Biology 305: Biostatistics

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Preface

Welcome to Biology 305 at the University of Nebraska at Kearney! Material in this class was designed by Dr. Melissa Wuellner and adapted by Dr. Jacob C. Cooper for use in R.

In this class, you will learn:

- 1. The basics of study design, the importance of understanding your research situation before embarking on a full study, and practice creating research frameworks based on different scenarios.
- 2. The basics of data analysis, including understanding what kind of variables are being collected, why understanding variable types are important, and basic tests to understand univariate distributions.
- 3. Basic multivariate statistics, including ANOVA, correlation, and regression, for comparing multiple different groups.
- 4. The basics of coding and working in R for performing statistical analyses.

This site will help you navigate different homework assignments to perform the necessary R tests. Furthermore, this GitHub repository contains all of the homework dataframes, so you will not have to manually enter assignments if you use R to complete your assignments.

Welcome to class!

Dr. Jacob C. Cooper, BHS 321

1 Intro to R

In this class, we will be using R to perform statistical analyses. R is a free software program designed for use in a myriad of statistical and computational scenarios. It can handle extremely large datasets, can handle spatial data, and has wrappers for compatibility with Python, Bash, and other programs (even Java!).

2 Setup

First, we need to download R onto your machine. We are also going to download RStudio to assist with creating R scripts and documents.

2.1 Installing *R*

First, navigate to the R download and install page. Download the appropriate version for your operating system (Windows, Mac, or Linux). **Note** that coding will be formatted slightly different for Windows than for other operating systems. If you have a Chromebook, you will have to follow the online instructions for installing both programs on Chrome.

Follow the installation steps for R, and verify that the installation was successful by searching for R on your machine. You should be presented with a coding window that looks like the following:

```
Copyright (C) 2024 The R Foundation for Statistical Computing Platform: aarch64-apple-darwin20

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and 'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or 'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.
```

R version 4.4.1 (2024-06-14) -- "Race for Your Life"

If that screen appears, congratulations! R is properly installed. If the install was not successful, please talk to Dr. Cooper and check with your classmates as well.

2.2 Installing RStudio

RStudio is a GUI (graphics user interface) that helps make R easier to use. Furthermore, it allows you to create documents in R, including websites (such as this one), PDFs, and even presentations. This can greatly streamline the research pipeline and help you publish your results and associated code in a quick and efficient fashion.

Head over the the *RStudio* download website and download "*RStudio* Desktop", which is free. Be sure to pick the correct version for your machine.

Open RStudio on your machine. You should be presented with something like the following:

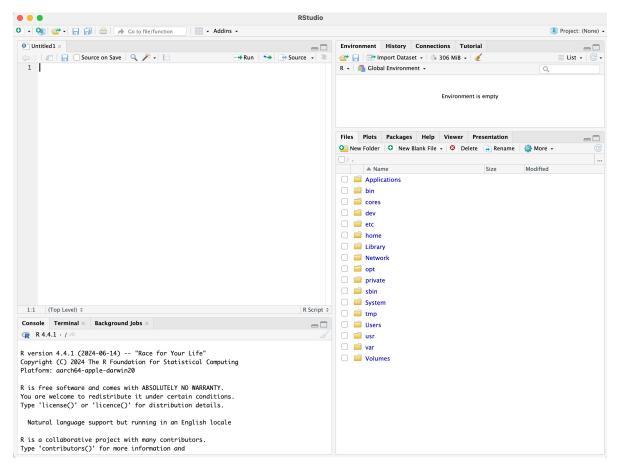


Figure 2.1: *RStudio* start window. Note that the screen is split into four different quadrants. Top left: *R* documents; bottom left: *R* program; top right: environment window; bottom right: plots, help, and directories.

In RStudio, the top left window is always going to be our coding window. This is where we will type all of our code and create our documents. In the bottom left we will see R executing the code. This will show what the computer is "thinking" and will help us spot any potential issues. The top right window is the "environment", which shows what variables and datasets are stored within the computers' memory. (It can also show some other things, but we aren't concerned with that at this point). The bottom right window is the "display" window. This is where plots and help windows will appear if they don't appear in the document (top left) window itself.

Now, we will create our first R document!

3 Creating an RMarkdown document

3.1 Setup

In this class, we will be creating assignments in what is called RMarkdown. This is a rich-text version of R that allows us to create documents with the code embedded. In RStudio, click the "+" button in the far top left to open the New Document menu. Scroll down this list and click on R Markdown.

A screen such as this will appear:

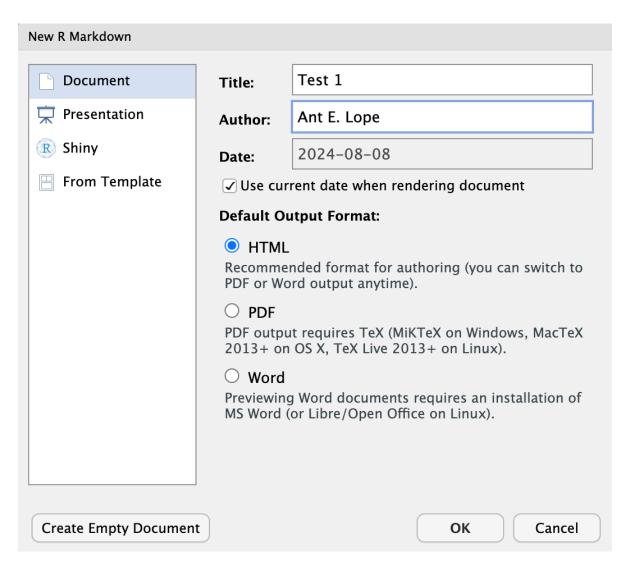


Figure 3.1: A new file window for an *RMarkdown* file.

After entering a title and your name and selecting document in the left hand menu, click OK.

```
title: "Test 1"
author: "Ant E. Lope"
date: "`r Sys.Date()`"
output: html_document
---

```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = TRUE)

R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring
HTML, PDF, and MS Word documents. For more details on using R Markdown see
<http://rmarkdown.rstudio.com>.

When you click the **Knit** button a document will be generated that includes both
content as well as the output of any embedded R code chunks within the document. You can
embed an R code chunk like this:

```{r cars}
summary(cars)

...
```

Figure 3.2: An example of a markdown script.

In the image above, we can see what a "default" *RMarkdown* script looks like after creating the file. At the top of the document, between all of the dashes, we have the yaml header that tells R what kind of document will be created, who the author is, and tells it to use today's date. In this class, we will be saving documents as html as they are the easiest documents to create and save. These documents will include all of your code, text, and even any plots you may create!

Plain text in the document will be rendered as plain text in the document. (I.e., whatever you type normally will become "normal text" in the finished document). Lines preceded with # will become headers, with ## being a second level header and ### being a third level header, etc. Words can also be made italic by putting an asterisk on each side of the word (*italic*) and bold by putting two asterisks on each side (**bold**). URLs are also supported, with <> on each side of a URL making it clickable, and words being hyperlinked by typing [words to show] (target URL).

We also have code "chunks" that are shown above. A code chunk can be manually typed out or inserted by pressing CTRL + ALT + I (Windows, Linux) or COMMAND + OPTION + I (Mac). Everything inside a "code chunk" will be read as R code and executed as such. Note that you can have additional commands in the R chunks, but we won't cover that for now.

3.2 Using code chunks

In your computer, erase all information except for the yaml header between the dashes on your computer. Save your file in a folder where you want your assignment to be located. It is important you do this step up front as the computer will sometimes save in random places if you don't specify a file location at the beginning. Don't forget to save your work frequently!

```
This is a test of the *RMarkdown* code.

```{r}
print("Hello world!")

```
```

Figure 3.3: Text to type in your *Rmarkdown* document.

After typing this into the document, hit knit near the top of the upper left window. R will now create an HTML document that should look like this:

Test 1

Ant E. Lope 2024-08-07

This is a test of the RMarkdown code.

```
print("Hello world!")

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

Figure 3.4: The output from the above code knitted into a document.

We can see now that the HTML document has the title of the document, the author's name, the date on which the code was run, and a greyed-out box with color coded R code followed by the output. Let's try something a little more complex. Create a new code chunk and type the following:

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

This will create a variable in R, x, that is sequentially each whole number between 1 and 10. We can see this by highlighting or typing only the letter x and running that line of code by clicking CTRL + ENTER (Windows / Linux) or COMMAND + ENTER (Mac).

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

If you look at the top right window, you will also see the value x in the environment defined as int [1:10] 1 2 3 4 5 6 7 8 9 10. This indicates that x is integer data spanning ten positions numbered 1 to 10. Since the vector is small, it displays every number in the sequence.

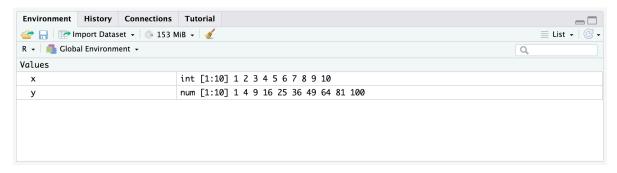


Figure 3.5: *RStudio* environment window showing saved objects. These are in the computer's memory.

Let's create another vector y that is the squared values of x, such that $y = x^2$. We can raise values to an exponent by using $\hat{}$.

```
y <- x<sup>2</sup>
y
```

[1] 1 4 9 16 25 36 49 64 81 100

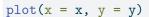
Now we have the value y in the environment that is the square of the values of x. This is a numeric vector of 10 values numbered 1 to 10 where each value corresponds to a square of the x value. We can raise things to any value however, including x^x !

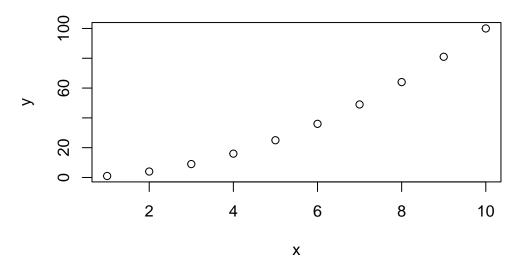
| x^x | | | | | | |
|-------|--------|----------|-----------|-------------|------|-------|
| | | | | | | |
| [1] | 1 | 4 | 27 | 256 | 3125 | 46656 |
| [7] | 823543 | 16777216 | 387420489 | 10000000000 | | |

As we can see, since I didn't "store" this value as a variable in R using \leftarrow , the value is not in the environment.

3.3 Plotting

Now, let's try creating a plot. This is easy in R, as we just use the command plot.





By specifying the y and x components in plot, we can quickly generate a point plot. We can alter the visual parameters of this plot using a few different commands. I will outline these below with inline notes. Inline notes in the code can be made by using a # symbol before them, which basically tells R to ignore everything after the #. For example:

```
print("Test")
```

[1] "Test"

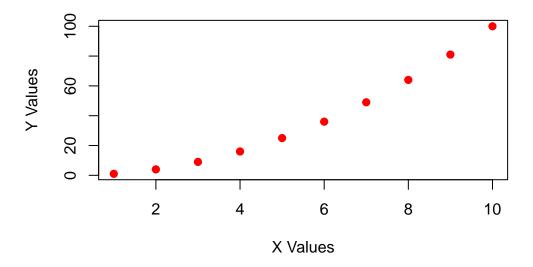
```
# print("Test 2")
```

This prints the word Test, but doesn't print Test 2.

Now let's make the plot with some new visual parameters.

```
plot(x = x, # specify x values
    y = y, # specify y values
    ylab = "Y Values", # specify Y label
    xlab = "X Values", # specify X label
    main = "Plot Title", # specify main title
    pch = 19, # adjust point style
    col = "red") # make points red
```

Plot Title



3.4 Tab complete

RStudio allows for "tab-completing" while typing code. Tab-completing is a way of typing the first part of a command, variable name, or file name and hitting "tab" to show all options with that spelling. You should use tab completing because it:

- reduces spelling mistakes
- reduces filepath mistakes
- increases the speed at which you code
- provides help with specific functions

3.5 Help

At any point in R, you can look up "help" for a specific function by typing ?functionname. Try this on your computer with the following:

?mean

4 Working with data

Throughout this course, we are going to have to work with datasets that are from our book or other sources. Here, we are going to work through an example dataset. First, we need to install *libraries*. A *library* is a collated, pre-existing batch of code that is designed to assist with data analysis or to perform specific functions. These *libraries* make life a lot easier, and create short commands for completing relatively complex tasks.

In this class, there are two libraries that you will need *almost every week*! First, we need to install the libraries. The main libraries we need for this class are:

- 1. curls: this package allows us to download things from URLs. We will be using this to download data files. Otherwise, you will have to enter all data by hand!
- 2. tidyverse: this package is actually a group of packages designed to help with data analysis, management, and visualization.

```
# run this code the first time ONLY
# does not need to be run every time you use R!

# curl allows for internet downloads
install.packages("curl")

# tidyverse has a bunch of packages in it!
# great for data manipulation
install.packages("tidyverse")

# if you ever need to update:
# leaving brackets open means "update everything"
update.packages()
```

After packages are installed, we will need to load them into our R environment. While we only need to install.packages once on our machine, we need to load libraries every time we restart the program!

```
library(curl)
```

Using libcurl 8.7.1 with LibreSSL/3.3.6

library(tidyverse)

```
-- Attaching core tidyverse packages ---
                                                               ---- tidyverse 2.0.0 --
v dplyr
             1.1.4
                                     2.1.5
                        v readr
v forcats
             1.0.0
                        v stringr
                                     1.5.1
v ggplot2
             3.5.1
                        v tibble
                                     3.2.1
v lubridate 1.9.3
                        v tidyr
                                     1.3.1
v purrr
             1.0.2
-- Conflicts -----
                                             -----ctidyverse_conflicts() --
x dplyr::filter()
                        masks stats::filter()
x dplyr::lag()
                        masks stats::lag()
x readr::parse_date() masks curl::parse_date()
i Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force all conflicts to become
```

You should an output like the above. What this means is:

- 1. The core packages that comprise the tidyverse loaded successfully, and version numbers for each are shown.
- 2. The conflicts basically means that certain commands will not work as they used to because R has "re-learned" a particular word.

To clarify the conflicts, pretend that you can only know one definition of a word at a time. You may know the word "cola" as a type of soda pop or as a drink in general. However, in Spanish, "cola" refers to a line or a tail. While we can learn both of these definitions and know which one is which because of context, a computer can't do that. In R, we would then have to specify which "cola" we are refferring to. We do this by listing the package before the command; in this case, english::cola would mean a soda pop and spanish::cola would refer to a line or tail. If we just type cola, the computer will assume one of these definitions but not even consider the other.

We won't have to deal with conflicts much in this class, and I'll warn you (or help you) if there is a conflict.

4.1 Downloading data

Now, we need to download our first data set. These datasets are stored on GitHub. We are going to be looking at data from Dr. Cooper's dissertation concerning Afrotropical bird distributions (Cooper 2021). This website is in the data folder on this websites' GitHub page, accessible here.

Alternatively, we can use the operator %% to simplify this process. %% means "take whatever you got from the previous step and pipe it into the next step". So, the following does the exact same thing:

```
ranges <- curl("https://raw.githubusercontent.com/jacobccooper/biol305_unk/main/datasets/lac-
read_csv()
```

Rows: 12 Columns: 10

Using the %>% is preferred as you can better set up a workflow and because it more closely mimics other coding languages, such as bash.

Let's view the data to see if it worked. We can use the command head to view the first few rows:

head(ranges)

```
# A tibble: 6 x 10
  species
                          combined_current_km2 consensus_km2 bioclim_current_km2
  <chr>
                                          <dbl>
                                                         <dbl>
                                                                              <dbl>
1 Batis diops
                                         25209.
                                                         6694.
                                                                             19241.
2 Chamaetylas_poliophrys
                                         68171.
                                                         1106.
                                                                             68158.
3 Cinnyris_regius
                                         60939.
                                                        13305.
                                                                             53627.
4 Cossypha_archeri
                                         27021.
                                                         6409.
                                                                             11798.
5 Cyanomitra alinae
                                         78680.
                                                        34320.
                                                                             63381.
6 Graueria_vittata
                                          8770.
                                                         861.
                                                                              8301.
# i 6 more variables: `2050_combined_km2` <dbl>, `2050_consensus_km2` <dbl>,
    `2070_combined_km2` <dbl>, `2070_consensus_km2` <dbl>,
#
    alltime_consensus_km2 <dbl>, past_stable_km2 <dbl>
```

We can perform a lot of summary statistics in R. Some of these we can view for multiple columns at once using summary.

summary(ranges)

```
species
                    combined_current_km2 consensus_km2
                                                            bioclim_current_km2
Length:12
                   Min.
                           : 8770
                                         Min.
                                                 : 861.3
                                                            Min.
                                                                   : 3749
Class :character
                    1st Qu.: 24800
                                         1st Qu.: 4186.2
                                                            1st Qu.: 10924
Mode :character
                   Median : 43654
                                         Median : 7778.1
                                                            Median : 31455
                   Mean
                           : 68052
                                         Mean
                                                 :18161.8
                                                            Mean
                                                                    : 42457
                    3rd Qu.: 70798
                                         3rd Qu.:18558.7
                                                            3rd Qu.: 62835
                   Max.
                           :232377
                                         Max.
                                                 :79306.6
                                                            Max.
                                                                    :148753
2050_combined_km2 2050_consensus_km2 2070_combined_km2
                                                          2070_consensus_km2
       : 1832
                               0.0
                                                  550.3
                                                                       0.0
Min.
                  Min.
                                      Min.
                                                          Min.
1st Qu.:
          6562
                  1st Qu.: 589.5
                                      1st Qu.: 6583.8
                                                          1st Qu.: 311.4
Median : 26057
                  Median: 6821.9
                                      Median: 24281.7
                                                          Median: 2714.6
Mean
       : 33247
                          :14418.4
                                      Mean
                                              : 31811.0
                                                                 : 8250.5
                  Mean
                                                          Mean
3rd Qu.: 40460
                  3rd Qu.:18577.1
                                      3rd Qu.: 38468.9
                                                          3rd Qu.:10034.4
Max.
       :132487
                  Max.
                          :79236.2
                                      Max.
                                              :129591.0
                                                          Max.
                                                                  :53291.8
alltime consensus km2 past stable km2
            0.0
                      Min.
                              :
1st Qu.: 790.9
                       1st Qu.:
                                  0.0
Median: 8216.8
                      Median:
                                  0.0
       :15723.3
                              : 127.3
Mean
                      Mean
3rd Qu.:19675.0
                       3rd Qu.:
                                  0.0
Max.
       :82310.5
                      Max.
                              :1434.8
```

As seen above, we now have information for the following statistics for each variable:

- Min = minimum
- 1st Qu. = 1st quartile
- Median = middle of the dataset
- Mean = average of the dataset
- 3rd Qu. = 3rd quartile
- Max. = maximum

We can also calculate some of these statistics manually to see if we are doing everything correctly. It is easiest to do this by using predefined functions in R (code others have written to perform a particular task) or to create our own functions in R. We will do both to determine the average of combined_current_km2.

4.2 Subsetting data

First, we need to select only the column of interest. In R, we have two ways of subsetting data to get a particular column.

- var[rows,cols] is a way to look at a particular object (var in this case) and choose a specific combination of row number and column number (col). This is great if you know a specific index, but it is better to use a specific name.
- var[rows,"cols"] is a way to do the above but by using a specific column name, like combined_current_km2.
- var\$colname is a way to call the specific column name directly from the dataset.

```
# using R functions
ranges$combined_current_km2
```

```
[1] 25209.4 68171.2 60939.2 27021.3 78679.9 8769.9 232377.2 17401.4 [9] 51853.5 35455.1 23570.3 187179.1
```

As shown above, calling the specific column name with \$ allows us to see only the data of interest. We can also save these data as an object.

```
current_combined <- ranges$combined_current_km2
current_combined</pre>
```

```
[1] 25209.4 68171.2 60939.2 27021.3 78679.9 8769.9 232377.2 17401.4 [9] 51853.5 35455.1 23570.3 187179.1
```

Now that we have it as an object, specifically a numeric vector, we can perform whatever math operations we need to on the dataset.

```
mean(current_combined)
```

[1] 68052.29

Here, we can see the mean for the entire dataset. However, we should always round values to the same number of decimal points as the original data. We can do this with round.

```
round(mean(current_combined),1) # round mean to one decimal
```

[1] 68052.3

Note that the above has a nested set of commands. We can write this exact same thing as follows:

```
# pipe mean through round
mean(current_combined) %>%
  round(1)
```

[1] 68052.3

Use the method that is easiest for you to follow!

We can also calculate the mean manually. The mean is $\frac{\sum_{i=1}^{n} x}{n}$, or the sum of all the values within a vector divided by the number of values in that vector.

```
# create function
# use curly brackets to denote function
# our data goes in place of "x" when finally run
our_mean <- function(x){
   sum_x <- sum(x) # sum all values in vector
   n <- length(x) # get length of vector
   xbar <- sum_x/n # calcualte mean
   return(xbar) # return the value outside the function
}</pre>
```

Let's try it.

```
our_mean(ranges$combined_current_km2)
```

[1] 68052.29

As we can see, it works just the same as mean! We can round this as well.

```
our_mean(ranges$combined_current_km2) %>%
  round(1)
```

[1] 68052.3

5 Your turn!

With a partner or on your own, try to do the following:

- 1. Create an $RMarkdown\ document$ that will save as an .html.
- 2. Load the data, as shown here, and print the summary statistics in the document.
- 3. Calculate the value of combined_current_km2 divided by 2050_combined_km2 and print the results.
- 4. knit your results, with your name(s) and date, as an HTML document.

Let me know if you have any issues.

6 Descriptive Statistics

6.1 Purposes of descriptive statistics

Descriptive statistics enable researchers to quickly and easily examine the "behavior" of their datasets, identifying potential errors and allowing them to observe particular trends that may be worth further analysis. Here, we will cover how to calculate descriptive statistics for multiple different datasets, culminating in an assignment covering these topics.

6.2 Preparing R

As with every week, we will need to load our relevant packages first. This week, we are using the following:

```
# allows for internet downloading
library(curl)
```

Using libcurl 8.7.1 with LibreSSL/3.3.6

```
# enables data management tools
library(tidyverse)
```

```
-- Attaching core tidyverse packages ----
                                                       ----- tidyverse 2.0.0 --
v dplyr
            1.1.4
                                     2.1.5
                       v readr
            1.0.0
                                     1.5.1
v forcats
                       v stringr
v ggplot2
            3.5.1
                       v tibble
                                     3.2.1
v lubridate 1.9.3
                       v tidyr
                                     1.3.1
v purrr
             1.0.2
-- Conflicts -----
                                                ----- tidyverse_conflicts() --
x dplyr::filter()
                       masks stats::filter()
x dplyr::lag()
                       masks stats::lag()
x readr::parse_date() masks curl::parse_date()
i Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force all conflicts to become
```

6.3 Downloading the data

For the example this week, we will be using the starbucks dataset, describing the number of drinks purchased during particular time periods during the day.

```
starbucks <- curl("https://raw.githubusercontent.com/jacobccooper/biol105_unk/main/datasets/
read_csv()</pre>
```

```
Rows: 9 Columns: 2
-- Column specification ------
Delimiter: ","
chr (1): Hour
dbl (1): Frap_Num

i Use `spec()` to retrieve the full column specification for this data.
```

i Specify the column types or set `show_col_types = FALSE` to quiet this message.

6.4 Descriptive statistics

Descriptive statistics are statistics that help us understand the shape and nature of the data on hand. These include really common metrics such as mean, median, and mode, as well as more nuanced metrics like quartiles that help us understand if there is any skew in the dataset. (Skew refers to a bias in the data, where more data points lie on one side of the distribution and there is a long tail of data in the other direction).

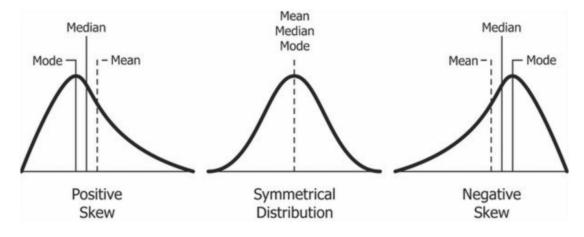


Figure 6.1: Examples of skew compared to a symmetrical, non-skewed distribution. Source: machinelearningparatodos.com

Note above that the relative position of the mean, median, and mode can be indicative of skew. Please also note that these values will rarely be exactly equal "in the real world", and thus you need to weigh differences against the entire dataset when assessing skew. There is a lot of nuance like this in statistics; it is not always an "exact" science, but sometimes involves judgment calls and assessments based on what you observe in the data.

Using the **starbucks** dataset, we can look at some of these descriptive statistics to understand what is going on.

6.4.1 Notation

As a quick reminder, we use Greek lettering for *populations* and Roman lettering for samples. For example:

- σ is a population, but s is a sample (both these variables refer to standard deviation).
- μ is a population, but \bar{x} is a sample (both of these variables refer to the mean).

6.4.2 Mean

The mean is the "average" value within a set of data, specifically, the sum of all values divided by the length of those values: $\frac{\sum_{i=1}^{n} x}{n}$.

head(starbucks)

```
# A tibble: 6 x 2
  Hour
            Frap_Num
  <chr>
                <dbl>
1 0600-0659
                    2
2 0700-0759
                    3
3 0800-0859
                    2
4 0900-0959
                    4
5 1000-1059
                    8
6 1100-1159
                    7
```

Here, we are specifically interested in the number of frappuccinos.

```
# get vector of frappuccino number
fraps <- starbucks$Frap_Num

# get mean of vector
mean(fraps)</pre>
```

[1] 6.222222

Note that the above should be rounded to a whole number, since we were given the data in whole numbers!

```
mean(fraps) %>%
  round(0)
```

[1] 6

```
# OR
round(mean(fraps),0)
```

[1] 6

We already covered calculating the average manually in our previous tutorial, but we can do that here as well:

```
# sum values
# divide by n, length of vector
# round to 0 places
round(sum(fraps)/length(fraps),0)
```

[1] 6

6.4.3 Range

The range is the difference between the largest and smallest units in a dataset. We can use the commands min and max to calculate this.

```
max(fraps) - min(fraps)
```

[1] 13

The range of our dataset is 13.

6.4.4 Median

The median is also known as the 50th percentile, and is the midpoint of the data when ordered from least to greatest. If there are an even number of data points, then it is the average point between the two center points. For odd data, this is the $\frac{n+1}{2}$ th observation. For even data, since we need to take an average, this is the $\frac{\frac{n}{2} + (\frac{n}{2} + 1)}{2}$. You should be able to do these by hand and by using a program.

```
median(fraps)
```

[1] 4

Now, to calculate by hand:

```
length(fraps)
```

[1] 9

We have an odd length.

```
# order gets the order
order(fraps)
```

[1] 1 3 7 2 4 6 5 9 8

```
# [] tells R which elements to put where
frap_order <- fraps[order(fraps)]
frap_order</pre>
```

[1] 2 2 2 3 4 7 8 13 15

```
# always use parentheses
# make sure the math maths right!
(length(frap_order)+1)/2
```

[1] 5

Which is the fifth element in the vector?

```
frap_order[5]
```

[1] 4

Now let's try it for an even numbers.

```
# remove first element
even_fraps <- fraps[-1]
even_fraps_order <- even_fraps[order(even_fraps)]
even_fraps_order</pre>
```

[1] 2 2 3 4 7 8 13 15

```
median(even_fraps)
```

[1] 5.5

Now, by hand: $\frac{\frac{n}{2} + (\frac{n}{2} + 1)}{2}$.

```
n <- length(even_fraps_order)

# get n/2 position from vector
m1 <- even_fraps_order[n/2]

# get n/2+1 position
m2 <- even_fraps_order[(n/2)+1]

# add these values, divide by two for "midpoint"
med <- (m1+m2)/2</pre>
med
```

[1] 5.5

As we can see, these values are equal!

6.4.5 Other quartiles and quantiles

We also use the 25th percentile and the 75th percentile to understand data distributions. These are calculated similar to the above, but the bottom quartile is only $\frac{1}{4}$ of the way between values and the 75th quartile is $\frac{3}{4}$ of the way between values. We can use the R function quantile to calculate these.

```
quantile(frap_order)
```

```
0% 25% 50% 75% 100%
2 2 4 8 15
```

We can specify a quantile as well:

```
quantile(frap_order, 0.75)
```

75% 8

We can also calculate these metrics by hand. Let's do it for the even dataset, since this is more difficult.

```
quantile(even_fraps_order)
```

```
0% 25% 50% 75% 100%
2.00 2.75 5.50 9.25 15.00
```

Note that the 25th and 75th percentiles are also between two different values. These can be calculated as a quarter and three-quarters of the way between their respective values.

```
# 75th percentile

n <- length(even_fraps_order)

# get position
p <- 0.75*(n+1)

# get lower value
# round down</pre>
```

```
m1 <- even_fraps_order[trunc(p)]

# get upper value
# round up
m2 <- even_fraps_order[ceiling(p)]

# position between
# fractional portion of rank
frac <- p-trunc(p)

# calculate the offset from lowest value
val <- (m2 - m1)*frac

# get value
m1 + val</pre>
```

[1] 11.75

Wait... why does our value differ?

R, by default, calculates quantiles using what is called Type 7, in which the quantiles are calculated by $p_k = \frac{k-1}{n-1}$, where n is the length of the vector and k refers to the quantile being used. However, in our book and in this class, we use Type 6 interpretation - $p_k = \frac{k}{n+1}$. Let's try using Type 6:

```
quantile(even_fraps_order, type = 6)
```

```
0% 25% 50% 75% 100%
2.00 2.25 5.50 11.75 15.00
```

Now we have the same answer as we calculated by hand!

This is a classic example of how things in R (and in statistics in general!) can depend on interpretation and are not always "hard and fast" rules.

In this class, we will be using Type 6 interpretation for the quantiles - you will have to specify this in the quantile function EVERY TIME! If you do *not* specify Type 6, you will get the questions incorrect and you will get answers that do not agree with the book, with Excel, or what you calculate by hand.

6.4.6 Mode

There is no default method for finding the mode in R. However, websites like Statology provide wraparound functions.

```
# Statology function
# define function to calculate mode
find_mode <- function(x) {
    # get unique values from vector
    u <- unique(x)
    # count number of occurrences for each value
    tab <- tabulate(match(x, u))
    # return the value with the highest count
    u[tab == max(tab)]
}
find_mode(fraps)</pre>
```

[1] 2

We can also do this by hand, by counting the number of occurrences of each value. This can be done in a stepwise fashion using commands in the above function.

```
# unique counts
u <- unique(fraps)
u</pre>
```

[1] 2 3 4 8 7 15 13

```
# which elements match
match(fraps,u)
```

[1] 1 2 1 3 4 5 1 6 7

```
# count them
tab <- match(fraps,u) %>%
  tabulate()
tab
```

[1] 3 1 1 1 1 1 1

Get the highest value.

```
u[tab==max(tab)]
```

[1] 2

Notice this uses ==. This is a logical argument that means "is equal to" or "is the same as". For example:

```
2 == 2
```

[1] TRUE

These values are the same, so TRUE is returned.

```
2 == 3
```

[1] FALSE

These values are unequal, so FALSE is returned. R will read TRUE as 1 and FALSE as ZERO, such that:

```
sum(2==2)
```

[1] 1

and

```
sum(2==3)
```

[1] 0

This allows you to find how many arguments match your condition quickly, and even allows you to subset based on these indices as well. Keep in mind you can use greater than <, less than >, greater than or equal to <=, less than or equal to >=, is equal to ==, and is not equal to != to identify numerical relationships. Other logical arguments include:

- &: both conditions must be TRUE to match (e.g., c(10,20) & c(20,10)). Try the following as well: fraps < 10 & fraps > 3.
- &&: and, but works with single elements and allows for better parsing. Often used with if. E.g., fraps < 10 && fraps > 3. This will not work on our multi-element frap vector.
- |: or, saying at least one condition must be true. Try: fraps > 10 | fraps < 3.
- ||: or, but for a single element, like && above.
- !: not, so "not equal to" would be !=.

6.4.7 Variance

When we are dealing with datasets, the variance is a measure of the total spread of the data. The variance is calculated using the following:

$$\sigma^2 = \frac{\sum (x_i - \bar{x})^2}{n - 1}$$

Essentially, this means that for every value of x, we are finding the difference between that value and the mean and squaring it, summing all of these quared differences, and dividing them by the number of samples in the dataset minus one. Let's do this for the frappuccino dataset.

frap_order

[1] 2 2 2 3 4 7 8 13 15

Now to find the differences.

Note that R is calculating the same thing for the entire vector! Since these are differences from the mean, they should sum to zero.

sum(diffs)

[1] 3.552714e-15

```
# square differences
diffs_sq <- diffs^2
diffs_sq</pre>
```

- [1] 17.8271605 17.8271605 17.8271605 10.3827160 4.9382716 0.6049383 3.1604938
- [8] 45.9382716 77.0493827

Now we have the squared differences. We need to sum these and divide by n-1.

```
n <- length(frap_order)
var_frap <- sum(diffs_sq)/(n-1)
var_frap</pre>
```

[1] 24.44444

Let's check this against the built-in variance function in R.

```
var(frap_order)
```

[1] 24.44444

They are identical! We can check this using a logical argument.

```
var_frap == var(frap_order)
```

[1] TRUE

Seeing as this is TRUE, we calculated it correctly.

6.4.8 Standard deviation

Another common measurement of spread is the standard deviation (σ) . As you remember from class (or may have guessed from the notation on this site), the standard deviation is just the square root of the variance.

```
sqrt(var_frap)
```

[1] 4.944132

We can test this against the built in sd function in R:

```
sqrt(var_frap) == sd(frap_order)
```

[1] TRUE

As you can see, we calculated this correctly!

6.4.9 Standard error

The standard error is used to help understand the spread of data and to help estimate the accuracy of our measurements for things like the mean. The standard error is calculated thusly:

$$SE = \frac{\sigma}{\sqrt{n}}$$

There is not built in function for the standard error in excel, but we can write our own:

```
se <- function(x){
  n <- length(x) # calculate n
  s <- sd(x) # calculate standard deviation
  se_val <- s/sqrt(n)
  return(se_val)
}</pre>
```

Let's test this code.

```
se(frap_order)
```

[1] 1.648044

Our code works! And we can see exactly how the standard error is calculate. We can also adjust this code as needed for different situations, like samples.

Remember, the standard error is used to help reflect our *confidence* in a specific measurement (*e.g.*, how certain we are of the mean, and what values we believe the mean falls between). We want our estimates to be as precise as possible with as little uncertainty as possible. Given this, does having more samples make our estimates more or less confident? Mathematically, what happens as our sample size *increases*?

6.4.10 Coefficient of variation

The coefficient of variation, another measure of data spread and location, is calculated by the following:

$$CV = \frac{\sigma}{\mu}$$

We can write a function to calculate this in R as well.

```
cv <- function(x){
  sigma <- sd(x)
  mu <- mean(x)
  val <- sigma/mu
  return(val)
}</pre>
```

[1] 0.7945927

Remember that we will need to round values.

6.4.11 Outliers

Outliers are any values that are outside of the 1.5 times the interquartile range. We can calculate this for our example dataset as follows:

```
lowquant <- quantile(frap_order,0.25,type = 6) %>% as.numeric()
hiquant <- quantile(frap_order,0.75,type = 6) %>% as.numeric()
iqr <- hiquant - lowquant
lowbound <- lowquant - (1.5*iqr)
hibound <- hiquant + (1.5*iqr)

# low outliers?
# select elements that match
# identify using logical "which"
frap_order[which(frap_order < lowbound)]</pre>
```

numeric(0)

```
# high outliers?
# select elements that match
# identify using logical "which"
frap_order[which(frap_order > hibound)]
```

numeric(0)

We have no outliers for this particular dataset.

6.5 Homework: Descriptive Statistics

Now that we've covered these basic statistics, it's your turn! For this week, you will be completing homework that involves methods from Chapter 4 in your book.

6.5.1 Homework instructions

Please create an RMarkdown document that will render as an .html file. You will submit this file to show your coding and your work. Please refer to the Introduction to R for refreshers on how to create an .html document in RMarkdown. You will need to do the following for each of these datasets:

- mean
- median
- range
- interquartile range
- variance
- standard deviation
- coefficient of variation
- standard error
- whether there are any "outliers"

Please show all of your work for full credit.

6.5.2 Data for homework problems

For each question, calculate the mean, median, range, interquartile range, variance, standard deviation, coefficient of variation, standard error, and whether there are any "outliers".

Please also write your own short response to the *Synthesis question* posed, which will involve thinking about the data and metrics you just analyzed.

6.5.2.1 1: UNK Nebraskans

Ever year, the university keeps track of where students are from. The following are data on the umber of students admitted to UNK from the state of Nebraska:

years nebraskans 10 2014 11 2013

Using these data, please calculate the mean, median, range, interquartile range, variance, standard deviation, coefficient of variation, standard error, and whether there are any "outliers" for the number of UNK students from Nebraska.

In order to do this, we will need to first select the column that denotes the number of Nebraskans from the dataset. Remember, we need to save this as an object in R to do the analyses. Here is a demonstration of getting the column to look at the mean, so that you can repeat this for the other questions. This relies heavily on the use of \$, used to get the data from a specific column.

```
# $ method
nebraskans <- nebraskans_years$nebraskans
nebraskans</pre>
```

[1] 5056 5061 5276 5244 5209 5262 5466 5606 5508 5540 5614

Now we can get the mean of this vector.

```
mean(nebraskans) %>%
  round(0) # don't forget to round!
```

[1] 5349

Synthesis question: Do you think that there are any really anomalous years? Do you feel data are similar between years? *Note* we are not looking at trends through time but whether any years are outliers.

6.5.2.2 2: Piracy in the Gulf of Guinea

The following is a dataset looking at oceanic conditions and other variables associated with pirate attacks within the region between 2010 and 2021 (Moura et al. 2023). Using these data, please calculate the mean, median, range, interquartile range, variance, standard deviation, coefficient of variation, standard error, and whether there are any "outliers" for distance from shore for each pirate attack (column Distance_from_Coast).

```
url_file <- curl("https://figshare.com/ndownloader/files/42314604")
pirates <- url_file %>% read_csv()
```

Synthesis question: Do you notice any patterns in distance from shore? What may be responsible for these patterns? *Hint*: Think about what piracy entails and also what other columns are available as other variables in the above dataset.

6.5.2.3 3: Patient ages at presentation

The following is a dataset on skin sores in different communities in Australia and Oceania, specifically looking at the amount of time that passes between skin infections (Lydeamore et al. 2020a). This file includes multiple different datasets, and focuses on data from children in the first five years of their life, on househould visits, and on data collected during targeted studies (Lydeamore et al. 2020b).

```
url_file <- curl("https://doi.org/10.1371/journal.pcbi.1007838.s006")
ages <- url_file %>% read_csv()

Rows: 17150 Columns: 2
-- Column specification -------
Delimiter: ","
chr (1): dataset
```

dbl (1): time_difference

- i Use `spec()` to retrieve the full column specification for this data.
- i Specify the column types or set `show_col_types = FALSE` to quiet this message.

Let's see what this file is like real fast. We can use the command dim to see the rows and columns.

```
dim(ages)
```

[1] 17150 2

As you can see, this file has only two columns but 17,150 rows! For the column time_difference, please calculate the mean, median, range, interquartile range, variance, standard deviation, coefficient of variation, standard error, and whether there are any "outliers".

Synthesis question: Folks will often think about probabilities of events being "low but never zero". What does that mean in the context of these data? What about these data make you feel like probabilities may decrease through time but never become zero?

7 Diagnosing data visually

7.1 The importance of visual inspection

Inspecting data visually can give us a lot of information about whether data are normally distributed and about whether there are any major errors or issues with our dataset. It can also help us determine if data meet model assumptions, or if we need to use different tests more appropriate for our datasets.

7.2 Sample data and preparation

First, we need to load our R libraries.

```
library(curl)
```

Using libcurl 8.7.1 with LibreSSL/3.3.6

-- Attaching core tidyverse packages -----

```
library(tidyverse)
```

```
v dplyr
       1.1.4
                    v readr
                                2.1.5
v forcats
           1.0.0
                    v stringr
                                1.5.1
v ggplot2 3.5.1
                    v tibble
                                3.2.1
v lubridate 1.9.3
                    v tidyr
                                1.3.1
v purrr
           1.0.2
-- Conflicts -----
                                       x dplyr::filter()
                    masks stats::filter()
x dplyr::lag()
                    masks stats::lag()
x readr::parse_date() masks curl::parse_date()
i Use the conflicted package (<a href="http://conflicted.r-lib.org/">http://conflicted.r-lib.org/</a>) to force all conflicts to become
```

----- tidyverse 2.0.0 --

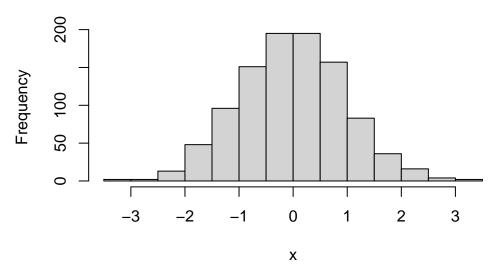
Next, we can download our data sample.

7.3 Histograms

A histogram is a frequency diagram that we can use to visually diagnose data and their distributions. We are going to examine a histogram using a random string of data. R can generate random (though, actually pseudorandom) strings of data on command, pulling them from different distributions. These distributions are pseudorandom because we can't actually program R to be random, so it starts from a wide variety of pseudorandom points.

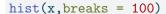
7.3.1 Histograms on numeric vectors

Histogram of x

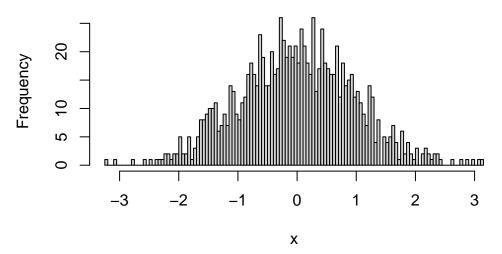


NOTE that a histogram can only be made on a vector of values. If you try to make a histogram on a data frame, *you will get an error and it will not work*. You have to specify which column you wish to use with the \$ operator. (For example, for dataframe xy with columns x and y, you would use hist(xy\$y)).

We can up the number of bins to see this better.



Histogram of x

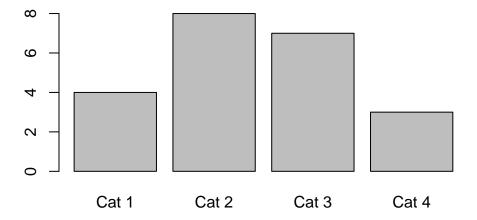


The number of bins can be somewhat arbitrary, but a value should be chosen based off of what illustrates the data well. R will auto-select a number of bins in some cases, but you can also select a number of bins. Some assignments will ask you to choose a specific number of bins as well.

7.3.2 Histograms on frequency counts

Say, for example, that we have a dataset where everything is already shown as frequencies. We can create a frequency histogram using barplot.

```
barplot(count_table$Count, # response variable, counts for histogram
    axisnames = T, # make names on plot
    names.arg = count_table$Category) # make these the names
```



7.3.3 ggplot histograms

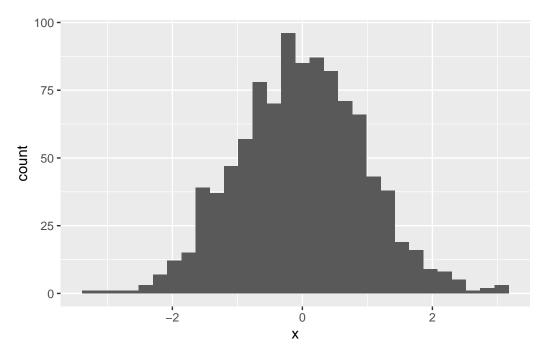
The following is an optional workthrough on how to make really fancy plots.

We can also use the program ggplot, part of the tidyverse, to create histograms.

```
# ggplot requires data frames
x2 <- x %>% as.data.frame()
colnames(x2) <- "x"

ggplot(data = x2, aes(x = x)) +
   geom_histogram()</pre>
```

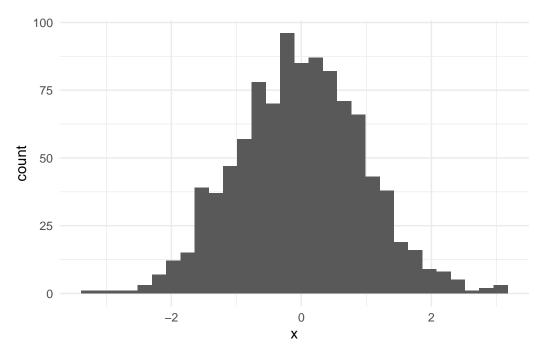
[`]stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



ggplot is nice because we can also clean up this graph a little.

```
ggplot(x2,aes(x=x)) + geom_histogram() +
   theme_minimal()
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



We can also do a histogram of multiple values at once in R.

```
x cat

1 -1.07435774 x

2 -0.82433014 x

3 0.76803047 x

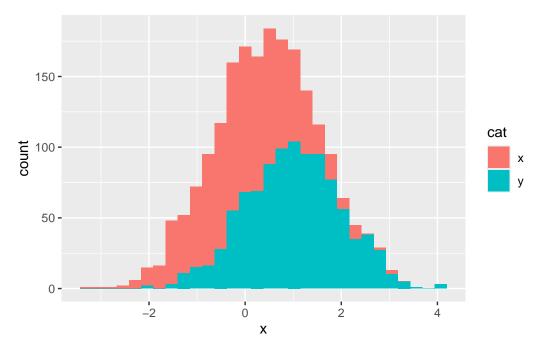
4 0.34248267 x

5 0.84234017 x

6 -0.00990365 x
```

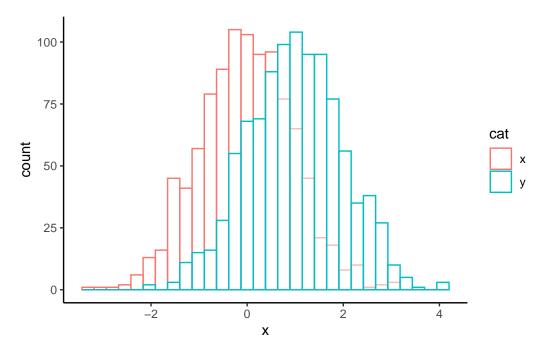
```
ggplot(xy,aes(x = x, fill = cat)) +
  geom_histogram()
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



We can also make this look a little nicer.

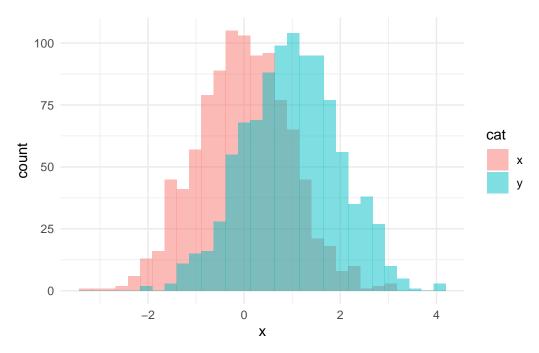
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



We can show these a little differently as well.

```
ggplot(xy, aes(x = x, fill = cat))+
  geom_histogram(position = "identity", alpha = 0.5) +
  theme_minimal()
```

`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.



There are lots of other commands you can incorporate as well if you so choose; I recommend checking sites like this one.

7.4 Skewness

Skew is a measure of how much a dataset "leans" to the positive or negative directions (*i.e.*, to the "left" or to the "right"). To calculate skew, we are going to use the moments library.

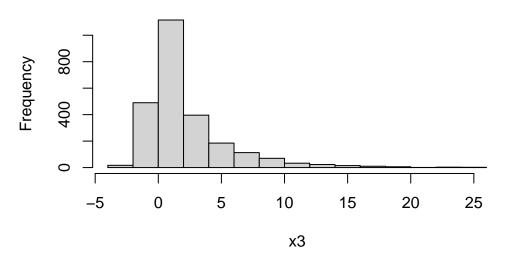
```
# don't forget to install if needed!
library(moments)
skewness(x)
```

[1] 0.04627593

Generally, a value between -1 and +1 for skewness is "acceptable" and not considered overly skewed. Positive values indicate "right" skew and negative values indicate a "left" skew. If something is too skewed, it may violate assumptions of normality and thus need non-parametric tests rather than our standard parametric tests - something we will cover later!

Let's look at a skewed dataset. We are going to artificially create a skewed dataset from our x vector.

Histogram of x3



skewness(x3)

[1] 2.303814

As we can see, the above is a heavily skewed dataset with a positive ("right") skew.

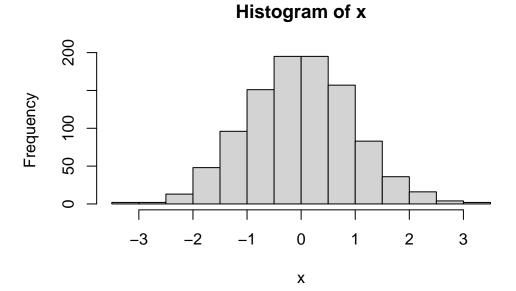
7.5 Kurtosis

Kurtosis refers to how sharp or shallow the peak of the distribution is (platykurtic vs. leptokurtic). Remember - platykyrtic are plateaukurtic, wide and broad like a plateau, and leptokurtic distributions are sharp. Intermediate distributions that are roughly normal are mesokurtic.

Much like skewness, **kurtosis values of** > 2 and < -2 are generally considered extreme, and thus not mesokurtic. This threshold can vary a bit based on source, but for this class, we will use a threshold of ± 2 for both skewness and kurtosis.

Let's see the kurtosis of x. **Note** that when doing the equation, a normal distribution actually has a kurtosis of 3; thus, we are doing kurtosis -3 to "zero" the distribution and make it comparable to skewness.

hist(x)



non-zeroed
kurtosis(x)

[1] 3.055901

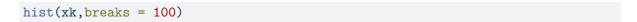
zeroed
kurtosis(x)-3

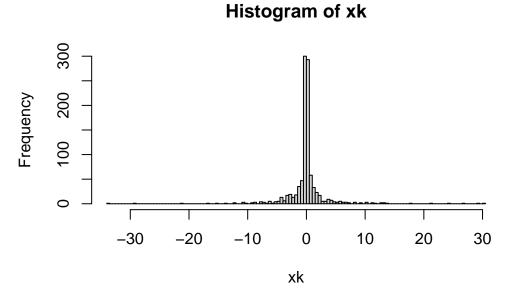
[1] 0.05590072

As expected, out values drawn from a normal distribution are not overly skewed. Let's compare these to a more kurtic distribution:

xk <- x^3
kurtosis(xk)-3</pre>

[1] 29.58594





As we can see, this is a very *leptokurtic* distribution.

7.6 Cumulative frequency plot

A *cumulative frequency plot* shows the overall spread of the data as a cumulative line over the entire dataset. This is another way to see the spread of the data and is often complementary to a histogram.

The following cumulative distribution plot is based on the method outlined in Geeks for Geeks.

7.6.1 If data are not in histogram/frequency format

You will need to create a frequency table to make them be in histogram format.

```
# declaring the break points
# make break points based on data
# always round UP with ceiling
range.x <- ceiling(max(x)-min(x))
range.x</pre>
```

[1] 7

```
range(x)
```

```
[1] -3.234498 3.110551
```

Based on this range, we need to create our bin sizes. We want our lowest bin to be below our lowest value, and our highest bin above our highest value. Here, I'm setting a step size of 1. You will have to examine your data and determine the best break size for your datasets.

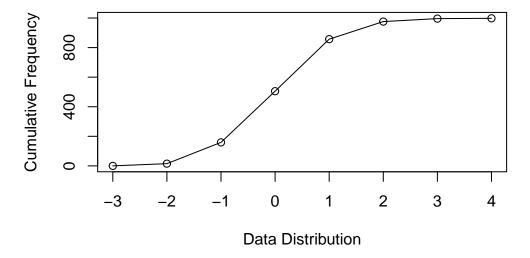
[1] "Frequency Table"

```
print(freq_table)
```

```
data_transform
[-3,-2) [-2,-1) [-1,0) [0,1) [1,2) [2,3) [3,4)
15 144 346 352 119 20 2
```

```
print(cumulative_freq)
```

```
[-3,-2) [-2,-1) [-1,0) [0,1) [1,2) [2,3) [3,4) 
0 15 159 505 857 976 996 998
```



7.6.2 If data are in histogram/frequency format

If you have a list of frequencies (say, for river discharge over several years), you only need to do the cumsum function. For example:

```
y <- c(1 ,2 ,4, 8, 16, 8, 4, 2, 1)

sum_y <- cumsum(y)

print(y)
```

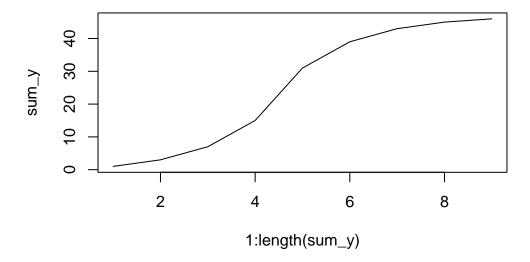
```
[1] 1 2 4 8 16 8 4 2 1
```

```
print(sum_y)
```

[1] 1 3 7 15 31 39 43 45 46

Now we can see we have out cumulative sums. Let's plot these. **NOTE** that this method will *not* have the x variables match the dataset you started with, it will only plot the curve based on the number of values given.

```
plot(x = 1:length(sum_y), # get length of sum_y, make x index
    y = sum_y, # plot cumulative sums
    type = "l") # make a line plot
```



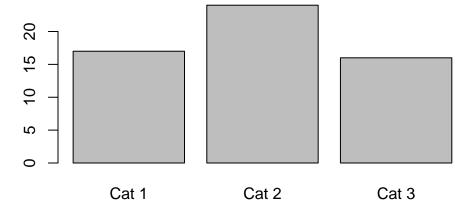
7.7 Homework: Chapter 3

From your book, complete problems 3.1, 3.4 & 3.5. Data for these problems are available on *Canvas* and in your book.

7.7.1 Helpful hint

HINT: For 3.5, consider just making a vector of the values of interest for a histogram. For example, see the following. For reference:

• c means "concatenate", or place things together in an object.



7.7.2 Directions

Please complete all computer portions in an rmarkdown document knitted as an html. Upload any "by hand" calculations as images in the HTML or separately on *Canvas*.

7.8 Addendum

With thanks to Hernan Vargas & Riley Grieser for help in formatting this page. Additional comments provided by BIOL 305 classes.

8 Normality & hypothesis testing

8.1 Normal distributions

A standard normal distribution is a mathematical model that describes a commonly observed phenomenon in nature. When measuring many different kinds of datasets, the data being measured often becomes something that resembles a standard normal distribution. This distribution is described by the following equation:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{\frac{(x-\mu)^2}{2\sigma^2}}$$

This equation is fairly well defined by the *variance* (σ^2), the overall spread of the data, and by the *standard deviation* (σ), which is defined by the square root of the variance.

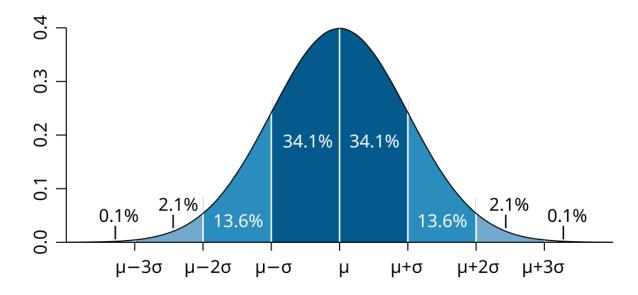


Figure 8.1: A standard normal distribution, illustrating the percentage of area found within each standard deviation away from the mean. By Ainali on Wikipedia; CC-BY-SA 3.0.

Standard normal distributions have a mean, median, and mode that are equal. The standard normal distribution is a density function, and we are interested in the "area under the curve" (AUC) to understand the relative probability of an event occurring. At the mean/median/mode, the probability on either side of the distribution is 50%. When looking at a normal distribution distribution, it is impossible to say the probability of a specific event occurring, but it is possible to state the probability of an event as extreme or more extreme than the event observed occurring. This is known as the p value.

8.1.1 Example in nature

In order to see an example of the normal distribution in nature, we are going to examine the BeeWalk survey database from the island of Great Britain (Comont 2020). We are not interested in the bee data at present, however, but in the climatic data from when the surveys were performed.

```
beewalk <- curl("https://figshare.com/ndownloader/files/44726902") %>%
  read_csv()
```

Note that this is another massive dataset - 306, 550 rows of data!

The dataset has the following columns:

colnames (beewalk)

```
[1] "RecordKey"
                             "Website.ID"
                                                      "Website.RecordKey"
                                                      "ViceCounty"
 [4] "SiteName"
                             "Site.section"
 [7] "established"
                             "GridReference"
                                                      "Projection"
                                                      "Transect.long"
[10] "Precision"
                             "Transect.lat"
[13] "transect.OS1936.lat"
                             "Transect.OS1936.long"
                                                     "transect_length"
                             "section_grid_ref"
                                                      "H1"
[16] "section_length"
                             "H3"
                                                      "H4"
[19] "H2"
[22] "habitat_description"
                             "T.1"
                                                      "L2"
```

```
[25] "land_use_description" "start_time"
                                                      "end_time"
[28] "sunshine"
                              "wind_speed"
                                                      "temperature"
[31] "TaxonVersionKey"
                              "species"
                                                      "latin"
[34] "queens"
                              "workers"
                                                      "males"
[37] "unknown"
                              "Comment"
                                                      "transect_comment"
[40] "flower_visited"
                              "StartDate"
                                                      "EndDate"
[43] "DateType"
                              "Year"
                                                      "Month"
[46] "Day"
                              "Sensitive"
                                                      "Week"
[49] "TotalCount"
```

We are specifically interested in temperature to determine weather conditions. Let's see what the mean of this variable is.

```
mean(beewalk$temperature)
```

[1] NA

Hmmm... we are getting an NA value, indicating that not every cell has data recorded. Let's view summary.

summary(beewalk\$temperature)

```
Min. 1st Qu. Median Mean 3rd Qu. Max. NA's 0.00 16.00 19.00 18.65 21.00 35.00 16151
```

As we can see, 16,151 rows do not have temperature recorded! We want to remove these NA rows, which we can do by using using na.omit.

```
beewalk$temperature %>%
  na.omit() %>%
  mean() %>%
  round(2) # don't forget to round!
```

[1] 18.65

Now we can record the mean.

Let's visualize these data using a histogram. *Note* I do not use na.omit as the hist function automatically performs this data-cleaning step!

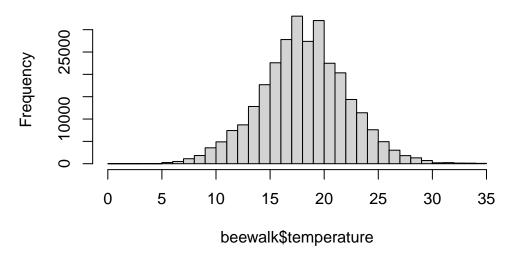
Histogram of beewalk\$temperature



Even with only five breaks, we can see an interesting, normal-esque distribution in the data. Let's refine the bin number.

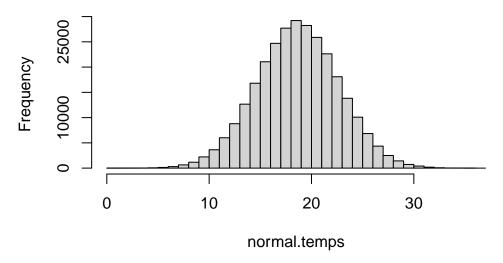
hist(beewalk\$temperature,breaks = 40)

Histogram of beewalk\$temperature



With forty breaks, the pattern becomes even more clear. Let's see what a *standard normal distribution* around these data would look like.

Histogram of normal.temps



As we can see, our normal approximation of temperatures is not too dissimilar from the distribution of temperatures we actually see!

Let's see what kind of data we have for temperatures:

```
# load moments library
library(moments)
skewness(temps)
```

[1] 0.02393257

Data do not have any significant skew.

```
kurtosis(temps)-3
```

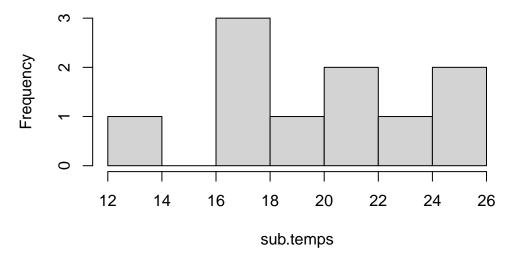
[1] 0.3179243

Data do not show any significant kurtosis.

8.1.2 Effect of sampling

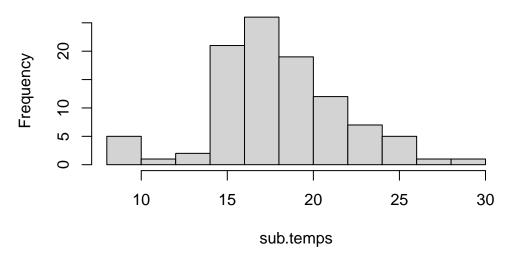
Oftentimes, we will see things approach the normal distribution as we collect more samples. We can model this by subsampling our temperature vector.

10 samples



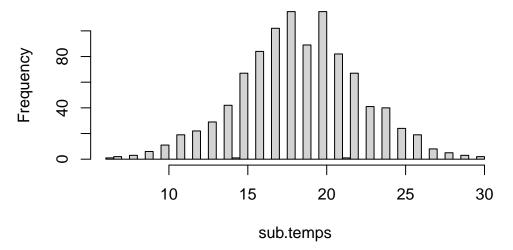
With only ten values sampled, we do not have much of a normal distribution. Let's up this to 100 samples.

100 samples



Now we are starting to see more of a normal distribution! Let's increase this to 1000 temperatures.

1000 samples



Now the normal distribution is even more clear. As we can also see, the more we sample, the more we approach the true means and distribution of the actual dataset. Because of this, we can perform experiments and observations of small groups and subsamples and make inferences about the whole, given that most systems naturally approach statistical distributions like the normal!

8.2 Hypothesis testing

Since we can define specific areas under the curve within these distributions, we can look at the percentage of area within a certain bound to determine how likely a specific outcome would be. Thus, we can begin to test what the *probability of observing an event* is within a theoretical, probabilistic space. A couple of important conceptual ideas:

- 1. We may not be able to know the probability of a specific event, but we can figure out the probability of events more extreme or less extreme as that event.
- 2. If the most likely result is the mean, then the further we move away from the mean, the less likely an event becomes.
- 3. If we look away from the mean at a certain point, then the area represents the chances of getting a result as extreme or more extreme than what we observe. This probability is known as the p value.

Once we have a p value, we can make statements about the event that we've seen relative to the overall nature of the dataset, but we do not have sufficient information to declare if this result is $statistically \ significant$.

8.2.1 Critical Values - α

In order to determine if something is significant, we compare things to a *critical value*, known as α . This value is traditionally defined as 0.05, essentially stating that we deem an event as significant if 5% or fewer of observed or predicted events are as extreme or more extreme than what we observe.

Your value should always set your α critical value before you do your experiments and analyses.

8.2.2 Visualizing a p value

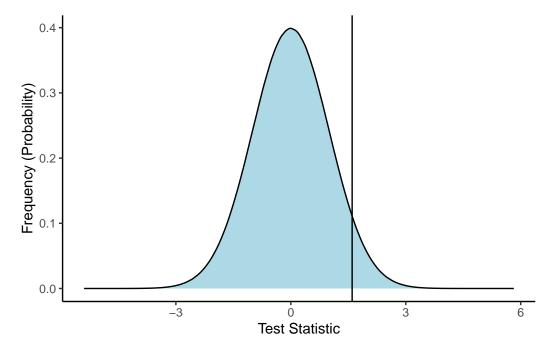
Let's say that we are looking at a dataset defined by a standard normal distribution with $\mu = 0$ and $\sigma = 1$. We draw a random value, x, with x = 1.6. What is the probability of drawing a number this extreme or more extreme from the dataset?

First, let's visualize this distribution:

```
###THIS WILL TAKE A WHILE TO RUN###
# create gigantic normal distribution dataset
# will be essentially normal for plotting
# rnorm gets random values
x \leftarrow rnorm(100000000)
# convert to data frame
x <- as.data.frame(x)
# rename column
colnames(x) <- c("values")</pre>
# thank you stack overflow for the following
# Creating density plot
p = ggplot(x,
           aes(x = values)
          ) +
  # generic density plot, no fill
  geom_density(fill="lightblue")
# Building shaded area
# create new plot object
p2 <- p + # add previous step as a "backbone"
  # rename axes
  geom_vline(xintercept = 1.6) +
```

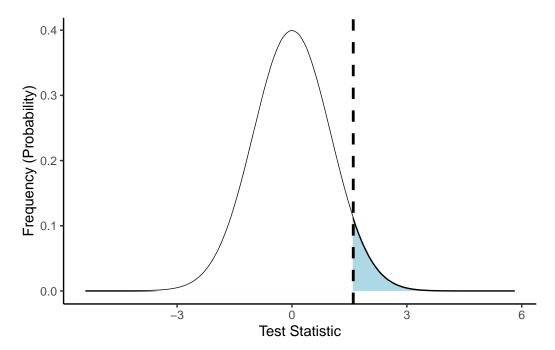
```
xlab("Test Statistic") +
ylab("Frequency (Probability)") +
# make it neat and tidy
theme_classic()

# plot it
# can use ggsave function to save
plot(p2)
```



Above, the solid black line represents x, with the illustrated standard normal distribution being filled in blue.

Let's see how much of the area represents values as extreme or more extreme as our value x.



Now we can see that it is only a portion of the distribution that is within our p value, but we don't know exactly what this area is! We can find out in one of two ways.

8.2.3 Calculating a p value

We have two different methods for calculating a p value:

8.2.3.1 Comparing to a z table

We can compare the z value we calculate to a z table, such as the one at ztable.net. On this webpage, you can scroll and find tables for positive and negative z scores. Note that normal distributions are symmetrical, so you can also transform from negative to positive to get an idea of the area as well. Given that a normal distribution is centered at 0, a z score of 0 will have a p value of 0.50.

On the z tables, you will find the tenths place for your decimal in the rows, and then go across to the columns for the hundredths place. For example, go to the website and find the p value for a z score of -1.33. You should find the cell marked 0.09176. Note the website uses naked decimals, which we do not use in this class.

For values that aren't on the z table, we can approximate its position between different points on the z table or, if it is extremely unlikely, denote that p < 0.0001.

8.2.3.2 Using R

In R, we can calculate a p value using the function pnorm. This function uses the arguments of p for our p value, mean for the mean of our distribution, sd for the standard deviation of our distribution, and also information on whether we want to log-transform p or if we are testing a specific hypothesis (lower tail, upper tail, or two-tailed). The function pnorm defaults to a standard normal distribution, which would be a z score, but it can also perform the z transformations for us if we define the mean and standard deviation.

For example, if we have a z score of -1.33:

pnorm(-1.33)

[1] 0.09175914

As we can see, we get the same result as our z table, just with more precision!

There are other functions in this family as well in R, including dnorm for quantiles, qnorm for determining the z score for a specific p value, and rnorm for getting random values from a normal distribution with specific dimensions. For now, we will focus on pnorm.

8.2.4 Workthrough Example

When this class was being designed, Hurricane Milton was about to make contact with Florida. Hurricane Milton is considered one of the strongest hurricanes of all time, so we can look at historical hurricane data to determine just how powerful this storm really was. We can get information on maximum wind speeds of all recorded Atlantic hurricanes as of 2024 from Wikipedia.

hurricanes <- read_csv("https://raw.githubusercontent.com/jacobccooper/biol305_unk/main/assignations.com/jacobccooper/b

```
Rows: 889 Columns: 1
-- Column specification ------
Delimiter: ","
dbl (1): Hurricane_Windspeed

i Use `spec()` to retrieve the full column specification for this data.
i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

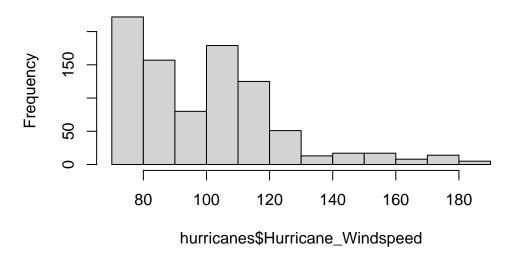
Above, we have loaded a .csv of one column that has all the hurricane speeds up to 2024. Hurricane Milton is the last row - the most recent hurricane. Let's separate this one out. We will use [,], which defines [rows,columns] to subset data.

```
milton <- hurricanes$Hurricane_Windspeed[nrow(hurricanes)]
other_hurricanes <- hurricanes[-nrow(hurricanes),]</pre>
```

We want to compare the windspeed of Milton (180 mph) to the overall distribution of hurricane speeds. We can visualize this at first.

```
# all hurricanes
hist(hurricane_Windspeed)
```

Histogram of hurricanes\$Hurricane_Windspeed



Windspeeds are more towards the lower end of the distribution, with strong storms being rarer.

For the sake of this class, we will assume we can use a normal distribution for these data, but if we were doing an official study we would likely need to use a non-parametric test (we will cover these later, but they cover non-normal data).

```
mu <- mean(other_hurricanes$Hurricane_Windspeed)
mu</pre>
```

[1] NA

Hmmm... we need to use na.omit to be sure we do this properly.

```
other_hurricanes_windspeed <- na.omit(other_hurricanes$Hurricane_Windspeed)
mu <- mean(other_hurricanes_windspeed)
mean(other_hurricanes_windspeed)</pre>
```

[1] 102.5254

Next, we need the standard deviation.

```
sd.hurricane <- sd(other_hurricanes_windspeed)
sd.hurricane</pre>
```

[1] 23.08814

Now, we can calculate our Z value.

```
Z <- (milton - mu)/sd.hurricane</pre>
```

[1] 3.355603

How significant is this?

```
pnorm(Z)
```

[1] 0.999604

This is greater than 0.5, so we need to do 1-p to figure things out.

```
1 - pnorm(Z)
```

[1] 0.000395961

This rounds to 0.0004, which means that this is an extremely strong hurricane.

8.2.4.1 Non-normality, for those curious

We can do a Shapiro-Wilk test of normality to see if this dataset is normal.

```
shapiro.test(other_hurricanes_windspeed)
```

```
Shapiro-Wilk normality test
```

```
data: other_hurricanes_windspeed
W = 0.88947, p-value < 2.2e-16</pre>
```

A p < 0.05 indicates that these data are non-normal.

We can do a Wilcoxon-Test since these data are extremely non-normal.

Wilcoxon rank sum test with continuity correction

data: other_hurricanes_windspeed and milton
W = 6.5, p-value = 0.08678
alternative hypothesis: true location shift is not equal to 0

Using non-normal corrections, we find that this is *not* an extremely strong hurricane, but it is near the upper end of what we would consider "normal" under historical conditions. Still an extremely bad hurricane!

8.3 Confidence Intervals

Because we can figure out the probability of an event occurring, we can also calculate *confidence intervals*. A *confidence interval* provides a range of numbers around a value of interest that indicates where we believe the mean of a population lies and our confidence that it lies within that range. **Note** that nothing is ever 100% certain, but this helps us determine where a mean is and demonstrates our confidence in our results.

Specifically, if the tails of the distribution, our α , are 0.05, then we have an area of 0.95 around the mean where we do not reject results. Another perspective on this area is that we can say with 95% certainty that a mean is within a certain area, and that if values fall within that confidence area then we do not reject the null hypothesis that the means are equal.

We will cover several different ways to calculate confidence intervals, but for normal distributions, we use the following equation, with the 0.95 confidence interval shown as an example:

$$CI = \bar{x} \pm Z_{1-\frac{\alpha}{2}} \cdot \frac{\sigma}{\sqrt{n}}$$

This interval gives us an idea of where the mean should lie. For example, if we are looking at the aforemention beewalk temperature data, we can calulate a 0.95 confidence interval around the mean.

```
temps <- na.omit(beewalk$temperature)

xbar <- mean(temps)
n <- length(temps)
sdtemp <- sd(temps)
# Z for 0.95 as P, so for 0.975, 0.025
# get value from P
Z <- qnorm(0.975)

CI <- Z*(sdtemp/sqrt(n))</pre>
CI
```

[1] 0.01458932

We have a very narrow confidence zone, because we have so many measurements. Let's round everything and present it in a good way.

If I want numbers to show up *in text* in RMarkdown, I can add code to a line of plain text using the following syntax:

```
# DO NOT RUN
# Format in plaintext
`r xbar`
```

Typing that into the <u>plaintext</u> should render as the following: `r xbar`. Then I can also type my answer as follows:

The \$95\$% Confidence Interval for the mean for this temperature dataset is `r round(xbar,2)` \$\pm\$ `r round(CI,2)`.

Figure 8.2: This is the "coded" version of the text below. Compare the above window to the text below this image.

Typing that into the plaintext should render as the following: 18.645963. Then I can also type my answer as follows:

The 95% Confidence Interval for the mean for this temperature dataset is 18.65 ± 0.01 .

Note that the above is rounded to two decimal places to illustrative purposes ONLY, and should be rounded to one decimal place if it was a homework assignment because the original data has only one decimal place.

8.4 Homework: Chapter 8

Please complete problems 8.1, 8.2, 8.3 & 8.6. Follow the directions as written in the book. Submit one html file, as derived from *RStudio*. For maximum clarity, create headings to separate your problems. (Remember, a header can be invoked by placing '#' in front of a line of text. For example: the header here is written as # Homework: Chapter 8).

9 Exam 2 practice

9.1 Exam 2 Practice

The following is practice for the exam. Please work through these problems and be ready to discuss them in class.

9.2 Question 1: Cyclones

Consider this short data set:

| Latitude band | Season | Number of cyclones |
|---------------|--------|--------------------|
| 40 - 49° S | Fall | 370 |
| 40 - 49° S | Winter | 452 |
| 40 - 49° S | Spring | 273 |
| 40 - 49° S | Summer | 422 |
| 50 - 59° S | Fall | 526 |
| 50 - 59° S | Winter | 624 |
| 50 - 59° S | Spring | 513 |
| 50 - 59° S | Summer | 1,059 |
| 60 - 69° S | Fall | 980 |
| 60 - 69° S | Winter | 1,200 |
| 60 - 69° S | Spring | 995 |
| 60 - 69° S | Summer | 1,751 |

All of the data was collected in the same year to determine whether the occurrences of cyclones differed by latitude and season around Antarctica. Use this data to answer the following questions.

- a) Classify each of the three variables above as either an explanatory or a response variable. Justify your answer.
- b) Classify each of the three variables above as either a nominal, ordinal, interval, or ratio variable. Justify your answer.

- c) State the null and alternative hypotheses for this scenario. Note: there are two sets of null/alternative hypotheses.
- d) Calculate the mean, median, and mode of your response variable. Based on your results, would you expect your data to be normally distributed or not? Justify your answer.
- e) Let's say now that you're testing the null hypothesis that the mean number of cyclones in this one year is similar to the average year. The mean number of cyclones per year across all years of data collected is 650 with a standard deviation of 425. Calculate the z-score and make a decision regarding the null hypothesis.

9.3 Question 2: Minnows

Consider this scenario: You have discovered a never-before-documented population of minnow in the Kearney Canal near campus. During your first sampling trip, you notice that the total length (i.e., measured from the tip of the snout to the very tip of the tail) of the fish you measure appear to be smaller than the average total length of the species as recorded among all known individuals across their range. The mean total length noted in one publication is 85 mm with a standard deviation of 4.50. Below is your data the data from 20 minnows that you captured during your first sampling trip to the Kearney Canal:

| Fish ID | Length (mm) |
|---------|-------------|
| 1 | 89 |
| 2 | 75 |
| 3 | 86 |
| 4 | 74 |
| 5 | 69 |
| 6 | 100 |
| 7 | 73 |
| 8 | 69 |
| 9 | 96 |
| 10 | 79 |
| 11 | 61 |
| 12 | 62 |
| 13 | 95 |
| 14 | 98 |
| 15 | 100 |
| 16 | 57 |
| 17 | 70 |
| 18 | 78 |
| 19 | 65 |
| 20 | 65 |

- a) State the null and alternative hypothesis for your study.
- b) Calculate the median, first and third quartiles, and interquartile range of your response variable. Create a boxplot. Based on your results, are there any outliers in your data? Explain.
- c) Calculate the z-score for this scenario. Please show all of your work.
- d) What is the probability that, by random chance alone, you would find your observed mean or something more extreme?
- e) Assume that you set your α for your study prior to your data collection to 0.05. Based on this information and the p-value you obtained for part c above, is your null hypothesis supported or rejected?

10 Probability distributions

10.1 Probability distributions

We rely on multiple different probability distributions to help us understand what probable outcomes are for a specific scenario. All of the tests that we are performing are comparing our results to what we would expect under perfectly random scenarios. For example, if we are flipping a coin, we are interested in whether the observation we have of the flips on our coin matches our expectation given the probability of getting heads or tails on a perfectly fair coin. While it is possible to get all heads or all tails on a coin flip, it is highly unlikely and may lead us to believe we have an unfair coin. The more trails we perform, the more confident we can be that out coin is atypical.

We perform similar comparisons for other distributions. If we are comparing sets of events, we can look at the probability of those events occurring if events are occurring randomly. If we are comparing counts, we can compare our counts to our expectation of counts if events or subjects are distributed randomly throughout the matrix or whether two sets of counts are likely under the same sets of assumptions.

Remember, for our specific tests, we are setting an α value in advance (traditionally 0.05, or 5%) against which we compare our p value, with p representing the probability of observing an event as extreme or more extreme than the event we observe given a specific probability distribution.

Previously, we talked about the *normal distribution*, which is used to approximate a lot of datasets in nature. However, several other probability distributions are also useful for biological systems, which are outlined here.

10.2 Binomial distribution

A binomial distribution is one in which only two outcomes are possible - often coded as 0 and 1 and usually representing failure and success, respectively. The binomial is described by the following function:

$$p(x) = \binom{n}{x} p^x (1-p)^{n-x}$$

where n = number of trials, x = the number of successes, and p = the probability of a success under random conditions.

In R, the binomial distribution is represented by the following functions:

- dbinom: the density of a binomial distribution
- pbinom: the distribution function, or the probability of a specific observation
- qbinom: the value at which a specific probability is found (the quantile function)
- rbinom: generates random values according to a binomial.

Let's see what this looks like. Let's consider a scenario where we flip a coin 10 times and get 9 heads. How likely is this outcome?

[1] 0.999

NOTE that the trailing 0 is dropped, such that the real answer is 0.9990. However, we mentioned before that the p value should be the probability of a result as extreme or more extreme, meaning that it should *always* be less than 0.5. If we are reporting a value of *greater* than 0.5, then we are comparing to the upper tail of the distribution. For a one-tailed α of 0.05, this would mean that we are looking for a value *greater than* 0.95 $(1 - \alpha)$.

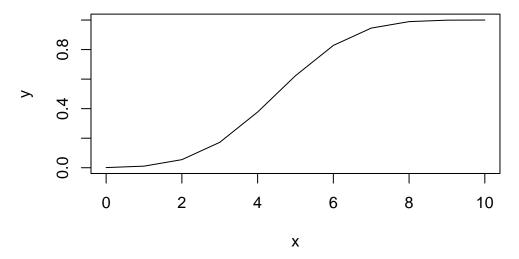
So, our real p is:

```
1-round(x,4)
```

[1] 0.001

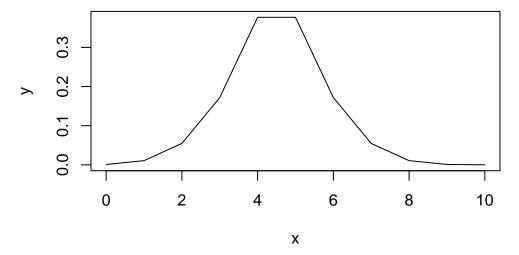
Again, the trailing zero is missing. Given that $p < \alpha$, we reject the null hypothesis that this is a fair coin.

How does this distribution look?



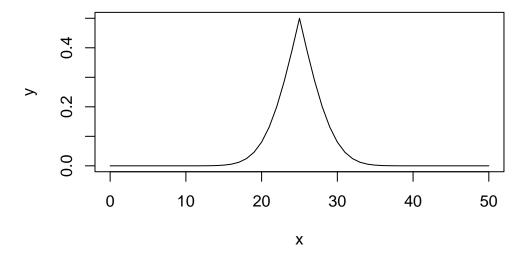
What about if we always have p less than 0.5 to reflect two tails?

```
# any value greater than 0.5 is subtracted from 1 y[y > 0.5] <-1 - y[y > 0.5] plot(x,y,type="l")
```



What if we do this with a bigger dataset, like for 50 flips?

```
# number of successes
# start at 0 for no heads
x <- 0:50
# cumulative probability to left of outcome
y <- pbinom(x,size = length(x), prob = 0.5, lower.tail = T)
# any value greater than 0.5 is subtracted from 1
y[y > 0.5] <- 1 - y[y > 0.5]
plot(x,y,type="l")
```



As we increase the number of flips, we can see that the probability of success forms a *normal distribution* centered on the outcome given the default probability. Thus, as we deviate from

our *expected outcome* (initial probability multiple by the number of trials), then our results become less likely.

10.3 Poisson distribution

The Poisson distribution is used to reflect random count data. Specifically, the Poisson is used to determine if success events are overdispersed (i.e., regularly spaced), random, or underdispersed (i.e., clustered). The Poisson introduces the variable lambda (λ) which represents the mean (μ) and the variance (σ^2), which are equal in a Poisson distribution. A Poisson distribution is described by the following function:

$$p(x) = \frac{\lambda^x e^{-\lambda}}{x!}$$

The Poisson is represented by the following functions in R which closely resemble the functions for the normal and binomial distributions:

- dpois: the log density function
- ppois: log distribution (probability) function
- qpois: quantile function
- rpois: random values from a Poisson.

Let's look at the probability of 0 to 10 successes when we have our $\lambda = 1$.

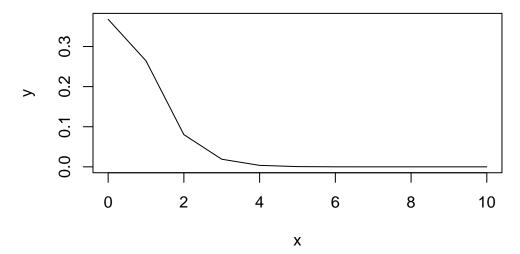
```
x \leftarrow 0:10

y \leftarrow ppois(x, lambda = 1)

# any value greater than 0.5 is subtracted from 1

y[y > 0.5] \leftarrow 1 - y[y > 0.5]

plot(x,y,type="l")
```



As we can see, the probability of rare events is high, whereas the probability quickly decreases as the number of successes increases.

10.4 χ^2 -squared distribution

 χ^2 -squared (pronounced "kai", and spelled "chi") is a distribution used to understand if count data between different categories matches our expectation. For example, if we are looking at students in the class and comparing major vs. number of books read, we would expect no association, however we may find an association for a major such as English which required reading more literature. The χ^2 introduces a new term degrees of freedom (df) which reflects the number of individuals in the study. For many tests, df are needed to reflect how a distribution changes with respect the number of individuals (and amount of variation possible) within a dataset. The equation for the χ^2 is as follows, with the χ^2 being a special case of the gamma (γ or Γ) distribution that is affected by the df, which is defined as the number of rows minus one multiplied by the number of columns minus one df = (rows - 1)(cols - 1):

$$f_n(x) = \frac{1}{2^{\frac{n}{2}}\Gamma(\frac{n}{2})} x^{\frac{n}{2-1}} e^{\frac{-x}{2}}$$

The χ^2 -squared distribution is also represented by the following functions, which perform the same things as the previous outlined equivalents for Poisson and binomial:

- dchisq
- pchisq
- qchisq
- rchisq

We can view these probabilities as well:

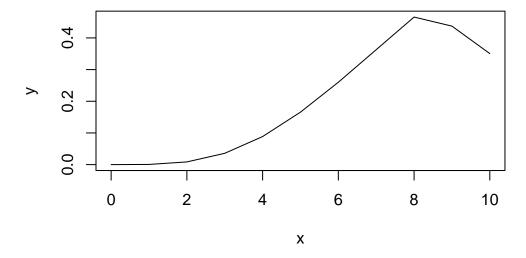
```
x \leftarrow 0:10

y \leftarrow pchisq(x, df = 9)

# any value greater than 0.5 is subtracted from 1

y[y > 0.5] \leftarrow 1 - y[y > 0.5]

plot(x,y,type="l")
```



10.4.1 Calculating the test statistic

We evaluate χ^2 tests by calculating a χ^2 value based on our data and comparing it to an expected χ^2 distribution. This *test statistic* can be evaluated by looking at a χ^2 table or by using R. **Note** that you need to know the degrees of freedom in order to properly evaluate a χ^2 test. We calculate our test statistic as follows:

$$\chi^2 = \Sigma \frac{(o-e)^2}{e}$$

where e = the number of expected individuals and o = the number of observed individuals in each category. Since we are squaring these values, we will only have positive values, and thus this will always be a one-tailed test.

10.4.2 χ^2 estimations in R

Usually when we use a χ^2 , we are looking at count data. Let's consider the following hypothetical scenario, comparing experience with R between English majors (who, in this theoretical scenario, do not regularly use R) and Biology majors who are required to take R for this class:

| Major | R experience | No R experience |
|---------|--------------|-------------------|
| English | 2 | 8 |
| Biology | 9 | 1 |

Intuitively, we can see a difference, but we want to perform a statistical test to see just how likely these counts would be if both groups were equally likely to use R:

```
chisq.test(x = c(2,8),
y = c(9,1))
```

Warning in chisq.test(x = c(2, 8), y = c(9, 1)): Chi-squared approximation may be incorrect

Pearson's Chi-squared test with Yates' continuity correction

```
data: c(2, 8) and c(9, 1)
X-squared = 0, df = 1, p-value = 1
```

Here we get p = 1, which reflects a one-tailed interpretation.

10.4.3 Calculating χ^2 by hand

10.5 Fisher's exact test

To be completed; involves calculations on smaller datasets.

10.6 Homework

10.6.1 Chapter 5

10.6.2 Chapter 7

11 Single population means testing

- 11.1 Introduction
- 11.2 *t*-distribution
- 11.3 *t*-tests
- 11.4 Wilcoxon tests
- 11.5 Confidence intervals
- 11.6 Homework: Chapter 9

12 Two sample tests

- 12.1 Introduction
- 12.2 *t*-tests
- 12.3 Mann-Whitney U tests
- 12.4 Error
- 12.5 Homework: Chapter 10

13 ANOVA: Part 1

- 13.1 Introduction
- 13.2 ANOVA: By hand
- 13.3 ANOVA: By *R*
- 13.4 Kruskal-Wallis tests
- 13.5 Homework: Chapter 11

14 ANOVA: Part 2

- 14.1 Two-way ANOVA
- 14.2 Designs
- 14.2.1 Randomized block design
- 14.2.2 Repeated measures
- 14.2.3 Factorial ANOVA
- 14.3 Friedman's test
- 14.4 Homework: Chapter 12

15 Correlation & regression

- 15.1 Introduction
- 15.2 Correlation
- 15.2.1 Pearson's
- 15.2.2 Spearman's
- 15.2.3 Other non-parametric methods
- 15.3 Correlation
- 15.3.1 Parametric
- 15.3.2 Non-parametric
- 15.4 Homework
- 15.4.1 Chapter 13
- 15.4.2 Chapter 14

16 Final exam & review

- 16.1 Pick the test
- 16.2 Final review

17 Conclusions

Parting thoughts about the course.

17.1

(pronounced doh-dah-dah-go-huh-ee) is a traditional Cherokee farewell. It does not mean goodbye, but rather reflects a parting of ways until a group of folks meet again.

I enjoyed getting to know all of you in class, and please feel free to reach out or stop by and say hi if you are ever passing through Kearney in the future or if you need help with something biology related.

Wishing you the best,

Dr. Cooper

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18 Glossary

18.1 Common Commands

The following are common useful commands used in R, with examples of their use.

 $\bullet~<-$ / = - save a value as an object

```
x <- 10
x
```

[1] 10

 $\bullet\,$ c - concatenate, place two values together

```
x <- c(10,11)
x
```

[1] 10 11

•