Hypotheses, Null Hypotheses, Independent and Dependent Variables, Controls and Statistical Analysis

By now you should know part of the scientific method includes proposing a hypothesis and testing that hypothesis. It sounds simple enough but there are road blocks to any approach to the scientific method. In reality, researchers often test the null hypothesis. A researcher looks to either accept or reject the null hypothesis.

Hypothesis

A hypothesis is a specific statement of prediction - or, an educated guess. The emphasis is on the word educated. Anyone may guess as to a problem's solution but if I were a betting person, my money would be on the person with a little expertise in the area. Anyone can propose a hypothesis as to the origin of the universe. However, given the choice between an astrophysicist's hypothesis and my sanitation engineer's hypothesis (my garbage man), I would choose to go with the hypothesis of the astrophysicist. However, in today's world, it could be possible for my sanitation engineer to be an out of work astrophysicist.

An example of a hypothesis is "Boys are more likely to be taller than girls." In other words, B>G. Always think in terms of two hypotheses: the hypothesis and the null hypothesis.

Null Hypothesis

In it's simplest form, a null hypothesis is the reverse of the hypothesis. In the above example, our null hypothesis would be boys are either less likely or equally as likely to be as tall as girls, or, B < G. You need to phrase the null hypothesis and the hypothesis in such a way there are no other possible outcomes.

In our experiment, we wish to see if (1) B < G and (2) B > G. The idea is to put forth two mutually exclusive predictions that collectively exclude all other possible outcomes. Second, the test must accept one of these predictions and reject the other. The null hypothesis is often associated with a statistical test called chi-square (or goodness-of-fit).

Variables

In science, a variable is any measured characteristic, quantity or attribute which differs from one subject to another. In our above example, in a room full of boys and girls, their ages may be different, their heights may be different, their hair color may be different, etc.

Independent Variables

An independent variable is any variable which the researcher may change. For example, if our null hypothesis is B < G, one independent variable the researcher may use is age. If the researcher only tests 12-14 year olds, but not 17 year olds, that's controlled by the researcher and therefore an independent variable. When graphing your results, independent variables are generally placed on the x-axis.

Dependent Variables

Dependent variables are affected by the independent variable. In our example, the heights can vary considerably from the age range of 12 to 14 where it might be fairly consistent by the sexes at one age, *e.g.* 15. When graphing your results, dependent variables should be on the y-axis.

Controls

Controls validate your experiment. How do you know your experiment is valid? In the previous case, what if you happen to choose as your test site a private high school with emphasis in basketball? Do you think you might get skewed results? Controls help determine the validity of your experiment.

Repeatability

An often overlooked feature to testing hypotheses is repeatability. When scientists make a discovery, they publish their results in refereed journals. Refereed journals have editorial boards who read the proposed paper for publishing and vote to either accept the paper as sound research or reject the paper. If accepted, the paper must be written in such a way that any scientist with interest in the topic can read your paper, repeat your experiment, and basically come up with the same results.

If I try to repeat an experiment published in a scientific journal and can't get the results indicated in the paper something is wrong. Either the paper was not written well enough to follow specific procedures, or I did something wrong, or of more concern, the original researcher made a mistake. It's repeatability that provides legitimacy to scientific research.

Conclusion

Throughout the semester, you will be asked to determine a hypothesis, null hypothesis, list the independent and dependent variables in the experiments you perform.

Elementary Statistical Analysis

How do you know if you were successful with your experiment? How do you know you didn't do something wrong? In a great many of your laboratories, you will be obtaining quantitative data (as opposed to qualitative data). These quantitative data may take two forms: count data and measurement data.

Count data (or discontinuous variables) are whole numbers. For example, if you are surveying the number of eggs in Flamingo nests, that is a discontinuous variable or count. Measurement data (or continuous variables) require measurements where fractional results may occur. For example, you may measure the absorbance of light of a solution using a spectrophotometer. The reading you obtain is a continuous variable.

In either case, mistakes can be made in the collection and measurement of data. If your instruments are not calibrated correctly, you could obtain false data. An individual could take the wrong reading even if the instrument is correctly calibrated. However, if you are careful and the instruments properly calibrated, then the chance of errors are small and will be randomly distributed around the measurements taken. This means, if you repeat the experiment several times, the true value will be closer to the mean than if you just performed the experiment once. That's why it is important to repeat experiments several times. In our laboratories, we don't have that luxury. However, if we have several students perform the experiments and then take the means of the data, we can get a pretty good idea of the validity of our data.

Bias occurs when an individual *consistently* misreads the data or the instrument has not been properly calibrated. Statistics cannot correct for bias. It is therefore critical bias not creep into your experimentation. Always make sure your instruments are properly calibrated and you are sure of your measurements.

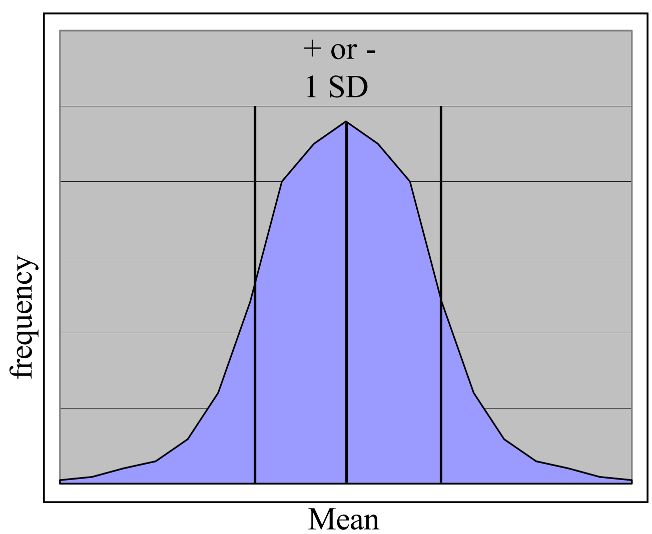
If you look around your classroom, you'll see variation is the central theme. Although we all belong to the same genus and species, *Homo sapiens sapiens*, we are all different. Even identical twins are not exactly alike. If we were to study the height of students in the class, we must take into consideration any variability that might skew our results. Statistical analysis allows us to cope with any random fluctuations in our measurements and in the properties we study.

Let's assume we are going to study the height of students in our class. Our procedure is simply to measure everyone's height in the class while they are in stocking feet. We'll use a meter stick and perform the measurements and record the results in centimeters. If we do the procedure correctly and have prevented any major errors taking place (incorrect readings, failure to remove shoes, etc.) there is still the probability some experimental error will creep into our results. For example, parallax effect can result in errors of reading the meter stick. So the results from our experiment may not be true. How can we make sure we are getting a clear indication of the heights of students in our class? One way is to repeat the experiment several times and take an average of the results. The average is called the mean. Using the mean eliminates any highs and lows due to chance.

Range is another tool in statistics which will give us some sense in the variability of measurement. Range, however, comes with its own set of problems. Let's say I'm interested in the heights of students in two different classes. I have one class determine their heights and a second class determine theirs. Look at the data obtained in *table 1*. Notice the range of heights is fairly different for the two classes although the class means are within 2.1 cm of each other. Unless one studied the data extensively, you would not realize that class 1 had some fairly short people in it. One way to provide more information about the variability of data sets is to provide variability estimates called variance and standard deviation. Standard deviation is calculated by determining the difference between *each* observation and the average. If a group of measurements, like our classes, are distributed randomly and symmetrically about the mean, the standard deviation

Table 1: Class Heights

Students	Class 1 (height in cm)	Class 2 (height in cm)
1	175.2	170
2	173.3	170.2
3	152.3	177.2
4	153.4	169.3
5	160.8	160.3
6	177.4	165.3
7	168.3	170.3
8	162.3	166.8
9	182.8	180.3
10	181.6	180.3
11	178.3	179.2
12	180.3	182.4
Sum Σ	2046	2071.6
N (# of students)	12	12
Mean	170.5	172.6
Range	152.3-182.8	160.3-182.4



defines a range of measurements in which 68% of the observed values fall. It should give us the classical bell curve.

The formula for variance is given below.

$$\text{variance} = \frac{\Sigma\text{ (measured value for each sample - mean)}^2}{\text{N-1 (one less than the number of observations)}}$$

The formula for standard deviation is

$$\sigma = \pm\sqrt{\text{variance.}}$$

Substituting the formula for variance, you get the following

$$\sigma = \pm\sqrt{\frac{\Sigma\,(x_o-\bar{x})^2}{(n-1)}}$$

where x_o is the observed value, $\bar{x}$ is the average of all observed values, and n is the number of observed values. Σ is the sum of. Remember, Σ means the sum of every one of the 12 students so in essence, you are looking at the sum of every student's height minus the mean, squared. Data table 2 shows you the calculations for Class 1.

$$\sigma = \pm\sqrt{\frac{1298.74}{11}}$$

If you take the square root, you come up with + 10.9. What this tells you is the data for class 1 has an average height of 170.5 cm + 10.9 cm which represents 68% of the heights in the class.

Data Table 2: Class 1 Standard Deviation

Students	**Class 1 (height in cm)**	**(x_o-mean)**	**(x_o-mean)^2**
1	175.2	4.7	22.09
2	173.3	2.8	7.84
3	152.3	-18.2	331.24
4	153.4	-17.1	292.41
5	160.8	-9.7	94.09
6	177.4	6.9	47.61
7	168.3	-2.2	4.84
8	162.3	-8.2	67.24
9	182.8	12.3	151.29
10	181.6	11.1	123.21
11	178.3	7.8	60.84
12	180.3	9.8	96.04
Sum	2046		1298.74
N (# of students)	12		
Mean	170.5		
Range	152.3-182.8		

Problem

Using the data for class 2, calculate the standard deviation for that class.

Procedure

I'm sure you don't do addition, substraction, multiplication or division by hand any more with the advent of calculators. To be honest, it is tedious and you have to be exceptionally careful about the order of operations. Remember PEMDAS from high school? Parenthesis, exponents, multiplication, division, addition and subtraction - in that order. Then you take the square root.

To make your life simpler, we'll use Excel spreadsheets to make the calculation.

1. Open up Excel spreadsheet software on the computer system in the lab.
2. For cell A1 type in the heading "Students".
3. for cells A2: A13 type in the data for class 2.
4. For A14 click on the symbol for sum (Σ) to add A2:A13.
5. For A15 click on the formula menu and use the formula for AVERAGE. Fill in the data A2:A13 to calculate the average.
6. For A16 use the formula menu to find the minimum (MIN) for A2:A13.
7. For A17 use the formula menu to find the maximum (MAX) for A2:13. The minimum and maximum are you range of data.
8. For A18 use the formula menu to find the standard deviation (STDEVA) for A2:A13.

Exercise A: Determining the Height of Students in Your Lab

In this exercise, you will determine the height of all the students in this lab. You will enter your data onto an Excel spreadsheet. Once you have collected the data, you will then calculate a mean height for each student in the lab and then calculate a standard deviation for each student's height. Every student must be measured six times (by the six tables in lab) and data entered on table 3 at the end of this lab packet.

Count Data

Later in the semester, you will be asked to count organisms and see if your results fit to expected outcomes. As an example, Mendelian genetics can be used to predict outcomes. The ability to roll your tongue lengthwise into a "U" shape is a dominant trait. Three quarters of the population are said to be tongue rollers. Twenty-five percent of the population cannot roll their tongue and are said to be recessive. So, out of a sample size of 100 people, 75 would be expected to be tongue rollers. The question becomes, what if in your sample of 100 people only 70 are tongue rollers. Is that simply due to chance or is there some other mechanism at work? One way to find out is to use a statistical test called chi-square (or goodness-of-fit). The chi square value generated in this test allows us the ability to reasonably predict the validity of our test.

Chi-square analysis involves the null hypothesis where the null hypothesis states there is no difference between the observed results and the predicted results. In other words, the differences you observed where 70 people instead of the predicted 75 people could roll their tongues is simply due to chance and not some error.

As you saw earlier, the null hypothesis is paired with the hypothesis (sometimes called the alternative hypothesis). If you statistically reject the null hypothesis then the hypothesis is true *by inference*. It does not directly prove the hypothesis if the null hypothesis is rejected.

In our case of tongue rolling, the null hypothesis would be "There is no significant difference between the observed results and predicted results of our simple dominant-recessive Mendelian pattern of inheritance." The hypothesis would be "The simple dominant-recessive Mendelian pattern of inheritance is not adequate to predict the outcomes of our experiment."

One thing to take into consideration in any count is the size of your sample. The larger the sample size, the better your data. Of course, your sample size can become too large and impractical but a greater problem is if your sample size is too small. That accentuates the possibility of poor results. In our case, we will have a very small sample (our labmates). We use the small sample simple to illustrate the process of chi-square analysis.

Exercise B: Chi-Square Analysis

We will sample the lab students to determine how many students can roll their tongue and how many cannot.

Procedure

1. One student at each table survey the other students at the table and count tongue rollers *vs* nontongue-rollers. All the tables will compile their data to provide a total class survey.

Data Table B-1: Frequency table of observed vs *expected results*

	Tongue Rollers	**Nontongue Rollers**	**Total**
Observed			
Expected			

2. If the lab has 24 students, then the expected number of tongue rollers would be 3/4 of 24 or 18 students and the number of nontongue rollers would be 6.

Chi-square (x^2) is calculated by using the following formula.

$$x^2 = \Sigma^n \frac{(O_i - E_i)^2}{E_i}$$

You should by now recognize the summation symbol. The letter *n* represents the number of experimental classes (in our case 2 - tongue rollers and nontongue rollers) and O_i represents the observed in class i and E_i represents the expected in class i, what ever i happens to be. In our case, i will stand for both tongue rollers and nontongue rollers.

Our formula now becomes:

$$x^2 = \frac{(O_{\text{tongue rollers}} - E_{\text{tongue rollers}})^2}{E_{\text{tongue rollers}}} + \frac{(O_{\text{nontonge rollers}} - E_{\text{nontongue rollers}})^2}{E_{\text{nontongue rollers.}}}$$

Now, calculate the specific value for x^2 based on the above formula.

What does this x^2 value mean? Is the number too large or too small to accept the null hypothesis? We must compare the x^2 value to a standard set of values for difference confidence levels and degrees of freedom.

Table B-2: Chi-Square values arranged according to chance occurance in percentage.

Possibility of Chance Occurence in Percentage (5% or less considered significant)									
Degrees of Freedom	**90%**	**80%**	**70%**	**50%**	**30%**	**20%**	**10%**	**5% (sig.)**	**1%**
1	0.016	0.064	0.148	0.455	1.074	1.642	2.706	3.841	6.635
2	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	11.341
4	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	13.277
5	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.070	15.086
6	2.204	3.070	3.828	5.348	7.231	8.558	10.645	12.592	16.812
7	2.833	3.822	4.671	6.346	8.383	9.803	12.017	14.067	18.475
8	3.490	4.594	5.527	7.344	9.524	11.030	13.362	15.507	20.090
9	4.168	5.380	6.393	8.343	10.656	12.242	14.684	16.919	21.666

Look at the category of degrees of freedom on the standard set of chi-square values. The degrees of freedom is typically one less the number of classes. In our case, we have two classes: tongue rollers and nontongue rollers, so our degrees of freedom is 1.

Follow row one across the standard set of values and located your calculated chi-square value. It should fall between two values. For example, is your calculated chi-square value is 2.613, your probability is between 20% and 10%. The way you state it is there is an 20% to 10% probability that the differences between observed and expected is due to something other than chance. It would be stated as follows: Statement of Probability: 20% > P > 10%. Is that good enough to accept the null hypothesis? Most scientists arbitrarily use the figure of 5% - if the deviation occurs in less than 5% of the trials, something other than chance is probably involved in the distribution. That tells scientists they need to revisit the problem, look for errors in calculations, counts, procedures, instrumentation, etc. Chi-square thus allows scientists to validate their results.

What was your statement of probability?
Statement of Probability: ________> P > ________.

Data Table 3: Student Heights, Means, and Standard Deviations

Student	Table 1 Height in Cm	Table 2 Height in cm	Table 3 Height in cm	Table 4 Height in cm	Table 5 Height in cm	Table 6 Height in cm	Mean Height	SD (σ)
1								
2								
3								
4								
5								
6								
7								
8								
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