AP Biology Lab #7 Genetics of Organisms

OVERVIEW:
In this lab you will use living organisms to do genetic crosses. You will learn how to collect and manipulate the organisms, collect data from F1 and F2 generations, and analyze the results form a monohybrid, dihybrid, or sex-linked cross.

OBJECTIVES:
Before doing this lab you should understand:
1) Chi-squared analysis of data.
2) The life cycle of diploid organisms useful in genetic studies.

After doing this lab you should be able to:
1) Investigate the independent assortment of two genes and determine whether the two genes are autosomal or sex-linked using a multigenerational experiment.
2) Analyze the data from your genetic crosses using chi-squared analysis techniques.

INTRODUCTION:
In this lab we will not breed fruit flies but will use data previously gathered from a similar experiment. The teacher will lead you through the exercises.

DATA:
Table 1: F1 Generation Data

Phenotype and Symbol	Females	Males

Table 2: F2 Generation Data

Phenotype and Symbol	Females	Males

Analysis of Results:
1. Describe and name the observed mutation(s).

2. Write a hypothesis that describes the mode of inheritance of the trait(s) you studies. This is your null hypothesis (will be described in the Statistical Analysis Section).

3. Construct two Punnett squares to predict the expected results of both the parental and F1 crosses from your null hypothesis.

4. Refer to the above Punnett squares. Record the expected ratios for the genotypes and phenotypes of the F1 and F2 crosses in the experiment.

	Expected Genotypic Ratio	Expected Phenotypic Ratio
F1		
F2		

5. Do the actual results deviate from what was expected? If so, explain how.

6. From the results, describe your cross.
      A. Sex-linked or Autosomal?
      B. Dominant or Recessive
      C. Monohybrid or Dihybrid?

7. Are the deviations for the phenotypic ratio of the F2 generation within the limits expected by chance? To answer this question, statistically analyze the data using the chi-squared analysis. Calculate the chi-square statistic for the F2 generation in the chart below.

Phenotype	# Observed (O)	# Expected (E)	$(O - E)$	$(O - E)^2$	$\dfrac{(O - E)^2}{E}$

$$X^2 =$$

      A. Calculate the chi-square value for these data.
          1. How many degrees of freedom are there?
          2. chi-square =
          3. Referring to the critical values chart, what is the probability value for this data?

      B. According to the probability value, can you accept or reject your null hypothesis? Explain why.

STATISTICAL ANALYSIS SECTION

      The chi-square test is used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories. Do the number of individuals or objects that fall in each category differ significantly from the number you would expect? Is this difference between the expected and observed due to sampling error, or is it a _real_ difference?

Chi-Square Test Requirements
      1. Quantitative data.
      2. One or more categories.
      3. Independent observations.
      4. Adequate sample size (at least 10).
      5. Simple random sample.

6. Data in frequency form.
7. All observations must be used.

Expected Frequencies

When you find the value for chi square, you determine whether the observed frequencies differ significantly from the expected frequencies.   You find the expected frequencies for chi square in three ways:

I . You hypothesize that **all the frequencies are equal** in each category. For example, you might expect that half of the entering freshmen class of 200 at Tech College will be identified as women and half as men. You figure the *expected* frequency by dividing the number in the sample by the number of categories. In this exam pie, where there are 200 entering freshmen and two categories, male and female, you divide your sample of 200 by 2, the number of categories, to get 100 (expected frequencies) in each category.

2. You **determine the expected frequencies** on the *basis of some prior knowledge*. Let's use the Tech College example again, but this time pretend we have prior knowledge of the frequencies of men and women in each category from last year's entering class, when 60% of the freshmen were men and 40% were women. This year you might expect that 60% of the total would be men and 40% would be women. You find the expected frequencies by multiplying the sample size by each of the hypothesized population proportions. If the freshmen total were 200, you would expect 120 to be men (60% x 200) and 80 to be women (40% x 200).

Now let's take a situation, find the expected frequencies, and use the chi-square test to solve the problem.

Situation

Thai, the manager of a car dealership, did not want to stock cars that were bought less frequently because of their unpopular color. The five colors that he ordered were red, yellow, green, blue, and white. According to Thai, the expected frequencies or number of customers choosing each color should follow the percentages of last year. She felt 20% would choose yellow, 30% would choose red, 10% would choose green, 10% would choose blue, and 30% would choose white. She now took a random sample of 150 customers and asked them their color preferences. The results of this poll are shown in Table 1 under the column labeled "observed frequencies."

Table 1 - Color Preference for 150 Customers for Thai's Superior Car Dealership

Category Color	Observed Frequencies	Expected Frequencies
Yellow	35	30
Red	50	45
Green	30	15
Blue	10	15
White	25	45

The expected frequencies in Table 1 are figured from last year's percentages.   Based on the percentages for last year, we would expect 20% to choose yellow.   Figure the expected frequencies for yellow by taking 20% of the 150 customers, getting an expected frequency of 30 people for this category. For the color red we would expect 30% out of 150 or 45 people to fall in this category. Using this method, Thai figured out the expected frequencies 30, 45, *15, 15,* and 45. Obviously, there are discrepancies between the colors preferred by customers in the poll taken by Thai and the colors preferred by the customers who bought their cars *last* year. Most striking is the difference in the green and white colors. If Thai were to follow the results of her poll, she would stock twice as many green cars than if she were to follow the customer color preference for green based on last year's sales. In the case of white cars, she would stock half as many this year. What to do??? Thai needs to know whether or not the discrepancies between last year's choices (expected frequencies) and this year's preferences on the basis of his poll (observed frequencies) demonstrate a _real_ change in customer color preferences. It could be that the differences are simply a result of the random sample she *chanced to* select. If so, then the population of customers really has not changed from last year as far as color preferences go. The *null hypothesis* states that there is no significant difference between the expected and observed frequencies. The *alternative hypothesis* states they *are* different.   The level of significance (the point at which you can say with 95% confidence that the difference is NOT due to chance alone) is set at **.05** (the standard for most science experiments.) The chi-square formula used on these data is

$$X^2 = \frac{(O - E)^2}{E}$$

where **O** is the <u>Observed Frequency</u> in each category

E is the <u>Expected Frequency</u> in the corresponding category

Σ is "<u>sum of</u>"

df is the "<u>degree of freedom</u>" (n-1)

X^2 is <u>Chi Square</u>

PROCEDURE

We are now ready to use our formula for X^2 and find out if there **is** a significant difference between the observed and expected frequencies for the customers in choosing cars. We will set up a worksheet; then you will follow the directions to form the columns and solve the formula.

1. *Directions for Setting Up Worksheet for Chi Square*

Category	O	E	(O - E)	$(O - E)^2$	$\frac{(O - E)^2}{E}$
yellow	35	30	5	25	0.83
red	50	45	5	25	0.56
green	30	15	15	225	15
blue	10	15	-5	25	1.67
white	25	45	-20	400	8.89

X^2 **= 26.95**

2. After calculating the Chi Square value, find the *"Degrees of Freedom."* (DO <u>NOT</u> SQUARE THE NUMBER YOU GET, NOR FIND THE SQUARE ROOT - THE NUMBER YOU GET FROM COMPLETING THE CALCULATIONS AS ABOVE IS CHI SQUARE.)

    Degrees of freedom (df) refers to the number of values that are free to vary after restriction has been placed on the data. For instance, if you have four numbers with the restriction that their sum has to be 50, then three of these numbers can be anything, they are free to vary, but the fourth number <u>definitely</u> is restricted. For example, the first three numbers could be 15, 20, and 5, adding up to 40; then the fourth number has to be 10 in order that they sum to 50. The degrees of freedom for these values are then three. The degrees of freedom here is defined as *N* - 1, the number in the group minus one restriction (4 - I).

3. Find the table value for Chi Square. Begin by finding the *df found* in step 2 along the left hand side of the table. Run your fingers across the proper row until you reach the predetermined level of significance (.05) at the column heading on the top of the table. The table value for Chi Square in the correct box of *4 df* and ***P=.05***

4. If the calculated chi-square value for the set of data you are analyzing (26.95) is equal to or greater than the table value (9.49), **reject** the null hypothesis. ***There IS a significant difference between the data sets that cannot be due to chance alone.*** If the number you calculate is LESS than the number you find on the table, than you can probably say that any differences are due to chance alone.

    In this situation, the rejection of the null hypothesis means that the differences between the expected frequencies (based upon last year's car sales) and the observed frequencies (based upon this year's poll taken by Thai) are **not** due to chance. That is, they are not due to chance variation in the sample Thai took; there is a real difference between them. Therefore, in deciding what color autos to stock, it would be to Thai's advantage to pay careful attention to the results of her poll.

The steps in using the chi-square test may be summarized as follows:

Chi-Square Test Summary

1. Write the observed frequencies in column **O**
2. Figure the expected frequencies and write them in column **E**.
3. Use the formula to find the chi-square value:
4. Find the **df**. (*N*-1)
5. Find the table value (consult the Chi Square Table.)
6. If your chi-square value is **equal to or greater than** the table value, reject the null hypothesis: *differences in your data are not due to chance alone*

For example, the reason observed frequencies in a fruit fly genetic breeding lab did not match expected frequencies could be due to such influences as:
- Mate selection (certain flies may prefer certain mates)
- Too small of a sample size was used
- Incorrect identification of male or female flies
- The wrong genetic cross was sent from the lab
- The flies were mixed in the bottle (carrying unexpected alleles)

Table E: Critical Values of Chi Square

df	.99	.98	.95	.90	.80	.70	.50	.30	.20	.10	.05	.02	.01	.001
1	.0002	.0006	.0039	.016	.064	.15	.46	1.07	1.64	2.71	3.84	5.41	6.64	10.83
2	.02	.04	.10	.21	.45	.71	1.39	2.41	3.22	4.60	5.99	7.82	9.21	13.82
3	.12	.18	.35	.58	1.00	1.42	2.37	3.66	4.64	6.25	7.82	9.84	11.34	16.27
4	.30	.43	.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	11.67	13.28	18.47
5	.55	.75	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	13.39	15.09	20.52
6	.87	1.13	1.64	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	15.03	16.81	22.46
7	1.24	1.56	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	16.62	18.48	24.32
8	1.65	2.03	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	18.17	20.09	26.12
9	2.09	2.53	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	19.68	21.67	27.88
10	2.56	3.06	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	21.16	23.21	29.59
11	3.05	3.61	4.58	5.58	6.99	8.15	10.34	12.90	14.63	17.28	19.68	22.62	24.72	31.26
12	3.57	4.18	5.23	6.30	7.81	9.03	11.34	14.01	15.81	18.55	21.03	24.05	26.22	32.91
13	4.11	4.76	5.89	7.04	8.63	9.93	12.34	15.12	16.98	19.81	22.36	25.47	27.69	34.53
14	4.66	5.37	6.57	7.79	9.47	10.82	13.34	16.22	18.15	21.06	23.68	26.87	29.14	36.12
15	5.23	5.98	7.26	8.55	10.31	11.72	14.34	17.32	19.31	22.31	25.00	28.26	30.58	37.70
16	5.81	6.61	7.96	9.31	11.15	12.62	15.34	18.42	20.46	23.54	26.30	29.63	32.00	39.25
17	6.41	7.26	8.67	10.08	12.00	13.53	16.34	19.51	21.62	24.77	27.59	31.00	33.41	40.79
18	7.02	7.91	9.39	10.86	12.86	14.44	17.34	20.60	22.76	25.99	28.87	32.35	34.80	42.31
19	7.63	8.57	10.12	11.65	13.72	15.35	18.34	21.69	23.90	27.20	30.14	33.69	36.19	43.82
20	8.26	9.24	10.85	12.44	14.58	16.27	19.34	22.78	25.04	28.41	31.41	35.02	37.57	45.32
21	8.90	9.92	11.59	13.24	15.44	17.18	20.34	23.86	26.17	29.62	32.67	36.34	38.93	46.80
22	9.54	10.60	12.34	14.04	16.31	18.10	21.34	24.94	27.30	30.81	33.92	37.66	40.29	48.27
23	10.20	11.29	13.09	14.85	17.19	19.02	22.34	26.02	28.43	32.01	35.17	38.97	41.64	49.73
24	10.86	11.99	13.85	15.66	18.06	19.94	23.34	27.10	29.55	33.20	36.42	40.27	42.98	51.18
25	11.52	12.70	14.61	16.47	18.94	20.87	24.34	28.17	30.68	34.38	37.65	41.57	44.31	52.62
26	12.20	13.41	15.38	17.29	19.82	21.79	25.34	29.25	31.80	35.56	38.88	42.86	45.64	54.05
27	12.88	14.12	16.15	18.11	20.70	22.72	26.34	30.32	32.91	36.74	40.11	44.14	46.96	55.48
28	13.56	14.85	16.93	18.94	21.59	23.65	27.34	31.39	34.03	37.92	41.34	45.42	48.28	56.89
29	14.26	15.57	17.71	19.77	22.48	24.58	28.34	32.46	35.14	39.09	42.56	46.69	49.59	58.30
30	14.95	16.31	18.49	20.60	23.36	25.51	29.34	33.53	36.25	40.26	43.77	47.96	50.89	59.70