

*"Nothing in Biology Makes Sense Except in the Light of Evolution" - Dobzhansky*

# 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), genetic drift, and natural selection** on HW equilibrium and microevolution.
- Understand the concept of **fitness**.

## Introduction:

Whether we study molecules or whole organisms, ecology or medicine there is no area of biology that is not 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.

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.

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## **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 G. 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 (be in **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
- 4 – There must be no Natural Selection
- 5 – The population must be infinitely large

NOTE: Random in condition 2 is a mathematical term meaning each possible combination is equally likely. It does not mean haphazard or careless mating.

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  $(p+q)^2$

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

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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**

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

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 (*Harvardensu bostonii*). We will be tracking the changes in allele and genotype frequencies of the you the predators (*H. clarkensii*).

#### *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* (which are represented by legumes in our exercise) it consumes. Pink *H. bostonii* pack more nutrition than do the yellow prey items:

**Pink → 3 gametes/ individual eaten**

**Yellow → 1 gametes/ individual eaten**

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**The Rules:**

- 1) Your class will be broken up into an equal number of males and females and each of you will be assigned a genotype such that your initial population is in Hardy-Weinberg Equilibrium.
  - ▶ Record the starting allele and genotype frequencies of the predator.
- 2) 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.
- 3) You will all gather around your population of prey and you will be given 10 seconds to consume as many prey items as possible, with the goal of being able to 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.
- 4) 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.
- 5) Once you have collected your gametes you should place them into the appropriate container. If you are a designated male you will dump them into the “Sperm Bucket” and if you are a designated female you will dump your beads into the “Egg Bucket.”
- 6) 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 round of predation.
- 7) Before predation can occur again, we must reconstitute our population of prey items. Return the legumes that were “eaten” in the previous round, and thoroughly mix them up.
- 8) Repeat steps 4 – 7 for 3 - 5 rounds.
  - ▶ **What happened to the frequency of each allele in the predator population, and why did you observe this pattern?**

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► 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?

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

## **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.

**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 the two forms of the allele. 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.

**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: Demonstrating Hardy-Weinberg Equilibrium in a population of M&Ms**

#### **A. Monohybrid Cross**

Start with a population of 10 individuals, each of which is heterozygous for the red and orange allele. 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). Randomly mate the population (this gives the F1 generation). Calculate the allelic, genotypic, and phenotypic frequency of the resulting population. Randomly mate the F1 generation to create the F2 generation. Calculate the allelic, genotypic, and phenotypic frequency of the resulting population.

► 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?

## **B. Dihybrid Cross**

Start with a population of 10 individuals, each of which are heterozygous for the red and orange allele and for a second allele Dominant Blue and recessive Green (Bg). Calculate the allelic, genotypic, and phenotypic frequency of the original population. Now perform a Dihybrid cross using the random mating rules. Randomly mate the F1 generation to create the F2 generation. Calculate the allelic, genotypic, and phenotypic frequency of the F1 and F2 Generations.

► 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?



## Genetic drift

Even populations under no selection may experience changing gene frequency due to stochastic processes. 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.

### Exercise 4: Founder Effect

Start with a population of 10 individuals, each of which are heterozygous for the red and orange allele and for a second allele Dominant Blue and recessive Green (Bg). Calculate the allelic, genotypic, and phenotypic frequency of the original population. Now perform a dihybrid cross using the random mating rules

Randomly select four individuals from the F1 generation. These randomly selected individuals were swept out to sea in a storm and ended up on an island. Calculate the allelic, genotypic, and phenotypic frequency of the original population.

The four “survivors” breed 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). Calculate the allelic, genotypic, and phenotypic frequency of the original population.

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

Look back at the F1 generation. Randomly select four individuals again and calculate their allelic frequency.

► Is it different from the first founder population? Is it different from the original population?

## 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?

## Sexual Selection

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 are potentially detrimental to 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!

## Exercise 5: Effects of Non-Random Mating (Sexual Selection)

Start with a population of 10 individuals, each of which is heterozygous for the red and orange allele. The allelic frequency is 10:10 (Red: Orange), genotypic frequency is 2:6:2 (RR:Ro:oo) and the phenotypic frequency is 8, 2 (Red feathers: Orange Feathers). The same mating rules from Exercise 2 apply with the following exceptions: 1) Homozygous recessive individuals are considered unattractive to the opposite sex and are not allowed to mate (feel free to eat those individuals, you vicious carnivore); 2) Each mating Pair generates three offspring instead of two; and 3) The population limit is still 10 individuals - so randomly select from the offspring the 10 who survive (or alternatively select the excess that do not).

Repeat this process three times (Generate an F1, F2, and F3 generation). Calculate the allelic, genotypic, and phenotypic frequency of the F1, F2 and F3 generations.

► Compare the frequencies in the three generations. How have they changed?

**Notes:**

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