2015

Using BioInteractive Resources to Teach

Mathematics and Statistics in Biology

Paul Strode, PhD
Fairview High School
Boulder, Colorado

Ann Brokaw
Rocky River High School
Rocky River, Ohio

Version: October 2015

Using BioInteractive Resources to Teach

Mathematics and Statistics in Biology

About This Guide

Many state science standards encourage the use of mathematics and statistics in biology education, including the newly designed AP Biology course, IB Biology, Next Generation Science Standards, and the Common Core. Several resources on the BioInteractive website (www.biointeractive.org), which are listed in the table at the end of this document, make use of math and statistics to analyze research data. **This guide is meant to help educators use these BioInteractive resources in the classroom by providing further background on the statistical tests used and step-by-step instructions for doing the calculations.** Although most of the example data sets included in this guide are not real and are simply provided to illustrate how the calculations are done, the data sets on which the BioInteractive resources are based represent actual research data.

This guide is not meant to be a textbook on statistics; it only covers topics most relevant to high school biology, focusing on methods and examples rather than theory. It is organized in four parts:

- Part 1 covers descriptive statistics, methods used to organize, summarize, and describe quantifiable data. The methods include ways to describe the typical or average value of the data and the spread of the data.
- Part 2 covers statistical methods used to draw inferences about populations on the basis of observations made on smaller samples or groups of the population—a branch of statistics known as inferential statistics.
- Part 3 describes other mathematical methods commonly taught in high school biology, including frequency and rate calculations, Hardy-Weinberg calculations, probability, and standard curves.
- Part 4 provides a chart of activities on the BioInteractive website that use math and statistics methods.

A first draft of the guide was published in July 2014. It has been revised based on user feedback and expert review, and this version was published in October 2015. The guide will continue to be updated with new content and based on ongoing feedback and review.

For a more comprehensive discussion of statistical methods and additional classroom examples, refer to John McDonald's *Handbook of Biological Statistics*, http://www.biostathandbook.com, and the College Board's *AP Biology Quantitative Skills: A Guide for Teachers*, http://apcentral.collegeboard.com/apc/public/repository/AP_Bio_Quantitative_Skills_Guide-2012.pdf.

Statistical Symbols and Equations

Listed below are the universal statistical symbols and equations used in this guide. The calculations can all be done using scientific calculators or the formula function in spreadsheet programs.

N: Total number of individuals in a population (i.e., the total number of butterflies of a particular species)

n: Total number of individuals in a sample of a population (i.e., the number of butterflies in a net)

df: The number of measurements in a sample that are free to vary once the sample mean has been calculated; in a single sample, df = $n - 1$

x_i: A single measurement

i: The ith observation in a sample

Σ: Summation

$\bar{x}$: Sample mean $\bar{x} = \frac{\sum x_i}{n}$

s^2: Sample variance $s^2 = \frac{\sum (x_i - \bar{x})^2}{n-1}$

s: Sample standard deviation $s = \sqrt{s^2}$

SE_x : Sample standard error, or standard error of the mean (SEM) $\mathrm{SE} = \frac{s}{\sqrt{n}}$

95% CI: 95% confidence interval 95% CI $= \frac{1.96s}{\sqrt{n}}$

t-test: $t_{\mathrm{obs}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$

Chi-square test (X^2): $X^2 = \sum \frac{(o-e)^2}{e}$

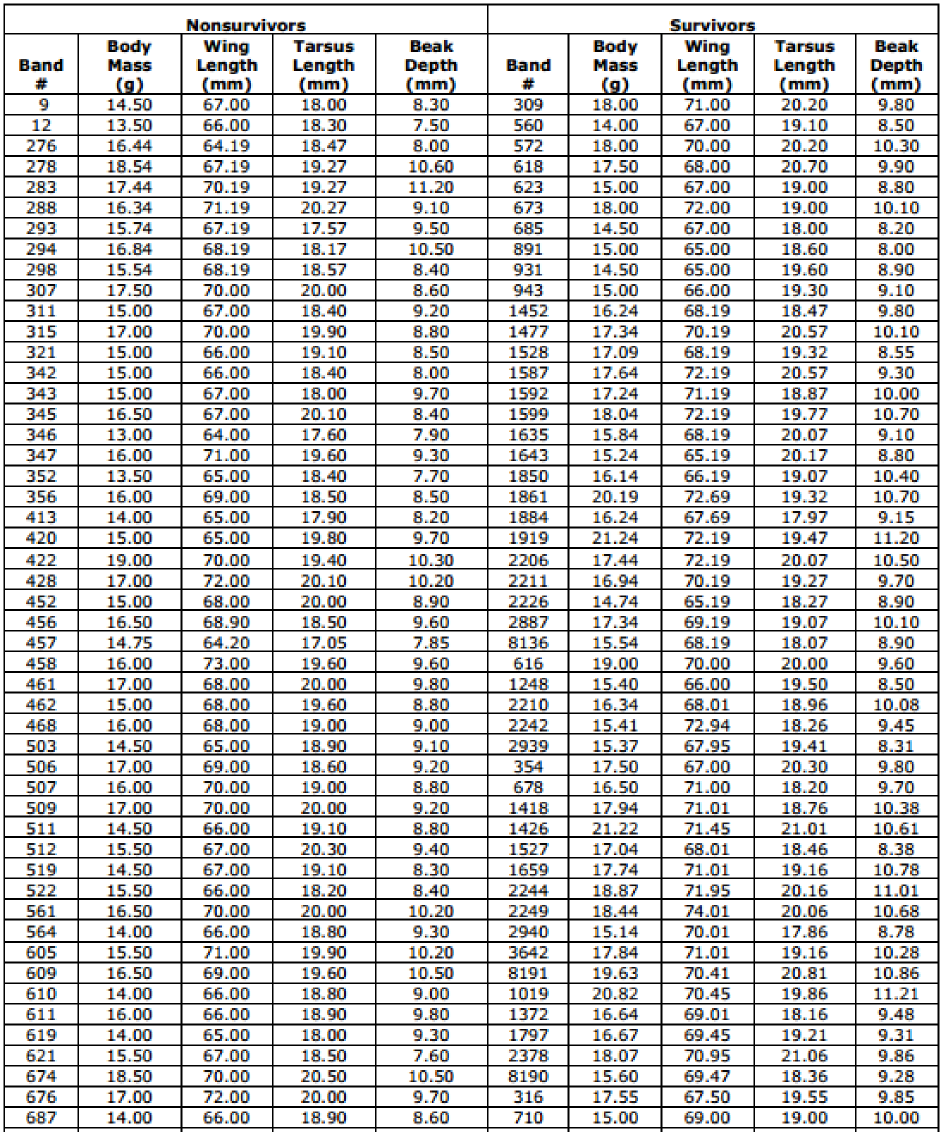
Linear regression test: $r = \frac{\sum_{i=1}^{n}\left(\frac{x_i - \bar{x}}{s_x}\right)\left(\frac{y_i - \bar{y}}{s_y}\right)}{n-1}$

Hardy-Weinberg principle: $p^2 + 2pq + q^2 = 1.0$

Part 1: Descriptive Statistics Used in Biology

Scientists typically collect data on a sample of a population and use these data to draw conclusions, or make inferences, about the entire population. An example of such a data set is shown in Table 1. It shows beak measurements taken from two groups of medium ground finches that lived on the island of Daphne Major, one of the Galápagos Islands, during a major drought in 1977. One group of finches died during the drought, and one group survived. (**These data were provided by scientists Peter and Rosemary Grant, and the complete data are available on the BioInteractive website at http://www.hhmi.org/biointeractive/evolution-action-data-analysis.**)

Table 1. Beak Depth Measurements in a Sample of Medium Ground Finches from Daphne Major

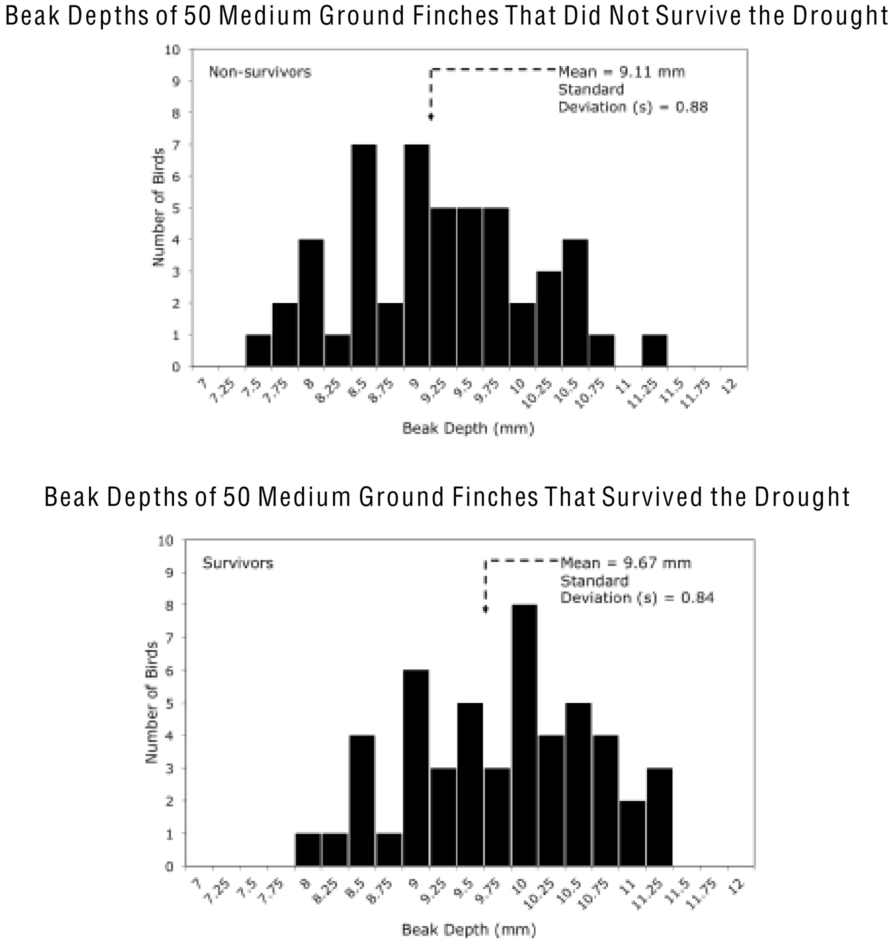


Nonsurvivors					Survivors				
Band #	Body Mass (g)	Wing Length (mm)	Tarsus Length (mm)	Beak Depth (mm)	Band #	Body Mass (g)	Wing Length (mm)	Tarsus Length (mm)	Beak Depth (mm)
9	14.50	67.00	18.00	8.30	309	18.00	71.00	20.20	9.80
12	13.50	66.00	18.30	7.50	560	14.00	67.00	19.10	8.50
276	16.44	64.19	18.47	8.00	572	18.00	70.00	20.20	10.30
278	18.54	67.19	19.27	10.60	618	17.50	68.00	20.70	9.90
283	17.44	70.19	19.27	11.20	623	15.00	67.00	19.00	8.80
288	16.34	71.19	20.27	9.10	673	18.00	72.00	19.00	10.10
293	15.74	67.19	17.57	9.50	685	14.50	67.00	18.00	8.20
294	16.84	68.19	18.17	10.50	891	15.00	65.00	18.60	8.00
298	15.54	68.19	18.57	8.40	931	14.50	65.00	19.60	8.90
307	17.50	70.00	20.00	8.60	943	15.00	66.00	19.30	9.10
311	15.00	67.00	18.40	9.20	1452	16.24	68.19	18.47	9.80
315	17.00	70.00	19.90	8.80	1477	17.34	70.19	20.57	10.10
321	15.00	66.00	19.10	8.50	1528	17.09	68.19	19.32	8.55
342	15.00	66.00	18.40	8.00	1587	17.64	72.19	20.57	9.30
343	15.00	67.00	18.00	9.70	1592	17.24	71.19	18.87	10.00
345	16.50	67.00	20.10	8.40	1599	18.04	72.19	19.77	10.70
346	13.00	64.00	17.60	7.90	1635	15.84	68.19	20.07	9.10
347	16.00	71.00	19.60	9.30	1643	15.24	65.19	20.17	8.80
352	13.50	65.00	18.40	7.70	1850	16.14	66.19	19.07	10.40
356	16.00	69.00	18.50	8.50	1861	20.19	72.69	19.32	10.70
413	14.00	65.00	17.90	8.20	1884	16.24	67.69	17.97	9.15
420	15.00	65.00	19.80	9.70	1919	21.24	72.19	19.47	11.20
422	19.00	70.00	19.40	10.30	2206	17.44	72.19	20.07	10.50
428	17.00	72.00	20.10	10.20	2211	16.94	70.19	19.27	9.70
452	15.00	68.00	20.00	8.90	2226	14.74	65.19	18.27	8.90
456	16.50	68.90	18.50	9.60	2887	17.34	69.19	19.07	10.10
457	14.75	64.20	17.05	7.85	8136	15.54	68.19	18.07	8.90
458	16.00	73.00	19.60	9.60	616	19.00	70.00	20.00	9.60
461	17.00	68.00	20.00	9.80	1248	15.40	66.00	19.50	8.50
462	15.00	68.00	19.60	8.80	2210	16.34	68.01	18.96	10.08
468	16.00	68.00	19.00	9.00	2242	15.41	72.94	18.26	9.45
503	14.50	65.00	18.90	9.10	2939	15.37	67.95	19.41	8.31
506	17.00	69.00	18.60	9.20	354	17.50	67.00	20.30	9.80
507	16.00	70.00	19.00	8.80	678	16.50	71.00	18.20	9.70
509	17.00	70.00	20.00	9.20	1418	17.94	71.01	18.76	10.38
511	14.50	66.00	19.10	8.80	1426	21.22	71.45	21.01	10.61
512	15.50	67.00	20.30	9.40	1527	17.04	68.01	18.46	8.38
519	14.50	67.00	19.10	8.30	1659	17.74	71.01	19.16	10.78
522	15.50	66.00	18.20	8.40	2244	18.87	71.95	20.16	11.01
561	16.50	70.00	20.00	10.20	2249	18.44	74.01	20.06	10.68
564	14.00	66.00	18.80	9.30	2940	15.14	70.01	17.86	8.78
605	15.50	71.00	19.90	10.20	3642	17.84	71.01	19.16	10.28
609	16.50	69.00	19.60	10.50	8191	19.63	70.41	20.81	10.86
610	14.00	66.00	18.80	9.00	1019	20.82	70.45	19.86	11.21
611	16.00	66.00	18.90	9.80	1372	16.64	69.01	18.16	9.48
619	14.00	65.00	18.00	9.30	1797	16.67	69.45	19.21	9.31
621	15.50	67.00	18.50	7.60	2378	18.07	70.95	21.06	9.86
674	18.50	70.00	20.50	10.50	8190	15.60	69.47	18.36	9.28
676	17.00	72.00	20.00	9.70	316	17.55	67.50	19.55	9.85
687	14.00	66.00	18.90	8.60	710	15.00	69.00	19.00	10.00

Note: “Band” refers to an individual’s identity—more specifically, the number on a metal leg band it was given. Fifty individuals died in 1977 (nonsurvivors) and 50 survived beyond 1977 (survivors), the year of the drought.

How would you describe the data in Table 1, and what does it tell you about the populations of medium ground finches of Daphne Major? These are difficult questions to answer by looking at a table of numbers.

One of the first steps in analyzing a small data set like the one shown in Table 1 is to graph the data and examine the distribution. Figure 1 shows two graphs of beak measurements. The graph on the top shows beak measurements of finches that died during the drought. The graph on the bottom shows beak measurements of finches that survived the drought.



Beak Depths of 50 Medium Ground Finches That Did Not Survive the Drought

Beak Depths of 50 Medium Ground Finches That Survived the Drought

Figure 1. Distributions of Beak Depth Measurements in Two Groups of Medium Ground Finches

Notice that the measurements tend to be more or less symmetrically distributed across a range, with most measurements around the center of the distribution. This is a characteristic of a **normal distribution**. Most statistical methods covered in this guide apply to data that are normally distributed, like the beak measurements above; other types of distributions require either different kinds of statistics or transforming data to make them normally distributed.

How would you describe these two graphs? How are they the same or different? **Descriptive statistics** allows you to describe and quantify these differences. The rest of Part 1 of this guide provides step-by-step instructions for calculating mean, standard deviation, standard error, and other descriptive statistics.

Measures of Average: Mean, Median, and Mode

In the two graphs in Figure 1, the center and spread of each distribution is different. **The center of the distribution can be described by the mean, median, or mode. These are referred to as measures of central tendency.**

Mean

You calculate the sample mean (also referred to as the average or arithmetic mean) by summing all the data points in a data set (Σ X) and then dividing this number by the total number of data points (*N*):

$$\bar{X} = \frac{\Sigma X}{N}$$

What we want to understand is the mean of the entire population, which is represented by μ. They use the sample mean, represented by $\bar{x}$, as an estimate of μ.

Application in Biology

Students in a biology class planted eight bean seeds in separate plastic cups and placed them under a bank of fluorescent lights. Fourteen days later, the students measured the height of the bean plants that grew from those seeds and recorded their results in Table 2.

Table 2. Bean Plant Heights

Plant No.	1	2	3	4	5	6	7	8
Height (cm)	7.5	10.1	8.3	9.8	5.7	10.3	9.2	8.7

To determine the mean of the bean plants, follow these steps:

I. Find the sum of the heights:

7.5 + 10.1 + 8.3 + 9.8 + 5.7 + 10.3 + 9.2 + 8.7 = 69.6 centimeters

II. Count the number of height measurements:

There are 8 height measurements.

III. Divide the sum of the heights by the number of measurements to compute the mean:

mean = 69.6 cm/8 = 8.7 centimeters

The mean for this sample of eight plants is 8.7 centimeters and serves as an estimate for the true mean of the population of bean plants growing under these conditions. In other words, if the students collected data from hundreds of plants and graphed the data, the center of the distribution should be around 8.7 centimeters.

Median

When the data are ordered from the largest to the smallest, the median is the midpoint of the data. It is not distorted by extreme values, or even when the distribution is not normal. For this reason, it may be more useful for you to use the median as the main descriptive statistic for a sample of data in which some of the measurements are extremely large or extremely small.

To determine the median of a set of values, you first arrange them in numerical order from lowest to highest. The middle value in the list is the median. If there is an even number of values in the list, then the median is the mean of the middle two values.

Application in Biology

A researcher studying mouse behavior recorded in Table 3 the time (in seconds) it took 13 different mice to locate food in a maze.

Table 3. Length of Time for Mice to Locate Food in a Maze

Mouse No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Time (sec.)	31	33	163	33	28	29	33	27	27	34	35	28	32

To determine the median time that the mice spent searching for food, follow these steps:

I. Arrange the time values in numerical order from lowest to highest:

27, 27, 28, 28, 29, 31, **32**, 33, 33, 33, 34, 35, 163

II. Find the middle value. This value is the median:

median = 32 seconds

In this case, the median is 32 seconds, but the mean is 41 seconds, which is longer than all but one of the mice took to search for food. In this case, the mean would not be a good measure of central tendency unless the really slow mouse is excluded from the data set.

Mode

The mode is another measure of the average. It is the value that appears most often in a sample of data. In the example shown in Table 3, the mode is 33 seconds.

The mode is not typically used as a measure of central tendency in biological research, but it can be useful in describing some distributions. For example, Figure 2 shows a distribution of body lengths with two peaks, or modes—called a **bimodal distribution**. Describing these data with a measure of central tendency like the mean or median would obscure this fact.

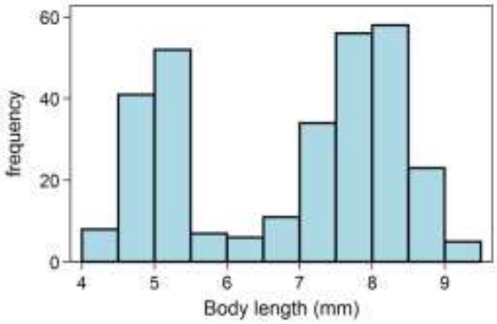


Figure 2. Graph of Body Lengths of Weaver Ant Workers (Reproduced from http://en.wikipedia.org/wiki/File:BimodalAnts.png.)

When to Use Which One

The purpose of these statistics is to characterize “typical” data from a data set. You use the mean most often for this purpose, but it becomes less useful if the data in the data set are not normally distributed. When the data are *not* normally distributed, then other descriptive statistics can give a better idea about the typical value of the data set. The median, for example, is a useful number if the distribution is heavily skewed. For example, you might use the median to describe a data set of top running speeds of four-legged animals, most of which are relatively slow and a few, like cheetahs, are very fast. The mode is not used very frequently in biology, but it may be useful in describing some types of distributions—for example, ones with more than one peak.

Measures of Variability: Range, Standard Deviation, and Variance

Variability describes the extent to which numbers in a data set diverge from the central tendency. It is a measure of how “spread out” the data are. **The most common measures of variability are range, standard deviation, and variance.**

Range

The simplest measure of variability in a sample of normally distributed data is the range, which is the difference between the largest and smallest values in a set of data.

Application in Biology

Students in a biology class measured the width in centimeters of eight leaves from eight different maple trees and recorded their results in Table 4.

Table 4. Width of Maple Tree Leaves

Plant No.	1	2	3	4	5	6	7	8
Width (cm)	7.5	10.1	8.3	9.8	5.7	10.3	9.2	8.7

To determine the range of leaf widths, follow these steps:

I. Identify the largest and smallest values in the data set:

largest = 10.3 centimeters, smallest = 5.7 centimeters

II. To determine the range, subtract the smallest value from the largest value:

range = 10.3 centimeters – 5.7 centimeters = 4.6 centimeters

A larger range value indicates a greater spread of the data—in other words, the larger the range, the greater the variability. However, an extremely large or small value in the data set will make the variability appear high. For example, if the maple leaf sample had not included the very small leaf number 5, the range would have been only 2.8 centimeters. The standard deviation provides a more reliable measure of the “true” spread of the data.

Standard Deviation and Variance

The standard deviation is the most widely used measure of variability. The **sample standard deviation** (*s*) is essentially the average of the deviation between each measurement in the sample and the sample mean ($\overline{x}$). The sample standard deviation estimates the standard deviation in the larger population.

The formula for calculating the sample standard deviation follows:

$$s = \sqrt{\frac{\sum(x_i - \overline{x})^2}{(n-1)}}$$

Calculation Steps

1. Calculate the mean ($\overline{x}$) of the sample.

2. Find the difference between each measurement (x_i) in the data set and the mean ($\overline{x}$) of the entire set:

$$(x_i - \overline{x})$$

3. Square each difference to remove any negative values:

$$(x_i - \overline{x})^2$$

4. Add up (sum, Σ) all the squared differences:

$$\Sigma\,(x_i - \overline{x})^2$$

5. Divide by the **degrees of freedom** (df), which is 1 less than the sample size (*n* – 1):

$$\frac{\sum(x_i - \overline{x})^2}{(n-1)}$$

Note that the number calculated at this step provides a statistic called **variance** (s^2). Variance is a measure of variability that is used in many statistical methods. It is the square of the standard deviation.

6. Take the square root to calculate the standard deviation (*s*) for the sample.

Application in Biology

You are interested in knowing how tall bean plants (*Phaseolus vulgaris*) grow in two weeks after planting. You plant a sample of 20 seeds (*n* = 20) in separate pots and give them equal amounts of water and light. After two weeks, 17 of the seeds have germinated and have grown into small seedlings (now *n* = 17). You measure each plant from the tips of the roots to the top of the tallest stem. You record the measurements in Table 5, along with the steps for calculating the standard deviation.

Table 5. Plant Measurements and Steps for Calculating the Standard Deviation

Plant No.	Plant Height (mm)	*Step 2:* $(x_i - \bar{x})$ (mm)	*Step 3:* $(x_i - \bar{x})^2$ (mm^2)
1	112	(112 – 103) = 9	9^2 = 81
2	102	(102 – 103) = (–1)	$(-1)^2$ = 1
3	106	(106 – 103) = 3	3^2 = 9
4	120	(120 – 103) = 17	17^2 = 289
5	98	(98 – 103) = (–5)	$(-5)^2$ = 25
6	106	(106 – 103) = 3	3^2 = 9
7	80	(80 – 103) = (–23)	$(-23)^2$ = 529
8	105	(105 – 103) = 2	2^2 = 4
9	106	(106 – 103) = 3	3^2 = 9
10	110	(110 – 103) = 7	7^2 = 49
11	95	(95 – 103) = (–8)	$(-8)^2$ = 64
12	98	(98 – 103) = (–5)	$(-5)^2$ = 25
13	74	(74 – 103) = (–29)	$(-29)^2$ = 841
14	112	(112 – 103) = 9	9^2 = 81
15	115	(115 – 103) = 12	12^2 = 144
16	109	(109 – 103) = 6	6^2 = 36
17	100	(100 – 103) = (–3)	$(-3)^2$ = 9
Step 1: Calculate mean.	$\bar{x}$ = 103 mm		*Step 4:* $\sum (x_i - \bar{x})^2$ = 2,205
		Variance, s^2	*Step 5:* $\sum (x_i - \bar{x})^2/(n-1)$ = 2,205/16 = 138
		Standard Deviation, *s*	*Step 6:* $\sqrt{s^2}$ = $\sqrt{138}$ = 11.7 mm

Note: The units for variance are squared units, which make variance less useful as a measure of dispersion.

The mean height of the bean plants in this sample is 103 millimeters ±11.7 millimeters. What does this tell us? **In a data set with a large number of measurements that are normally distributed, 68.3% (or roughly two-thirds) of the measurements are expected to fall within 1 standard deviation of the mean and 95.4% of all data points lie within 2 standard deviations of the mean on either side (Figure 3).** Thus, in this example, if you assume that this sample of 17 observations is drawn from a population of measurements that are normally distributed, 68.3% of the measurements in the population should fall between 91.3 and 114.7 millimeters and 95.4% of the measurements should fall between 80.1 millimeters and 125.9 millimeters.

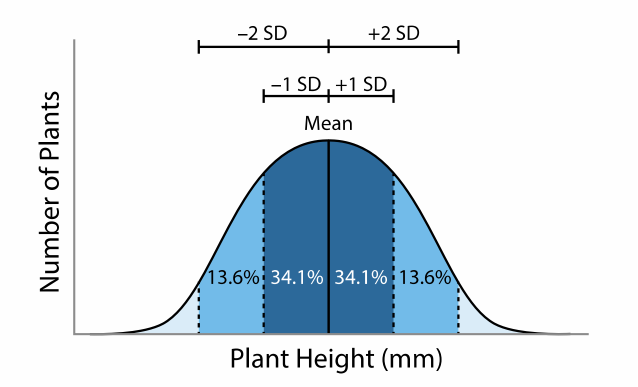


Figure 3. Theoretical Distribution of Plant Heights. For normally distributed data, 68.3% of data points lie between ±1 standard deviation of the mean and 95.4% of data points lie between ±2 standard deviations of the mean.

We can graph the mean and the standard deviation of this sample of bean plants using a bar graph with error bars (Figure 4). **Standard deviation bars summarize the variation in the data—the more spread out the individual measurements are, the larger the standard deviation.** On the other hand, error bars based on the standard error of the mean or the 95% confidence interval reveal the uncertainty in the sample mean. They depend on how spread out the measurements are *and* on the sample size. (These statistics are discussed further in "Measures of Confidence: Standard Error of the Mean and 95% Confidence Interval".)

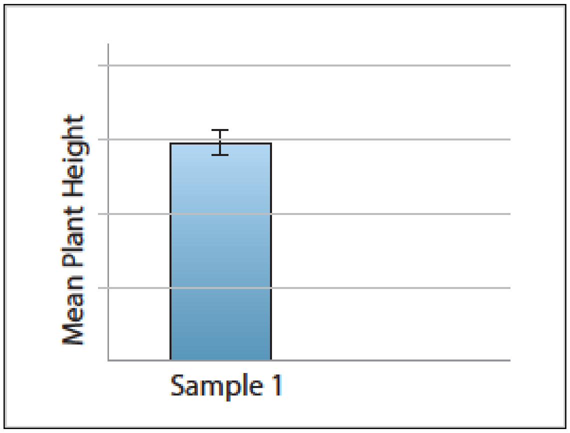


Figure 4. Mean Plant Height of a Sample of Bean Plants and an Error Bar Representing ±1 Standard Deviation. Roughly two-thirds of the measurements in this population would be expected to fall in the range indicated by the bar.

A common misconception is that standard deviation decreases with increasing sample size**. As you increase the sample size, standard deviation can either increase or decrease depending on the measurements in the sample.** However, with a larger sample size, standard deviation will become a more accurate estimate of the standard deviation of the population.

Understanding Degrees of Freedom

Calculations of sample estimates, such as the standard deviation and variance, use degrees of freedom instead of sample size. The way you calculate degrees of freedom depends on the statistical method you are using, but for calculating the standard deviation, it is defined as 1 less than the sample size (n − 1).

To illustrate what this number means, consider the following example. Biologists are interested in the variation in leg sizes among grasshoppers. They catch five grasshoppers (n = 5) in a net and prepare to measure the left legs. As the scientists pull grasshoppers one at a time from the net, they have no way of knowing the leg lengths until they measure them all. In other words, all five leg lengths are "free" to vary within some general range for this particular species. The scientists measure all five leg lengths and then calculate the mean to be x = 10 millimeters. They then place the grasshoppers back in the net and decide to pull them out one at a time to measure them again. This time, since the biologists already know the mean to be 10, only the first four measurements are free to vary within a given range. If the first four measurements are 8, 9, 10, and 12 millimeters, then there is no freedom for the fifth measurement to vary; it has to be 11. Thus, once they know the sample mean, the number of degrees of freedom is 1 less than the sample size, $\mathrm{df} = 4$.

Measures of Confidence: Standard Error of the Mean and 95% Confidence Interval

The standard deviation provides a measure of the spread of the data from the mean. A different type of statistic reveals the *uncertainty* in the calculation of the mean.

The sample mean is not necessarily identical to the mean of the entire population. In fact, every time you take a sample and calculate a sample mean, you would expect a slightly different value. In other words, the sample means themselves have variability. This variability can be expressed by calculating the standard error of the mean (abbreviated as $\mathrm{SE}_{\bar{x}}$ or SEM).

To illustrate this point, assume that there is a population of a species of anole lizards living on an island of the Caribbean. If you were able to measure the length of the hind limbs of every individual in this population and then calculate the mean, you would know the value of the population mean. However, there are thousands of individuals, so you take a sample of 10 anoles and calculate the mean hind limb length for that sample. Another researcher working on that island might catch another sample of 10 anoles and calculate the mean hind limb length for this sample, and so on. The sample means of many different samples would be normally distributed. **The standard error of the mean represents the standard deviation of such a distribution and estimates how close the sample mean is to the population mean.**

The greater each sample size (i.e., 50 rather than 10 anoles), the more closely the sample mean will estimate the population mean, and therefore the standard error of the mean becomes smaller.

To calculate $\mathrm{SE}_{\bar{x}}$ or SEM divide the standard deviation by the square root of the sample size:

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

$$\mathrm{SE}_{\bar{x}} = \frac{s}{\sqrt{n}}$$

What the standard error of the mean tells you is that about two-thirds (68.3%) of the sample means would be within ±1 standard error of the population mean and 95.4% would be within ±2 standard errors.

Another more precise measure of the uncertainty in the mean is the 95% confidence interval (95% CI). For large sample sizes, 95% CI can be calculated using this formula: $\frac{1.96s}{\sqrt{n}}$, which is typically rounded to $\frac{2s}{\sqrt{n}}$ for ease of calculation. In other words, 95% CI is about twice the standard error of the mean.

The actual formula for calculating 95% CI uses a statistic called the *t*-value for a significance level of 0.05, which is explained in Table 8 in Part 2. For large sample sizes, this *t*-value is 1.96. Since *t*-values are not typically covered in high school biology, in this guide we estimate the 95% CI by using 2 x SEM, but note that this is just an approximation.

Note about Error Bars: Many bar graphs include error bars, which may represent standard deviation, SEM, or 95% CI. When the bars represent SEM, you know that if you took many samples only about two-thirds of the error bars would include the population mean. This is very different from standard deviation bars, which show how much variation there is among *individual observations* in a sample. When the error bars represent 95% confidence intervals in a graph, you know that in about 95% of cases the error bars include the population mean. If a graph shows error bars that represent SEM, you can estimate the 95% confidence interval by making the bars twice as big—this is a fairly accurate approximation for large sample sizes, but for small samples the 95% confidence intervals are actually more than twice as big as the SEMs.

Application in Biology—Example 1

Seeds of many weed species germinate best in recently disturbed soil that lacks a light-blocking canopy of vegetation. Students in a biology class hypothesized that weed seeds germinate best when exposed to light. To test this hypothesis, the students placed a seed from crofton weed (*Ageratina adenophora*, an invasive species on several continents) in each of 20 petri dishes and covered the seeds with distilled water. They placed half the petri dishes in the dark and half in the light. After one week, the students measured the combined lengths in millimeters of the radicles and shoots extending from the seeds in each dish. Table 6 shows the data and calculations of variance, standard deviation, standard error of the mean, and 95% confidence interval. The students plotted the data as two bar graphs showing the standard error of the mean and 95% confidence interval (Figure 5).

Table 6. Combined Lengths of Crofton Weed Radicles and Shoots after One Week in the Dark and the Light

Petri Dishes	**Dark (x_1) (mm)**	**Light (x_2) (mm)**	**Dark $(x_i - \bar{x}_1)^2$ (mm^2)**	**Light $(x_i - \bar{x}_2)^2$ (mm^2)**
1 and 2	12	18	$(12 – 9.6)^2$ = 5.8	$(18 – 18.4)^2$ = 0.16
3 and 4	8	22	$(8 – 9.6)^2$ = 2.6	$(22 – 18.4)^2$ = 12.96
5 and 6	15	17	$(15 – 9.6)^2$ = 29.1	$(17 – 18.4)^2$ = 1.96
7 and 8	13	23	$(13 – 9.6)^2$ = 11.5	$(23 – 18.4)^2$ = 21.16
9 and 10	6	16	$(6 – 9.6)^2$ = 13.0	$(16 – 18.4)^2$ = 5.76
11 and 12	4	18	$(4 – 9.6)^2$ = 31.4	$(18 – 18.4)^2$ = 0.16
13 and 14	13	22	$(13 – 9.6)^2$ = 11.6	$(22 – 18.4)^2$ = 12.96
15 and 16	14	12	$(14 – 9.6)^2$ = 19.3	$(12 – 18.4)^2$ = 40.96
17 and 18	5	19	$(5 – 9.6)^2$ = 21.1	$(19 – 18.4)^2$ = 0.36
19 and 20	6	17	$(6 – 9.6)^2$ = 13.0	$(17 – 18.4)^2$ = 1.96
			$\sum (x_i - \bar{x}_1)^2$ = 158.4	$\sum (x_i - \bar{x}_2)^2$ = 98.4
Mean ($\bar{x}$)	$\bar{x}_1$ = 9.6 (10) mm	$\bar{x}_2$ = 18.4 (18) mm	$\frac{\sum (x_i - \bar{x})^2}{(n-1)} = \frac{158.4}{9}$	$\frac{\sum (x_i - \bar{x})^2}{(n-1)} = \frac{98.4}{9}$
		Variance (s^2)	s_1^2 = 17.6	s_2^2 = 10.93
Standard Deviation, $s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)}}$			s = 4.20 mm	s = 3.31 mm
Standard Error of the Mean, $\mathrm{SE}_{\bar{x}} = \frac{s}{\sqrt{n}}$			$\mathrm{SE}_{\bar{x}} = \frac{4.20}{\sqrt{10}}$ = 1.33	$\mathrm{SE}_{\bar{x}} = \frac{3.31}{\sqrt{10}}$ = 1.05
$95\%\ \mathrm{CI} = \frac{\boldsymbol{2s}}{\sqrt{\boldsymbol{n}}}$			$95\%\ \mathrm{CI} = \frac{2(4.20)}{\sqrt{10}}$ = 2.7	$95\%\ \mathrm{CI} = \frac{2(4.74)}{\sqrt{10}}$ = 2.1

Note: The number of replicates (i.e., sample size, *n*) = 10. Means in parentheses, that is, (10) and (18), are to the nearest millimeter.

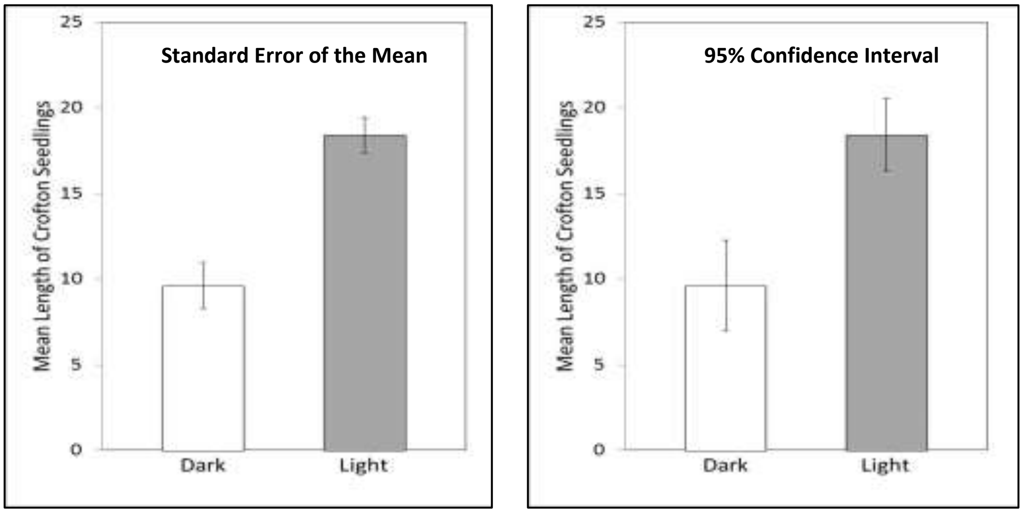


Figure 5. Mean Length of Crofton Seedlings after One Week in the Dark or in the Light. The standard error of the mean graph shows the $\mathbf{SE}_{\bar{x}}$ as error bars, and the 95% confidence interval graph shows the $\mathbf{95\%\ CI}$ as error bars. (Note that in these calculations we approximated 95% CI as about twice the SEM.)

The calculations in Table 6 show that although the students don't know the actual mean combined radicle and shoot length of the entire population of crofton plants in the dark, it is likely to be a number around the sample mean of 9.6 millimeters ± 1.3 millimeters. For the light treatment it is likely to be 18.4 millimeters ± 1 millimeter. The students can be even more certain that the population mean would be 9.6 millimeters ±2.6 millimeters for the dark treatment and 18.4 millimeters ± 2.1 millimeters for the light treatment.

Note: By looking at the bar graphs, you can see that the means for the light and dark treatments are different. Because the 95% confidence interval error bars do not overlap, this suggests that the true population means are also different. However, in order to determine whether this difference is significant, you will need to conduct another statistical test, the Student's *t*-test, which is covered in "Comparing Averages" in Part 2 of this guide.

Application in Biology—Example 2

A teacher had five students write their names on the board, first with their dominant hands and then with their nondominant hands. The rest of the class observed that the students wrote more slowly and with less precision with the nondominant hand than with the dominant hand. The teacher then asked the class to explain their observations by developing testable hypotheses. They hypothesized that the dominant hand was better at performing fine motor movements than the nondominant hand. The class tested this hypothesis by timing (in seconds) how long it took each student to break 20 toothpicks with each hand. The results of the experiment and the calculations of variance, standard deviation, standard error of the mean, and 95% confidence interval are presented in Table 7. The students then illustrated the data and uncertainty with two bar graphs, one showing the standard error of the mean and the other showing the 95% confidence interval (Figure 6).

Table 7. Number of Seconds It Took for Students to Break 20 Toothpicks with Their Nondominant (ND) and Dominant (D) Hands (number of replicates [n] = 14)

Students	**ND (x_1) (sec.)**	**D (x_2) (sec.)**	**ND $(x_i - \bar{x}_1)^2$**	**D $(x_i - \bar{x}_2)^2$**
Josh	33	37	$(33 - 33)^2 = 0$	$(37 - 35)^2 = 4$
Bobby	24	22	$(24 - 33)^2 = 81$	$(22 - 35)^2 = 169$
Qing	35	37	$(35 - 33)^2 = 4$	$(37 - 35)^2 = 4$
Julie	33	28	$(33 - 33)^2 = 0$	$(28 - 35)^2 = 49$
Lisa	42	50	$(42 - 33)^2 = 81$	$(50 - 35)^2 = 225$
Akash	36	36	$(36 - 33)^2 = 9$	$(36 - 35)^2 = 1$
Hector	31	36	$(31 - 33)^2 = 4$	$(36 - 35)^2 = 1$
Viviana	40	46	$(40 - 33)^2 = 49$	$(46 - 35)^2 = 121$
Brenda	28	26	$(28 - 33)^2 = 25$	$(26 - 35)^2 = 81$
Jane	24	28	$(24 - 33)^2 = 81$	$(28 - 35)^2 = 49$
Asa	23	22	$(23 - 33)^2 = 100$	$(22 - 35)^2 = 169$
Eli	44	52	$(44 - 33)^2 = 121$	$(52 - 35)^2 = 289$
Adee	35	29	$(35 - 33)^2 = 4$	$(29 - 35)^2 = 36$
Jenny	36	37	$(36 - 33)^2 = 9$	$(37 - 35)^2 = 4$
			$\sum (x_i - \bar{x}_1)^2 = 568$	$\sum (x_i - \bar{x}_2)^2 = 1{,}200$
Mean ($\bar{x}$)	$\bar{x}_1 = 33$	$\bar{x}_2 = 35$	$\frac{\sum (x_i - \bar{x}_1)2}{n-1} = \frac{568}{13}$	$\frac{\sum (x_i - \bar{x}_2)2}{n-1} = \frac{1{,}200}{13}$
		Variance, s^2	$s_1^2 = 44$	$s_2^2 = 92$
Standard Deviation, $s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)}}$			$s = 6.6$ sec.	$s = 9.6$ sec.
Standard Error of the Mean, $\mathrm{SE}_{\bar{x}} = \frac{\boldsymbol{s}}{\sqrt{\boldsymbol{n}}}$			$\mathrm{SE}_{\bar{x}} = \frac{6.6}{\sqrt{14}} = 1.8$ sec.	$\mathrm{SE}_{\bar{x}} = \frac{9.6}{\sqrt{14}} = 2.6$ sec.
95% CI = $\frac{\boldsymbol{2s}}{\sqrt{\boldsymbol{n}}}$			$95\%\ \mathrm{CI} = \frac{2(6.84)}{\sqrt{14}} = 3.5$	$95\%\ \mathrm{CI} = \frac{2(9.6)}{\sqrt{14}} = 5.1$

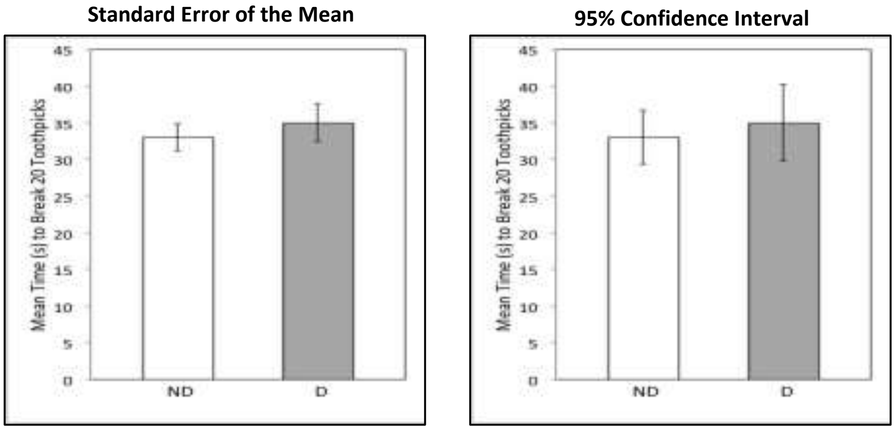


Figure 6. Mean Number of Seconds for Students to Break 20 Toothpicks with their Nondominant Hands (ND) and Dominant Hands (D). The standard error of the mean graph shows the $\mathbf{SE}_{\bar{x}}$ as error bars, and the 95% confidence interval graph shows the $\mathbf{95\%\ CI}$ as error bars.

The calculations indicate that it takes about 31.2 seconds (33 − 1.8) to 34.8 seconds (33 + 1.8) for the nondominant hand to break toothpicks and about 32.4 to 37.6 seconds for the dominant hand. You can be more certain that the average for the nondominant hand would fall somewhere between 29.5 seconds (33 – 3.5) and 36.5 seconds (33 + 3.5) and for the dominant hands falls somewhere between 29.9 seconds (35 – 5.1) and 40.1 seconds (35 + 5.1).

This ends the part on descriptive statistics. Going back to the finch data set in Table 1 and Figure 1 of Part 1, how would you calculate the sample means for beak sizes of the survivors and nonsurvivors? Is there more variability among survivors or nonsurvivors? What is the uncertainty in your sample mean estimates? To find the answers to these questions, see the "Evolution in Action: Data Analysis" activities at http://www.hhmi.org/biointeractive/evolution-action-data-analysis.

Part 2: Inferential Statistics Used in Biology

Inferential statistics tests statistical hypotheses, which are different from experimental hypotheses. To understand what this means, assume that you do an experiment to test whether "nitrogen promotes plant growth." This is an experimental hypothesis because it tells you something about the biology of plant growth. To test this hypothesis, you grow 10 bean plants in dirt with added nitrogen and 10 bean plants in dirt without added nitrogen. You find out that the means of these two samples are 13.2 centimeters and 11.9 centimeters, respectively. Does this result indicate that there is a difference between the two populations and that nitrogen might promote plant growth? Or is the difference in the two means merely due to chance? A statistical test is required to discriminate between these possibilities.

Statistical tests evaluate statistical hypotheses. The statistical null hypothesis (symbolized by H_0 and pronounced H-naught) is a statement that you want to test. In this case, if you grow 10 plants with nitrogen and 10 without, the null hypothesis is that there is no difference in the mean heights of the two groups and any observed difference between the two groups would have occurred purely by chance. The alternative hypothesis to H_0 is symbolized by H_1 and usually simply states that there is a difference between the populations.

The statistical null and alternative hypotheses are statements about the data that should follow from the experimental hypothesis.

Significance Testing: The α (Alpha) Level

Before you perform a statistical test on the plant growth data, you should determine an acceptable significance level of the null statistical hypothesis. That is, ask, when do I think my results and thus my test statistic are so unusual that I no longer think the differences observed in my data are simply due to chance? This significance level is also known as "alpha" and is symbolized by α.

The significance level is the probability of getting a test statistic rare enough that you are comfortable rejecting the null hypothesis (H_0). (See the "Probability" section of Part 3 for further discussion of probability.) The widely accepted significance level in biology is 0.05. If the probability (p) value is less than 0.05, you reject the null hypothesis; if p is greater than or equal to 0.05, you don't reject the null hypothesis.

Comparing Averages: The Student's *t*-Test for Independent Samples

The Student's *t*-test is used to compare the means of two samples to determine whether they are statistically different. For example, you calculated the sample means of survivor and nonsurvivor finches from Table 1 and you got different numbers. What is the probability of getting this difference in means, if the population means are really the same?

The *t*-test assesses the probability of getting a result more different than the observed result (i.e., the values you calculated for the means shown in Figure 1) if the null statistical hypothesis (H_0) is true. Typically, the null statistical hypothesis in a *t*-test is that the mean of the population from which sample 1 came (i.e., the mean beak size of survivors) is equal to the mean of the population from which sample 2 came (i.e., the mean beak size of the nonsurvivors), or $\mu_1 = \mu_2$. Rejecting H_0 supports the alternative hypothesis, H_1, that the means are significantly different ($\mu_1 \neq \mu_2$). In the finch example, the *t*-test determines whether any observed differences between the means of the two groups of finches (9.67 millimeters versus 9.11 millimeters) are statistically significant or have likely occurred simply by chance.

A *t*-test calculates a single statistic, *t*, or t_{obs}, which is compared to a critical *t*-statistic (t_{crit}):

$$t_{\text{obs}} = \frac{|\bar{x}_1 - \bar{x}_2|}{SE}$$

To calculate the standard error (SE) specific for the *t*-test, we calculate the sample means and the variance (s^2) for the two samples being compared—the sample size (*n*) for each sample must be known:

$$\text{SE} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

Thus, the complete equation for the *t*-test is

$$t_{\text{obs}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Calculation Steps

1. Calculate the mean of each sample population and subtract one from the other. Take the absolute value of this difference.

2. Calculate the standard error, SE. To compute it, calculate the variance of each sample (s^2), and divide it by the number of measured values in that sample (*n*, the sample size). Add these two values and then take the square root.

3. Divide the difference between the means by the standard error to get a value for *t*. Compare the calculated value to the appropriate critical *t*-value in Table 8. Table 8 shows t_{crit} for different degrees of freedom for a significance value of 0.05. **The degrees of freedom is calculated by adding the number of data points in the two groups combined, minus 2.** Note that you do not have to have the same number of data points in each group.

4. If the calculated *t*-value is greater than the appropriate critical *t*-value, this indicates that you have enough evidence to support the hypothesis that the means of the two samples are significantly different at the probability value listed (in this case, 0.05). If the calculated *t* is smaller, then you cannot reject the null hypothesis that there is no significant difference.

Table 8. Critical *t*-Values for a Significance Level α = 0.05

Degrees of Freedom (df)	t_{crit} (α = 0.05)
1	12.71
2	4.30
3	3.18
4	2.78
5	2.57
6	2.45
7	2.36
8	2.31
9	2.26
10	2.23
11	2.20
12	2.18
13	2.16
14	2.14
15	2.13
16	2.12
17	2.11
18	2.10
19	2.09
20	2.09
21	2.08
22	2.07
23	2.07
24	2.06
25	2.06
26	2.06
27	2.05
28	2.05
29	2.04
30	2.04
40	2.02
60	2.00
120	1.98
Infinity	1.96

Note: There are two basic versions of the *t*-test. The version presented here assumes that each sample was taken from a different population, and so the samples are therefore independent of one another. For example, the survivor and nonsurvivor finches are different individuals, independent of one another, and therefore considered **unpaired**. If we were comparing the lengths of right and left wings on all the finches, the samples would be classified as **paired**. Paired samples require a different version of the *t*-test known as a paired *t*-test, a version to which many statistical programs default. The paired *t*-test is not discussed in this guide.

Application in Biology

After a small population of crayfish was accidentally released into a shallow pond, biologists noticed that the crayfish had consumed nearly all of the underwater plant population; aquatic invertebrates, such as the water flea (*Daphnia* sp.), had also declined. The biologists knew that the main predator of *Daphnia* is the goldfish, and they hypothesized that the underwater plants protected the *Daphnia* from the goldfish by providing hiding places. The *Daphnia* lost their protection as the underwater plants disappeared. The biologists designed an experiment to test their hypothesis. They placed goldfish and *Daphnia* together in a tank with underwater plants, and an equal number of goldfish and *Daphnia* in another tank without underwater plants. They then counted the number of *Daphnia* eaten by the goldfish in 30 minutes. They replicated this experiment in nine additional pairs of tanks (i.e., sample size = 10, or n = 10, per group). The results of their experiment and their calculations of experimental error (variance, s^2) are in Table 9.

Experimental hypothesis: The underwater plants protect *Daphnia* from goldfish by providing hiding places.
Experimental prediction: By placing *Daphnia* and goldfish in tanks with and without plants, you should see a difference in the survival of *Daphnia* in the two tanks.
Statistical null hypothesis: There is no difference in the number of *Daphnia* in tanks with plants compared to tanks without plants: any difference between the two groups occurs simply by chance.
Statistical alternative hypothesis: There is a difference in the number of *Daphnia* in tanks with plants compared to tanks without plants.

Table 9. Number of *Daphnia* Eaten by Goldfish in 30 Minutes in Tanks with or without Underwater Plants

Tanks	Plants (sample$_1$)	No Plants (sample$_2$)	Plants $(x_i - \bar{x}_1)^2$	No Plants $(x_i - \bar{x}_2)^2$
1 and 2	13	14	$(9.6 – 13)^2 = 11.56$	$(14.4 – 14)^2 = 0.16$
3 and 4	9	12	$(9.6 – 9)^2 = 0.36$	$(14.4 – 12)^2 = 5.876$
5 and 6	10	15	$(9.6 – 10)^2 = 0.16$	$(14.4 – 15)^2 = 0.436$
7 and 8	10	14	$(9.6 – 10)^2 = 0.16$	$(14.4 – 14)^2 = 0.16$
9 and 10	7	17	$(9.6 – 7)^2 = 6.76$	$(14.4 – 17)^2 = 6.76$
11 and 12	5	10	$(9.6 – 5)^2 = 21.16$	$(14.4 – 10)^2 = 19.37$
13 and 14	10	15	$(9.6 – 10)^2 = 0.16$	$(14.4 – 15)^2 = 0.36$
15 and 16	14	15	$(9.6 – 14)^2 = 19.34$	$(14.4 – 15)^2 = 0.36$
17 and 18	9	18	$(9.6 – 9)^2 = 0.36$	$(14.4 – 18)^2 = 12.96$
19 and 20	9	14	$(9.6 – 9)^2 = 0.36$	$(14.4 – 14)^2 = 0.16$
			$\sum (x_i - \bar{x}_1)^2 = 60.4$	$\sum (x_i - \bar{x}_2)^2 = 46.4$
Mean, $\bar{x}$	$\bar{x}_1 = 9.6$	$\bar{x}_2 = 14.4$	$\frac{\sum (x_i - \bar{x}_1)^2}{n-1} = \frac{60.4}{9}$	$\frac{\sum (x_i - \bar{x}_1)^2}{n-1} = \frac{46.4}{9}$
		Variance, s^2	$s_1^2 = 6.71$	$s_2^2 = 5.16$

To determine whether the difference between the two groups was significant, the biologists calculated a *t*-test statistic, as shown below:

$$\mathrm{SE} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} = \sqrt{\frac{6.71}{10} + \frac{5.16}{10}} = 1.089$$

The mean difference (absolute value) = $|\bar{x}_1 - \bar{x}_2|$ = |9.6 − 14.4| = 4.8

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\mathrm{SE}} = \frac{4.8}{1.089} = 4.41$$

There are (10 + 10 − 2) = 18 degrees of freedom, so the critical value for p = 0.05 is 2.10 from Table 8. The calculated *t*-value of 4.41 is greater than 2.10, so the students can reject the null hypothesis that the differences in the numbers of *Daphnia* eaten in the presence or absence of underwater plants were accidental.

So what can they conclude? It is possible that the goldfish ate significantly more *Daphnia* in the absence of underwater plants than in the presence of the plants.

Analyzing Frequencies: The Chi-Square Test

The *t*-test is used to compare the sample means of two sets of data. The chi-square test is used to determine how the observed results compare to an expected or theoretical result.

For example, you decide to flip a coin 50 times. You expect a proportion of 50% heads and 50% tails. Based on a 50:50 probability, you predict 25 heads and 25 tails. These are the *expected* values. You would rarely get exactly 25 and 25, but how far off can these numbers be without the results being significantly different from what you expected? After you conduct your experiment, you get 21 heads and 29 tails (the observed values). Is the difference between observed and expected results purely due to chance? Or could it be due to something else, such as something might be wrong with the coin? The chi-square test can help you answer this question. The statistical null hypothesis is that the observed counts will be equal to that expected, and the alternative hypothesis is that the observed numbers are different from the expected.

Note that this test must be used on raw categorical data. Values need to be simple counts, not percentages or proportions. The size of the sample is an important aspect of the chi-square test—it is more difficult to detect a statistically significant difference between experimental and observed results in a small sample than in a large sample. Two common applications of this test in biology are in analyzing the outcomes of a genetic cross and the distribution of organisms in response to an environmental factor of interest.

To calculate the chi-square test statistic (χ^2), you use the equation

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

o = observed values
e = expected values
χ^2 = chi-square value
Σ = summation

Calculation Steps

1. Calculate the chi-square value. The columns in Table 10 outline the steps required to calculate the chi-square value and test the null hypothesis, using the coin-flipping example discussed above. The equations for calculating a chi-square value are provided in each column heading.

Table 10. Coin-Toss Chi-Square Value Calculations

Side of Coin	**Observed (*o*)**	**Expected (*e*)**	**(*o* − *e*)**	**$(o-e)^2$**	**$(o-e)^2/e$**
Heads	21	25	(−4)	16	0.64
Tails	29	25	4	16	0.64

$\chi^2 = \sum (o-e)^2/e \rightarrow \quad \chi^2 = 1.28$

2. Determine the degrees of freedom value as follows:

$$\text{df} = \text{number of categories} - 1$$

In the example above, there are two categories (heads and tails):

$$\text{df} = (2 - 1) = 1$$

3. Use the critical values table (Table 11) to determine the probability (*p*) value. A *p*-value of 0.05 (which is shown in red in Table 11) means there is only a 5% probability of getting the observed difference between observed and expected values by chance, if the null hypothesis is true (i.e., there is no real difference).

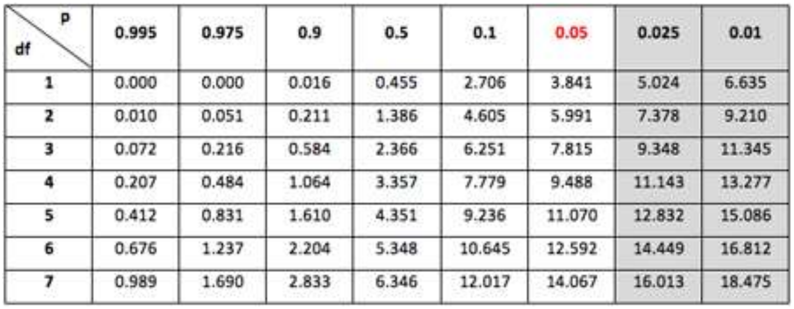
For example, for df = 1, there is a 5% probability (*p*-value = 0.05) of obtaining a χ^2-value of 3.841 or larger by chance. If the χ^2-value obtained was 4.5, then you can reject the null hypothesis that there is no real difference between observed and expected data. The difference between observed and expected data is likely real and is considered statistically significant.

If the χ^2-value was 3.1, then you cannot reject the null hypothesis. The difference between observed and expected data may be accidental and is not statistically significant.

Significance testing in biology typically uses a *p*-value of 0.05, which is also referred to as the alpha value (see “Significance Testing: The α (Alpha) Level” in Part 2). **A result with the *p*-value of 0.05 or lower is deemed a statistically significant result.**

To use the critical values table (Table 11), locate the calculated χ^2-value in the row corresponding to the appropriate number of degrees of freedom. For the coin-flipping example, locate the calculated χ^2-value in the df = 1 row. The χ^2-value obtained was 1.28, which falls between 0.455 and 2.706 and is smaller than 3.841 (the χ^2-value at the p = 0.05 cutoff); in other words, the result was likely to happen between 10% and 50% of time. Therefore, you cannot reject the null hypothesis that the results have likely occurred simply by chance, at an acceptable significance level.

Table 11. Critical Values Table for Different Significance Levels and Degrees of Freedom



df \ p	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01
1	0.000	0.000	0.016	0.455	2.706	3.841	5.024	6.635
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475

Application in Biology—Example 1

Students just learned in their biology class that pill bugs use gill-like structures to breathe oxygen. The students hypothesized that the pill bugs’ gills require them to live in wet environments for their survival. To test the hypothesis, they wanted to determine whether pill bugs show a preference for living in wet or dry environments.

The students placed 15 pill bugs on the dry side of a two-sided choice chamber, and 15 pill bugs on the wet side of the chamber. Fifteen minutes later, 26 pill bugs were on the wet side and 4 on the dry side. The data are shown in Table 12.

Table 12. Pill Bug Locations on Two-Sided Chamber

Elapsed Time (min.)	Pill Bugs on Wet Side (no.)	Pill Bugs on Dry Side (no.)

0	15	15
15	26	4

Experimental hypothesis: Pill bugs' gills require them to live in wet environments for survival.
Experimental prediction: If you place pill bugs in a two-sided chamber that is dry on one side and wet on the other, they will show a preference for the wet side.
Statistical null hypothesis: There is no difference in the numbers of pill bugs on the dry and wet sides of the chamber; any difference between the two sides occurred purely by chance.
Statistical alternative hypothesis: There is a difference in the numbers of pill bugs on the dry and wet sides of the chamber.

Table 13. Pill Bug Location Chi-Square Value Calculations

Side of Chamber	Observed (*o*)	Expected (*e*)	$(o - e)$	$(o - e)^2$	$(o - e)^2/e$
Wet	26	15	11	121	8.07
Dry	4	15	−11	121	8.07

$\chi^2 = \sum (o - e)^2/e$ → $\chi^2 = 16.14$

In Table 13, the degrees of freedom (df) = (2 − 1) = 1. The χ^2-value is 16.14, which is much greater than the critical value of 3.841 (from the critical values table [Table 11] for a *p*-value of 0.05). This means that there is a statistically significant difference between expected and observed data, and it may indicate that the pill bugs prefer one side of the chamber to the other.

Note that an alternative hypothesis is never proven true with any statistical test like the chi-square Test. This statistical test only tells you whether the null hypothesis can or cannot be rejected. There is always a chance, however small, that the observed difference could have occurred by chance even if the null hypothesis is true. Likewise, failing to reject the null hypothesis does not necessarily mean that it is true. There might be a difference between the observed and expected data that was too small to detect with the sample size of the experiment.

Application to Biology—Example 2

One common application for the chi-square test is a genetic cross. In this case, the statistical null hypothesis is that the observed results from the cross are the same as those expected, for example, the 3:1 ratio or 1:2:1 ratio for a Mendelian trait.

Dr. William Cresko, a researcher at the University of Oregon, conducted several crosses between marine stickleback fish and freshwater stickleback fish. All marine stickleback fish have spines that protrude from the pelvis, which presumably serve as protection from larger predatory fish. Many freshwater stickleback populations lack pelvic spines. Dr. Cresko wanted to find out whether the presence or absence of pelvic spines behaves like a Mendelian trait, meaning that it is likely to be controlled mainly by a single gene.

In one cross, marine stickleback with spines were crossed with stickleback from Bear Paw Lake, which don't have pelvic spines. All the progeny fish from this cross, the so-called F_1 generation, had pelvic spines. Dr. Cresko then took the F_1 offspring and conducted several crosses between them to produce the F_2 generation. The results of the F_2 crosses are shown in Table 14.

Table 14. F_2 Generation: Cross of F_1 Generation Individuals

Cross Number	Total Number of F_2 Fish	F_2 Fish with Spines	F_2 Fish without Spines
1	98	71	27
2	79	62	17
3	62	49	13
4	34	28	6
5	29	24	5
6	23	17	6
7	21	17	4
8	19	18	1
9	15	11	4
10	12	10	2
11	12	10	2
12	4	3	1
Total	408	320	88

Source: Cresko, William A., A. Amores, C. Wilson, J. Murphy, M. Currey, P. Phillips, M. Bell, C. Kimmel, and J. Postlethwait. "Parallel Genetic Basis for Repeated Evolution of Armor Loss in Alaskan Threespine Stickleback Populations." *Proceedings of the National Academy of Sciences of the United States of America* 101 (2004): 6050–6055.

If the presence of pelvic spines is controlled by a single gene and the presence of pelvic spines is the dominant trait as suggested by the F_1 results, you would expect a ratio of 3:1 for fish with pelvic spines to fish without pelvic spines in the F_2 generation. For a total of 408 fish, the expected results would be 306:102. The results from Dr. Cresko's crosses are 320:88.

The null hypothesis is that there is no real difference between the expected results and the observed results, and that the difference that we see occurred purely by chance. The statistical alternative hypothesis is that there is a real difference between observed and expected results.

Table 15. Stickleback Spine Chi-Square Value Calculations

Phenotype	**Observed (*o*)**	**Expected (*e*)**	**$(o - e)$**	**$(o - e)^2$**	**$(o - e)^2/e$**
Spines present	320	306	14	196	0.64
Spines absent	88	102	−14	196	0.64

$\chi^2 = \sum (o - e)^2/e \quad \rightarrow \quad \chi^2 = 1.28$

The χ^2-value is 1.28, which is less than the critical value of 3.841 (from the critical values table [**Table 11**] for a *p*-value of 0.05 and a df of 1). This means that the difference between expected and observed data is not statistically significant. Based on this calculation, we cannot reject the null hypothesis and conclude that any difference between observed and expected results may have occurred simply by chance.

The chi-square example above is provided in the BioInteractive activity "Using Genetic Crosses to Analyze a Stickleback Trait," http://www.hhmi.org/biointeractive/using-genetic-crosses-analyze-stickleback-trait. Another application of chi-square to genetics is available in the activity "Mapping Genes to Traits in Dogs Using SNPs," http://www.hhmi.org/biointeractive/mapping-traits-in-dogs.