

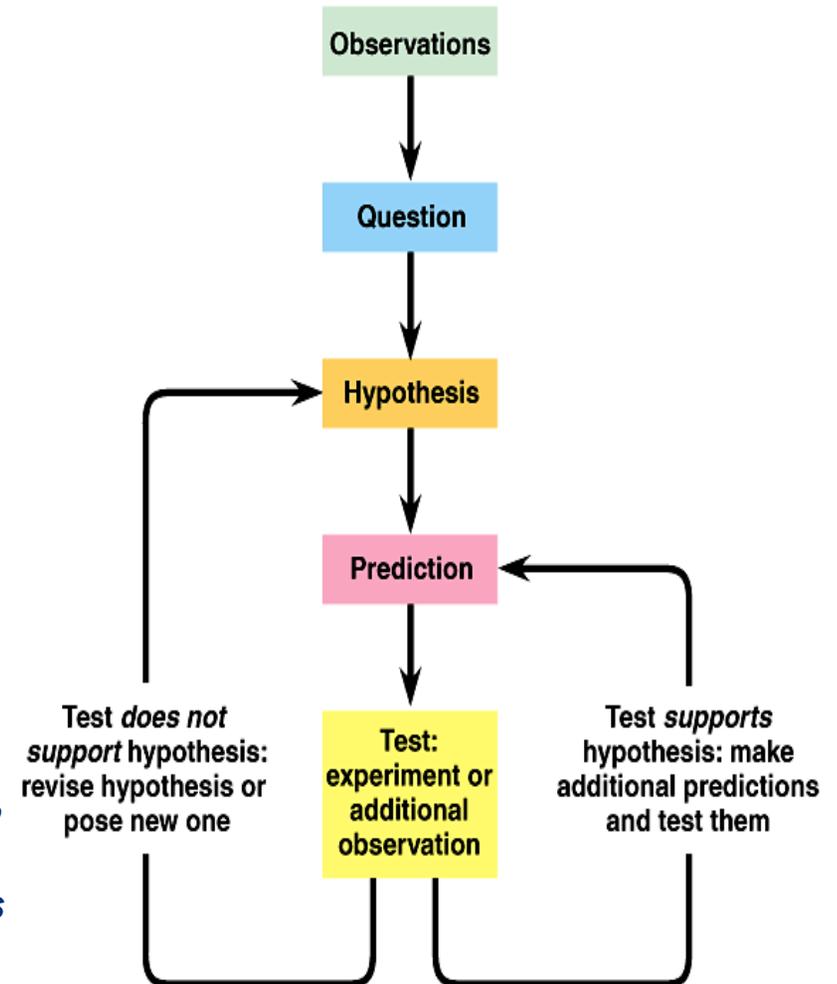


χ^2 [CHI SQUARE] TEST

“Testing Goodness of Fit”

Recall the steps of the scientific method

- Experiments are run to test a **predication** [*if..then..statements*] based on your hypothesis
 - ◆ The hypothesis is an explanatory statement of a phenomenon.
 - ◆ Hypothesis are not questions. They are tentative **EXPLANATIONS**
- *“Female students who get 8 hours of sleep outperform female students who get 4 hours of sleep on a mathematical task”*
- *“When crossing two heterozygote Pea Plants with purple flowers, there is a 25% chance of the offspring displaying the recessive white flower trait if this species follows a Mendelian Complete Dominance inheritance pattern.”*

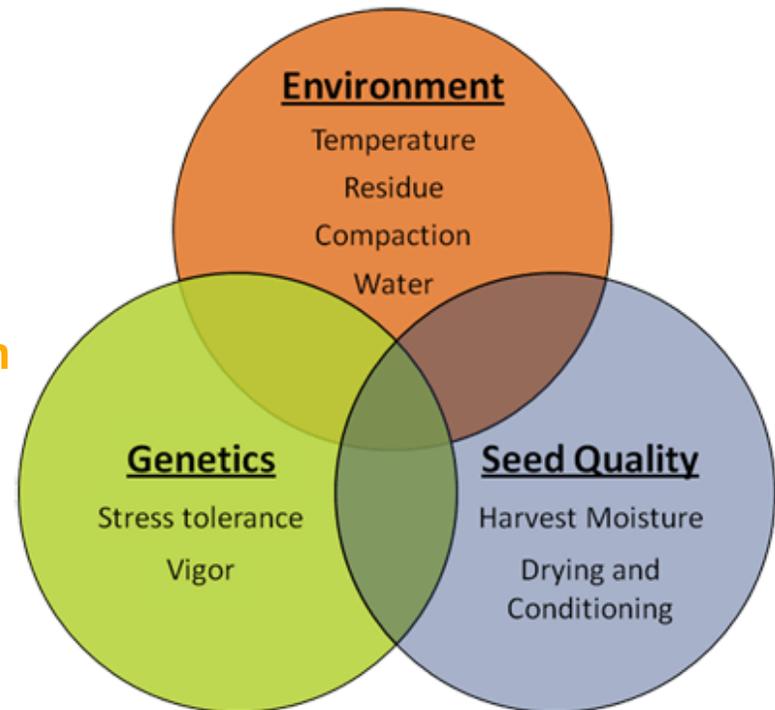


Recall the steps of the scientific method

- Many variables may influence a phenomenon.
 - ◆ For Ex: Many variables influence the length of time it takes for a species of plant seed to germinate → **dependent variable**

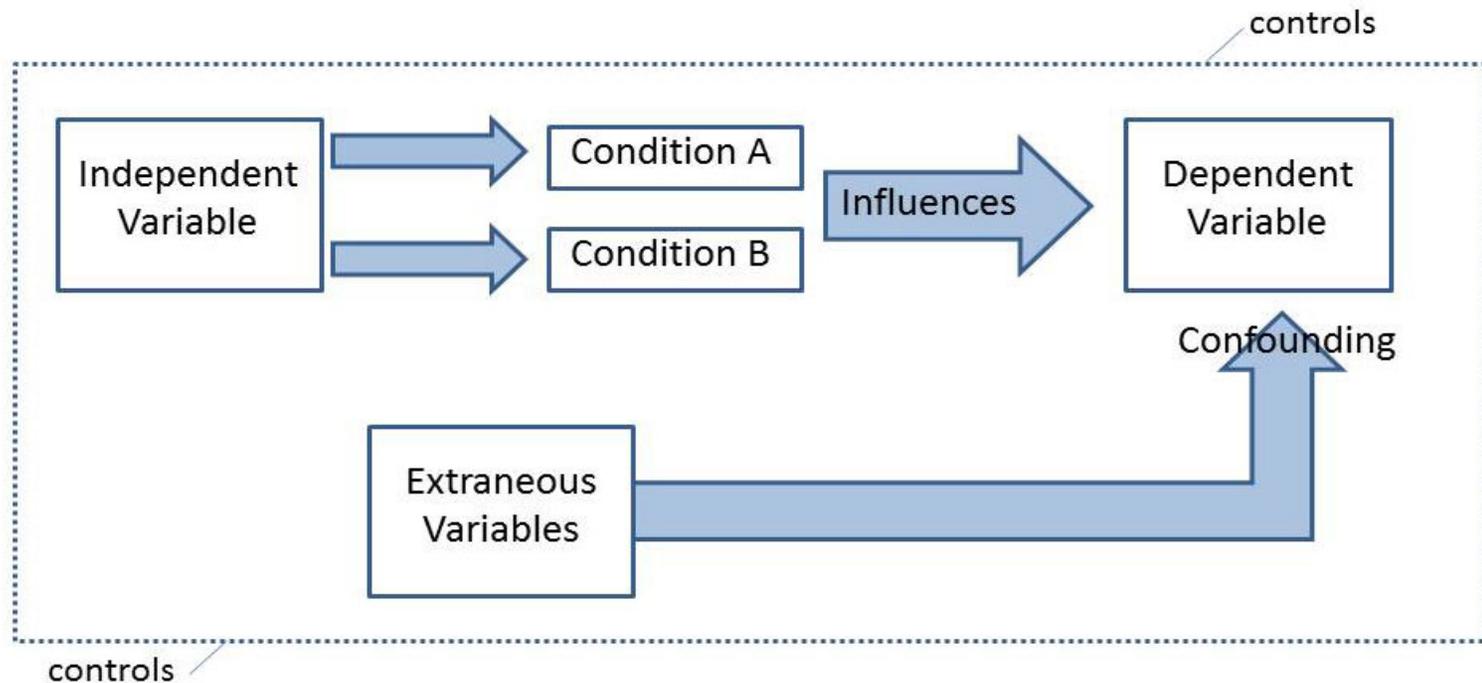
Possible independent variables

- the pH of the soil
- the soil composition
- soil nutrient content
- the amount of water received
- the number of times watered daily
- the time of day when water is given
- the amount of sunlight received
- the wavelength of light exposed to
- the air temperature
- the soil temperature
- the biotic factors in the soil etc..



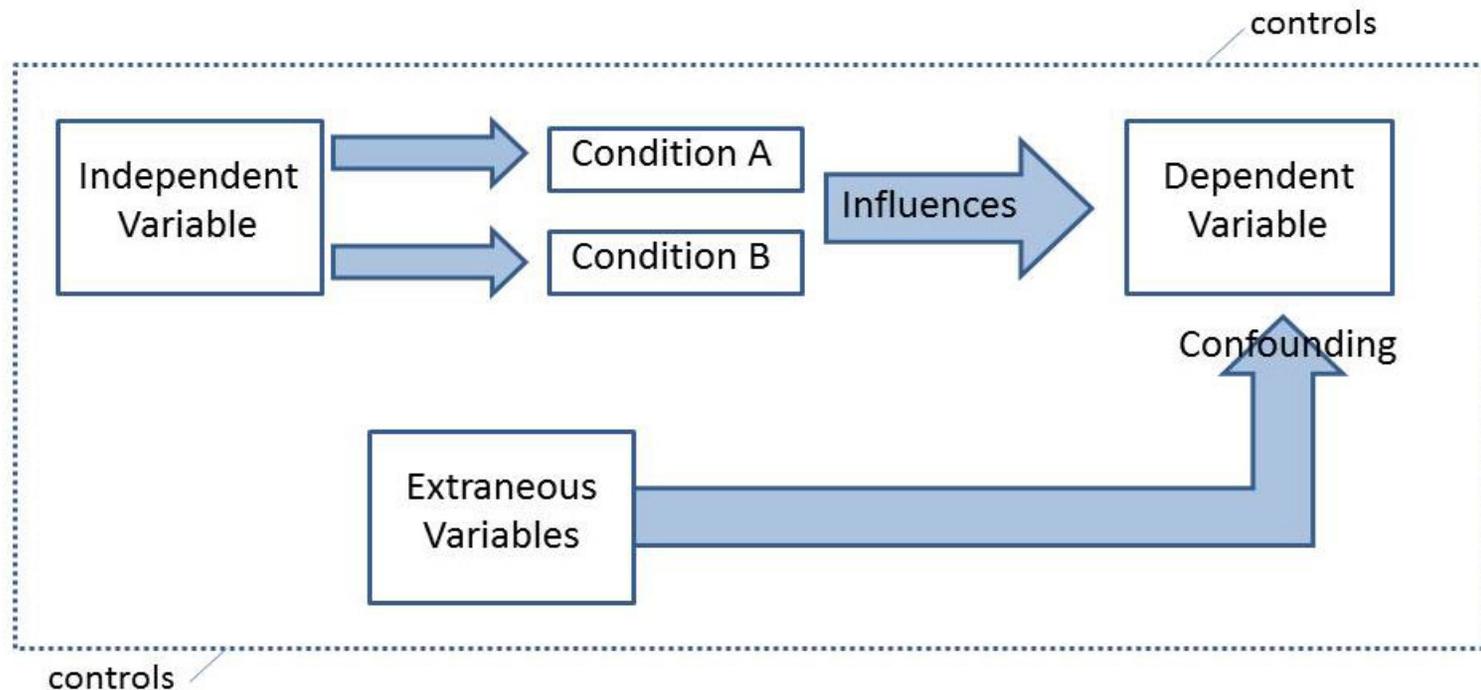
The Need for Experiments

- You **cannot** just assume one variable influences another in a certain way without **EVIDENCE** to back up your assertion.
 - ◆ You must run a **controlled experiment** to see if the data supports your tentative explanation linking two variables together *[as you state in a hypothesis]*
 - **Ex: “Adding water to soil shortens the time it takes for seeds to germinate”**
 - ◆ You must run your experiment to see IF there is a link between these two variables: amount of water & time until seeds germinate



The Need for Experiments

- **With an experiment, you will test if your treatments (certain amounts/levels of an independent variable) influence/effect your dependent variable.**
 - ◆ Of course, other variables [extraneous variables] may influence the dependent variable too



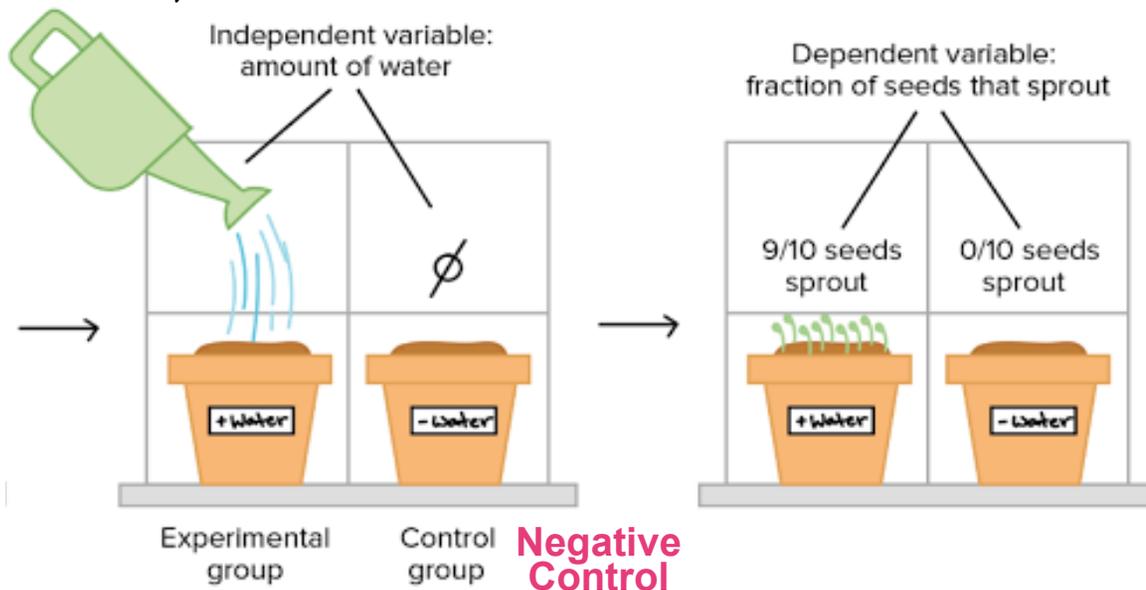
Controlled Experiments



- To know that your independent variable 'being watered,' *instead of another variable [soil pH, amount of light, wavelength of light etc...],* influences the dependent variable, 'germination time,' we set up a controlled experiment
 - ◆ We control extraneous variables making them controlled variables or CONSTANTS
 - Both the Experimental/Treatment Group and the Control Group [**THE CONTROL**] receive equal amounts of the constants (soil pH, amount of light, wavelength of light) or...
 - When that isn't possible - *like when running an experiment in nature and not the lab making it hard to keep all variables from fluctuating over time* - the constants are allowed to fluctuate in both the Experimental/Treatment and the Control groups equally and in the same way

Controlled Experiments - Positive & Negative Controls

- To know that your independent variable 'being watered' influences the dependent variable 'germination time,' we set up a controlled experiment
 - ◆ Unlike the constants, the value of the independent variable is made to **differ** between the Experimental/Treatment Group & the Control/Control Group.
 - The Treatment/Experimental Group receives a certain amount of the independent variable = **GETS A LEVEL OF TREATMENT**
 - ◆ You may have multiple experimental groups, each receiving a difference level of your independent variable (**TREATMENTS**)
 - The Control Group does not receive that amount = **DOES NOT GET THE TREATMENT** (though it may still experience some level of the Indep. Variable) OR **GETS A TREATMENT THAT WE ALREADY KNOW HAS A CERTAIN EFFECT!!!**

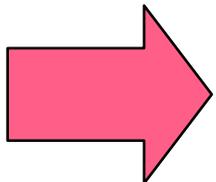


If the data collected shows a difference in the Dependent Variable of the treatment group and not the control group, then it is valid to state that the I.V. influences the D.V.

Controlled Experiments - Positive & Negative Controls

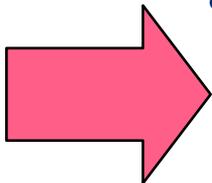
■ Negative Controls

- ◆ A control group uses a treatment that isn't expected to produce an effect on the dependent variable
 - Uses the almost same procedures as the treatment/experimental group experiences except the I.V. treatment level, but on a different group of subjects
 - Subjects in Negative Control group experience no treatment, a placebo (fake treatment), or the condition that would be experienced naturally in the organism's natural environment
 - ◆ D.V. = Amount of flowers produced by plant
 - ◆ I.V. = Amount of sunlight
 - ◆ Treatments (levels of I.V.) in Experimental Group = **Longer or shorter exposure to light daily than what plant normally receives**
 - ◆ Negative Control = No sunlight (24 hours of dark) **OR**
 - ◆ Negative Control = The natural amount of sunlight that organism would receive in nature (ex: 12 hours of light and 12 hours of dark)
 - Negative controls are used to eliminate the alternate explanations for the effects on the D.V. produced by the treatment level of I.V. applied in the experimental/treatment group



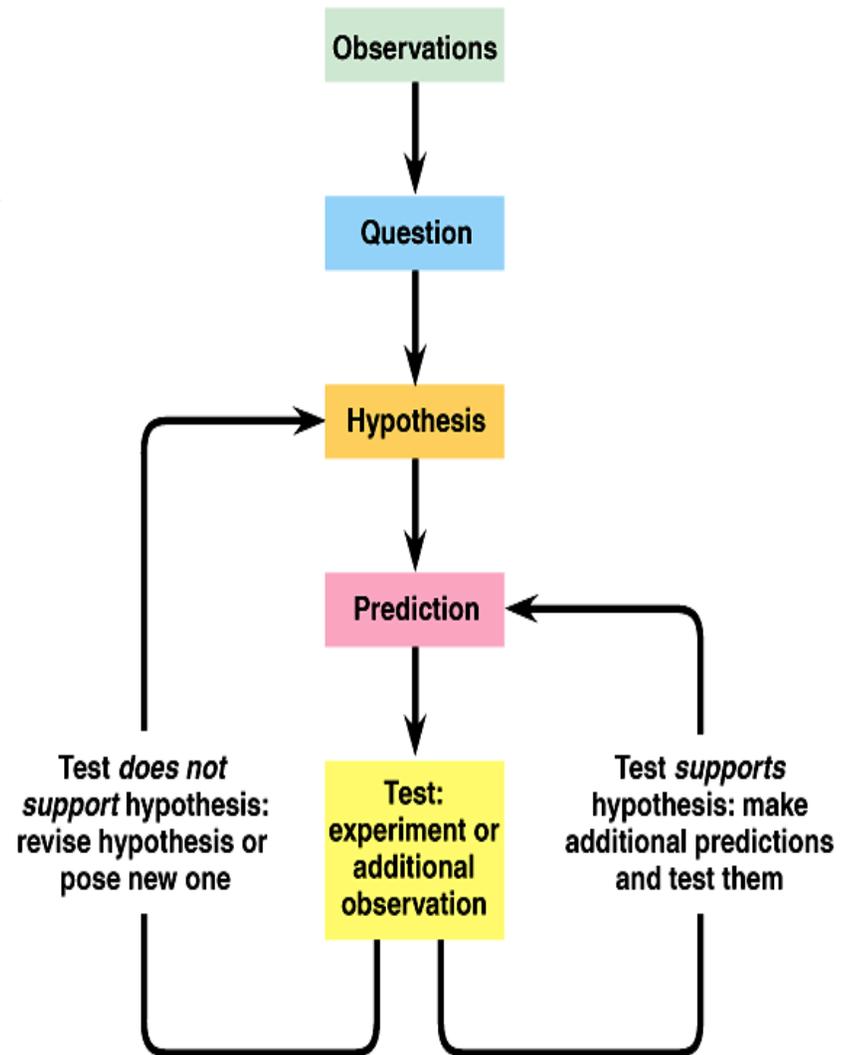
■ Positive Controls

- ◆ A control group uses a treatment that **IS ALREADY KNOWN** to produce a specific effect on the dependent variable
 - Uses the same procedures as the treatment/experimental group experiences on a different group of subjects
 - Subjects in **Positive Control** group experience **a treatment that is known to produce results similar to those predicted in your hypothesis about the effects of your independent variable**
 - ◆ D.V. = Extent of acne on face (# of pimples, for example)
 - ◆ I.V. = New acne medication
 - ◆ Treatments (levels of I.V.) in Experimental Group = **Different doses of new acne medication applied to face once a day**
 - **Negative Control** = Placebo (“fake medication”) applied to face daily that does not have any biological effect on acne
 - ◆ Used to **eliminate the alternate explanation** that psychological status or process of applying cream is the actual reason acne may decrease
 - **Positive Control** = Group of subjects given different, real medication already known to decrease acne
 - ◆ Used to **eliminate worry that there is a problem with your overall experimental design** (to detect any problem with the experimental design)
 - If the **known** acne medication didn't work to reduce acne, there may be some aspect of your experiment that is interfering with medication effectiveness that may also be interfering with your new acne medication effectiveness too: **you are not getting valid results regarding your new medication effectiveness in your experimental/ treatment groups**



So, to review Experimental Design Basics

- The organisms you conduct your experiment on are your subjects.
 - ◆ Remember, in your experimental design you will divide your subjects into, at minimum, two groups of (*ideally at least 30 subjects each*):
 - The Control Groups
 - ◆ Do NOT receive the treatment [*altered levels of I.V.*]
 - ◆ And, sometimes, receive a treatment with KNOWN effect on Dependent Variable
 - The Treatment Groups
 - ◆ DO receive one of more variations of the treatment [*altered levels of I.V.*]
- Besides the I.V., all other variables that may influence your D.V. are kept constant between the groups. [Always list at least 3 constants on the AP Exam!!!]



There are always TWO hypotheses

- The validity of your hypothesis depends on the data you collect by conducting experiment.
- ◆ Two kinds of hypotheses exist in an experiment:
 1. Null hypothesis: *Often the “default” condition.* This hypothesis states that there is **NO** relationship between the independent and dependent variables; that the treatment [IV] does *not* have an effect on the dependent variable measured -
Ho: “The observed matches the expected”
 - In experiments: A does **NOT** affect B
 - In genetic crosses: the null hypothesis is that the inheritance pattern of a gene is complete dominance/Mendelian (gene is autosomal) **UNLESS** they tell you that the gene is thought to be non-Mendelian (sex-linked, incompletely dominant etc)
 - The null hypothesis is what is **tested** in an experiment
 - The null hypothesis is what will be **supported or rejected**

There are always TWO hypotheses

- The validity of your hypothesis depends on the data you collect by conducting experiment.
- ◆ Two kinds of hypotheses exist in an experiment:
 1. Null hypothesis: *Often the “default” condition.*

Ho: “The observed matches the expected”
 2. Alternate hypotheses: This hypothesis states that there **IS** an effect on the dependent variable by the independent variable; that there exists a relationship between the IV treatment and the DV

HA: “The observed does **NOT** match the expected”

 - In experiments: A **DOES** affect B
 - In genetic crosses: Inheritance pattern of a gene is **NOT** Mendelian (gene is autosomal/completely dominant/genes are **not** linked) if the default was that is what Mendelian, or, if we said the gene is non-Mendelian, the gene is **NOT** non-Mendelian

The need for statistical analysis...

- When you collect data to test your prediction, **you are testing the validity of your Null Hypothesis - H_0** *[the treatment has no effect on the dependent variable, gene follows Mendelian inheritance pattern, two genes assort independently etc...]*
 - ◆ **Two outcomes are possible based on the data collected...**
 - Based on the data collected, you may **fail to reject** your H_0 *[and therefore have no evidence to believe your H_A may be a valid alternate explanation of the phenomena you see]*
 - ◆ **OR...**
 - Based on the data collected, you may **reject** your H_0 *[causing you to believe that some relationship between the treatment and dependent variable measured does exist, one of these possible explanation being H_A]*
 - ◆ **BUT...**
- **When do you decide that your H_0 is no longer “supported” by the data?**
 - ◆ When do you feel that the data is strong enough to allow you to reject the notion that the independent variable/the treatment has no effect on the dependent variable, for example?

Comparing the Observed to the Expected

- Let's look at an example with dice....

- If I roll a die, how many outcomes are possible?

6

- If I roll a die 60 times, how many of each outcome do you **EXPECT** [theoretically]?



- What is my Null Hypothesis?

I get the expected
 $1/6:1/6:1/6:1/6:1/6:1/6$

- What is my Alternate Hypothesis?

I do **NOT** get the expected
 $1/6:1/6:1/6:1/6:1/6:1/6$ ratio

- What is my Prediction that I will test if I run the experiment

[what is the if...then statement]?

If I roll the die 60 times, then I should get
10 of each of the possible 6 results

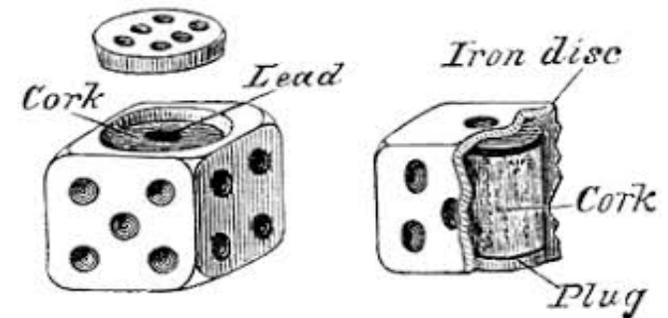
- If I actually roll the die 60 times and collect data, will my **OBSERVED** data match my expectation? *Will it be an exact match?*

Maybe, but I may also not get exactly 10 of each of the 6 results.

What if the Observed and Expected don't match!!!!

- After 60 die rolls, what if my **OBSERVED** data does not exactly match my **EXPECTED**?
 - ◆ Do I reject or fail to reject [“support”] my Null Hypothesis that I should get 1/6 of my throws yielding 1 pip & 1/6 yielding 2 pips, & 1/6 yielding 3 pips etc...? **Not necessarily...**
- Some difference between the expected and the observed is normal, even expected, just due to **CHANCE VARIATIONS!**
 - ◆ **But...** as the difference between the expected and observed grows, you may start to doubt if chance alone is affecting that difference observed and wonder if some other factor might be the cause for the difference you witness.

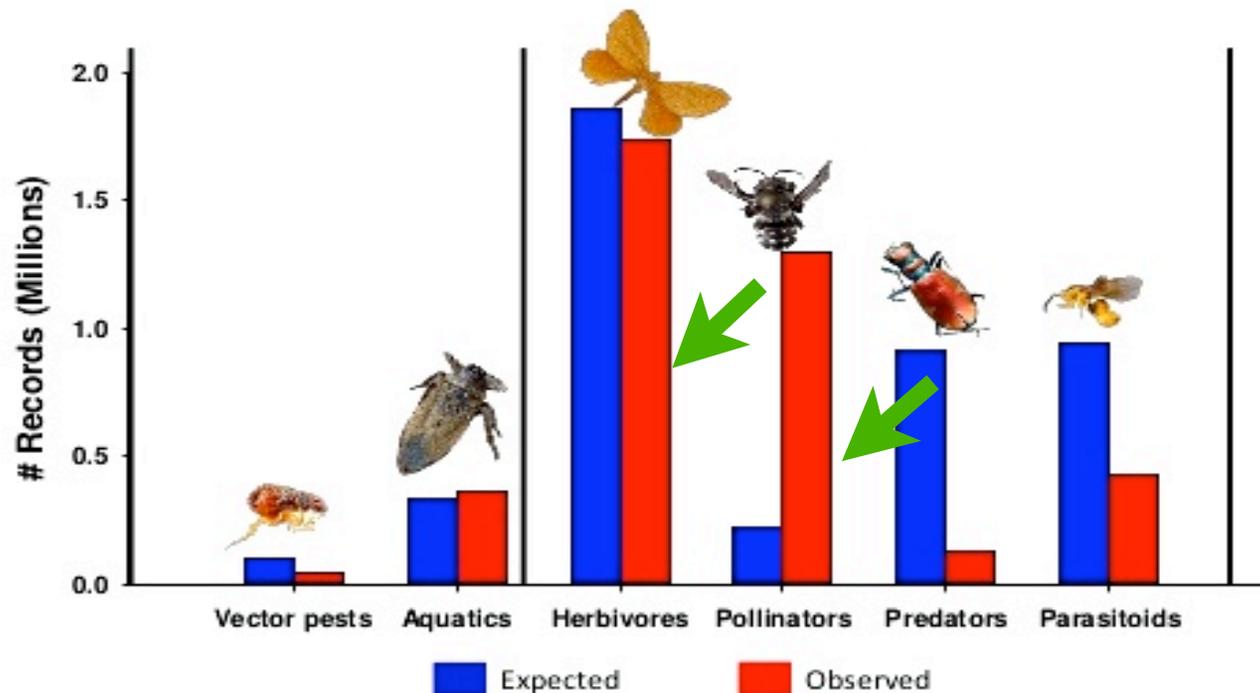
- Might this be a trick die that favors some numbers over others?
- **When is the difference between expected and observed large enough chance alone becomes an improbable explanation for what you witness????????????????????**



What if the Observed and Expected don't match?

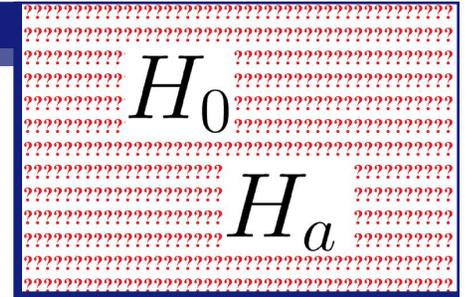
- We cannot leave it up to each individual to subjectively decide when chance is or is not causing the difference between observed and expected results, and if something else is causing the difference detected.

Ecological Distribution: Expected vs Observed Records



- ◆ Are the number of herbivores observed different enough from the number expected because of **ANOTHER VARIABLE** or is this just due to **CHANCE**?
- ◆ Do the number of pollinators observed differ from the expected because of random **CHANCE** or is some **OTHER FACTOR** in the environment causing the greater observed pollinator number than what was expected?

Chi-square test: Testing if a hypothesis is statistically significant.



- The Chi Square Tests is a “goodness of fit” test.
 - ◆ It answers the question of how well the experimental [observed] data fits the expected - YOUR H_0 .
- The chi-squared (X^2) test evaluates the likelihood that the variation observed in your experimental data compared to the expected results was due to chance alone!
 - ◆ It cannot tell you whether the variation was because your independent variable caused the difference you see between expected and observed [it does NOT prove your H_A correct], but it can be used as evidence to rule out an - most likely - invalid null hypothesis H_0
 - The X^2 Test helps you decide when to reject or not reject your Null Hypothesis!!!!!!

The Chi-square tests the validity of H_0

Your **Null Hypothesis** is the explanatory statement that says “the observed matches the expected,” the expected being that, for example, in an experiment, the treatment/I.V. does **not** have an effect on the D.V. or that gene inheritance patterns follow of a certain

- If the variance between observed and expected numbers seems plausibly due to “random chance” = **Fail to Reject the Null** [sometimes that’s a mistake - O.J. Simpson found innocent?]
- If the variance between observed and expected numbers does **not** seem plausibly due to “random chance” alone so there appears to be another factor influencing the data observed... = **Reject the Null** [more often you will be correct - Casey Anthony is not innocent of murder]



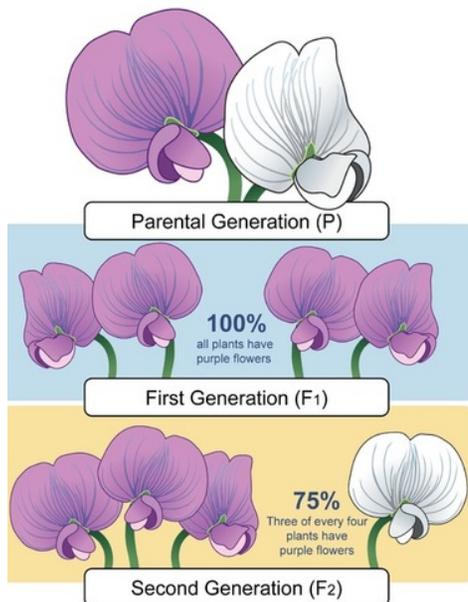
I was found
“not guilty”
But I’m sure not
Innocent!



I was found
“guilty”
So I’m sure not
Innocent!

Writing Hypotheses in Biology - THE NULL HYPOTHESIS IS THE HYPOTHESIS THAT IS BEING TESTED!

- Now, let's look at an example of a problem in biology instead of dice..A fundamental problem in genetics is determining whether the experimentally determined observed data fits the results expected from theory (*i.e. Mendel's laws as expressed by the Punnett Square*).
 - How can you tell if the observed offspring are legitimately the result of a given cross?
 - Let's take a cross between two pea plants heterozygous for purple flowers. *What do you expect in the offspring?*



- The Null Hypothesis = The observed #'s of purple and white pea plant offspring match the expected # of purple and white pea plants given Mendel's law of segregation and Complete Dominance (or "Offspring show a 3/4 purple to 1/4 white phenotypic ratio")
- The Alternate Hypothesis = The observed #'s of purple and white pea plant offspring do NOT match the expected # based on Mendel's laws of segregation and Complete Dominance (or "Offspring do NOT show a 3/4 purple to 1/4 white phenotypic ratio" or "NOT Ho")
 - Conclusion if we reject the Null Hypothesis = maybe complete dominance is not the inheritance pattern or maybe the parents are not both heterozygotes

X² in Biology

- Let's say you run the experiment and actually cross your two heterozygous pea plants. What do you **expect** for your phenotype ratios?
- Lets say, you **observe** 290 purple flowers and 110 white flowers in the offspring. This is pretty close to a 3 purple : 1 white ratio or 3/4 purple to 1/4 white, but is it close enough to fail to reject the Null Hypothesis and say complete dominance **IS** taking place?
 - ◆ First, we must determine how many of each phenotype would be **expected** given basic genetic theory, following the *assumption that the baseline inheritance patterns always follow Mendel's Laws of Segregation and Independent Assortment if nothing else is stated explicitly in the problem.*
 - ◆ Then calculate the chi-square statistic using this formula:

		pollen ♂	
		B	b
pistil ♀	B	BB	Bb
	b	Bb	bb

AP Bio

Observed individuals with a given phenotype

Expected individuals with a given phenotype

Greek letter "chi"

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

Summation => add together a term for each condition

Finding the Observed vs Expected

- In this example... you count offspring, and **observe** 290 purple and 110 white flowers. **This is a total of 400 offspring.**
- We **expect** a 3/4 purple : 1/4 white ratio. We need to calculate the expected numbers to compare expected to observed. **(You MUST always use the numbers of offspring, NEVER the proportion/percentage/frequency!!!)**
 - ◆ **This is done by multiplying the total offspring by the expected proportions.**
 - **This we expect $400 * 3/4 = 300$ purple & $400 * 1/4 = 100$ white.**
 - ◆ For purple, **observed = 290 & expected = 300.**
 - ◆ For white, **observed = 110 & expected = 100.**

Calculating the X^2 value

- Now it's just a matter of plugging into the formula:

$$X^2 = \sum \frac{(obs - exp)^2}{exp}$$

$$\begin{aligned} X^2 &= (290 - 300)^2 / 300 + (110 - 100)^2 / 100 \\ &= (-10)^2 / 300 + (10)^2 / 100 \\ &= 100 / 300 + 100 / 100 \\ &= 0.333 + 1.000 \\ &= 1.333 \end{aligned}$$

- **1.333 is our X^2 value (Chi Square value)**
 - ◆ What does this mean and how do we use it?

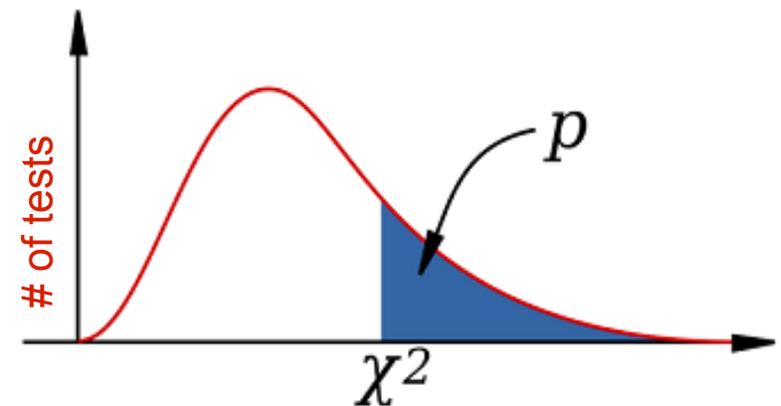
The Chi-Square Distribution

- Let's say we do the same experiment 1000 times - the same self-pollination of a Pp heterozygote - which should give the 3/4 : 1/4 ratio in offspring phenotypes.
 - ◆ For each experiment we calculate the chi-square value, then plot them all on a graph.
 - The x-axis lists all chi-square values calculated from the formula.
 - The y-axis lists the number of individual experiments that got that specific chi-square value (frequency)
- **You see that there is a range in the graph:** if the results of a Pp x Pp cross were perfect [and your observed offspring numbers matches your expected exactly] you get a chi-square value of 0 (obs = exp).

This rarely happens!!!!!!

- ◆ Sometimes your obs #s are very different from exp #'s: the long tail on the graph =====>

- The probability of getting a big difference between obs & exp by chance alone though is very small even if you repeat the experiment an infinite number of times [the blue area - p - under the curve represents that probability]



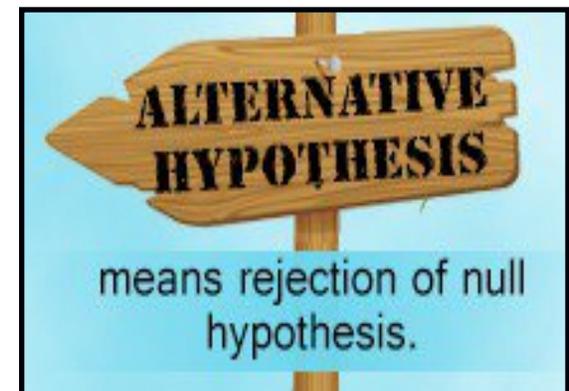
The Chi-Square Test for Goodness of Fitness

- Really odd things occasionally do happen by chance alone (*for instance, you might win the lottery*).
 - ◆ Most often though, unexpected things happen because another factor is influencing the observed result [*there's an ALTERNATE explanation for the observation seen; the expectation was wrong!!!*].
- You can never tell for certain that a given result you got was completely impossible based on the theory you used.
 - ◆ *All we can do is determine whether a given result is likely or unlikely to occur.*
 - The Chi Square calculation emphasizes the DIFFERENCE between the observed # and the expected #
 - ◆ If the the # expected and # observed are identical, then the Chi Square value is 0.
 - There is a perfect fit between what we hypothesized in our Ho and what we observed in our data.
 - ◆ The more the observed # differs from the expected #, the larger the Chi Square value becomes
 - Is this difference observed due to chance alone or is there another reason for this difference seen?



Why might you get high Chi-Square Values?

- There are 2 reasons for getting a high chi-square values:
 1. The variation between observed and expected varied by chance
 - ◆ Your Null Hypothesis is valid still despite difference between your observed and expected values
 2. The variation between observed and expected varied because the Null Hypothesis may be incorrect!
 - ◆ An Alternate Explanation is warranted.
 - ◆ You should Reject your Null in favor of your Alternate Hypothesis



The Chi-Square Distribution

	p	p		P	p
P	Pp	Pp	P	PP	Pp
p	pp	pp	p	Pp	pp

- Using our Pea Plant, how can you tell if your 290 purple: 110 white offspring ratio really fits a 3/4 : 1/4 ratio (*as expected from mating a heterozygote*) or whether the results were more likely caused by an unknown mistake in the mating - a mating between a heterozygous purple with a homozygous recessive plant instead? (*which theoretically should give you a 1/2 purple : 1/2 white ratio, but in real life could still result in 290 purple and 110 white offspring by chance!!!*).
- ◆ You can't be certain, but you can at least determine whether your observed result is reasonable given the null hypothesis that assumes two heterozygotes are mating.
 - Usually, an observed result is said to not differ significantly enough from expectations to warrant thinking the null hypothesis is wrong if the difference seen between expected and observed results happens at least 5% [or more] of the time by chance alone!!!!
 - ◆ **Meaning:** If the difference between the observed results and the expected results is small enough that such difference would occur at least 1 out of every 20 or more times due to chance alone, we “fail to reject” the null hypothesis. The deviations between expected and observed numbers witnessed can reasonably happen because of chance.
 - ◆ “1 time in 20” can be written as a probability value **p = 0.05**



Degrees of Freedom

- Degrees of freedom = the number of independent categories - 1
 - ◆ In our example with two purple heterozygotes mating, the degrees of freedom is the number of phenotypic classes of offspring [purple vs white] minus 1 so $d.f. = 2 - 1 = 1$



Using the Critical Value for Chi-Square

To decide when your calculated Chi Square value is too large and you should **not** reasonable attribute such a large deviation between expected and observed to chance alone, you must **compare your X^2 value calculated to another X^2 value we call the CRITICAL VALUE.**

- ◆ The Critical Value is a pre-calculated Chi-square values found on tables, sorted by degrees of freedom and probability levels. Be sure to use $p = 0.05$ unless they tell you to use $p = 0.01$.
 - If your calculated chi-square is **greater** than the critical value [x^2 from the table], you **“reject the null hypothesis.”**
 - ◆ You expect such a large deviation between the observed and expected to occur due to chance alone less than 5% (or 1%) of the time
 - There is a good possibility something else is causing the observed to differ so much from your expected - **Reject your Null Hypothesis**
- ◆ If your calculated chi-square is **less** than the critical value, you **“fail to reject the null hypothesis.”**
 - ◆ You expect the deviation between your observed and your expected due to chance alone at least 5% (or 1%) of the time due to chance alone.
 - Your explanation is valid - **Fail to Reject (“Support”) your Null Hypothesis**

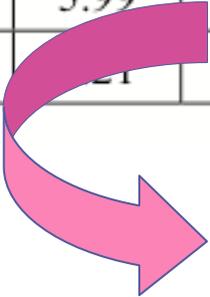
Finding the Critical Value in your Chi Square Table

p value = Probability that the difference between the observed & expected is due to chance alone assuming the Null Hypothesis is correct

Chi-Square Table

df = Calculated Degrees of Freedom

<i>p</i> value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09



The Critical Value [that you will compare your calculated X^2 value to in order to determine if you should Reject or Fail to Reject your Null Hypothesis]

Using the X^2 Table

- In our example of 290 purple to 110 white observed, we **calculated a chi-square value of 1.333**, with 1 degree of freedom.
 - ◆ Looking at the table, 1 d.f. is the first column, and $p = 0.05$ is the first row. Here we find the critical [chi-square] value of 3.841.
 - At critical value above 3.841, the difference between obs and exp is expected to occur by chance alone **less than 5% of the time!** *Something else is most likely causing this difference!*

Chi-Square Table

p value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09

Using the X² Table

- In our example of 290 purple to 110 white observed, we calculated a chi-square value of 1.333, with 1 degree of freedom.

Chi-Square Table

<i>p</i> value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09

- ◆ Since our calculated chi-square, 1.333, is less than the critical value, 3.841, we **“fail to reject”** the null hypothesis. *(Our difference between obs & exp can occur due to chance 5% or more of the time so the Ho is still valid)*
 - Conclusion: An observed ratio of 290 purple to 110 white is a good fit to a 3/4 to 1/4 [3:1] expected ratio
 - ◆ **Our null hypothesis failed to be falsified/rejected - “Ho was supported”**

The Chi Square table is found on the AP Bio Exam formula sheet...

- To decide whether to reject or fail to reject the Null Hypothesis, **calculate the χ^2** at p-value 0.05 (that the H_0 is valid and we expect to see a deviation this large from expected at least 5% of the time due to **CHANCE** alone)
 - If your **calculated χ^2** for your data is **larger** than the **CRITICAL VALUE χ^2** in the table at p-value 0.05, reject the H_0 and accept the H_A . *(This means, at a p-value of 0.05 there is a less than 5% probability of seeing the deviation from expected you see in your observed by chance alone - **something else must be causing your observed to differ so greatly from expected**)*
 - If your **calculated χ^2** for your data is **smaller** than the **CRITICAL VALUE χ^2** in the table at p-value 0.05, fail to reject H_0 (**the expected was valid**) *(The deviation of the observed from expected **could have reasonably still occurred BY CHANCE**).*
 - ◆ **THERE IS NO STATISTICAL DIFFERENCE BETWEEN YOUR OBSERVED AND THE EXPECTED!!!**

Chi-Square Table

<i>p</i> value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09

Steps To
Solving a X^2
Problem

STEPS TO SOLVING CHI SQUARE PROBLEMS

1. State your H_0 (**Exp = Obs**) and H_A (not **Exp = Obs**)
2. Calculate the # you **expect** to see based on your Null Hypothesis/ explanation.
3. Count the # you **observe**.
4. Calculate your **Degrees of Freedom** ($df = \# \text{ of categories} - 1$)
5. Look up your **Critical Value** in the X^2 Table on your Formula Sheet.
This is a theoretical X^2 value at which there is a 5% probability that the difference between the observed and expected used to produce this theoretical value happened by chance.
6. Calculate your own **X^2 Value** using the X^2 formula from your Formula Sheet.
7. **Compare** your calculated **X^2 Value** to the **Critical Value**.
 - ▶ If your calculated X^2 is **larger** than the Critical Value, state that you reject your H_0 .
 - ★ There **IS A STATISTICAL DIFFERENCE** between observed and expected.
 - ➔ If you reject H_0 , give a reason why the observed numbers were so different from expected quantities – provide a possible Alternate Hypothesis [**the H_A**] to explain the observed
 - ▶ If you calculate X^2 is **smaller** than the Critical Value, state that you fail to reject your H_0 .
 - ★ There is **NO STATISTICAL DIFFERENCE** between observed and expected.

Helpful Hints

Helpful Hints When Doing Chi Square Hypothesis Designs & Testing

Remember, **you always test and, therefore, reject or fail to reject your NULL HYPOTHESIS.** Whatever you write for the Alternate is just a plausible alternate explanation if your Null turns out to be wrong, but the validity of your Null is the hypothesis you are actually testing in a Chi Square Test for Goodness of Fit.

In general, the H_0 Hypothesis states that "There is **NO** statistical difference between your expected and observed values."

Your H_A states that "There **IS** a statistical difference between your expected and observed values."

- When there is a statistical difference [large enough difference] between the number of expected and the number actually observed, the chance of this variation being due to chance alone is not believable. Something else must be influencing the differences observed. You reject your H_0 that states that the difference between observed and expected is due to chance alone. You may then be asked to come up with some possible reasons for the difference if you no longer believe it to be because of chance. These are your alternates.

The Chi square test helps you decide when to reject the H_0 or when to support the H_0 , which is your final conclusion in this type of problems - when to say any variation between observed and expected is due to chance and when it is most likely not because of chance alone.

Remember when doing Chi Square problems you have to plug into your formula the # of individuals (integers) NEVER %'s or fractions or decimals.

What to do in different scenarios - KNOW THIS WELL!!!

Scenario #1 - Evolutionary Question

If you are not told which evolutionary mechanism is occurring, assume NO evolutionary mechanism is occurring for your H_0 [that the population is in H-W Equilibrium]. You cannot after all just assume one of the five mechanism (genetic drift due to small population size, nonrandom mating, natural selection, mutation, or gene flow) must be occurring because you think it makes sense - in science, you must find evidence first to make such a claim.

Let's say you start with an H_0 that states that "all colors of worms are equally likely to be eaten in nature" [or "there is NO statistical difference between the expected and the observed number of worms being eaten"]. H_A then states that "not all colors have an equal chance of being eaten" [or "there IS a statistical difference between the expected and the observed number of worms being eaten"]

- If you Reject H_0 , then you could discuss possible reasons why the colors are not equally likely to be eaten...These explanations are your Alternate Hypotheses. You can conclude that maybe natural selection is taking place, for instance, because of a prey that relies on eyesight to hunt and some colors being easier to discern in the environment where these colored worms live compared to other colors that help the organism camouflage better.
- If instead you "Support" or Fail to Reject your H_0 then indeed any variation in worm color eaten is purely because of chance and no selective pressure is being placed by the predator on the prey based on the prey's color.

Scenario #2 - Experimental Question

If you are dealing with a controlled experiment with a control group and one or more treatment groups (who receive the independent variable “treatment”), you assume for your H_0 that there is NO effect of the Independent Variable/Treatment on the Dependent Variable (that “there will be NO statistical difference between your control and treatment group data”). H_A then will state that there IS an effect of the Independent Variable on the Dependent Variable (that “there IS a statistically significant difference between the control group and treatment group”). After all, in a research experiment, you cannot assume X causes Y without evidence. You always start with the assumption that X does not cause Y **unless** the problem you are given already tells you that the researcher has explained the behavior in some way and wants to test this new explanation, in which case the explanation becomes the new Null Hypothesis to test.

- Again, you are comparing the # of subjects in the Control to the # of subjects in the Treatment Group.

So, if, for example, you are given an experiment to find out if fruit flies prefer sugar water over regular water, and you set up an experiment to test if adding sugar to water causes fruit flies to drink more water than water without sugar, your null is that there is no affect by the presence of sugar on the amount of water a fruit fly drinks.

Scenario #3 - Heredity Question - Unknown Inheritance Pattern

If you do not know what type of inheritance pattern is occurring you will **ALWAYS** assume an H_0 that states that the inheritance pattern is Mendelian (Complete dominance with, if relevant, multiple genes following the law of Independent Assortment – being located on different types of chromosomes). You cannot assume linkage of genes for different characters, sex-linkage of a gene on an X or Y chromosome, incomplete dominance, epistasis, co-dominance etc... If you think something is inherited by Mendelian mechanisms, you can figure out then also which ratio of phenotypes or genotypes you expect in your offspring of a mating.

- From a Punnett Square, and the resulting phenotype [or genotype] ratios, you can figure out the #s of organism with each phenotype [or genotype] expected.
 - *Remember, Chi Square can only be performed with the observed vs the expected #'s, not with %'s, frequencies, or ratio's.*

Your H_A will state that Mendelian and Complete Dominance does **NOT** occur or that Independent Assortment does **NOT** occur or that the ratio of phenotypes or genotypes expected will **NOT** be observed. You will then compare the # of individuals observed to the # expected to do your Chi Square and Reject or Fail to Reject [“Support”] your H_0 .

- This type of set up tests whether the inheritance pattern observed follows Mendelian Laws: Independent Assorts of two or more genes when relevant & Complete Dominance of one allele over another whether you are dealing with one gene or more than one gene.

Scenario #4 - Heredity Question - Known Inheritance Pattern

If the problem, however, already tells you that the researchers **EXPECT** a certain pattern or result - like sex-linkage or some specific phenotypic ratio in offspring, then this expectation [to test] becomes your H_0 . This becomes what you assume to be your **EXPECTED** values.

Your H_A then is that the inheritance pattern is **NOT** sex-linked or that the expected phenotypic ratio does **NOT** occur the way the researcher claims they did.

- This type of set up tests whether a gene is indeed sex-linked, or whether two genes are linked, or whether incomplete or co-dominance is the inheritance pattern as stated etc...



Any Questions??
