Statistical Intuition for Modern Biologists

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Preface

This is a Quarto book.

To learn more about Quarto books visit https://quarto.org/docs/books.

```
1 + 1
```

```
[1] 2
```

Part I

Necessary Introduction

1 Introduction to RNA-seq

This is a book created from markdown and executable code.

See Knuth (1984) for additional discussion of literate programming.

```
1 + 1
```

```
[1] 2
```

2 Introduction to R

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

3 Introduction to Probability

Why collect replicates of the same exact data? The (in)famous definition of insanity, "Doing something over and over again and expecting a different result", would seem to classify replication as crazy. Except, anyone who has ever collected any kind of data has an intuition for why replication is important: each replicate is always different from the last. Somehow, despite following a precise protocol in a particular lab with the same reagents, pipettes, measuring devices, etc., every replicate yields a slightly different result. **There is randomness in your data**.

This randomness throws a wrench in naive data analyses. In biology, we are often measuring something (e.g., the concentration of an interesting molecule, the viability of an organism, etc.) in two or more "conditions" (e.g., with and without drug) and assessing whether or not any of our measurements differ from one another. If every replicate of an experiment yielded the same data, this task would be trivial. Just take one measurement in each condition, compare the measurements, and draw conclusions. If the measurements differ, the thing you are measuring differs. If the measurements are identical, the thing you are measuring is unaffected by your perturbations. The randomness of data forces us to consider an alternative conclusion. What if our measurements differ, not because the thing we are measuring is changing, but because our measurements fluctuate from experiment-to-experiment? How do we know what differences are real and what differences are the result of this randomness?

We will start answering this question with the help of **probability theory**. Probability theory is a field of mathematics that gives us the tools to think about and quantify this randomness. This chapter will introduce you to the key instrument provided by this field: the probability distribution. With this tool, we will be able to describe and better understand the random fluctuations that plague our data.

4 The Many Flavors of Randomness

To understand how statisticians think about randomness, consider an analogy. In biology, everything is complicated and every system we study seems unique. To tackle this complexity, we group things into bins based on important characteristics they share, and then we try to understand the patterns that govern each bin. Bins in biology may be determined by the type of organism being studied (e.g., entymology, mammology, microbiology, etc.), the activities of molecular machines you investigate (e.g., phosphorylating subtrates, regulating transcription, intracellular transport, etc.), and much more. Similarly in statistics, every kind of data seems to possess unique sources and types of variance. Work with enough data though and you will notice that there are patterns in the types of randomness we commonly see. We thus focus on understanding these common patterns and apply them to understanding our unique data.

The patterns in randomness that we observe are coined "probability distributions". Think of them as functions, which take as input a number, and provide as output the probability of observing that number. These functions could take any shape you dream up, but as mentioned, particular patterns crop up all of the time. In the following exercises, we are going to explore these patterns and understand what gives rise to them.

4.1 The basics

To illustrate some general facts about randomness, we will start by working with two functions in R: `rbinom()` and `rnorm()`. These are two of many "random number generators" (RNGs) in R. As we will learn in these exercises, there isn't just one kind of randomness, so there isn't just one RNG.

`rbinom` and `rnorm` both have 3 parameters. Both have a parameter called `n`, which sets the number of random numbers to generate. `rbinom` has two other parameters called `size` and `prob`. `size` can be any integer >= 0. `prob` can be any number between 0 and 1 (including 0 and 1). `rnorm`'s two additional parameters are called `mean` and `sd`. `mean` can be any number, and `sd` can be any positive number. Take some time to play with both of these functions. As you do so, consider the following:

1. In what ways are their output similar?
2. In what ways are their output distinct?
3. How do `prob` and `size` impact the output of `rbinom`?
4. How do `mean` and `sd` impact the output of `rnorm`?

5. Make histograms with different numbers of random numbers (i.e., different `n`). What do you see as `n` increases? Now repeat this but with different parameters for the relevant function. What changes?

4.1.1 Example exercise:

Generated 5 draws from each to compare general output:

```
print("rbinom output:")
```

```
[1] "rbinom output:"
```

```
rbinom(10, size = 100, prob = 0.5)
```

```
 [1] 55 45 45 53 50 55 57 62 50 58
```

```
print("rnorm output:")
```

```
[1] "rnorm output:"
```

```
rnorm(10)
```

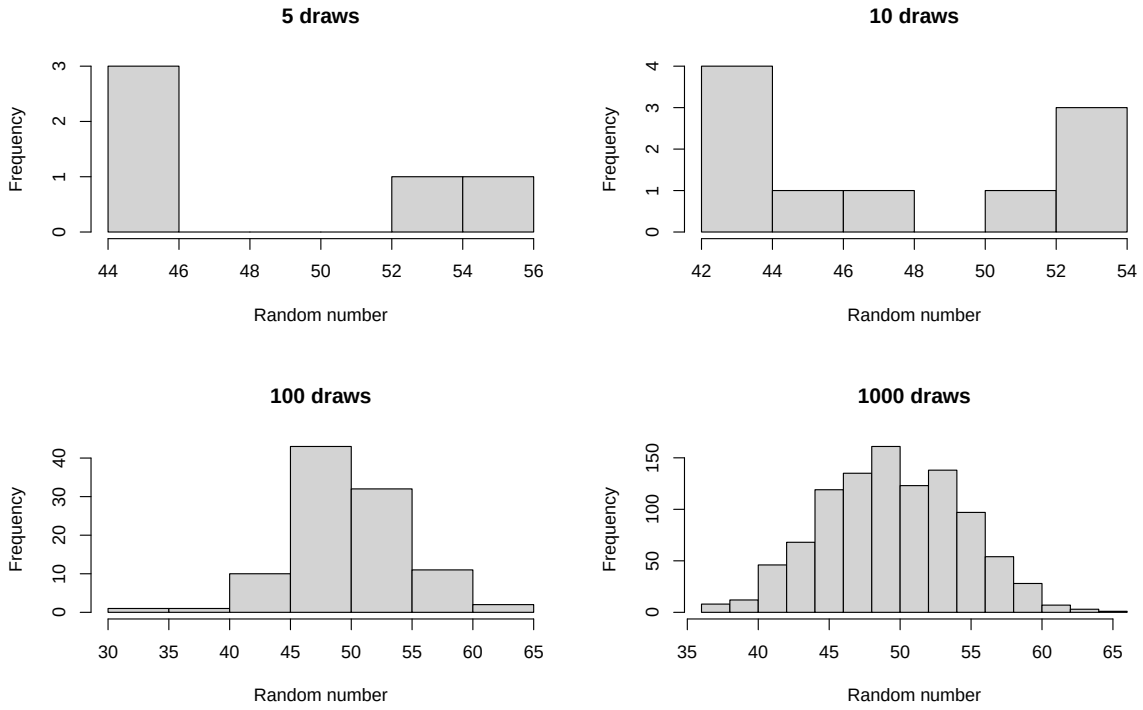
```
 [1] -1.1970764  0.6093135 -0.3263831  0.4662279 -1.3589269 -0.1343390
 [7]  0.4270621 -0.8600750  0.6860104  0.3292209
```

Observations:

1. `rbinom` seems to only output exact integers. rnorm's output has lots of decimal places
2. As expected, output for both usually differs from run to run
3. `rnorm` output seems to always differ while `rbinom` might spit out the same number from time to time.

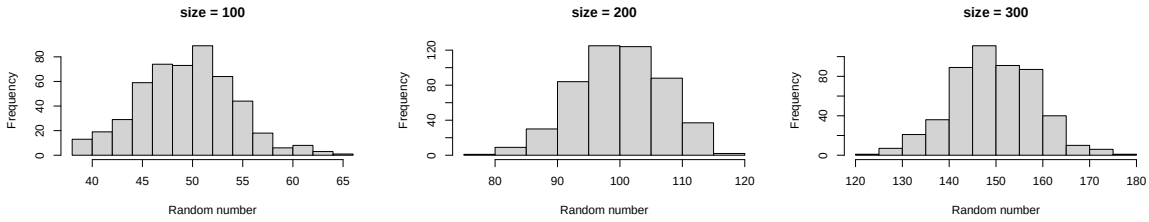
Taking more draws reveals patterns in the randomness that is not as evident from less draws:

```r
b5 <- rbinom(n = 5, size = 100, prob = 0.5)
b50 <- rbinom(n = 10, size = 100, prob = 0.5)
b500 <- rbinom(n = 100, size = 100, prob = 0.5)
b5000 <- rbinom(n = 1000, size = 100, prob = 0.5)


hist(b5, main = "5 draws", xlab = "Random number")
hist(b50, main = "10 draws", xlab = "Random number")
hist(b500, main = "100 draws", xlab = "Random number")
hist(b5000, main = "1000 draws", xlab = "Random number")
```



Varying size of rbinom:

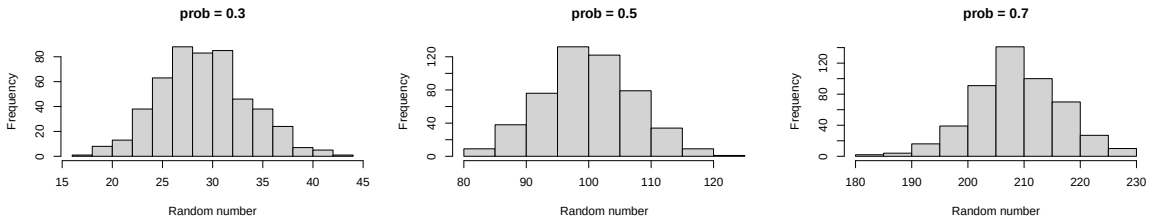
```r
b100 <- rbinom(500, size = 100, prob = 0.5)
b200 <- rbinom(500, size = 200, prob = 0.5)
b300 <- rbinom(500, size = 300, prob = 0.5)

hist(b100, main = "size = 100", xlab = "Random number")
hist(b200, main = "size = 200", xlab = "Random number")
hist(b300, main = "size = 300", xlab = "Random number")
```



Higher size generates larger random numbers. In fact, distribution of random numbers seems to increase in proportion to increase in size (i.e., doubling size doubles the average random number generated)

Varying prob of `rbinom`

```
b0.3 <- rbinom(500, size = 100, prob = 0.3)
b0.5 <- rbinom(500, size = 200, prob = 0.5)
b0.7 <- rbinom(500, size = 300, prob = 0.7)

hist(b0.3, main = "prob = 0.3", xlab = "Random number")
hist(b0.5, main = "prob = 0.5", xlab = "Random number")
hist(b0.7, main = "prob = 0.7", xlab = "Random number")
```



Higher prob has a similar effect to higher size, increasing the average magnitude of random numbers generated. There also seems to be a similar linear proportionality.

4.1.2 Takeaways

1. There are two kinds of randomness: discrete and continuous.
2. Discrete randomness is randomness where the output can be described with integers.
3. Continuous randomness is randomness where the output can be described with real numbers.
4. Randomness is described by its patterns. Any individual data point may be random but collect enough of them from a given process/source and a pattern will emerge. Some values are more likely to occur, some are less likely, and some may even never occur.

4.1.3 Properties of random variables

4.1.3.1 Mean

The mean of a distribution is a measure of its "central tendencies". Usually, values closer to the mean are more common than values further away from the mean. The mean of a set of random numbers can be calculated in R using the `mean()` function:

```
b <- rbinom(100, size = 100, prob = 0.5)
n <- rnorm(100, mean = 5)

mean(b)
```

```
[1] 49.54
```

```
mean(n)
```

```
[1] 4.928811
```

Because of the randomness inherent in RNGs, the mean that you will get for any finite sample will often vary from sample to sample:

```
b1 <- rbinom(100, size = 100, prob = 0.5)
b2 <- rbinom(100, size = 100, prob = 0.5)
b3 <- rbinom(100, size = 100, prob = 0.5)

mean(b1)
```

```
[1] 49.94
```

```
mean(b2)
```

```
[1] 50.46
```

```
mean(b3)
```

```
[1] 49.17
```

Every distribution has some inherent mean given its set of parameters. You can think of this as the mean you would get if you took a really large sample. For example, the `mean` parameter in `rnorm` is exactly this large sample mean. You can see how the mean of a given sample tends to gets closer to this value as you increase the sample size:

```
n10 <- rnorm(10, mean = 5)
n100 <- rnorm(100, mean = 5)
n1000 <- rnorm(1000, mean = 5)

mean(n10)
```

```
[1] 4.82812
```

```
mean(n100)
```

```
[1] 5.04991
```

```
mean(n1000)
```

```
[1] 5.011704
```

You can calculate the mean yourself with some simple R code; it is the sum of all your data points divided by the number of data points:

```
n100 <- rnorm(100, mean = 5)

mean(n100)
```

```
[1] 4.941502
```

```
sum(n100)/length(n100)
```

```
[1] 4.941502
```

For any distribution, you can look up its mean (as well as the other properties discussed below) on sites like Wikipedia:

1. Binomial distribution
2. Normal distribution

4.1.3.2 Variance/standard deviation

A key feature of randomness is the amount of variability from outcome to outcome of a random process. As we will see when we start using models of data randomness to draw conclusions about said data, figuring out how variable our data is represents a key challenge.

We often describe the variability of a random sample using the variance and standard deviation. These can be calculated in R using the `var` and `sd` functions, respectively:

```
b <- rbinom(100, size = 100, prob = 0.5)
n <- rnorm(100, mean = 5)

sd(b)
```

```
[1] 5.02538
```

```
sd(n)
```

```
[1] 0.930266
```

```
var(b)
```

```
[1] 25.25444
```

```
var(n)
```

```
[1] 0.8653949
```

Close inspection will reveal that the output of `var` is just the output of `sd` squared:

```
b <- rbinom(100, size = 100, prob = 0.5)
n <- rnorm(100, mean = 5)

sd(b)^2
```

```
[1] 21.68929
```

```r
var(b)
```

```
[1] 21.68929
```

```r
sd(n)^2
```

```
[1] 0.7655041
```

```r
var(n)
```

```
[1] 0.7655041
```

The variance measures the squared distance of each data point from the mean, and sums up all of these squared distances:

```r
b <- rbinom(100, size = 100, prob = 0.5)
n <- rnorm(100, mean = 5)

var(b)
```

```
[1] 26.06828
```

```r
sum( ( b - mean(b) )^2 )/(length(b) - 1)
```

```
[1] 26.06828
```

```r
var(n)
```

```
[1] 1.029502
```

```r
sum( ( n - mean(n) )^2 )/(length(n) - 1)
```

```
[1] 1.029502
```

Why the square? When assessing variability, we don't care if data is above or below the mean, so we want to score data points greater than the mean equally to how we score those below the mean. The square of the mean - 1 is the same as the square of the mean + 1, and both are positive values, because squaring a real number always yields a positive number. Why not the absolute value? Or the fourth power of the distance to the mean? Or any other definitively positive value? Convenience and mathematical formalism. In other words, don't worry about it.

NOTE: EXTRA DETAIL ALERT

The other mystery of the calculation of the standard deviation/variance is the fact that the sum of the deviations from the mean are divided by the number of data points **minus 1**, rather than just the number of data points, as in the mean. Why is this? Again, mathematical formalism, but there is an intuitive explanation to be discovered.

When calculating the mean, each data point is its own entity that contributes equally to the calculation. I can't calculate the mean without all n data points. When calculating the standard deviation though, each data point is compared to the mean, which is itself calculated from all of the data points.

If I tell you the mean though, I only need to tell you n - 1 data points for you to infer the final data point. For example, if I have 3 numbers that I tell you average to 2, and two of them are 1 and 3, what is the third? It has to be 2. Thus, when calculating the distance from each point to the mean, there are only n - 1 unique pieces of information. I tell you n - 1 data points and the mean, and you can tell me the standard deviation (since you can infer the missing data point). We say that there are "n - 1 degrees of freedom". Thus, the effective average of the differences from the mean are all of the squared differences divided by n - 1, the number of "non-redundant" data points that remain after calculating the mean.

END OF THE EXTRA INFO ALERT

4.1.3.3 Higher order "moments" (BONUS CONTENT)

The mean and the variance are related to what are called (who knows why) moments of a distribution. A moment is the average of some function of the random data. For example, the mean is the average of the data itself, which you could call data passed through the function f(x) = x. The variance is related to this moment, as well as the so-called second moment, which is the average of the square of the random data (f(x) = x^2). In fact, this leads to an equivalent way to calculate the variance:

```
b <- rbinom(100, size = 100, prob = 0.5)
n <- rnorm(100, mean = 5)

var(b)
```

```
[1] 22.5304
```

```
mean(b^2) - mean(b)^2
```

```
[1] 22.3051
```

```
var(n)
```

```
[1] 1.000763
```

```
mean(n^2) - mean(n)^2
```

```
[1] 0.9907555
```

Well not fully equivalent, but they get closer to one another as the sample size increases:

```
n100 <- rnorm(100, mean = 5)
n1000 <- rnorm(1000, mean = 5)
n10000 <- rnorm(10000, mean = 5)
```

```
n = 100:
var(x):  1.181613
(x^2) - mean(x)^2:  1.169797

n = 1000:
var(x):  1.04252
(x^2) - mean(x)^2:  1.041478

n = 10000:
var(x):  1.002371
x^2) - mean(x)^2:  1.00227
```

Various other moments or functions of moments enjoy some level of popularity in specific circles. We won't talk about them here, but you'll see them on Wikipedia sites for distributions, things like the "skewness" and "excess kurtosis" are examples of these additional moments, or functions of moments.

4.1.3.4 Density/mass functions

Earlier, I said "Think of [probability distributions] as functions, which take as input a number, and provide as output the probability of observing that number". Fleshing out what I mean by this will help you understand one of the key concepts in probability theory: probability density/mass functions.

Take a look at what a normalized histogram of draws from `rbinom()` looks like as we increase `n`. "Normalized" here means that instead of the y-axis representing the number of draws in a given bin (what is plotted by `hist()` by default), it is this divided by the total number of draws.

```
b5 <- rbinom(n = 10, size = 100, prob = 0.5)
b50 <- rbinom(n = 100, size = 100, prob = 0.5)
b500 <- rbinom(n = 1000, size = 100, prob = 0.5)
b5000 <- rbinom(n = 10000, size = 100, prob = 0.5)


# freq = FALSE will plot normalized counts
hist(b5, main = "10 draws", xlab = "Random number", freq = FALSE)
hist(b50, main = "100 draws", xlab = "Random number", freq = FALSE)
hist(b500, main = "1000 draws", xlab = "Random number", freq = FALSE)
hist(b5000, main = "10000 draws", xlab = "Random number", freq = FALSE)
```

As you increase `n`, a pattern begins to emerge. The y-axis is the fraction of the time a random number ended up in a particular bin. It can thus be interpreted as the probability that the random number falls within the bounds defined by that bin. What you may notice is that these probabilities seem to converge to particular values as `n` gets large. This pattern of probabilities is known as the binomial distributions "probability mass function". If the output of the RNG is continuous real numbers, then this is referred to as the "probability density function".

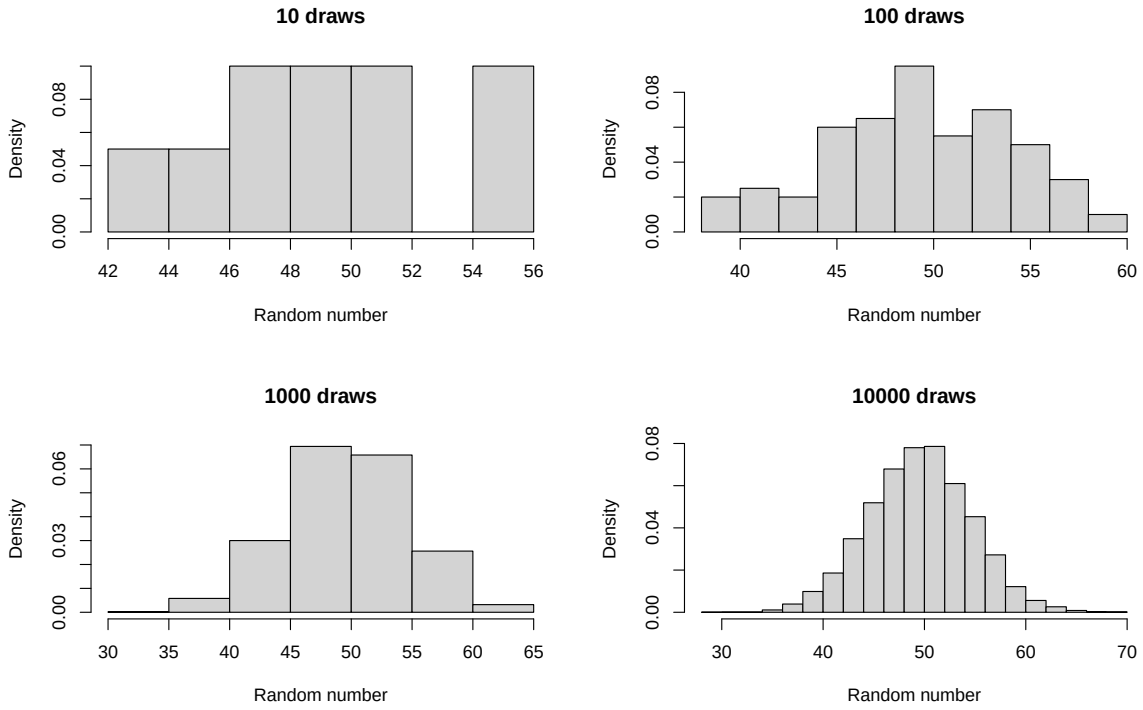
For all of the distributions that we can draw random numbers from in R, we can similarly use R to figure out its probability mass/density function. The function will have the form `d<distribution>`, where `d` denotes density function:

```
dbinom(x = 50, size = 100, prob = 0.5)
```

```
[1] 0.07958924
```

```
dbinom(x = 10, size = 100, prob = 0.5)
```

```
[1] 1.365543e-17
```




```
dbinom(x = 90, size = 100, prob = 0.5)
```

```
[1] 1.365543e-17
```

```
dbinom(x = 110, size = 100, prob = 0.5)
```

```
[1] 0
```

```
dbinom(x = 50.5, size = 100, prob = 0.5)
```

```
Warning in dbinom(x = 50.5, size = 100, prob = 0.5): non-integer x = 50.500000
```

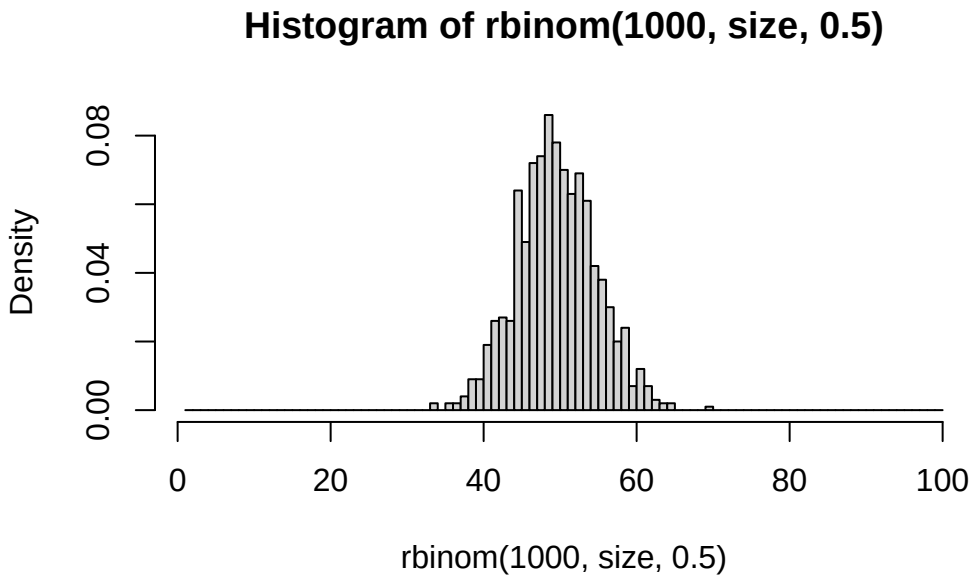
```
[1] 0
```

4.1.3.4.1 Exercise

Prove to yourself that `dbinom` yield probabilities similar to what you saw in your normalized histograms.

Hint:

```
### Make bins of size 1 so as to match up more cleanly with `dbinom()`

# Maximum value
size <- 100

# Plot with bin size of 1
hist(rbinom(1000, size, 0.5), freq = FALSE, breaks = 1:size)
```



EXAMPLE EXERCISE:

```
library(ggplot2)
library(dplyr)
```

```
Warning: package 'dplyr' was built under R version 4.3.2
```

```
Attaching package: 'dplyr'
```

```
The following objects are masked from 'package:stats':

    filter, lag

The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

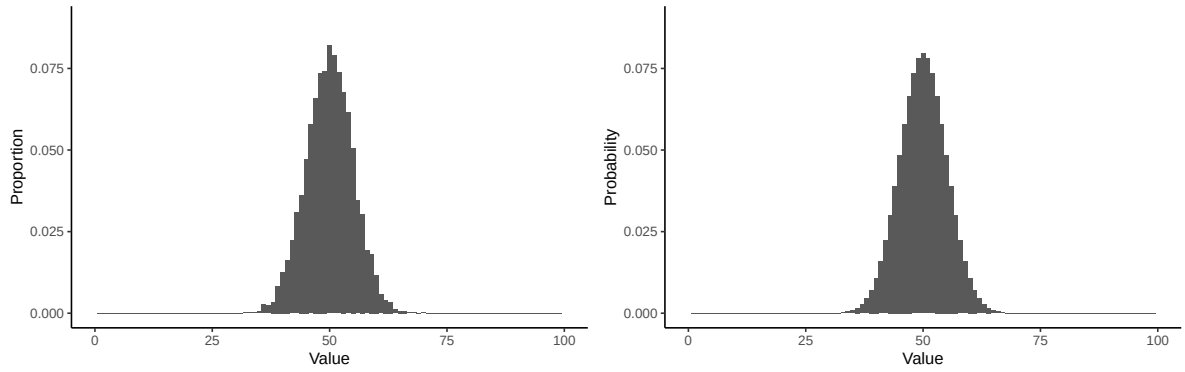
```r
# Histogram
rns <- rbinom(10000, 100, 0.5)

# dbinom
values <- 0:100
probs <- dbinom(values, size = 100, prob = 0.5)

tibble(x = rns) %>%
  ggplot(aes(x = x)) +
  theme_classic() +
  geom_histogram(binwidth = 1, aes(y=..count../sum(..count..))) +
  xlab("Value") +
  ylab("Proportion") +
  xlim(c(0, 100)) +
  ylim(c(0, max(probs + 0.01)))
```

```
Warning: The dot-dot notation (`..count..`) was deprecated in ggplot2 3.4.0.
i Please use `after_stat(count)` instead.
```

```
Warning: Removed 2 rows containing missing values (`geom_bar()`).
```

```r
tibble(x = values,
       y = probs) %>%
  ggplot(aes(x = x, y = y)) +
  geom_bar(stat = "identity") +
  theme_classic() +
  xlab("Value") +
  ylab("Probability") +
  xlim(c(0, 100)) +
  ylim(c(0, max(probs + 0.01)))
```

```
Warning: Removed 2 rows containing missing values (`geom_bar()`).
```



END OF EXAMPLE EXERCISE

In the case of discrete random variables, this exercise shows that the output of `d<distribution>()` has a clear interpretation: its the probability of seeing the specified value given the specified distribution parameters. In the case of continuous random variables though, the interpretation is a bit trickier. For example, consider an example output of `dnorm()`:

```
dnorm(0, mean = 0, sd = 0.25)
```

```
[1] 1.595769
```

It's greater than 1! How could a probability be greater than 1?? The answer is that the output isn't a probability in the same way as in the discrete case. All that matters for this class is that this number is *proportional* to a probability. The distinction here is a bit unintuitive, but there are some great resources for fleshing out what this means. See for example 3blue1brown's video on the topic.

BEGINNING OF BONUS CONTENT

Here I will briefly explain what the output of `dnorm()` represents.

The output of `dnorm()` represents a "probability density", hence the name "probability density function". What is a "probability density"? The analogy to an object's mass and density is fitting. If you want to get something's mass given its density, you need to multiply its density by that object's volume. To get probability (mass) from a density (output of `dnorm()`) you need to specify how large of a bin to consider.

```
dnorm(0, mean = 0, sd = 0.25)*0.001
```

```
[1] 0.001595769
```

This can be interpreted as the probability of a number generated by `rnorm()` falling between -0.0005 (i.e., 0 - 0.001/2) and 0.0005. This is only an approximation, as the density (output of `dnorm()`) is not constant between -0.0005 and 0.0005:

```
dnorm(-0.0005, mean = 0, sd = 0.25)
```

```
[1] 1.595766
```

```
dnorm(0, mean = 0, sd = 0.25)
```

```
[1] 1.595769
```

Choose a small enough bin though, and it will be pretty close to the probability of a number ending up in that bin. The exact answer to this question comes from integrating the probability density function within the bin of interest:

$$\mathrm{P}(\mathrm{x} \in [L, U]) = \int_{L}^{U} \mathrm{dnorm}(\mathrm{x},\ \mathrm{mean} = 0,\ \mathrm{sd} = 0.25)\ \mathrm{dx}$$

$\mathrm{P}(\mathrm{x} \in [L, U])$ should be read as "the probability that a random variable x drawn from `rnorm()` falls between L and U".

!!END OF LECTURE 1!!

4.2 Discrete random variables

4.2.1 Modeling two outcomes: the binomial distribution

You have already played around with the binomial distribution, as that is the name given to the distribution of numbers generated by `rbinom`. Now it is time to tell its story. All distributions have a story, or rather, a generative model. A generative model is a process that would give rise to data following a particular distribution.

The binomial's story goes like this: imagine you have an event that can result in two possible outcomes. For example, flipping a coin (an event) can result in either a heads or a tails (two possible outcomes). One thing has to be true about this process for it to be well described by a binomial distribution: the probability of a particular outcome must be exactly the same from event to event. For example, if every time you flip a coin, it has a 50% chance of coming up heads and a 50% chance of coming up tails, then the number of heads is well described by a binomial distribution.

The `size` parameter in the `rbinom` function sets the number of events you want to simulate. The `prob` parameter sets the probability of an outcome termed the "success". Success here has a misleading connotation; it might represent an outcome you are happy about, it might represent an outcome that you displeased by, it might represent an outcome that you are completely indifferent to. Statisticians name things in funny ways…

4.2.1.1 Exercise

Take some time to explore the properties and output of `rbinom()`. Questions to consider include:

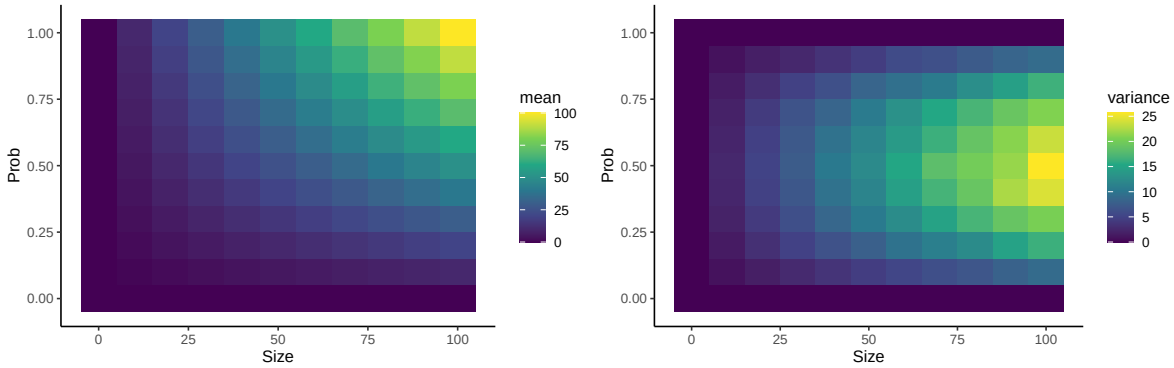
1. How does the mean depend on `size`?
2. How does the mean depend on `prob`?
3. How does the variance depend on `size`? `prob`?
4. What is the most likely outcome for a given `size` and `prob`?
5. What is an aspect of RNA-seq data that you could model with a binomial distribution?

4.2.1.2 Example exercise:

Going hardcore: inspect properties of distribution as function of size and prob

```
library(dplyr)
library(ggplot2)

nsims <- 5000

probs <- seq(from = 0, to = 1, by = 0.1)
sizes <-  seq(from = 0, to = 100, by = 10)

Lp <- length(probs)
Ls <- length(sizes)


means <- rep(0, times = Lp*Ls)
vars <- means

count <- 1
for(p in seq_along(probs)){

  for(s in seq_along(sizes)){
```

```
    simdata <- rbinom(nsims, size = sizes[s], prob = probs[p])

    means[count] <- mean(simdata)
    vars[count] <- var(simdata)
    count <- count + 1

  }

}


sim_df <- dplyr::tibble(prob = rep(probs, each = Ls),
                        size = rep(sizes, times = Lp),
                        mean = means,
                        variance = vars)

sim_df %>%
  ggplot(aes(x = size, y = prob, fill = mean)) +
  geom_tile() +
  scale_fill_viridis_c() +
  theme_classic() +
  xlab("Size") +
  ylab("Prob")
sim_df %>%
  ggplot(aes(x = size, y = prob, fill = variance)) +
  geom_tile() +
  scale_fill_viridis_c() +
  theme_classic() +
  xlab("Size") +
  ylab("Prob")
```



Observations:

1. Confirms that higher size and prob = higher average number
2. Interesting to see that variance increases as a function of size, but that prob = 0.5 leads to the highest variance.
3. Setting prob to 0 or 1 causes variance to go exactly 0, regardless of what size is set to. Similarly, setting prob to 0 causes the mean to go to exactly 0, regardless of what size is set to.

4.2.2 Modeling > 2 outcomes: the multinomial distribution

When exploring the binomial distribution, we considered data with two possible outcomes. What if many of the same assumptions hold, but there are now more than 2 possible results? That is where the multinomial distribution comes in.

Generative model: Imagine you have an event that can result in one of N outcomes, where N is some integer. Each outcome has some probability, p_{i} (i denoting the ith outcome, for i between 1 and N, inclusive) of occurring, and all of the p_{i} remain constant from event to event.

Example case: When rolling a die, there are 6 outcomes of what number shows face up when the die stops rolling (1, 2, 3, 4, 5, or 6). If each face is equally likely to show up with each roll, then p_{i} = 1/6 for all i. This can be simulated in R with `rmultinom()`:

```
rmultinom(5, size = 1, prob = rep(1/6, times = 6))
```

```
     [,1] [,2] [,3] [,4] [,5]
[1,]    1    1    0    0    0
[2,]    0    0    0    0    0
[3,]    0    0    0    1    1
[4,]    0    0    0    0    0
[5,]    0    0    0    0    0
[6,]    0    0    1    0    0
```

The output will be a matrix with `n` columns and number of rows equal to the length of `prob`. The value in the ith row and jth column is the number of times event i was observed in simulation j. In this case, the rows can be interpreted as the number a die came up, and each columns represents the result of a single role of a single die.

4.2.2.1 Exercise

Use `rmultinom()` and a bit of ancillary code to simulate the sequence of an RNA produced from a gene. This doesn't need to be a real gene, just a random sequence of nucleotides.

4.2.2.2 Example exercise

```
### Parameters for simulation

# Number of nucleotides in RNA
length <- 100

# Nucleotide proportions
ATprop <- 0.4
CGprop <- 0.6

### Generate random nucleotides
nucleotides <- rmultinom(length, size = 1,
                         prob = c(ATprop/2, ATprop/2,
                                  CGprop/2, CGprop/2))

### Determine sequence
nucleotide_types <- c("A", "U", "C", "G")
nucleotide_ids <- 1:4

# Cute matrix algebra trick
  # IDs will be row ID for which value of matrix was 1
IDs <- t(nucleotides) %*% matrix(1:4, nrow = 4, ncol = 1)
sequence <- paste(nucleotide_types[IDs], collapse = "")


print(sequence)
```

```
[1] "ACCAGUCACUAGCCGUCGGUCUCGCUCCUCGUGUCAGCUAGGGCUGGUUAUAAGCCCCUGAGCCCUCCGGGAUACCCGAUAGCGAGC
```

4.2.3 Modeling "counts": the Poisson distribution

4.2.4 Modeling time to first "success": the geometic distribution

4.2.5 Modeling time to nth "success": the negative binomial distribution

4.2.6 Sampling with replacement: the hypergeometric distribution

!!END OF LECTURE 2!!

4.3 Continuous random variables

4.3.1 Pure randomness: the uniform distribution

Play around with the `runif()` function in R. This is one of many "random number generators" (RNGs) in R. As we will learn in these exercises, there isn't just one kind of randomness, so there isn't just one RNG.

`runif()` has 3 parameters of note. The first is the same for all RNGs and is called `n`. It represents the number of random numbers it spits out. The other two are called `min` and `max`. Both of these can be any number you want, as long as `min` <= `max`. Generate some random numbers with `runif` and observe there properties:

4.3.2 Modeling the time until an event: the exponential distribution

4.3.3 Generalizing the exponential distribution: the gamma distribution

4.3.4 Modeling probabilities: the beta distribution

4.3.5 All distributions lead here: the normal distribution

4.4 Connections between the distributions

4.4.1 Poisson + gamma = negative binomial

4.4.2 The Central Limit Theorem (CLT)

!!END OF LECTURE 3!!

4.5 Problem set: Simulating data with distributions

4.5.1 RNA-seq data

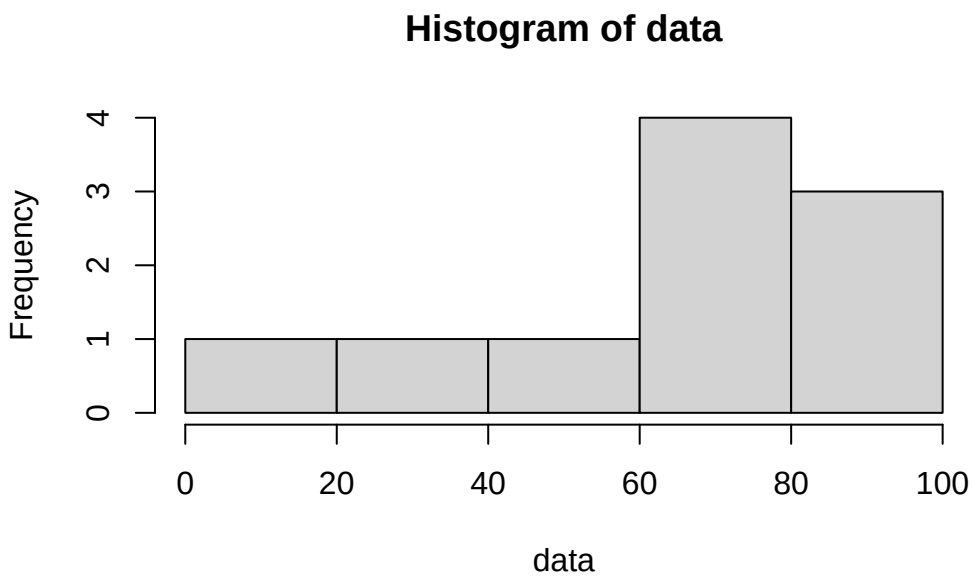
4.5.2 Poisson Process

4.5.3 NR-seq data

Appendix: Probability Distributions

When I claim that "there is randomness in your data", what does that mean? For some people, the term "randomness" implies complete unpredictability. Such people would interpret my claim to mean that every time you collect a new replicate, any value for the thing you are measuring is fair game and equally likely. "You got 100 reads from the MYC gene in your last RNA-seq dataset? Well don't be surprised if you get 1000, or 10000, or 0 reads next time!" You may call this **uniform randomness**. You can generate such data right here in R, using the `runif()` function:

```
data <- runif(n = 10, min = 0, max = 100)

hist(data)
```

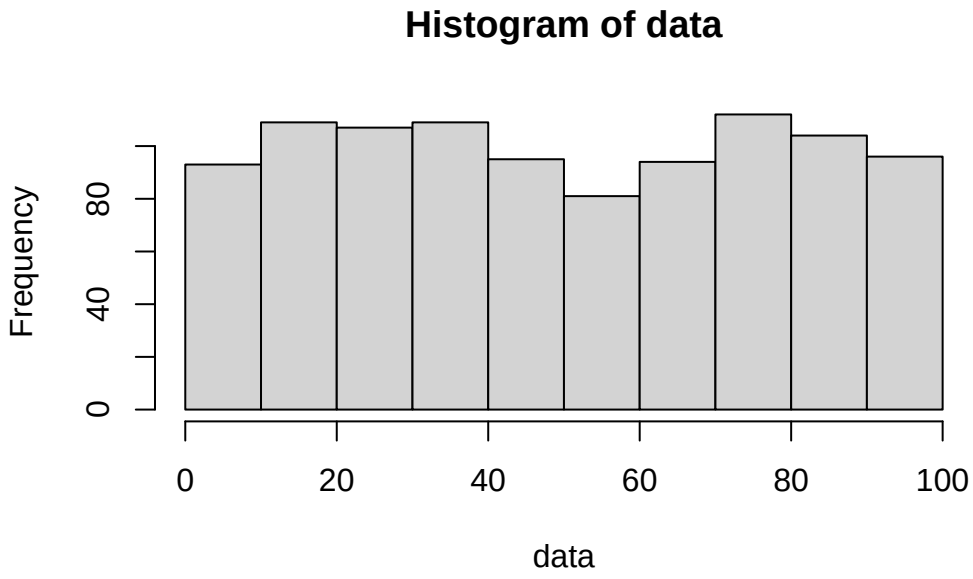


```
# Check out the individual data points
print(data)
```

```
 [1] 38.066508 76.229025 51.275148 97.291396 70.399628 94.961856  3.576121
 [8] 63.458378 89.713443 66.285285
```

`runif()` has three parameters: 1) `n` specifies the number of numbers to generate, 2) `min` specifies the minimum number it could possibly generate, and 3) `max` specifies the maximum number it could possibly generate. In the above example, I am thus creating 10 numbers between 0 and 100, with every number in between being equally likely to pop out. Generate a lot more numbers and this uniform pattern of appearance becomes much more clear:

```
data <- runif(n = 1000, min = 0, max = 100)

hist(data)
```



While this definition of randomness is intuitive, it can't be the only type of randomness. If RNA-seq data were this random, it would be useless! There is nothing to learn from measurements that can take on any value with equal probability.

Thus, to describe all of the kinds of randomness we see in the real world, it is important to expand our definition beyond uniform randomness. Enter the **probability distribution**. A

probability distribution is like a function in R. It takes as input a number (or maybe a set of numbers), and provides as output, the probability of seeing that number. For uniformly random data this might look something like:

```
uniform_distribution <- function(data, min = 0, max = 100){

  if(data >= min & data <= max){

    output <- 1

  }else{

    output <- 0

  }

  return(output)

}
```

That is to say, as long as the data is within the bounds of what is possible, it has the same probability of occurring; you get the same number out from this function. This function would make mathematicians

5 Introduction to Statistical Modeling

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

Part II

Popular Methods

6 Hypothesis Testing

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

7 Linear Modeling

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

8 Dimensionality Reduction

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

9 Clustering and Mixture Modeling

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

Part III

Statistics in the Wild

10 Practical Bioinformatics

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

11 Analysis of an RNA-seq dataset

In summary, this book has no content whatsoever.

```
1 + 1
```

```
[1] 2
```

References

Knuth, Donald E. 1984. "Literate Programming." *Comput. J.* 27 (2): 97–111. https://doi.org/10.1093/comjnl/27.2.97.