>> ids = [humanhits[0][1], flyhits[0][1]]
>>> ids
[u'P31749', u'Q8INB9']
>>> swissrecs = [pipeline.get_swissrec(i) for i in ids]
>>> seqs = [pipeline.swissrec2seqrec(i) for i in swissrecs]
>>> seqs[0]
SeqRecord(seq=Seq('MSDVAIVKEGWLHKRGEYIKTWRPRYFLKNDGTFIGYKERPQDVHQREAPLNN
...GTA', IUPACProtein()), id='P31749', name='AKT1_HUMAN', description='
RecName: Full=RAC-alpha serine/threonine-protein kinase; EC=2.7.11.1;
AltName: Full=Protein kinase B; Short=PKB; AltName: Full=Protein kinase
B alpha; Short=PKB alpha; AltName: Full=Proto-oncogene c-Akt; AltName:
Full=RAC-PK-alpha;', dbxrefs=[])
>>> seqs[1]
SeqRecord(seq=Seq('MNYLPFVLQRRSTVVASAPAGSASRIPESPTTGNSNIINIIYSQSTHPNSSPT
...SMQ', IUPACProtein()), id='Q8INB9', name='AKT1_DROME', description='
RecName: Full=RAC serine/threonine-protein kinase; Short=DAkt; Short=
DRAC-PK; Short=Dakt1; EC=2.7.11.1; AltName: Full=Akt; AltName: Full=
Protein kinase B; Short=PKB;', dbxrefs=[])
>>> seqs.append(prot1) # add our original protein sequence to the list
>>> pipeline.write_fasta(seqs, 'unknown1-plus-human-fly.fasta')
```

13.3.6. Multiple sequence alignment via MAFFT

We've now generated a new FASTA file that includes our original protein sequence and the sequences for the human and fly BLAST best hits. We will use MAFFT to perform a multiple alignment. Biopython has built in code to simplify command line usage of common alignment programs like CLUSTALW, MAFFT, and MUSCLE. However I'll show you how to do this with your own code using the subprocess module. Knowing how the subprocess module works is useful because it allows you to interface with any command line program from within Python.

The subprocess module allows your Python code to start other programs (child processes) and send/get input and output from those same processes. When we use the subprocess module we're putting the Unix design element of 'Everything is a file or process' to use. Here's a simple example:

```
>>> import subprocess
>>> subprocess.call(["ls", "-l"])
# on windows the equivalent command is
# subprocess.call(["dir"], shell=True)
# output is NOT shown in ipython notebook, instead
# a return code (0 if the command worked) is shown
total 11696
-rw-r--r-- 1 pmagwene staff 93514 Nov 22 19:36 prot1-fly-blast.out
-rw-r--r-- 1 pmagwene staff 109635 Nov 22 19:35 prot1-hum-blast.out
-rw-r--r-- 1 pmagwene staff 109635 Nov 22 19:19 prot1_blast.out
-rw-r--r-- 1 pmagwene staff 2308 Nov 22 20:07 unknown1-plus-human-
```

```

fly.fasta
-rw-r--r-- 1 pmagwene staff      854 Nov 22 16:46 unknown1-protein.fasta
-rwx----- 1 pmagwene staff      2535 Nov 22 15:38 unknown1.fas
-rw-r--r--@ 1 pmagwene staff     24849 Nov 22 16:25 unknown2.fas
-rw-r--r-- 1 pmagwene staff     8331 Nov 22 16:46 unknowns-protein.fasta

```

The above code uses a convenience function `call()` in the `subprocess` module. We'll use the same function to run MAFFT:

```

import subprocess

def mafft_align(infile, outfile):
    ofile = open(outfile, 'w')
    retcode = subprocess.call(["mafft", infile], stdout=ofile)
    ofile.close()
    if retcode != 0:
        raise Exception("Possible error in MAFFT alignment")

```

And we put it to use as follows:

```

In [8]: reload(pipeline)
In [8]: pipeline.mafft_align('unknown1-plus-human-fly.fasta', 'unknown1-
alignment.fasta')

```

If all went well this should have created the file `unknown1-alignment.fasta` in your directory. Open this alignment using JalView to examine the alignment in more detail.

13.3.7. Searching for protein domains using HMMER and Pfam

As the final step of our pipeline we'll use HMMER and the Pfam database to search for known protein domains in our original protein. This assumes you have the HMMER binaries and Pfam database installed as demonstrated in last weeks exercises and that you've already run `hmmpress` against the Pfam database. Again we write a small wrapper function using the `subprocess` module. This time we'll use the `Popen` class to illustrate how we can capture the output produced by `hmmpfam`. Note that if you haven't installed the HMMER binaries to one of the standard locations you might need to specify the full path to the `hmmscan` executable in the code below.

```

def hmmer_pfam(infilename, outfilename, pfamdb):
    pipe = subprocess.Popen(["hmmscan", pfamdb, infilename],
                           stdout=subprocess.PIPE).stdout
    output = pipe.read() # this gives us the output of our command
    outfile = open(outfilename, 'w')
    outfile.write(output)
    outfile.close()

```

This function can be called like this:

```

# change the last argument to match the path to your Pfam database.
>>> pipeline.hmmer_pfam('unknown1-protein.fasta', 'unknown1-domains.out', '/Users/pmagwene/tmp/Pfam-A.hmm')

```

As before this search may take several minutes.

13.3.8. Putting it all together

We've generated a variety of functions that take care of the major steps of our pipeline. It's time to put the steps together to automate the entire process.

```
def oneseq_pipeline(infilename, pfamdb=None,
                     compareto=['Homo sapiens', 'Drosophila melanogaster'],
                     skipHMMER = True, extension="XX"):

    # translate nucleotide sequence to protein seq
    protout = 'protein-' + infilename + extension
                # add the extension so all generated files have
                # different extension than input files

    translate_fasta(infilename, protout)

    # run blastp on protein sequence against swissprot and extract best
    # hits
    protrec = parse_fasta(protout)[0]
    blastout ='blast-' + protout
    besthitids = []
    for organism in compareto:
        equery = '(%s[ORGN])' % organism # create the entrez organism query
        brecord = blastp(protrec, blastout, database='swissprot',
                         entrez_query=equery)
        bhits = summarize_blastoutput(brecord)
        besthitids.append(bhits[0][1])

    # download corresponding records from Swiss-Prot
    swissrecs = [get_swissrec(i) for i in besthitids]
    seqs = [swissrec2seqrec(i) for i in swissrecs]
    seqs.append(protrec)

    # write FASTA file with best hits plus original protein sequence
    plusout = 'blasthits-' + protout + '.XML'
    write_fasta(seqs, plusout)

    # do multiple alignment via mafft
    mafft_align(plusout, 'aligned-' + protout)

    # search for domains via HMMER/Pfam
    if not skipHMMER:
        if pfamdb is not None:
            hmmerout = 'hmmer-' + protout
            hmmer_pfam(protout, hmmerout, pfamdb)
```

Our function can take as input a FASTA file with a single sequence or with multiple sequences. In the case of a multiple sequences it assumes that the 'target' sequence for the search is the first sequence in the file. Also, note the `skipHMMER` argument included in the function. The HMMER search takes a relatively long time and doing it sequence by sequence is not very efficient so by default the pipeline will skip this

step. If you want to include the HMMER step than specify the Pfam database file and set skipHMMER=False.

Testing out the pipeline

To test out the function we do:

```
>>> reload(pipeline)
>>> pipeline.oneseq_pipeline('unknown1.fas')
```

This will create four new FASTA files:

- 1) protein-unknown1.fasXX
- 2) blast-protein-unknown1.fasXX.XML
- 3) blasthits-protein-unknown1.fasXX
- 4) aligned-protein-unknown1.fasXX

These respectively contain:

- 1) the amino acid sequence translated from the nucleotide sequence given as input
- 2) the XML output of the qblast query to NCBI
- 3) the amino acid sequences for the BLAST hits returned from NCBI
- 4) the MAFFT multiple alignment of the protein sequences.

Let's now test the pipeline using an alternate set organisms:

```
>>> pipeline.oneseq_pipeline('unknown1.fas', compareto=["Homo sapiens", "Mus
musculus", "Caenorhabditis elegans"])
```

For completeness let's also test the pipeline with the HMMER step included:

```
>>> pipeline.oneseq_pipeline('unknown1.fas', '/home/pmagwene/tmp/Pfam-A.hmm
',
skipHMMER=False)
```

Extending the pipeline to deal with multiple inputs

Now that we're confident out single sequence pipeline function works it can be easily adapted to deal with multiple input files:

```
def multiseq_pipeline(inext, pfamdb=None,
                      compareto=['Homo sapiens', 'Drosophila melanogaster'],
                      skipHMMER=True):
    inout = inout_pairs(inext, 'XX')
    infilenames = [i[0] for i in inout]
    for filename in infilenames:
        print "Processing %s" % filename
        oneseq_pipeline(filename, pfamdb, compareto, skipHMMER)
```

To test the complete multi-sequence pipeline delete all the generated files (so that only unknown1.fas and unknown2.fas are in the unknowns directory) and try the following:

```
>>> pipeline.multiseq_pipeline('.fas')
```

Given our example data this function will process just two input files. However, you can add an arbitrary number of additional '.fas' files to the directory and the pipeline will process those as well with exactly the same command.

There are a number of ways the pipeline could be sped up. One obvious improvement would be to utilize a local installation of BLAST and the respective databases. However, optimization is often a complex task. The pipeline we developed here doesn't require us to install BLAST (which can be somewhat involved) and provides adequate performance for a modest number of sequences. It is possible to turn this set of Python functions into a program that you could run from the command line (rather than the Python interpreter) just like any other Unix program.

13.4. The `pipeline.py` module

The pages that follow give the complete code listing for the `pipeline.py` module.

```
"""
pipeline.py -- An illustrative example of a bioinformatics pipeline.
Requires Python 2.6+ and BioPython 1.53+
(c) Copyright by Paul M. Magwene, 2009-2011 (mailto:paul.magwene@duke.edu)
"""

from Bio import Seq, SeqIO, SeqRecord
from Bio import ExPASy, SwissProt
from Bio.Blast import NCBIWWW, NCBIXML

import glob, subprocess

def read_fasta(infile):
    """Read a single sequence from a FASTA file"""
    rec = SeqIO.read(infile, 'fasta')
    return rec

def parse_fasta(infile):
    """Read multiple sequences from a FASTA file"""
    recs = SeqIO.parse(infile, 'fasta')
    return [i for i in recs]

def write_fasta(recs, outfile):
    ofile = open(outfile, 'w')
    SeqIO.write(recs, ofile, 'fasta')

def translate_recs(seqrecs):
    """ nucleotide SeqRecords -> translated protein SeqRecords """
    proteins = []
    for rec in seqrecs:
        aaseq = rec.seq.translate()
        protrec = SeqRecord.SeqRecord(aaseq, id=rec.id, name=rec.name,
                                      description=rec.description)
        proteins.append(protrec)
    return proteins

def translate_fasta(infile, outfile):
    """ nucleotide fasta file -> protein fasta file """
    nrecs = parse_fasta(infile)
    precs = translate_recs(nrecs)
    write_fasta(precs, outfile)

def inout_pairs(insuffix, outsuffix):
    """ Files in directory with given suffix -> list of tuples w/ (infile,
    outfile) """
    infiles = glob.glob('*'+insuffix)
    pairs = []
    for infile in infiles:
        inprefix = infile[:-len(insuffix)]
```

```
outfile = inprefix + outsuffix
pairs.append((infile,outfile))
return pairs

def blastp(seqrec, outfile, database='nr', entrez_query='(none)'):
    handle = NCBIWWW.qblast('blastp', database, seqrec.seq.tostring(),
                             entrez_query=entrez_query)
    results = handle.read()
    sfile = open(outfile, 'w')
    sfile.write(results)
    sfile.close()
    bout = open(outfile, 'r')
    brecord = NCBIXML.read(bout)
    return brecord

def summarize_blastoutput(brecord):
    hits = []
    for alignment in brecord.alignments:
        expect = alignment.hsps[0].expect
        accession = alignment.accession
        hits.append((expect,accession))
    hits.sort() # will sort tuples by their first value (i.e. expect)
    return hits

def get_swissrec(accession):
    handle = ExPASy.get_sprot_raw(accession)
    record = SwissProt.read(handle)
    return record

def swissrec2seqrec(record):
    seq = Seq.Seq(record.sequence, Seq.IUPAC.protein)
    s = SeqRecord.SeqRecord(seq, description=record.description,
                           id=record.accessions[0], name=record.entry_name)
    return s

def mafft_align(infile, outfile):
    ofile = open(outfile,'w')
    retcode = subprocess.call(["mafft",infile], stdout=ofile)
    ofile.close()
    if retcode != 0:
        raise Exception("Possible error in MAFFT alignment")

def hmmer_pfam(infilename, outfilename, pfamdb):
    pipe = subprocess.Popen(["hmmscan", pfamdb, infilename],
                          stdout=subprocess.PIPE).stdout
    output = pipe.read() # this gives us the output of our command
    outfile = open(outfilename, 'w')
    outfile.write(output)
    outfile.close()
```

```
def oneseq_pipeline(infilename, pfamdb=None,
                    compareto=['Homo sapiens', 'Drosophila melanogaster'],
                    skipHMMER = True, extension="XX"):
    # translate nucleotide sequence to protein seq
    protout = 'protein-' + infilename + extension
        # add the extension so all generated files have
        # different extension than input files

    translate_fasta(infilename, protout)

    # run blastp on protein sequence against swissprot and extract best
    # hits
    protrec = parse_fasta(protout)[0]
    blastout ='blast-' + protout + '.XML'
    besthitids = []
    for organism in compareto:
        equery = '(%s[ORGN])' % organism # create the entrez organism query
        brecord = blastp(protrec, blastout, database='swissprot',
                         entrez_query=equery)
        bhits = summarize_blastoutput(brecord)
        besthitids.append(bhits[0][1])

    # download corresponding records from Swiss-Prot
    swissrecs = [get_swissrec(i) for i in besthitids]
    seqs = [swissrec2seqrec(i) for i in swissrecs]
    seqs.append(protrec)

    # write Fasta file with best hits plus original protein sequence
    plusout = 'blasthits-' + protout
    write_fasta(seqs, plusout)

    # do multiple alignment via mafft
    mafft_align(plusout, 'aligned-' + protout)

    # search for domains via HMMER/Pfam
    if not skipHMMER:
        if pfamdb is not None:
            hmmerout = 'hmmer-' + protout
            hmmer_pfam(protout, hmmerout, pfamdb)

def multiseq_pipeline(inext, pfamdb=None,
                      compareto=['Homo sapiens', 'Drosophila melanogaster'],
                      skipHMMER=True):
    inout = inout_pairs(inext, 'XX')
    infiles = [i[0] for i in inout]
    for filename in infiles:
        print "Processing %s" % filename
        oneseq_pipeline(filename, pfamdb, compareto, skipHMMER)
```

A. The Unix Command Line

A.1. Platform specific issues

OS X

If your computer has Linux or Mac OS X installed you already have a native Unix environment. All of the command line tools we'll use in this tutorial are already available to you.

Unix on Windows

If your computer runs Windows you can have access to a Unix-like environment by installing a program called Cygwin (<http://www.cygwin.com>). Cygwin is free, open source, and provides a convenient installer for common Unix programs. Download the installer program (`setup.exe`) and place it in a `c:\cygwin` directory (I recommend that you use this directory as the installation directory as well).

During the installation you will have the choice of installing additional tools. I recommend you install the following packages (use the search box to find them):

- curl
- mintty

`curl` is a command line tool for transferring data over networks. It makes it easy to download packages and source code with a single text command. `mintty` is a terminal window program for Cygwin that provides a nicer interface than the standard Windows terminal.

The first time you run Cygwin I recommend you start it with the default terminal emulator (the Cygwin shortcut on your desktop will link to the default). After that you can interface with the Cygwin tools via mintty. During the Cygwin installation a mintty shortcut was put in your Start Menu (under the Cygwin folder). Since you'll be using the terminal frequently I recommend you pin the mintty shortcut to your taskbar (available via right clicking the mintty icon on Windows 7; copy the shortcut and drag the copy to your taskbar on older version of Windows).

Directory structure under Cygwin

NOTE: all the commands that follow should be executed from within the cygwin bash shell (i.e. in the terminal window you get when you click the Cygwin or mintty shortcuts).

Cygwin creates a set of subdirectories that mirrors the standard Unix file system (`/bin`, `/usr`, `/var`, `/home`, etc). When you start the bash shell you will be in your home directory (`/home/<username>`).

Cygwin will treat the directory where you installed it (`c:\cygwin` if you followed my instructions above) as if that was the ‘root’ directory of the Unix file system. So when you type `cd /home` from the shell in Cygwin, you’re really in `c:\cygwin\home`. To access the standard Windows drive names, Cygwin provides a mapping through a directory called `/cygdrive`. For example, to list the contents of `c:\Python27` from Cygwin you would type:

```
$ ls /cygdrive/c/Python27
```

Setting up symbolic links and aliases in bash under Cygwin

The bash shell (the default shell under Cygwin) can be customized and configured to suit your needs. Let’s start by creating a convenient link between your cygwin home directory and your Windows home directory.

```
$ cd ~ # makes sure you're in your home directory  
$ ln -s /cygdrive/c/<username> ~/winhome
```

`ln -s` is a command that creates a “symbolic” or “soft” link between files and directories. This makes it convenient to quickly navigate from your cygwin home directory as so:

```
$ cd ~/winhome  
$ ls  
... list of files and directories in your home directory...
```

A.2. The Unix philosophy

Doug McIlroy who invented the concept of the Unix ‘pipe’ (discussed below) summarized the Unix philosophy as follows:

“This is the Unix philosophy: Write programs that do one thing and do it well. Write programs to work together. Write programs to handle text streams, because that is a universal interface.”

This is not some rigid set of specifications, but rather an approach to writing simple programs that can be tied together in useful ways to accomplish larger, more complex tasks. Most of the standard Unix commands are written with this philosophy in mind. The scripts you will develop over the next three class session will follow the same philosophy (and take advantage of other software tools that also use the Unix approach).

A.3. The Unix command line

A Unix/Linux command line environment is the de-facto standard for building bioinformatics pipelines. While the command line may not be particularly user friendly, various aspects of how Unix is designed make it very powerful for constructing analysis pipelines. We'll review some of those design aspects here.

A.3.1. Unix tools

You'll need the following tools. Under Cygwin these are all easily installed using the GUI installation interface (or installed by default). On Linux or OS X many of these are already installed by default.

- less - a 'pager' (convenient for viewing files)
- curl - a package for retrieving files using a variety of Internet protocols; found under 'Net' in the cygwin installer.
- gzip - a file compression utility
- tar - a file archiving utility (usually used in conjunction with gzip)
- awk - a text processing programming language.

Basic Unix commands

You should familiarize yourself with the basic unix commands covered in the UNIX Tutorial for Beginners (see link on class website). Here are some of the more common ones you'll need to navigate around your file system:

- ls - list the content of a directory e.g. `ls /home/`
- cd - change directory. e.g. `cd /home/pmagwene/tmp`
- pwd - display the name of the present working directory.
- mv - move a file. e.g. `mv myfile newfile`
- rm - remove (delete) a file. Be careful with this one! e.g. `rm tempfile`
- find - find files that match a given pattern. e.g. `find . -name "*.txt"` (matches all files in the current directory that end with '.txt').
- man - show the manual pages for a command. e.g. `man ls`
- less - show the contents of a file, displaying one page at a time. e.g. `less somefile.txt` (use the space bar to advance, b to go back, q to quit)

The bash shell

The bash shell is the default shell on most Linux systems, Cygwin, and recent versions of OS X. The shell itself provides a useful framework for interacting with the operating system. Shell scripts can be written to make the shell environment even more powerful. We'll explore how to do this in today's exercises.

Here's a few efficiency tips to keep in mind when working in the bash shell:

- Scroll backwards and forwards through your command history using the up and down arrow keys
- Use the tab key to invoke command and file-name completion to keep your typing to a minimum
- Use <ctrl-r> and then start typing a word or phrase to search on; this invokes the history search mode to do a reverse incremental search of previous commands
 - Once you've found the command you were searching for hit <Enter> to execute it or <ctrl-j> to retrieve the command for further editing.
 - Use <ctrl-g> or <ctrl-c> to cancel the history search mode

Everything is a file or a process

One of the aspects of Unix that makes it easy to tie programs together is that the operating systems treats pretty much everything as either a *file* or a *process*. There are three categories of files in Unix: plain files (e.g. text files, image files, word documents, video files, the code for a program, etc.), directories (e.g. your home directory, the root directory), and devices (e.g. the keyboard, a printer, a display screen, etc). The same basic set of commands can be applied to all three types of files.

A *process* is an instance of a running program. Everytime you start a program the operating system creates a process ID (PID) that is associated with that process. Processes typically operate on data in the form of files (of any of the three types) and return data that is sent to a file (again, any of the three file types). Any given process can start multiple subprocesses (also called child processes), however a process can only have one parent. For example, when you logon to a Unix system you are typically working in a shell process (common shells include bash, tcsh, csh, etc.). When you type a command like `ls` this creates a child process. The parent process is temporarily suspended until the child process returns its output. The `ls` process takes a file as input (the current directory by default), and returns it's output to the display associated with the shell (represented by a device file).

Redirection and Pipes

Because Unix treats everything as a file or process, it's easy to change the source of input and the destination for output. There are several special operators that allow one to change the source/destination of input and output from a process. These are:

- > (redirect output operator)
- >> (append output operator)
- < (redirect input operator)
- | (pipe)

We'll give a few example of redirection and pipes using the commands `ls` (list directory contents), and `grep` (find lines matching a pattern):

```
$ ls -l
total 4184
drwx-----+ 43 pmagwene staff      1462 Nov 13 18:14 Desktop
drwx-----+ 20 pmagwene staff      680 Sep 23 12:32 Documents
... output truncated ...
$ ls -l > ex1.out # redirect output to file
```

[bash]

In the example above we redirected the output of the `ls -l` command to a file named `ex1.out`. Open the file to confirm this. Now we'll 'pipe' the output of `ls` to the `grep` command.

```
$ ls -l | grep 'Nov'
drwx-----+ 43 pmagwene staff      1462 Nov 13 18:14 Desktop
drwx-----+ 1285 pmagwene staff    43690 Nov 13 18:48 Downloads
drwx-----+ 14 pmagwene staff      476 Nov 10 11:51 Pictures
-rw-r--r--  1 pmagwene staff     1427 Nov 14 14:13 ex1.out
-rw-r--r--  1 pmagwene staff    604976 Nov  1 12:21 rolland-etal-2000-cAMP.pdf
```

In this example we used `grep` to show all the lines of the `ls` output that have the string 'Nov' in them.

Now we'll combine those two commands and redirect the output to another file.

```
$ ls -l | grep 'Nov' > ex2.out
```

Finally, let's use the append output operator to append to our file lines with 'Oct' as well.

```
$ ls -l | grep 'Oct' >> ex2.out
```

If we had used the redirection operator rather than append than it would have overwritten the previous contents of the file rather than adding the output to what was already there. Open `ex2.out` to confirm that your commands worked as expected.

Review chapter 3 of the UNIX Tutorial for Beginners (see link on class website) for more examples illustrating the use of redirection and pipes. We'll be using pipes and redirection throughout these hands-on exercises.

A.3.2. Using curl to retrieve files from the net

`curl` is a command line tool for transferring data to or from a server using a variety of different protocols including FTP, HTTP, SCP, etc. `curl` is available by default in recent versions of OS X. If you're running Cygwin on Windows you may have to install it from the "Net" subdirectory in the Cygwin installer.

Using `curl` is relatively straightforward. Here, we'll use it to download a file that we're going to use for today's exercises:

```
$ curl -O http://downloads.yeastgenome.org/curation/chromosomal_feature/  
saccharomyces_cerevisiae.gff
```

Type `man curl` or check out an online version of the manual for more information on using `curl`: <http://curl.haxx.se/docs/manual.html>.

A.3.3. The GFF3 File Format

GFF3 (GENERIC FEATURE FORMAT VERSION 3) is a text-based format for representing genomic features. It is widely used by the genomics research community for representing sequence features associated with genome projects. All of the major genome databases provide data in GFF3 format and most of the software tools used by the research community can parse GFF3 formatted files.

You can read the details of the GFF3 format here: <http://www.sequenceontology.org/gff3.shtml>. Notice that a GFF3 file consists 9 columns, separated by tabs. Read the above web page to understand what each of these 9 columns represents.

A.4. Tools for manipulating text

Many types of data, including GFF3 files, are structured text files. Because of this it's useful to have a handle on some of the major tools that Unix provides for manipulating such files.

A.4.1. head and tail

`head` and `tail` respectively show the first n and last n lines of a file (default $n = 10$). These can be useful for quickly checking out what's in a file. `tail` is especially useful for looking at log files to see the last few entries entered in a log.

```
$ head saccharomyces_cerevisiae.gff  
... < output truncated > ...  
$ tail saccharomyces_cerevisiae.gff  
... < output truncated > ...
```

Use the `-n` argument to specify the number of lines you'd like to see:

```
$ head -n 3 saccharomyces_cerevisiae.gff  
##gff-version 3  
#date Mon Nov 15 19:50:13 2010  
#
```

A.4.2. less

`less` is 'pager' program that allows you to scroll through a file (or standard input) page by page.

```
$ less saccharomyces_cerevisiae.gff
```

From within `less` you can scroll forward by hitting the space bar, or the 'f' key, backward by typing 'b'. To search for a particular word or pattern in the file type '/' followed by the word of interest and then hit return. All the instances of that word / pattern will be highlighted. For example, from within `less` type `/gene<RET>`, where <RET> means hit the enter or return key, to find all instances of the word 'gene' in the file. Type q to quit `less`.

A.4.3. echo

`echo` simply writes it's string argument to standard output (i.e. it echos what you type).

```
$ echo "hello, world"  
hello, world  
$ echo "These are the times that try men's souls"  
These are the times that try men's souls
```

A.4.4. cat

`cat`, short for 'concatenate', is a utility for concatenating and printing text. Here are some examples of it's use:

```
$ echo "some text here" > file1.txt  
$ echo "some more text" > file2.txt  
$ cat file1.txt file2.txt > file1plus2.txt  
$ cat file1plus2.txt  
some text here  
some more text
```

A.4.5. wc

`wc` is a program that counts the number of words, lines, and characters in a file. You can also specify you only want one of those counts using options like `-l` (count only lines).

```
$ wc saccharomyces_cerevisiae.gff  
168490 299825 18871650 saccharomyces_cerevisiae.gff  
$ wc -l saccharomyces_cerevisiae.gff  
168490 saccharomyces_cerevisiae.gff
```

A.4.6. cut

`cut` is a utility for subsetting words, bytes or columns of a text file. For example:

```
$ cut -f1-3 saccharomyces_cerevisiae.gff | less
```

In the above we use `cut` to show the first three fields of the file, and then we pipe it to `less` to examine one page of text at a time. The default field delimiter in `cut` is a tab (\t), but you can specify other delimiters with the `-d` option. You don't have to use adjacent columns with `cut`. For example,

```
$ cut -f1,3-5,7 saccharomyces_cerevisiae.gff | less
```

This allows us to look at the first column, and columns 3-5 and 7, corresponding to the seqid (=chromosome), the feature type, the feature start and stop coordinates (1-based), and the strand on which the feature is defined.

Notice how in addition to the fields, `cut` also gave use the header information at the beginning of the file. We can use the `-s` option to suppress lines that don't have the field delimiter character:

```
$ cut -s -f1,3-5,7 saccharomyces_cerevisiae.gff > out.txt
```

Notice this time we redirected the output of the command to a file, `out.txt`.

A.4.7. sort

The `sort` utility sorts lines of text. By default `sort` interprets an entire line of text as the key for sorting and sorts in dictionary order. For example, to see the default sorting:

```
$ sort out.txt | less
```

We can use the `-k` option to specify the field to sort on. For example, this is how we can sort on the second column of `out.txt`:

```
$ sort -k2 out.txt | less
```

Another useful option to sort is `-u` which tells `sort` to output only the first instance of a set of identical keys. Try and figure out what the following command does before running it:

```
$ cut -s -f3 saccharomyces_cerevisiae.gff | sort -u
```

A.4.8. grep

`grep` is a tool for doing regular expression matching on lines of a file. Regular expressions are a way to specify search patterns in strings. The simplest type of regular expression is to just search for a specific word, as illustrated here:

```
$ grep "gene" out.txt | less
```

The above command simply returns all the lines in `out.txt` that have the word "gene" in them. Let's use this in a slightly different way to count instances of different features in the file:

```
$ grep "gene" out.txt | wc -l  
6720  
$ grep "pseudogene" out.txt | wc -l
```

```
21  
$ grep "telomere" out.txt | wc -l  
32
```

NOTE: the numbers of matches may change somewhat between releases of the curated yeast genome. If the numbers you get above or below are *slightly* different from what is shown here don't worry.

We can get a little fancier if we use the “extended” grep syntax (specified using the -E option). Here’s how we can search for lines that match on any of a set of terms (the vertical bar | indicates an “OR” operator):

```
$ grep -E "tRNA|rRNA|snRNA|snoRNA" out.txt | wc -l  
409
```

Note that we have to be careful about what grep matches, for example:

```
$ grep "chr01" out.txt | grep "gene" | wc -l  
121
```

Note how we piped two grep commands together to get the equivalent of AND (“chr01” AND “gene”). However, there’s a very subtle problem with this command as constructed. We search on the word “gene” but “gene” is also a substring of “psuedogene” and hence “pseudogene” features also generate matches. What we really want is whole word matches. We can do that as follows:

```
$ grep "\<chr01\>" out.txt | grep "\<gene\>" | wc -l  
117
```

This uses what are called “POSIX character classes” to match possible sets of characters. A list of the POSIX character classes is linked to on the course wiki. Here’s the equivalent call for counting genes on chromosome IV:

```
$ grep "\<chr04\>" out.txt | grep "\<gene\>" | wc -l  
836
```

We’ve only just scratched the surface of regular expressions. Regular expressions are a very powerful tool and there are whole books on the topic. I’ll post a number of links on the course wiki to online tutorials on grep and regular expressions.

A.4.9. tr

tr is a utility for translating characters within a text stream. tr can be useful for converting delimiters from one file type to another. For example, let’s say we wanted to analyze the file out.txt in a program that expected comma separated values (csv) instead of tab-delimited fields. tr makes that conversion easy:

```
$ cat out.txt | tr "\t" "," > out.csv
```

Note that tr only reads from standard input so we used the cat program to feed the lines of text to tr.

A.4.10. awk

awk is a programming language designed for processing structured text files. You can use it to write short one liners or to write full blown programs. It turns out that some form of text file manipulation is often a necessary first step in most bioinformatics analyses, so awk often comes in very handy. We'll use awk to illustrate how you might transitions from simple command line usage into slightly more complicated scripts.

One simple thing we can do with awk is to use it to re-order fields in a structured data file:

```
awk '{print $2, $1, $4, $5}' out.txt | less
```

In the command above the the dollar signs followed by numbers refer to the fields of the file. With it's default setting awk operates line by line, so you can interpret the above statement as saying: "for each line, print the fields 2, 1, 4 and 5".

The basic syntax of awk is often depicted in the form pattern {action}. The above command only specified an action, so it was applied to every line. By contrast, in the example below we specify a pattern. The pattern can be read as - "if the 3rd field is 'chromosome'". For all lines that match that pattern the correspoding action is applied; in this case "print fields 1 and 5" (the chromosome name and its length):

```
awk '$3=="chromosome" {print $1, $5}' saccharomyces_cerevisiae.gff
```

Here's another pattern {action} pair that shows how we could find all gene features with length less than 300:

```
$ awk '$3 == "gene" && ($5 - $4) < 300 {print $0 }' \
    saccharomyces_cerevisiae.gff | wc -l
446
```

&& is the AND operator. Read this as "if the 3rd fields is 'gene' AND the the 5th field minus the 4th field is less than 300."

In this last example we added one more condition - we looked for the word 'Dubious' in the 9th field. The results indicate that a significant proportion of these small genes are classified as 'Dubious'.

```
$ awk '$3 == "gene" && ($5 - $4) < 300 && match($9, "Dubious") {print $0
}' saccharomyces_cerevisiae.gff | wc -l
163
```