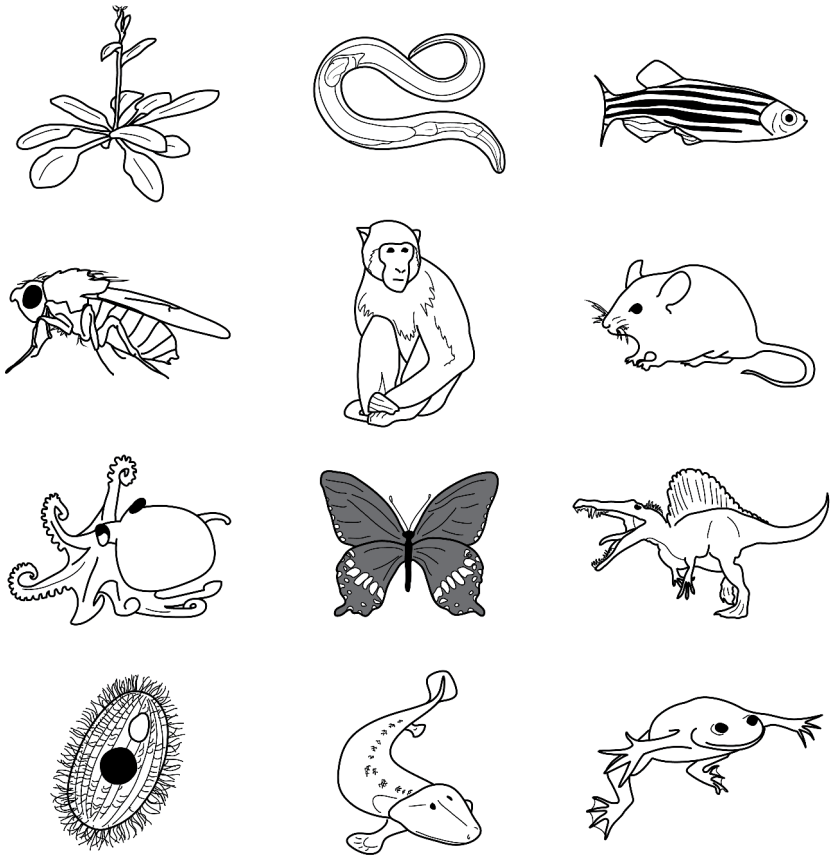
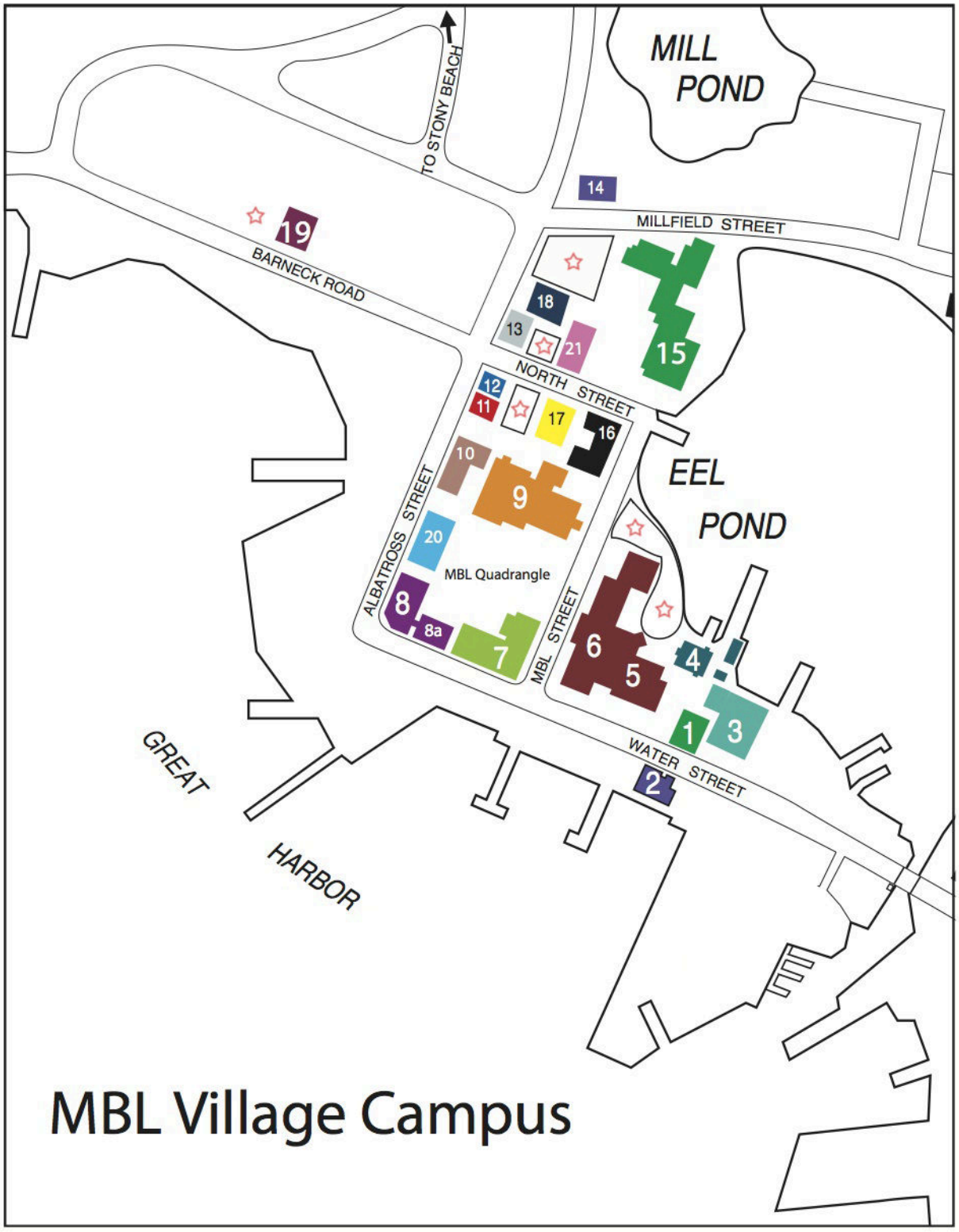
THE UNIVERSITY OF
CHICAGO BIOLOGICAL SCIENCES

BSD qBio^2 Boot Camp @ MBL



September 6–14, 2016
Marine Biological Laboratories
Woods Hole, MA



1 Candle House (Administration)
2 100 Water Street (Pierce Exhibit Center, Satellite Club, MBL Club)
3 Marine Resources Center
4 Collection Support Facility
5 Crane Wing (Labs, Shipping)
6 Lillie Laboratory (Labs, Service Shops, MBLWHOI Library)
7 Rowe Laboratory (Labs, Speck Auditorium)
8 Environmental Sciences Laboratory
8a Homestead Administration (Human Resources, Education Dept.)
9 Loeb Laboratory (Research and Teaching Labs, Lecture Rooms)
10 Brick Apartment House
11 Veeder House Dormitory
12 David House Dormitory
13 Broderick House (IT)
14 Crane House
15 Swope Center (Registration, Cafeteria, Dormitory, Meigs Room)
16 Ebert Hall Dormitory
17 Drew House Dormitory
18 15 North Street (Carpentry Shop)
19 Smith Cottage and Barneck Road Property
20 C.V. Starr Environmental Sciences Laboratory
21 11 North Street
☆ MBL Parking

MBL | Biological Discovery in Woods Hole

INFORMATION SHEET

http://www.mbl.edu/

EMERGENCY

Call boxes can be found in the Brick Apartment Building, and outside of Swope.

From cell: *508-289-7911* calls MBL security who will respond and notify Falmouth police or fire. Put this on the favorites list of you cell while you are here

MBL security non-emergency: *508-289-7217*

AIRPORTS Logan International Airport www.massport.com/logan-airport Boston, MA.) or **TF Green Airport** www.pvdairport.com (Providence, RI.)

ALCOHOL No one under the age of 21 is allowed to consume alcoholic beverages. This is strictly enforced.

ATM MACHINE 22 Luscombe Ave Woods Hole, MA. OR Bank of Woods Hole, 2 Water Street, Woods Hole, MA. There is also an ATM next to the Woods Hole Market.

BADGES and LANYARDS ID Cards and *MBL* lanyards are for registered guests and employees on the MBL campus. They must be worn at all times for identification purposes, entry into buildings and to attend meals. Please swipe them slowly as you are entering the building as the machines are sensitive.

BIKE PATH Shining Sea Bikeway is 10.7 miles which hugs the coast from Falmouth to Woods Hole and then beyond to North Falmouth. It is ideal for casual biking, which passes through Sippewissett Marsh, Cranberry bogs and overlooks Chapoquoit Beach crisscrossing through the Salt Pond Bird Sanctuary. For bike rental information try Corner Cycle Cape Cod (508-540-4195) http://www.cornercycle.com/about/rental-info-pg60.htm. An indoor bicycle storage area is available in the basement of Ebert Hall for a nominal fee. Bicycles may not be stored in dormitories or laboratory buildings. For more information about bikes visit http://www.mbl.edu/parking-program/

BIOLOGICAL SAFETY: All needles, syringes, razor blades should be placed in "sharp" boxes prior to disposal. The safety department supplies these boxes to your lab x 7641 Safety Office.

CAMPUS MAPS Copies of the campus map are provided within your orientation packet. They can also be found at the Swope Front Desk.

CHEMICAL SAFETY safety data sheets (SDSs) can be found on the website: http://www.mbl.edu/services/safety/ . Please contact x 7424 for further assistance.

FALMOUTH TAXI Falmouth Taxi may be contacted should you need transportation outside the MBL. They are often located at the ferry terminal as well. Please call 508-548-3100 for service.

FIRST AID KITS are available in hallways adjacent to emergency showers in Loeb classrooms and MBL Laboratories. AEDs are found at the entrance to most of the main buildings.

FOOD ALLERGIES On your registration form or program application and during orientation you will have opportunities to notify the MBL about food allergies. It is also recommended to inform your servers in the dining hall so that proper accommodations can be made. If severe allergies exist, please set up a meeting with our dining services by contacting Erin Hummetoglu at 508-457-9084.

GIFT SHOP HOURS - The MBL gift shop is open in July-August 10:00 am - 4:30 pm, MWF. Hours vary in other months. See http://giftshop.mbl.edu

HISTORY OF MBL The Marine Biological Laboratory (MBL) history project seeks to document the wonderfully rich history of the MBL. Historians from across America (http://history.archives.mbl.edu/info/about#team) are pouring over the archive to bring us more information on the significant contributions that the people of the MBL have made to scientific discovery See http://history.archives.mbl.edu

LAUNDRY Laundry facilities are located in Swope and in the basement of Ebert Hall and the Brick Apartment dorms. Those staying in the cottages may use the Pilot House in Devil's Lane, access is your cottage key. All laundry facilities have credit card-operated washers and dryers

LOCAL TRANSPORTATION Options for local transportation to and from the Marine Biological Laboratory can use
Peter Pan Bus peterpanbus.com, and the schedule
http://peterpanbus.com/commuter/commuter-bus-schedule-Woods-Hole-Boston.pdf
Green Shuttle gogreenshuttle.com/# or, **White Tie Limo** whitetie.imadevsvr.com or Falmouth Taxi is available 24hrs a day and located at the ferry terminal - call 508-548-3100

LUGGAGE Upon check out (10am) there is a small hut outside Swope for your luggage. Please do not bring luggage with you into classrooms, labs or other indoor areas.

MAIL Please register with the MBL Mail Room upon arrival located in the basement of Lillie. Your address will be (Your Name) Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543.

MBL ALERT emergency.mbl.edu/mblalert
a high speed, automated emergency notification system to all registered scientists, staff, visitors and students conveying vital safety and security information quickly and accurately.

MBL LIBRARY The MBL library is located in the Lillie Building, on the 2nd floor. It is staffed and open to the public 8 am-5pm Monday-Friday. The Library is closed from 11 pm - 7 am everyday. See: http://www.mblwhoilibrary.org to locate journal titles, databases, and the library catalog. Call: 508-289-7002.

NOISE ORDINANCE: Loud noises/music/ conversation that carry more than 150 feet are prohibited after 10 pm. This will be enforced by MBL security.

PARKING Parking passes must be obtained from the Swope Check-In desk. Parking is off campus in Devil's Lane Lot. Special permission for passes to Lillie, Loeb, and Swope lots must be obtained through education@mbl.edu . Street parking is metered and cars will be towed from meters between 2 am-7 am. MBL parking lots are available after hours (between 4 pm-7 am) and on weekends.

PIERCE EXHIBIT CENTER located at 100 Water Street gives the history and story of the MBL and its impact of scientific discovery. Hours of operation 11am - 4pm (Spring), 10am - 4pm (Summer).

POISON CONTROL from campus phone 9-1-800-222-1222 (cell: 800-222-1222).

PRINTING AND PHOTOCOPYING
Students have 24/7 access to two photocopying machines located on the 2nd floor lobby of the Lillie library. You must use your student ID card to access this building. Printing is functional only from library workstations. Faculty may obtain a copy code from program organizer: 0.10 black and white and 0.30. color copies.

RECYCLING The MBL has an active recycling program that includes electronic equipment, paper, packaging material, glass, and plastic. Recycling containers are positioned and recycling notices are posted throughout the campus. Contact Bob Kaski (508-289-7326) for information.

RESPONSIBLE CONDUCT RESEARCH
(http://www.mbl.edu/ethics/) and the MBL Code of Conduct (http://www.mbl.edu/hr/staff-toolbox/marine-biological-laboratory-policy-manual/code-of-conduct/)

SMOKING The MBL provides a smoke-free environment for all investigators, faculty, students and visitors. As such, smoking is prohibited in the interiors of all MBL facilities including housing, vehicles and all exterior doorways.

SPORTS EQUIPMENT Tennis rackets & balls, volleyball, football and other sporting equipment available to sign-out at the Swope front desk.

STONEY BEACH Stoney beach is located near the intersection of Millfield street and Albatross (map), a short walk from the MBL campus. Beach hours are 8 am – 10 pm.

SWOPE DINING HALL HOURS: SUMMER
Breakfast: 7:00 am - 9:30 am
Lunch: 11:30 am - 1:30 pm
Dinner: 5:00 pm - 7:30 pm

TECHNOLOGY SUPPORT
Information regarding common technology questions can generally be found online at www.mbl.edu/it. However, if you are unable to find answers to your questions online or if you wish to report a technical problem or request technical assistance please call the IT Help Desk at 508-289-7654 or x7654 from an internal phone, or submit a request to: helpdesk@mbl.edu.

WIRELESS ACCESS The *MBL-GUEST* (https://intranet.mbl.edu/display/IN/MBL-Guest) wireless network is an easy access encrypted wireless network designed to provide access to the internet. Please refer to wireless access signs posted throughout the campus for additional connectivity information.

BSD qBio^2 @ MBL

General Schedule

Tuesday, September 6

2:45-5:00	Check-in
5:00-6:30	Dinner
8:15-9:00	Introduction – Allesina and Palmer

Wednesday, September 7

8:30-10:00	Free time (*M.mulatta M.musculus O.bimaculoides P.polytes*)
	Imaging (*S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Trip on the Gemma (*A.thaliana C.elegans*)
	Visit Marine Resources Center (*D.melanogaster D.rerio*)
10:00-10:30	Coffee break
10:30-12:00	Free time (*M.mulatta M.musculus O.bimaculoides P.polytes*)
	Imaging (*S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Trip on the Gemma (*D.melanogaster D.rerio*)
	Visit Marine Resources Center (*A.thaliana C.elegans*)
12:00-12:45	Lunch
12:45-1:00	MBL Orientation
1:00-2:30	Basic/Advanced computing I (*A.thaliana C.elegans D.melanogaster D.rerio M.mulatta M.musculus*)
	Math. Foundations I (*O.bimaculoides P.polytes S.aegyptiacus T.roseae T.thermophila X.laevis*)
2:30-2:45	Coffee break
2:45-4:30	Basic/Advanced computing I (*A.thaliana C.elegans D.melanogaster D.rerio M.mulatta M.musculus*)
	Math. Foundations I (*O.bimaculoides P.polytes S.aegyptiacus T.roseae T.thermophila X.laevis*)
5:00-6:30	Dinner
7:15-8:00	Welcome – Nishi; Intro to Grad School – Prince

Thursday, September 8

8:30-10:00	Free time (*S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Imaging (*A.thaliana C.elegans D.melanogaster D.rerio*)
	Trip on the Gemma (*M.mulatta M.musculus*)
	Visit Marine Resources Center (*O.bimaculoides P.polytes*)
10:00-10:30	Coffee break
10:30-12:00	Free time (*S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Imaging (*A.thaliana C.elegans D.melanogaster D.rerio*)
	Trip on the Gemma (*O.bimaculoides P.polytes*)

	Visit Marine Resources Center (*M.mulatta M.musculus*)
12:00-1:00	Lunch
1:00-2:30	Basic/Advanced computing I (*O.bimaculoides P.polytes S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Math. Foundations I (*A.thaliana C.elegans D.melanogaster D.rerio M.mulatta M.musculus*)
2:30-2:45	Coffee break
2:45-4:30	Basic/Advanced computing I (*O.bimaculoides P.polytes S.aegyptiacus T.roseae T.thermophila X.laevis*)
	Math. Foundations I (*A.thaliana C.elegans D.melanogaster D.rerio M.mulatta M.musculus*)
5:00-6:30	Dinner
7:15-8:00	MBL Talk – Jennifer Morgan

Friday, September 9

8:30-10:00	Free time (*A.thaliana C.elegans D.melanogaster D.rerio*)
	Imaging (*M.mulatta M.musculus O.bimaculoides P.polytes*)
	Trip on the Gemma (*S.aegyptiacus T.thermophila*)
	Visit Marine Resources Center (*T.roseae X.laevis*)
10:00-10:30	Coffee break
10:30-12:00	Free time (*A.thaliana C.elegans D.melanogaster D.rerio*)
	Imaging (*M.mulatta M.musculus O.bimaculoides P.polytes*)
	Trip on the Gemma (*T.roseae X.laevis*)
	Visit Marine Resources Center (*S.aegyptiacus T.thermophila*)
12:00-1:00	Lunch
1:00-2:30	Basic/Advanced computing II (*A.thaliana D.rerio M.mulatta O.bimaculoides S.aegyptiacus T.roseae*)
	Math. Foundations II (*C.elegans D.melanogaster M.musculus P.polytes T.thermophila X.laevis*)
2:30-2:45	Coffee break
2:45-4:30	Basic/Advanced computing II (*A.thaliana D.rerio M.mulatta O.bimaculoides S.aegyptiacus T.roseae*)
	Math. Foundations II (*C.elegans D.melanogaster M.musculus P.polytes T.thermophila X.laevis*)
5:00-6:30	Dinner
7:15-8:00	MBL Talk – Roger Hanlon

Saturday, September 10

8:30-10:00	Basic/Advanced computing II (*C.elegans D.melanogaster M.musculus P.polytes T.thermophila X.laevis*)
	Math. Foundations II (*A.thaliana D.rerio M.mulatta O.bimaculoides S.aegyptiacus T.roseae*)
10:00-10:30	Coffee break
10:30-12:00	Basic/Advanced computing II (*C.elegans D.melanogaster M.musculus P.polytes T.thermophila X.laevis*)
	Math. Foundations II (*A.thaliana D.rerio M.mulatta O.bimaculoides S.aegyptiacus T.roseae*)
12:00-8:00	Free time

Sunday, September 11

8:30-10:00	Workshop Cobey (*A.thaliana C.elegans D.rerio*)
	Workshop Novembre (*O.bimaculoides P.polytes S.aegyptiacus*)
	Workshop Osborne (*T.roseae T.thermophila X.laevis*)
	Workshop Vander Griend (*D.melanogaster M.mulatta M.musculus*)
10:00-10:30	Coffee break
10:30-12:00	Workshop Cobey (*A.thaliana C.elegans D.rerio*)
	Workshop Novembre (*O.bimaculoides P.polytes S.aegyptiacus*)
	Workshop Osborne (*T.roseae T.thermophila X.laevis*)
	Workshop Vander Griend (*D.melanogaster M.mulatta M.musculus*)
12:00-1:00	Lunch
1:00-2:30	Workshop Cobey (*D.melanogaster O.bimaculoides T.thermophila*)
	Workshop Novembre (*C.elegans M.mulatta X.laevis*)
	Workshop Osborne (*D.rerio M.musculus S.aegyptiacus*)
	Workshop Vander Griend (*A.thaliana P.polytes T.roseae*)
2:30-2:45	Coffee break
2:45-4:30	Workshop Cobey (*D.melanogaster O.bimaculoides T.thermophila*)
	Workshop Novembre (*C.elegans M.mulatta X.laevis*)
	Workshop Osborne (*D.rerio M.musculus S.aegyptiacus*)
	Workshop Vander Griend (*A.thaliana P.polytes T.roseae*)
5:00-6:30	Dinner
6:30-7:10	Life after PhD a) (*D.rerio T.thermophila*)
	Life after PhD b) (*D.melanogaster S.aegyptiacus*)
	Navigating first year a) (*A.thaliana X.laevis*)
	Navigating first year b) (*C.elegans T.roseae*)
	Publishing primer a) (*M.mulatta P.polytes*)
	Publishing primer b) (*M.musculus O.bimaculoides*)
7:20-8:00	Life after PhD a) (*A.thaliana X.laevis*)
	Life after PhD b) (*C.elegans T.roseae*)
	Navigating first year a) (*M.mulatta P.polytes*)
	Navigating first year b) (*M.musculus O.bimaculoides*)
	Publishing primer a) (*D.rerio T.thermophila*)
	Publishing primer b) (*D.melanogaster S.aegyptiacus*)
8:10-8:50	Life after PhD a) (*M.mulatta P.polytes*)
	Life after PhD b) (*M.musculus O.bimaculoides*)
	Navigating first year a) (*D.rerio T.thermophila*)
	Navigating first year b) (*D.melanogaster S.aegyptiacus*)
	Publishing primer a) (*A.thaliana X.laevis*)
	Publishing primer b) (*C.elegans T.roseae*)

Monday, September 12

8:30-10:00	Workshop Cobey (*M.mulatta S.aegyptiacus T.roseae*)
	Workshop Novembre (*A.thaliana M.musculus T.thermophila*)
	Workshop Osborne (*C.elegans D.melanogaster P.polytes*)
	Workshop Vander Griend (*D.rerio O.bimaculoides X.laevis*)
10:00-10:30	Coffee break
10:30-12:00	Workshop Cobey (*M.mulatta S.aegyptiacus T.roseae*)
	Workshop Novembre (*A.thaliana M.musculus T.thermophila*)
	Workshop Osborne (*C.elegans D.melanogaster P.polytes*)
	Workshop Vander Griend (*D.rerio O.bimaculoides X.laevis*)
12:00-1:00	Lunch
1:00-2:30	Workshop Cobey (*M.musculus P.polytes X.laevis*)
	Workshop Novembre (*D.melanogaster D.rerio T.roseae*)
	Workshop Osborne (*A.thaliana M.mulatta O.bimaculoides*)
	Workshop Vander Griend (*C.elegans S.aegyptiacus T.thermophila*)
2:30-2:45	Coffee break
2:45-4:30	Workshop Cobey (*M.musculus P.polytes X.laevis*)
	Workshop Novembre (*D.melanogaster D.rerio T.roseae*)
	Workshop Osborne (*A.thaliana M.mulatta O.bimaculoides*)
	Workshop Vander Griend (*C.elegans S.aegyptiacus T.thermophila*)
5:00-6:30	Dinner
7:15-8:00	MBL Talk – David Remsen

Tuesday, September 13

8:30-10:00	Workshop Munro/Rust
10:00-10:30	Coffee break
10:30-12:00	Workshop Munro/Rust
12:00-1:00	Lunch
1:00-2:30	Workshop Munro/Rust
2:30-2:45	Coffee break
2:45-4:30	Workshop Munro/Rust
5:00-6:30	Dinner
5:00-6:00	Wrapping up
6:00-8:00	BBQ

Wednesday, September 14

10:00-10:30	Departure for BOS

Tutorials

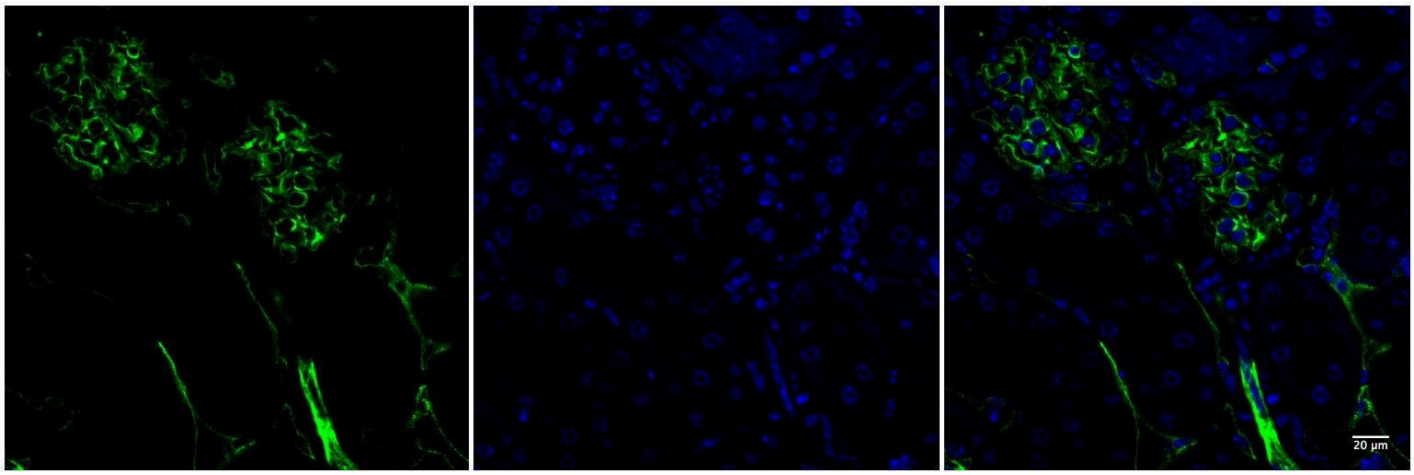
Imaging
Mathematical Foundations I
Mathematical Foundations II
Basic Computing I
Basic Computing II
Advanced Computing I
Advanced Computing II

Image Processing with ImageJ Exercises

Using the information in the ImageJ Tutorials and the built-in ImageJ Command Finder tool (open ImageJ / Fiji and type L to get it to pop up), complete the following exercises. You may not be able to complete these exercises in the time you have, but do your best.

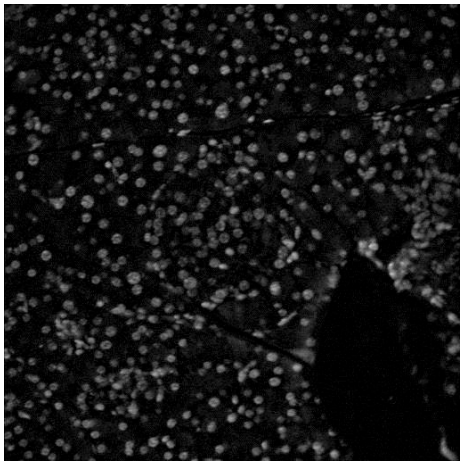
Exercise 1

Use the cd31 glomeruli.tif file to create the montage shown below. The image comes as two images grouped together in a stack, so your first job is to figure out how to separate them. The pixel size for the image is 0.25 microns. Don't forget the 20um scale bar in the lower right hand corner!



Exercise 2

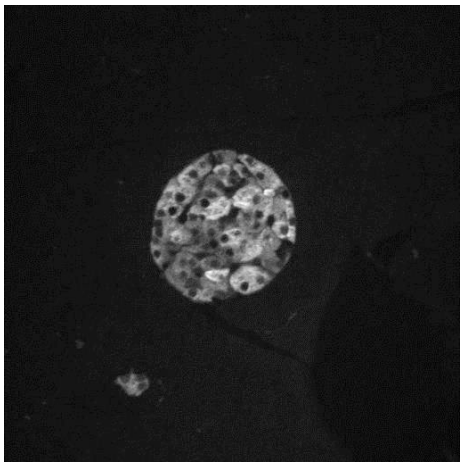
Use the pancreatic_islet.tif to answer the following questions:



a) How many cells are in the entire image? One nucleus per cell is marked by the DAPI stain in the first image.

b) What is the average nuclear area for all the cells in the image? Readout should be in um^2 NOT cm^2!

c) How would you create a data table that includes the mean gray value for every nucleus in the image? They are not all 255!



d) How many beta cells are in the image? Beta cells are represented by the insulin (green) stain in the second image. Hint: you can still count the nuclei if you find a way to restrict your count to nuclei in an islet.

Bonus Questions (if you have time)

Bonus 1: if there were multiple islets in the pancreatic_islet.tif image, how could you count the number of cells PER islet?

Bonus 2: Create a macro to automate the creation of the cd31 glomeruli montage.

Basic Computing 1 – Introduction to R

Stefano Allesina

Basic Computing 1

- **Goal:** Introduce the stastical software `R`, and show how it can be used to analyze biological data in an automated, replicable way. Showcase the `RStudio` development environment, illustrate the notion of assignment, present the main data structures available in `R`. Show how to read and write data, how to execute simple programs, and how to modify the stream of execution of a program through conditional branching and looping.
- **Audience:** Biologists with little or no background in programming.
- **Installation:** To complete the tutorial, we will need to install `R` and `RStudio`. To install `R`, follow instructions at `cran.rstudio.com`. Then install `Rstudio` following the instructions at `goo.gl/a42jYE`.

Motivation

When it comes to analyzing data, there are two competing paradigms. First, one could use point-and-click software with a graphical user interface, such as Excel, to perform calculations and draw graphs; second, one could write programs that can be run to perform the analysis of the data, the generation of tables and statistics, and the production of figures automatically.

This latter approach is to be preferred, because it allows for the automation of analysis, it requires a good documentation of the procedures, and is completely replicable.

A few motivating examples:

- You have written code to analyze your data. You receive from your collaborators a new batch of data. With simple modifications of your code, you can update your results, tables and figures automatically.
- A new student joins the laboratory. The new student can read the code and understand the analysis without the need of a lab mate showing the procedure step-by-step.
- The reviewers of your manuscript ask you to slightly alter the analysis. Rather than having to start over, you can modify a few lines of code and satisfy the reviewers.

Here we introduce `R`, which can help you write simple programs to analyze your data, perform statistical analysis, and draw beautiful figures.

What is R?

`R` is a statistical software that is completely programmable. This means that one can write a program (script) containing a series of commands for the analysis of data, and execute them automatically. This approach is especially good as it makes the analysis of data well-documented, and completely replicable.

`R` is free software: anyone can download its source code, modify it, and improve it. The `R` community of users is vast and very active. In particular, scientists have enthusiastically embraced the program, creating thousands of packages to perform specific types of analysis, and adding many new capabilities. You can find

a list of official packages (which have been vetted by R core developers) at goo.gl/SOSDWA; many more are available on GitHub and other websites.

The main hurdle new users face when approaching R is that it is based on a command line interface: when you launch R, you simply open a console with the character > signaling that R is ready to accept an input. When you write a command and press Enter, the command is interpreted by R, and the result is printed immediately after the command. For example,

```
1 + 1
```

```
## [1] 2
```

A little history: R was modeled after the commercial statistical software S by Robert Gentleman and Ross Ihaka. The project was started in 1992, first released in 1994, and the first stable version appeared in 2000. Today, R is managed by the *R Core Team*.

RStudio

For this introduction, we're going to use RStudio, an Integrated Development Environment (IDE) for R. The main advantage is that the environment will look identical irrespective of your computer architecture (Linux, Windows, Mac). Also, RStudio makes writing code much easier by automatically completing commands and file names (simply type the beginning of the name and press Tab), and allowing you to easily inspect data and code.

Typically, an RStudio window contains four panels:

- **Console** This is a panel containing an instance of R. For this tutorial, we will work mainly in this panel.
- **Source code** In this panel, you can write a program, save it to a file pressing Ctrl + S and then execute it by pressing Ctrl + Shift + S.
- **Environment** This panel lists all the variables you created (more on this later); another tab shows you the history of the commands you typed.
- **Plots** This panel shows you all the plots you drew. Other tabs allow you to access the list of packages you have loaded, and the help page for commands (just type help(name_of_command) in the Console) and packages.

How to write an R program

An R program is simply a list of commands, which are executed one after the other. The commands are witten in a text file (with extension .R). When R executes the program, it will start from the beginning of the file and proceed toward the end of the file. Every time R encounters a command, it will execute it. Special commands can modify this basic flow of the program by, for example, executing a series of commands only when a condition is met, or repeating the execution of a series of commands multiple times.

Note that if you were to copy and paste (or type) the code into the **Console** you would obtain exactly the same result. Writing a program is advatageous, however, because it can be automated and shared with other researchers. Moreover, after a while you will have a large code base, so that you can recycle much of your code in several programs.

The most basic operation: assignment

The most basic operation in any programming language is the assignment. In `R`, assignment is marked by the operator `<-`. When you type a command in `R`, it is executed, and the output is printed in the **Console**. For example:

```
sqrt(9)
```

```
## [1] 3
```

If we want to save the result of this operation, we can assign it to a variable. For example:

```
x <- sqrt(9)
x
```

```
## [1] 3
```

What has happened? We wrote a command containing an assignment operator (`<-`). `R` has evaluated the right-hand-side of the command (`sqrt(9)`), and has stored the result (`3`) in a newly created variable called `x`. Now we can use `x` in our commands: every time the command needs to be evaluated, the program will look up which value is associated with the variable `x`, and substitute it. For example:

```
x * 2
```

```
## [1] 6
```

Types of data

`R` provides different types of data that can be used in your programs. The basic data types are:

- `logical`, taking only two possible values: `TRUE` and `FALSE`

```
v <- TRUE
class(v)
```

```
## [1] "logical"
```

- `numeric`, storing real numbers (actually, their approximations, as computers have limited memory and thus cannot store numbers like π, or even 0.2)

```
v <- 3.77
class(v)
```

```
## [1] "numeric"
```

- `integer`, storing whole numbers

```r
v <- 23L # the L signals that this should be stored as integer
class(v)
```

```
## [1] "integer"
```

- `complex`, storing complex numbers (i.e., with a real and an imaginary part)

```r
v <- 23 + 5i # the i marks the imaginary part
class(v)
```

```
## [1] "complex"
```

- `character`, for strings, characters and text

```r
v <- 'a string' # you can use single or double quotes
class(v)
```

```
## [1] "character"
```

In `R`, the type of a variable is evaluated at runtime. This means that you can recycle the names of variables. This is very handy, but can make your programs more difficult to read and to debug (i.e., find mistakes). For example:

```r
x <- '2.3' # this is a string
x
```

```
## [1] "2.3"
```

```r
x <- 2.3 # this is numeric
x
```

```
## [1] 2.3
```

Operators and functions

Each data type supports a certain number of operators and functions. For example, numeric variables can be combined with `+` (addition), `-` (subtraction), `*` (multiplication), `/` (division), and `^` (exponentiation). A possibly unfamiliar operator is the modulo (`%%`), calculating the remainder of an integer division:

```r
5 %% 3
```

```
## [1] 2
```

meaning that `5 %/% 3` (5 integer divided by three) is 1 with a remainder of 2. The modulo operator is useful to determine whether a number is divisible for another: if y is divisible by x, then `y %% x` is 0.

Numeric types also support many built-in functions, such as:

- `abs(x)` absolute value
- `sqrt(x)` square root
- `round(x, digits = 3)` round `x` to three decimal digits
- `cos(x)` cosinus (also supported are all the usual trigonometric functions)
- `log(x)` natural logarithm (use `log10` for base 10 logarithms)
- `exp(x)` calculating e^x

Similarly, `character` variables have their own set of functions, such as

- `toupper(x)` make uppercase
- `nchar(x)` count the number of characters in the string
- `paste(x, y, sep = "_")` concatenate strings, joining them using the separator `_`
- `strsplit(x, "_")` separate the string using the separator `_`

Calling a function meant for a certain data type on another will cause errors. If sensible, you can convert a type into another. For example:

```
v <- "2.13"
class(v)
```

```
## [1] "character"
```

```
# if we call v * 2, we get an error.
# to avoid it, we can convert v to numeric:
as.numeric(v) * 2
```

```
## [1] 4.26
```

If sensible, you can use the comparison operators `>` (greater), `<` (lower), `==` (equals), `!=` (differs), `>=` and `<=`, returning a logical value:

```
2 == sqrt(4)
```

```
## [1] TRUE
```

```
2 < sqrt(4)
```

```
## [1] FALSE
```

```
2 <= sqrt(4)
```

```
## [1] TRUE
```

Similarly, you can concatenate several comparison and logical variables using `&` (and), `|` (or), and `!` (not):

```
(2 > 3) & (3 > 1)
```

```
## [1] FALSE
```

```
(2 > 3) | (3 > 1)
```

```
## [1] TRUE
```

Data structures

Besides these simple types, `R` provides structured data types, meant to collect and organize multiple values.

Vectors

The most basic data structure in `R` is the vector, which is an ordered collection of values of the same type. Vectors can be created by concatenating different values with the function `c()` (concatenate):

```
x <- c(2, 3, 5, 27, 31, 13, 17, 19)
x
```

```
## [1]  2  3  5 27 31 13 17 19
```

You can access the elements of a vector by their index: the first element is indexed at 1, the second at 2, etc.

```
x[3]
```

```
## [1] 5
```

```
x[8]
```

```
## [1] 19
```

```
x[9] # what if the element does not exist?
```

```
## [1] NA
```

`NA` stands for Not Available. Other special values are `NaN` (Not a Number, e.g., `0/0`), `Inf` (Infinity, e.g., `1/0`), and `NULL` (variable not set). You can test for special values using `is.na(x)`, `is.infinite(x)`, etc.

Note that in `R` a single number (string, logical) is a vector of length 1 by default. That's why if you type `3` in the console you see `[1] 3` in the output.

You can extract several elements at once (i.e., create another vector), using the colon (`:`) command, or by concatenating the indices:

```
x[1:3]
```

```
## [1] 2 3 5
```

```
x[4:7]
```

```
## [1] 27 31 13 17
```

```
x[c(1,3,5)]
```

```
## [1]  2  5 31
```

You can also use a vector of logical variables to extract values from vectors. For example, suppose we have two vectors:

```
sex <- c("M", "M", "F", "M", "F") # sex of Drosophila
weight <- c(0.230, 0.281, 0.228, 0.260, 0.231) # weigth in mg
```

and that we want to extract only the weights for the males.

```
sex == "M"
```

```
## [1]  TRUE  TRUE FALSE  TRUE FALSE
```

returns a vector of logical values, which we can use to subset the data:

```
weight[sex == "M"]
```

```
## [1] 0.230 0.281 0.260
```

Given that `R` was born for statistics, there are many statistical functions you can perform on vectors:

```
length(x)
```

```
## [1] 8
```

```
min(x)
```

```
## [1] 2
```

```
max(x)
```

```
## [1] 31
```

```
sum(x) # sum all elements
```

```
## [1] 117
```

```
prod(x) # multiply all elements
```

```
## [1] 105436890
```

```
median(x) # median value
```

```
## [1] 15
```

```
mean(x) # arithmetic mean
```

```
## [1] 14.625
```

```
var(x) # unbiased sample variance
```

```
## [1] 119.4107
```

```
mean(x ^ 2) - mean(x) ^ 2 # population variance
```

```
## [1] 104.4844
```

```
summary(x) # print a summary
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    2.00    4.50   15.00   14.62   21.00   31.00
```

You can generate vectors of sequential numbers using the colon command:

```
x <- 1:10
x
```

```
##  [1]  1  2  3  4  5  6  7  8  9 10
```

For more complex sequences, use `seq`:

```
seq(from = 1, to = 5, by = 0.5)
```

```
## [1] 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
```

To repeat a value or a sequence several times, use `rep`:

```
rep("abc", 3)
```

```
## [1] "abc" "abc" "abc"
```

```
rep(c(1,2,3), 3)
```

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

Exercise:

- Create a vector containing all the even numbers between 2 and 100 (inclusive) and store it in variable `z`.
- Extract all the elements of `z` that are divisible by 12. How many elements match this criterion?
- What is the sum of all the elements of `z`?
- Is it equal to $51 \cdot 50$?
- What is the product of elements 5, 10 and 15 of `z`?
- Create a vector `y` that contains all numbers between 0 and 30 that are divisible by 3. Find the five elements of `y` that are also elements of `z`.
- Does `seq(2, 100, by = 2)` produce the same vector as `(1:50) * 2`?
- What happens if you type `z ^ 2`?

Matrices

A matrix is a two-dimensional table of values. In case of numeric values, you can perform the usual operations on matrices (product, inverse, decomposition, etc.):

```
A <- matrix(c(1, 2, 3, 4), 2, 2) # values, nrows, ncols
A
```

```
##      [,1] [,2]
## [1,]    1    3
## [2,]    2    4
```

```
A %*% A # matrix product
```

```
##      [,1] [,2]
## [1,]    7   15
## [2,]   10   22
```

```
solve(A) # matrix inverse
```

```
##      [,1] [,2]
## [1,]   -2  1.5
## [2,]    1 -0.5
```

```
A %*% solve(A) # this should return the identity matrix
```

```
##      [,1] [,2]
## [1,]    1    0
## [2,]    0    1
```

```
B <- matrix(1, 3, 2) # you can fill the whole matrix with a single number (1)
B
```

```
##      [,1] [,2]
## [1,]    1    1
## [2,]    1    1
## [3,]    1    1
```

```
B %*% t(B) # transpose
```

```
##      [,1] [,2] [,3]
## [1,]    2    2    2
## [2,]    2    2    2
## [3,]    2    2    2
```

```
Z <- matrix(1:9, 3, 3) # by default, matrices are filled by column
Z
```

```
##      [,1] [,2] [,3]
## [1,]    1    4    7
## [2,]    2    5    8
## [3,]    3    6    9
```

To determine the dimensions of a matrix, use `dim`:

```
dim(B)
```

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

```
dim(B)[1]
```

```
## [1] 3
```

```
nrow(B)
```

```
## [1] 3
```

```
dim(B)[2]
```

```
## [1] 2
```

```
ncol(B)
```

```
## [1] 2
```

Use indices to access a particular row/column of a matrix:

```
Z
```

```
##      [,1] [,2] [,3]
## [1,]    1    4    7
## [2,]    2    5    8
## [3,]    3    6    9
```

```
Z[1, ] # first row
```

```
## [1] 1 4 7
```

```
Z[, 2] # second column
```

```
## [1] 4 5 6
```

```
Z [1:2, 2:3] # submatrix with coefficients in first two rows, and second and third column
```

```
##      [,1] [,2]
## [1,]    4    7
## [2,]    5    8
```

```
Z[c(1,3), c(1,3)] # indexing non-adjacent rows/columns
```

```
##      [,1] [,2]
## [1,]    1    7
## [2,]    3    9
```

Some operations use all the elements of the matrix:

```
sum(Z)
```

```
## [1] 45
```

```
mean(Z)
```

```
## [1] 5
```

Arrays

If you need tables with more than two dimensions, use arrays:

```
M <- array(1:24, c(4, 3, 2))
M
```

```
## , , 1
##
##      [,1] [,2] [,3]
## [1,]    1    5    9
## [2,]    2    6   10
## [3,]    3    7   11
## [4,]    4    8   12
##
## , , 2
##
##      [,1] [,2] [,3]
## [1,]   13   17   21
## [2,]   14   18   22
## [3,]   15   19   23
## [4,]   16   20   24
```

You can still determine the dimensions using:

```
dim(M)
```

```
## [1] 4 3 2
```

and access the elements as done for matrices. One thing you should be paying attention to: `R` drops dimensions that are not needed. So, if you access a "slice" of a 3-dimensional array:

```
M[,,1]
```

```
##      [,1] [,2] [,3]
## [1,]    1    5    9
## [2,]    2    6   10
## [3,]    3    7   11
## [4,]    4    8   12
```

you obtain a matrix:

```
dim(M[,,1])
```

```
## [1] 4 3
```

This can be problematic, for example, when your code expects an array and `R` turns your data into a matrix (or you expect a matrix but find a vector). To avoid this behavior, add `drop = FALSE` when subsetting:

```
dim(M[,,1, drop = FALSE])
```

```
## [1] 4 3 1
```

Lists

Vectors are good if each element is of the same type (e.g., numbers, strings). Lists are used when we want to store elements of different types, or more complex objects (e.g., vectors, matrices, even lists of lists). Each element of the list can be referenced either by its index, or by a label:

```
mylist <- list(Names = c("a", "b", "c", "d"), Values = c(1, 2, 3))
mylist
```

```
## $Names
## [1] "a" "b" "c" "d"
##
## $Values
## [1] 1 2 3
```

```
mylist[[1]] # access first element using index
```

```
## [1] "a" "b" "c" "d"
```

```
mylist[[2]] # access second element by index
```

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

```
mylist$Names # access second element by label
```

```
## [1] "a" "b" "c" "d"
```

```r
mylist[["Names"]] # another way to access by label
```

```
## [1] "a" "b" "c" "d"
```

```r
mylist[["Values"]][3]  # access third element in second vector
```

```
## [1] 3
```

Data frames

Data frames contain data organized like in a spreadsheet. The columns (typically representing different measurements) can be of different types (e.g., a column could be the date of measurement, another the weight of the individual, or the volume of the cell, or the treatment of the sample), while the rows typically represent different samples.

When you read a spreadsheet file in R, it is automatically stored as a data frame. The difference between a matrix and a data frame is that in a matrix all the values are of the same type (e.g., all numeric), while in a data frame each column can be of a different type.

Because typing a data frame by hand would be tedious, let's use a data set that is already available in R:

```r
data(trees) # Girth, height and volume of cherry trees
str(trees) # structure of data frame
```

```
## 'data.frame':    31 obs. of  3 variables:
##  $ Girth : num  8.3 8.6 8.8 10.5 10.7 10.8 11 11 11.1 11.2 ...
##  $ Height: num  70 65 63 72 81 83 66 75 80 75 ...
##  $ Volume: num  10.3 10.3 10.2 16.4 18.8 19.7 15.6 18.2 22.6 19.9 ...
```

```r
ncol(trees)
```

```
## [1] 3
```

```r
nrow(trees)
```

```
## [1] 31
```

```r
head(trees) # print the first few rows
```

```
##   Girth Height Volume
## 1   8.3     70   10.3
## 2   8.6     65   10.3
## 3   8.8     63   10.2
## 4  10.5     72   16.4
## 5  10.7     81   18.8
## 6  10.8     83   19.7
```

```
trees$Girth # select column by name
```

```
##  [1]  8.3  8.6  8.8 10.5 10.7 10.8 11.0 11.0 11.1 11.2 11.3 11.4 11.4 11.7
## [15] 12.0 12.9 12.9 13.3 13.7 13.8 14.0 14.2 14.5 16.0 16.3 17.3 17.5 17.9
## [29] 18.0 18.0 20.6
```

```
trees$Height[1:5] # select column by name; return first five elements
```

```
## [1] 70 65 63 72 81
```

```
trees[1:3, ] #select rows 1 through 3
```

```
##   Girth Height Volume
## 1   8.3     70   10.3
## 2   8.6     65   10.3
## 3   8.8     63   10.2
```

```
trees[1:3, ]$Volume # select rows 1 through 3; return column Volume
```

```
## [1] 10.3 10.3 10.2
```

```
trees <- rbind(trees, c(13.25, 76, 30.17)) # add a row
trees_double <- cbind(trees, trees) # combine columns
colnames(trees) <- c("Circumference", "Height", "Volume") # change column names
```

Exercise:

- What is the average height of the cherry trees?
- What is the average girth of those that are more than 75 ft tall?
- What is the maximum height of trees with a volume between 15 and 35 ft^3?

Reading and writing data

In most cases, you will not generate your data in `R`, but import it from a file. By far, the best option is to have your data in a comma separated value text file or in a tab separated file. Then, you can use the function `read.csv` (or `read.table`) to import your data. The syntax of the functions is as follows:

```
read.csv("MyFile.csv") # read the file MyFile.csv
read.csv("MyFile.csv", header = TRUE) # The file has a header.
read.csv("MyFile.csv", sep = ';') # Specify the column separator.
read.csv("MyFile.csv", skip = 5) # Skip the first 5 lines.
```

Note that columns containing strings are typically converted to *factors* (categorical values, useful when performing regressions). To avoid this behavior, you can specify `stringsAsFactors = FALSE` when calling the function.

Similarly, you can save your data frames using `write.table` or `write.csv`. Suppose you want to save the data frame `MyDF`:

```
write.csv(MyDF, "MyFile.csv")
write.csv(MyDF, "MyFile.csv", append = TRUE) # Append to the end of the file.
write.csv(MyDF, "MyFile.csv", row.names = TRUE) # Include the row names.
write.csv(MyDF, "MyFile.csv", col.names = FALSE) # Do not include column names.
```

Let's look at an example: Read a file containing data on the 6th chromosome for a number of Europeans (Data adapted from Stanford HGDP SNP Genotyping Data by John Novembre):

```
ch6 <- read.table("../data/H938_Euro_chr6.geno", header = TRUE)
```

where `header = TRUE` means that we want to take the first line to be a header containing the column names. How big is this table?

```
dim(ch6)
```

```
## [1] 43141     7
```

we have 7 columns, but more than 40k rows! Let's see the first few:

```
head(ch6)
```

```
##   CHR        SNP A1 A2 nA1A1 nA1A2 nA2A2
## 1   6  rs4959515  A  G     0    17   107
## 2   6   rs719065  A  G     0    26    98
## 3   6  rs6596790  C  T     0     4   119
## 4   6  rs6596796  A  G     0    22   102
## 5   6  rs1535053  G  A     5    39    80
## 6   6 rs12660307  C  T     0     3   121
```

and the last few:

```
tail(ch6)
```

```
##       CHR        SNP A1 A2 nA1A1 nA1A2 nA2A2
## 43136   6 rs10946282  C  T     0    16   108
## 43137   6  rs3734763  C  T    19    56    48
## 43138   6   rs960744  T  C    32    60    32
## 43139   6  rs4428484  A  G     1    11   112
## 43140   6  rs7775031  T  C    26    56    42
## 43141   6 rs12213906  C  T     1    11   112
```

The data contains the number of homozygotes (`nA1A1`, `nA2A2`) and heterozygotes (`nA1A2`), for 43,141 single nucleotide polymorphisms (SNPs) obtained by sequencing European individuals:

- `CHR` The chromosome (6 in this case)
- `SNP` The identifier of the Single Nucleotide Polymorphism
- `A1` One of the alleles
- `A2` The other allele
- `nA1A1` The number of individuals with the particular combination of alleles.

Exercise:

- How many individuals were sampled? Find the maximum of the sum `nA1A1 + nA1A2 + nA2A2`. Note: you can access the columns by index (e.g., `ch6[,5]`), or by name (e.g., `ch6$nA1A1`, or also `ch6[,"nA1A1"]`).
- Try using the function `rowSums` to obtain the same result.
- For how many SNPs do we have that all sampled individuals are homozygotes (i.e., all `A1A1` or all `A2A2`)?
- For how many SNPs, are more than 99% of the sampled individuals homozygous?

Conditional branching

Now we turn to writing actual programs in the **Source code** panel. To start a new `R` program, press `Ctrl + Shift + N`. This will open an `Untitled` script. Save the script by pressing `Ctrl + S`: save it as `conditional.R` in the directory `basic_computing_1/code/`. To make sure you're working in the directory where the script is contained, on the menu on the top choose `Session -> Set Working Directory -> To Source File Location`.

Now type the following script:

```
print("Hello world!")
x <- 4
print(x)
```

and execute the script by pressing `Ctrl + Shift + S`. You should see `Hello World!` and `4` printed in your console.

As you saw in this simple example, when `R` executes the program, it starts from the top and proceeds toward the end of the file. Every time it encounters a command (for example, `print(x)`, printing the value of `x` into the console), it executes it.

When we want a certain block of code to be executed only when a certain condition is met, we can write a conditional branching point. The syntax is as follows:

```
if (condition is met){
  # Execute this block of code
} else {
  # Execute this other block of code
}
```

For example, add these lines to the script `conditional.R`, and run it again:

```
print("Hello world!")
x <- 4
print(x)
if (x %% 2 == 0){
  my_message <- paste(x, "is even")
} else {
  my_message <- paste(x, "is odd")
}
print(my_message)
```

We have created a conditional branching point, so that the value of `my_message` changes depending on whether `x` is even (and thus the remainder of the integer division by 2 is 0), or odd. Change the line `x <- 4` to `x <- 131` and run it again.

Exercise: What does this do?

```
x <- 36
if (x > 20){
  x <- sqrt(x)
} else {
  x <- x ^ 2
}
if (x > 7) {
  print(x)
} else if (x %% 2 == 1){
  print(x + 1)
}
```

Looping

Another way to change the flow of the program is to write a loop. A loop is simply a series of commands that are repeated a number of times. For example, you want to run the same analysis on different data sets that you collected; you want to plot the results contained in a set of files; you want to test your simulation over a number of parameter sets; etc.

`R` provides you with two ways to loop over blocks of commands: the `for` loop, and the `while` loop. Let's start with the `for` loop, which is used to iterate over a vector (or a list): for each value of the vector, a series of commands will be run, as shown by the following example, which you can type in a new script called `forloop.R`.

```
myvec <- 1:10 # vector with numbers from 1 to 10

for (i in myvec) {
  a <- i ^ 2
  print(a)
}
```

In the code above, the variable `i` takes the value of each element of `myvec` in sequence. Inside the block defined by the `for` loop, you can use the variable `i` to perform operations.

The anatomy of the `for` statement:

```
for (variable in list_or_vector) {
  execute these commands
} # automatically moves to the next value
```

For loops are used when you know that you want to perform the analysis using a given set of values (e.g., run over all files of a directory, all samples in your data, all sequences of a fasta file, etc.).

The `while` loop is used when the commands need to be repeated while a certain condition is true, as shown by the following example, which you can type in a script called `whileloop.R`:

```
i <- 1

while (i <= 10) {
  a <- i ^ 2
  print(a)
```

```
  i <- i + 1
}
```

The script performs exactly the same operations we wrote for the `for` loop above. Note that you need to update the value of `i`, (using `i <- i + 1`), otherwise the loop will run forever (infinite loop—to terminate click on the stop button in the top-right corner of the console). The anatomy of the `while` statement:

```
while (condition is met) {
  execute these commands
} # beware of infinite loops: remember to update the condition!
```

You can break a loop using the command `break`. For example:

```
i <- 1

while (i <= 10) {
  if (i > 5) {
    break
  }
  a <- i ^ 2
  print(a)
  i <- i + 1
}
```

Exercise: What does this do? Try to guess what each loop does, and then create and run a script to confirm your intuition.

```
z <- seq(1, 1000, by = 3)
for (k in z) {
  if (k %% 4 == 0) {
     print(k)
  }
}
```

```
z <- readline(prompt = "Enter a number: ")
z <- as.numeric(z)
isthisspecial <- TRUE
i <- 2
while (i < z) {
  if (z %% i == 0) {
     isthisspecial <- FALSE
     break
  }
  i <- i + 1
}
if (isthisspecial == TRUE) {
  print(z)
}
```

Useful Functions

We conclude with a list of useful functions that will help you write your programs:

- `range(x)`: minimum and maximum of a vector `x`
- `sort(x)`: sort a vector `x`
- `unique(x)`: remove duplicate entries from vector `x`
- `which(x == a)`: returns a vector of the indices of `x` having value `a`
- `list.files("path_to_directory")`: list the files in a directory (current directory if not specified)
- `table(x)` build a table of frequencies

Exercises: What does this code do? For each snippet of code, first try to guess what will happen. Then, write a script and run it to confirm your intuition.

```
v <- c(1,3,5,5,3,1,2,4,6,4,2)
v <- sort(unique(v))
for (i in v){
  if (i > 2){
    print(i)
  }
  if (i > 4){
    break
  }
}
```

```
x <- 1:100
x <- x[which(x %% 7 == 0)]
```

```
my_files <- sort(list.files("../data/Saavedra2013/", full.names = TRUE))
for (f in my_files){
  M <- read.table(f)
  print(paste("The file", basename(f), "contains a matrix with", nrow(M),
  "rows and ", ncol(M), "columns. There are", sum(M == 1),
  "coefficients that are 1 and", sum(M == 0), "that are 0."))
}
```

```
my_amount <- 10
while (my_amount > 0){
  my_color <- NA
  while(is.na(my_color)){
    tmp <- readline(prompt="Do you want to bet on black or red? ")
    tmp <- tolower(tmp)
    if (tmp == "black") my_color <- "black"
    if (tmp == "red") my_color <- "red"
    if (is.na(my_color)) print("Please enter either red or black")
  }
  my_bet <- NA
  while(is.na(my_bet)){
    tmp <- readline(prompt="How much do you want to bet? ")
    tmp <- as.numeric(tmp)
    if (is.numeric(tmp) == FALSE){
      print("Please enter a number")
    } else {
      if (tmp > my_amount){
        print("You don't have enough money!")
      } else {
        my_bet <- tmp
```

```
        my_amount <- my_amount - tmp
      }
    }
  }
  lady_luck <- sample(c("red", "black"), 1)
  if (lady_luck == my_color){
    my_amount <- my_amount + 2 * my_bet
    print(paste("You won!! Now you have", my_amount, "gold dobloons"))
  } else {
    print(paste("You lost!! Now you have", my_amount, "gold dobloons"))
  }
}
print("I told you this was not a good idea...")
```

Exercises in groups

Nobel nominations

The file `../data/nobel_nominations.csv` contains the nominations to the Nobel prize from 1901 to 1964. There are three columns (the file has no header): a) the field (e.g. `Phy` for physics), b) the year of the nomination, c) the id and name of the nominee.

- Take your favorite field. Who received most nominations?
- Who received nominations in more than one field?
- Take the field of physics. Which year had the largest number of nominees?
- What is the average number of nominees for each field? Calculate the average number of nominee for each field across years.

Hints

- You will need to subset the data. To make operations clearer, you can give names to the columns. For example, suppose you stored the data in in a data frame called `nobel`. Then `colnames(nobel) <- c("Field", "Year", "Nominee")` will do the trick.
- The simplest way to obtain a count from a vector is to use the command `table`. The command `sort(table(my_vector))` produces a table of the occurrences in `my_vector` sorted from few to many counts.
- You can build a table using more than one vector. For example, store the Nobel nominations in the data frame `nobel`, and name the columns as suggested above. The command `head(table(nobel$Nominee, nobel$Field))` will build a table with the number of nominations in each field (column) for each nominee (rows).

Basic Computing 2 – Packages, Functions, Documenting code

Stefano Allesina

Basic Computing 2

- **Goal:** Show how to install, load and use the many freely available `R` packages. Illustrate how to write user-defined functions and how to organize code. Showcase basic plotting functions. Introduce the package `knitr` for writing beautiful reports.
- **Audience:** Biologists with basic knowledge of `R`.
- **Installation:** To produce well-documented code, we need to instal the package `knitr`. We will also use the package `MASS` for statistics.

Packages

`R` is the most popular statistical computing software among biologists due to its highly specialized packages, often written from biologists for biologists. You can contribute a package too! The `RStudio` support (`goo.gl/harVqF`) provides guidance on how to start developing `R` packages and Hadley Wickham's free online book (`r-pkgs.had.co.nz`) will make you a pro.

You can find highly specialized packages to address your research questions. Here are some suggestions for finding an appropriate package. The Comprehensive R Archive Network (CRAN) offers several ways to find specific packages for your task. You can either browse packages (`goo.gl/7oVyKC`) and their short description or select a scientific field of interest (`goo.gl/OWdIcu`) to browse through a compilation of packages related to each discipline.

From within your `R` terminal or `RStudio` you can also call the function `RSiteSearch("KEYWORD")`, which submits a search query to the website `search.r-project.org`. The website `rseek.org` casts an even wider net, as it not only includes package names and their documentation but also blogs and mailing lists related to `R`. If your research interests relate to high-throughput genomic data, you should have a look the packages provided by Bioconductor (`goo.gl/7dwQlq`).

Installing a package

To install a package type

```
install.packages("name_of_package")
```

in the Console, or choose the panel **Packages** and then click on *Install* in `RStudio`.

Loading a package

To load a package type

```
library(name_of_package)
```

or call the command into your script. If you want your script to automatically install a package in case it's missing, use this boilerplate:

```
if (!require(needed_package, character.only = TRUE, quietly = TRUE)) {
    install.packages(needed_package)
    library(needed_package, character.only = TRUE)
}
```

Example

For example, say we want to access the dataset `bacteria`, which reports the incidence of *H. influenzae* in Australian children. The dataset is contained in the package `MASS`.

First, we need to load the package:

```
library(MASS)
```

Now we can load the data:

```
data(bacteria)
bacteria[1:3,]
```

```
##   y ap hilo week  ID     trt
## 1 y  p   hi    0 X01 placebo
## 2 y  p   hi    2 X01 placebo
## 3 y  p   hi    4 X01 placebo
```

Do shorter titles lead to more citations?

To keep learning about `R`, we study a simple problem: do papers with shorter titles have more citations? This is what claimed by Letchford *et al.*, who in 2015 analyzed 140,000 papers (dx.doi.org/10.1098/rsos.150266) finding that shorter titles correlated with a larger number of citations.

In the `data/citations` folder, you find information on all the articles published between 2004 and 2013 by three top disciplinary journals (*Nature Neuroscience*, *Nature Genetics*, and *Ecology Letters*), which we are going to use to explore the robustness of these findings.

We start by reading the data in. This is a simple `csv` file, so that we can use

```
papers <- read.csv("../data/citations/nature_neuroscience.csv")
```

to load the data. However, running `str(papers)` shows that all the columns containing text have been automatically converted to `Factor` (categorical values, which is good when performing regressions). Because we want to manipulate these columns (for example, count how many characters are in a title), we want to avoid this automatic behavior. We can accomplish this by calling the function `read.csv` with an extra argument:

```
papers <- read.csv("../data/citations/nature_neuroscience.csv", stringsAsFactors = FALSE)
```

Next, we want to take a peek at the data. How large is it?

```
dim(papers)
```

```
## [1] 2000    7
```

Let's see the first few rows:

```
head(papers, 3)
```

```
##            Authors                                    Title Year
## 1 Logothetis, N.K.                Francis crick 1916-2004. 2004
## 2       Narain, C. Object-specific unconscious processing. 2005
## 3       Narain, C.                       Going down BOLDly. 2006
##           Source.title Page.start Page.end Cited.by
## 1  Nature neuroscience       1027     1028        0
## 2 Nature neuroscience.       1288       NA        0
## 3  Nature neuroscience        474       NA        0
```

Now, we want to test whether papers with longer titles do accrue fewer (or more) citations than those with shorter titles. The first step is therefore to add another column to the data, containing the length of the title for each paper:

```
papers$TitleLength <- nchar(papers$Title)
```

Basic statistics in R

In the original paper, Letchford *et al.* used rank-correlation: rank all the papers according to their title length and the number of citations. If the Kendall's Tau (rank correlation) is positive, then longer titles are associated with more citations; if Tau is negative, longer titles are associated with fewer citations. In R you can compute rank correlation using:

```
kendall_cor <- cor(papers$TitleLength, papers$Cited.by, method = "kendall")
kendall_cor
```

```
## [1] 0.04528715
```

To perform a significance test, use

```
cor.test(papers$TitleLength, papers$Cited.by, method = "kendall")
```

```
##
##  Kendall's rank correlation tau
##
## data:  papers$TitleLength and papers$Cited.by
## z = 3.0023, p-value = 0.002679
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##        tau
## 0.04528715
```

showing that the correlation between the ranks is positive and significant. We have found the opposite effect than Letchford *et al.*—longer titles are associated with **more** citations!

Now we are going to examine the data in a different way, to test whether these results are robust.

Basic plotting in R

To plot the title length vs. number of citations, we need to learn about plotting in R. To produce a simple scatterplot using the base functions for plotting, simply type:

```
plot(papers$TitleLength, papers$Cited.by)
```

It is hard to detect any trend in this plot, as there are a few papers with many more citations than the rest. We can transform the data by plotting on the y-axis the log10 of citations + 1 (so that papers with zero citations do not cause errors):

```
plot(papers$TitleLength, log10(papers$Cited.by + 1))
```

Again, it's hard to see any trend in here. Maybe we should plot the best fitting line and overlay it on top of the graph. To do so, we first need to learn about regressions in R.

Regressions in R

R was born for statistics — the fact that it's very easy to fit a linear regression is not surprising! To build a linear model, simply write

```
# model y = a + bx + error
my_model <- lm(y ~ x)
```

Because it's more convenient to call the code in this way, let's add a new column to the data frame with the log of citations:

```
papers$LogCits <- log10(papers$Cited.by + 1)
```

And perform a linear regression:

```
model_cits <- lm(papers$LogCits ~ papers$TitleLength)
# This is the best fitting line
model_cits
```

```
##
## Call:
## lm(formula = papers$LogCits ~ papers$TitleLength)
##
## Coefficients:
##        (Intercept)  papers$TitleLength
##           1.358195            0.006193
```

```
# This is a summary of all the statistics
summary(model_cits)
```

```
##
## Call:
## lm(formula = papers$LogCits ~ papers$TitleLength)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.68022 -0.25261  0.02803  0.29188  1.82838
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.3581953  0.0445997   30.45   <2e-16 ***
## papers$TitleLength 0.0061927  0.0005339   11.60   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4522 on 1998 degrees of freedom
## Multiple R-squared:  0.06309,    Adjusted R-squared:  0.06263
## F-statistic: 134.6 on 1 and 1998 DF,  p-value: < 2.2e-16
```

And plotting

```
# plot the points
plot(papers$TitleLength, log10(papers$Cited.by + 1))
# add the best fitting line
abline(model_cits, col = "red")
```

Again, we find a positive trend. One thing to consider, is that in the database we have papers spanning a decade. Naturally, older papers have had more time to accrue citations. In our models, we would like to control for this effect. First, let's plot the distribution of citations for a few years. To produce an histogram in R, use

```
hist(papers$LogCits)
# increase the number of breaks
hist(papers$LogCits, breaks = 15)
```

Alternatively, estimate the density using

```
plot(density(papers$LogCits))
```

Let's plot the density for years 2004, 2009, 2013:

```
# plot the density for the older papers:
plot(density(papers$LogCits[papers$Year == 2004]))
lines(density(papers$LogCits[papers$Year == 2009]), col = "red")
lines(density(papers$LogCits[papers$Year == 2013]), col = "blue")
```

As expected, younger papers have fewer citations. We can account for this fact in our regression model:

```
model_year_length <- lm(papers$LogCits ~ as.factor(papers$Year) + papers$TitleLength)
summary(model_year_length)
```

```
##
## Call:
## lm(formula = papers$LogCits ~ as.factor(papers$Year) + papers$TitleLength)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.88880 -0.21178  0.01232  0.25186  1.51362
##
## Coefficients:
##                               Estimate Std. Error t value Pr(>|t|)
## (Intercept)                  1.6953101  0.0499837  33.917  < 2e-16 ***
## as.factor(papers$Year)2005 -0.0277435  0.0424764  -0.653 0.513735
## as.factor(papers$Year)2006 -0.1137399  0.0431189  -2.638 0.008409 **
## as.factor(papers$Year)2007 -0.1603601  0.0435023  -3.686 0.000234 ***
## as.factor(papers$Year)2008 -0.1630806  0.0442859  -3.682 0.000237 ***
## as.factor(papers$Year)2009 -0.2373747  0.0430316  -5.516 3.91e-08 ***
## as.factor(papers$Year)2010 -0.2824792  0.0433906  -6.510 9.48e-11 ***
## as.factor(papers$Year)2011 -0.4927523  0.0425956 -11.568  < 2e-16 ***
## as.factor(papers$Year)2012 -0.6278381  0.0423156 -14.837  < 2e-16 ***
## as.factor(papers$Year)2013 -0.6539580  0.0419966 -15.572  < 2e-16 ***
## papers$TitleLength          0.0056728  0.0004645  12.213  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3912 on 1989 degrees of freedom
## Multiple R-squared:  0.302,  Adjusted R-squared:  0.2985
## F-statistic: 86.06 on 10 and 1989 DF,  p-value: < 2.2e-16
```

Using `as.factor(papers$Year)` we have fitted a model in which each year has a different baseline, and the title length influences this baseline. Again, we find that longer titles are associated with more citations.

Random numbers

As a reminder, the Kendall's τ takes as input two rankings x and y, both of length n. It calculates the number of "concordant pairs", in which if $x_i > x_j$ then $y_i > y_j$ and "discordant pairs". Then,

$$\tau = \frac{\text{num. concordant} - \text{num. discordant}}{\frac{n(n-1)}{2}}$$

If x and y were completely independent, we would expect τ to be distributed with a mean of 0. The variance of the null distribution of τ (and hence the p-value calculated above) depends on the data, and is typically approximated as a normal distribution. If you want to have a stronger result, you can use randomizations to approximate the p-value. Simply, compute τ for the actual data, and for many "fake" datasets obtained randomizing the data. Your p-value is well approximated by the proportion of τ values for the randomized sets that exceed the τ value for the actual data.

To perform this randomization, or any simulation, we typically need to draw random numbers. `R` has functions to sample random numbers from very many different statistical distributions. For example:

```r
runif(5) # sample 5 numbers from the uniform distribution between 0 and 1
```

```
## [1] 0.6003503 0.7980935 0.4715618 0.4650129 0.8497015
```

```r
runif(5, min = 1, max = 9) # set the limits of the uniform distribution
```

```
## [1] 3.804503 5.591121 5.969146 8.640388 5.850124
```

```r
rnorm(3) # three values from standard normal
```

```
## [1]  0.16605088  0.00876875 -1.12075603
```

```r
rnorm(3, mean = 5, sd = 4) # specify mean and standard deviation
```

```
## [1]  6.295445 10.881698  2.360098
```

To sample from a set of values, use `sample`:

```r
v <- c("a", "b", "c", "d")
sample(v, 2) # without replacement
```

```
## [1] "c" "d"
```

```r
sample(v, 6, replace = TRUE) # with replacement
```

```
## [1] "a" "b" "c" "c" "c" "b"
```

```r
sample(v) # simply shuffle the elements
```

```
## [1] "a" "b" "d" "c"
```

Let's try to write a randomization to calculate p-value associated with the τ observed for year 2006.

```r
# first, we subset the data
papers_year <- papers[papers$Year == 2006, ] # get all rows matching the year
# compute original tau
tau_original <- cor(papers_year$TitleLength, papers_year$Cited.by, method = "kendall")
tau_original
```

```
## [1] 0.1140872
```

Now we want to calculate it on the "fake" data sets. To have confidence in the first two decimal digits, we should perform about ten thousand randomizations. This and similar randomization techniques are known as "bootstrapping".

```
num_randomizations <- 10000
pvalue <- 0 # initialize at 0
for (i in 1:num_randomizations){
  # calculate cor on shuffled data
  tau_shuffle <- cor(papers_year$TitleLength,
                     sample(papers_year$Cited.by), # scramble the citations at random
                     method = "kendall")
  if (tau_shuffle >= tau_original){
    pvalue <- pvalue + 1
  }
}
# calculate proportion
pvalue <- pvalue / num_randomizations
pvalue
```

```
## [1] 0.0074
```

Note that the p-value is different (and in fact smaller) than that calculated using the normal approximation:

```
cor.test(papers_year$TitleLength, papers_year$Cited.by, method = "kendall")
```

```
##
##  Kendall's rank correlation tau
##
## data:  papers_year$TitleLength and papers_year$Cited.by
## z = 2.3902, p-value = 0.01684
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##       tau
## 0.1140872
```

Whenever possible, use randomizations, rather than relying on classical tests. They are more computationally expensive, but they allow you to avoid making assumptions about your data.

Writing functions

We have written code that analyzes one year of data. If we wanted to repeat the analysis on a different year, we would have to modify the code slightly. Instead of doing that, we can write a function that allows us to select a given year, and randomize the data. To do so, we need to learn about functions.

The R community provides about 7,000 packages. Still, sometimes there isn't an already made function capable of doing what you need. In these cases, you can write your own functions. In fact, it is in general a good idea to always divide your analysis into functions, and then write a small "master" program that calls the functions and performs the analysis. In this way, the code will be much more legible, and you will be able to recycle the functions for your other projects.

A function in R has this form:

```
my_function_name <- function(arguments of the function){
  # Body of the function
  # ...
  #
  return(return_value) # this is optional
}
```

A few examples:

```
sum_two_numbers <- function(a, b){
  apb <- a + b
  return(apb)
}
sum_two_numbers(5, 7.2)
```

```
## [1] 12.2
```

You can set a default value for some of the arguments: if not specified by the user, the function will use these defaults:

```
sum_two_numbers <- function(a = 1, b = 2){
  apb <- a + b
  return(apb)
}
sum_two_numbers()
```

```
## [1] 3
```

```
sum_two_numbers(3)
```

```
## [1] 5
```

```
sum_two_numbers(b = 9)
```

```
## [1] 10
```

The return value is optional:

```
my_factorial <- function(a = 6){
  if (as.integer(a) != a) {
    print("Please enter an integer!")
  } else {
    tmp <- 1
    for (i in 2:a){
      tmp <- tmp * i
    }
    print(paste(a, "! = ", tmp, sep = ""))
  }
}
my_factorial()
```

```
## [1] "6! = 720"
```

```
my_factorial(10)
```

```
## [1] "10! = 3628800"
```

You can return **only one** object. If you need to return multiple values, organize them into a vector/matrix/list and return that.

```r
order_two_numbers <- function(a, b){
  if (a > b) return(c(a, b))
  return(c(b,a))
}
order_two_numbers(runif(1), runif(1))
```

```
## [1] 0.3678014 0.2407045
```

Having learned a little about functions, we want to write one that takes as input a vector of citations, a vector of title lengths, and a number of randomizations to perform. The function returns the value of τ as well as the associated p-value. In R, we can write:

```r
tau_citations_titlelength <- function(citations, titlelength, num_randomizations = 1000){
  tau_original <- cor(titlelength, citations, method = "kendall")
  pvalue <- 0 # initialize at 0
  for (i in 1:num_randomizations){
    # calculate cor on shuffled data
    tau_shuffle <- cor(titlelength,
                       sample(citations), # scramble the citations at random
                       method = "kendall")
    if (tau_shuffle >= tau_original){
      pvalue <- pvalue + 1
    }
  }
  # calculate proportion
  pvalue <- pvalue / num_randomizations
  # return a list
  return(list(tau = tau_original,
              pvalue = pvalue))
}
```

We can write a loop that calls in turn the function for each year separately:

```r
all_years <- sort(unique(papers$Year))
for (my_year in all_years){
  tmp <- tau_citations_titlelength(papers$Cited.by[papers$Year == my_year],
                                   papers$TitleLength[papers$Year == my_year],
                                   1000)
  print(paste(my_year, "-> Tau:", round(tmp$tau, 3), "pvalue:", tmp$pvalue))
}
```

```
## [1] "2004 -> Tau: 0.006 pvalue: 0.467"
## [1] "2005 -> Tau: 0.054 pvalue: 0.133"
## [1] "2006 -> Tau: 0.114 pvalue: 0.009"
## [1] "2007 -> Tau: 0.01 pvalue: 0.437"
## [1] "2008 -> Tau: 0.065 pvalue: 0.101"
## [1] "2009 -> Tau: 0.012 pvalue: 0.38"
## [1] "2010 -> Tau: -0.052 pvalue: 0.873"
## [1] "2011 -> Tau: 0.145 pvalue: 0"
## [1] "2012 -> Tau: 0.114 pvalue: 0.005"
## [1] "2013 -> Tau: 0.045 pvalue: 0.188"
```

Organizing and running code

Now we would like to be able to automate the analysis, such that we can repeat it for each journal. This is a good place to pause and introduce how to go about writing programs that are well-organized, easy to write, and easy to debug.

1. Take the problem, and divide it into its basic building blocks
2. Write the code for each building block separately, and test it thoroughly.
3. Extensively document the code, so that you can understand what you did, how you did it, and why.
4. Combine the building blocks into a master program.

For example, let's say we want to write a program that takes as input the name of a file containing the data on titles, years and citations for a given journal. The program should first run the linear model:

```
log(citations + 1) ~ Year (categorical) + TitleLength
```

And output the coefficient associated with `TitleLength` as well as its p-value.

Then, the program should run the Kendall's test for each year separately, again outputting τ and the p-value obtained with the normal approximation for each year.

Dividing it into blocks, we need to wite:

- code to load the data, calculate title lengths and log citations
- a function to perform the linear model
- a function to perform the Kendall's test
- a master code putting it all together

Our first function:

```
load_data <- function(filename){
  papers <- read.csv(filename, stringsAsFactors = FALSE)
  papers$TitleLength <- nchar(papers$Title)
  papers$LogCits <- log10(papers$Cited.by + 1)
  return(papers)
}
```

Make sure that everything is well by testing our function on the data:

```
for (my_file in list.files("../data/citations", full.names = TRUE)){
  print(my_file)
  print(basename(my_file))
  papers <- load_data(my_file)
}
```

```
## [1] "../data/citations/ecology_letters.csv"
## [1] "ecology_letters.csv"
## [1] "../data/citations/nature_genetics.csv"
## [1] "nature_genetics.csv"
## [1] "../data/citations/nature_neuroscience.csv"
## [1] "nature_neuroscience.csv"
```

Now the function to fit the linear model:

```
linear_model_year_length <- function(papers){
  my_model <- summary(lm(LogCits ~ as.factor(Year) + TitleLength, data = papers))
  # Extract the coefficient and the pvalue
  estimate <- my_model$coefficients["TitleLength","Estimate"]
  pvalue <- my_model$coefficients["TitleLength","Pr(>|t|)"]
  return(list(estimate = estimate,
              pvalue = pvalue))
}
```

Let's run this on all files:

```
for (my_file in list.files("../data/citations", full.names = TRUE)){
  print(basename(my_file))
  papers <- load_data(my_file)
  linear_model <- linear_model_year_length(papers)
  print(paste("Linear model -> coefficient",
        round(linear_model$estimate, 5),
        "pvalue", round(linear_model$pvalue, 5)))
}
```

```
## [1] "ecology_letters.csv"
## [1] "Linear model -> coefficient -3e-04 pvalue 0.45814"
## [1] "nature_genetics.csv"
## [1] "Linear model -> coefficient 0.00276 pvalue 0"
## [1] "nature_neuroscience.csv"
## [1] "Linear model -> coefficient 0.00567 pvalue 0"
```

Now the function that calls the Kendall's test for each year: we write two functions. One subsets the data, and the other simply runs the test.

```
Kendall_test <- function(a, b){
  my_test <- cor.test(a, b, method = "kendall")
  return(list(estimate = as.numeric(my_test$estimate),
              pvalue = my_test$p.value))
}

call_Kendall_by_year <- function(papers){
  all_years <- sort(unique(papers$Year))
  for (yr in all_years){
    my_test <- Kendall_test(papers$TitleLength[papers$Year == yr],
                 papers$Cited.by[papers$Year == yr])
    print(paste("Year", yr, "-> estimate", my_test$estimate, "pvalue", my_test$pvalue))
  }
}
```

Now a master function to test that the program is working:

```
analyze_journal <- function(my_file){
  # First, the linear model
  print(basename(my_file))
  papers <- load_data(my_file)
  linear_model <- linear_model_year_length(papers)
```

```
  print(paste("Linear model -> coefficient",
        round(linear_model$estimate, 5),
        "pvalue", round(linear_model$pvalue, 5)))
  # Then, Kendall year by year
  call_Kendall_by_year(papers)
}
analyze_journal("../data/citations/nature_genetics.csv")
```

```
## [1] "nature_genetics.csv"
## [1] "Linear model -> coefficient 0.00276 pvalue 0"
## [1] "Year 2004 -> estimate 0.045610559310653 pvalue 0.370327813824674"
## [1] "Year 2005 -> estimate -0.0550279205871904 pvalue 0.267581272430449"
## [1] "Year 2006 -> estimate 0.0596670158288517 pvalue 0.213567208478809"
## [1] "Year 2007 -> estimate -0.0390688441353209 pvalue 0.418061571895653"
## [1] "Year 2008 -> estimate 0.0674234973583218 pvalue 0.149431264169447"
## [1] "Year 2009 -> estimate 0.0297023932900025 pvalue 0.524960049608212"
## [1] "Year 2010 -> estimate -0.158944978390644 pvalue 0.00176578355758891"
## [1] "Year 2011 -> estimate 0.130352296718688 pvalue 0.00961339158713348"
## [1] "Year 2012 -> estimate 0.168763320248576 pvalue 0.000111079233389155"
## [1] "Year 2013 -> estimate 0.0792087074318355 pvalue 0.0880890674881281"
```

Finally, let's analyze all the journals!

```
for (my_file in list.files("../data/citations", full.names = TRUE)){
  analyze_journal(my_file)
}
```

```
## [1] "ecology_letters.csv"
## [1] "Linear model -> coefficient -3e-04 pvalue 0.45814"
## [1] "Year 2004 -> estimate -0.0330650126212166 pvalue 0.613150449110409"
## [1] "Year 2005 -> estimate 0.027544573550034 pvalue 0.671742234063287"
## [1] "Year 2006 -> estimate -0.0639728739951933 pvalue 0.334992423839896"
## [1] "Year 2007 -> estimate 0.130805775658087 pvalue 0.0792684296315682"
## [1] "Year 2008 -> estimate -0.0946065886996697 pvalue 0.185851799603582"
## [1] "Year 2009 -> estimate -0.00528034747952401 pvalue 0.932130040345834"
## [1] "Year 2010 -> estimate 0.0478717663229855 pvalue 0.429380872167305"
## [1] "Year 2011 -> estimate -0.103776346604215 pvalue 0.0989632669799205"
## [1] "Year 2012 -> estimate 0.0708774786316527 pvalue 0.205434298051716"
## [1] "Year 2013 -> estimate -0.0532355687009939 pvalue 0.326025326942473"
## [1] "nature_genetics.csv"
## [1] "Linear model -> coefficient 0.00276 pvalue 0"
## [1] "Year 2004 -> estimate 0.045610559310653 pvalue 0.370327813824674"
## [1] "Year 2005 -> estimate -0.0550279205871904 pvalue 0.267581272430449"
## [1] "Year 2006 -> estimate 0.0596670158288517 pvalue 0.213567208478809"
## [1] "Year 2007 -> estimate -0.0390688441353209 pvalue 0.418061571895653"
## [1] "Year 2008 -> estimate 0.0674234973583218 pvalue 0.149431264169447"
## [1] "Year 2009 -> estimate 0.0297023932900025 pvalue 0.524960049608212"
## [1] "Year 2010 -> estimate -0.158944978390644 pvalue 0.00176578355758891"
## [1] "Year 2011 -> estimate 0.130352296718688 pvalue 0.00961339158713348"
## [1] "Year 2012 -> estimate 0.168763320248576 pvalue 0.000111079233389155"
## [1] "Year 2013 -> estimate 0.0792087074318355 pvalue 0.0880890674881281"
## [1] "nature_neuroscience.csv"
```

```
## [1] "Linear model -> coefficient 0.00567 pvalue 0"
## [1] "Year 2004 -> estimate 0.00589142291037872 pvalue 0.918745470907139"
## [1] "Year 2005 -> estimate 0.0535867457573801 pvalue 0.243552261640925"
## [1] "Year 2006 -> estimate 0.114087173434659 pvalue 0.0168412989934459"
## [1] "Year 2007 -> estimate 0.00966171293776095 pvalue 0.843003041215137"
## [1] "Year 2008 -> estimate 0.0652257952013621 pvalue 0.200992557539214"
## [1] "Year 2009 -> estimate 0.012124492386758 pvalue 0.79906576984121"
## [1] "Year 2010 -> estimate -0.0518768931100408 pvalue 0.28616709714268"
## [1] "Year 2011 -> estimate 0.145346619264126 pvalue 0.00174156563082106"
## [1] "Year 2012 -> estimate 0.114380879075143 pvalue 0.0125050217924556"
## [1] "Year 2013 -> estimate 0.0446967410995934 pvalue 0.318724166924561"
```

Discussion: How many significant results we should expect, when citations and title lengths are completely independent?

Documenting the code using `knitr`

> *Let us change our traditional attitude to the construction of programs: Instead of imagining that our main task is to instruct a computer what to do, let us concentrate rather on explaining to humans what we want the computer to do.*
>
> Donald E. Knuth, Literate Programming, 1984

When doing experiments, we typically keep track of everything we do in a laboratory notebook, so that when writing the manuscript, or responding to queries, we can go back to our documentation to find exactly what we did, how we did it, and possibly why we did it. The same should be true for computational work.

`RStudio` makes it very easy to build a computational laboratory notebook. First, create a new `R Markdown` file (choose `File -> New File -> R Markdown` from the menu).

The gist of it is that you write a text file (`.Rmd`). The file is then read by an iterpreter that transforms it into an `.html` or `.pdf` file, or even into a Word document. You can use special syntax to render the text in different ways. For example,

```
***********

*Test* **Test2**

# Very large header

## Large header

### Smaller header

## Unordered lists

* First
* Second
    + Second 1
    + Second 2

1. This is
```

```
2. A numbered list

You can insert `inline code`

-----------
```

The code above yields:

Test **Test2**

Very large header

Large header

Smaller header

Unordered lists

- First
- Second
  - Second 1
  - Second 2

1. This is
2. A numbered list

You can insert `inline code`

The most important feature of `R Markdown`, however, is that you can include blocks of code, and they will be interpreted and executed by `R`. You can therefore combine effectively the code itself with the description of what you are doing.

For example, including

```
```r
print("hello world!")
```
```

will become

```r
print("hello world!")
```

```
## [1] "hello world!"
```

If you don't want to run the `R` code, but just display it, use `{r, eval = FALSE}`; if you want to show the output but not the code, use `{r, echo = FALSE}`.

You can include plots, tables, and even render equations using LaTeX. In summary, when exploring your data or writing the methods of your paper, give `R Markdown` a try!

You can find inspiration in the notes for the Boot Camp: both the notes for Basic and Advanced Computing are written in `R MarkDown`.

Exercises in groups

Google Flu Trends

Preis & Moat (2014, `dx.doi.org/10.1098/rsos.140095`) showed that Google Flu Trends are correlated with outpatient visits due to influenza-like illness. You can find the data, along with its description, in `data/GoogleFlu`.

- Read the data
- Plot number of visits vs. `GoogleFluTrends`
- Calculate the correlation using `cor`
- The data spans 2010-2013. In Aug 2013 Google Flu changed their algorithm. Did this lead to improvements? Compare the data from Aug to Dec 2013 with the same months in 2010, 2011, and 2012. For each, calculate the correlation, and see whether the correlation is higher for 2013.

Hints You will need to extract the year from a string for each row. To do so, you can use `substr(google$WeekCommencing, 1,4)`.

Advanced Computing 1 – Data wrangling and visualization

Stefano Allesina

Data Wrangling and Visualization

- **Goal:** learn how to manipulate large data sets by writing efficient, consistent, and compact code. Introduce the use of `dplyr`, `tidyr`, and the "pipeline" operator `%>%`. Produce beautiful graphs and figures for scientific publications using `ggplot2`.
- **Audience:** experienced `R` users, familiar with the data type `data.frame`, loops, functions, and having some notions of data bases.
- **Installation:** the following packages need to be installed: `ggplot2`, `dplyr`, `tidyr`, `lubridate`, `ggthemes`

Data wrangling

As biologists living in the XXI century, we are often faced with tons of data, possibly replicated over several organisms, treatments, or locations. We would like to streamline and automate our analysis as much as possible, writing scripts that are easy to read, fast to run, and easy to debug. Base `R` can get the job done, but often the code contains complicated operations (think of the cases in which you used `lapply` only because of its speed), and a lot of `$` signs and brackets.

We're going to learn about the packages `dplyr` and `tidyr`, which can be used to manipulate large data frames in a simple and straightforward way. These tools are also much faster than the corresponding base `R` commands, are very compact, and can be concatenated into "pipelines".

To start, we need to import the libraries:

```
library(dplyr)
library(tidyr)
```

Then, we need a dataset to play with. We take a dataset containing all the Divvy bikes trips in Chicago in July 2014:

```
divvy <- read.csv("../data/Divvy_Trips_July_2014.csv")
```

A new data type, `tbl`

This is now a data frame:

```
is.data.frame(divvy)
```

`dplyr` ships with a new data type, called a `tbl`. To convert from data frame, use

```
divvy <- tbl_df(divvy)
divvy
```

The nice feature of `tbl` objects is that they will print only what fits on the screen, and also give you useful information on the size of the data, as well as the type of data in each column. Other than that, a `tbl` object behaves very much like a `data.frame`. In some rare cases, you want to transform the `tbl` back into a `data.frame`. For this, use the function `as.data.frame(tbl_object)`.

We can take a look at the data using one of several functions:

- `head(divvy)` shows the first few (10 by default) rows
- `tail(divvy)` shows the last few (10 by default) rows
- `glimpse(divvy)` a summary of the data (similar to `str` in base R)
- `View(divvy)` open in spreadsheet-like window

Selecting rows and columns

There are many ways to subset the data, either by row (subsetting the *observations*), or by column (subsetting the *variables*). For example, suppose we want to count how many trips (of the $>$ 410k) are very short. The column `tripduration` contains the length of the trip in seconds. Let's select only the trips that lasted less than 3 minutes:

```
filter(divvy, tripduration < 180)
```

You can see that "only" 11,099 trips lasted less than three minutes. We have used the command `filter(tbl, conditions)` to select certain observations. We can combine several conditions, by listing them side by side, possibly using logical operators.

> **Exercise:** what does this do?
>
> ```
> filter(divvy, gender == "Male", tripduration > 60, tripduration < 180)
> ```

We can also select particular variables using the function `select(tbl, cols to select)`. For example, select `from_station_name` and `from_station_id`:

```
select(divvy, from_station_name, from_station_id)
```

How many stations are represented in the data set? We can use the function `distinct(tbl)` to retain only the rows that differ from each other:

```
distinct(select(divvy, from_station_name, from_station_id))
```

Showing that there are 300 stations, once we removed the duplicates.

Other ways to subset observations:

- `sample_n(tbl, howmany, replace = TRUE)` sample `howmany` rows at random with replacement
- `sample_frac(tbl, proportion, replace = FALSE)` sample a certain proportion (e.g. `0.2` for 20%) of rows at random without replacement

- `slice(tbl, 50:100)` extract the rows between `50` and `100`
- `top_n(tbl, 10, tripduration)` extract the first `10` rows, once ordered by `tripduration`

More ways to select columns:

- `select(divvy, contains("station"))` select all columns containing the word `station`
- `select(divvy, -gender, -tripduration)` exclude the columns `gender` and `tripduration`
- `select(divvy, matches("year|time"))` select all columns whose names match a regular expression

Creating pipelines using %>%

We've been calling nested functions, such as `distinct(select(divvy, ...))`. If you have to add another layer or two, the code would become unreadable. `dplyr` allows you to "un-nest" these functions and create a "pipeline", in which you concatenate commands separated by the special operator `%>%`. For example:

```
divvy %>% # take a data table
  select(from_station_name, from_station_id) %>% # select two columns
  distinct() # remove duplicates
```

does exactly the same as the command above, but is much more readable. By concatenating many commands, you can create incredibly complex pipelines while retaining readability.

Producing summaries

Sometimes we need to calculate statistics on certain columns. For example, calculate the average trip duration. We can do this using `summarise`:

```
divvy %>% summarise(avg = mean(tripduration))
```

which returns a `tbl` object with just the average trip duration. You can combine multiple statistics (use `first`, `last`, `min`, `max`, `n` [count the number of rows], `n_distinct` [count the number of distinct rows], `mean`, `median`, `var`, `sd`, etc.):

```
divvy %>% summarise(avg = mean(tripduration),
                    sd = sd(tripduration),
                    median = median(tripduration))
```

Summaries by group

One of the most useful features of `dplyr` is the ability to produce statistics for the data once subsetted by *groups*. For example, we would like to measure whether men take longer trips than women. We can then group the data by `gender`, and calculate the mean `tripduration` once the data is split into groups:

```
divvy %>% group_by(gender) %>% summarise(mean = mean(tripduration))
```

showing that women tend to take longer trips than men.

> **Exercise:** count the number of trips for Male, Female, and unspecified gender.

Ordering the data

To order the data according to one or more variables, use `arrange()`:

```
divvy %>% select(trip_id, tripduration) %>% arrange(tripduration)
divvy %>% select(trip_id, tripduration) %>% arrange(desc(tripduration))
```

Renaming columns

To rename one or more columns, use `rename()`:

```
divvy %>% rename(tt = tripduration)
```

Adding new variables using mutate

If you want to add one or more new columns, use the function `mutate`:

```
divvy %>% select(from_station_id, to_station_id) %>%
  mutate(mylink = paste0(from_station_id, "->", to_station_id))
```

use the function `transmute()` to create a new column and drop the original columns. You can also use `mutate` and `transmute` on grouped data:

```
# A more complex pipeline
divvy %>%
  select(trip_id, gender, tripduration) %>%  # select only three columns
  rename(t = tripduration) %>% # rename a column
  group_by(gender) %>% # create a group for each gender value
  mutate(zscore = (t - mean(t) ) / sd(t)) %>% # compute z-score for t, according to gender
  ungroup() %>% # remove group information
  arrange(desc(t), zscore, gender) %>% # order by t (decreasing), zscore, and gender
  head(20) # display first 20 rows
```

Data visualization

The most salient feature of scientific graphs should be clarity. Each figure should make crystal-clear a) what is being plotted; b) what are the axes; c) what do colors, shapes, and sizes represent; d) the message the figure wants to convey. Each figure is accompanied by a (sometimes long) caption, where the details can be explained further, but the main message should be clear from glancing at the figure (often, figures are the first thing editors and referees look at).

Many scientific publications contain very poor graphics: labels are missing, scales are unintelligible, there is no explanation of some graphical elements. Moreover, some color graphs are impossible to understand if printed in black and white, or difficult to discern for color-blind people.

Given the effort that you put in your science, you want to ensure that it is well presented and accessible. The investment to master some plotting software will be rewarded by pleasing graphics that convey a clear message.

In this section, we introduce `ggplot2`, a plotting package for `R` This package was developed by Hadley Wickham who contributed many important packages to `R` (including `dplyr`). Unlike many other plotting

systems, `ggplot2` is deeply rooted in a "philosophical" vision. The goal is to conceive a grammar for all graphical representation of data. Leland Wilkinson and collaborators proposed The Grammar of Graphics. It follows the idea of a well-formed sentence that is composed of a subject, a predicate, and an object. The Grammar of Graphics likewise aims at describing a well-formed graph by a grammar that captures a very wide range of statistical and scientific graphics. This might be more clear with an example – Take a simple two-dimensional scatterplot. How can we describe it? We have:

- **Data** The data we want to plot.
- **Mapping** What part of the data is associated with a particular visual feature? For example: Which column is associated with the x-axis? Which with the y-axis? Which column corresponds to the shape or the color of the points? In `ggplot2` lingo, these are called *aesthetic mappings* (`aes`).
- **Geometry** Do we want to draw points? Lines? In `ggplot2` we speak of *geometries* (`geom`).
- **Scale** Do we want the sizes and shapes of the points to scale according to some value? Linearly? Logarithmically? Which palette of colors do we want to use?
- **Coordinate** We need to choose a coordinate system (e.g., Cartesian, polar).
- **Faceting** Do we want to produce different panels, partitioning the data according to one (or more) of the variables?

This basic grammar can be extended by adding statistical transformations of the data (e.g., regression, smoothing), multiple layers, adjustment of position (e.g., stack bars instead of plotting them side-by-side), annotations, and so on.

Exactly like in the grammar of a natural language, we can easily change the meaning of a "sentence" by adding or removing parts. Also, it is very easy to completely change the type of geometry if we are moving from say a histogram to a boxplot or a violin plot, as these types of plots are meant to describe one-dimensional distributions. Similarly, we can go from points to lines, changing one "word" in our code. Finally, the look and feel of the graphs is controlled by a theming system, separating the content from the presentation.

Basic `ggplot2`

`ggplot2` ships with a simplified graphing function, called `qplot`. In this introduction we are not going to use it, and we concentrate instead on the function `ggplot`, which gives you complete control over your plotting. First, we need to load the package. While we are at it, let's also load a package extending its theming system:

```
library(ggplot2)
library(ggthemes)
```

And then, let's get a small data set, containing the data on the Divvy stations:

```
divvy_stations <- read.csv("../data/Divvy_Stations_July_2014.csv")
```

A particularity of `ggplot2` is that it accepts exclusively data organized in tables (a `data.frame` or a `tbl` object). Thus, all of your data needs to be converted into a data frame format for plotting.

Let's look at the data:

```
head(divvy_stations)
```

For our first plot, we're going to plot the position of the stations, using the latitude (y axis) and longitude (x axis). First, we need to specify a dataset to use:

```
ggplot(data = divvy_stations)
```

As you can see, nothing is drawn: we need to specify what we would like to associate to the x axis, and what to the y axis (i.e., we want to set the *aesthetic mappings*):

```
ggplot(data = divvy_stations) + aes(x = longitude, y = latitude)
```

Note that we concatenate pieces of our "sentence" using the + sign! We've got the axes, but still no graph... we need to specify a geometry. Let's use points:

```
ggplot(data = divvy_stations) + aes(x = longitude, y = latitude) + geom_point()
```

You can now see the outline of Chicago, with the lake on the right (east), the river separating the Loop from the West Loop, etc. As you can see, we wrote a well-formed sentence, composed of **data** + **mapping** + **geometry**. We can add other mappings, for example, showing the capacity of the station using different point sizes:

```
ggplot(data = divvy_stations) +
  aes(x = longitude, y = latitude, size = dpcapacity) +
  geom_point()
```

or colors

```
ggplot(data = divvy_stations) +
  aes(x = longitude, y = latitude, colour = dpcapacity) +
  geom_point()
```

Scatterplots

Using `ggplot2`, one can produce very many types of graphs. The package works very well for 2D graphs (or 3D rendered in two dimensions), while it lack capabilities to draw proper 3D graphs, or networks.

The main feature of `ggplot2` is that you can tinker with your graph fairly easily, and with a common grammar. You don't have to settle on a certain presentation of the data until you're ready, and it is very easy to switch from one type of graph to another.

For example, let's calculate the median `tripduration` by `birthdate`, to see whether older people tend to take longer or shorter trips:

```
duration_byyr <- divvy %>%
  filter(is.na(birthyear) == FALSE) %>% # remove records without birthdate
  filter(birthyear > 1925) %>% # remove ultra centenarian people (probably, errors)
  group_by(birthyear) %>% # group by birth year
  summarise(median_duration  = median(tripduration)) # calculate median for each group

pl <- ggplot(data = duration_byyr) + # data
  aes(x = birthyear, y = median_duration) + # aesthetic mappings
  geom_point() # geometry

pl # or show(pl)
```

We can add a smoother by typing

```
pl + geom_smooth() # spline by default
pl + geom_smooth(method = "lm", se = FALSE) # linear model, no standard errors
```

> **Exercise:** repeat the plot of the median, but grouping the data by `gender` as well as `birthyear`. Set the aesthetic mapping `colour` to plot the results by gender.

Histograms, density and boxplots

How many trips did each bike take? We can plot a histogram showing the number of trips per bike:

```
ggplot(data = divvy, aes(x = bikeid)) + geom_histogram(binwidth = 50)
```

showing a quite uniform density. Speaking of which, we can draw a density plot:

```
ggplot(data = divvy, aes(x = bikeid)) + geom_density()
```

Similarly, we can produce boxplots, for example showing the tripduration for men and women (in `log10`, as the distribution is close to a lognormal):

```
ggplot(data = divvy, aes(x = gender, y = log10(tripduration))) + geom_boxplot()
```

It is very easy to change geometry, for example switching to a violin plot:

```
ggplot(data = divvy, aes(x = gender, y = log10(tripduration))) + geom_violin()
```

Duration by weekday

Now we're going to test whether the trip duration varies considerably by weekday. To do so, we load the package `lubridate`, which contains many excellent functions for manipulating dates and times.

```
library(lubridate)
```

we then create a new variable, `tripday` specifying the day of the week when the trip was initiated. First, we want to transform the string `starttime` into a date:

```
head(divvy) %>% mutate(tripday = mdy_hm(starttime)) #mdy_hm specifies the date format
```

then we can call `wday` with `label = TRUE` to have a label specifying the day of the week:

```
head(divvy) %>% mutate(tripday = wday(mdy_hm(starttime), label = TRUE))
```

Looks good! Let's perform this operation on the whole set:

```
divvy <- divvy %>% mutate(tripday = wday(mdy_hm(starttime), label = TRUE))
```

> **Exercises:**
>
> - Produce a barplot (`geom_bar`) showing the number of trips by day
> - Calculate the median trip duration per weekday. Then plot it with the command:

```
ggplot(medianbyweekday, aes(x = tripday, y = mediantrip)) +
       geom_bar(stat = "identity")
```

the command `stat = "identity"` tells `ggplot2` to interpret the `y` aesthetic mapping as the height of the barplot.

Scales

We can use scales to determine how the aesthetic mappings are displayed. For example, we could set the x axis to be in logarithmic scale, or we can choose how the colors, shapes and sizes are used. `ggplot2` uses two types of scales: `continuous` scales are used for continuos variables (e.g., real numbers); `discrete` scales for variables that can only take a certain number of values (e.g., colors, shapes, sizes).

For example, let's plot a histogram of `tripduration`:

```
ggplot(divvy, aes(x = tripduration)) + geom_histogram() # no transformation
ggplot(divvy, aes(x = tripduration)) + geom_histogram() +
  scale_x_continuous(trans = "log")
ggplot(divvy, aes(x = tripduration)) + geom_histogram() +
  scale_x_continuous(trans = "log10")
ggplot(divvy, aes(x = tripduration)) + geom_histogram() +
  scale_x_continuous(trans = "sqrt", name = "Duration in minutes")
ggplot(divvy, aes(x = tripduration)) + geom_histogram() + scale_x_log10() # shorthand
```

We can use different color scales. We can convert the capacity to a factor, to use discrete scales:

```
pl <- ggplot(data = divvy_stations) +
  aes(x = longitude, y = latitude, colour = as.factor(dpcapacity)) +
  geom_point()
pl + scale_colour_brewer()
pl + scale_colour_brewer(palette = "Spectral")
pl + scale_colour_brewer(palette = "Blues")
pl + scale_colour_brewer("Station Capacity", palette = "Paired")
```

Or use the capacity as a continuous variable:

```
pl <- ggplot(data = divvy_stations) +
  aes(x = longitude, y = latitude, colour = dpcapacity) +
  geom_point()
pl + scale_colour_gradient()
pl + scale_colour_gradient(low = "red", high = "green")
pl + scale_colour_gradientn(colours = c("blue", "white", "red"))
```

Similarly, you can use scales to modify the display of the shapes of the points (`scale_shape_contintuous`, `scale_shape_discrete`), their size (`scale_size_continuous`, `scale_size_discrete`), etc. To set values manually (useful typically for discrete scales of colors or shapes), use `scale_colour_manual`, `scale_shape_manual` etc.

Themes

Themes allow you to manipulate the look and feel of a graph with just one command. The package `ggthemes` extends the themes collection of `ggplot2` considerably. For example:

```
library(ggthemes)
pl <- ggplot(divvy, aes(x = tripduration)) +
  geom_histogram() +
  scale_x_continuous(trans = "log")
pl + theme_bw() # white background
pl + theme_economist() # like in the magazine "The Economist"
pl + theme_wsj() # like "The Wall Street Journal"
```

Faceting

In many cases, we would like to produce a multi-panel graph, in which each panel shows the data for a certain combination of parameters. In `ggplot` this is called *faceting*: the command `facet_grid` is used when you want to produce a grid of panels, in which all the panels in the same row (column) have axis-ranges in common; `facet_wrap` is used when the different panels do not have axis-ranges in common.

For example:

```
pl <- ggplot(data = divvy, aes(x = log10(tripduration))) + geom_histogram(binwidth = 0.1)
show(pl)
ggplot(data = divvy, aes(x = log10(tripduration), group = gender)) +
  geom_histogram(binwidth = 0.1) + facet_grid(~gender)
ggplot(data = divvy, aes(x = log10(tripduration), group = gender)) +
  geom_histogram(binwidth = 0.1) + facet_grid(gender~.)
```

Now faceting by `tripday` and `gender`

```
ggplot(data = divvy, aes(x = log10(tripduration),
                         colour = gender,
                         fill = gender,
                         group = tripday)) +
  geom_histogram(binwidth = 0.1) +
  facet_grid(tripday~gender)
```

Setting features

Often, you want to simply set a feature (e.g., the color of the points, or their shape), rather than using it to display information (i.e., mapping some aestethic). In such cases, simply declare the feature outside the `aes`:

```
pl <- ggplot(data = divvy_stations, aes(x = longitude, y = latitude))
pl + geom_point()
pl + geom_point(colour = "red")
pl + geom_point(shape = 3)
pl + geom_point(alpha = 0.5)
```

Saving graphs

You can either save graphs as done normally in `R`:

```
# save to pdf format
pdf("my_output.pdf", width = 6, height = 4)
print(my_plot)
dev.off()
# save to svg format
svg("my_output.svg", width = 6, height = 4)
print(my_plot)
dev.off()
```

or use the function `ggsave`

```
# save current graph
ggsave("my_output.pdf")
# save a graph stored in ggplot object
ggsave(plot = my_plot, filename = "my_output.svg")
```

Multiple layers

You can overlay different plots. To do so, however, they must share some of the aesthetic mappings. The simplest case is that in which you have only one dataset:

```
ggplot(data = divvy_stations, aes(x = longitude, y = latitude)) +
  geom_density2d() +
  geom_point()
```

in this case, the `geom_density2d` and `geom_point` shared the `aes`, and were taken from the same dataset.

Let's build a more complicated example:

```
# Capacity of stations in Michigan Avenue
data1 <- divvy_stations %>%
  filter(grepl("Michigan", as.character(name))) %>%
  select(name, dpcapacity) %>%
  rename(value = dpcapacity)
data1

# Number of trips leaving stations in Michigan Ave
data2 <- divvy %>%
  filter(grepl("Michigan", as.character(from_station_name))) %>%
  mutate(name = from_station_name) %>%
  select(name) %>%
  group_by(name) %>%
  summarise(value = n())
data2
```

Now we want to plot the capacity:

```
pl <- ggplot(data = data1, aes(x = name, y = value)) +
    geom_point() +
    scale_y_log10() +
    theme(axis.text.x=element_text(angle=90, hjust=1)) # rotate labels
```

And overlay the other data set:

```
pl + geom_point(data = data2, colour = "red")
```

which is allowed, as the two datasets have the same `aes`. Note that Divvy should increase the capacity of the station at Michigan & Oak!

Tidying up data

The best data to plot is the one in *tidy form*, meaning that a) each variable has its own column, and b) each observation has its own row. When data is not in tidy form, you can use the package `tidy` to reshape it.

For example, suppose we want to produce a table in which we have the number of trips departing a certain station by gender. First, we create a summary:

```
station_gender <- divvy %>%
  group_by(from_station_name, gender) %>%
  summarise(tot_trips = n()) %>%
  filter(gender == "Male" | gender == "Female") %>%
  ungroup()
```

Now we would like to create two columns (for `Male` and `Female`), containing the number of trips. To do so, we **spread** the column gender:

```
# Syntax:
# my_tbl %>% spread(COL_TO_SPREAD, WHAT_TO_USE_AS_VALUE, OPTIONAL: fill = NA)
station_gender <- station_gender %>% spread(gender, tot_trips)
station_gender
```

Having reshaped the data, we can see that the station with the highest proportion of women is in Hyde Park:

```
station_gender %>%
  mutate(proportion_female = Female / (Male + Female)) %>%
  arrange(desc(proportion_female))
```

In the data, we have a column from the station of departure and one for that of arrival. Suppose that for our analysis we would need only one column for the station name, and a separate column detailing whether this is the start or the end of the trip:

```
all_stations <- divvy %>% select(from_station_name, to_station_name, tripduration)
```

We can **gather** the two columns creating a column specifying whether it's a from/to station, and one containing the name of the station:

```
# Syntax:
# my_tbl %>% gather(NAME_NEW_COL, NAME_CONTENT, COLS_TO_GATHER)
all_stations %>% gather("FromTo", "StationName", 1:2)
```

Finally, sometimes we need to split the content of a column into several columns. We can use **separate** to do this quickly:

```
divvy %>% select(starttime) %>% separate(starttime, into = c("Day", "Time"), sep = " ")
```

Joining tables

If you have multiple data frames or `tbl` objects with columns in common, it is easy to join them (as in a database). To showcase this, we are going to create a map of all the trips in the data. First, we count the number of trips from/to each pair of stations:

```
num_trips <- divvy %>% group_by(from_station_id, to_station_id) %>% summarise(trips = n())
# remove trips starting and ending at the same point, for easier visualization
num_trips <- num_trips %>% filter(from_station_id != to_station_id)
```

Now we use `inner_join` to combine the data from `num_trips` and `divvy_stations`, creating the columns `x1` and `y1` containing the coordinates of the starting station. If we rename the columns so that their names match, the join is done automatically:

```
only_id_lat_long <- divvy_stations %>% select(id, latitude, longitude)

# Join the coordinates of the starting station
num_trips <- inner_join(num_trips,
                        only_id_lat_long %>%
                          rename(from_station_id = id,
                                 x1 = longitude,
                                 y1 = latitude))
# Join the coordinates of the ending station
num_trips <- inner_join(num_trips,
                        only_id_lat_long %>%
                          rename(to_station_id = id,
                                 x2 = longitude,
                                 y2 = latitude))

num_trips$trips <- as.numeric(num_trips$trips)

# Now we can plot all the trips!
ggplot(data = num_trips,
       aes(x = x1, y = y1, xend = x2, yend = y2,
           alpha = trips / max(trips)) )+
  geom_curve() + scale_alpha_identity() + theme_minimal()
```

Project: network analysis of Divvy data

Now that we have an overview of the methods available, we are going to perform some simple analysis on the data. First of all, we are going to create a matrix of station-to-station flows, where the rows are the starting stations, the columns the ending stations, and coefficients in the matrix measure the number of trips.

For this, we can use a combination of `dplyr` and `tidyr`:

```
flows <- divvy %>%
  select(from_station_id, to_station_id) %>%
  group_by(from_station_id, to_station_id) %>%
```

```
  summarise(trips = n())
# transform into a matrix
flows_mat <- flows %>% spread(to_station_id, trips, fill = 0) %>% as.matrix()
# remove the first col (use it for row name)
rownames(flows_mat) <- flows_mat[,1]
flows_mat <- flows_mat[,-1]
# see one corner of the matrix
flows_mat[1:10, 1:10]
```

Now we're going to rank stations according to their PageRank, the algorithm at the heart of Google's search engine. The idea of PageRank is to simulate a random walk on a set of web-pages: at each step, the random walker can follow a link (with a probability proportional its weight), or "teleport" to another page at random (with small probability). The walk therefore describes a Markov Chain, whose stationary distribution (Perron eigenvector) is the PageRank score for all the nodes. This value indicates how "central" and important a node in the network is.

Mathematically, we want to calculate the Perron eigenvector of the matrix:

$M' = (1 - \epsilon)M + \epsilon U$

Where M is a nonnegative matrix with columns summing to 1, and U is a matrix with all coefficients being 1. ϵ is the teleport probability.

First, we construct the matrix M, by dividing each row for the corresponding row sum, and transposing:

```
M <- t(flows_mat / rowSums(flows_mat))
```

Then, we choose a "teleport probability" (here $\epsilon = 0.01$), and build M':

```
U <- matrix(1, nrow(M), ncol(M))
epsilon <- 0.01
M_prime <- (1 - epsilon) * M + epsilon * U
```

and calculate the PageRank

```
ev <- eigen(M_prime)$vectors[,1]
# normalize ev
ev <- ev / sum(ev)
page_rank <- data.frame(station_id = as.integer(rownames(M_prime)), pagerank = Re(ev))
```

Which stations are the most "central" Divvy stations in Chicago? Let's plot them out:

```
st_pr <- inner_join(divvy_stations, page_rank, by = c("id" = "station_id"))
st_pr <- st_pr %>% mutate(lab = replace(name, pagerank < 0.0055, NA))
ggplot(st_pr,
       aes(x = longitude, y = latitude, colour = pagerank,
           size = pagerank, label = lab)) +
  geom_point() + geom_text(colour = "black",hjust=0, vjust=0)
```

Exercises in groups

The file `data/Chicago_Crimes_May2016.csv` contains a list of all the crimes reported in Chicago in May 2016. Form small groups and work on the following exercises:

- **Crime map** write a function that takes as input a crime's `Primary Type` (e.g., `ASSAULT`), and draws a map of all the occurrences. Mark a point for each occurrence using `Latitude` and `Longitude`. Set the `alpha` to something like 0.1 to show brighter colors in areas with many occurrences.
- **Crimes by community** write a function that takes as input a crime's `Primary Type`, and produces a barplot showing the number of crimes per `Community area`. The names of the community areas are found in the file `data/Chicago_Crimes_CommunityAreas.csv`. You will need to `join` the tables before plotting.
- **Violent crimes** add a new column to the dataset specifying whether the crime is considered violent (e.g., `HOMICIDE`, `ASSAULT`, `KIDNAPPING`, `BATTERY`, `CRIM SEXUAL ASSAULT`, etc.)
- **Crimes in time** plot the number of violent crimes against time, faceting by community areas.
- **Dangerous day** which day of the week is the most dangerous?
- **Dangerous time** which time of the day is the most dangerous (divide the data by hour of the day).
- **Correlation between crimes** which crimes tend to have the same pattern? Divide the crimes by day and type, and plot the correlation between crimes using `geom_tile` and colouring the cells according to the correlation (see `cor` for a function that computes the correlation between different columns).

Advanced Computing 2 – UNIX shell and Regular Expressions

Stefano Allesina

Advanced Computing 2

- **Goal:** Learn to write pipelines for data manipulation and analysis using the `UNIX` shell. Show how to interface the `UNIX` shell and `R`. Introduce the use of regular expressions.
- **Audience:** experienced `R` users, familiar with `dplyr` and `ggplot2`.
- **Installation:** Windows users need to install a UNIX shell emulator (e.g., `Git Bash`); for regular expressions, we are going to use the `R` package `stringr`.

The UNIX Shell

`UNIX` is an operating system (i.e., the software that lets you interface with the computer) developed in the 1970s by a group of programmers at the AT&T Bell laboratories. Among them were Brian Kernighan and Dennis Ritchie, who also developed the programming language `C`. The new operating system was an immediate success in academic circles, with many scientists writing new programs to extend its features. This mix of commercial and academic interest led to the many variants of `UNIX` available today (e.g., OpenBSD, Sun Solaris, Apple OS X), collectively denoted as *nix systems. Linux is the open source `UNIX` clone whose "engine" (kernel) was written from scratch by Linus Torvalds with the assistance of a loosely-knit team of hackers from across the internet.

All *nix systems are multi-user, network-oriented, and store data as plain text files that can be exchanged between interconnected computer systems. Another characteristic is the use of a strictly hierarchical file system.

Why use UNIX?

Many biologists are not familiar with coding in *nix systems and, given that the learning curve is initially fairly steep, we start by listing the main advantages of these systems over possible alternatives.

First, `UNIX` is an operating system written by programmers for programmers. This means that it is an ideal environment for developing your code and storing your data.

Second, hundreds of small programs are available to perform simple tasks. These small programs can be strung together efficiently so that a single line of `UNIX` commands can perform complex operations, which otherwise would require writing a long and complex program. The possibility of creating these pipelines for data analysis is especially important for biologists, as modern research groups produce large and complex data sets, whose analysis requires a level of automation that would be hard to achieve otherwise. For instance, imagine working with millions of files by having to open each one of them manually to perform an identical task, or try opening your single 80Gb whole-genome sequencing file in a software with a graphical user interface! In `UNIX`, you can string a number of small programs together, each performing a simple task, and create a complex pipeline that can be stored in a script (a text file containing all the commands). Then, you can let the computer analyze all of your data while you're having a cup of coffee.

Third, text is the rule: almost anything (including the screen, the mouse, etc.) in `UNIX` is represented as a text file. Using text files means that all of your data can be read and written by any machine, and without

the need for sophisticated (and expensive) proprietary software. Text files are (and always will be) supported by any operating system and you will still be able to access your data decades from today (while this is not the case for most commercial software). The text-based nature of `UNIX` might seem unusual at first, especially if you are used to graphical interfaces and proprietary software. However, remember that `UNIX` has been around since the early 1970s and will likely be around at the end of your career. Thus, the hard work you are putting into learning `UNIX` will pay off over a lifetime.

The long history of `UNIX` means that a large body of tutorials and support web sites are readily available online. Last but not least, `UNIX` is very stable, robust, secure, and—in the case of Linux—freely available.

In the end, entirely avoiding to work in a `UNIX` "shell" is almost impossible for a professional scientist: basically all resources for High-Performance Computing (computer clusters, large workstations, etc.) run a `UNIX` or Linux operating system. Similarly, the transfer of large files, websites, and data between machines is typically accomplished through command-line interfaces.

Directory structure

In `UNIX` we speak of "directories", while in a graphical environment the term "folder" is more common. These two terms are interchangeable and refer to a structure that may contain sub-directories and files. The `UNIX` directory structure is organized hierarchically in a tree. As a biologist, you can think of this structure as a phylogenetic tree. The common ancestor of all directories is also called the "root" directory and is denoted by an individual slash (`/`). From the root directory, several important directories branch:

- `/bin` contains several basic programs.
- `/etc` contains configuration files.
- `/dev` contains the files connecting to devices such as the keyboard, mouse and screen.
- `/home` contains the home directory of each user (e.g., `/home/yourname`; in OS X, your home directory is stored in `/Users/yourname`).
- `/tmp` contains temporary files.

You will typically work in your home directory. From there, you can access the Desktop, Downloads, Documents, and other directories you are likely familiar with. When you navigate the system you are in one directory and can move deeper in the tree or upward towards the root.

Using the terminal

Terminal refers to the interface that you use to communicate with the kernel (the core of the operating system). The terminal is also called *shell*, or *command-line interface* (CLI). It processes the commands you type, translates them for the kernel, and shows you the results of your operations. There are several shells available. Here, we concentrate on the most popular one, the `bash` shell, which is the default shell in both Ubuntu and OS X.

In Ubuntu, you can open a shell by pressing `Ctrl + Alt + t` or by opening the dash (press the `Super` key) and typing `Terminal`. In OS X, you want to open the application `Terminal.app`, which is located in the folder *Utilities* within *Applications*. Alternatively, you can type `Terminal` in *Spotlight*. In either system, the shell will automatically start in your home directory. Windows users can launch `Git Bash` or another terminal emulator.

The command line prompt ends with a "dollar" (`$`) sign. This means the terminal is ready to accept your commands. Here, a `$` sign at the beginning of a line of code signals that the command has to be executed in your terminal. You do not need to type the `$` sign in your terminal, just copy the command that follows it.

In `UNIX`, you can use the `Tab` key to reduce the amount you have to type, which in turn reduces errors caused by typos. When you press `Tab` in a (properly configured) shell, it will try to automatically complete your

command, directory or file name (if multiple completions are possible, you can display them all by hitting the `Tab` key twice). Additionally, you can navigate the history of commands you typed by using the up/down arrows (you do not need to re-type a command that you recently executed). There are also shortcuts that help when dealing with long lines of code:

- `Ctrl + A` Go to the beginning of the line
- `Ctrl + E` Go to the end of the line
- `Ctrl + L` Clear the screen
- `Ctrl + U` Clear the line before the cursor position
- `Ctrl + K` Clear the line after the cursor
- `Ctrl + C` Kill the command that is currently running
- `Ctrl + D` Exit the current shell
- `Alt + F` Move cursor forward one word (in OS X, `Esc + F`)
- `Alt + B` Move cursor backward one word (in OS X, `Esc + B`)

Mastering these and other keyboard shortcuts will save you a lot of time. You may want to print this list and keep it next to your keyboard—in a while you will have them all memorized and will start using them automatically.

Basic UNIX commands

Here we introduce some of the most basic (and most useful) `UNIX` commands. We write the commands in `fixed-width font` and specific, user-provided input is capitalized in square brackets. Again, the brackets and special formatting are not required to execute a command in your terminal.

Many commands require some arguments (e.g., copy which file to where), and all can be modified using the several options available. Typically, options are either written as a dash followed by a single letter (older style, e.g., `-f`) or two dashes followed by words (newer style, e.g., `--full-name`). A command, its options and arguments are separated by a space.

How to get help in UNIX

`UNIX` ships with hundreds of commands. As such, it is impossible to remember them all, let alone all their possible options. Fortunately, each command is described in detail in its manual page, which can be accessed directly from the shell by typing `man [COMMAND OF YOUR CHOICE]` (not available in `Git Bash`). Use arrows to scroll up and down and hit `q` to close the manual page. Checking the exact behavior of a command is especially important, given that the shell will execute any command you type without asking whether you know what you're doing (such that it will promptly remove all of your files, if that's the command you typed). You may be used to more forgiving (and slightly patronizing) operating systems in which a pop-up window will warn you whenever something you're doing is considered dangerous. In `UNIX`, it is always better to consult the manual rather than improvising.

If you want to interrupt the execution of a command, press `Ctrl + C` to halt any command that is currently running in your shell.

Navigating the directory system

You can navigate the hierarchical `UNIX` directory system using these commands:

- `pwd` print the path of the current working directory.
- `ls` list the files and sub-directories in the current directory. `ls -a` list all (including hidden) files. `ls -l` return the long list with detailed information. `ls -lh` provide file sizes with units (B, M, K, etc.).}

- `cd [NAMEOFDIR]` change directory. `cd ..` move one directory up; `cd /` move to the root directory; `cd ~` move to your home directory; `cd -` go back to the directory you visited previously (like "Back"" in a browser).

Handling directories and files

Create and delete files or directories using the following commands:

- `cp [FROM] [TO]` copy a file. The first argument is the file to copy. The second argument is where to copy it (either a directory or a file name).
- `mv [FROM] [TO]` move or rename a file. Move a file by specifying two arguments: the file, and the destination directory. Rename a file by specifying the old and the new file name in the same directory.
- `touch [FILENAME]` Update the date of last access to the file. Interestingly, if the file does not exist, this command will create an empty file.
- `rm [TOREMOVE]` remove a file. `rm -r` deletes the contents of a directory recursively (i.e., including all files and sub-directories in it; use with caution!). Similarly, `rm -f` removes the file and suppresses any prompt asking whether you are sure you want to remove the file.
- `mkdir [DIRECTORY]` make a directory. To create nested directories, use the option `-p` (e.g., `mkdir -p d1/d2/d3`).
- `rmdir [DIRECTORY]` remove an empty directory.

Printing and modulating files

`UNIX` was especially designed to handle text files, which is apparent when considering the multitude of commands dealing with text. Here are a few popular ones:

- `less [FILENAME]` progressively print a file on the screen (press `q` to exit). Funny fact: there is a command called `more` that does the same thing, but with less flexibility. Clearly, in `UNIX`, `less` is `more`.
- `cat [FILENAME]` concatenate and print files.
- `wc [FILENAME]` line, word, and byte (character) count of a file.
- `sort [FILENAME]` sort the lines of a file and print the result to the screen.
- `uniq [FILENAME]` show only unique lines of a file. The file needs to be sorted first for this to work properly.
- `file [FILENAME]` determine the type of a file.
- `head [FILENAME]` print the `head` (i.e., first few lines of a file).
- `tail [FILENAME]` print the tail (i.e., last few lines of a file).
- `diff [FILE1] [FILE2]` show the differences between two files.

Exercise To familiarize yourself with these commands, try the following:

- Go to the `data` directory for this tutorial.
- How many lines are in file `Marra2014_data.fasta`?
- Go back to the `code` directory.
- Create the empty file `toremove.txt`.
- List the content of the directory.
- Remove the file `toremove.txt`.

Miscellaneous commands

- `echo "[A STRING]"` print the string `[A STRING]`.
- `time` time the execution of a command.

- `wget [URL]` download the webpage at `[URL]`. (Available in Ubuntu; for OS X look at `curl`, or install `wget`).
- `history` list the last commands you executed.

Advanced UNIX commands

Redirection and pipes

So far, we have printed the output of each command (e.g., `ls`) directly to the screen. However, it is easy to direct the output to a file (*redirect*) or use it as the input of another command (*pipe*). Stringing commands together in pipes is the real power of `UNIX`—the ability to perform complex processing of large amounts of data in a single line of commands. First, we show how to redirect the output of a command into a file:

```
$ [COMMAND] > [FILENAME]
```

Note that if the file `[FILENAME]` exists, it will be overwritten. If instead we want to append to an existing file, we can use the `>>` symbol as in the following line:

```
$ [COMMAND] >> [FILENAME]
```

When the command is very long and complex, we might want to redirect the content of a file as input to a command, "reversing" the flow:

```
$ [COMMAND] < [FILENAME]
```

To run a few examples, let's start by moving to our `code` directory:

```
$ cd ~/BSD-QBio2/tutorials/advanced_computing2/code
```

The command `echo` can be used to print a string on the screen. Instead of printing to the screen, we redirect the output to a file, effectively creating a file containing the string we want to print:

```
$ echo "My first line" > test.txt
```

We can see the result of our operation by printing the file to the screen using the command `cat`:

```
$ cat test.txt
```

To append a second line to the file, we use `>>`:

```
$ echo "My second line" >> test.txt
$ cat test.txt
```

We can redirect the output of any command to a file. For example, it is quite common to have to determine how many files are in a directory. The files could have been created by an instrument or provided by a collaborator. Before analyzing the data, we want to get a sense of how many files will we need to process. If there are thousands of files, it is quite time consuming to count them by hand or even open a file browser that can do the counting for us. It is much simpler and faster to just type a command or two. To try this, let's create a file listing all the files contained in `data/Saavedra2013`:

```
$ ls ../data/Saavedra2013 >> filelist.txt
$ cat filelist.txt
```

Now we want to count how many lines are in the file. We can do so by calling the command `wc -l` (count only the lines):

```
$ wc -l filelist.txt
$ rm filelist.txt
```

However, we can skip the creation of the file by creating a short pipeline. The pipe symbol `|` tells the shell to take the output on the left of the pipe and use it as the input of the command on the right of the pipe. To take the output of the command `ls` and use it as the input of the command `wc` we can write:

```
$ ls  ../data/Saavedra2013 | wc -l
```

We have created our first, simple pipeline. In the following sections, we are going to build increasingly long and complex pipelines. The idea is always to start with a command and progressively add one piece after another to the pipeline, each time checking that the result is the desired one.

Selecting columns using `cut`

When dealing with tabular data, you will often encounter the Comma Separated Values (CSV) Standard File Format. The CSV format is platform and software independent, making it the standard output format of many experimental devices. The versatility of the file format should also make it your preferred choice when manually entering and storing data.

The main `UNIX` command you want to master for comma-, space-, tab-, or character-delimited text files is `cut`. To showcase its features, we work with data on generation time of mammals published by Pacifici *et al.*. First, let's make sure we are in the right directory (`advanced_computing2/data`). Then, we can print the header (the first line, specifying the content of each column) of the CSV file using the command `head`, which prints the first few lines of a file on the screen, with the option `-n 1`, specifying that we want to output only the first line:

```
$ head -n 1 Pacifici2013_data.csv
TaxID;Order;Family;Genus;Scientific_name;...
```

We now pipe the header to `cut`, specify the character to be used as delimiter (`-d ';'`), and use the `head` command to extract the name of the first column (`-f 1`), or the names of the first four columns (`-f 1-4`):

```
$ head -n 1 Pacifici2013_data.csv | cut -d ';' -f 1
TaxID

$ head -n 1 Pacifici2013_data.csv | cut -d ';' -f 1-4
TaxID;Order;Family;Genus
```

Remember to use the `Tab` key to auto-complete file names and the arrow keys to access your command history.

In the next example, we work with the file content. We specify a delimiter, extract specific columns, and pipe the result to the `head` command—to display only the first few elements:

```
$ cut -d ';' -f 2 Pacifici2013_data.csv  | head -n 5
Order
Rodentia
Rodentia
Rodentia
Macroscelidea

$ cut -d ';' -f 2,8 Pacifici2013_data.csv  | head -n 3
Order;Max_longevity_d
Rodentia;292
Rodentia;456.25
```

Now, we specify the delimiter, extract the second column, skip the first line (the header) using the `tail -n +2` command (i.e., return the whole file starting from the second line), and finally display the first five entries:

```
$ cut -d ';' -f 2 Pacifici2013_data.csv  | tail -n +2 | head -n 5
Rodentia
Rodentia
Rodentia
Macroscelidea
Rodentia
```

We pipe the result of the previous command to the `sort` command (which sorts the lines), and then again to `uniq`, (which takes only the elements that are not repeated). Effectively, we have created a pipeline to extract the names of all the Orders in the database, from Afrosoricida to Tubulidentata (a remarkable Order, which today contains only the aardvark).

```
$ cut -d ';' -f 2 Pacifici2013_data.csv  | tail -n +2 | sort | uniq
Afrosoricida
Carnivora
Cetartiodactyla
...
```

This type of manipulation of character-delimited files is very fast and effective. It is an excellent idea to master the `cut` command in order to start exploring large data sets without the need to open files in specialized programs (if you don't want to modify the content of a file, you should not open it in an editor!).

Exercise:

- If we order all species names (fifth column) of `Pacifici2013_data.csv` in alphabetical order, which is the first species? Which the last?
- How many families are represented in the database?

Substituting characters using `tr`

We often want to substitute or remove a specific character in a text file (e.g., to convert a comma-separated file into a tab-separated file). Such a one-by-one substitution can be accomplished with the command `tr`. Let's look at some examples in which we use a pipe to pass a string to `tr`, which then processes the text input according to the search term and specific options.

Substitute all characters `a` with `b`:

```
$ echo 'aaaabbb' | tr 'a' 'b'
bbbbbbb
```

Substitute every number in the range `1` through `5` with `0`:

```
$ echo '123456789' | tr 1-5 0
000006789
```

Substitute lower-case letters with upper-case (note the one-to-one mapping):

```
$ echo 'ACtGGcAaTT' | tr actg ACTG
ACTGGCAATT
```

We achieve the same result using bracket expressions that provide a predefined set of characters. Here, we use the set of all lower-case letters `[:lower:]` and translate into upper-case letters `[:upper:]`:

```
$ eecho 'ACtGGcAaTT' | tr [:lower:] [:upper:]
ACTGGCAATT
```

We can also indicate ranges of characters to substitute:

```
$ echo 'aabbccddee' | tr a-c 1-3
112233ddee
```

Delete all occurrences of `a`:

```
$ echo 'aaaaabbbb' | tr -d a
bbbb
```

"Squeeze" all consecutive occurrences of `a`:

```
$ echo 'aaaaabbbb' | tr -s a
abbbb
```

Note that the command `tr` reads standard input, and does not operate on files directly. However, we can use pipes in conjunction with `cat`, `head`, `cut`, etc. to create input for `tr`:

```
$  tr ' ' '\t' < [INPUTFILE] > [OUTPUTFILE]
```

In this example we input `[INPUTFILE]` to the `tr` command to replace all spaces with tabs. Note the use of quotes to specify the space character. The tab is indicated by `\t` and is called a "meta-character". We use the backslash to signal that the following character should not be interpreted literally, but rather is a special code referring to a character that is difficult to represent otherwise.

Now we can apply the command `tr` and the commands we have showcased earlier to create a new file containing a subset of the data contained in `Pacifici2013_data.csv`, which we are going to use in the next section.

First, we change directory to the `code`:

```
$ cd ../code/
```

Now, we want to create a version of `Pacifici2013_data.csv` containing only the `Order`, `Family`, `Genus`, `Scientific_name`, and `AdultBodyMass_g` (columns 2-6). Moreover, we want to remove the header, sort the lines according to body mass (with larger critters first), and have the values separated by spaces. This sounds like an awful lot of work, but we're going to see how this can be accomplished piping a few commands together.

First, let's remove the header:

```
$ tail -n +2 ../data/Pacifici2013_data.csv
```

Then, take only the columns 2-6:

```
$ tail -n +2 ../data/Pacifici2013_data.csv | cut -d ';' -f 2-6
```

Now, substitute the current delimiter (`;`) with a space:

```
$ tail -n +2 ../data/Pacifici2013_data.csv | cut -d ';' -f 2-6 | tr -s ';' ' '
```

To sort the lines according to body size, we need to exploit a few of the options for the command `sort`. First, we want to sort numbers (option `-n`); second, we want larger values first (option `-r`, reverse order); finally, we want to sort the data according to the sixth column (option `-k 6`):

```
$ tail -n +2 ../data/Pacifici2013_data.csv |cut -d ';' -f 2-6 | tr -s ';' ' ' | sort -n -r -k 6
```

That's it. We have created our first complex pipeline. To complete the task, we redirect the output of our pipeline to a new file called `BodyM.csv`.

```
$ tail -n +2 ../data/Pacifici2013_data.csv | cut -d ';' -f 2-6 | tr -s
  ';' ' ' | sort -r -n -k 6 > BodyM.csv
```

You might object that the same operations could have been accomplished with a few clicks by opening the file in a spreadsheet editor. However, suppose you have to repeat this task many times, e.g., to reformat every file that is produced by a laboratory device. Then it is convenient to automate this task such that it can be run with a single command.

Similarly, suppose you need to download a large CSV file from a server, but many of the columns are not needed. With `cut`, you can extract only the relevant columns, reducing download time and storage.

Selecting lines using `grep`

`grep` is a powerful command that finds all the lines of a file that match a given pattern. You can return or count all occurrences of the pattern in a large text file without ever opening it. `grep` is based on the concept of regular expressions, which we will cover just below (but using `R`, in which the syntax is slightly different).

We will test the basic features of `grep` using the file we just created. The file contains data on thousands of species:

```
$ wc -l BodyM.csv
5426 BodyM.csv
```

Let's see how many wombats (family Vombatidae) are contained in the data. First we display the lines that contain the term "Vombatidae":

```
$ grep Vombatidae BodyM.csv
Diprotodontia Vombatidae Lasiorhinus Lasiorhinus krefftii 31849.99
Diprotodontia Vombatidae Lasiorhinus Lasiorhinus latifrons 26163.8
Diprotodontia Vombatidae Vombatus Vombatus ursinus 26000
```

Now we add the option `-c` to count the lines:

```
$ grep -c Vombatidae BodyM.csv
3
```

Next, we have a look at the genus `Bos` in the data file:

```
$ grep Bos BodyM.csv
Cetartiodactyla Bovidae Bos Bos sauveli 791321.8
Cetartiodactyla Bovidae Bos Bos gaurus 721000
Cetartiodactyla Bovidae Bos Bos mutus 650000
Cetartiodactyla Bovidae Bos Bos javanicus 635974.3
Cetartiodactyla Bovidae Boselaphus Boselaphus tragocamelus 182253
```

Besides all the members of the `Bos` genus, we also match one member of the genus `Boselaphus`. To exclude it, we can use the option `-w`, which prompts `grep` to match only full words:

```
$ grep -w Bos BodyM.csv
Cetartiodactyla Bovidae Bos Bos sauveli 791321.8
Cetartiodactyla Bovidae Bos Bos gaurus 721000
Cetartiodactyla Bovidae Bos Bos mutus 650000
Cetartiodactyla Bovidae Bos Bos javanicus 635974.3
```

Using the option `-i` we can make the search case-insensitive (it will match both upper- and lower-case instances):

```
$ grep -i Bos BodyM.csv
Proboscidea Elephantidae Loxodonta Loxodonta africana 3824540
Proboscidea Elephantidae Elephas Elephas maximus 3269794
Cetartiodactyla Bovidae Bos Bos sauveli 791321.8
Cetartiodactyla Bovidae Bos Bos gaurus 721000
...
```

Sometimes, we want to know which lines precede or follow the one we want to match. For example, suppose we want to know which mammals have body weight most similar to the gorilla (*Gorilla gorilla*). The species are already ordered by size, thus we can simply print the two lines before the match using the option `-B 2` and the two lines after the match using `-A 2`:

```
$ grep -B 2 -A 2 "Gorilla gorilla" BodyM.csv
Cetartiodactyla Bovidae Ovis Ovis ammon 113998.7
Cetartiodactyla Delphinidae Lissodelphis Lissodelphis borealis 113000
Primates Hominidae Gorilla Gorilla gorilla 112589
Cetartiodactyla Cervidae Blastocerus Blastocerus dichotomus 112518.5
Cetartiodactyla Iniidae Lipotes Lipotes vexillifer 112138.3
```

Use option `-n` to show the line number of the match. For example, the gorilla is the 164th largest mammal in the database:

```
$ grep -n "Gorilla gorilla" BodyM.csv
164:Primates Hominidae Gorilla Gorilla gorilla 112589
```

To print all the lines that do not match a given pattern, use the option `-v`. For example, to get the other species of the genus *Gorilla* with the exception of *Gorilla gorilla*, we can use:

```
$ grep Gorilla BodyM.csv | grep -v gorilla
Primates Hominidae Gorilla Gorilla beringei 149325.2
```

To match one of several strings, use `grep "[STRING1]\|[STRING2]"`

```
$ grep -w "Gorilla\|Pan" BodyM.csv
Primates Hominidae Gorilla Gorilla beringei 149325.2
Primates Hominidae Gorilla Gorilla gorilla 112589
Primates Hominidae Pan Pan troglodytes 45000
Primates Hominidae Pan Pan paniscus 35119.95
```

You can use `grep` on multiple files at a time! Simply, list all the files to use instead of just one file.

Interfacing `R` and the `UNIX` shell

Note: this will work if you're using Mac OSX or UNIX; in Windows options are much more limited.

Calling `R` from the command line

In `R`, you typically work in "interactive" mode — you type a command, it gets executed, you type another command, and so on. Often, we want to be able to re-run a script on different data sets or with different parameters. For that purpose you can store all the commands in a text file (typically, with extension `.R`), and then re-run the analysis by typing in the `UNIX` command line

```
$ Rscript my_script_file.R
```

To properly automate our analysis and figure generation, however, we can additionally pass command-line arguments to `R`. This allows us for instance to perform the analysis using a specific input file, or save the figure using a specific file name.

`Rscript` accepts command-line arguments, that need to be parsed within `R`. The code at the beginning of the following script shows how this is accomplished:

```r
# Get all the command-line arguments
args <- commandArgs(TRUE)
# Assign each argument to a variable,
# making sure to convert it to the right
# type of variable (string by default)

# check the number of arguments
num.args <- length(args)
print(paste("Number of command-line arguments:", num.args))
# print all the arguments
if (num.args > 0) {
```

```
    for (i in 1:num.args) {
        print(paste(i, "->", args[i]))
    }
}

# We can initially set to default values
# (but pay attention to the order,
# the optional arguments should be at the end)
input.file <- "test.txt"
number.replicates <- 10
starting.point <- 3.14

if (num.args >= 1) {
    input.file <- args[1]
}
if (num.args >= 2) {
    number.replicates <- as.integer(args[2])
}
if (num.args >= 3) {
    starting.point <- as.double(args[3])
}

print(c(input.file, number.replicates, starting.point))

# Save this script as my_script.R
# Run the script in bash with different arguments
# Rscript my_script.R abc.txt 5 100.0
# Rscript my_script.R abc.txt 5
# Rscript my_script.R abc.txt
# Rscript my_script.R
```

Calling the command line from R

You can call the operating system from within R (assuming you're in /advanced_computing_2/code):

```
system("wc -l < ../../basic_computing_1/data/H938_Euro_chr6.geno")
```

You can also capture the output from the shell commands and save it into R. Everything is treated as text (convert to numeric if necessary):

```
numlines <- system("wc -l < ../../basic_computing_1/data/H938_Euro_chr6.geno",
                   intern = TRUE)
numlines
```

```
## [1] "43142"
```

You can also use a combination of shell commands and read.table to capture more complex output:

```
mydf <- system("grep rs125283 ../../basic_computing_1/data/H938_Euro_chr6.geno",
                     intern = TRUE)
mydf <- read.table(file = textConnection(mydf))
mydf
```

```
##   V1         V2 V3 V4 V5 V6  V7
## 1  6 rs12528302  G  A 26 59  39
## 2  6 rs12528322  G  A  0 21 103
## 3  6 rs12528313  G  T  1 25  98
## 4  6 rs12528341  C  T  3 31  90
```

Regular expressions in R

Sometimes data is hidden in free text. Think of citations in a manuscript, mentions of DNA motifs in tables, etc. You could copy and paste data from these unstructured texts yourself, but if you have much text, the task is very boring and error-prone. What you need is a way to describe a text pattern to a computer, and then have it extract the data automatically. Regular Expressions do exactly that.

Because you want to describe a text pattern using text, a level of abstraction is inevitable. What you want to do is to construct a pattern using **literal** characters and **metacharacters**.

For all our examples, we will use the package `stringr`, which makes the regular expression syntax consistent (there are many *dialects*), and provides a set of easy-to-use functions:

```
library(stringr)
```

All the functions have a common structure. For example, `str_extract` extracts text matching a pattern: `str_extract(text, pattern)`. The simplest possible expression is one in which the pattern is described literally (i.e., we want to find exactly the text we're typing):

```
str_extract("a string of text", "t")
```

```
## [1] "t"
```

```
str_extract_all("a string of text", "t")
```

```
## [[1]]
## [1] "t" "t" "t"
```

Of course, you need to be able to describe much more general patterns. Use the following metacharacters:

- `\d` Match a digit character (`0-9`)
- `\D` Match any character that is not a digit
- `\n` Match a newline
- `\s` Match a space
- `\t` Match a `Tab`
- `\b` Match a "word boundary"
- `\w` Match a "word" character (alphanumeric)
- `.` Match any character

Some examples (note that to escape characters, you want to use two backslashes — you need to escape the backslash itself!):

```
# find the first digit
str_extract("123.25 grams", "\\d")
```

```
## [1] "1"
```

```
# find word separator + word character + word separator
str_extract("Albert Einstein was a genius", "\\b\\w\\b")
```

```
## [1] "a"
```

```
# find all digits
str_extract_all("my cell is 773 345 6789", "\\d")
```

```
## [[1]]
##  [1] "7" "7" "3" "3" "4" "5" "6" "7" "8" "9"
```

```
# extract all characters
str_extract_all("for example, this and that", ".")
```

```
## [[1]]
##  [1] "f" "o" "r" " " "e" "x" "a" "m" "p" "l" "e" "," " " "t" "h" "i" "s"
## [18] " " "a" "n" "d" " " "t" "h" "a" "t"
```

Of course, you don't want to type `\\w` fifteen times, in case you are looking for a string that is 15 characters long! Rather, you can use quantifiers:

- `*` Match zero or more times. Match as many times as possible.
- `*?` Match zero or more times. Match as few times as possible.
- `+` Match one or more times. Match as many times as possible.
- `+?` Match one or more times. Match as few times as possible.
- `?` Match zero or one times. In case both zero and one time match, prefer one.
- `??` Match zero or one times, prefer zero.
- `{n}` Match exactly `n` times.
- `{n,}` Match at least `n` times. Match as many times as possible.
- `{n,m}` Match between `n` and `m` times.

Exercise

What does this do? Try to guess, and then type the command into `R`

```
str_extract_all("12.06+3.21i", "\\d+\\.?\\d+")
my_str <- "most beautiful and most wonderful have been, and are being, evolved."
str_extract(my_str, "\\b\\w{6,10}\\b")
str_extract(my_str, "\\b\\w+\\b")
str_extract(my_str, "w\\w*")
str_extract(my_str, "b\\w*")
str_extract(my_str, "b\\w+?")
str_extract(my_str, "\\s\\wn\\w+")
```

What if you want to match the characters `?`, `+`, `*`, `.`? You will need to escape them: for example, `\\.` matches the "dot" character.

You can specify anchors to signal that the match has to be in certain special positions in the text:

- `^` Match at the beginning of a line.
- `$` Match at the end of a line.

```r
str_extract("Ah, ba ba ba ba Barbara Ann", "\\w{2,}$")
```

```
## [1] "Ann"
```

```r
str_extract("Ah, ba ba ba ba Barbara Ann", "^\\w{2,}")
```

```
## [1] "Ah"
```

To match one of several characters, list them between brackets:

```r
str_extract("01234567890", "[3120]+")
```

```
## [1] "0123"
```

```r
str_extract("01234567890", "[3-5]+")
```

```
## [1] "345"
```

```r
str_extract("supercalifragilisticexpialidocious", "[a-i]{3,}")
```

```
## [1] "agi"
```

To match either of two patterns, use alternations:

```r
str_extract_all("The quick brown fox jumps over the lazy dog", "fox|dog")
```

```
## [[1]]
## [1] "fox" "dog"
```

If you need more complex alternations, use parentheses to separate the patterns.

Parentheses can also be used to define **groups**, which are used when you want to capture unknown text that is however flanked by known patterns. For example, suppose you want to save the user name of a UofC email:

```r
str_match_all("sallesina@uchicago.edu mjsmith@uchicago.edu",
              "\\b([a-zA-Z0-9]*?)@uchicago.edu")
```

```
## [[1]]
##      [,1]                     [,2]
## [1,] "sallesina@uchicago.edu" "sallesina"
## [2,] "mjsmith@uchicago.edu"   "mjsmith"
```

Note that you have to use `str_match` or `str_match_all` to obtain information on the groups.

Useful functions

Many functions have a similar `str_***_all` version, returning all matches.

- `str_detect(strings, pattern)` do the `strings` contain the `pattern`? Returns a logical vector.
- `str_locate(strings, pattern)` find the character position of the pattern
- `str_extract(strings, pattern)` extracts the first match
- `str_match(strings, pattern)` like `extract` but capture groups defined by parentheses
- `str_replace(strings, pattern, newstring)` replaces the first matched `pattern` with `newstring`

Exercises in groups

Extract primers and polymorphic sites

In the file `data/Ptak_etal_2004.txt` you find a text version of the supplementary materials of Ptak *et al.* (PLoS Biology 2004, doi:10.1371/journal.pbio.0020155).

- Take a look at the file. You see that it first lists the primers used for the study (e.g., `TAP2-17-3' -> CTTGGATATAACACCAAACGCA`), and then the polymorphic sites for 24 chimpanzees (e.g., .
- Read the text as a single string, intervaled by new lines

```
my_txt <- paste(readLines("../data/Ptak_etal_2004.txt"), collapse="\n")
```

- Use regular expressions to produce a data frame containing the primers used in the study:

```
head(primers)
```

```
         ID               Sequence
1 TAP2-1-5' GAGAATCACTTGAACCTGGGAG
2 TAP2-2-5' TTGTCCACAGTGTACCACATGA
3 TAP2-3-5' TATTTCTTCCTGGGGTTTCCTT
4 TAP2-4-5' CATGATGTGTCATGCTGAATTG
5 TAP2-5-5' ATAGAACAAGAACCAAAGCCCA
6 TAP2-6-5' GGACAACAGATAAAGTTGCCCT
```

- Write another regular expression to extract the polymorphic region for each chimp. Use `str_replace_all` to remove extra spaces and newlines. The results should look like:

```
  Chimp                                                Sequence
1   311 ATACCCTGGAGGCAAGAATCTTCCGATAGACGCCAGTCCCTAGTTGT...
2   312 GCACCCTGGAGGCAAGAGTCTTCCGATAGACGCCAGTCCCTAGTTGC...
3   313 GCACCCTGGAGGCAAGAGTCTTCCGATAGACGCCAGTCCCCAGTTGT...
4   314 GCACCCTGGAGGCAAGAGTCTTCCGATAGACCCCAGTCCCCAGTTGC...
5   317 GCACCCTGGAGGTGGGGGCCCCCCAGGAGGCCCCAGCCCCCGGTCGT...
6   320 GCACCCTGGAGATAAGGGCCCCCCAGGAGGCCCCAGCCCCCGGTCGT...
```

A map of *Science*

Where does science come from? This question has fascinated researchers for decades and even led to the birth of the field of "Science of Science", where researchers use the same tools they invented to investigate nature to gain insights on the development of science itself. In this exercise, you will build a map of *Science*, showing where articles published in *Science* magazine have originated. You will find two files in the directory `data/MapOfScience`. The first, `pubmed_results.txt`, is the output of a query to PubMed listing all the papers published in Science in 2015. You will extract the US ZIP codes from this file, and then use the file `zipcodes_coordinates.txt` to extract the geographic coordinates for each ZIP code.

- Read the file `pubmed_results.txt`, and extract all the US ZIP codes.
- Count the number of occurrences of each ZIP code using `dplyr`.
- Join the table you've created with the data in `zipcodes_coordinates.txt`
- Plot the results using `ggplot2` (either use points with different colors/alphas, or render the density in two dimensions)

Workshops

Workshop Cobey
Workshop Novembre
Workshop Osborne
Workshop Vander Griend
Workshop Munro & Rust

Unraveling dynamics from time series

Instructors: Sarah Cobey and Sylvia Ranjeva

BSD Bootcamp on Quantitative Biology @ MBL

Goal

The aim of this workshop is to show how mathematical models can be used to investigate dynamical systems. We will begin by constructing and analyzing simple models of the spread of an infectious disease. We'll work with pencil and paper (or the whiteboard versions) before simulating the dynamics in R. We'll see that small nonlinearities can generate complex dynamics, which we can summarize with bifurcation diagrams. Finally, we'll discuss the use of likelihood in model fitting and review the criteria by which to judge a model.

At the end of the workshop, students should have facility analyzing simple models analytically and simulating systems of differential equations in R. They should also understand the steps involved in creating bifurcation diagrams. Conceptually, they should understand the spectrum of "mechanistic" to "statistical" models and the limitations of analyses based on correlations and linear assumptions. They should also be able to define the concept of the likelihood and rank factors to consider in judging the strength of a model.

Audience

This workshop is intended for biologists investigating nonlinear dynamical systems. The material is thus relevant to molecular and cell biologists, population geneticists, ecologists, immunologists, etc. The lecture assumes some familiarity with ordinary differential equations (ODEs), and the exercises assume familiarity with R. Although both ODEs and R will be covered to some extent in the tutorials, if you are eager to test your knowledge, you can inspect the code and suggested readings below.

Installation

Please have a recent version of R installed before the workshop. We will also use the deSolve package. It would be helpful if you could try installing this package before the workshop.

Workshop resources

- Slides
- Code
- Data: We will not be analyzing any real data as part of the workshop. However, if you have a time series in mind that you would like to focus on, please contact the instructors beforehand.

Readings and additional resources

These readings are not mandatory, but they review some of the techniques and models we will cover in the workshop.

- Lotka-Volterra Model (Wikipedia)
- Compartmental SIR Models (Wikipedia)
- Chaos theory (Wikipedia)
- May 1976: "Simple mathematical models with very complicated dynamics"
- Earn et al. 2000: "A simple model for complex dynamical transitions in epidemics"
- Shrestha et al. 2011: "Statistical inference for multi-pathogen systems"

Population genetics workshop

Welcome

This excercise is going to expose you to several basic ideas in probability and statistics as well as show you the utility of using R for basic statistical analyses. We'll do so in the context of a basic population genetic analysis.

The scenario

As a biologist, you will learn what are the major patterns that are expected when the data you work with is clean. Using that expertise will save you from the mistake of misinterpreting error-prone data. In population genetics, there are a number of patterns that we expect to see immediately in our datasets. In this exercise you will explore one of those major patterns. Rather than give it away — let's begin some analysis and see what we find. In the narative that follows, we'll refine our thinking as we go.

Introductory terminology

- Single-nucleotide polymorphism (SNP): A nucleotide basepair that is *polymorphic* (i.e. it has multiple types or *alleles* in the population)
- Allele: A particular variant form of DNA (e.g. A partciular SNP may have the "A-T" allele in one DNA copy and "C-G" in another; We typically define a reference strand of the DNA to read off of, and then denote the alleles according to the reference strand base - so for example, these might be called simply the "A" and "C" alleles. In many cases we don't care about the precise base, so we might call these simply the A_1 and A_2 alleles, or the A or a alleles, or the 0 and 1 alleles.)
- Minor allele: The allele that is more rare in a population
- Major allele: The allele that is more common in a population
- Genotype: The set of alleles carried by an individual (E.g. AA, AC, CC; or AA, AA, and aa; or 0/0, 1/1, 2/2)
- Genotyping array: A technology based on hybridization with probes and florensence that allows genotype calls to be made at 100s of thousands of SNPs per individual at an affordable cost.

The data-set and basic pre-processing

We will look at Illumina 650Y genotyping array data from the CEPH-Human Genome Diversity Panel. This sample is a global-scale sampling of human diversity with 52 populations in total.

The data were first described in Li et al (Science, 2008) and the raw files are available from the following link: http://hagsc.org/hgdp/files.html. These data have been used in numerous subsequent publications (e.g Pickrell et al, Genome Research, 2009) and are an important reference set. A few technical details are that the genotypes were filtered with a GenCall score cutoff of 0.25 (a quality score generated by the basic genotype calling software). Individuals with a genotype call rate <98.5% were removed, with the logic being that if a sample has many missing genotypes it may due to poor quality of the source DNA, and so none of the genotypes should be trusted. Beyond this, to prepare the data for the workshop, we have filtered down the indiviudals to a set of 938 unrelated individuals. (For those who are interested, the data are available as plink-formatted files `H938.bed` `H938.fam`, `H938.bim` from this link: http://bit.ly/1aluTln). We have also extracted the basic counts of three possible genotypes.

The files with these genotype frequencies are your starting points.

Note about logistics

We will use some of functions from the `dplyr` and `ggplot2` and `reshape2` libaries so first let's load them:

```
library(dplyr)
library(ggplot2)
library(reshape2)
```

If you get an error message saying there is no package titled `dplyr`,`ggplot2`, or `reshape2` you may need to first run `install.packages("dplyr")`,`install.packages("ggplot2")`, or`install.packages("reshape2")` to install the appropriate package.

We will not be outputting files - but you may want to set your working directory to the `sandbox` sub-directory in case you want to output some files.

The `MBL_WorkshopJN.Rmd` file has the R code that you can run. I provide code for most steps, but some you will need ot devise for yourselves to answer the questions that are part of the workshop narrative.

Initial view of the data

Read in the data table:

```
g <- read.table("../Data/H938_chr15.geno", header=TRUE)
```

It will be read in as a dataframe in R.

And use the "head" command to see the beginning of the dataframe:

```
head(g)
```

You should see that there are columns each with distinct names.

```
CHR        SNP A1 A2 nA1A1 nA1A2 nA2A2
```

- CHR: The chromosome number. In this case they are all from chromosome 2.
- SNP: The rsid of a SNP is a unique identifier for a SNP and you can use the rsid to look up information about a SNP using online resource such as dbSNP or SNPedia.
- A1: The minor allele at the SNP
- A2: The major allele
- nA1A1 : The number of A1/A1 homozygotes
- nA1A2 : The number of A1/A2 homozygotes
- nA2A2 : The number of A2/A2 homozygotes

Calculate the number of counts at each locus

Next compute the total number of observations by summing each of the three possible genotypes. Here we use the `mutate` function from the `dplyr` library to do the addition and add a new column to the dataframe in one nice step. (Note: You could also use the `colSums` function from the base R library).

```
g <- mutate(g, nObs = nA1A1 + nA1A2 + nA2A2)
```

Run `head(g)` and confirm your dataframe `g` has a new column called `nObs`.

Now use the `summary` function to print a simple summary of the distribution:

```
summary(g$nObs)
```

The `ggplot2` library has the ability to make "quick plots" with the command `qplot`. If we pass it a single column it will make a histogram of the data for that column. Let's try it:

```
qplot(nObs, data = g)
```

Our data are from 938 individuals. When the counts are less than this total, it's because some individuals had array data that was difficult to call a genotype for and so no genotype was reported.

Question: Do most of the SNPs have complete data?
Question: What is the lowest count observed? Is this number in rough agreement with what we know about how the genome-wide missingness rate filter was set to >98.5% of all SNPs

Calculating genotype and allele frequencies

Let's move on to calculating genotype and allele frequencies. For allele A_1 we will denote its frequency among all the samples as p_1, and likewise for A_2 we will use p_2.

```
# Compute genotype frequencies
g <- mutate(g, p11 = nA1A1/nObs , p12 = nA1A2/nObs, p22 = nA2A2/nObs )
# Compute allele frequencies from genotype frequencies
g <- mutate(g, p1 = p11 + 0.5*p12, p2 = p22 + 0.5*p12)
```

Question: With a partner or group member, discuss whether the equations in the code for p_1 and p_2 are correct and if so, why?

Run `head(g)` again and confirm `g` now has the extra columns for the genotype and allele frequencies.

And let's plot the frequency of the major allele (A2) vs the frequency of the minor allele (A1). The `ggplot2` library has the ability to make "quick plots" with the command `qplot`. Let's try it here:

```
qplot(p1, p2, data=g)
```

Notice that $p_2 > p_1$ (be careful to inspect the axes labels here) This makes sense because A_1 is supposed to be the minor (less frequent) allele. Note also that there is a linear relationship between p_2 and p_1

Question: What is the equation decribing this relationship?

The relationship exists because there are only two alleles - and so their proportions must sum to 1. The linear relationship you found exists because of this constraint. It also provides a nice check on our work (if p_1 and p_2 didn't sum to 1 it would suggest something is wrong with our code!).

Plotting genotype on allele frequencies

Let's look at an initial plot of genotype vs allele frequencies. We could use the base plotting functions, but the following uses the `ggplot2` commands. These are a little trickier, but end up being very compact (we need fewer lines of code overall to achieve our desired plot). To use `ggplot2` commands effectively our data need to be what statisticans call "tidy" (in this case, that means with one row per pair of points we will plot).

To do this, first we subset the data on the columns we'd like (using the `select` command and listing the set of columns we want), then we pass this (using the `%>%` operator) to the `melt` command which will reformat the data for us, and output it as `gTidy`:

```
gTidy <- select(g, c(p1,p11,p12,p22)) %>% melt(id='p1',value.name="Genotype.Proportion")
head(gTidy)
ggplot(gTidy) + geom_point(aes(x = p1,
                               y = Genotype.Proportion,
                               color = variable,
                               shape = variable))
```

Now let's look at the graph produced. There is some scatter in the relationship between genotype proportion and allele frequency for any given genotype, but at the same time there is a very regular underlying relationship between these variables.

Question: What are approximate relationships between p_{11} vs p_1, p_{12} vs p_1, and p_22 vs p_1? (Hint: These look like parabolas, which suggests are some very simple quadratic functions of p_1).

You might start to recognize that these are the classic relationships that are taught in introductory biology courses. If you recall, under assumptions that there is no mutation, no natural selection, infinite population size, no population substructure and no migration, then the genotype frequencies will take on a simple relationship with the allele frequencies. That is: $p_{11} = p_1^2$, $p_{12} = 2p_1(1-p_1)$ and $p_{22} = (1-p_1)^2$. In your basic texts, they typicaly use p and q for the frequencies of allele 1 and 2, and present these *Hardy-Wienberg proportions* as: p^2,$2pq$, and q^2.

Another way to think of the Hardy-Weinberg proportions is in the following way. If the state of an allele (A_1 vs A_2) is *independent* within a genotype, then the probability of a particular genotype state (such as A_1A_1) will be determined by taking the product of the alleles within it (so $p_{11} = p_1p_1$ or p_1^2).

Let's add to the plot lines that represent Hardy-Weinberg proportions:

```
ggplot(gTidy)+
  geom_point(aes(x=p1,y=Genotype.Proportion,color=variable,shape=variable))+
  stat_function(fun=function(p) p^2, geom="line", colour="red",size=2.5) +
  stat_function(fun=function(p) 2*p*(1-p), geom="line", colour="green",size=2.5) +
  stat_function(fun=function(p) (1-p)^2, geom="line", colour="blue",size=2.5)
```

On average, the data follow the classic theoretical expectations fairly well. It is pretty remarkable that such a simple theory has some bearing on reality!

By eye, we can see that the fit isn't perfect though. There is a systematic deficiency of heterozygotes and excess of homozygotes. Why?

Let's look at this more closely and more formally...

Testing Hardy Weinberg

Pearson's χ^2-test is a basic statistical test that can be used to see if count data conform to a particular expectation. It is based on the X^2-test statistic:

$$X^2 = \sum_i \frac{(o_i - e_i)^2}{e_i}$$

which follows a χ^2 distribution under the null hypothesis that the data are generated from a multinomial distribution with the expected counts given by e_i.

Here we compute the test statistic and obtain its associated p-value (using the `pchisq` function). We keep in mind that there is 1 degree of freedom (because we have 3 observations per SNP, but then they have to sum to a single total sample size, and we have to use the data once to get the estimated allele frequency, which reduces us down to 1 degree of freedom).

```
g <- mutate(g, X2 = (nA1A1-nObs*p1^2)^2 /(nObs*p1^2) +
            (nA1A2-nObs*2*p1*p2)^2 / (nObs*2*p1*p2) +
            (nA2A2-nObs*p2^2)^2 / (nObs*p2^2))
g <- mutate(g,pval = 1-pchisq(X2,1))
```

The problem of multiple testing

Let's look at the top few p-values:

```
head(g$pval)
```

How should we interpret these? A p-value gives us the frequency at which that the observed departure from expectations (or a more extreme departure) would occur if the null hypothesis is true. As an agreed upon standard (of the frequentist paradigm for statistical hypothesis testing), if the data are relatively rare under the null (e.g. p-value $< 5\%$), we reject the null hypothesi, and we would infer that the given SNP departs from Hardy-Weinberg expectations. This is problematic here though. The problem is that we are testing many, many SNPs (Use `dim(g)` to remind yourself how many rows/SNPs are in the dataset). Even if the null is universally true, 5% of our SNPs would be expected to be rejected using the standard frequentist paradigm. This is called the multiple testing problem. As an example, if we have 50,000 SNPs, that all obey the null hypothesis, we would on average naively reject the null for ~2500 SNPs based on the p-values < 0.05.

We clearly need some methods to deal with the "multiple testing problem". Two frameworks are the Bonferroni approach and false-discovery-rate (FDR) approaches. We will not say more about these here. Instead, we will do two simple checks to see though if our data are globally consistent with the null.

First, let's see how many tests have p-values less than 0.05. Is it much larger than the number we'd expect on average given the total number of SNPs and a 5% rate of rejection under the null?

```
sum(g$pval < 0.05, na.rm = TRUE)
```

Wow - we see many more. This is our first sign that though by eye these data show qualitiative similarities to HW, statistically they are not fitting Hardy-Weinberg well enough.

Let's look at this another way. A classic result from Fisher is that under the null hypothesis the p-values of a well-designed test should be distributed uniformly between 0 and 1. What do we see here?

```
qplot(pval, data = g)
```

The data show an enrichment for small p-values relative to a uniform distribution. Notice how the whole distribution is shifted towards small values - The data appear to systematically depart from Hardy-Weinberg.

Plotting expected vs observed heterozygosity

To understand this more clearly, let's make a quick plot of the expected vs observed heterozygosity (the proportion of heterozygotes):

```
qplot(2*p1*(1-p1), p12, data = g) + geom_abline(intercept = 0,
                                                slope=1,
                                                color="red",
                                                size=1.5)
```

Most of the points fall below the y=x line. That is, we see a systematic deficiency of heterozygotes (and this implies a concordant excess of homozygotes). This general pattern is contributing to the departure from HW seen in the X^2 statistics.

Discussion: Population subdivision and departures from Hardy-Weinberg expectations

We might wonder why the departure from Hardy-Weinberg proportional is directional, in that, on average, we are seeing a deficiency of heterozygotes (and excess of homozygotes). One enlightening way to understand this is by thinking about what Sewall Wright (a former eminent University of Chicago professor) called "the correlation of uniting gametes". To produce an A_1A_1 individual we need an A_1-bearing sperm and an A_1-bearing egg to unite. If these events were independent of each other, we would expect A_1A_1 individuals at the rate predicted by multiplying probabilites, that is, p_1^2 (an idea we introduced above). However, what if uniting gametes are positively correlated, in that an A-bearing sperm is more likely to join with an A-bearing egg? In this case we will have more A_1A_1 individuals than predicted by $2p_1^2$, and conversely fewer A_1A_2 individuals than predicted by $2p_1p_2$. If our population is structured somehow such that A_1 sperm are more likely to meet with A_1 eggs, then we will have such a positive correlation of uniting gametes, and the resulting excess of homozygotes and deficiency of heterozygotes.

Given the HGDP data is from 52 sub-populations from around the globe, and alleles have some some probability of clustering within populations, a good working hypothesis for the deficiency of heterozygotes in this dataset is the presence of some population structure.

While statistically significant, the population structure appears to be subtle in absolute terms — based on our plots, we have seen the genotype proportions are not wildly off from HW proportions.

Question: As an exercise, compute the average deficiency of heterozygotes relative to the expectated proportion. This is the average of

$$\frac{2p_1(1-p_1) - p_{12}}{2p_1(1-p_1)}$$

What is this number for this data-set? A common "rule-of-thumb" for this deficiency in a global sample of humans is approximately 10%. Do you find this to be true from the data?

A ~10% difference between expected and observed seems pretty remarkable given these samples are taken from across the globe. It is a reminder that human populations are not very deeply structured. Most of the alleles in the sample are globally widespread and not sufficiently geographically clustered to generate correlations among the uniting alleles. This is because all humans populations derived from an ancestral population in Africa around 100-150 thousand years ago, which is relatively small amount of time for variation across populations to accumulate.

Finding specific loci that are large departures from Hardy-Weinberg

Now, let's ask if we can find any loci that are wild departures from HW proportions. These might be loci that have erroneous genotypes, or loci that cluster geographically in dramatic ways (such that they have few heterozygotes relative to expectations).

To find these loci, we'll compute the same relative deficiency you computed above, but let's look at it per SNP. This number is referred to as F by Sewall Wright and has connections directly to correlation coefficients (advanced exercise: Try to work this out!). If we assume there is no inbreeding within populations, this number is an estimator of F_{ST} (a quantity that appears often in population genetics).

Let's add this value to our dataframe and plot how it's value changes across the chromosome from one end to another:

```
g <- mutate(g, F = (2*p1*(1-p1)-p12) / (2*p1*(1-p1)))
plot(g$F, xlab = "SNP number")
```

There are a few interesting SNPS that show either a very high or low F value.

Now, here's a trick. When a high or low F value is due to genotyping error, it likely only effects a single SNP. However, when there is some population genetic force acting on a region of the genome, it likely effects multiple SNPs in the region. So let's try to take a local average in a sliding window of SNPs across the genome, computing an average F over every 5 consecutive SNPs (in real data analysis we might use 100kb or 0.1cM windows).

The `stats::filter` command below calls the `filter` function from the `stats` library. The code above instructs the function to take 5 values centered on a focal SNP, weighting them each by 1/5 and then taking the sum. In this way it produces a local average in a sliding window of 5 SNPs. Let's define the `movingavg` function and then make a plot of its values:

```
movingavg <- function(x, n=5){stats::filter(x, rep(1/n,n), sides = 2)}
plot(movingavg(g$F), xlab="SNP number")
```

Wow — there appears to be one large spike where the average F is approximately 60% in the dataset!

Let's extract the SNP id for the largest value, and look at the dataframe:

```
outlier=which (movingavg(g$F) == max(movingavg(g$F),na.rm=TRUE))
g[outlier,]
```

Question: Which SNP is returned? By inserting the rs id into the UCSC genome browser (https://genome.ucsc.edu/), and following the links, find out what gene this SNP resides near. The gene names should start with "SLC.." What gene is it?

Question: Carry out a literature search on this gene using the term "positive selection" and see what you find. It's thought the high F value observed here is because natural selection led to a geographic clustering of alleles in this gene region. Discuss with your partners why this might or might not make sense.

Discussion: The outlier reigon

The region you've found is one of the most differentiated betwen human populations that is known. Notice in your literature search, how it is known to affect skin pigmentation and is thought to contribute to differences in skin pigmentation that are seen between human populations. Finding strong population structure for alleles that affect external morhological phenotypes is not uncommon when looking at other chromosomes. Some of the most differentiated genes that exist in humans are those that involve morphological phenotypes - such as skin pigmentation, hair color/thickness, and eye color (the genes OCA2/HERC2, SCL45A2, KITLG, EDAR all come to mind). Many of these are thought to have arisen due to direct or indirect effects of adaptation to local selective pressures (e.g. adaptation to varing levels of UV exposure, local pathogens, local diets, local mating preferences), though in most cases we still do not yet have a fully convincing understanding of their evolutionary histories. Regardless of the reasons, it is notable that many of the features that humans see externally in each other (i.e. the morphological differences) are controlled by genes that are outliers in the genome. At most variant SNPs, the patterns of variation are much closer to those of a single random mating populations than they are at variant sites like EDAR. Put another way, a genomic perspective shows us many of the differences people see in each other are in a sense, just skin-deep.

Wrap-up

Modern population genetics has a lot of additional tools on its workbench, but here using relatively simple and classical ideas combined with genomic-scale data, we have been able to observe and interpret some major features of human genetic diversity. We have also revisited some basic concepts of probablity and statistics such as indepedence vs correlation, the χ^2 test, and the problems of multiple testing. One remarkable thing we saw is that a very simple mathematical model based on assuming independence of alleles of genotypes can predict genotype proportions within ~10% of the true values. This gives us a hint of how simple mathematical models may be useful even in the face of biological complexity. Finally, we have gained more familiarity with R. We didn't discuss how genotyping errors that might create Hardy-Weinberg departures, but if we were doing additional analyses, we could use Hardy-Weinberg departures to filter them from our data. It's common practice to do so, but with a Bonferonni correction and using data from within populations to do the filtering.

Follow-up activities

In the 'addons' folder, we are including data files that you can explore to gain more experience. These include global data for other chromosomes (`H938_chr*.geno`) and the same data but limited to European populations (`H938_Euro_chr*.geno`). Here are a few suggested follow-up activities. It may be wise to split the activities across class members and reconvene after carrying them out.

Follow-up actvity: Look at a chromosome from the European-restricted data - is the global deficiency in heterozygosity as strong as it was on the global scale? Before you begin, what would you expect to see?

Follow-up activity Using the European data, do you find any regions of the genome that are outliers for F on chromosome 2? Using genome browsers and/or literature searches, can you find what is the likely locus under selection for that region?

Follow-up activity: Using the global data or the European data, analyze other chromosomes – do you find other loci that show high F values?

References

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Characterizing Neural Responses: feature selectivity, spiking statistics, and information

Leslie Osborne, Matthew Macellaio

Contents

Opening the black box

When you drop an electrode into the brain, you can detect the tiny electrical currents emitted by neurons as they communicate. The currents are typically brief pulses termed "spikes", and it is the temporal pattern of spikes and the intervening silences that constitute the code by which neurons transmit information. Reading that code has been, and continues to be, one of the central challenges of the field. In this tutorial you will learn how to analyze the responses of a sensory neuron to determine what features of the sensory stimulus trigger spikes, how variable that response is, and how much information about stimulus features those spikes encode – in other words, how to (begin to) answer the question, "what does this neuron do?"

You will be analyzing the responses of a neuron in extrastriate cortical area MT, a visual area in which many neurons respond selectively to visual motion stimuli. The recording used an extracellular electrode to detect voltage fluctuations near the membrane surface while visual stimuli were displayed on a monitor. We have filtered that electrical signal and isolated the action potentials (spikes) for you.

Before we start, let's get our R environment set up. Enter the following command into your RStudio console to set RStudio's working directory to our workshop folder.

You can copy and paste any commands given here directly into your **Console** window, or type them in yourself. Anything that comes after a # sign is what we call a comment: we use it to clarify to users how the code works, but the # tells R to ignore it. You can paste comments into the console without them affecting the calculations. At any time, if you wish to clear your workspace, type in `rm(list=ls())`.

```
setwd("~/GitHub/BSD-QBio/Workshops/Osborne/Code")
```

Load the data file `MTneuron.RData` into R, and display its contents.

```
load("../Data/MTneuron.RData")
print(ls())
```

You should see a data array:

```
RFmap
```

Inspect the size of `RFmap`.

```
dim(RFmap)
```

Notice that `RFmap` has dimensions of y–position (10) by x–position (15) by repetition (16) by spike times (maximum number 24). We'll explain the experiment below, but the x,y dimensions are positions on the screen for a visual stimulus, the repeats indicate how many times we presented the stimulus in a location, and the spikes are the time stamps of the recorded action potentials (in milliseconds) relative to stimulus motion onset. The motion starts at 0ms and lasts for 250 ms, so none of the numbers will be greater than 250 ms.

Neural responses are variable. Although we repeated the stimulus presentations at the same location on the screen, for each presentation the neuron fires a different number of spikes. Look at one entry in the array, say (3,10,16).

```
RFmap(3,10,16)
```

On this trial the neuron fired 24 spikes, which turns out to be the most it ever fired during this experiment. If you look at a different stimulus location or different repeat, you will see fewer numbers indicating spike times and zeros which pad the empty spaces in the array. To count the number of spikes fired at each location and on each repeat, you need to count the non-zero values in the array.

Exercise 1. Map the receptive field of a neuron in cortical area MT

An experimenter's first task is to locate the neuron's receptive field (RF) within the "visual field" so that stimuli can be properly configured to drive the neuron. The visual field refers to space with respect to the fovea of the retina — the sweet spot where you have the highest density of photoreceptors and therefore the greatest spatial acuity . It is customary to define the size of a visual image in angular units as if the eye is sitting at the center of a sphere. One degree of visual angle is about the apparent size of your thumb if you hold out your arm.

We searched for the location of the neuron's RF by presenting small patches of moving dots at different screen locations and recording the activity of the neuron. The array `RFmap` represents the neuron's response to the moving dot patterns with respect to location on the screen. The random dot patterns moved within a 2° by 2° (square) aperture that was positioned on a 15 x 10 grid with 2° spacing. The center of the screen is located at grid position ($x = 12, y = 9$) with the grid position (1,1) in the upper left corner of the screen. The entire stimulus set tiled a 30° by 20° field of view, from $-22°$ to $+6°$ in x and $-2°$ to $+16°$ in y with respect to the center of gaze. The stimulus moved for 250 ms and was repeated 16 times at each location.

The receptive field of a neuron is usually defined as the part of the visual field to which the neuron responds most strongly. We can determine the receptive field by counting how many spikes the neuron fires as a function of where the stimulus is shown on the screen (the grid positions). This exercise involves counting the spikes at each spatial location across

all repeats. Here, we will loop through each x and y position on the grid and count the spikes this cell fired in response to each position.

```
#at each grid position
for (ypos in 1:10){
    for (xpos in 1:15){

# count the spikes (non-zero entries) in the 3rd (repeats) and 4th (spike times) dimensions of RFmap
#If you want to select all values along a dimension of your array, leave a space between the commas indicating the dimensions
        inds <- sum(RFmap[ypos,xpos, ,] !=0)

        #store the sum as a function of x,y grid location
        numspks[ypos, xpos] <- inds
    } #don't forget to close your loops
}
```

Question: Neural activity is often described by a "firing rate", i.e. the frequency with which spikes are generated for a particular stimulus. What is the average firing rate (i. e. spikes per second) of the neuron?

How to plot the a 2D color density map of the neural responses: If $\vec{x}$ and $\vec{y}$ are vectors indicating locations in the stimulus grid, you can use the R command `image` to plot the average spike count at each location. The command `seq` generates a sequence from a minimum value, a maximum value, and an increment size.

```
x<- seq(-14,14,2)
y<- seq(9,-9,2)
nTrials <- dim(RFmap)[3]
#create a color density plot of the mean number of spikes fired per trial
image(x, y, numspks / nTrials)
```

You will get a tidier figure if you only include stimulus driven spikes, in this case, spikes that occur later than 50ms after the onset of the stimulus. Why? This neuron, like most, has a latency period before it will begin to fire spikes. This neuron's latency is about 50ms. So if you get spikes that happened later than 45ms then you are pretty sure to be counting all of the relevant spikes and ignoring the irrelevant spikes. Run the loop above again, using only the relevant spikes by substituting in the below code.

```
> numspks[ypos, xpos]<- sum(RFmap[ypos, xpos, , ] >45)
```

You might add a fixation point as well, to indicate the center of gaze and to see where the receptive field is located with respect to the fovea. You can make the plot full-screen by

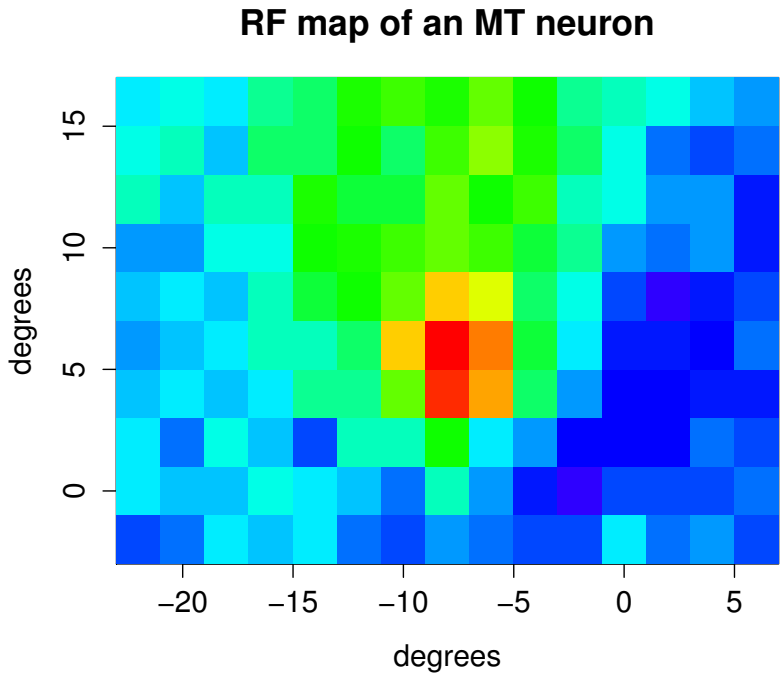


Figure 1: The receptive field map showing spike count as a function of location, with red indicating high spike count and blue indicating low.

clicking the **Zoom** button in your **Plots** tab. A more publication-ready plotting method is the function `filled.contour`, which interpolates and smooths between the sampled points.

```
filled.contour(x, y, numspks/nTrials, nlevels = 30,
              plot.title = title(main = "RF map of an MT neuron",
                                 xlab = "degrees", ylab = "degrees"),
              # choose colors
              col = rev(rainbow(30,start=0,end=0.7)),
              # add a point
              plot.axes={
                axis(1); # plot the x−axis
                axis(2); # plot the y axis
                # The center of the visual field of the monkey was at (7.5, −7.5)
                # Let's put a + sign to mark the spot
```

```
            points(0, 0, pch = "+", cex = 2, col = "white", font = 2)
               }
        )
```

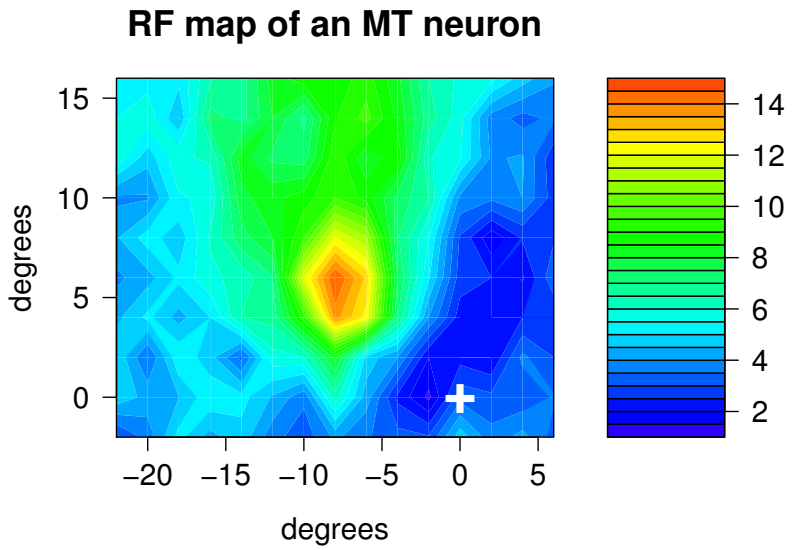


Figure 2: The RF map counting only spikes occurring > 45ms post stimulus onset. The white cross indicates the direction of gaze. Spike count values have been smoothed.

The script file `plot_RFmap_v2.R` is a complete algorithm. Consult it for help, more comments, or run it to check your work.

Exercise 2. Plot a direction tuning curve

Many sensory neurons are "tuned" for a particular feature of a stimulus, meaning that the firing rate changes smoothly as a function of that value. In MT, many neurons are tuned for the direction and the speed of motion with the highest firing rate for a preferred direction and preferred speed. We have given you data of 8 MT neurons responding to a range of motion directions and speeds to show their direction and speed tuning. Clear your workspace, then load the data from these neurons.

```
rm(list=ls())

load("../Data/MTneurons8.RData")
print(ls())
```

The data array `spikes` contains the responses of all 8 MT neurons to motion in 24 different directions, spaced about the circle in 15° incremements. The visual stimulus consisted of random patterns of dots that moved coherently (all had the same direction and speed) in an aperture window centered on each neuron's RF. Each trial in the experiment consisted of a 200ms motion step in a single direction at one speed. Inspect the array `spikes` to see how the data is organized.

```
dim(spikes)
```

The array `spikes` has the dimensions directions (24) by speeds (8) by trials (32) by time (200 milliseconds) by cells (8). Instead of spike times as before, we now have placed a "1" into each time bin in which a spike occurred on that trial for a specific stimulus, and bins with no spikes indicated by a 0. The stimuli for each cell were repeated a different number of times, so we have also included the array `nReps`, a vector containing the number of repetitions recorded for each cell. If a cell was recorded for less than 32 trials, we have padded the trials with NaN (stands for "not a number", an indicator that there is no data point there). The vectors `directions` and `speeds` contain the recorded directions and speeds of motion, and `dates` contains the information of when each cell was recorded.

Let's start by applying the skills you learned in Exercise 1 to find the average number of spikes fired for each motion direction from the array `spikes` and then plot that value as a function of direction. The direction values are stored in the vector `directions`), and the speed values are stored in the vector `speeds`.

Refer to `plot_tunecurve_v3.R` for more tips if needed.

Type

```
nReps[1]
```

to display the number of repeats for each motion direction for neuron 1. You can see that this cell was recorded for 32 trials at each direction. Let's collect the spikes for only the first cell, and only on the trials that we recorded.

```
cellnum<-1
spikes_cell<-spikes[,,1:nReps[cellnum],,cellnum]
```

How many spikes did the cell fire on a trial to a specific stimulus? As an example, sum the number of spikes on the first trial to direction #13 and speed #4: 15°, 8°/sec:

```
sum(spikes_cell[13,4,1,])
```

You should find that the cell fired 11 spikes in this time window on this trial. To calculate the mean spike count for all directions, we sum over time, then take the mean across trials. We use the function `rowSums` to sum across the 4th dimension (time) by telling the function to keep the first three dimensions. We then can use `rowMeans` in a similar fashion to take the mean along the 3rd dimension (trials):

```
spikecount_sum<-rowSums(spikes_cell,dims=3)
spikecount_mean<-rowMeans(spikecount_sum,dims=2)
```

You should now have an array `spikecount_mean` with dimensions 24 by 8, containing mean spike count for each combination of direction and speed stimuli presented. To find the direction tuning curve, collapse across speeds by taking the mean along the 2nd dimension, and scale by the amount of time recorded (200ms) to get tuning in units of spikes/second.

```
mean_rates_dir<-rowMeans(spikecount_mean)*(1000/200)

# Plot the tuning curve
print(
  plot(directions, mean_rates_dir,
       xlab = 'direction (degrees)', # x label
       ylab = 'Average firing rate (spikes/s)',
#set title
       main = paste('Direction tuning curve'),
#set y axis limits to fit everything
       ylim=c(0,70)
  )
)
```

To add error bars, calculate the standard deviation across trials first. First, make an empty vector to concatenate your standard deviation values for each direction, then add lines indicating error bars to your plot:

```
sddir<-vector()
for (dir in 1:length(directions)){
#c(x,y) combines x and y into a single vector
#sd(x) calculates standard deviation of a vector
  sddir<-c(sddir,sd(spikecount_sum[dir,,]*(1000/200) ))
}

#plot vertical segments of errorbars
segments(directions, mean_rates_dir-sddir,directions, mean_rates_dir+
   sddir)
#width of top/bottom of errorbars
segwidth = 2
segments(directions- segwidth,mean_rates_dir-sddir,directions+ segwidth,
   mean_rates_dir-sddir)
segments(directions- segwidth,mean_rates_dir+sddir,directions+ segwidth,
   mean_rates_dir+sddir)
```

You will generate a figure that looks like this:

We call the direction that elicits the highest firing rate for the cell the "preferred direction" of this cell. We can identify this cell's preferred direction by identifying the

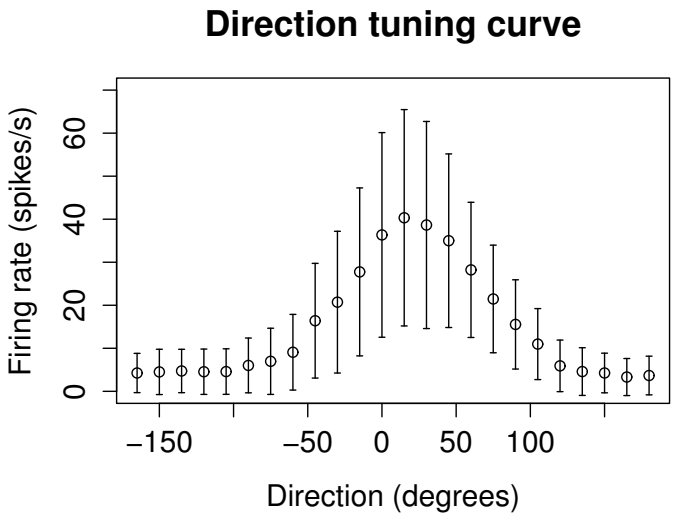


Figure 3: The firing rate in spikes per second for each motion direction around the circle. Markers indicate the mean over repeats, and errorbars indicate SD. Here we pooled data with different stimulus speeds.

stimulus eliciting the maximum spike count:

```
maxdir_index<-which(mean_rates_dir==max(mean_rates_dir)
directions[maxdir_index]
```

Question: What is the background firing rate of this cell? Tip: use spikes occurring at times earlier than 60ms after motion onset. Remember that neurons have a response latency. For the cell you are working with now, the response latency is approximately 70ms. Spikes occurring at earlier times cannot be driven by the motion of the stimulus. Rather, they occur from background network activity. How does the level of background activity affect your ability to detect a motion stimulus from this neuron's spikes?

Question: How large is the difference between the firing rate at the preferred direction and at the opposite direction?

Exercise 3: Plot a speed tuning curve and direction-speed tuning

This recording also includes data about this cell's responses to speed, so we can calculate the speed tuning in the same way as we calculated direction tuning. Notice that speed tuning is plotted in log_2 units: plotting (and sampling) in log units allows us to understand patterns at the wide range of speeds to which MT neurons respond.

```
mean_rates_spd<-colMeans(spikecount_mean)*(1000/200)

print(
  plot(log2(speeds),
       mean_rates_spd,
       xlab = 'log2(speed) (degrees per second)', # x label
       ylab = 'Average firing rate (spikes/s)',
       main = paste('Speed tuning curve'),
       ylim=c(0,80)
  )
)

sdspeed<-vector()
for (speed in 1:length(speeds)){
  sdspeed<-c(sdspeed,sd(spikecount_sum[,speed,]*(1000/200) ))
}

#plot vertical segments of errorbars
segments(log2(speeds), mean_rates_spd-sdspeed,log2(speeds), mean_rates_
    spd+sdspeed)
#width of top/bottom of errorbars
segwidth = 0.02
segments(log2(speeds)-segwidth,mean_rates_spd-sdspeed,log2(speeds)+
    segwidth,mean_rates_spd-sdspeed)
segments(log2(speeds)-segwidth,mean_rates_spd+sdspeed,log2(speeds)+
    segwidth,mean_rates_spd+sdspeed)
```

You may notice that the error bars are quite large, so it may seem difficult to distinguish what speed was presented to the cell based on the cell's firing rate. Since cells in MT modulate their firing rates to both direction and speed, try plotting speed tuning at the cell's preferred direction. You can alter this code to also plot direction tuning at a single speed to compare to direction tuning at all directions.

Here, we use the plot command `points` to plot the speed tuning for one direction on the same plot as we previously plotted speed tuning for all directions. The last argument in the `print` command functions to change the color.

```
mean_rates_1spd<-as.vector(spikecount_mean[13,])*(1000/200)

print(
  points(log2(speeds),
       mean_rates_1spd,
       xlab = 'log2(speed) (degrees per second)', # x label
```

```
      ylab = 'Average firing rate (spikes/s)',
      main = paste('Speed tuning curve'),
      ylim=c(0,80),
      col='red'
  )
)

#get sd again
sd1speed<-vector()
for (speed in 1:length(speeds)){
  sd1speed<-c(sd1speed,sd(spikecount_sum[20,speed,]*(1000/200) ))
}

#plot vertical segments of errorbars
segments(log2(speeds), mean_rates_1spd-sd1speed,log2(speeds), mean_rates
    _1spd+sd1speed,col='red')
#width of top/bottom of errorbars
segwidth = 0.02
segments(log2(speeds)-segwidth,mean_rates_1spd-sd1speed,log2(speeds)+
    segwidth,mean_rates_1spd-sd1speed,col='red')
segments(log2(speeds)-segwidth,mean_rates_1spd+sd1speed,log2(speeds)+
    segwidth,mean_rates_1spd+sd1speed,col='red')
```

You can add more speed tuning curves at different directions to this plot to visualize how speed tuning can change as a function of direction.

We can move one step further by plotting the two-dimensional tuning curve for this cell: how firing rate changes as a function of both direction and speed. One easily visual way to do so is to plot a heat map, as we did with receptive field mapping. We've calibrated the colors so that blue indicates low spike counts, and red indicates high spike counts.

```
image(directions,log2(speeds),spikecount_mean, col=rev(rainbow(30,start
    =0,end=0.7)))
```

```
filled.contour(directions,log2(speeds),spikecount_mean,nlevels=30,
             plot.title = title(main = 'spike count for an MT neuron',
                                xlab = 'direction (degrees)',
                                ylab = 'log(speed) (degrees per second)',)
                                   ,
             col=rev(rainbow(30,start=0,end=0.7))
)
```

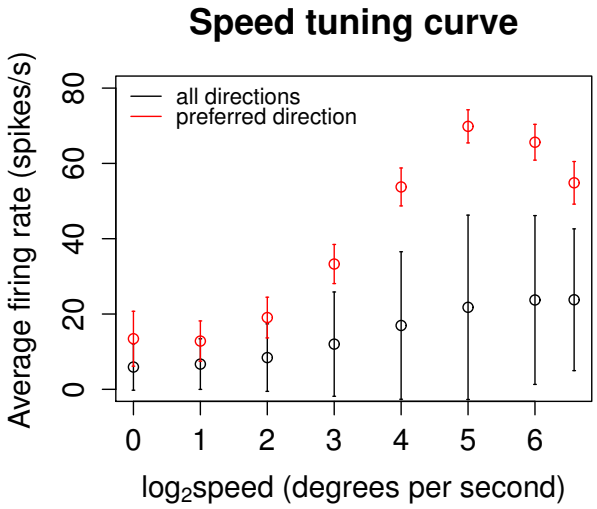
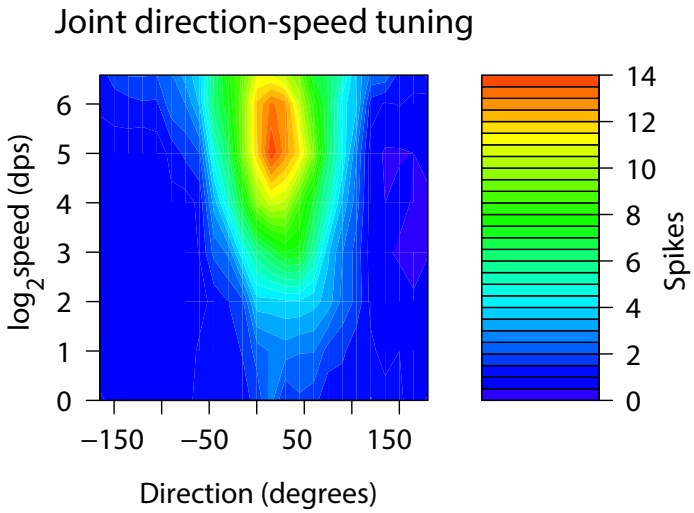


Figure 4: Firing changes changes smoothly with speed. Black circles indicate the direction-averaged rate. Red circles indicate firing rate for $\theta = 15°$



Exercise 4: Plot the firing rate as a function of time and direction

By summing across time, we're losing a great deal of information that the neuron is communicating by changing its firing rate over time. In this exercise you will be forming a peri–stimulus time histogram (PSTH) to visualize those changes and uncover clues to more complex interactions between neurons.

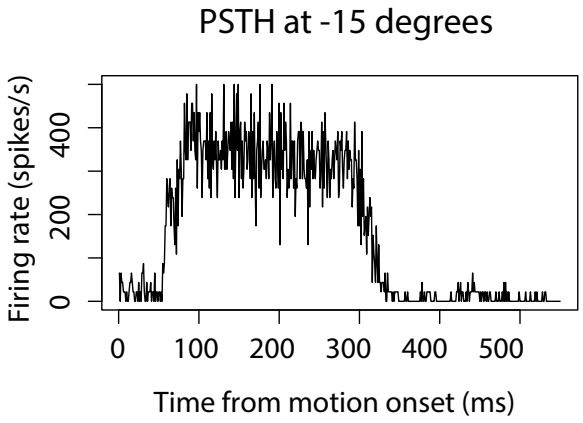


Figure 5: Average firing rate of an MT neuron over time to motion direction of -15 degrees.

The PSTH is the firing rate of the neuron, determined by averaging the response across repeats of the stimulus. If you compute the PSTH for each direction and plot what you get in direction and time, you'll be making this plot. In practice, you want to create an array called PSTH that is directions (24) by time (550) (or vice versa) and use the `plot` command to plot the PSTH for one direction or the `image` to plot the result for all directions as a 2D color density plot. Now load data from another neuron with many repeats to see how direction tuning changes over time. In this experiment, the stimulus moved for the first 250ms, then stopped moving while we continued to record the neuron's responses for another 300ms.

If you need to jump ahead or check your work, the script `plot_2Dpsth_v3.R` for the full code.

First, we'll load the data and make an empty array called PSTH with dimensions 550 (time) by 24 (directions) to fill in with the mean spike rate for each time bin.

```
load("../Data/MTneuron_long.RData")
maxTime<-550
nDirs<-length(directions)
PSTH <- array(0, c(maxTime, nDirs))
```

The array `spikes_long` contains spikes as before: there is a 1 in any time 1–ms bin in which a spike was fired. You should also have a variable `nReps_long` that indicates the number of repeats for each motion direction. Compute the PSTH, which is the neuron's trial–averaged response as a function of time for each motion direction.

```
 for (n in 1:nDirs){
#average just over the actual trials for each direction, then multiply by 1000 to get
    units of spikes per second
PSTH[ ,n] <- rowMeans(mydata[, n, 1:(nReps[n])])*1000
 }
```

Now, we can display the neuron's time-varying firing rate by plotting the PSTH at the preferred direction. You can prove to yourself using code from earlier that direction #12 is this neuron's preferred direction.

```
plot(PSTH[,12],
     type = "l",     # plot lines instead of points for a cleaner figure
     xlab = 'time from motion onset (ms)',  # x label
     ylab = 'Average firing rate (spikes/s)', # y label
     main = paste('PSTH of MT neuron response to ', directions[12], '
         degrees') #title
)
```

This neuron shows a clear preference for motion over a static stimulus. You can also see that the neuron's firing rate is higher at the beginning of the response, peaking around 100ms. We call that the "transient" response, and it quickly decays to a slightly lower firing rate for the rest of the duration of the stimulus: the "sustained" response. If we plot the PSTH for all directions on the same figure, even more interesting timing dynamics come to light. We'll use `filled.contour` again to display those.

```
print(
  filled.contour(1:maxTime, directions, (PSTH),
    nlevels = 30,
                  plot.title = title(main = 'PSTH vs direction for an MT
                      neuron',
                   xlab = 'time since motion onset (ms)'),
     ylab = 'Direction (degrees)',
    # choose colors
    col = rev(rainbow(28,start=0,end=2/3))
  )
)
```

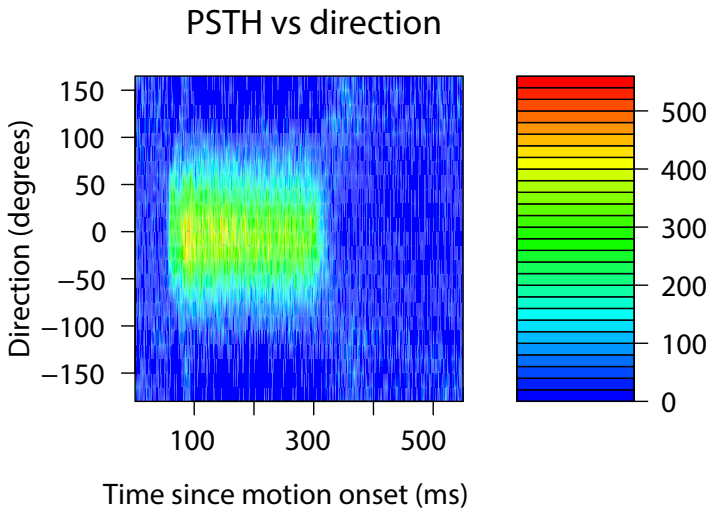


Figure 6: Average firing rate over time of an MT neuron.

Click the **Zoom** button at the top of your **Plots** tab to make this plot full-screen. What features of the cell's response do you see? Compare the background firing rate (spikes before 50ms) to the firing rate while the stimulus is on. Try plotting the PSTH for single directions to highlight these firing rate changes.

Are there any times while the stimulus is moving, or after it stops moving, that the firing rate appears to be lower than the background firing rate? Why might that happen? What effect could that have on perception?

Exercise 5: Direction and speed tuning over time

Let's now return to our other set of neurons modulated by directions and speeds, and apply what we've seen about timing dynamics to see how direction and speed tuning change and interact at different points in the neural response. A trick we can use to smooth out some of the variability in spiking behavior is to bin spikes over small time windows. We can select specific time bins, sum spikes over those and take the mean across trials.

To simplify our computations, let's also reshape the `spikes_cell` array to put the time (4th) dimension first, using the `aperm` function. In this way, we can easily take the mean across trials while keeping the time dimension unaffected. A helpful R command to quickly sum spikes in each time bin is `colSums`, which takes the sum of an array along its first dimension (in this case, time), leaving us with an array of total spikes fired for each repetition of each stimulus direction presentation. While we're doing this, we can find out

the maximum spikes per time bin to use for our plot axis in the next step.

```
binsize<-10
spikes_rot<-aperm(spikes_cell,c(4,1,2,3))
#create this array now so you can fill it during the loop
spikect_binmean<-array(0,c(dim(spikes_rot)[1]-binsize,length(directions)
    ,length(speeds)))

for (timebin in 1:(dim(spikes_rot)[1]-binsize)){
  spikect_binsum<-colSums(spikes_rot[timebin:(timebin+binsize),,,],dims
      =1)
  spikect_binmean[timebin,,]<-rowMeans(spikect_binsum,dims=2)
}
maxsps<-max(spikect_binmean)
```

Now we have a variable, `spikect_binmean`, that has the mean spike count in 10–millisecond sliding time windows for each direction and speed. We can cycle through this, plot each time bin using `filled.contour` heat maps, and see the spike count change over time.

Question: Does the preferred speed or preferred direction change over time? What does this mean for how the brain might "read out" or interpret the activity of visual neurons to identify the stimulus?

```

#toString to convert numbers to strings
#Sys.sleep(seconds) pauses the calculations and plotting
#You can speed up the animation by changing the increment value in
#     seq or by reducing the Sys.sleep time
#press the escape (esc) key to break out of the loop and animation

numlevels<-30
for (timebin in seq(1,(dim(spikes_rot)[1]-binsize),1)){
  filled.contour(directions,log2(speeds),spikect_binmean[timebin,,],
      levels=seq(0,maxsps,(maxsps/numlevels)),
                plot.title = title(main = paste('spike count from',
                    toString(timebin),'to',toString(timebin+binsize),'ms')
                    ,
                                  xlab = 'direction (degrees)',
                                  ylab = 'log(speed) (degrees per second)'
                                      ,),
                col=rev(rainbow(numlevels,start=0,end=0.7)))
  Sys.sleep(0.1)
}
```

Try using different bin sizes. At what point does it seem like there is no change in the dynamics over time?

Question: Consider the challenge of trying to read the spikes. The time scale over which you count spikes will determine what you think the motion direction and speed are when tuning is dynamic. What are the trade offs between sampling on very short (ms) timescales vs. have a longer integration time? What if you are trying to "listen" for this neuron in a background of noise where it is harder to resolve individual spikes? Will that affect will that have on your choice of time bin?

You can also use the tricks from the PSTH exercise to plot just the time—direction PSTH or the time—speed PSTH to uncover patterns in the data.

Don't forget that there are 7 more cells in the `spikes` variable. You can take a look at any of those by entering

```
spikes_cell<- spikes[, ,1:nReps[k] , , k]
```

for cell k, then running the code in this exercise, to see the variability in different cells' timing dynamics.

The statistics of neural responses

How reliable are the neuron's responses to repeated stimuli? If we controlled all of the inputs to the neuron, the answer is that its outputs would be extremely reliable (e. g. Mainen ZF, Sejnowski TJ (1995) Reliability of spike timing in neocortical neurons. Science 268: 1503-1506). Therefore, the cellular mechanism of generating spikes is not inherently noisy. However, while recording from a neuron embedded in the brain, the responses to repeated presentations of visual stimuli are variable because we control only a limited number of experimental parameters that can modulate a neuron's input. In this exercise you will characterize the variability in an MT neuron's response from the experimenter's point of view. How much of that variability constitutes noise that affects the brain's estimate of motion from that neuron's responses is an open (and interesting) question.

To get a visual sense of the variability in spiking to repeated motion stimuli, run the script `plot_MTraster_v2.R` to create a raster plot of the spike times on different trials for this data set. You may be able to see patterns in the data that you didn't see with the PSTH in the last exercise.

Type

```
speed<-4
```

(for example) to plot the raster of all directions for the 4th speed in the list of speeds. Each row of a raster plot indicates a spike time on one stimulus presentation with a dot or line. The rows indicate the neuron's responses on different trials. Color indicates the different direction values in the same order as directions. Remember that the motion stimulus begins at time 0 and lasts for 200 ms. What is the latency of this neuron's response to motion? Does the latency depend on direction? What do you notice about the variability of spiking during driven vs. spontaneous firing periods?

The time at which spikes are fired relative to the onset of visual motion varies from trial to trial, as does the number of spikes fired. Suppose that MT neurons encode motion direction with the number of spikes fired in a certain time window. In that case, variability in the spike count will create variability in the estimates of motion direction for any downstream decoding of this neuron's responses.

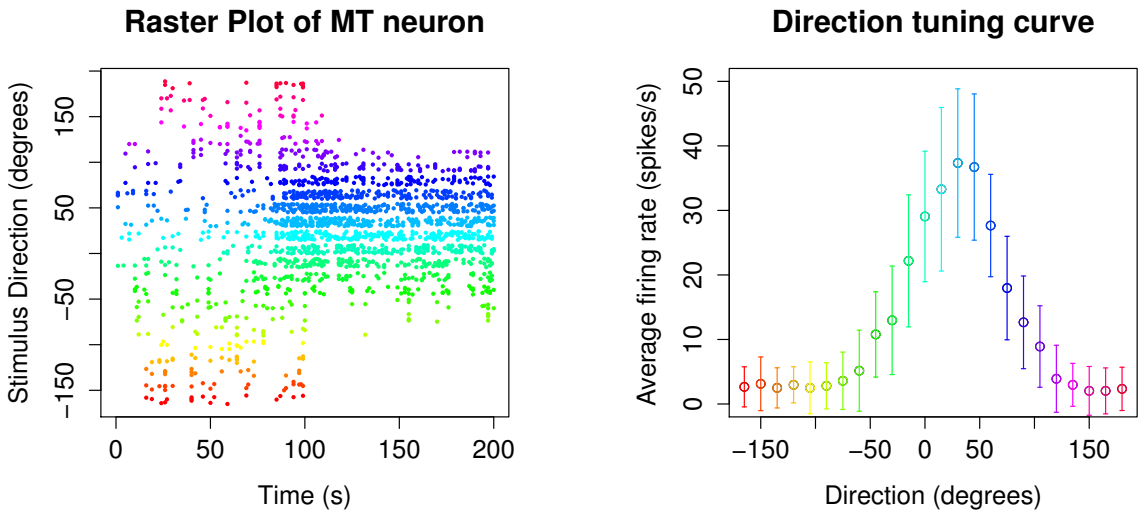


Figure 7: Left panel: Each dot represents a spike. Each color indicates a different motion direction. Right panel: Mean firing rate, colored to correspond to directions in left panel.

Exercise 6. Calculate the probability of spiking for a range of directions

What is the probability of observing a particular count value in a time window of duration T, given the motion direction, θ? i. e. $P_T(n \mid \theta = \theta_i)$. How does the shape of the distribution change with direction (and thus with the average firing rate)? Choose the preferred direction and an off-preferred direction and compare the count distributions by making a figure: you can find these directions from the tuning curve plot you made, or the 2D PSTH plot that shows the firing rate change with direction. Choose a time window of 200 ms from motion onset, and then try other time windows if you have time. The objective of this exercise is to gain experience in computing a probability distribution and to gain insight about spike count variability.

A useful R command for measuring the fraction of trials is `hist(array, breaks)`, which outputs a histogram, computing the number of data points contained in bins, whose edges are defined by `breaks.hist(array, breaks)` returns a structure, and you will want the variable `counts` within that structure, which contains the counts within each bin. To access variables within a structure, you use the $ operator, as shown below.

```
#set up variables again to make sure
load("../Data/MTneurons8.RData")
cellnum<-1
spikes_cell<-spikes[,,1:nReps[cellnum],,cellnum]
spikecount_sum<-rowSums(spikes_cell,dims=3)
```

```
spikehist<-array(0,c(length(directions),length(speeds),(max(spikecount_
    sum)+1)))

#bins to separate spike count (including 0 spikes)
bin_edges<-seq(0,max(spikecount_sum)+1,1)-0.5

for (d in 1:length(directions)){
  for (s in 1:length(speeds)){
    #hist: defining bin edges to bin spike counts
    #hist gives a list of components and plots histogram
    #we extract "counts" component by following hist command with $counts
    #suppress plot by plot=FALSE
    spikehist[d,s,]<-hist(spikecount_sum[d,s,],bin_edges,plot=FALSE)$
        counts
  }
}
```

At this point, you can compute the probability of the count given the motion direction in your time window, i. e. $P_T(n \mid \theta)$. The probability is like a frequency or a fraction — how many times did you observe a count of n, out of all the repetitions?

```
PnGstim<-spikehist/nReps[cellnum]
```

Make a 1D plot of $P(n \mid \theta = \theta_i)$ (probability of observing counts of different values given that the motion was in a particular direction, θ_i and a particular speed, v_i.

```
theta<-13
v<-6
print(plot(1:dim(PnGstim)[3],PnGstim[theta,v,],
     type = "l", # plot lines
     xlab = 'Spike count',
     ylab = expression(paste('P(n| ',theta,')')),ylim=c(0,0.5))
)
```

Do this for the preferred direction and speed, which elicits the highest firing rate, and an off-preferred direction with a lower firing rate. Use color or line type to denote the 2 different directions as we did before. How does the likelihood of observing a particular count value change as a function of direction? To really visualize this you could step through all the directions, but you will have a crowded figure unless you just choose a few!

Is the mapping between count and direction unique? These distributions are the answer to the question, "Given the direction, what is the most likely spike count". The problem that the brain faces is better phrased, "Given the spike count, what is the most likely direction?" That distribution is $P(\theta \mid n)$. We can generate this probability distribution by

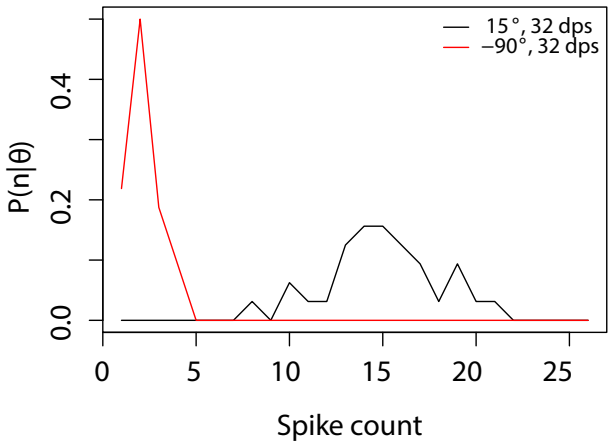


Figure 8: Distributions of the probability of spike count given two different stimulus directions.

taking our array `spikehist` for each spike count n and dividing by the number of times any stimulus presentation resulted in that spike count.

If the cell did not fire any spikes in the repetitions recorded for a specific stimulus, we will be dividing by zero. A safeguard against this is to add epsilon (accessed by `.Machine$double.eps`) to the denominator, which is the minimum value that can be represented by your machine. Epsilon is typically $2.2\text{x}10^{-16}$, so even though it is infinitesimally small, it avoids the issue of dividing by zero but does not affect your calculations.

```
for (n in 1:(max(spikecount_sum)+1)){
PstimGn[,,n]<-spikehist[,,n]/(sum(spikehist[,,n])+.Machine$double.eps)
}
```

Inspect your probability distributions by stepping through each spike count and plotting a heat map of $P(\{\theta, v\} \mid n)$.

```
for (n in 1:(max(spikecount_sum)+1)){


  filled.contour(directions,log2(speeds),PstimGn[,,n],levels=seq
      (0,0.2,0.2/30),
                plot.title = title(main = paste('stimulus probability
                    from',toString(n-1),'spikes')),
```

```
                                    xlab = 'direction (degrees)',
                                    ylab = 'log(speed) (degrees per second)'
                                        ,
8                 col=rev(rainbow(30,start=0,end=0.7))
                  )

11 Sys.sleep(0.1)
}
```

How is this affected by different spike count binning? Try changing `bin_edges` argument in the

```
spikehist[d,s,]<-hist(spikecount_sum[d,s,],bin_edges,plot=false)$counts
```

command a few steps above. Consider that a stimulus that elicits 10 spikes on average would also have a standard deviation (SD) of $\pm\sqrt{10} = 3$ spikes. What might happen with bin edges of [0,2,4,7,10,14,18,22,27,32], so that bin size increases proportional to the SD?

A legitimate complaint at this point would be that the brain can use a large population of neurons to generate estimates, so we may want to evaluate our uncertainty of estimating the stimulus when we use multiple cells. Use the below code to repeat the process above for all cells in the spikes array.

```

2 numcells<-8
  spikecount_sum_allcells<-array(0,c(length(directions),length(speeds),max
     (nReps),numcells))
  spikecount_mean_allcells<-array(0,c(length(directions),length(speeds),
     numcells))
5
  for (cellnum in 1:numcells){
    spikes_cell<-spikes[,,1:nReps[cellnum],,cellnum]
8   spikecount_sum_allcells[,,1:nReps[cellnum],cellnum]<-rowSums(spikes_
       cell,dims=3)
    spikecount_mean_allcells[,,cellnum]<-rowMeans(spikecount_sum_allcells
       [,,1:nReps[cellnum],cellnum],dims=2)
  }
11
  bin_edges<-seq(0,max(spikecount_sum_allcells)+1,1)-0.5
  spikehist_allcells<-array(0,c(length(directions),length(speeds),length(
     bin_edges)-1,numcells))
14 PstimGn_allcells<-array(0,c(length(directions),length(speeds),length(bin
     _edges)-1,numcells))

```

```
for (cellnum in 1:numcells){
  #calculate histograms
  for (d in 1:length(directions)){
    for (s in 1:length(speeds)){
      spikehist_allcells[d,s,,cellnum]<-hist(spikecount_sum_allcells[d,s
          ,1:nReps[cellnum],cellnum],bin_edges,plot=FALSE)$counts
    }
  }
  #normalize for each spike count bin
  for (n in 1:dim(spikehist_allcells)[3]){
    PstimGn_allcells[,,n,cellnum]<-spikehist_allcells[,,n,cellnum]/(sum(
        spikehist_allcells[,,n,cellnum])+.Machine$double.eps)
  }
  Sys.sleep(1)
}
```

What are the relative probabilities for the estimate of a stimulus given the response from all cells? Try direction index 16, speed index 4 as an example. Step through cells to combine the $P(\{\theta, v\} \mid n)$ distributions for the (rounded) mean spike count for each cell. The cell-averaged distribution is $P(\text{stimulus}_{\text{estimated}} \mid \text{stimulus}_{\text{actual}})$. You can also wrap this code in `for`loops to cycle through the direction and speed indices and watch how the variance of the estimate changes.

Question: How much is the estimate variance affected by the preferred directions and speeds of cells we have sampled? Which neurons are the most and least informative about the stimulus? What does this suggest about integration of spikes across a pool of neurons in the brain?

```

#for each stimulus value, make empty stimprob_estim to generate distribution
   stimprob_estim<-array(0,c(length(directions),length(speeds)))
      for (cellnum in 1:numcells){
        spikect_estim<-round(spikecount_mean_allcells[dirindex,spdindex,
            cellnum])
        if (sum(PstimGn_allcells[,,spct_est,cellnum])>0){
          stimprob_estim<-stimprob_estim+PstimGn_allcells[,,spikect_
              estim,cellnum]
         }
       }

stimprob_estim<- stimprob_estim/sum(stimprob_estim) #normalization

filled.contour(directions,log2(speeds),stimprob_estim,levels=
```

```
14              seq(0,max(stimprob_estim),max(stimprob_estim)/30),
                    plot.title = title(main = 'stimulus probability'),
                    xlab = 'direction (degrees)',
17                  ylab = 'log(speed) (degrees per second)',
                    col=rev(rainbow(30,start=0,end=0.7))
    )
20

Sys.sleep(1)
```

How do these estimates change depending on how much of the response you use? Go back to your array `spikecount_sum` and run through this code again while only using the transient response, for example. Is there much difference in your ability to estimate the stimulus as time elapses and more spikes are available?

Critical and quantitative analyses of next generation sequencing data

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BSD Bootcamp on Quantitative Biology @ MBL

Goal:

Next generation sequencing is becoming mainstream and learning how to analyze and critically evaluate sequencing data will be crucial for nearly every field of study. Also, it's a quick way to obtain data for your project with no bench time! In this 3hr workshop we will cover the fundamentals of analyzing next generation sequencing (NGS) data, from raw Fastq sequencing files to quantative data analyses. The ***overall objective*** of this workshop is to provide a basic understanding of NGS data analysis, experimental considerations for both sequencing and data analyses, and how to access public data for use in their research. First, we will use the Galaxy platform and some simple datasets to cover key concepts which include experimental design, quality control analyses, read trimming, utilization and considerations of reference genomes, alignment processes, file types (i.e. BAM and GTF), FPKM and RPKM values, and false discovery rates. Second, we'll utilize the UCSC visualizing browser to introduce students to visualizing their sequencing data, and overlaying their data with publically-available resources such as ENCODE. Third and finally, we will cover major sequencing initiatives (TCGA, Broad, ENCODE, 1000 genomes) and how to access and analyze publically-available datasets for use in their own research.

Audience:

This workshop is intended for all biologists interested in understanding, and more so for using, next-generation sequencing approaches for their research. Given how commonplace sequencing has become, and understanding of the fundamentals will enhance any research project and provide a foundation for additional learning if desired.

Installation:

No software is necessary for this course, as we will be using a cloud computing platform. Please go to https://usegalaxy.org/ and set up an account.

Workshop Resources:

1. My powerpoint slides
2. Two simple datasets to play with
3. Understanding of how to access many more dataset

Readings and Additional Resources:

These readings are a good starting point after the workshop is completed to begin your NGS analyses journey.

1. Rodriguez-Ezpeleta et al. (Editors). Bioinformatics for High Throughput Sequencing. Springer 2012. Download for free from here: http://link.springer.com/book/10.1007%2F978-1-4614-0782-9
2. Garber et al. Computational methods for transcriptome annotation and quantification using RNA-seq. Nature Methods 2011, PMID 21623353.
3. Trapnell et al. Differential analysis of gene regulation at transcript resolution with RNA-seq. Nature Biotechnology, PMID 23222703.
4. Sims et al. Sequencing depth and coverage: key considerations in genomic analyses. Nature Reviews Genetics, PMID 24434847.

Quantitative Bootcamp Dynamical Systems Tutorial

Ed Munro and Mike Rust

August 12, 2016

Contents

0.1 Dynamical analysis of cell cycle progression

In this tutorial, we will explore how one can turn simple biochemical circuit diagrams into mathematical equations and then analyse those equations, both graphically and numerically, to get insights into circuit's dynamics.

We will focus on one of the most intensively studied biochemical circuits in all of biology, the circuit that controls cell cycle progression in eukaryotic cells. The state of the cell cycle is encoded by the activity of cyclin-dependent kinases (CDKs). CDKs complex with proteins called Cyclins, which undergo cycles of synthesis and destruction over time. When complexed with Cyclin, Cdk can become active and phosphorylate different target proteins to regulate diverse cell cycle functions.

In some cells, such as early embryonic cells,the cell cycle is a freely running oscillator, in which Cyclin levels, and CDK activity, go up and down with clock-like regularity. Other cells progress through the cell cycle in a series of steps, involving switch-like transitions in CDK activity (low $\rightarrow$ high or high $\rightarrow$ low), that are controlled by external inputs like nutrient availability or by internal checkpoint controls that monitor the completion of key events like DNA replication or chromosome alignment. As you will see, switch-like transitions and oscillatory dynamics are intimately connected.

0.2 A biochemical circuit for cell cycle control.

Figure 1 summarizes a set of basic facts about the core biochemical circuit that is thought to generate cell cycle oscillations. Cyclin protein is synthesized at a constant rate. It forms a complex with Cdk1 (Cdk1-Cyclin) which is rapidly and constitutively activated by the Cdk-activating kinase (CAK, not shown).

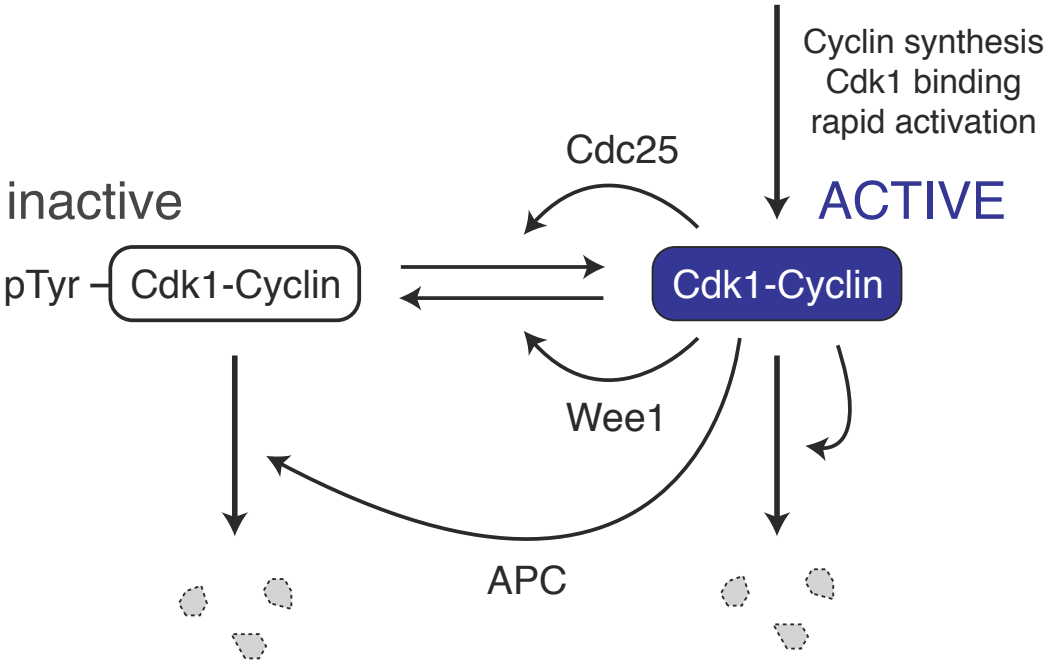

Figure 0.1 Biochemical circuit view of the core cell cycle oscillator

Active Cdk1-Cyclin then participates in three feedback loops: (1) It inhibits a tyrosine kinase called Wee1, which phosphorylates and inactivates Cdk1; (2) It activates a protein phosphatase called Cdc25, which dephosphorylates and reactivates Cdk1 and (3) It promotes the activity of the APC complex which in turn promotes the destruction of Cdk1-Cyclin complexes (both active and inactive).

Question: A feedback loop is called positive if it is self-reinforcing (e.g. active Cdk1-Cyclin promotes more active Cdk1-Cyclin) and negative if it is self-antagonizing. Which of these feedback loops is positive and which is negative?

In this tutorial, you will explore whether and how these feedback loops can account for two key properties of cell cycle control observed in experiments:

- The ability to convert slow changes in Cyclin levels into switch-like (all-or-none)

changes in Cdk1 activity.

- The ability to produce free-running oscillations in the absence of any additional inputs.

Session 1

Analysis of a very simple circuit

Lets start by analyzing a very simple circuit in which the Cdk1-Cyclin complex undergoes rapid conversion between inactive and active states at constant rates.

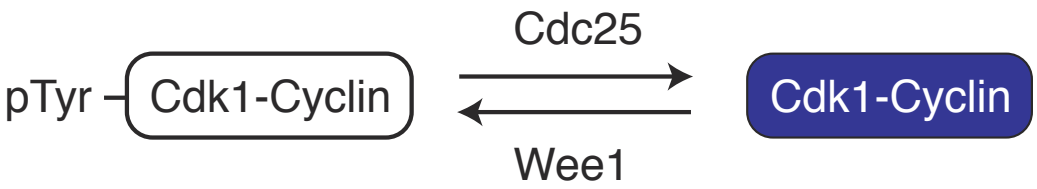

Figure 1.1 Simple interconversion of active and inactive Cdk1 at constant rates

From experimental studies, we know that Cyclin binds Cdk1 with very high affinity and that Cdk1 is always much more abundant than Cyclin. We will assume throughout this tutorial that all available Cyclin is complexed with Cdk1, and therefore that the total amount of the complex is the same as the total amount of Cyclin. From now on, we will refer to the Cdk1-Cyclin complex simply as Cdk1.

We want to write an equation that describes how the rate of change in active Cdk1 depends on the current amounts of active and inactive Cdk1. If we let C represent the total amount of Cyclin protein, and A represent the amount of active Cdk1, then the amount of inactive Cdk1 is $(C - A)$.

The total rate of change in active Cdk1 is equal to the rate at which inactive Cdk1 becomes active minus the rate at which active Cdk1 becomes inactive. If each molecule of inactive Cdk1 becomes active with a fixed probability per unit time described by k_{act}, then the total activation rate is $k_{act}(C - A)$. Likewise, if each molecule of active Cdk1 becomes

inactive with a probability per unit time described by k_{inact}, then the inactivation rate is $k_{inact}A$.

Putting these together, we can write the following differential equation:

$$\frac{dA}{dt} = k_{act}(C - A) - k_{inact}A \tag{1.1}$$

Equation 1.1 specifies how the rate of change in active Cdk1 depends on its current level and on the three parameters: C, k_{act} and k_{inact}. You could solve this equation with pencil and paper if you knew the values of all three parameters. However, this is almost never true for more complicated circuits. Here you will learn approaches to solving such equations that do not require pencil and paper. We will focus on two complementary approaches: graphical and computational.

1.1 Graphical approach: Flux balance analysis

Lets start with a simple graphical approach called flux balance analysis. The basic idea is to divide the right hand side of Equation 1 into a positive contribution, the positive flux and a negative contribution, the negative flux. As you will see, plotting the positive and negative fluxes as a function of A can tell us almost everything we want to know about the dynamics of this circuit. Lets start by defining a list of parameters in R and specifying provisional values:

Here and throughout the tutorial, when you encounter a piece of R code, your response should be to type (or copy and paste) the code into an R terminal window and execute:

```
params <- list (k_act=0.1 , k_inact=0.1 , C=100 )
```

Now we define R functions for the positive and negative flux contributions:

```
posflux <- function(A, params) {
    return (params\$k_act * (params\$C-A) )
}

negflux <- function(A, params) {
    return (params\$k_inact * A )
}
```

Now plot the positive and negative fluxes as a function of A:

```
A = seq (0 , 100 , 1 )
plot(A,posflux(A,params), type="l", col="red", main="Flux balance", xlim
    =c(0, 100), ylim=c(0, 10))

lines(A,negflux(A,params), col="blue")
```

The interpretation of this plot is very simple: Every possible state of the system is represented by a point on the horizontal axis, corresponding to a particular amount of A. The sum of the positive and negative flux equals dA/dt, the total rate of change in A. So dA/dt is positive when the positive flux is greater than the negative flux; it is negative when the positive flux is less than the negative flux and it is 0 (i.e. the system is at steady state) when the two fluxes are equal. This means that from the flux balance plot, for any current value of A, we can read off immediately how A will change in the next instant of time!

Note that there is a unique steady state point at which the positive and negative curves cross one another, and therefore where $dA/dt = 0$. The molecular meaning of this steady state point is that Cdk1 molecules are becoming activated exactly as fast as they are becoming inactivated, so the overall state of the system is constant.

Exercise 1.1

Question: Do you think this steady state is stable or unstable? That is, if you pushed the value of active Cdk1 up or down a little, would it return to the steady state value or move further away?

Here is a simple way to answer this question: Reproduce the above plot on a sheet of paper. Beneath the plot, at different points along the active Cdk1 (A) axis, draw arrows whose direction represents the sign of the difference between the positive and negative flux, and whose magnitude represents the magnitude of that difference. These arrows tell you, for a given value of active Cdk1, in what direction and how fast the amount of active Cdk1 is changing. Reading these arrows, is the steady state point stable or unstable?

1.2 Numerical approach

An complementary approach is to solve Equation 1 numerically, using a computer to solve for $A(t)$ which tells us how the state of the system changes as a function of time. To do so, we need to define a function that represents the right hand side of Equation 1; we need to supply particular values for the parameters k_{act}, k_{inact} and C; an initial value for A, and a sequence of time points at which to evaluate the solution.

Lets start by importing a package called desolve, which defines the relevant functions:

```
## Import the ‘ deSolve‘ package
2
library (deSolve)
```

We have already defined the list of parameters above. Now we define the right hand side function:

```
rhs <- function(t, A, params) {
    return (with (params, list (k_act * (C-A) - k_inact*A)))
}
```

We define a starting value for A:

```
A0 <- 0.0
```

and we define a sequence of time points at which to evaluate the solutions:

```
t <- seq (0 , 100 , 1 )
```

Now we call a numerical ODE solver function called ode to solve the equations, passing it the initial value A0, the time sequence t, the right hand side function rhs, and the list of parameters params:

```
out <- ode (A0, t, rhs, params)
```

The ode solver returns the solution in the form of an array of values of A at the specified time points contained in t.

We can plot this solution as follows:

```
plot(out, lwd=2, main="Simple Circuit", xlim=c(0, 100), ylim=c(0, 100))
```

Of course, it is very easy to evaluate the solution for a different initial value of A and add that solution to our plot:

```
A0=10
out <- ode (A0, t, rhs, params)
lines (out, lwd=2 )
```

Exercise 1.2

Try repeating this for a number of different initial conditions. How does the long term (steady state) behavior of this circuit depend on the initial conditions? Does this agree with what you learned from the graphical analysis?

1.3 Plotting the steady state level of Cdk1 as a function of Cyclin input

A key question that we would like to answer is: How does the steady state level of active Cdk1 vary as the amount of Cyclin changes? Again, there are two complementary ways to address this question: graphical and numerical.

Graphical construction of steady state response to cyclin levels:

The graphical approach is a simple extension of the flux balance analysis: For each value of C, the flux balance plot tells us what the steady state value of A will be. Note that in Equation 1, only the positive flux (the activation rate) depends on C. This suggests the following simple approach: Plot the negative flux once, and then plot the positive flux for a sequence of different values of C and observe how the position of the crossing (the steady state value of A) varies with C.

Here is a start:

```
A = seq (0 , 100 , 1 )
plot(A,negflux (A,params), type="l" , col="red" , main="Flux balance
    plot" ,   xlim=c (0 , 100 ), ylim=c (0 , 10 ))

params\$C=0
lines(A,posflux(A,params), col=blue)

params\$C=10
lines(A,posflux(A,params), col=blue)
```

Exercise 1.3

Now continue for a sequence of increasing values of C. On a separate sheet of paper, sketch a graph that shows (qualitatively) the relationship between the steady state level of Cdk1 and the input level of Cyclin.

Question: What is the shape of this graph?

Question: How does the fraction of active Cyclin/Cdk1 relative to the amount of Cyclin change as the total amount of Cyclin increases?

Actually, a slicker way to do this would be to use a simple for loop:

```
A = seq (0 , 100 , 1 )
plot (A,negflux (A,params), type="l" , col="red" , main="Flux balance
    plot" ,    xlim=c (0 , 100 ), ylim=c (0 , 10 ))

for (C in seq (0 ,200 ,10 )){
    params\$C=C
    lines (A,posflux (A,params), col="blue" )
}
```

1.4 Numerical construction of steady state response to cyclin levels:

Here is another way to construct the same graph numerically: Start with C = 0. Set the initial value of A to 0 and solve Equation 1 numerically to find the steady state level of A.

```
    plot(x=NULL,y=NULL,xlim=c(0,100),ylim=c(0,100),xlab="Cyclin level",
        ylab="Steady State Cdk1")
    params\$C=0
    A0 = 0
    out <- ode (A0, t, rhs, params)
    points(params\$C,out[101,2])
```

Now increase C by a small amount; use the previous steady state value for A as a new initial value and solve again for steady state.

```
    params\$C=10
    A0 = out[101,2]
    out <- ode (A0, t, rhs, params)
    points(params\$C,out[101,2])
```

Exercise 1.4

Repeat this process many times as you step params$C from 0 to some very high level (say params$C = 200), adding each steady state value to your plot.

Hint: try using a simple for loop to accomplish the this task.

Question: How does this plot compare to the qualitative plot you constructed on paper using flux balance analysis?

Important note: For this very simple scenario, we could have gotten this answer much more directly by setting $dA/dt = 0$ in Equation 1 and then solving algebraically for A in terms of C, k_{act} and k_{inact} to obtain:

$$0 = k_{act}(C - A) - k_{inact}A \implies A = \frac{k_{act}C}{k_{act} + k_{inact}} \tag{1.2}$$

However, this is only possible because the right hand side of Equation 1 is very simple. As soon as we add more complicated feedback, the equations we write down become impossible to solve this way!

Challenge: Above you constructed a response curve in which the input is the level of Cyclin and the response is the steady state level of Cdk1. Use the same approaches to construct a response curve in which the input is the rate of Cdk1 activation k_{act}. Now do the same for the rate of Cdk1 inactivation k_{inact}.

Session 2

How does positive feedback lead to switch-like activation of Cdk1?

For the simple circuit that you analyzed above, the steady state level of active Cdk1 deponds linearly on the level of Cyclin input. Now let's ask how this steady state response changes when we add the two feedback loops in which (a) active Cdk-1 activates Cdc-25 and Cdc-25 promotes activation of Cdk-1, (b) active Cdk-1 inhibits Wee-1 and Wee-1 promotes inactivation of Cdk-1.

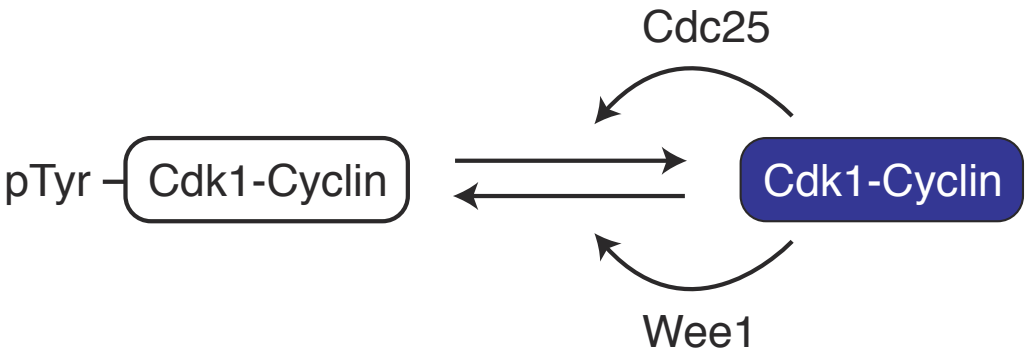

Figure 2.1 Interconversion of active and inactive Cdk1 with feedback control of activation and inactivation rates

Now the rate of Cdk1 activation is proportional to the product of Cdc25 enzyme activity and inactive Cdk1, and the rate of inactivation is proportional to the product of Wee1 enzyme activity and active Cdk1:

$$\frac{dA}{dt} = Cdc25(A) \cdot (C - A) - Wee1(A) \cdot A \tag{2.1}$$

We write Cdc25(A) and Wee1(A) in Equation 2 to reflect the fact that Cdc25 and Wee1 activities themselves depend on how phosphorylated these enzymes are (through the feedback loops involving Cdk1): Phosphorylated Cdc25 is active; phosphorylated Wee1 is inactive. To build our model, we will use empirical data showing what these functions look like. The dependencies of Cdc25 and Wee1 phosphorylation on Cdk1 activity have been measured experimentally in cytoplasmic extracts and they look like this:

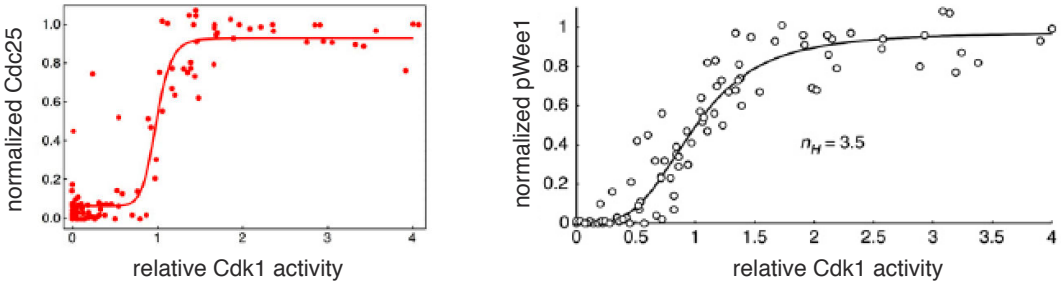

Figure 2.2 Normalized levels of phosphorylated (active) Cdc25 and phosphorylated (inactive) Wee1 as a function of Cdk1 activity

This kind of S-shaped, or sigmoidal dependence is very common in biology. The sharp rise in output level above some threshold value of the input is called ultrasensitivity. Ultrasensitive responses can arise in many ways in biochemical circuits, for example through cooperative binding, or multisite phosphorylation, or substrate competition (see Ferrell and Ha, 2013 for a nice discussion of this). The saturation of the response at high levels of input reflects the fact that there exists a maximum number of Cdc25 or Wee1 molecules that can become active.

Hill functions are a very useful way to represent sigmoidal responses in mathematical models. Originally invented by Archibald Hill to describe the sigmoidal binding curve for O_2 to hemoglobin, The equation for a Hill function looks like this:

$$\mathsf{Hill}(x, K, n) = \frac{x^n}{K^n + x^n} \tag{2.2}$$

where x is the input variable, and K and n are parameters.

Lets define the Hill function in R and graph it for a particular choice of K and n values.

```
hill <- function(X,K,n) {
    return(X^n/(K^n + X^n)
}

x = seq(0,4,0.1)
plot(x,hill(x,1,4),xlim=c(0,4),ylim=c(0,1),xlab=X,ylab=Hill(X))
```

How do the parameters K and n control the shape of the Hill function? Lets plot $\mathsf{Hill}(x, K, n)$ for a fixed value of K ($K = 1$) and a range of different n values:

```
    plot(x=NULL,y=NULL,xlim=c(0,4),ylim=c(0,1),xlab="X",ylab="Hill(X)")
    for (n in seq(1,10,1)) {
        lines(x,hill(x,2,n))
    }
```

Exercise 2.1

Now repeat the above, but this time plot the Hill function for a fixed value of n (say n = 4), and a range of K values.

Question: What key features of the Hill functions shape do K and n control?

The solid lines in Figure 4 above show the results of fitting Hill functions to the experimental measurements of phosphorylated (active) Cdc25 and Wee1 vs Cdk, yielding Hill coefficients n = 3.5 for Wee1 and n = 11 for Cdc25. These fits justify the use of Hill functions to represent Cdc25(A) and Wee1(A) in Equation 2 above. That is, we can write:

$$Cdc25(A) = a_{Cdc25} + f_{Cdc25}\frac{A^{11}}{K_{Cdc25}^{11} + A^{11}} \tag{2.3}$$

where a_{Cdc25} represents the basal level of Cdc25 activity, f_{Cdc25} is the strength of Cdk1 feedback, and K_{Cdc25} represents the threshold for this feedback.

Similarly, we can write:

$$Wee1(A) = a_{inact} + f_{inact}\frac{K_{inact}^{3.5}}{K_{inact}^{3.5} + A^{3.5}} \tag{2.4}$$

where a_{inact} represents the minimum level of Wee1 activity when fully inhibited, f_{inact} is the additional activity when uninhibited, and K_{inact} is the threshold for inhibitory feedback.

Importantly, the values of a_{Cdc25}, a_{inact}, f_{Cdc25}, f_{inact}, K_{Cdc25}, and K_{inact} have all been estimated by fitting equations 2.3 and 2.4 to experimental data (see Xiong and Ferrell, 2013).

Using these values, and substituting our expressions for $Cdc25(A)$ and $Wee1(A)$ into Equation 2 above gives:

$$\frac{dA}{dt} = \left(0.05 + 1.2\frac{A^{11}}{34^{11} + A^{11}}\right) \cdot (C - A) - \left(0.08 + 0.4\frac{A^{3.5}}{33^{3.5} + A^{3.5}}\right) \cdot A \tag{2.5}$$

This equation may look complicated, but it is built in straightforward way out of the pieces discussed above. If it looks overwhelming go back and see where each piece came from. Note here that we've suppressed the units for protein concentrations for simplicity. In Equation 4, the rate of change in A depends only on A itself, and on the level of Cyclin (C). However, unlike the simple circuit, we can no longer solve Equation 4 with pencil and paper. Here is where flux balance analysis and numerical solutions become really useful! Lets apply the same tools we used for the simple circuit above to analyze the more complicated dynamics of this feedback circuit. As before, we will first characterize active Cdk1 dynamics for a particular level of Cyclin; then well explore how the steady state Cdk1 levels vary with Cyclin levels.

2.1 Flux balance analysis of the positive feedback circuit

Lets start by defining a list of parameters and assigning their values from the published data:

```
feedback_params <- list(a_act = 0.16, f_act = 0.8, K_act = 34, n_act =
    11, a_inact = 0.08, f_inact = 0.4, K_inact = 33, n_inact = 3.5, C =
    60)
```

Note that we have assigned a nominal value for Cyclin ($C = 60$).

Exercise 2.2

Modify the flux balance analysis approach that we used in Section 1.1.1 to identify the steady states for this system and determine their stability: i.e. first define new positive and negative flux functions based on terms from the right hand side of Equation 4; then plot these functions vs A.

Question: How many steady state crossings are there? which of these do you think are stable and which are unstable?

As you did for the simpler case above, answer this question by reproducing the flux balance plot and drawing arrows below the Cdk1 axis that indicate the magnitude and direction of the rate of change in Cdk1 for different values of Cdk1.

Exercise 2.3

Numerical analysis of the positive feedback circuit

Now verify your conclusions from the flux balance analysis by solving Equation 4 numerically for different starting values of Cdk1. Following the approach we used in Section 1.1.2, define a new function for the right hand side of Equation 4, and define a sequence of time values at which to evaluate the solutions. Now choose a bunch of different starting values for A, and for each one, solve the equations numerically and plot the solutions.

Question: How many different stable steady state points are there?

Question: For what range of initial A values does A approach each of these stable steady states?

Question: What happens if you try to pick starting values closer and closer to an unstable steady state?

Question: Do you think the circuit could remain at this point for very long if it were operating in a real cell? Why or why not?

Summary: A key take-home message of this analysis is that, at least for some Cyclin

levels, this circuit can exhibit what we call bistable dynamics - it can home in on and remain stably at one of two possible states, depending on initial conditions. How do bistable dynamics shape the response of the circuit to changing levels of Cyclin?

Exercise 2.4

Map the steady steady response to changing levels of Cyclin.

We are particularly interested in asking how steady state levels of Cdk1 activity change as the levels of Cyclin slowly increase. Can positive feedback generate a switch-like response in Cdk1 activity to slow increases in Cyclin, as observed during cell cycle progression in living cells? Again we can answer these questions using a combination of graphical and numerical approaches:

Graphical construction of steady state response to cyclin levels: Lets start by adapting the graphical approach that we used for the simple circuit above. As for the simper circuit, only the positive flux depends on the value of C. So again, you can plot the negative flux vs A once, and then plot the positive flux vs A for a range of different values of C. Modify the code we used in Section 1.1.3 to do this.

Of course now, for some values of C, there are multiple stable and unstable steady states. As you vary C, these steady states move continuously along the A axis. This is more complicated than the simple circuit, but you can still make sense of whats happening:

As before, on a separate sheet of paper, construct a graph in which the horizontal axis represents the Cyclin level (C) and the vertical axis represents steady state levels of Cdk1(A). Draw a curve with a solid line to indicate the changing positions of stable steady states and curves with a dashed line to indicate the positions of unstable steady states.

We call this graph a bifurcation diagram.

Question: What does this bifurcation diagram tell you about the steady state response to changing Cyclin levels? How do you make sense of this when there are multiple possible steady state values for some levels of Cyclin?

Take a few moments to ponder these questions and discuss with your partners before going to the next step.

2.2 Numerical construction of steady state response to cyclin levels

Your analysis shows that for an intermediate range of C values, the circuit exhibits bistable dynamics: I.e. for some values of C, the circuit can adopt one of two possible stable steady

states, depending on initial conditions. This suggests that the steady state response of this circuit to changing Cyclin levels will depend on the history of prior Cyclin levels.

Exercise 2.5

During cell cycle progression, Cyclin and active Cdk1 levels both start out low, and steady state Cdk1 activity levels respond continuously to slow increases in Cyclin levels. Revise the numerical approach that we used for the simple circuit in Secrtion 1.1.4 above to determine the steady state Cdk1 activity responds to slowly increasing Cyclin levels.

Start with low values for C and A; solve to find a steady state value for C. Now increase C in a series of steps. At each step solve the equations numerically, using the previous steady state level of A as a new initial value, and then plot the steady state value of Cdk1 (A) vs the level of input Cyclin.

Question: How does this plot differ from the one you constructed earlier for the simple circuit lacking positive feedback? How does it relate to the bifurcation diagram you constructed in the last step?

Exercise 2.6

Now repeat the same exercise, but this time start with a very high level of Cyclin, and map out the steady state Cdk1 response as you decrease the Cyclin level stepwise towards zero.

Now, on the same graph, plot the steady state Cdk1 values that you obtained by increasing Cyclin slowly from a low value and by decreasing Cyclin slowly from a high value.

Question: How do these two plots differ and why? How do these two plots relate to the bifurcation diagram?

Optional Challenge: What is the point of having two feedback loops instead of just one?

One possibility is that two feedback loops make the switchlike behavior of the circuit more robust with respect to variation in parameter values. Here is a way to test this idea:

Looking at the bifurcation diagram you constructed above, choose an intermediate value of Cyclin for which the circuit exhibits bistable dynamics.

First, consider the parameter f_{inact}, which controls the strength of feedback through Wee1 and effectively controls the maximum rate of Cdk1 inactivation. Use flux balance analysis, ask: For what range of values of f_{inact} does the circuit continue to exhibit bistable dynamics?

Now consider a reduced circuit in which there is no feedback through Wee1, i.e the rate of Cdk1 inactivation is controlled by a constant parameter k_{inact}, as in Equation 1. Again use flux balance analysis to ask: For what range of values of k_{inact} does the circuit continue to exhibit bistable dynamics? What version of the circuit tolerates a larger range of variation in Cdk1 inactivation rates?

Session 3

From switch-like transitions to free-running oscillations: Adding negative feedback.

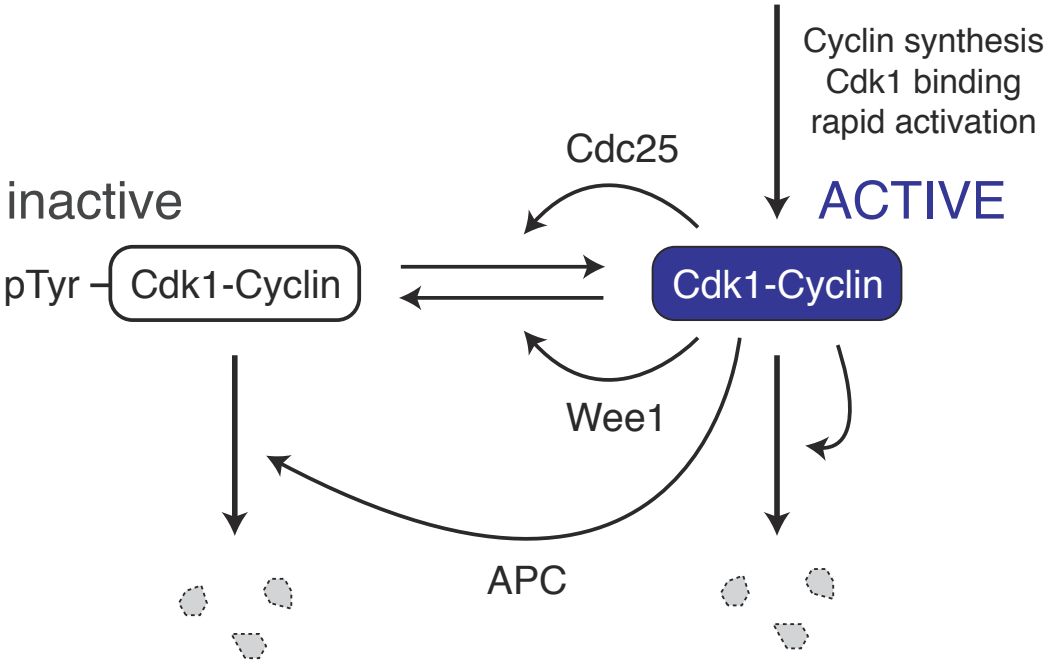

Figure 3.1 The full circuit with negative feedback

So far we have considered Cyclin levels as an external input or control variable. In real cells, Cyclin synthesis and degradation are coupled to Cdk1 activity: As Cyclin builds up above a threshold, Cdk1 becomes active; active Cdk1 in turn promotes APC-dependent degradation of Cyclin, causing Cyclin levels to fall rapidly again. Figure 3.1 again is a schematic view of the Cyclin/Cdk1 circuit with this additional negative feedback.

Could switch-like activation of Cdk1 and negative feedback in which active Cdk1 promotes APC-dependent Cyclin degradation lead to robust free-running oscillations in Cyclin and Cdk1 activity? Again, we can use simple mathematical models to explore how this works. Lets start with a simple thought experiment.

Exercise 3.1

A simple thought experiment: Consider the bifurcation diagram and the response curves for steady state Cdk1 vs Cyclin that you constructed in Part 2. Discuss with your partners how negative feedback in which Cdk1 activates APC and Cyclin degradation could drive the rise and fall of Cyclin levels and Cdk1 activity.

Now lets test your intuition by adding negative feedback to our circuit model, based on the arrows in Figure 5 above. We need to write an additional equation for the rate of change in Cyclin due to synthesis and degradation:

$$\frac{dC}{dt} = k_{syn} - k_{deg}(A) \cdot C \tag{3.1}$$

Here, we have written $k_{deg}(A)$ to reflect the fact that the degradation rate is a function of APC activity which in turn depends on the level of Cdk1 activity. Recent experimental measurements from the James Ferrells lab show that, like Cdc25 and Wee1, APC activity is a switch-like function of Cdk1 activity, which is well-fit by a Hill function with Hill coefficient $n = 17$:

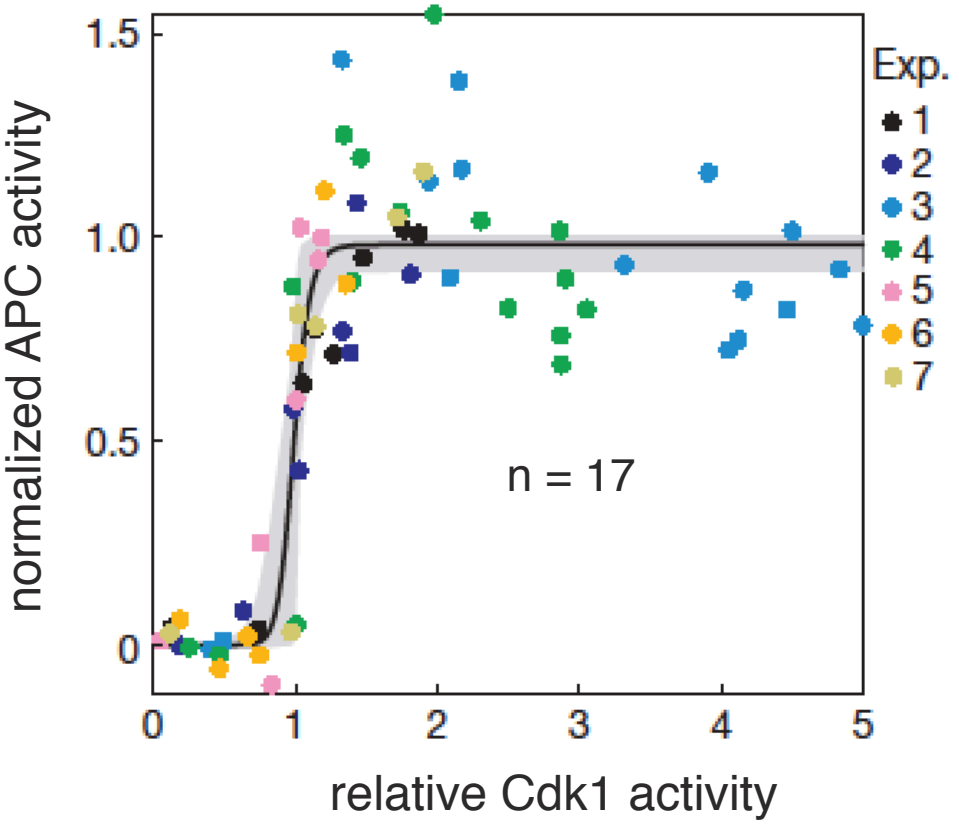

Figure 3.2 Dependence of APC activity on Cdk1

Again, we can represent the dependence of Cyclin degradation rates on Cdk1 using a function of the form:

$$k_{deg}(A) = a_{deg} + f_{deg}\frac{A^{n_{deg}}}{K_{deg}^{n_{deg}} + A^{n_{deg}}} \tag{3.2}$$

where $a_{deg} = 0.01$, $f_{deg} = 0.04$, $K_{deg} = 32$, and $n_{deg} = 17$ are estimated from experiments.

Plugging into Equation 3.1, and combining with our previous equation for active Cdk1 (A), we now have a pair of differential equations:

$$\frac{dA}{dt} = \left(0.16 + 0.8\frac{A^{11}}{35^{11} + A^{11}}\right) \cdot (C - A) - \left(0.08 + 0.4\frac{A^{3.5}}{30^{3.5} + A^{3.5}}\right) \cdot A$$
$$\frac{dC}{dt} = k_{synth} + \left(0.01 + 0.04\frac{A^{17}}{32^{17} + A^{17}}\right) A \qquad (3.3)$$

Again, we can analyze the dynamics this system using a combination of graphical and numerical approaches:

3.1 Graphical

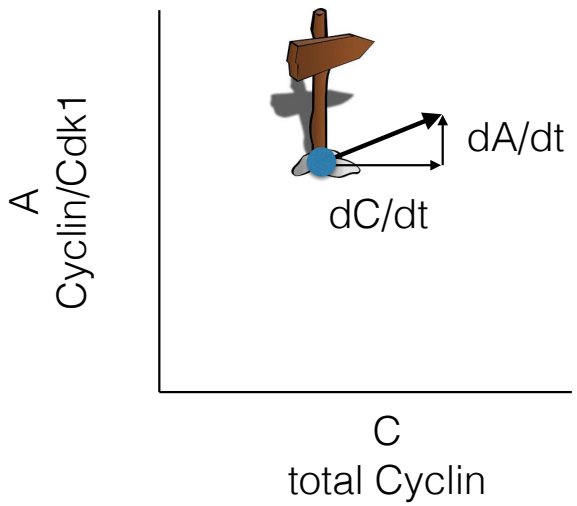

Figure 3.3 A phase plane

The state of this system is described by two values: the concentration of Cyclin and the concentration of active Cdk1. We can think of the state of the system, at any moment in time, as being defined by a point in a 2D "phase plane". The position of this point on the A-axis indicates how much active Cyclin/Cdk1 there is in the cell at that moment, and the position along the C-axis indicates the total amount of Cyclin. The differential equations for dC/dt and dA/dt above are rules that tell you, given the current values of A and C, how much the system will move in the C direction and A direction in the next instant in time. We can think of these rules as arrows attached to each point in the 2D plane, together constituting what we call a flow field. The flow along these arrows defines what will happen to the system.

Analyzing the behavior of a system with two dynamical variables in the phase plane is more difficult than the 1D examples from before. More kinds of behavior are possible, but we can still use graphical tools to think intuitively about what will happen.

First, we need to load a library that defines some useful tools for graphical "phase plane analysis"

```
## Loading the 'phaseR' library
library(phaseR)
```

Now lets define a function that computes the right hand sides of our pair of differential equations:

```
RHS_2D <- function(t, y, parameters) {
    with(as.list(parameters), {
        C = y[1]
        A = y[2]
        dc=k_syn-(a_deg + f_deg*A^n_deg/(K_deg^n_deg + A^n_deg))*C
        pda = (a_act+f_act*A^n_act/(K_act^n_act+A^n_act))*(C-A)
        nda = (a_inact+f_inact*A^n_inact/(K_inact^n_inact+A^n_inact))
            *A
        return(list(c(dc,pda-nda)))
    })
}
```

The arguments to this function are the current time t, a list y containing the current values of C (y[1]) and A (y[2]) and a list of paramater values. The function returns a list of two values corresponding to dC/dt and dA/dt

Finally, let's define the list of parameters and assign their values:

```

full_pars = list(k_syn=1.0,a_deg=0.01,f_deg=0.04,K_deg=32,n_deg=17,a_act
    =0.16,f_act=0.8,K_act=35,n_act=11,a_inact=0.08,f_inact=0.4,K_inact
    =30,n_inact=3.5)
```

OK, now we can use a predefined function (part of the phaseR library) that computes and displays the flow field:

```
flowField(RHS_2D, x.lim = c(0, 100),
y.lim = c(0, 100),
xlab="C", ylab="A",
parameters = full_pars,
points = 15, add = FALSE)
```

Question: What can you infer about the dynamics of this circuit by looking at the flow field?

Recall that for the 1D system, it was very useful to know where the steady states are. How do we accomplish this in 2D? Instead of plotting positive and negative fluxes and locating steady states where these two curves cross, we will instead plot two nullclines" and locate steady states where these cross. The A nullcline is the set of (A,C) value pairs for which dA/dt = 0, and the C nullcline is the set of (A,C) value pairs for which dC/dt = 0. Where these cross, both dA/dt and dC/dt must be 0. To think intuitively about the meaning of the nullclines, notice that when the system is on the A-nullcline, the next instantaneous step must be in the C direction (and vice versa). Thus, the arrows in the flow field must point along the C direction on the A-nullcline.

For this particular system of equations, you could find the A and C nullclines algebraically with pencil and paper. Feel free to do that now if you really want to show off your algebra chops. However, if you just want to get the nullclines quickly with minimal effort, you can use this built in R function to plot the nullclines over the flowfield you plotted a moment ago:

```
nullclines(FHN, x.lim = c(0, 100),
y.lim = c(0, 100),
parameters = full_pars,
points = 500)
```

Exercise 3.2

Question: How many steady states are there? Do you think they are stable or unstable? How could you tell?

3.2 Numerical

The short answer to the above question is that you cannot determine if a steady state is stable or unstable just by looking at the shapes of the nullclines (except in very special cases). A very straightforward (and quite powerful) way to investigate the dynamics of this circuit is to solve the equations numerically for different starting values of A and C and then plot the solutions in the A/C plane.

```
trajectory(FHN, y0 = c(10,10), t.end = 500,
parameters = full_pars)
```

Exercise 3.3

Repeat this for a series of different starting values. Does the long term behavior of this circuit depend on the initial conditions? If so, how?

3.3 A closer look at the limit cycle dynamics

Plot A(t) and C(t) vs time. Does the system go to a steady state? If a system does not approaches a steady state point, but instead stably cycles through the same states over and over, we say that the system is attracted to a "limit cycle". Relate the time series to the limit cycle view in the phase plane. Infer that there is slow movement along the upper and lower branches of the Cyc nullcline and fast jumps between branches.

A mathematical model of the cell cycle like this of course simplifies many molecular details and leaves out some of the biology. The power of a description like this is that it now allows to ask which features of the molecular circuit are crucial for the observed behavior. Now that you have assembled these tools, you can computationally manipulate the cell cycle circuit to see how the behavior changes.

3.4 Challenges

Challenge: What is the importance of switch-like dependence of Cyclin degradation on Cdk1?

Set n = 1 in the differential equation for Cyc and in the equation for the Cyc nullcline.

Again plot nullclines and plot solutions for a variety of different initial conditions. What is the behavior of this system?

Is it possible to get oscillatory dynamics to return by tuning e.g. the threshold or strength of negative feedback?

Challenge: How tolerant is the cell cycle oscillator to changes in the threshold for activation of Cyclin degradation by Cdk1? How does this tolerance change as you vary the steepness of the feedback (i.e. the exponent n)?

Challenge: Explore what happens as you vary Cyclin synthesis and degradation rates. How does the period of the oscillation change? Why?

Challenge: The original form of the circuit had two feedback loops on active Cycle/Cdk1: one acted on the phosphatase Cdc25, and one acted on the kinase Wee1. If you computationally remove the feedback loop involving Wee1, what happens to oscillations? Can you get a different answer if you change the parameters describing the feedback loop through Cdc25? It may help to go back to the single differential equation describing dA/dt and reanalyze it without the Wee1 feedback loop to look for multiple steady states.