Name: ______________________________
Date: _________ Pd: _________

**Objectives**

**Before doing this lab you should understand:**

Chi-square analysis of data & the life cycle of diploid organisms useful in genetics studies.

**After doing this lab you should be able to:**

Investigate the independent assortment of two genes and determine whether the two genes are autosomal or sex-linked using a multigenerational experiment, and analyze the data from your genetic crosses using chi-square analysis techniques.

**Statistical Analysis Section - Example 1**

Statistics can be used to determine if differences among groups are significant, or simply the result 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. This test can be used to determine if deviations from the expected values are due to chance alone, or to some other circumstance. For example, consider corn seedlings resulting from an F1 cross between parents that are heterozygous for color.

A Punnett square of the F1 cross Gg x Gg would predict the expected proportion of green:albino seedlings would be 3:1. Use this information to fill in the Expected (e) column and the (o-e) column in Table 7.1.

**Table 7.1**

| Phenotype | Genotype | Observed (o) | Expected (e) | (o-e) |
|---|---|---|---|---|
| Green | GG or Gg | 72 | | |
| Albino | gg | 12 | | |
| | **Total:** | 84 | | |

There is a small difference between the observed and expected results, but are these data close enough that the difference can be explained by random chance or variation in the sample?

To determine if the observed data fall within the 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 3:1, an **alternative hypothesis** is accepted (or you fail to reject the null).

The formula for chi-square is $\chi^2 = \Sigma \frac{(o-e)^2}{E}$ where o = observed number of individuals
e = expected number of individuals

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

**Critical Values Table**

| | Degrees of Freedom (df) | | | | |
|---|---|---|---|---|---|
| **Probability (p)** | **1** | **2** | **3** | **4** | **5** |
| 0.05 | 3.84 | 5.99 | 7.82 | 9.49 | 11.1 |
| 0.01 | 6.64 | 9.21 | 11.3 | 13.2 | 15.1 |
| 0.001 | 10.8 | 13.8 | 16.3 | 18.5 | 20.5 |

1. This statistical test will examine the null hypothesis, which predicts that the data from the experimental cross above will be expected to fit the 3:1 ratio.

2. Use the data from Table 7.1 to complete table 7.2.

**Table 7.2**

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(o-e)^2$ | $\frac{(o-e)^2}{e}$ |
|---|---|---|---|---|---|
| Green | 72 | | | | |
| Albino | 12 | | | | |

Degrees of Freedom: ___________________ $X^2$ value = ____________________

Conclusion:

**Example 2**

In a study of incomplete dominance in tobacco seedlings, the counts in table 7.3 were made from a cross between two heterozygous plants. Draw the Punnett square in the box to the right.

**Table 7.3**

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(o-e)^2$ | $\frac{(o-e)^2}{e}$ |
|---|---|---|---|---|---|
| Green | 22 | | | | |
| Yellow Green | 50 | | | | |
| Albino | 12 | | | | |

Degrees of Freedom: ___________________ $X^2$ value = ____________________

A Punnett square for this cross indicates an expected ratio of 1 green:2 yellow green:1 albino. Calculate the expected for each and fill out the Table 7.3.

According to the critical value of $\chi^2$, can you accept or reject the null hypothesis (does the data fit the expected 1:2:1 ratio?)

**Practice Problem**

An investigator observes that when pure-breeding, long-winged fruit flies are mated with pure-breeding, short-winged flies, the $F_1$ offspring have an intermediate wing length. When several intermediate-winged flies are allowed to interbreed, the following results are obtained:

***230 long-winged flies, 510 intermediate winged flies, 260 short winged flies.***

a. What is the genotype of the $F_1$ intermediate-wing-length flies?

b. Write a hypothesis describing the mode of inheritance of wing length in fruit flies (this is your null hypothesis).

c. Complete Table 7.4

**Table 7.4**

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(o-e)^2$ | $\frac{(o-e)^2}{e}$ |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |

Degrees of Freedom: ___________________ $X^2$ value = ____________________

g. Can you accept or reject the null hypothesis? Explain why.

**Procedure: Monohybrid Cross**

1. Go to the website: [http://www.sciencecourseware.com/vcise/drosophila/](http://www.sciencecourseware.com/vcise/drosophila/) and enter as a guest.
2. "Order" a pair of flies: a male wild type for wing size and a female with vestigial wing size. Put them in your "Shopping Cart". Check out.
3. Breed the flies, click on the "Mating Jar" and record the Data in Table 7.5.
4. Sort flies and then send data to computer.
5. Click on analyze results and record your data below. Do not click ignore sex!

**Table 7.5-$F_1$ Generation**

| Phenotype and Symbol | # Females | # Males |
|---|---|---|
| | | |
| | | |

6. Return to Menu, then return to lab, click on the incubator, and then click on the F1 generation mating jar.
7. Zoom in on the pile of females and then click on use in new mating, and then zoom out.
8. Zoom in on the pile of males and then click on use in new mating, and then zoom out.
9. Click return to lab.
10. Click the mating jar to mate the flies.
11. Breed these flies and record the $F_2$ generation data in Table 7.6.

**Table 7.6-$F_2$ Generation**

| Phenotype and Symbol | Females | Males |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

12. Write a hypothesis that describes the mode of inheritance of the trait you studied. This is your null hypothesis.

13. Construct Punnett squares to predict the expected results of both parental and $F_1$ generational crosses from your null hypothesis.

| **Parental Cross** | **$F_1$ Cross** |
|---|---|
| | |

14. Refer to the Punnett squares above. In the table below, record the *expected* ratios for the genotypes and phenotypes of the $F_1$ and $F_2$ crosses in the experiment.

| | **Expected Genotypic Ratio** | **Expected Phenotypic Ratio** |
|---|---|---|
| **$F_1$ generation** | | |
| **$F_2$ generation** | | |

15. From the results, describe your cross.
    Is the mutation sex-linked or autosomal? ________________
    Is the mutation dominant or recessive? ________________

16. Perform a chi-square test on your $F_2$ results to see if the deviations are within limits expected by chance.

| **Phenotype** | **Observed (o)** | **Expected (e)** | **(o-e)** | **$(o-e)^2$** | **$\frac{(o-e)^2}{e}$** |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Degrees of Freedom: ________________ $X^2$ value = ________________

17. Can you accept or reject your null hypothesis? Explain why.

**Procedure: Monohybrid Cross**

1. Return to the lab and click the "trash" to clear the lab of flies. (Click on the trash can to do this).
2. "Order" a pair of flies: a male wild type body color and a female with a yellow body. Check out
3. Breed the flies, click on the "Mating Jar" and record the Data in Table 7.7.

**Table 7.7-$F_1$ Generation**

| **Phenotype and Symbol** | **Females** | **Males** |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

4. Add one female and one male from the $F_1$ Generation and put them into a new mating jar. Breed them and record the $F_2$ generation data in Table 7.8

**Table 7.8-$F_2$ Generation**

| **Phenotype and Symbol** | **Females** | **Males** |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

5. Write a null hypothesis that describes the mode of inheritance of the trait you studied.

6. Construct Punnett squares to predict the expected results of both parental and $F_1$ generational crosses from your null hypothesis.

| Parental Cross | $F_1$ Cross |
|---|---|
| | |

7. Refer to the Punnett squares above. In the table below, record the *expected* ratios for the genotypes and phenotypes of the $F_1$ and $F_2$ crosses in the experiment.

| | Expected Genotypic Ratio | Expected Phenotypic Ratio |
|---|---|---|
| **$F_1$ generation** | | |
| **$F_2$ generation** | | |

8. From the results, describe your cross.
   Is the mutation sex-linked or autosomal? __________________
   Is the mutation dominant or recessive? __________________

9. Perform a chi-square test on your $F_2$ results to see if the deviations are within limits expected by chance.

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(o\text{-}e)^2$ | $\frac{(o\text{-}e)^2}{e}$ |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Degrees of Freedom: ___________________ $X^2$ value = ____________________

10. Can you accept or reject your null hypothesis? Explain why?

**Procedure: Dihybrid Cross**

1. Return to the lab and click the "trash" to clear the lab of flies.
2. Order a wild type female and order a male with sepia eyes and black body.
3. Breed the flies, click on the "Mating Jar" and record the Data in Table 7.9.

**Table 7.9-$F_1$ Generation**

| Phenotype and Symbol | Females | Males |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

4. Add one female and one male from the $F_1$ Generation and put them into a new mating jar. Breed them and record the $F_2$ generation data in Table 7.10

**Table 7.10-$F_2$ Generation**

| Phenotype and Symbol | Females | Males |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

5. Write a null hypothesis that describes the mode of inheritance of the traits you studied.

6. Construct Punnett squares to predict the expected results of both parental and $F_1$ generational crosses from your null hypothesis.

| Parental Cross | $F_1$ Cross |
|---|---|
| | |

7. Refer to the Punnett squares above. In the table below, record the *expected* ratios for the genotypes and phenotypes of the $F_1$ and $F_2$ crosses in the experiment.

| | Expected Genotypic Ratio | Expected Phenotypic Ratio |
|---|---|---|
| **$F_1$ generation** | | |
| **$F_2$ generation** | | |

8. From the results, describe your cross.
   Are the mutations sex-linked or autosomal? ___________________
   Are the mutations dominant or recessive? ___________________

9. Perform a chi-square test on your $F_2$ results to see if the deviations are within limits expected by chance.

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(o-e)^2$ | $\frac{(o-e)^2}{e}$ |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Degrees of Freedom: ____________________ $X^2$ value = _____________________

10. Can you accept or reject your null hypothesis? Explain why?