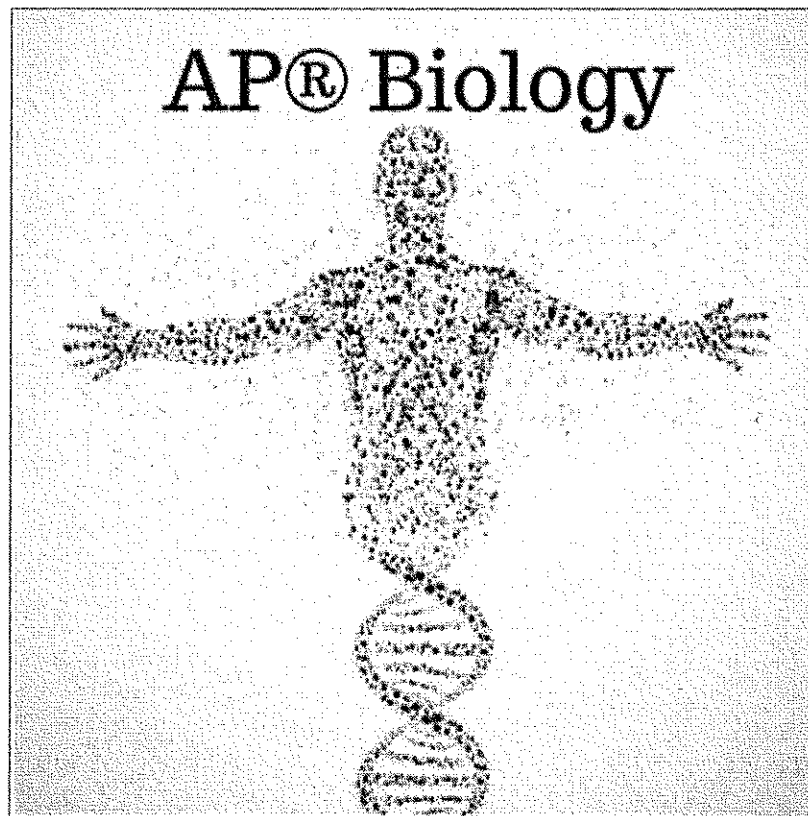




# WHITE STATION HIGH SCHOOL

Dedicated To Excellence



## Summer Assignment

**Instructor: Chikezie O. Madu, Ph.D.**

Text @wshapbo14 to (615)212-2686  
To opt-out of messages at any time by replying,  
"unsubscribe@wshapbo14"

**33% of overall Lab grade**

### Heading

Full Name:

Grade:

Contact Phone Number:

Responses must be typed, scanned, and emailed to [cemadu1@gmail.com](mailto:cemadu1@gmail.com)

Deadline: 11:59 PM on 08/01/2018

\*\*\*\*Hard copy will be turned in on the first day of the school year\*\*\*\*

To contact instructor, email [cemadu1@gmail.com](mailto:cemadu1@gmail.com). Please do not call or text.

Dear AP Biology Students,  
Welcome to AP Biology!

I am excited about working with you as you continue to expand your scientific understanding. Advanced Placement courses are reasonably arduous and AP Biology is no exception. We cover a two-semester college course in addition to a lab course. Occasionally, you will be asked to stretch yourself and some task will seem overwhelming. However, I will work with you to make it less stressful. While the course may be challenging, it will be worthwhile! Your summer assignment begins by:

# **Task One:**

Due Date-05/30

- 1. Sign up for REMIND 101 Text @wshapbio14 to (615)212-2686**

# Task Two:

Due Date-06/04

<https://sites.google.com/a/providenceday.org/apbiology/class-resources/graphing>

<https://www.youtube.com/watch?v=9dbl6YZaT5A>

<https://www.youtube.com/watch?v=Xi79jGeQ9H0>

<https://www.youtube.com/watch?v=Rvzqkw1DNq0>

[https://www.youtube.com/watch?v=Rflug\\_pB4JY](https://www.youtube.com/watch?v=Rflug_pB4JY)

<https://www.youtube.com/watch?v=biK0YDaaS8o>

"Biological concepts and models are becoming more quantitative, and biological research has become critically dependent on concepts and methods drawn from other scientific disciplines. The connections between the biological sciences and the physical sciences, mathematics, and computer science are rapidly becoming deeper and more extensive." BIO2010 report of the National Research Council (2003)

Therefore, it is imperative that today's students develop and apply quantitative skills as part of their exploration into biology. A good grasp of quantitative methodology and reasoning is particularly important in the laboratory experience. Visit these websites and others you may find, and become familiar with the following statistic concepts:

1. Mean
2. Standard deviation
3. Standard error of mean
4. Chi square

<https://www.youtube.com/watch?v=lgqYISKoXak>

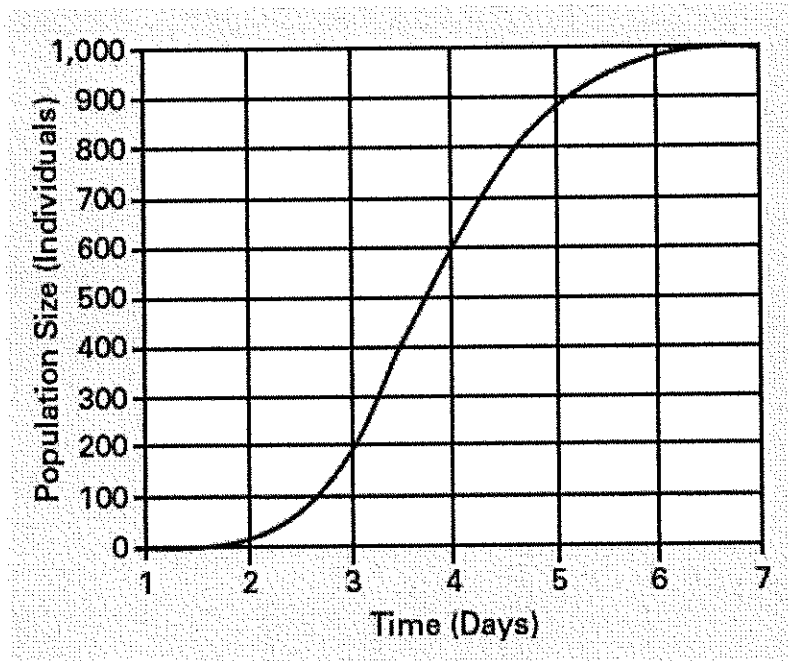
<http://www.bozemanscience.com/chi-squared-test/>

<http://www.bozemanscience.com/standard-deviation/>

<http://www.bozemanscience.com/standard-error/>

**You will take a quiz on this during the first week of school.**

### Sample Grid-In Question Using Graphing



Use the graph above to calculate the mean rate of population growth (individuals per day) between day 3 and day 5. Give your answer to the nearest whole number.

### Sample Free-Response Question Using Graphing

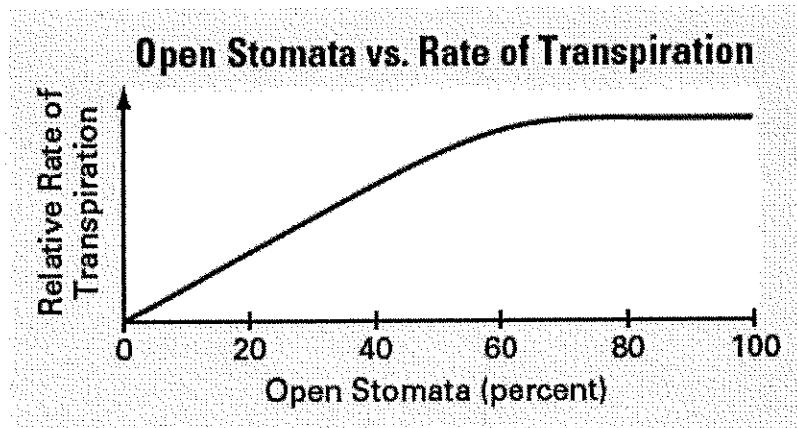
Plants lose water from their aboveground surfaces in the process of transpiration. Most of this water is lost from stomata, microscopic openings in the leaves. Excess water loss can have a negative effect on the growth, development, and reproduction of a plant. Severe water loss can be fatal. Environmental factors have a major impact on the rate of plant transpiration.

Transpiration Rate Versus Temperature

Temperature (°C)	20	23	27	28
Transpiration Rate (mmol/m <sup>2</sup> .sec)	1.5	3	5	4.5

- Using the data above and the axes provided, **draw** a graph showing the effect of temperature change on the rate of transpiration. **Explain** the shape of the curve from 23 degrees to 28 degrees.
- Humidity is another environmental factor that affects transpiration rate. Using the axes provided, **draw** a curve that illustrates what you predict would be the rate of transpiration with increasing humidity and constant temperature. **Justify** the shape of the curve based on your prediction.

- (c) The curve below illustrates the rate of transpiration related to the percent of open stomata on the leaf of a particular plant. **Explain** why the curve levels off with increasing percentage of open stomata per area of the leaf.



- (d) The data below show the density of stomata on the leaf surfaces of three different species of plants. **Describe** the environments in which each plant most likely evolved. **Justify** your descriptions.

Plant	Stomata Density (# of stomata/mm <sup>2</sup> )	
	In Upper Epidermis	In Lower Epidermis
Anacharis	0	0
Water Lily	420	0
Black Walnut	2	465

# **Task Three:**

**Due Date-06/15**

## Chi-square Statistical Analysis on Data from Genetics Experiments

### Using the Chi-Square Test for Statistical Analysis of Experimental Data

Statistics can be used to determine if differences among groups are significant, or simply the results of predictable error. The statistical test most frequently used to determine whether data obtained experimentally provide a good fit or approximation to the expected or theoretical data is the **chi-square test** (abbreviated as  $\chi^2$ ). This test can be used to determine if deviations from the expected values are due to chance alone, or to some other circumstance. Examples are presented below that explain how to use chi-square.

The formula for chi-square is:  $\chi^2 = \sum \frac{(o - e)^2}{e}$

where **o** = **observed** number of individuals

**e** = **expected** number of individuals

$\Sigma$  = the **sum of the values** (in this case, the differences, *squared*, divided by the number expected)

To determine if the observed data fall within acceptable limits, a chi-square analysis is performed to test the validity of a **null hypothesis** (that there is no statistically significant difference between the observed and expected data). If the chi-square analysis indicates that the data vary too much from the expected ratio, an **alternative hypothesis** is proposed.

#### Example 1

In the following example two heterozygous green (*Gg*) plants were crossed. The chi-square analysis will determine whether or not to reject the null hypothesis, which predicts that the data from the experimental cross will fit a 3:1 ratio.

Phenotype	# Observed (o)	# Expected (e)	(o - e)	(o - e) <sup>2</sup>	$\frac{(o - e)^2}{e}$
Green	72	63	9	81	1.29
Albino	12	21	-9	81	3.86
Calculated chi-square value = 5.15					5.15

Table of Chi-Square Values

Degrees of Freedom (n)	Probability Values (P) Deviation from Hypothesis Not Significant								Deviation Significant	Deviation Highly Significant
	0.99	0.95	0.90	0.75	0.50	0.25	0.20	0.10	0.05	0.01
1	0.0002	0.004	0.016	0.102	0.455	1.323	1.642	2.706	3.841	6.635
2	0.020	0.103	0.211	0.575	1.386	2.773	3.219	4.605	5.991	9.210
3	0.115	0.352	0.584	1.213	2.366	4.108	4.642	6.251	7.815	11.345
4	0.297	0.711	1.064	1.923	3.357	5.385	5.989	7.779	9.488	13.277
5	0.554	1.146	1.610	2.675	4.352	6.626	7.289	9.236	11.070	15.086
6	0.872	1.635	2.204	3.455	5.348	7.841	8.558	10.645	12.592	16.812
7	1.239	2.167	2.833	4.255	6.346	9.037	9.803	12.017	14.067	18.475
8	1.647	2.732	3.490	5.071	7.344	10.219	11.030	13.362	15.507	20.090
Chi square value consistent with hypothesis									Not consistent	



### How to Use and Interpret the Table of Chi-Square Values

1. You must first determine the **degrees of freedom (df)** for your experiment. The df value is the number of phenotypic classes in your cross minus 1. Since there are two possible phenotypes (green and white), for this experiment  $df = 1$  (2 phenotypes - 1).

#### To calculate degrees of freedom for different types of crosses:

- **monohybrid cross:** expect 2 different phenotype classes, so 1 degree of freedom.
  - **dihybrid cross:** expect 4 different phenotype classes, so 3 degrees of freedom.  
(for both these crosses, do not separate males and females into a separate class).
  - **sex-linked cross:** depends on the cross; be sure to separate different types of males and females as a separate class. (ex. a cross that resulted in offspring recorded as red-eyed males, white-eyed males, and red-eyed females would have 3 classes, and thus 2 degrees of freedom in your chi-square calculations)
2. You can now find the probability (**p**) value. The p-value is located on the top row of the chart. This value will tell you the percentage of times you should have a deviation from what is expected in a cross such as yours. If your p-value is 0.10 (10%) or higher (0.10 to 0.95 on the chart), this indicates that the deviation from what you expected is not significant, and it was due to chance alone. If your p-value is 0.05 or lower, the deviation is significant, and the null hypothesis must be rejected; the deviation is most likely not due to chance alone,

If calculated  $\chi^2$  is **less than** the critical table value at the 0.05 level, **do not reject the null hypothesis;** differences **are due to chance alone.**

If calculated  $\chi^2$  is **more than** the critical table value at the 0.05 level, **reject the null hypothesis;** differences are significant, and **are not due to chance alone.**

**Note that you do not state that you “accept the null hypothesis.” The chi square tests a hypothesis based on data but does not prove it to be true.**

To find your p-value, find the degrees of freedom for your cross, and look across in that row for where your calculated value of chi-square would fall. In our example, the chi-square value is 5.15. If you look in the row next to 1 degree of freedom, you will find that this value falls between 3.8 and 6.6. Now look up to the corresponding p-value, and you will see that it is somewhere between 0.05 and 0.01. This means that for our example the chi-square value indicates that the deviation is significant at the 0.05 level. These results mean only 5% of the time would you expect to get deviations this great in a similar type of cross. In other words, 95% of the time that you performed a similar cross you would expect to get deviations less than what you obtained.

Since these data do not fit the expected 3:1 ratio, you must consider reasons for this variation. Additional experimentation would be necessary. Perhaps the sample size is too small, or errors were made in the data collection. In this example, perhaps the albino seedlings are underrepresented because they died before the counting was performed.

### SUMMARY

If calculated  $\chi^2$  is **less than** the critical table value at the 0.05 level, **do not reject the null hypothesis;** differences **are due to chance alone.**

If calculated  $\chi^2$  is **more than** the critical table value at the 0.05 level, **reject the null hypothesis;** differences are significant, and **are not due to chance alone.**

**Practice Chi-square Problem with Coins:**

A coin was flipped 50 times and the number of heads and tails were recorded. Perform a chi square analysis on the following data:

Heads	Tails
30	20

State your null hypothesis:

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Use the table below to calculate the Chi-square value.

Outcome	# Observed (o)	# Expected (e)	(o - e)	(o - e) <sup>2</sup>	$\frac{(o - e)^2}{e}$
Heads					
Tails					

Degrees of freedom = \_\_\_\_\_

Based on your calculated chi square value, what is the probability value (p value) and what does this p value tell you about the data collected on this experiment?

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Using .05 as the critical value, do you reject or not reject the null hypothesis? Explain your answer.

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**Independent Practice:****Perform a chi square analysis on the following data.**

For 1000 shoppers donating blood at a local mall, the frequencies of blood types were as shown in the table below.

Blood type	O	A	B	AB	Total
Number of people	465	394	96	45	1000

Theory says that these blood types should be in the ratio of 9:8:2:1. For example, the fraction of people having blood O blood should be  $9/20$ . □

1. State your null hypothesis

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2. Use the table below to calculate the Chi-square value. (*Note: there may be more rows in this table than are necessary for the number of classes*)

Class	# Observed (o)	# Expected (e)	(o - e)	(o - e) <sup>2</sup>	$\frac{(o - e)^2}{e}$
Chi-square value					

3. Degrees of freedom = \_\_\_\_\_

4. Based on your calculated chi square value, what is the probability value (p value) and what does this p value tell you about the data collected on this experiment?

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5. Using .05 as the critical value, do you reject or not reject the null hypothesis? Explain your answer.

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## AP Biology Scientific Practices

AP science courses incorporate six overarching practices that capture important aspects of the work of scientists. Science practices describe the knowledge and skills that students should learn and demonstrate to reach a goal or complete a learning activity. They are listed below for your reference.

### *Science Practice 1 – Concept Explanation*

Explain biological concepts, processes, and models presented in written format.

### *Science Practice 2 – Visual Representations*

Analyze visual representations of biological concepts and processes.

### *Science Practice 3 – Questions and Methods*

Determine scientific questions and methods.

### *Science Practice 4 – Representing and Describing Data*

Represent and describe data.

### *Science Practice 5 – Statistical Tests and Data Analysis*

Perform statistical tests and mathematical calculations to analyze and interpret data.

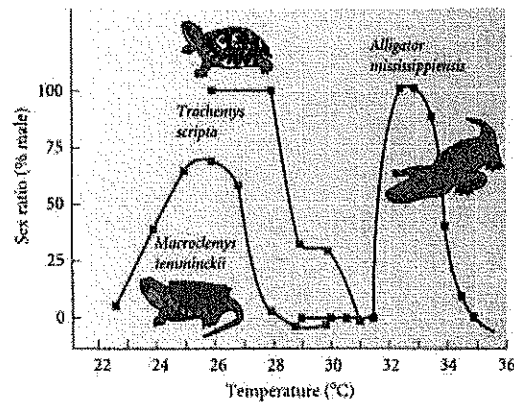
### *Science Practice 6 – Argumentation*

Develop and justify scientific arguments using evidence.

## Science Practice 1 – Concept Explanation

Explain biological concepts, processes, and models presented in written format.

1. Describe biological concepts and/or processes.
  2. Explain biological concepts and/or processes.
  3. Explain biological concepts, processes, and/or models in applied contexts.
- 



1. Describe the effect of temperature on sex determination for each of the three species of reptiles depicted above:

a. *Macrolemys temminckii*:

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b. *Trachemys scripta*:

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c. *Alligator mississippiensis*:

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2. As temperatures continue to rise, hypothesize the impact on each of the three reptile populations. Think beyond statements such as “more or less males.” (so... then what?) And think on a global scale ☺

a. 

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b. 

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c. 

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## Science Practice 2 – Visual Representations

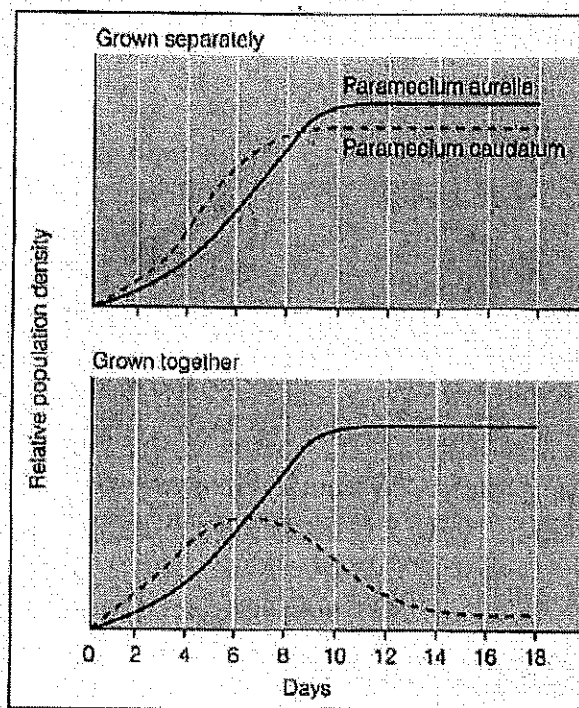
**Analyze visual representations of biological concepts and processes.**

1. Describe characteristics of a biological concept, process, or model represented visually.
2. Explain relationships between different characteristics of biological concepts, processes, or models represented visually.
  - a. In theoretical contexts.
  - b. In applied contexts.
3. Explain how biological concepts or processes represented visually relate to larger biological principles, concepts, processes, or theories.
4. Represent relationships within biological models, including
  - a. Mathematical models
  - b. Diagrams
  - c. Flow charts

A theory used in AP Biology is the Law of Competitive Exclusion. Review the graphs of *Paramecium aurelia* (solid line) and *Paramecium caudatum* (dashed line) below to learn more:

**Figure 2.14.**

Population growth of *Paramecium aurelia* and *Paramecium caudatum* when grown separately and together.



1. Describe the results of the experiment depicted in graph 1.

2. Describe the results of the experiment depicted in graph 2.

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3. Based on these “trends,” postulate what the Law of Competitive Exclusion states regarding population growth of these two species of paramecium.

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# Task Four:

Due Date-06/20



## Science Practice 3 – Questions and Methods

**Determine scientific questions and methods.**

1. Identify or pose a testable question based on an observation, data, or a model.
  2. State the null and alternative hypotheses, or predict the results of an experiment.
  3. Identify experimental procedures that are aligned to the question, including
    - a. Identifying dependent and independent variables.
    - b. Identifying appropriate controls.
    - c. Justifying appropriate controls.
  4. Make observations, or collect data from representations of laboratory setups or results
  5. Propose a new/next investigation based on
    - a. An evaluation of the evidence from an experiment
    - b. An evaluation of the design/methods.
- 

**Part I:** Identify the independent (IV) and dependent (DV) variables for each of the experiments below.

1. Guinea pigs are kept at different temperatures for 6 weeks. Percent weight gain is recorded.

IV= \_\_\_\_\_, DV= \_\_\_\_\_

2. The diversity of algal species is calculated for a coastal area before and after an oil spill.

IV= \_\_\_\_\_, DV= \_\_\_\_\_

3. The light absorption by a pigment is measured for red, blue, green and yellow light.

IV= \_\_\_\_\_, DV= \_\_\_\_\_

4. Batches of seeds are soaked in salt solutions of different concentrations, and germination is counted for each batch.

IV= \_\_\_\_\_, DV= \_\_\_\_\_

5. An investigator hypothesizes that the adult weight of a dog is higher when it has fewer littermates.

IV= \_\_\_\_\_, DV= \_\_\_\_\_

**Part II:** Identify a control group for each of the following examples. Remember, control group = **without** the IV.

1. An investigator studies the amount of alcohol produced by yeast when it is incubated with different types of sugar.

\_\_\_\_\_

2. The effect of light intensity on photosynthesis is measured by collecting oxygen produced by a plant.

\_\_\_\_\_

3. The effect of sweetener on tumor development in lab rats.

\_\_\_\_\_

**Part III:** Read the following description of experiments. Identify the IV, DV, control, and constants. Report one way to improve each experiment. Remember – a constant is something kept the same IN EVERY GROUP.

1. A group of students hypothesizes that the amount of alcohol produced in fermentation depends on the amount of glucose supplied to yeast. They want to use 5%, 10%, 15%, 20%, 25% and 30% glucose solutions.

IV = \_\_\_\_\_, DV = \_\_\_\_\_

What control treatment should be used?

\_\_\_\_\_

What constants should be used?

\_\_\_\_\_

2. A group of students wants to study the effect of temperature on bacterial growth. To get bacteria, they leave Petri dishes of nutrient agar open on a shelf. They then put the dishes in different places: an incubator ( $37^{\circ}\text{C}$ ), a refrigerator ( $10^{\circ}\text{C}$ ), and a freezer ( $0^{\circ}\text{C}$ ). Bacterial growth is measured by estimating the percentage of each dish covered by bacteria at the end of a 3 - day growth period.

IV = \_\_\_\_\_, DV = \_\_\_\_\_

What control group should be used?

\_\_\_\_\_

What constants should be used?

\_\_\_\_\_

3. A team of scientists is testing a new drug, XYZ, on AIDS patients. They expect patients to develop fewer AIDS-related illnesses when given the drug, but they don't expect XYZ to cure AIDS.

What hypothesis are they testing?

\_\_\_\_\_

IV = \_\_\_\_\_, DV = \_\_\_\_\_

What control group should be used?

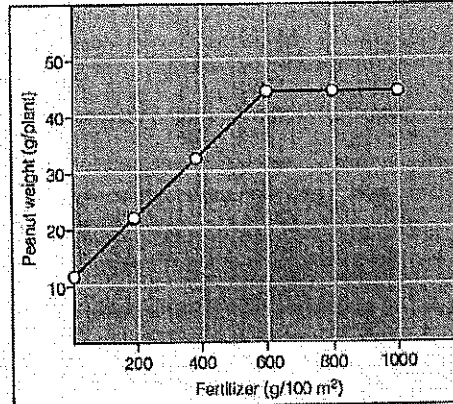
\_\_\_\_\_

What constants should be used?

\_\_\_\_\_

The graph below shows the effect of fertilizer on peanut plant growth.

**Figure 2.6.**  
Graph of peanut weight vs.  
amount of fertilizer applied.



**Figure 1.** Weight of peanuts produced per plant when amount of fertilizer applied is varied. (Average seed weight per plant in 100 m² plots, 400 plants/plot.)

1. Describe the trends shown in the graph:

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2. Develop a testable question and hypothesis that would explain the data (note: you will design an experiment for this hypothesis in the next section)

a. Testable question: \_\_\_\_\_

b. Hypothesis: \_\_\_\_\_

3. Using the testable question and hypothesis you developed above, design an experiment. Be sure to include specific data collection strategies.

a. Independent variable: \_\_\_\_\_

b. Dependent variable: \_\_\_\_\_

c. Constants: \_\_\_\_\_

d. Control Group: \_\_\_\_\_

e. Data to collect / how you will collect: \_\_\_\_\_

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## Science Practice 4 – Representing and Describing Data

**Represent and describe data.**

## 1. Construct a graph, plot or chart

a. Orientation

b. Labeling

c. Units

d. Scaling

e. Plotting

f. Type

g. Trend line

## 2. Describe data from a table or graph, including

a. Identifying specific data points.

b. Describing trends and/or patterns in data.

c. Describing relationships between variables

An investigation was carried out to measure the rate of activity of catalase, an enzyme that breaks down hydrogen peroxide. Five 40-mL solutions of the enzyme at concentrations of 20%, 40%, 60%, 80%, and 100% were prepared. A filter paper disk was placed in each enzyme solution. Each soaked disk from the different enzyme concentrations was then added to different cups containing 30 mL of 1% hydrogen peroxide. The rate of catalase activity was inferred from measurements of how fast the disks rose from the bottom to the top of each cup. The following data were obtained: 40%–12.1 seconds, 80%–5.8 seconds, 100%–4.1 seconds, 20%–15.8 seconds, and 60%–9.9 seconds.

**Directions:** Organize the data by completing the data table, according to the directions below.

1. Label the second column of the data table with an appropriate heading and record that label on the  $y$ -axis of the graph. [Be sure to include units.]

2. Complete the data table so that the percent enzyme *increases* from the top to the bottom of the table.

3. Make a line graph of the data on the grid below.

Enzyme Concentration (percent)	

**Be sure your graph has:**

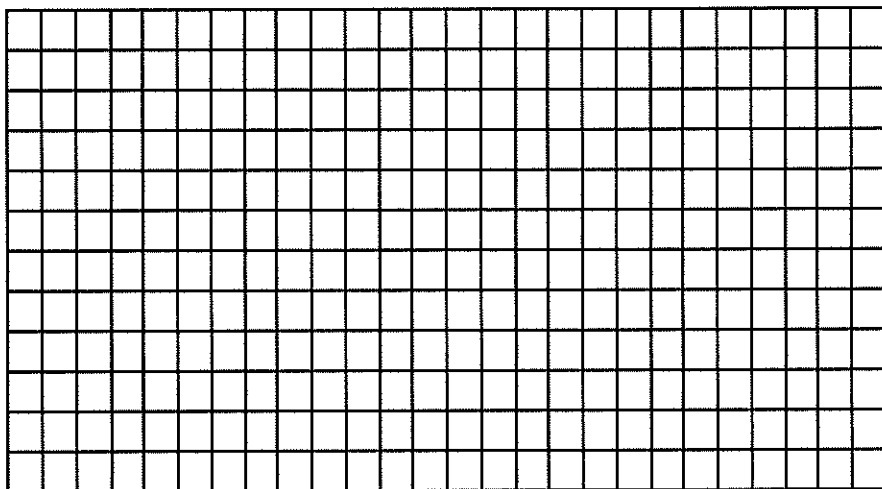
Title

Axes labeled

Units on axis

Clear data points

Best fit line/curve



The independent variable in the experiment is: \_\_\_\_\_

The dependent variable in the experiment is: \_\_\_\_\_

State one valid conclusion that relates enzyme concentration to reaction rate:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

[illegible][illegible][illegible]

## Science Practice 5 – Statistical Tests and Data Analysis

Perform statistical tests and mathematical calculations to analyze and interpret data.

- Perform mathematical calculations, including
  - Mathematical equations in the curriculum
  - Means.
  - Rates.
  - Ratios.
  - Percentages.
- Use confidence intervals and/or error bars (both determined using standard errors) to determine whether sample means are statistically different.
- Perform chi-square hypothesis testing.
- Use data to evaluate a hypothesis (or prediction), including
  - Rejecting or failing to reject the null hypothesis.
  - Supporting or refuting the alternative hypothesis.

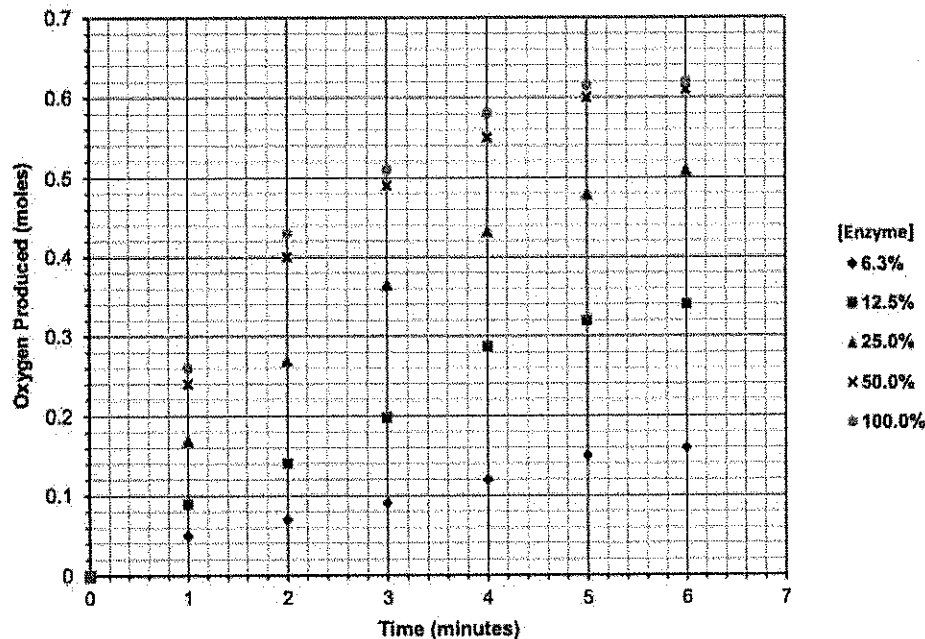
The enzyme peroxidase was isolated from fresh turnips. This enzyme is a slight variation on the catalase you have used in your protein study. In both cases each enzyme breaks down hydrogen peroxide to oxygen gas and water. In this case, however, the oxygen produced reacts with guaiacol, bringing about a color change.



By using a spectrophotometer (or colorimeter), the amount of oxygen produced can be quantified by measuring the increasing amount of brown tetraguaiacol produced. Thus the amount of oxygen produced can be recorded by measuring the absorbance of the brown pigment. Using a standard curve, this can be converted to moles of oxygen. You have done similar measurements using colorimeters in chemistry.

### Experiment C

This study was repeated, but this time the amount of enzyme was varied by the same dilution process. The oxygen produced was recorded for 6 minutes. The data from these trials are graphed below. Complete the graph by putting in best-fit curves.



Using your curves of best fit, calculate the initial rate of reaction and record in the table below.

Science Practice 6 – Argumentation

Develop and justify scientific arguments using evidence.

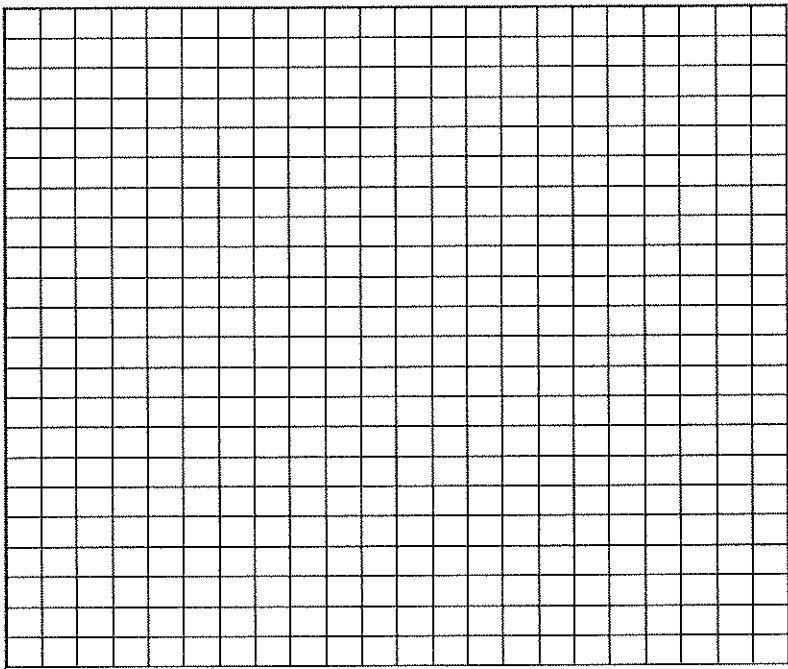
- 1. Make a scientific claim.
- 2. Support a claim with evidence from biological principles, concepts, processes, and/or data.
- 3. Provide reasoning to justify a claim by connecting evidence to biological theories.
- 4. Explain the relationship between experimental results and larger biological concepts, processes, or theories.
- 5. Predict the causes or effects of a change in, or disruption to, one or more components in a biological system based on
  - a. Biological concepts or processes.
  - b. A visual representation of a biological concept, process, or model.
  - c. Data.

Germination Rates of Pinto Beans

Day	% Germination (15° C)	% Germination (20° C)	% Germination (25° C)
0	0	0	0
2	2	10	10
4	10	30	50
6	20	40	80
8	20	60	90
10	35	70	90

Table 4

Construct an appropriately labeled graph of the data in Table 4.



1. What conclusions can you draw from the graph?

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2. Give one suggestion for improvement for this experiment.

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# **TASK Five:**

**Due Date-06/25**

## Thinking about data collection...

## Part 1: Qualitative vs Quantitative Data

For each of the theoretical experiments below, **list** and **describe** one source of *qualitative* and one source of *quantitative* data that you could collect if you were doing this experiment. Do not try to hypothesize or predict the results of the experiment, only think about what kinds of data you could collect if you were trying to answer the experimental question.

## Sample

A researcher is trying to determine if a new type of fungicide is effective at preventing fungus infections on a certain crop

## Qualitative

1. Presence or absence of fungus on the plant
2. Color of the plant leaves (yellow or brown indicates fungal infection)

## Quantitative

1. Size of fungus spots on a plant
2. Number of fungus spots on plant

1. A researcher notices that in a population of moths, some have a light gray color and some have a very dark gray, almost black color. She wonders if the coloring might be a form of camouflage.

Qualitative –

Quantitative –

2. A researcher is comparing the effectiveness of two different medications in treating flu symptoms.

Qualitative –

Quantitative –

3. A researcher believes that a local chemical production plant might secretly be dumping waste into a nearby river.

Qualitative –

Quantitative –

4. A student working for a pet food company is testing different flavors of cat food in order to develop new flavors to sell.

Qualitative –

Quantitative –

## Part 2: Determining what evidence supports a claim

For this part, **list** and **describe** two pieces of evidence (qualitative, quantitative, or one of each) that would *support* the researchers claim or hypothesis. First consider **how** you will measure change, then **what** change you would expect to see.

### Sample

A scientist notices that some individuals in a population of fish have an extra dorsal fin. He hypothesizes that this extra fin might give those individuals an advantage when trying to escape predators.

1. The fish with the extra fin are faster than the fish without, meaning they could likely outswim predators.
  2. At the end of the summer a higher percentage of fish with the dorsal fin survived than fish without the extra fin meaning fewer of them were eaten.
- 
1. A researcher hypothesizes that eating certain foods that are high in Vitamin C interferes with a human's ability to absorb calcium from other foods.
  2. A researcher believes that when working out, doing more repetitions at a low weight stimulates more muscle growth than doing fewer repetitions at a high weight.
  3. A researcher is excited about the potential of a new natural substance that may help prevent damage to DNA from ultraviolet light from the sun.
  4. Mr. Pilgrim thinks that his cats prefer the chicken-flavored cat food over the tuna-flavored cat food.

## Null and Alternative Hypotheses

### A new way of thinking about experiments

#### Background:

In prior science courses you have been taught to make **hypotheses** – predictions – about the outcome of experiments that you conduct. You did in order to have a sense of direction during your experiment and what to focus on as you made observations.

In real-world experiments, scientists rarely explicitly predict their outcomes. Instead, they ask questions as you have learned to do and then brainstorm possible outcomes, including ones that they think are unlikely. This allows them to keep an open mind about their observations.

For example, if you think that experimental data will show one thing but it ends up showing the opposite thing, you might think your experiment was flawed somehow (the classic “we messed up”). If you open your mind to other possibilities as scientists do you might gain knowledge you otherwise wouldn’t have.

#### Null hypothesis:

One common way that scientists force themselves to keep an open mind is through a technique called a **null hypothesis**. Null means “invalid” or “having no value”. You already know that a hypothesis is a prediction, so a null hypothesis is a hypothesis that says that **nothing will happen**, or more properly that the **independent variable** of the experiment **has no effect** on the **dependent variable**.

Think back to the mealworm lab. Most of the scientific questions that were generated had this form:

“Do the mealworms prefer A or B?”

That question does not account for the possibility that the mealworms have NO preference!

Also, we expect that when we do experiments that something will happen. In fact, surveys of published and unpublished experiments suggest that nearly 80% of experiments show no or weak results! But that is okay! Finding no effect of an independent variable on a dependent variable is still new information that we did not have before.

#### Writing null hypotheses:

To construct a null hypothesis, first identify the **independent** and **dependent** variables in the experiment, then indicate that the independent variable **has no effect** on the dependent variable.

“(IV) has no effect on (DV).”

Ex. A scientist is trying to determine if a new chemical is effective at preventing fungus growth on plant leaves.

Independent variable (IV): chemical

Dependent variable (DV): fungus growth

Null hypothesis: The chemical **has no effect** on fungus growth.

Spoiler alert: Chemical and pharmaceutical companies often test hundreds of compounds and formulas before finding one that works. Most of their experiments end with them finding no effect! Back to the drawing board!

Practice writing null hypotheses:

For Examples 1-3 below, identify the independent and dependent variables, then write a null hypothesis for the experiment.

1. A gardener is testing different types of soil (organic, sandy, clay, etc.) to determine which is best for growing her vegetables.
  - a. IV –
  - b. DV –
  - c. Null hypothesis –
  
2. A teacher finds a new activity and is trying to see if it helps his students understand a concept better and do better on a test.
  - a. IV –
  - b. DV –
  - c. Null hypothesis –
  
3. A student is trying to organize their papers in a different way to try to help them not lose as many papers and assignments.
  - a. IV –
  - b. DV –
  - c. Null hypothesis –

Alternative hypotheses:

Of course, scientists do not do experiments unless they think that there might be some effect. They do not want to waste their time! In addition to writing a null hypothesis, scientists will also write an **alternative hypothesis**, which is what you have already learned as just a “hypothesis”. An alternative hypothesis states that the independent variable **has an effect** on the dependent variable. When writing an alternative hypothesis, **do not state what you think the effect will be or explain why**, simply state that there will be an effect (see example below)

Ex. A scientist is trying to determine if a new chemical is effective at preventing fungus growth on plant leave.

Independent variable (IV): chemical

Dependent variable (DV): fungus growth

Alternative hypothesis: The chemical **has an effect** on fungus growth.

**\*\*Note that the alternative hypothesis simply states: “(IV) has an effect on (DV).”, it does not state what that effect will be (that is what we call a “prediction”).**

For Examples 4-6 below (same scenarios as null hypothesis section), rewrite the null hypothesis, then write an alternative hypothesis.

4. A gardener is testing different types of soil (organic, sandy, clay, etc.) to determine which is best for growing her vegetables.
  - a. Null hypothesis –
  - b. Alternative hypothesis –
5. A teacher finds a new activity and is trying to see if it helps his students understand a concept better and do better on a test.
  - a. Null hypothesis –
  - b. Alternative hypothesis –
6. A student is trying to organize their papers in a different way to try to help them not lose as many papers and assignments.
  - a. Null hypothesis –
  - b. Alternative hypothesis –

#### More practice

7. A nutritionist theorizes that diets rich in fruits and vegetables may have a positive effect on human digestive system bacterial species growth and survival.
  - a. Null hypothesis –
  - b. Alternative hypothesis –
8. Epidemiologists (scientists that study disease transmission, causes, and methods of control) believe that using antibacterial hand soaps have actually increased the number and virulence (harmfulness) of antibiotic-resistant bacteria in the world.
  - a. Null hypothesis –
  - b. Alternative hypothesis –

9. Wildlife biologists noticed that when wolves in Yellowstone National Park became protected from hunting by federal laws, average tree height in Yellowstone increased by 20% in 10 years.
- Null hypothesis –
  - Alternative hypothesis –

# **Task Six:**

**Due Date-06/30**



## 95% Confidence Intervals / $\pm 2SE_x$

### Background

When an experiment is conducted there are often sources of **error** – you call it “messing up”, but really error is any place in an experiment where, if you were to do the experiment again, some variation might occur that could affect your data. This is why it is generally considered good practice to “redo” investigations and gather multiple pieces of data that can be looked at as a whole. Seemingly insignificant choices may cause variation or error in results. Consider the following possibilities:

- You use old chemicals that may not be as effective (some chemicals undergo spontaneous reactions and so are only good for a certain amount of time)
- Errors / inaccuracies in measurements / equipment (we often use the least expensive beakers and pipettes 🤖)
- Judging qualitative data (colors, levels of activity, “low”, “high” – different people have different judgements)
- Different experimental groups with different characteristics (like living organisms)
- Environmental conditions - ambient air temperature, humidity, etc. may affect results

All of these small factors may add up to create uncertainty in your results.

### Communicating Confidence

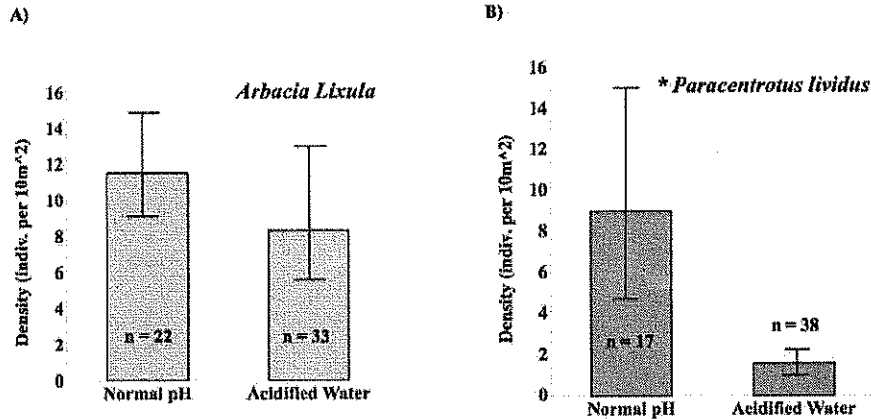
Scientists use a variety of ways to communicate how confident they are in their experimental results. The simplest way, and one that you have done, is to list possible **sources of error**. This lets other scientists know where the experimenters possibly made errors and how those errors may have affected their results. Also, scientists conduct investigations more than once, or they have many different samples of the same experiment running at the same time. If the data they get from each sample or each run of the investigation is similar, the scientist can be pretty confident in their results. If the range of data is large, then they probably have less confidence because they are getting lots of different values for their data.

In addition to **describing** sources of error, scientists can **quantify (calculate)** their error and, therefore, their confidence in their data. One way that scientists do this is by calculating the **standard error of the mean**. This is a statistical analysis method that outputs an estimate of the error in the data. They then use this to create a **95% confidence interval**, visualized as an **error bar**, on their graph. A 95% confidence interval is a scientist saying, “Here are my results. I know there is probably some uncertainty and error in my data. I am 95% certain that even with the uncertainty, the data falls within this range.” 95% confidence intervals are used on **graphs that show quantitative data**. They are easy, visual ways for scientists to show the uncertainty in their data.

Calculating 95% confidence intervals involves statistical equations and theory that are beyond the scope of what you will need to do in this course; however, you do need to be able to look at a graph that shows 95% confidence intervals and explain what the intervals indicates about the reliability of the data and any conclusions that can be drawn based on the data. One way that confidence intervals are calculated is by using a calculation standard error of the mean (abbreviated as  $\pm 2SE_x$ ). If you see “standard error of the mean” or “ $\pm 2SE_x$ ”, these are referring to confidence intervals.

95% confidence intervals are shown by drawing **error bars** on graphs. Any graph that shows quantitative data can have error bars. You will see bar graphs, line graphs, and scatter plots that all have error bars.

### Looking at 95% Confidence Intervals



Take a look at Graph A (*Arbacia lixula*).

1. What is the dependent variable of the experiment? \_\_\_\_\_
2. What is the independent variable of the experiment? \_\_\_\_\_

The shaded (pink and gray) bars show the scientists' data from their experiment.

3. What is the density of *A. lixula* in normal pH water? \_\_\_\_\_
4. What is the density of *A. lixula* in acidified water? \_\_\_\_\_

The lines extending upwards and downwards from the top of the bars show the 95% confidence interval, calculated from the standard error of the mean, for the scientists' data. It considers possible sources of error in the experiment and shows the possible range of data for each trial. **Large error lines indicate a wider range of possible results, small lines indicate a smaller range of possible results, but all error bars show the range of results with 95% confidence.**

5. What are the highest and lowest possible densities for *A. lixula* in normal pH water? \_\_\_\_\_
6. What are the highest and lowest possible densities for *A. lixula* in acidified water? \_\_\_\_\_

Look at Graph B (*Paracentrotus lividus*).

7. What is the density of *P. lividus* in normal pH water? \_\_\_\_\_
8. What is the range of possible densities of *P. lividus* in normal pH water? \_\_\_\_\_
9. What is the density of *P. lividus* in acidified water? \_\_\_\_\_

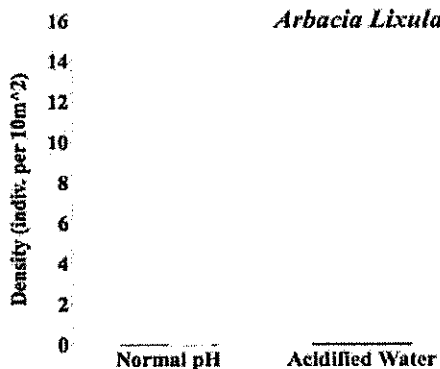
10. What is range of possible densities of *P. lividus* in acidified water? \_\_\_\_\_

### What does it mean?????

The error bars show the **possible range of the data** with 95% confidence. For example, for *A. lixula* in normal pH water the density was measured to be **12**, but because of sources of error (maybe the machine was calibrated incorrectly, the bacteria are reeeeealllly hard to see, or they used cheap beakers), the actual density could be anywhere from **9** to **15** (the range shown by the error bars). That's a pretty wide range! Similarly, for *A. lixula* in acidified water the density was measured to be **8** but because of error and uncertainty could be anywhere between **6** and **14**. Again, a very wide range!

Say that the machine was in fact calibrated incorrectly. When the scientists took their measurements, they measured the density of *A. lixula* in normal pH water as 12 (shown on the graph) but in reality, the density was 9. Now imagine that because of errors the scientists measured the density of *A. lixula* in acidified water as 8 but in reality, the density was 13. What would the graph of the **real** values look like? **Draw it below:**

A)



11. Based on this data, what would the scientists conclude about the effect of acid on the density of *A. lixula*?

12. How would this differ from the conclusion they would draw from the data in the original graph on the previous page?

### \*\*Takeaways

- When two sets of data have **overlapping** error bars, the difference in the data **is not significant** and conclusions **cannot** be drawn from that data. There is too much uncertainty!
- When two sets of data have error bars that **do not overlap**, the difference in the data **is significant** and conclusions **can** be drawn from the data.
- ALWAYS LOOK AT ERROR BARS IN GRAPHS
- Looking at the scientists' data in Graph A, are you confident in the scientists' results? Why or why not? Explicitly talk about the error bars in your answer.
- Now look at the data in Graph B, especially the error bars. How do they differ from the error bars in Graph A?
- What does this mean for any conclusions or statements that the scientists might make based on this data?

## Investigation Design – Controls, Constants, and Groups

### Background

This activity will introduce and solidify your understanding of a few concepts related to investigation design – control groups, experimental groups, and constants. Every investigation has these and it is important to be able to identify each of these when examining or designing an investigation.

### Control Groups

Control groups (or just “controls”) are those groups in an investigation that serve as comparisons for the rest of your investigation. They allow you to compare the results of your investigation to some known values to see if there is a difference. This helps you determine if the change you made (your independent variable) actually had an effect on what you are measuring (your dependent variable).

There are two types of controls: Negative and positive

#### *Negative controls*

A negative control group is the group in your investigation that **lacks the independent variable**. For example, if you are investigating the effects of cat food flavoring on how much a cat eats at a meal, the negative control could be cat food with **no** flavoring. If you are investigating the effects of differing types of fertilizer on tomato plant growth, a good negative control group would be a few plants that are given **no** fertilizer. Now you can compare what happens when you give different fertilizers with the plants that were given no fertilizer.

1. Identify a possible negative control for the following investigations:
  - a. A teacher finds a new activity and is trying to see if it helps his students understand a concept better and do better on a test.
  - b. A researcher hypothesizes that eating certain foods that are high in Vitamin C interferes with a human's ability to absorb calcium from other foods.
  - c. A researcher is comparing the effectiveness of two different medications in treating flu symptoms.

Positive controls are a bit trickier and likely a concept you have not experienced before. *A positive control group is the group in an investigation that already has a **known result**.* It has two purposes: 1) as a comparison for the other groups (like a negative control), and 2) to make sure that your experimental design does not have any flaws. For example, let's go back to the tomato plant investigation. A positive control for that experiment might be a group that is given **250 mL** of water each day because you know from some previous investigations that if you give a tomato plant 250 mL of water each day, after 30 days it will be about 20 cm tall. If you do an experiment and find that a tomato plant given 250 mL of water each day doesn't grow at all, it is an indication that some other part of your investigative procedure might be off (maybe bad soil or old seeds in this case).

Let's say you are working in a lab and developing a new chemical fertilizer that is supposed to help plants grow. You do an investigation. Your negative control is plants with no fertilizer, and you have a second group of plants that are grown with the new fertilizer. How do you know if the new fertilizer is better than the fertilizers already available? Sure, it might be better than no fertilizer, but if it's not better than what is already out there nobody will buy it! A **positive control**, like a group with an available fertilizer that you know increases growth by 50%, would be very useful. If your new fertilizer increases growth by 25%, it would not be worth developing and trying to sell. If your fertilizer increases growth by 75%, then that is an improvement! Without the positive control you would never know if your fertilizer is better than what is already available.

Positive controls are not **always** necessary in an experiment but they are sometimes helpful.

2. In the following investigations, identify the positive control:

- a. Researchers are testing the effectiveness of 5 new antibiotics to see if any are better at preventing bacterial growth than amoxicillin, a common antibiotic already in use.
  
  
  
  
  
  
  
  
  
  
- b. Researchers are testing new materials for headphones to see if they block outside sounds better than existing materials.

**Experimental Groups**

Related to negative and positive controls, experimental groups are any group in an experiment that is **not** either a positive or negative control. From example 2a above, the experimental groups would be the 5 new antibiotics. In 2b, the experimental groups are the new materials.

3. Identify the control group and the experimental groups in the following investigation: testing the effect of different pH's (3, 5, 6, 8, 9, and 11) on the activity of an enzyme as compared to neutral pH (7).
  - a. Control group =
  - b. Experimental groups =

**Constants**

Constants are those parts of an investigation which are **kept the same** across **every group, including the control and experimental groups**. They are NOT controls. Remember that you want to keep as much the same as possible so that you can be certain that any differences in your dependent variable (measured results) are because of your independent variable and not something else. Examples of constants include:

- Using the same measuring equipment (yes, even highly accurate tools like graduated cylinders and balances can vary between individual ones).
- In an investigation testing the effectiveness of different antibiotics, keeping the volume of each antibiotic used the same and measuring their effectiveness over the same period of time (also maybe keeping temperature the same, testing on the same type of bacteria, etc.).

4. For each investigation below, identify TWO possible constants:
  - a. Testing the effectiveness of different fertilizers on plant growth.
    - 1.
    - 2.
  - b. Testing the effect of temperature on a certain chemical reaction between two substances.
    - 1.
    - 2.
  - c. Testing how quickly different types of sugar get absorbed in the body and enter the bloodstream.
    - 1.
    - 2.

# Task Seven:

Due Date-07/01

For each of the following experiments, state the following

1. A Hypothesis (if any)
2. A Null Hypothesis
3. Dependent variable (if any)
4. Independent variable (if any)
5. Relationship between the DV and IV (if any)
6. Control(s) (if any)
7. Constants (if any)
8. Conclusions

## Passage VI

Three studies compared the effects of 5 sweeteners (Sweeteners Q–U) on food consumption by rats and on the concentrations of *leptin* and *ghrelin* (hormones that regulate appetite) in the blood of rats. Sweeteners Q–U differ only in the percent by mass of fructose and of glucose (see Table 1).

Table 1		
Sweetener	Percent by mass of:	
	fructose	glucose
Q	0	100
R	42	58
S	50	50
T	55	45
U	100	0

### Study 1

Each of 5 groups (Groups 1–5) of rats was assigned a solution having a 100 g/L concentration of 1 of the 5 sweeteners. Each rat was placed in a separate cage and provided unlimited access to the assigned sweetener solution and to solid food for 56 days. Table 2 shows, for each group, the amounts of sweetener solution and solid food consumed per rat per day. On Day 56, blood was collected from each rat for analysis in Studies 2 and 3.

Table 2			
Group	Sweetener	Amount consumed per rat per day	
		sweetener solution (mL)	solid food (g)
1	Q	73	9
2	R	55	14
3	S	52	16
4	T	48	18
5	U	29	23

Table 2 adapted from Heather R. Light et al., "The Type of Caloric Sweetener Added to Water Influences Weight Gain, Fat Mass, and Reproduction in Growing Sprague-Dawley Female Rats." ©2009 by the Society for Experimental Biology and Medicine.

### Study 2

A 1 mL blood sample from each rat was placed in a separate test tube containing 0.2 mL of *Indicator N* (which reacts with leptin to form a blue dye). The concentration of blue dye in each tube was directly proportional to the leptin concentration in the blood sample. Table 3 shows the leptin concentration per sample for each group.

Table 3		
Group	Sweetener	Leptin concentration per sample (pM*)
1	Q	804
2	R	622
3	S	553
4	T	475
5	U	251
*picomolar		

### Study 3

Study 2 was repeated, except that *Indicator P* (which reacts with ghrelin to form a yellow dye) was used instead of *Indicator N*. The concentration of yellow dye in each tube was directly proportional to the ghrelin concentration in the blood sample (see Table 4).

Table 4		
Group	Sweetener	Ghrelin concentration per sample (pM)
1	Q	852
2	R	1,125
3	S	1,279
4	T	1,450
5	U	1,758

Tables 3 and 4 adapted from Andreas Lindqvist, Annemie Baetmans, and Charlotte Erlanson-Albertsson, "Effects of Sucrose, Glucose and Fructose on Peripheral and Central Appetite Signals." ©2008 by Elsevier B.V.



# PASSAGE VII

Bacteria can be categorized by how they respond, as indicated by reproduction and growth, to certain temperatures. They are grouped into four categories—psychrophiles, psychrotrophs, mesophiles, and thermophiles—based on their growth response to certain temperatures. Minimal growth temperature is the lowest point at which the bacteria will reproduce. Optimum growth point is the temperature at which the bacteria reproduce most efficiently. Maximum growth point is the very highest temperature to which the bacteria will respond, beyond which the bacteria will not reproduce at all. Table 1 lists the types of bacteria as well as the growth points for each.

Table 2 represents a list of common bacteria and their growth points.

Table 1			
Growth points or ranges (°C)			
Classifications	Minimum	Optimum	Maximum
Psychrophile	below 0	10–15	below 20
Psychrotroph	0–5	15	30
Mesophile	5–25	18–45	30–50
Thermophile	25–45	50–60	60–90

Table 2			
Cardinal growth points (°C)			
Bacteria name	Minimum	Optimum	Maximum
<i>Anoxybacillus flavithermus</i>	30	60	72
<i>Bacillus flavothermus</i>	30	60	72
<i>Clostridium perfringens</i>	15	45	50
<i>Escherichia coli</i>	10	37	45
<i>Listeria monocytogenes</i>	1	34	45
<i>Micrococcus cryophilus</i>	0	15	30
<i>Staphylococcus aureus</i>	10	37	45
<i>Streptococcus pyogenes</i>	20	37	40
<i>Streptococcus pneumoniae</i>	25	37	42

## PASSAGE II

Researchers conducted trials on a certain prescription drug delivered in immediate-release capsules and extended-release capsules.

Figure 1 shows the mean concentration (nanograms per milliliter [ng/mL]) of the two active ingredients of the prescription drug in patients' blood plasma over time (hr).

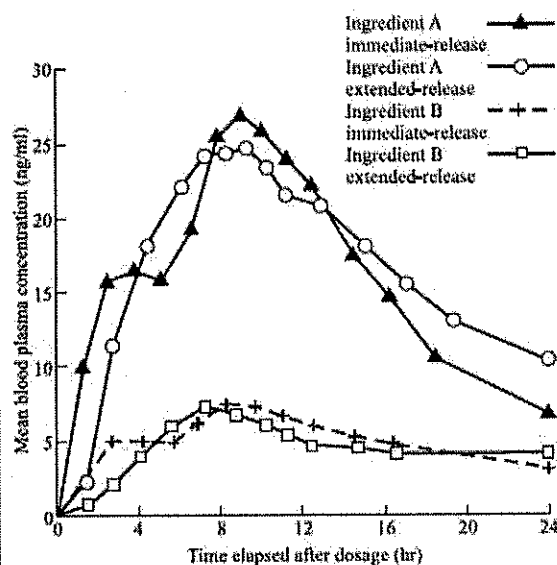


Figure 1

In clinical trials of the prescription drug, subjects given the prescription drug were interviewed at regular intervals about the symptoms the prescription drug is meant to relieve. After each interview, the subjects were assigned a symptom score. A high symptom score corresponds to high intensity of symptoms, and a low symptom score indicates low intensity of symptoms. Figure 2 shows the mean symptom score over time (hr) for subjects who took the prescription drug.

In the clinical trials, some subjects were given the prescription drug and some subjects were given a placebo (an inactive pill). Table 1 shows the percentage of subjects from both groups who reported various adverse side effects.

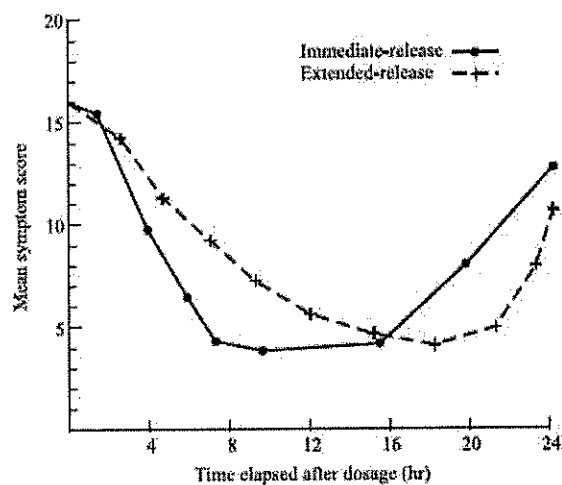


Figure 2

Table 1			
Body system	Side effect	Prescription drug group (%)	Placebo group (%)
General	Feeling of weakness	6	5
	Headache	26	14
Digestive system	Loss of appetite	32	5
	Diarrhea	8	0
	Dry mouth	31	5
	Nausea	14	0
Nervous system	Anxiety	7	4
	Dizziness	9	0
	Insomnia	25	11
	Irritability	11	4
Cardiovascular system	Rapid heart rate	10	2
Nutritional	Weight gain	15	0

## PASSAGE V

*Tenebrio molitor* is an arthropod insect which, like 90% of all insects, undergoes the process of complete metamorphosis, meaning that it passes through four life stages: egg, larva, pupa, and adult. In the larval stage the insect is commonly known as a mealworm; as a full adult it is a darkling beetle. Figure 1 shows the four stages of the *T. molitor* life cycle (x-axis), as well as data for minimum and maximum days spent in each stage of metamorphosis (y-axis) for *T. molitor* that were raised by students in a lab.

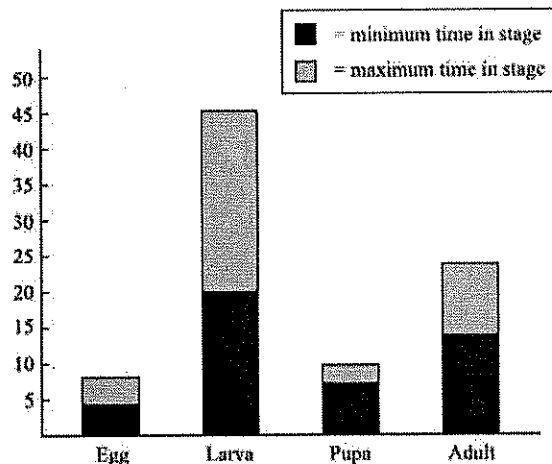


Figure 1

Table 1 includes data recorded for four different colonies of *T. molitor* raised by students in a lab, including the type of food each colony was given, beginning larval length, duration of time in larval and pupal stages, and final adult length. It was decided that the colonies would be given only one type of food source: either a fruit, a vegetable, or one of two whole grains. Apple was chosen as the fruit, carrot for the vegetable, and oats and wheat for the two whole grains.

Colony	Diet	Avg. larval size (mm)	Avg. duration in larval stage (days)	Avg. duration in pupal stage (days)	Avg. adult size (mm)
1	Apple	25.8	36.9	7.5	19.3
2	Carrot	24.5	39.4	8.4	19.5
3	Oat	24.9	49.1	9.2	20.6
4	Wheat	25.3	57.2	10.8	21.3

## PASSAGE II

Certain species of flowers attract more bees than others with the scent of their pollen. The pollen is found on a structure within the flower called the *anther*, which is located on top of another structure called the *stamen*. Flowers typically have multiple anthers and stamens.

Bees carry the pollen from the flowers on their legs. The bees move from flower to flower while collecting pollen. Some of the pollen falls from their legs as they land on another flower. This depositing of pollen causes cross-pollination to occur (fertilization of the other flowers). Three studies were conducted to study this process.

### Study 1

For two flower species (A and B), pollen quantity per anther in milligrams (mg), anther quantity per flower in number, and percentage of stamens covered with pollen were recorded (see Table 1).

Table 1			
Flower species	Pollen quantity (mg) per anther	Anther quantity per flower	Stamens covered with pollen (%)
A	4.9	12	27
B	7.6	19	27

### Study 3

The researchers hand-pollinated flowers from a third species, Species C. They also observed the Species C plants being cross-pollinated by the bees in the area. All flowers were observed for 2 years. The scientists recorded the results in Table 3.

Table 3		
Cross-pollination of Species C flowers	Results from:	
	Hand-pollinated flowers	Bee-pollinated flowers
Flowers that reproduced	31	12
Flowers reproducing after 1 year	10	34
Flowers reproducing after 2 years	8	15
Total flowers produced after 2 years	50	43

## PASSAGE V

Gregor Mendel is known for his work in genetics. He is credited with discovering how traits (characteristics) are passed from one generation to the next. After his observations of inherited traits, Mendel concluded that each organism carries two sets of information about a certain trait. If the two sets differ about the same trait, one set dominates the other. That way, information can be passed on through the generations, even if the trait is not expressed.

It has since been determined that the presence of certain traits is attributed to *genes*, and the different forms that genes can take, known as *alleles*. Dominant alleles (*D*) produce dominant characteristics; recessive alleles (*d*) produce recessive characteristics. Dominant alleles are expressed whenever present (*DD*, *Dd*) but recessive alleles are expressed only when the dominant allele is absent (*dd*).

A study was done in which the independence of two traits was tested. In this study, a rabbit with long black hair was mated with a rabbit with short white hair. The dominant trait for hair length is short (*H*). The dominant trait for hair color is black (*B*). If the two initial rabbits (level 1 in the figure below) are *homozygous* for their traits, meaning that the two alleles for each trait are the same, breeding them will result in offspring that have both a dominant and recessive allele for each trait. Such a pairing of alleles is known as *heterozygous*. If, as in level 2 of the figure, two heterozygous rabbits are bred, the chart (level 3) contains all the possibilities for their offspring.

### Study 2

Three study sites were established to determine the pollen collection rate of one species of bee for the flowers used in Study 1. In Site 1, Species A flowers were absent. In Site 2, Species B flowers were absent. In Site 3, both Species A and B flowers were absent.

Two pollen containers were placed at each site: one containing 50 mg Species A pollen and one containing 50 mg Species B pollen. The containers were left in place for 36 hours and the amount of pollen that was taken from the containers was measured. The results are recorded in Table 2.

Table 2			
Site	Flower species absent	Amount of Pollen (mg) removed from dishes containing pollen from:	
		Species A	Species B
1	A	26	13
2	B	12	35
3	A and B	2	4

#### Dominant Traits

- Black color (BB)
- Short hair (HH)

#### Recessive Traits





- White color (bb)
- Long hair (hh)

Level 1: Long hair (hh) × Short hair (HH)  
Black (BB) White (bb)

Level 2: Short hair (Hh) Short hair (Hh)  
Black (Bb) Black (Bb)

Level 3: Hh Bb × Hh Bb

	HB	Hb	hB	hb
HB	HHBB	HHBb	HhBB	HhBb
Hb	HHBb	HHbb	HhBb	Hhbb
hB	HhBB	HhBb	hhBB	hhBb
hb	HhBb	Hhbb	hhBb	hhbb

Length	Color
	= short black
	= short white
	= long black
	= long white

## PASSAGE V

Aphids are small plant-eating insects known to feed on rosebushes. In the cultivation of roses, certain pesticides are often applied when the presence of aphids is detected. However, sometimes the flowers that are treated with the pesticides are not as vibrant or fragrant as those that did not receive the pesticide treatment. Two experiments were conducted to study the effects of certain pesticides on rosebushes.

### Experiment 1

A gardener filled 125 pots with Soil Type 1. No pesticide was added to the soil in 25 pots. The other pots were divided into four groups of 25 and the soils in each group were treated with 5, 15, 25, or 35 parts per million (ppm) of either Pesticide A or Pesticide B. All other factors were held constant. Fully grown rosebushes with buds but no flowers were planted after the pesticide was placed in the soil. After 30 days the rosebushes were uprooted, sun-dried, and the total number of petals produced by the bushes was counted. The results are shown in Table 1.

Table 1		
Pesticide dose (ppm)	Number of petals	
	Pesticide A	Pesticide B
5	12	15
15	2	7
25	9	14
35	5	7
None	14	14

### Experiment 2

Experiment 1 was repeated with 100 pots of Soil Type 1 and 100 pots of Soil Type 2. The same pesticide doses and type and number of rosebushes were used. All other factors were held constant. After 30 days the rosebushes were uprooted and weighed. The results are shown in Table 2.

Information on the composition of the two soil types used is given in Table 3.

Table 3			
Solid type	pH level	Organic matter (%)	Clay (%)
1	4.1	3.0	12.5
2	3.9	6.5	6.3

Table 2				
Pesticide dose (ppm)	Average weight of rosebush (oz)			
	Soil type 1		Soil type 2	
	Pesticide A	Pesticide B	Pesticide A	Pesticide B
5	47.5	51.4	52.7	61.2
15	37.1	42.3	40.3	51.7
25	27.5	32.9	31.1	40.3
35	19.7	22.1	23.6	29.7
Note: Average plant weight with untreated Soil Type 1 was 42.1 oz; average plant weight with untreated Soil Type 2 was 24.7 oz.				

# PASSAGE VII

In nature, different types of organisms often form *symbiotic* (mutually beneficial) relationships with each other. One such example of this is between certain types of fungi and plants; this relationship is known as a *mycorrhiza*. The association provides the fungus with food through access to sugars from photosynthesis in the plant. In return, the plant gains the use of the fungi's surface area to absorb mineral nutrients from the soil. It is believed that without the assistance of fungi, these plants would not be able to absorb crucial nutrients, including phosphates, from the soil. Two experiments were performed to study the effect that the plant-fungi relationship has on plant growth.

## Experiment 1

For 6 weeks, several specimens of three different types of plants, selected from among four different types of plants, were grown in a greenhouse. The average growth of each type of plant was recorded every two weeks. The soil used for the plants was treated to remove any trace of fungi to establish expected growth without the plant-fungi association. The results are shown in Table 1.

Table 1			
Plant type	Average plant growth (in)		
	Week 2	Week 4	Week 6
1	1.2	2.8	3.7
3	0.6	1.7	2.0
4	0.9	2.6	3.5

## Experiment 2

In this experiment, several specimens of four different types of plants were grown in a greenhouse for six weeks, and the average growth of each type of plant was recorded every two weeks. This time, however, untreated soil that contained fungi was used. The results are shown in Table 2.

Table 2			
Plant type	Average plant growth (in)		
	Week 2	Week 4	Week 6
1	2.6	3.8	5.1
2	2.9	4.1	5.9
3	1.9	3.3	5.4
4	1.7	3.4	4.9

Information on the plant types used is given in Table 3.

Table 3			
Plant type	Root structure	Native climate type	Leaf type
1	Diffuse	Prairie	Grass-like
2	Taproot	Northern forest	Evergreen needle
3	Taproot	Prairie	Broad
4	Diffuse	Tropical forest	Broad

36. The results of Experiment 1 indicate that during what time frame did all of the plant types studied experience the greatest increase in growth rate?
  - F. 0–2 weeks
  - G. 2–4 weeks
  - H. 4–6 weeks
  - J. Cannot be determined from the given information.
37. A plant from which climate type was NOT studied in Experiment 1?
  - A. Prairie
  - B. Tropical forest
  - C. Northern forest
  - D. All climate types were studied in Experiment 1.
38. Based on the results of Experiment 1, which plant type experienced the most total growth between Week 2 and Week 6?
  - F. Plant Type 1
  - G. Plant Type 3
  - H. Plant Type 4
  - J. Each plant type experienced the same total growth.

## PASSAGE IV

Students wanted to test the effects of nutrition on the growth of guinea pigs. Two experiments were conducted using different feeds and vitamin supplements. For both experiments, four groups of 10 guinea pigs each were given a different type of feed over an 8-week period. Each group received the same quantity of food and was provided with fresh water daily. The guinea pigs were measured and weighed weekly. The guinea pigs in each group had an average starting weight of 50 grams (g) and an average starting length of 20 centimeters (cm).

### Experiment 1

Group 1 was fed a high-protein feed (Feed P).

Group 2 was fed a grain-based feed with vitamin supplements (Feed Q).

Group 3 (control group) was fed a grain-based feed without supplements (Feed R).

Group 4 was fed a grain-based feed without supplements plus fruits and vegetables (Feed S).

The results and average measurements are recorded in Table 1 below.

Table 1		
Group	Average weight after 8 weeks (g)	Average length after 8 weeks (cm)
1	93	32.50
2	82	29.00
3	74	25.25
4	76	23.00

### Experiment 2

Group 5 was fed a high-protein feed plus fruits and vegetables (Feed V).

Group 6 was fed a grain-based feed with vitamin supplements plus fruits and vegetables (Feed W).

Group 7 (control group) was fed a grain-based feed without supplements (Feed X).

Group 8 was fed a grain-based feed without supplements plus fruits only (Feed Y).

The results and average measurements are recorded in Table 2 below.

Table 2		
Group	Average weight after 8 weeks (g)	Average length after 8 weeks (cm)
5	98	38.25
6	85	30.50
7	75	25.00
8	74	23.25



29. Based on the results of the study, what is the order of the suspected mutagens, from the substance with the *least* potential to be mutagenic to the substance with the *most* potential to be mutagenic?
- P, M, N, L
  - P, L, M, N
  - N, L, P, M
  - N, M, L, P

30. In the study, the scientists tested the effect of Substance P at a concentration of  $5 \times 10^{-9}$  g/mL. After the study, the scientists repeated their test of the effect of Substance P, but at 3 other concentrations. The 3 concentrations and their corresponding results are shown in the table below.

Concentration of Substance P	Number of colonies
$10 \times 10^{-9}$ g/mL	14
$50 \times 10^{-9}$ g/mL	54
$100 \times 10^{-9}$ g/mL	114

What is the relationship, if any, between the concentration of Substance P and its potential to cause mutations?

- As the concentration of Substance P increases, its potential to cause mutations increases only.
- As the concentration of Substance P increases, its potential to cause mutations decreases only.
- As the concentration of Substance P increases, its potential to cause mutations first decreases and then increases.
- There is no relationship between the concentration of Substance P and its potential to cause mutations.

31. Before bacteria were added to it, the dish that was intended to serve as the control dish in the study lacked which of the substances listed below?

- Histidine
- Nutrient agar
- Suspected mutagen

- II only
- III only
- I and II only
- I and III only

32. Which of the following statements about the numbers of bacteria that regained the ability to synthesize histidine is consistent with the results of the study for Dishes 2 and 3? The number of bacteria that became His<sup>+</sup> revertants after exposure to:

- Substance M was about 2 times the number of bacteria that became His<sup>+</sup> revertants after exposure to Substance L.
- Substance L was about 2 times the number of bacteria that became His<sup>+</sup> revertants after exposure to Substance M.
- Substance M was about 4 times the number of bacteria that became His<sup>+</sup> revertants after exposure to Substance L.
- Substance L was about 4 times the number of bacteria that became His<sup>+</sup> revertants after exposure to Substance M.

33. The particular strain of *S. typhimurium* chosen for the study lacks normal DNA repair mechanisms. Which of the following statements gives the most likely reason this particular strain was chosen? The scientists:

- did not want the bacteria in the study to synthesize any DNA.
- did not want the bacteria in the study to synthesize any proteins.
- wanted the bacteria in the study to be able to repair the mutations caused by the substances.
- wanted the bacteria in the study to be unable to repair the mutations caused by the substances.

# Task Eight:

Due Date-07/05

Article Reading

# Frequency-dependent Batesian mimicry

Predators avoid look-alikes of venomous snakes only when the real thing is around.

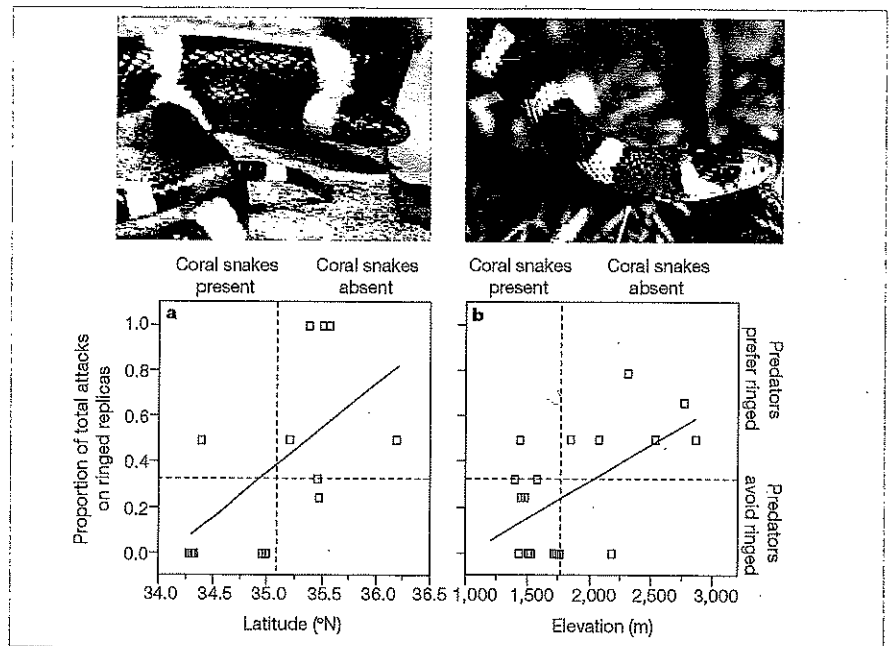
Batesian mimicry holds that palatable species look like dangerous species because both are then protected from predation<sup>1-5</sup>. But this protection should break down where the dangerous model is absent, when predators would not be under selection to recognize the model or any other species resembling it as dangerous<sup>2,4,5</sup>. Here we provide experimental evidence to support this critical prediction of Batesian mimicry by demonstrating that predators avoid harmless look-alikes of venomous coral snakes only in areas that are inhabited by these deadly snakes.

Many coral snakes and non-venomous kingsnakes possess red, yellow (or white), and black ringed markings<sup>6</sup>, which predators avoid<sup>7</sup>, though often without prior experience<sup>8</sup>. To determine whether this avoidance depends on the model's presence in the vicinity, we constructed snake replicas<sup>7</sup> (1.5 cm × 18 cm cylinders of pre-coloured, non-toxic plasticine threaded onto an S-shaped wire) with a tricolour ringed pattern, a striped pattern with identical colours and proportions as the ringed replicas, or a plain brown pattern.

Ringed replicas conformed to the local mimic: scarlet kingsnakes (*Lampropeltis triangulum elapsoides*), which resemble eastern coral snakes (*Micrurus fulvius*)<sup>9</sup>, or sonoran mountain kingsnakes (*L. pyromelana*), which resemble western coral snakes (*Micruroides euryxanthus*)<sup>10</sup>; striped and brown replicas served as controls. We arranged three different replicas (triplets) 2 m apart in natural habitat (each was used once only). At each site, 10 triplets were placed 75 m apart in a line. After collection, a person without knowledge of the replica's location scored attacks by noting any impressions corresponding to a predator<sup>7</sup>.

We tested whether predators avoid *L. t. elapsoides* only in areas inhabited by *Micrurus* by placing 10 triplets at eight sympatric sites (sites with mimic and model) and eight allopatric sites (sites with only the mimic) in North and South Carolina, USA (480 replicas; allopatric sites were more than 80 km outside *Micrurus*'s range<sup>9,11</sup>; sites were 16–420 km apart). After 4 weeks, 25 (5.2%) replicas had been attacked by carnivores. The mean ( $\pm$  s.e.m.) proportion of ringed replicas attacked was significantly greater in allopatry ( $0.654 \pm 0.107$ ) than in sympatry ( $0.083 \pm 0.116$ ;  $P = 0.009$ , 2-tailed Wilcoxon two-group test).

We next investigated whether predators avoid *L. pyromelana* only in sympatry with *Micruroides* by placing 10 triplets at 24 sites (720 replicas) along an elevational gradient



**Figure 1** Frequency-dependent mimicry. The proportion of carnivore attacks on ringed replicas of scarlet kingsnakes (top left; a mimic of eastern coral snakes) and sonoran mountain kingsnakes (top right; a mimic of western coral snakes) increased with **a**, latitude ( $y = -13.314 + 0.391x$ ,  $P < 0.035$ ,  $R^2 = 0.345$ ) and **b**, elevation ( $y = -0.329 + 0.00032x$ ,  $P < 0.014$ ,  $R^2 = 0.310$ ). Horizontal dashed line: proportion of attacks on ringed replicas expected under randomness. Vertical dashed line: maximum latitude and elevation for coral snakes in North Carolina and Arizona, respectively.

(1,204–2,866 m) near Portal, Arizona (*Micruroides* only occur at altitudes below 1,770 m (ref. 10); there were 14 sympatric and 10 allopatric sites 3–100 km apart). After 2 weeks, 49 (6.8%) replicas had been attacked by carnivores.

The mean proportion of ringed replicas attacked was significantly greater in allopatry ( $0.496 \pm 0.078$ ) than in sympatry ( $0.138 \pm 0.060$ ;  $P = 0.006$ ). Moreover, in sympatry, the proportion of ringed replicas attacked (0.138) was significantly less than randomness (0.33;  $P = 0.010$ , 2-tailed Wilcoxon signed-rank test). By contrast, attacks were random in allopatry ( $P = 0.188$ ). Thus, predators avoid coral snake mimics only in sympatry with the model.

Coral snakes become increasingly rare with increasing latitude (Spearman  $\rho = -0.57$ ,  $P = 0.014$ )<sup>11</sup> and elevation ( $\rho = -0.77$ ,  $P = 0.026$ ; our unpublished results). Consequently, selection to avoid ringed patterns should weaken with increasing latitude and elevation. As expected, the proportion of ringed replicas attacked increased gradually with latitude and elevation (Fig. 1), suggesting that selection to avoid ringed patterns is indeed sensitive to the abundance of coral snakes.

Our results do not fully resolve why mimetic patterns occur where models are absent<sup>6,9-11</sup>. Possibly selection for mimicry

in sympatry, coupled with gene flow between sympatric and allopatric populations<sup>12</sup>, maintains mimetic patterns in both regions. Nevertheless, our results verify the critical prediction of Batesian mimicry and demonstrate that the benefits of mimicry depend on abundance of the model.

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## Inquiry Figure 2.2: *What Creates 'Devil's Gardens' in the Rain Forest?*

### Introduction—The Article and Phenomenon Under Study

Tropical rain forests have many species of trees, with such a great diversity that it is rare to have several individuals of the same species next to each other. A notable exception is in the Amazonian 'devil's gardens,' which consist of large stands of a single species of tree, *Duroia hirsuta*. What creates these 'devil's gardens'? Inquiry Figure 2.2 in Campbell/Reece *Biology*, Eighth Edition, presents experimental data testing possible chemical mechanisms from the following paper:

Megan E. Frederickson, Michael J. Greene, and Deborah M. Gordon, 'Devil's gardens' bedevilled by ants, *Nature* 437:495–496 (22 September 2005).

### Guiding Questions for Reading This Article

#### A. About the Article

1. Give the name of the journal and the year in which this article was published.

2. What are the last names of the three authors? What are their universities?

3. Specialized vocabulary: Write a brief definition of each term.

*Defined in the article:*

allelopathy

domatia

*Not defined in the article:*

formic acid

herbicide

host plant

mutualism

necrosis

niche

## 8 Inquiry in Action: Interpreting Scientific Papers

4. Write out the full genus and species name of the ant in this study. Why is this scientific name written in italics in the article?
5. What is the abbreviated genus and species name for the tree in this study?

### B. About the Study

6. Why are these regions of the Amazonian forest called 'devil's gardens'?
7. What are two alternate hypotheses to explain why these single-species patches of trees occur in an otherwise species-rich tropical forest?
8. How were saplings of the tropical cedar species *C. odorata* used in this experimental manipulation?
9. In what two ways did investigators measure damage to the *C. odorata* cedar saplings?
10. One testable hypothesis is that the formation of 'devil's gardens' involves ants, independent of allelopathy. The null hypothesis states that ants have no effect. Consider hypothesis 1 here, and fill in this chart with your predictions about leaf damage to cedar saplings, yes or no.

Hypothesis 1: *M. schumanni* ant defense keeps other plants out of the garden by increasing leaf damage to cedar saplings.  
Null hypothesis: There is no relationship between presence of ants and amount of leaf damage to cedar saplings.

Predictions Under Hypothesis 1	Will leaf damage occur if ants are excluded?	Will leaf loss occur if ants are not excluded?
Cedar saplings planted inside the garden (ants present)	A	B
Cedar saplings planted outside the garden (ants not present)	C	D

11. An alternate testable hypothesis is that *Duroia* allelopathy accounts for the formation of ‘devil’s gardens,’ independent of ant defense. Consider hypothesis 2 here, and fill in the chart with your predictions about leaf damage to cedar saplings, yes or no.

Hypothesis 2: *D. hirsuta* tree allelopathy keeps other plants out of the garden by increasing leaf damage to cedar saplings. Null hypothesis: There is no relationship between presence of *D. hirsuta* trees and amount of leaf damage to cedar saplings.

Predictions Under Hypothesis 2	Will leaf loss occur if ants are excluded?	Will leaf loss occur if ants are not excluded?
Cedar saplings planted inside the garden (ants present)	A	B
Cedar saplings planted outside the garden (ants not present)	C	D

12. The four cells (A–D) of the table represent four treatments of the tropical cedar saplings. Why did the investigators use *more than one* sapling in each of the four treatment groups?
13. Look at the results of Figure 2 in the article (p. 495). Which of the four treatment groups of tropical cedar *C. odorata* (nonhost plant) suffered the greatest leaf necrosis after one day? Which treatment group had the greatest percentage of leaf shedding after one week?
14. Compare these results to your predictions in question 10. Do these results agree with your predictions under hypothesis 1? Do these results allow you to reject that null hypothesis?
15. Compare these results to your predictions in question 11. Do these results agree with your predictions under hypothesis 2? Do these results allow you to reject that null hypothesis?
16. Although the experimental results in Figure 2 allow you to reject hypothesis 2, they do not “prove” hypothesis 1 and do not explain how the ants cause leaf loss. How did the authors test whether the leaf damage was caused by formic acid?

**10 Inquiry in Action: Interpreting Scientific Papers**

17. Is this an *observational study*, in which quantitative, observational data are taken but no experimental manipulation made? Or, is this an *experimental study*, in which researchers make manipulations by which the effects of different variables are tested, one at a time?
18. Is this a *field study*, with data collected on organisms in their natural habitat, or is this a *lab study*, in which plants are studied under controlled conditions in the laboratory or greenhouse?

**C. General Conclusions and Extensions of the Work**

19. Explain how the behavior of *M. schumanni* ants lead to the formation of 'devil's gardens.'
20. Imagine that you were a member of this research team and involved in these experiments. What could be a possible follow-up test that extends this work? Briefly state another experiment or measurement you would do within the search system.

## BRIEF COMMUNICATIONS

## 'Devil's gardens' bedevilled by ants

An ant species uses herbicidal weaponry to secure its own niche in the Amazonian rainforest.

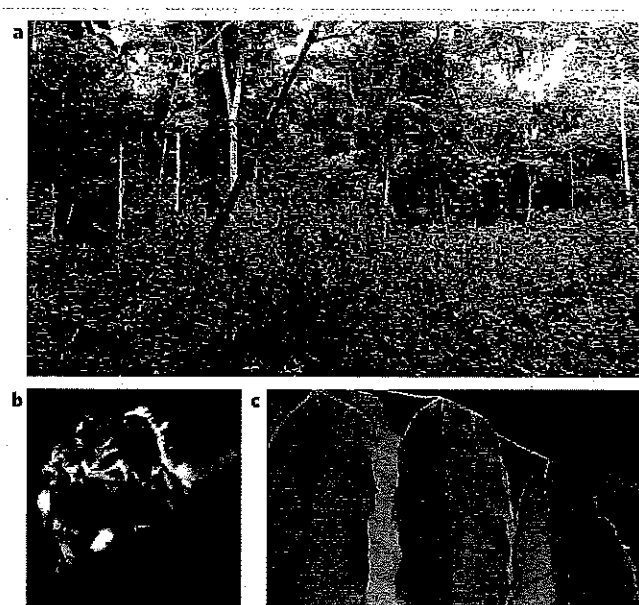
'Devil's gardens' are large stands of trees in the Amazonian rainforest that consist almost entirely of a single species, *Duroia hirsuta*<sup>1–5</sup>, and, according to local legend, are cultivated by an evil forest spirit. Here we show that the ant *Myrmelachista schumanni*, which nests in *D. hirsuta* stems, creates devil's gardens by poisoning all plants except its host plants with formic acid. By killing these other plants, *M. schumanni* provides its colonies with abundant nest sites — a long-lasting benefit as colonies can live for 800 years.

*M. schumanni* lives in the hollow, swollen stems (domatia) of *D. hirsuta*, the tree species that dominates devil's gardens (Fig. 1a). Previous studies of the mutualism between *D. hirsuta* and *M. schumanni* indicated that devil's gardens result from allelopathy, which is the local inhibition of plant growth by another plant, by *D. hirsuta*<sup>2–5</sup>. However, studies of a different ant–plant mutualism — between an unidentified species of *Myrmelachista* and the ant-plants *Tococa guianensis* and *Clidemia heterophylla* — indicated that *Myrmelachista* may create stands comprising only its host plants by using herbicide<sup>6,7</sup>.

We did an ant-exclusion experiment to determine whether the selective killing of plants inside devil's gardens is due to the activity of *M. schumanni* workers or to allelopathy by *D. hirsuta*. We planted saplings of a common Amazonian tree, the cedar *Cedrela odorata*, inside and outside devil's gardens, and either excluded or did not exclude ants from the saplings (for methods, see supplementary information).

We found that the *M. schumanni* workers

**Figure 1 | The ant *M. schumanni* creates devil's gardens by killing all plants other than its host tree, *D. hirsuta*.** **a**, A devil's garden, or mono-specific stand of *D. hirsuta*, in the foreground contrasts with the species-rich rainforest in the background. **b**, A worker *M. schumanni* ant attacking a plant: the ant bites a small hole in the leaf tissue, inserts the tip of its abdomen into the hole and releases formic acid. **c**, Leaves develop necrosis along primary veins within hours of the attack.



M. DOHRN © BBC

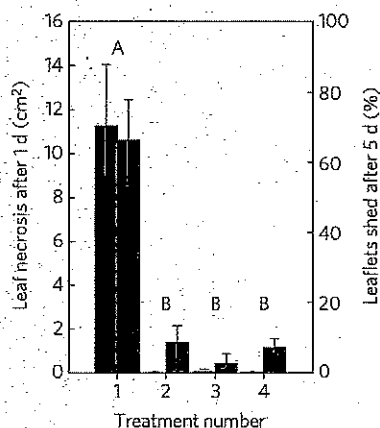
promptly attacked the saplings in devil's gardens from which ants had not been excluded, injecting a poison into their leaves (Fig. 1b), which developed necrosis within 24 hours (Fig. 1c). Most of the leaflets on these saplings were lost within five days, and the proportion lost was significantly higher than on saplings from which ants were excluded (Fig. 2). We also found that ant-free *C. odorata* inside devil's gardens fared as well as *C. odorata* planted outside devil's gardens. These results show that devil's gardens are produced by *M. schumanni* workers, rather than by *D. hirsuta* allelopathy.

In a second experiment, we investigated

whether *M. schumanni* attacks only plants that are not its host plants and whether the ant uses domatia to recognize its host. We planted *C. odorata* saplings with and without artificial domatia and *D. hirsuta* saplings with and without domatia in devil's gardens. After 24 h, there was significant leaf necrosis on all *C. odorata* plants (mean area on plants with artificial domatia: 39.7 cm<sup>2</sup>; s.e., 26.4–55.6 cm<sup>2</sup>; on plants without artificial domatia: 14.2 cm<sup>2</sup>; s.e., 9.2–20.3 cm<sup>2</sup>), whereas there was no leaf necrosis at all on any *D. hirsuta* plants, irrespective of the presence of domatia (analysis of variance,  $F_{3,20} = 57.03$ ,  $P < 0.0001$ ). We conclude that *M. schumanni* attacks only non-host plants such as *C. odorata* and that it does not rely on the presence of domatia to discriminate between its hosts and other plant species.

Chemical analysis revealed that the poison glands of *M. schumanni* contain formic acid ( $0.43 \pm 0.12$   $\mu$ l per worker); no other compounds were detected. Treatment of leaves with formic acid induced leaf necrosis on all the plants we tested. (For details, see supplementary information.) Many formicine ants produce formic acid: to our knowledge, this is the first record of an ant using formic acid as a herbicide — although it is known to have bactericidal and fungicidal properties<sup>8</sup>.

Devil's gardens covered 4.5% of our study plot and grew by  $0.7 \pm 0.3\%$  per year. Using this growth rate, we estimate that the largest



**Figure 2 | *M. schumanni* ants, and not allelopathy, create devil's gardens.** Saplings of the non-host plant *C. odorata* were subjected to different treatments: 1, planted inside a devil's garden, ants not excluded; 2, planted inside a devil's garden, ants excluded; 3, planted outside devil's gardens, ants not excluded; and 4, planted outside devil's gardens, ants excluded. Only saplings exposed to ants inside devil's gardens developed significant necrosis within one day (average  $\pm$  s.e.; blue bars) and shed a significant percentage of their leaflets within five days (average  $\pm$  s.e.; red bars). Multivariate analysis of variance results: Pillai trace, 0.88,  $F_{6,72} = 9.41$ ,  $P < 0.0001$ . Analysis of variance (ANOVA) results (necrosis):  $F_{3,36} = 52.78$ ,  $P < 0.0001$ . ANOVA results (leaflets shed):  $F_{3,36} = 17.19$ ,  $P < 0.0001$ . Bars marked A are significantly different ( $P < 0.001$ ) by Tukey post-hoc tests from bars marked B.



## BRIEF COMMUNICATIONS

NATURE | Vol 437 | 22 September 2007

devil's garden in our plot, with 351 plants, is 807 years old (95% confidence interval, 446–4,234 years old). A devil's garden is tended by a single *M. schumanni* colony (our unpublished results) comprising as many as 3 million workers and 15,000 queens; the presence of many queens undoubtedly contributes to colony longevity.

The cultivation of devil's gardens by *M. schumanni* is an example of niche construction<sup>9</sup>. By killing plants of other species, the ant promotes the growth and establishment of *D. hirsuta*, thereby gaining more nest sites. A devil's garden begins when an *M. schumanni* queen colonizes a single *D. hirsuta* tree: over time, *D. hirsuta* saplings become estab-

lished within the vegetation-free area created by the ants, and the ant colony expands to occupy them. The devilry of *M. schumanni* today provides homes for ants in the future.

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Supplementary information accompanies this communication on Nature's website.  
Competing financial interests: declared none.  
doi:10.1038/437495a

## Inquiry Figure 9.15: *Is the Rotation of the Internal Rod in ATP Synthase Responsible for ATP Synthesis?*

### Introduction—The Article and Phenomenon Under Study

ATP is made from ADP and inorganic phosphate using the energy from proton flow through the membrane protein complex known as ATP synthase. A particular subunit of the protein complex acts as a rotary motor in the production of ATP. Inquiry Figure 9.15 in Campbell/Reece *Biology*, Eighth Edition, shows how investigators provided direct evidence for this mechanism of ATP synthesis by means of rotation in the following article:

Hiroyasu Itoh, Akira Takahashi, Kengo Adachi, Hiroyuki Noji, Ryohei Yasuda, Masasuke Yoshida, and Kazuhiko Kinosita Jr., Mechanically driven ATP synthesis by F1-ATPase, *Nature* 427:465–468 (29 January 2004).

### Guiding Questions for Reading This Article

#### A. About the Article

1. What is the name of the first author of this research team?
2. Most of the authors of this paper work in major research laboratories in what nation? What is the name of the author affiliated with the Cold Spring Harbor Laboratory in the United States?
3. What is the name of the journal in which this article was published? This paper was published in what month and year?

14 Inquiry in Action: Interpreting Scientific Papers

4. This research was supported in part by grants from what organization? (See Acknowledgements, p. 468.)
5. The article's abstract (p. 465, first paragraph, in bold print) summarizes the major contributions of the study. Write one sentence from the abstract that states the basic concept for which the study provides direct evidence.
6. Specialized vocabulary: Write a brief definition of each term.
  - chemiluminescence
  - hydrolysis
  - luciferase
  - photon

**B. About the Study**

7. Was this an *in vivo study* (in intact cells, in living organisms) or an *in vitro study* (literally, "in glass," meaning in tubes or in laboratory containers)?
8. The action of isolated F1 particles of ATP synthase appeared to be reversible. (a) The central subunit rotates *anticlockwise* (that is, *counterclockwise*) when what happens—A or B? (A) ATP is synthesized; (B) ATP is hydrolyzed (broken down to ADP and inorganic P). (b) The central subunit rotates *clockwise* when what happens—A or B? (A) ATP is synthesized; (B) ATP is hydrolyzed (broken down to ADP and inorganic P).
9. The researchers manipulated a single subunit of the ATP synthase complex, the  $\gamma$  (gamma) subunit. Prior to this study, what aspect of the  $\gamma$  subunit in F1 reversal had not yet been tested?

10. One end of the F1 test complex was attached to a glass slide by modifying two cysteine amino acids of one end of the complex. The other end of the F1 test complex, the  $\gamma$  end, was attached to a magnetic bead using a specific attachment protein (streptavidin). Examine the experimental setup in Figure 1 (p. 466). Although the drawing in Figure 1a is not to scale, which is the larger element, the F1 protein complex or the magnetic bead? How did the investigators manipulate this system to cause the  $\gamma$  end of the complex to rotate?
11. The investigators noted that during ATP hydrolysis, the complex rotated in one direction. What reaction did they hypothesize would occur if the complex was rotated in the opposite direction?
12. The investigators used photons of light produced in a chemiluminescent luciferin-luciferase system as an indicator of ATP synthesis. When ATP is present, this enzyme system will hydrolyze ATP to produce ADP and inorganic phosphate and release photons of light. (Thus, it serves as a “reporter enzyme,” as mentioned in Inquiry Figure 9.15 of Campbell/Reece *Biology*, Eighth Edition.) What does the y-axis on the left side of Figures 3a and 3b represent? This is an indirect measure of the rate of which rotation-driven process—ATP synthesis or ATP hydrolysis?
13. Figure 3a shows the number of photons detected with respect to rotation of the  $\gamma$ -subunit of the ATP synthase. N, S, and H refer to what three rotation states? In Figure 3a, the relative number of photons detected is highest at which type of rotation—N, S, or H?
14. In the control experiments graphed in Figure 3b, the broken lines show the lowest number of photons detected. What was different about those experiments to produce such low numbers? Why did this omission serve as a control for the experiment?

# TASK Nine:

Due Date-07/15

## Second Task- due date 06/04/18

Read chapter 1 and 2 of Campbell and Reece's *Biology 9<sup>th</sup> edition, AP edition textbook*. And pay attention to the objectives included in the packet. A copy of the textbook can be found on my webpage and the link can be accessed through the school web page. Along with the assigned reading, you will be required to complete the guided reading and activities before August 1, 2016. You will take an assessment on this chapter and the entire packet the first Friday of the school year.

### CHAPTER 1-INTRODUCTION: THEMES IN THE STUDY OF LIFE

After reading this chapter,

1. Briefly describe, in your own words, unifying themes that pervade the science of biology, and suggest why they are considered unifying themes.
2. Explain how the properties of life emerge from complex organization.
3. Describe five emergent properties associated with life, and suggest why they are essential.
4. Distinguish between holism and reductionism, using analogies.
5. Explain how technological breakthroughs contributed to the formulation of the cell theory and our current knowledge of the cell.
6. Using a Venn diagram, distinguish between prokaryotic and eukaryotic cells.
7. Explain, in your own words, what is meant by "form fits function." Describe five organs or cell that can be used to explain this.
8. List the five kingdoms of life and use a Venn diagram to compare and contrast them.
9. Distinguish between inductive and deductive reasoning using nonscientific and scientific examples.
10. Explain how science and technology are interdependent using several appropriate examples.

### CHAPTER 2 THE CHEMICAL CONTEXT OF LIFE

After reading this chapter,

1. State four elements essential to life that make up 96% of living matter, and propose why they are essential.
2. Describe the structure of an atom and the importance the structure plays in its properties and function.
3. Explain how electron configuration influences the chemical behavior of an atom.
4. Define electronegativity and explain how it influences the formation of chemical bonds.
5. Distinguish among nonpolar covalent, polar covalent and ionic bonds using an analogy.
6. Describe the formation of a hydrogen bond and explain how it differs from a covalent or ionic bond.
7. Explain why weak bonds are important to living organisms and give an example of how it plays a role in life.
8. Describe how the relative concentrations of reactants and products affect a chemical reaction.

# TASK Ten:

Due Date-07/20

## Integrity Policy

**"Plagiarism.** Failure to acknowledge ideas, phrases, data, images, or other intellectual property gained from a preexisting body of work. This includes self-plagiarism, or the submission of one piece of work in more than one course without the explicit permission of the instructors involved."<sup>1</sup>

**"Cooperative or collaborative effort in coursework without acknowledgment and explicit permission of the instructor.** Assume that acknowledgement is necessary any time you collaborate and/or cooperate, unless you are expressly informed that it is not."<sup>2</sup>

**"Cheating.** The submission of work as one's own that has been prepared by another person."<sup>3</sup>

1-3-<https://www.hamilton.edu/student-handbook/studentconduct/honor-code>

"I affirm that I have not given or received any unauthorized help on this assignment, and that this work is my own."

Signature \_\_\_\_\_