

## Lab 4: Testing Hypotheses about Patterns of Inheritance

### How do we talk about genetic information?

Each cell in living organisms contains DNA. **DNA** is made of nucleotide subunits arranged in very long strands. By winding around structural proteins, the strands become condensed into compact units called **chromosomes**. Regions in the DNA, known as **genes**, carry specific instructions for making proteins. Genes represent unique combinations of nucleotides that provide information for the genetically-based traits organisms display.

While all humans have the same genes (that is, each human has a gene that codes for eye color, etc), humans are not genetically identical. Think of all the variation in the human population. We have different colors of hair, eyes, different heights, different tendencies towards disease, etc. The reason for the variation we see is that organisms, like humans, have different forms of genes, which we term **alleles**. These alleles code for different proteins, and therefore result in the expression of different traits or **phenotypes**.

A **dominant allele** is expressed phenotypically when present on either a single chromosome or on both homologous chromosomes (that is, when it's present on either the chromosome inherited from the mother, or the father, or both). A **recessive allele** is masked by a dominant allele and is expressed only when paired with another recessive allele on the homologous chromosome (that is, it is only expressed when the chromosomes inherited from both parents have the recessive allele). A pair of identical alleles (*AA* or *aa*) at a gene locus represents the **homozygous condition**. Two different alleles (*Aa*) at a gene locus represent the **heterozygous condition**. For example, eye color in humans is determined by a single gene locus (although other loci can modify its effects). If the alleles at that locus are homozygous dominant (*AA*) or heterozygous (*Aa*), the eyes will be brown. If the alleles are homozygous recessive (*aa*), the eyes will be blue. Alleles for brown eyes are said to be dominant over alleles for blue eyes.

### How is genetic material passed on from generation to generation?

Alleles are passed on from parent to offspring through reproduction, specifically through **meiosis**. Meiosis is the process of cell division that happens only in sex cells and is responsible for creating gametes (sperm and egg). Sperm and egg cells are unique from other body (somatic) cells in that each sperm or egg cell contains exactly half of the genetic material from each parent. *Why do you think this is the case?*

To learn more about meiosis please visit the "Cells Alive" site (<http://www.cellsalive.com/meiosis.htm>). Watch the animations for the cell and as you do, track the locations of the chromosomes as they move through the phases of meiosis. In addition, compare the beginning cells to the end products of meiosis. How many cells does meiosis begin with? How many cells does meiosis end with? Are the numbers and types of chromosomes the same in both cases?

### Using experiments to test patterns of inheritance

Modern genetics experiments began with the work of Gregor Mendel, an Austrian monk with an inquisitive mind. To understand the framework for our lab activity today, please view the Discovery Education Video (<http://www.youtube.com/watch?v=0Dzmdtb8P-0>) found on YouTube about Mendel's Peas and Morgan's Fruit flies. You may need to type in the address above depending on the browser you use. You can begin the video at 2:20 seconds and stop watching at the end of the clip (yes, it abruptly ends, but that's okay!).

Mendel and Morgan performed experimental crosses, or matings between organisms with the same or different characteristics, such as round or wrinkled peas. A **monohybrid cross** is one in which the pattern of inheritance of a single pair of alternative alleles is studied (e.g., eye color being either red or white in fruit flies). In contrast, a **dihybrid cross** involves parents that are identical except for two independent traits (e.g., color and shape of peas). In both Mendel's and Morgan's experiments, it was important to begin with parents that are said to be **true breeding**. This means that the parents are homozygous for the traits of interest for the study. Because the genetic makeup is known for the starting organisms, this enables scientists to track the genotypes (or the genetic makeup of the organisms) through generations.

Mendel discovered that certain traits can mask one another (i.e. dominant traits mask recessive ones). Morgan built upon Mendel's findings through his discovery that traits may be **sex-linked**, meaning the gene is carried on the X chromosome. Females have two copies of the X chromosome, and therefore carry two alleles of sex-linked genes, one on each X chromosome. Males, however, only have one X chromosome and therefore only carry one allele of a sex-linked gene. This means that whatever allele is passed on to males on the X chromosome will be expressed regardless of whether it is dominant or recessive. Since females inherit two alleles for sex-linked genes, expression of the alleles follows the dominant/recessive model described above. Any genes that are present on the other 22 pairs of chromosomes (that is, not on the X or Y chromosomes) are said to be **autosomal**. Mendel's pea plant traits are inherited on autosomal chromosomes.

**Reciprocal crosses** were important to Morgan's discovery about sex-linked genes. A reciprocal cross is a cross between parents that is opposite the original cross. For example, Morgan initially crossed a red-eyed female with a white-eyed male. For the reciprocal cross, Morgan used different organisms with opposite traits, in this case, a white-eyed female and a red-eyed male. The patterns of traits in the offspring were different through these two crosses (the original and reciprocal) and as a result, Morgan was able to determine that this eye-color trait in fruit flies was sex-linked.

Finally, traits that are naturally common in the wild (e.g. red eyes in fruit flies) are known as **wild-type** traits. Less common traits (e.g. white eyes in fruit flies) are known as **mutant** traits. These are derived from characteristics that are expressed naturally in the wild. *It's important to note that wild-type traits are not always dominant, nor are mutant traits always recessive.*

### **Hypotheses about Inheritance Methods**

The laws of Mendelian genetics allow you to make predictions about the outcome of crosses before you perform an experiment, and then determine whether or not the patterns you observe in an experiment match what you would expect if that hypothesis was true. Under each model, you can predict what traits offspring will have if the model is true (expected values), and compare these data to actual experiments (observed values).

There are four models that explain the inheritance of most traits: sex-linked dominant, sex-linked recessive, autosomal dominant, and autosomal recessive. When considering 1 or 2 traits, these possible models may be combined to create as many as 8 hypotheses about patterns of inheritance. These are the hypotheses we will be working to test today.

Our **hypothesis** is that there is a difference between expected and observed phenotypes and the difference may be that...

### Monohybrid Hypotheses

- 1) Mutant trait is autosomal recessive
- 2) Mutant trait is autosomal dominant
- 3) Mutant trait is sex-linked dominant
- 4) Mutant trait is sex-linked recessive

### Dihybrid Hypotheses

- 5) Mutant trait 1 is autosomal recessive and mutant trait 2 is autosomal recessive
- 6) Mutant trait 1 is autosomal dominant and mutant trait 2 is autosomal dominant
- 7) Mutant trait 1 is autosomal dominant and mutant trait 2 is autosomal recessive
- 8) Mutant trait 1 is autosomal recessive and mutant trait 2 is autosomal dominant

There are several ways to construct predictions, or expected data assuming a hypothesized mode of inheritance is true. We will use the **Punnett square** method because it is an easy-to-remember algorithm based on simple bookkeeping. For the types of traits we will study in lab, it is necessary to predict the results of parental crosses (producing the first (F1) generation) and of F1 crosses (producing the second (F2) generation). The predicted phenotypic ratios among the F2 generation are converted to expected results and will be used to compare with the results of real (or simulated) crosses to test your hypotheses.

### Using Punnett Squares to Predict Inheritance

The Punnett square technique involves six basic steps and is a careful way of predicting how the chromosomes bearing a trait of interest may sort during meiosis into gametes, then combine at fertilization. These steps corresponding with an example are described below.

*You have a cross and are interested in determining how the trait for Brown eyes is inherited. You hypothesize it may be autosomal dominant, and will use a Punnett square to determine the expected phenotype ratio for this hypothesis.*

Step 1: Set up the parental cross based on one hypothesis.

Determine if the cross is a monohybrid or dihybrid cross. Assign a different letter to symbolize each trait: the hypothesis you are using will determine which you assign to the wild type and which you assign to the mutant trait

Step 1: Establishing the Parents for the Cross

Both parents are true breeding (and express opposite traits)

Male = AA (Brown eyes)    Female = aa (Blue eyes)

Step 2: Use the Punnett square to create the F1 generation of the parental cross.

The genotypes of the gametes of the parents are placed at the head of each column (for the male parent) and row (for the female parent). The remaining boxes in the Punnett square represent the genotypes of the offspring produced by combining the parental genotypes of one type of sperm and one type of egg. Fill in the progeny genotypes by writing in the genotype of the mother's gametes (row) and the father's gametes (column) corresponding to each square in the body of the table. These entries are the expected genotypes of the F1 generation.

Step 2: Cross 1 (cross the true breeding parents to create the possible F1 offspring)

	A	A
a	Aa	Aa
a	Aa	Aa

Step 3: Analyze the F1 progeny to determine the genotypic and phenotypic ratios. To determine the **genotypic ratios** of the F1s, simply count the number of squares represented by each genotype. Then, convert the number to a ratio of total genotypes. To determine the **phenotypic ratios**, refer back to the basic hypothesis being analyzed, and determine the ratio of offspring that express the phenotypes.

Step 3: Analyze the F1 generation

All offspring are heterozygous (Aa) and would have brown eyes.  
(Genotype ratio and phenotype ratio are 100%)

Step 4: Construct a new Punnett square to predict the genotypes of the progeny of the F1 cross. Follow the same procedures used in Step 2, using the genotypes of the F1 male and female parents. F1 male alleles will go in the boxes heading each column and F1 female alleles will go in the boxes heading each row. Continue to fill in the rest of the boxes as you did in Step 2.

Step 4: Cross 2 (cross two offspring from F1 to create the possible F2 offspring)

	A	a
A	AA	Aa
a	Aa	aa

Step 5: Determine the genotypic and phenotypic ratios among the F2, the progeny of the F1 cross. Use the same procedures and reasoning you used in Step 3. The F2 genotypic and phenotypic ratios you generate in this step are predictions about what the F2 ratios should be if the basic hypothesis is true. *This is the set of predictions you can test against a real cross to evaluate if the hypothesis might be true.*

Step 5: Analyze the F2 generation

When the trait of interest (brown eyes) is hypothesized to be autosomal dominant, our prediction shows that the F2 offspring will display the brown and blue phenotypes in a 3:1 ratio (3 brown-eyed offspring for every 1 blue-eyed offspring). All possible combinations of A and a alleles are represented in the genotypes of the F2 offspring. For every homozygous dominant (AA) offspring, we expect to see 2 heterozygote (Aa) offspring, and 1 homozygous recessive (aa). The final phenotypic and genotypic ratios are represented as:

1 AA : 2 Aa : 1 aa = genotype ratio

3 brown eye : 1 blue eye = phenotype ratio

Step 6: Convert the ratios to expected values, assuming that we are working with 100 offspring. You will need to scale up the ratio to determine the actual number of offspring out of 100 that will bear that particular phenotype.

**Step 6: Expected values**

To determine expected values, we convert the ratio to a percentage. For example,  $\frac{1}{4}$  of the offspring (25%) will be homozygous dominant,  $\frac{1}{2}$  of the offspring (50%) will be heterozygous, and the remaining  $\frac{1}{4}$  (25%) will be homozygous recessive. Next, we multiply the percentages by the total number of offspring, in this case 100, to determine the actual number of offspring we might expect if this hypothesis is true.  $0.25 \times 100 = 25$  offspring will be homozygous dominant, and express brown eyes.  $0.50 \times 100 = 50$  offspring will be heterozygous and express brown eyes.  $0.25 \times 100 = 25$  offspring will be homozygous recessive and express blue eyes.

Each model of inheritance (i.e. alternative hypothesis) has a unique F2 generation prediction, and therefore the results of experiments can be compared to the predicted value to determine how traits are passed on from parents to offspring. The best model will be the one whose predictions do not differ statistically from the experimental values. We will use the Chi-Squared Test Statistic (see Appendix B) to determine whether or not our experimental data actually matches our predictions.

**What you will do**

In today's lab, you will test hypothesized patterns of inheritance using a computer simulation of fruit flies (<http://www.biosci.msu.edu/FlyWeb/>). First, you will make observations of patterns of inheritance in offspring resulting from true breeding parents. Then, you will eliminate competing hypotheses about how the trait(s) of interest are inherited, and test selected hypotheses with the Chi-Square statistic.

**Laboratory Objectives**

As a result of participating in this laboratory activity, you will

1. Make predictions about patterns of inheritance
2. Practice using Punnett Squares as predictive tools
3. Identify expected outcomes (predictions) of hypotheses and relate these to predictions
4. Evaluate competing hypotheses about modes of inheritance
5. Use the Chi-Square test to evaluate support for hypotheses
6. Draw conclusions about modes of inheritance and hypothesis testing

**Methods:**

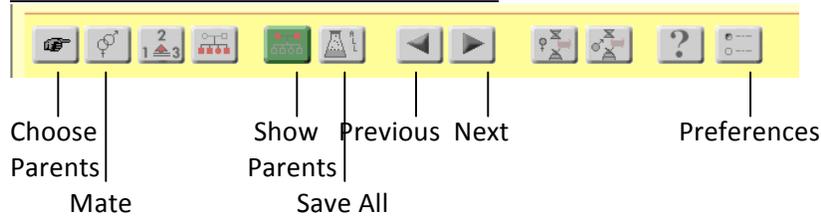
**Testing Hypotheses of Inheritance**

Using the Fly Web computer simulation, work in groups to:

1. Perform each cross indicated in the instructions that follow
2. Use your data to eliminate hypotheses from the 8 alternatives posed above. Choose the hypothesis for the mode of inheritance that best predicts your data
3. Test your hypothesis using a Chi-Square Test for Goodness of Fit
4. Draw conclusions from the Chi-Square test to determine whether or not your hypothesis can be accepted or rejected.
5. Work with your group to complete the questions throughout the lab activity. Turn in your completed questions (1 set per group) as your post-lab assignment for today.

## Part 1. Fly Web Lab

### LEGEND for the COMPUTER SIMULATION



### Possible traits of *Drosophila*:

Sex: Male (short body with sex combs) or Female (long body without sex combs)  
Eye Color: Red (Wild Type), Purple, Dwarf Red Eye, Sepia, or White  
Body Color: Brown (Wild Type), Yellow, or Blackish Grey  
Wings: See visualizer – TAs will have pictures

### Hypotheses for Elimination:

#### Monohybrid Hypotheses

- 1) Mutant trait is autosomal recessive
- 2) Mutant trait is autosomal dominant
- 3) Mutant trait is sex-linked dominant
- 4) Mutant trait is sex-linked recessive

#### Dihybrid Hypotheses

- 5) Mutant trait 1 is autosomal recessive and mutant trait 2 is autosomal recessive
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- 7) Mutant trait 1 is autosomal dominant and mutant trait 2 is autosomal recessive
- 8) Mutant trait 1 is autosomal recessive and mutant trait 2 is autosomal dominant

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- Step 1 Go to <http://www.biosci.msu.edu/FlyWeb>
- Step 2 Click on "Fly Cross #1"  
(You may have to Install Active X and Shockwave)  
a. Once the main screen loads, click the green arrow at the bottom  
b. Click "ok" in the Parent Dialog box
- Step 3 Record the phenotypes of each parent in **Table 1** (Males have a short body, Females have a long body)

- Step 4 Click on the "Preferences" icon. Check the "Show Generations" box then "ok" (You will need to do this before EVERY cross)
- Step 5 Mate the flies by clicking the "Mate" icon.
- Step 6 Record the phenotypes and numbers of resulting offspring (F1 generation) [You should ALWAYS get a total of 100 offspring]

**Table 1 Cross #1**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				

**1) Based on your data in Table 1, are there any hypotheses (of the 8 possible hypotheses) that you can eliminate? Explain.**

Step 7 Perform the reciprocal cross. Click on "Fly Cross #2" (use the back button to get to Fly Cross 2)

Step 8 Mate the parental flies and record the phenotypes and numbers of the resulting offspring in **Table 2**

**Table 2 Reciprocal Cross of #1**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				

**2) Based on the data in Tables 1 & 2, how is the mutant trait inherited (remember, wild type is Red Eyes, Brown Body and Normal Wings)? How do you know?**

**3) What is the expected outcome (ratio) of the F2 offspring, *if your hypothesis is true?***

Step 9 Repeat Steps 2-8 for Fly Cross #3 and Fly Cross #4 and record the observed results in **Tables 3 & 4**.

**Table 3 Cross #3**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				

**4) Based on your data in Table 3, are there any hypotheses that you can eliminate? Explain.**

**Table 4 Reciprocal Cross of #3**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				

**5) Based on the data in Tables 3 & 4, how is the mutant trait inherited (remember, wild type is Red Eyes, Brown Body and Normal Wings)? How do you know?**

**6) What is the expected outcome (ratio) of the F2 offspring, *if your hypothesis is true?***

Step 10 Repeat steps 2-8 for Fly Cross #5 and Fly Cross #6. Record observations in **Tables 5 & 6**.

Step 11 Save all of your offspring by clicking the “Save All Offspring” icon

Step 12 Click the “Show Parents” icon

Step 13 In order to produce the F2 generation, you must mate the F1 generation:

- a. Click the “Choose Parents” icon
- b. Click on one of the F1 flies and click “Choose” when the dialog box appears
- c. Click on the other F1 fly and choose it as the second parent.

Step 14 Click the “show parents” icon and then click “Mate” to show the F2 generation  
 (You will have to use the green “next” arrow to see all 100 offspring)

Step 15 Record all phenotypes and numbers of the resulting offspring in **Table 5 & 6**.

Do you have 100 offspring? You should! Use the green arrows to find all offspring.

**Table 5 Cross #5**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				
F2:					

**7) After just this first cross, are there any hypotheses that you can eliminate? Explain.**

**Table 6 Reciprocal of Cross #5**

Fly Generation	Sex	Eye Color	Body Color	Wings	Number of Individuals
Parental:	F				XXXXXXXX
Parental:	M				XXXXXXXX
F1:	F				
F1:	M				
F2:					

**8) Based on the above Tables 5 & 6, how are the mutant traits inherited (Remember, Wild Type is Red Eyes, Brown Body and Normal Wings)? How do you know? (2 pts)**

Step 16: Next, you will test the hypothesis you just came up with in #8 above, using a Chi-Square test. Follow the directions below to complete your test.

**13) What are the expected values for your hypothesis? That is, if your hypothesis is true, how many F2 offspring (out of 100) would you expect to have of each phenotype? Show your work! (2 pts)**

**14) Complete the Chi-Square table, comparing your observed and expected values. The observed values come from the F2 generation of cross 5 (data found in Table 5). (2 pts)**

Phenotypes	Observed	Expected	$(O-E)^2$	$(O-E)^2/E$
<b>sum</b>				

Degrees of freedom =

**15) What are your conclusions? Is the mode of inheritance different from the hypothesis that you chose? How do you know? (2 pts)**

## Part 2. Modeling Meiosis:

You have just completed a lab activity where you were crossing fruit flies to determine how traits were inherited. Remember that each parent fruit fly produces gametes with genetic material carried on chromosomes, and it is the combination of these gametes to form a zygote that is responsible for inheritance of genetic information. To check your understanding of meiosis, use the following fly scenario to model meiosis:

In fruit flies, eye color and wing shape are unrelated; that is, eye color is coded for by a gene on a separate chromosome than is wing shape. Red eyes (R) are dominant; purple eyes (r) are recessive. Normal wing shape is recessive (n) and wrinkled wings are dominant (N). The female fruit fly is heterozygous for eye color, and has normal wings. The male fruit fly has purple eyes and is heterozygous for wing shape.

1. What is the genotype of the female fruit fly? \_\_\_\_\_ (1 pt)
2. What is the genotype of the male fruit fly? \_\_\_\_\_ (1 pt)
  
3. Model the chromosomes for the female fruit fly at: (10 pts)
  - (a) G1
  - (b) G2
  - (c) Metaphase of Meiosis 1
  - (d) Metaphase of Meiosis 2, and
  - (e) Products of meiosis (gametes)

Be sure to label all alleles, homologous chromosomes, and sister chromatids.

### **Appendix A. Chi-Square Test Statistic (specific to Mendelian Genetics)**

This test compares actual (observed) and predicted (expected) outcomes of an experiment. For each type of outcome (in our case, the number of F2 generation organisms of a particular phenotype) in the experiment, there are both observed and expected numbers of occurrences. The Chi-Square statistic is used in this test:

$$X^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

Statisticians have determined the probability distribution of the Chi-Square statistic (Table 1). You will use Table 1 for the Chi-Square statistic to analyze your results. This table will let you decide if the difference between observed and expected values is small enough to be due to chance (meaning the hypothesis may be accepted) or is too large to be due to chance (meaning the hypothesis produces a poor prediction of your results and is unlikely to be accepted).

**Table 1. Chi Square Probability Table**

Degrees of freedom	Reject the hypothesis if $X^2$ is greater than:
1	3.841
2	5.991
3	7.815
4	9.488
5	11.070

**Table 2. A simple way to manage the Chi-Square statistic**

Phenotype or Genotype	Observed (O) #	Expected (E) #	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> /E
Homozygous brown		25			
Heterozygous brown		50			
Homozygous blue		25			
	-----	----	---	---	SUM this column

In Table 2, the observed numbers will come from the data you collect during lab while the expected numbers will be derived from your hypotheses (the values here come from our above example of Punnett Squares). To determine the degrees of freedom (df) you simply take the number of phenotypes (or rows in the table) and subtract 1. In our case,  $df = 3 - 1 = 2$ . Now use Table 1 to look at the row for the appropriate degrees of freedom (in our case, 2) to get a Chi-Square probability value. Compare this number to the value you obtained in Table 2 and determine whether or not the hypothesis you were testing holds true. If the number you calculated is greater than the number you pulled from Table 1, you fail to accept (i.e. reject) the hypothesis you were testing. However, if the value you calculated for Chi-Squared is smaller than the number in Table 1, you will not reject the hypothesis you tested.

#### **Works Cited**

Elzinga, C., S. Michaud Lawrence, L. M. Leege, M. K. Heidemann, and D. O. Straney. 2007. Biological Sciences 110: Laboratory Manual. 12<sup>th</sup> Edition. Plymouth, MI: Hayden-McNeil Pub.