

# Microevolution & Hardy-Weinberg Equilibrium

## Objectives:

- Know the terms **evolution, macroevolution, microevolution, gene, locus, allele, genotype, homozygous, heterozygous, phenotype**, and how these are interrelated.
- Understand the principles of **Hardy-Weinberg Equilibrium**.
- Be able to calculate allele and genotype frequencies for a population.
- Understand the principles of **Hardy-Weinberg Equilibrium**.
- Understand the influences of **mutation, migration, non-random mating (sexual selection and phenotypic assortment), genetic drift, and natural selection** on HW equilibrium and microevolution.
- Be able to calculate allele and genotype frequencies for a population.
- Understand the concept of **fitness**.

## Introduction:

Whether we study molecules or whole organisms, ecology or medicine, every area of biology is much better understood by having a firm grasp on the principles of evolution. Introduction to Organismal Biology will take a strong evolutionary perspective on the study of biology.

The simplest definition of evolution is lasting change over time in biological populations. There are two different scales of evolutionary change: **macroevolutionary** and **microevolutionary**. Macroevolution deals with changes in species composition over large amounts of time, whereas microevolution is the changes that occur in allele frequency within a population over a span of generations.

In this series of evolution labs you will get familiar with some important terms and concepts that will be used repeatedly throughout the semester, learn the Hardy-Weinberg equation and the causes of microevolution, and gain an understanding of both the drivers of and outcomes of macroevolution.

## **Hardy-Weinberg Equilibrium:**

Prior to the discovery of meiotic segregation and Mendelian inheritance, Scientists were unable to explain the persistence of genetic variability. If selection always favored optimal forms, then any new genetic variant that arose would either be quickly extinguished (if less optimal) or spread to uniformity in the population (if it were more advantageous). In 1908, G.H. Hardy (a British Mathematician) and W. Weinberg (a German doctor) independently solved the puzzle of why genetic variation persists in nature. Using basic Mendelian inheritance, they demonstrated that the original proportions of alleles in the population would remain unchanged from generation to generation (a condition now known as **Hardy-Weinberg equilibrium**) if the following conditions were met:

- 1 – There must be no mutation
- 2 – There must be no emigration or immigration
- 3 – Mating must be completely random, meaning that all possible mating combinations are equally likely
- 4 – There must be no Natural Selection
- 5 – The population must be infinitely large

As you have probably already guessed, it is highly unlikely that all of these conditions will be met. Therefore evolution is very likely to occur. In the exercises you complete today you will look at the impact of violating each of these assumptions on allele frequencies and you will also investigate how these assumptions are interrelated.

The **Hardy-Weinberg equation** can be used to calculate allele frequencies and/or genotype frequencies in sexually reproducing populations. Consider a population of 100 Guppies characterized by a single trait controlled by two alleles. There are 84 Red Guppies and 16 Blue Guppies. Statistically speaking, Reds are 84% of the population or a frequency of 0.84. Blue Guppies are 16% (0.16) of the population.

Given what we know about Mendelian inheritance, can we calculate the allelic frequency of the population? If we assume that the Blues are homozygous for the recessive blue allele ( $bb$ ), and the Reds are either Homozygous dominant ( $RR$ ) for the red allele or heterozygous ( $Rb$ ), then we can calculate the allelic frequencies.

Assume  $p$  is the frequency of one allele, and  $q$  is the other allele. Since there are only two alleles, the frequency of  $p$  and  $q$  must equal one:

$$p + q = 1$$

The Hardy-Weinberg equation can now be expressed as a binomial expansion  $(p+q)^2$

$$p^2 + 2pq + q^2 = 1$$

If  $q^2 = 0.16$  (frequency of blue alleles), then  $q = 0.4$  (square root of 0.16). Given that  $p$  and  $q$  must sum to 1, then  $p = 0.6$ .

We can now calculate the Genotype frequencies:  $p^2 = (0.6)^2 = 0.36 \times 100$  (the number of Guppies in the total population) = 36 Homozygous Dominant individuals. Heterozygotes have both alleles and so are  $2pq$  in the population ( $2 \times 0.6 \times 0.4$ ) = 48. And of course, there are 16 homozygous recessive guppies (our original blues).

**Exercise 1:**  
**Calculating allele, genotype and phenotype frequencies (work individually)**

Suppose you have a population of frogs that have a single gene that codes for skin color. This gene has two alleles **G** for **green** and **g** for **albino**. Homozygous GG individuals are green, Heterozygous individuals are green, and Homozygous gg individuals are albino. You are told that the frequency of the g allele in this population of frogs is **0.1**. Answer the following questions:

- ▶ What is the frequency of G? \_\_\_\_\_
- ▶ What is the frequency of Homozygous GG individuals? \_\_\_\_\_
- ▶ What is the frequency of Heterozygous (Gg) individuals? \_\_\_\_\_
- ▶ What is the frequency of Homozygous gg individuals? \_\_\_\_\_
- ▶ What is the frequency green frogs? \_\_\_\_\_
- ▶ What is the frequency of albino frogs? \_\_\_\_\_

## Natural Selection

When people are discussing evolution, they are very often referring to **evolution by natural selection**. The process of evolution by natural selection was elegantly laid out in Darwin's (1859) *The Origin of Species*. Simply stated, if a trait allows an organism to have more offspring, then that trait will be present in a greater proportion in the following generation. If natural selection acts in the same direction over several generations then that trait will become more and more prevalent and possibly even **fixed** in that population (fixed means that there is no variation in the population with respect to that trait). Remember conditions can change over time, and what was once a beneficial trait could become neutral or even detrimental/deleterious. Also keep in mind that the strength of natural selection can vary over generations as well.

### Exercise 2: Effects of Natural Selection (with genotypes listed on the board and class results pooled)

In this exercise, you are a member of a predatory species that is endemic to WSU (*Harvardophagus worcesteri*), whose diet consists entirely of a species that originated at Harvard (*Harvardensius bostonii*). We will be tracking the changes in allele and genotype frequencies of the you the predators (*H. worcesteri*).

#### *The Predators (Harvardophagus worcesteri)*

*H. worcesteri* are small creatures that can only eat a single *H. bostonii* at a time. They must compete furiously for the limited number of prey items and consume enough to produce gametes (eggs and sperm). They live for a short time, spawn and then promptly die.

The clarity of their vision and their ability to discriminate colors is affected by two alleles (isn't that convenient!). **R** is the dominant allele (represented by a Red Pop Bead), and it codes for "red vision," **y** is the recessive allele (represented by a Yellow Pop Bead) and it codes for "clear vision." The genotypes and resultant phenotypes are listed below:

**RR → Red Vision**  
**Ry → Red Vision**  
**yy → Clear Vision**

The number of gametes that any individual can produce is controlled by the number and color of *H. bostonii* (represented in our exercise by legumes-- split peas) that it consumes. The pink *H. bostonii* pack more nutrition than do the yellow prey items:

**Pink → 3 gametes/ individual eaten**  
**Yellow → 1 gametes/ individual eaten**

## **2A. Setting up the Initial Predator Population (Generation 0):**

A large container of yellow and red "pop beads" will be set up to serve as a "sperm pool" (or "sperm bank"), and another container will be set up as an "egg pool." (Together, these two gamete pools represent the "**gene pool**" of the population.) To begin the exercise, each student must, without looking, select one red or yellow allele from the sperm pool and one red or yellow allele from the egg pool. These alleles determine your genotype (RR, Ry, or yy), as specified above. **Record your genotype** on the front board, and add up the numbers of each genotype for the entire class. **Calculate the genotype frequencies and the allele frequencies for this Initial Population (Generation 0). Record these results.** Also, empty the egg pool and sperm pool of all pop beads.

## **2B. Selection phase:**

- 1) If you are RR or RY you must wear a pair of Red Vision goggles. If you are YY you do not need to wear any goggles.
- 2) You will all gather around your population of prey and you will each be given 10 seconds to consume as many prey items (legumes) as possible, with the goal of being able to maximize your fitness (produce the most gametes when you reproduce). You are only allowed to use your thumb and forefinger on one hand to pick up the prey items, and you can only pick up a single prey item at a time (No scooping!). You will be given a "stomach" (a cup) into which you will deposit your prey items.
- 3) At the end of a round of predation you should calculate the number of gametes you can produce (based on the prey values above) and collect the appropriate number of colored beads. (Note – if you can produce an odd number of gametes, you should round UP to the next even number). If you are homozygous, all your gametes will be the same color bead, but, if you are heterozygous, half of your gametes will be red and half will be yellow. If you are male, place all your gametes into the "sperm pool" container; if you are female, place all your gametes into the "egg pool" container. Also, return all prey items (legumes) to the selection area and thoroughly mix them up.
- 4) Random mating will now occur! Each student will go and randomly draw a bead from the "Sperm Bucket" and a bead from the "Egg Bucket" to determine their genotype and phenotype for the next generation. **Record your genotype** on the front board, and add up the numbers of each genotype for the entire class. **Calculate the genotype frequencies and the allele frequencies for this New Generation. Record all results.**

**REPEAT THE SELECTION PHASE for at least three generations.**

► Record what happened to the frequency of each allele in the predator population over time. What forces or processes caused the frequencies that you observed?

► Which allele was the dominant allele in the predator population, and which allele was better to possess? Were these the same allele? What does it mean if an allele is dominant? Discuss this with your lab partners if necessary.

► What do you think the point was of tracking the number of gametes produced by each predator instead of survival?

► In what ways was your class exercise a realistic model of a natural predator population? In what ways was the model unrealistic?

## **M&M EXERCISES!!!**

To demonstrate effects of the Hardy-Weinberg equilibrium and violating the assumptions, we will use hypothetical organisms with traits that are represented by different alleles.

**CAUTION:** Please keep your fingers clean and dry during this lab. The M&M's will get handled by multiple people and are therefore not safe to eat.

**Materials:** M&M's (a quirky species known for its tastiness to predators, unusual vocal patterns, and resistance to thermal stress except in the presence of salivary digestive enzymes). It is easiest to use Plain M&M's to represent recessive traits, and Peanut M&M's to represent dominant traits, and to use different colors to represent different alleles. The specific colors used are not important, so long as the traits represented by the colors are kept clear. For Example, a trait (say feather color) takes two forms Red (dominant) and Orange (recessive). Use Red Peanut M&M's and orange plain M&M's to represent the two alleles. Follow instructions on the board regarding colors.

**Breeding:** Unless otherwise specified, probabilities are 50% and can be determined by a simple coin flip. More complex probabilities can be simulated using polyhedral dice (available at gaming shops in 4-, 6-, 8-, 10-, 12-, and 20-sided varieties). A standard 6-sided die (d6) can simulate probabilities of 50% (1-3, 4-6), 33% (1-2, 3-4, 5-6) and 16.6%(1 of 6). In order to simulate random mating, numbers can be drawn to pair individuals (or one can use a d10). All individuals mate, all produce 2 offspring, and all individuals are semelparous (breed once, then die). Therefore, there will be NO population growth under default conditions. Each parent has a 0.5 probability of passing each allele to its offspring. Calculate this probability separately for each offspring. Thus, a parent who is homozygous may give the same allele to each offspring OR give one of each. The probability in each case is 0.5 and they are independent of one another.

### **Exercise 3: Hardy-Weinberg Equilibrium in a population of M&Ms (work in groups of 4-5; post your results up front on the board)**

Start with a population of 10 individuals, each of which is heterozygous for alleles represented by two different colors (say, red and orange). The allelic frequency is 10:10 (Red: Orange), genotypic frequency is 0:10:0 (RR:Ro:oo) and the phenotypic frequency is 10, 0 (Red feathers: Orange Feathers). To obtain the F1 generation, randomly mate the population as follows: from a container containing 10 gametes of each color, select a male and a female gamete, record the resulting genotype, and return the gametes to the gene pool (sampling with replacement). Repeat this procedure until a new population of 10 individuals has been picked, then calculate the allelic, genotypic, and phenotypic frequency of the resulting population. Adjust the gene pool to reflect the composition of the F1 population, then repeat the procedure to obtain an F2 population of 10 new individuals. Calculate the allelic, genotypic, and phenotypic frequency of the resulting population. Also, report your parental, F1, and F2 results on the front board and calculate the pooled results for the entire class.

► Compare the frequencies in the three generations. Was the Hardy-Weinberg equilibrium satisfied? If not, what condition may have been violated? Now, sum the data for the entire class. With the larger sample size, was the Hardy-Weinberg equilibrium satisfied?

### **Exercise 4: Demonstrating Genetic Drift (work in groups as in Ex. 3)**

Even populations under no selection may experience changing gene frequency due to stochastic processes (involving chance events). Population size can strongly affect the evolution of a population. The importance of population size is that changes in allele frequencies due to chance alone (**genetic drift**) are more likely when a population is small. It is important to remember that changes that occur as a result of genetic drift are completely random; in other words, the changes are NOT a result of one allele being any better than another allele. These changes are NOT the result of natural (or sexual) selection. In this exercise, we will demonstrate the **founder effect**, a particular type of genetic drift that occurs in a population that is temporarily small, as in a population of any size derived from the breeding of a small number of founder individuals.

Start with a gene pool containing 10 gametes of one color and 10 of another color. Calculate the allelic, genotypic, and phenotypic frequency of this original parent population. Now randomly select just four individuals in the manner that you did previously. Assume that these randomly selected individuals were swept out to sea in a storm and ended up on an island. Calculate the allelic, genotypic, and phenotypic frequencies of this "founder" (F1) population, and adjust your gene pool to reflect the gametes (8 in all) of these founders.

Breed the four "survivors" (founders) in the same fashion as before, except that each pair produces FOUR offspring (there's lots of room and lots of food on the island), so that the population doubles to 8 individuals in the F2 generation (children of the F1 founders). Calculate the allelic, genotypic, and phenotypic frequency of the F2 population, then readjust the gene pool to match the new frequencies and repeat the procedure to obtain an F3 population of 16 individuals representing the grandchildren of the founders.

► Are the allelic frequencies in the new island population different from the original population? Is this a result of selection?

- ▶ Compare your results with one other group. Was their group of four "founders" the same as yours or different? After three generations, were their genotype frequencies the same as yours or different? If they were different, what accounts for the differences?

## **Selection & Fitness**

In discussions of Natural Selection and Evolution, **fitness** does not refer to your BMI, or how fast you can run a mile. Rather, it is a measure of an individual's relative contribution to the gene pool of the next generation.

- ▶ Which individual is more fit? An extraordinarily attractive person who never had any children, or a person of average attractiveness who had three children? Why?

## **Exercise 5: Effects of Non-Random Mating (Sexual Selection)** **(work in groups as in Ex. 3-4)**

Some genes will code for traits that have no bearing on mating success. Alleles for those genes will not evolve via sexual selection because with respect to these alleles mating is entirely random. However, other genes code for traits that have a direct impact on whether or not that individual is likely to mate. For example, individuals with traits that are attractive to the opposite sex are more likely to reproduce. Individuals that have more offspring than another individual have greater evolutionary **fitness**. Sexual selection is treated as a separate force from natural selection because it deals only with traits relevant to the reproductive behavior of that species, and because it often drives the evolution of traits that may not always benefit the individual. For example, the bright, big, showy tail of a peacock is very attractive to peahens, but it also makes them far more visible to predators!

Start with a population of 10 individuals, each of which is heterozygous (Ro) for the alleles represented by two different colors such as red and orange, so the allelic frequency is

10:10 (Red R: Orange o). This time, perform the matings as follows:

- 1) select two gametes from the gene pool to represent one parent;
- 2) homozygous recessive individuals are considered unattractive to the opposite sex and are not allowed to mate, so if you have selected a homozygous recessive, put their genes aside (out of the gene pool) and try again;
- 3) if you have selected an individual with at least one dominant allele, choose one of the two alleles (without looking) to contribute to the next generation's gene pool. (Write down what allele you picked, then return the M&M's to the gene pool that they came from.)
- 4) Repeat steps 1-3 for the other parent and record the genotype of the new offspring.
- 5) Now keep repeating steps 1-4 until you have recorded the genotypes of a new generation of 10 diploid individuals (20 alleles in all). Calculate the allelic, genotypic, and phenotypic frequency of the F1 generation.
- 6) Adjust the gene pool of M&Ms to match the composition of the F1, then repeat all the above steps to generate an F2 population. Do this yet again to generate an F3 population. Calculate the allelic, genotypic, and phenotypic frequency of the F2 and F3 generations.

► Compare the frequencies in the three generations. How have they changed? How do you explain the changes?

## Hardy-Weinberg - Follow-up Homework

Instead of a full lab report, please type your answers to the six questions below.

1) Is a dominant allele always a good allele? Give evidence from your lab exercises.

**Answer:**

2) In the Natural Selection Exercise, what was the point of having the ability to produce gametes affected by the quantity and quality of food you were able to eat?

**Answer:**

3) When you did Exercise 3, was Hardy-Weinberg equilibrium maintained? Why or why not?

**Answer:**

4) What happened to allele frequencies in Exercise 4? If a change occurred, what was the driving force that caused the change?

**Answer:**

5) What happened to allele frequencies in Exercise 5? If a change occurred, what was the driving force that caused the change?

**Answer:**

6) You are studying field mice. In the following table are your records for mouse size, how long each mouse lived, and how many offspring per year each mouse had.

Name	Weight (g)	Years Lived	Number of Offspring Produced Per Year
Harry	25	3	2
Sally	20	5	2
Ren	15	1	7
Stimpy	22	2	4
Pinky	10	3	4
The Brain	12	5	1

Which mouse had the greatest fitness? Explain why.

**Answer:**