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, especially the last two. Therefore evolution is very likely to occur. In the exercises you complete today you will look at the impact of violating some 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:

$$\mathbf{p + q = 1}$$

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

$$\mathbf{p^2 + 2pq + q^2 = 1}$$

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: $p^2 = (0.6)^2 = 0.36$ x 100 (the number of Guppies in the total population) = 36 Homozygous Dominant individuals. Heterozygotes have both alleles and so are 2*pq* in the population (2 x 0.6 x 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, then check your results with a neighbor)

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 (*Harvardensus 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. **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 or lentils) 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):

First, make sure that each lab bench has at least two males and two females; recruit students from another bench to switch to yours if necessary. 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 immediately put it back) and one red or yellow allele from the egg pool (and put it back). ("Sampling with replacement" ensures that everyone is choosing from the same population.) 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 as follows:**

Number of RR Number of Ry Number of yy Total
p = (2*RR + 1*Ry) /Total q = (1*Ry + 2*yy) /Total

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. Gather around your population of prey and you will each be given 10 seconds to consume as many prey items (legumes such as lentils) 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.

2) At the end of a round of predation, calculate the number of gametes you can produce (3 for each pink lentil, 1 for each yellow lentil) and collect the appropriate number of colored beads. (Note – if you get an odd number, add 1 to 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 bank" container; if you are female, place all your gametes into the "egg basket" container. Also, return all prey items (legumes) to the selection area and thoroughly mix them up.

3) Wait until all students have calculated their scores and deposited their gametes. Random mating will now occur! Each student will go and randomly draw a bead from the Sperm Bank and a bead from the Egg Basket to determine their genotype and phenotype for the next generation, as you did for step 2A, above. **Replace each bead into the container from which you got it** (sampling with replacement); **then record your new 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** as you did for step 2A, above.

4) After recording the allele frequencies for Generation 1 (after 1 round of selection), **REPEAT THE SELECTION PHASE (2B)** for a second generation of selection and then a third. Stop after you have recorded the frequencies for Generation 3.

► Record what happened to the frequency of each allele in the predator population over time. Did the frequencies change over time? What forces or processes contributed to any observed change?

► 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?

EXERCISES WITH COLORED BEADS

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. We will use plastic beads of different colors; **be careful** not to let them roll away.
[**NOTE:** In past years, we used M&M candies. If M&Ms are used, please keep your fingers clean and dry. Above all, **do not eat the M&Ms,** because they have been in contact with laboratory surfaces and handled by multiple people and are therefore not safe to eat.**]**

Materials: Use bowls containing two different colors of beads, plus an empty container in which to mix and select them. Any two colors will do, but choose a suitable notation for the two alleles, such as *G* for green (dominant) and *g* for clear (recessive). Assume that the alleles control a trait like feather color

Breeding: Assume that all individuals mate once in a lifetime (semelparous) and produce 2 offspring. There will be NO population growth under these default conditions.

Exercise 3: Genetic Drift in a population of colored beads. (Work as a separate group for each lab bench; record all your results.)

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.

3A. Pick two different colors of beads and designate one of them as dominant. Start with a population of 10 individuals, each of which is heterozygous. Thus, for the initial population (Generation 0), the genotypic ratio is 0:10:0 (GG: Gg: gg) and the allele frequencies are p=0.5, q=0.5.

3B. To obtain the F1 generation, randomly mate the population as follows: from a container containing 10 gametes of each color, select (without looking) 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 4 diploid individuals has been picked, then calculate the genotypic ratio and allele frequencies of the resulting population.

3C. Now change the gene pool to contain 8 gametes or alleles that reflect the composition of the F1 population that you calculated. Randomly mate this population as you did before until you have chosen 4 new diploid individuals to make an F2. Record all your results.

3D. Repeat procedure 3C to obtain an F3. Record all your results, then stop.

► Compare your group's frequencies in the three generations. Was the Hardy-Weinberg equilibrium satisfied? If not, what condition may have been violated? Did the allele frequencies change, or were they stable? If there were any changes, were they consistent or erratic? Did any changes result from selection, or were they stochastic (random)?

► Compare your results with one other lab group. After three generations, were their allele frequencies the same as yours or different? How do you account for any differences?

Exercise 4: Effects of Non-Random Mating (Sexual Selection). (Work again in one group for each lab bench; record all your results.)

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 a unique force that deals only with traits relevant to reproductive success, and it may drive the evolution of bizarre traits at some individual cost or risk. 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!

4A. Start with two different colors of beads and designate one of them as dominant. Start your initial population with 10 diploid individuals, each of them heterozygous (Gg) for the two alleles, by placing 10 beads of each color in a bowl. Thus, for the initial population (Generation 0), the genotypic ratio is 0:10:0 (GG: Gg: gg) and the allele frequencies are p=0.5, q=0.5.

4B. This time, perform the matings as follows:

1) Without looking, one student selects two alleles 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 for the remainder of this generation) and try again by selecting two new alleles.

3) A second student selects two alleles, without looking, from the gene pool to represent the other parent. Again, if you select a homozygous recessive, put the genes aside and try again.

4) Once you have selected a parent with at least one dominant allele, conceal one allele in each hand and ask a third student to choose one of the two alleles to contribute to the next generation's gene pool. Write down what allele you picked, but return both beads to the current generation's gene pool that they came from. Do this also for the other parent, and record the two chosen alleles as a new diploid genotype.

5) Repeat steps 1-4 until you have recorded the genotypes of a new generation of 10 diploid individuals (20 alleles in all). Calculate the number of dominant homozygotes, heterozygotes, and recessive homozygotes; then calculate the allele frequencies (p and q) of the F1 generation. (You can calculate $\underline{p}$ = dominant alleles / total of 20 alleles, and $\underline{q}$ = recessive alleles / total of 20 alleles.) Set up a new gene pool for the next generation by selecting 20 beads to match the composition of the F1.

4C. Now repeat all the steps of 4B to generate an F2 population. Do this yet again to generate an F3 population. For each generation, calculate the number of dominant homozygotes, heterozygotes, and recessive homozygotes; then calculate the allele frequencies $\underline{p}$ and $\underline{q}$.

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

Thought questions: 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 (or rich, or talented) person who never had any children, or a person of average attractiveness (or wealth, or talent) who had three children? Why?

Homework exercise: see next page

Hardy-Weinberg – Follow-up Homework

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

1) Is a dominant allele always a beneficial allele? **Give evidence** from you 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 a Hardy-Weinberg equilibrium maintained? Why or why not? If a change occurred, was it consistent or erratic? What force or forces caused the change?

   Answer:

4) What happened to allele frequencies in Exercise 4? If a change occurred, what force or forces caused the change?

   Answer:

5) 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: