Name _____ Lab section _____

# Lab 2. Forces of evolution

## Forces of evolution

Evolution is defined quite simply as a **change in allele (gene) frequencies** from one generation to the next and is caused by several different forces, which produce and redistribute variation. They are known as the **four forces of evolution**. They include:

1. **Natural selection**
2. **Mutation**
3. **Genetic drift** (two particular types of genetic drift are *founder effect* and *bottlenecks*)
4. **Gene flow**

Before we start, here are some terms you should familiarize yourself with:

**Allele(s)** – alternate forms of the same gene (often used synonymously). Alleles are located at the same *locus* (place) on paired chromosomes (one inherited from each parent), and carry genetic information for the same trait.

**Genotype** – the genetic makeup of an individual. It can refer to the entirety of an individual's genetic makeup (aka *genome*), or to specific alleles at a particular locus.

**Phenotype** – the observable physical characteristics of an organism.

**Dominant vs. recessive alleles** – as alleles come in pairs (one half of the pair is inherited from the biological mother, one from the biological father), whether an allele is dominant or recessive determines which one is expressed in the phenotype. A *dominant allele is always expressed when present*, whether the individual has two copies of the allele (one from each parent), or just one (from either parent). A recessive allele is masked from expression if paired with a dominant allele, so *two copies of the recessive allele (from both mom and dad) are required for it to be expressed in the phenotype*.

**Homozygous vs. heterozygous** – an organism is *homozygous* (either homozygous dominant or homozygous recessive) if it has *the same allele on both of the paired chromosomes.* An organism is *heterozygous* if it has *two different alleles at the same locu*s, one on each of the paired chromosomes.

**Hardy-Weinberg equilibrium** – a hypothetical state of a population where *no evolution is occurring* (i.e., the population is infinitely large, the mating is random, there is no selection, gene flow, genetic drift or mutation). It doesn't really happen in reality, but it provides a mathematical model of *expected allele frequencies* to which we can compare the *observed allele frequencies*.

## Population genetics

Population genetics is the study of allele frequency distribution and change under the influence of the **four evolutionary forces**: natural selection, genetic drift, mutation, and gene flow. In this lab we will do hands-on experiments that will illustrate how allele frequencies change as a result of three different evolutionary forces: selection, gene flow, and genetic drift. You will also examine how these forces can act on the frequencies of certain alleles in a population. When you complete this lab you should:

1. Understand the forces of evolution
2. Be able to give examples of how alleles have changed under the forces of evolution

To better understand the forces of evolution, we will use red and white beads to represent different alleles in a population. The red beads will represent a dominant allele, **R**, and the white beads will represent a recessive allele, **r**.

For this exercise, you will need one container with 24 white beads and 24 red beads (48 beads total). In each exercise, shake the container several times to make sure the beads are mixed thoroughly. Once the beads are mixed, you will draw out two beads. These two beads represent a hypothetical new individual (24 individuals total). This individual has inherited one allele from each parent. The beads represent these alleles (48 alleles total). For example, if you draw a red bead and a white bead, you will have formed an individual with the genotype **Rr.** This individual is a heterozygote, with the dominant phenotype. If you draw two white beads, you will have formed an individual with the genotype **rr**, who is a homozygote with the recessive phenotype.

We will use the Hardy-Weinberg equilibrium to calculate the expected allele frequencies and to compare the observed frequencies with. We will also compare genotype and phenotype frequencies. The easiest way to visualize this is with a Punnet square using our two alleles, the dominant allele **R** and recessive allele **r**, to represent gametes in order to calculate possible genotype combinations in the next generation:

| | **R** | **r** |
|---|---|---|
| **R** | RR | Rr |
| **r** | Rr | rr |

Possible genotype combinations include one dominant homozygote (**RR**), two heterozygotes (**2×Rr**) and one recessive homozygote (**rr**).

By convention, in the Hardy-Weinberg formula different alleles are represented by letters **p** and **q**. If we replace **R** and **r** with **p** and **q**, respectively, we obtain the following:

| | **p** | **q** |
|---|---|---|
| **p** | pp | pq |
| **q** | pq | qq |

The possible genotype combinations again include one dominant homozygote (**pp**, or $\mathbf{p^2}$), two heterozygotes (**2×pq**) and one recessive homozygote (**qq,** or $\mathbf{q^2}$).

Since there are only two alleles at this locus (**p** and **q**), their cumulative frequencies must add up to 1, or 100%. So it follows that:

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

[p=f(R); q=f(r)]

If we cross two organisms carrying the alleles **p** and **q**, we get:

$$(\mathbf{p}+\mathbf{q})\times(\mathbf{p}+\mathbf{q}) = (\mathbf{p}+\mathbf{q})^2 = \mathbf{p^2} + \mathbf{2pq} + \mathbf{q^2}$$

$[p^2=f(RR); 2pq=f(Rr); q^2=f(rr)]$

This is the same result we got in our Punnet square! These mathematical expressions enable us to easily calculate genotype and phenotype frequencies from allele frequencies, and vice versa. Here are some examples using our alleles **R** and **r** to replace the conventional p and q:

**Example 1:**
Allele frequencies **p**=0.5 → **f(R)** = 0.5 (aka 50%) **q**=0.5 →**f(r)** = 0.5 (aka 50%)

Expected **allele frequencies**

$$\mathbf{p}+\mathbf{q} = 1$$
$$\mathbf{f(R)} + \mathbf{f(r)} = 1$$
$$0.5+0.5 = 1$$

Expected **genotype frequencies**

$$\mathbf{p^2} + \mathbf{2pq} + \mathbf{q^2} = 1 \rightarrow \mathbf{f(RR)} + \mathbf{f(Rr)} + \mathbf{f(rr)} = 1$$
$$0.5^2 + 2\times0.5\times0.5 + 0.5^2 = 1 \rightarrow 0.25 + 0.50 + 0.25=1$$
$$\mathbf{f(RR)} = 0.25, \mathbf{f(Rr)} = 0.50, \mathbf{f(rr)} = 0.25$$

Expected **genotype counts** (or how many individuals in the population have each of the expected genotypes, where **N** is the total number of individuals)

If **N**=24, then:

$$\mathbf{RR} = \mathbf{f(RR)} \times \mathbf{N} \rightarrow \mathbf{RR} = 0.25 \times 24 = 6 \text{ individuals}$$
$$\mathbf{Rr} = \mathbf{f(Rr)} \times \mathbf{N} \rightarrow \mathbf{Rr} = 0.50 \times 24 = 12 \text{ individuals}$$
$$\mathbf{rr} = \mathbf{f(rr)} \times \mathbf{N} \rightarrow \mathbf{rr} = 0.25 \times 24 = 6 \text{ individuals}$$

Expected **allele counts** (or how many **R** and **r** alleles are there in the population, since each individual has 2 alleles, where **n** is the total number of alleles)

$$\mathbf{R} = 2\times\mathbf{RR} + 1\times\mathbf{Rr} \rightarrow \mathbf{R} = 12+12 = 24 \text{ alleles}$$
$$\mathbf{r} = 2\times\mathbf{rr} + 1\times\mathbf{Rr} \rightarrow \mathbf{r} = 12+12 = 24 \text{ alleles}$$
$$\mathbf{n} = \mathbf{R}+\mathbf{r} =24+24 = 48 \text{ alleles}$$
$$\mathbf{n}=2\times\mathbf{N}$$

**Example 2**:
If we reverse this scenario, and instead start with the observed genotype counts (**RR**, **Rr** and **rr**), we can calculate genotype frequencies and expected allele counts and frequencies from there.

Observed **genotype counts**:

**RR**=6, **Rr**=12, **rr**=12 individuals
**N**= 6+12+6 = 24 individuals

Observed **genotype frequencies**:

**f(RR)** = **RR** ÷ **N** → **f(RR)** = 6/24 = 0.25
**f(Rr)** = **Rr** ÷ **N** → **f(Rr)** = 12/24 = 0.50
**f(Rr)** = **Rr** ÷ **N** → **f(rr)** = 6/24 = 0.25

Expected **allele counts**:

**R** = 2×**RR** + 1×**Rr** → **R** = 12+12 = 24 alleles **r**
= 2×**rr** + 1×**Rr** → **r** = 12+12 = 24 alleles

Expected **allele frequencies**:

**f(R)** = **R**/**n** → **f(R)** = 24/48 = 0.5
**f(r)** = **r**/**n** → **f(r)** = 24/48 = 0.5
**f(R)** + **f(r)** = 1
0.5+0.5 = 1

Remember, **genotype and phenotype frequencies may change between generations without a concomitant change in allele frequencies**! If there was no change in allele frequencies from one generation to the next, *no evolution has occurred*. For example:

| **Genotypes** | | | **Allele frequencies** | |
|---|---|---|---|---|
| **RR** | **Rr** | **rr** | **f(R)** | **f(r)** |
| 6 | 12 | 6 | 0.5 | 0.5 |
| 12 | 0 | 12 | R/n=(2×RR+1×Rr)/2N= =(2×12+1×0)/48=0.5 | r/n=(2×rr+1×Rr)/2N= =(2×12+1×0)/48=0.5 |
| 0 | 24 | 0 | R/n=(2×RR+1×Rr)/2N= =(2×0+1×24)/48=0.5 | r/n=(2×rr+1×Rr)/2N= =(2×0+1×24)/48=0.5 |

**IMPORTANT**: For each part of this exercise **please read all of the instructions first** before you begin drawing beads from the container!

**Exercise 1. Genetic drift**

In this exercise we will simulate two generations of reproduction in a population. We will track changes in genotype and allele frequencies between generations caused by genetic drift. In a theoretical population that is in Hardy-Weinberg equilibrium, allele frequencies will not change between generations and no evolution will occur. However, no population is infinitely large. In finite populations in the absence of gene flow, mutation, and selection it is **expected** that allele frequencies will not change between generations, but because of **random sampling effects** allele frequencies may **drift** up and down between generations. This is like flipping a coin. If you flip a coin 10 times you expect to get heads five times. In reality, you may get heads 6 times or 4 times or 8 times, etc. Unlike flipping a coin, though, the expectation changes with each generation.

Start with a container filled with 24 red and 24 white beads. Red will represent the dominant allele **R**, white will represent the recessive allele **r**. The population size is then 24 individuals (**N**) and 48 total alleles (**n**). First calculate the **expected genotype and allele frequencies** for generation 1 from the starting allele frequencies using the HardyWeinberg formula and record these in the table below.

Now create generation 1 by making 24 draws from the container of beads. Each draw will simulate a birth. For each draw select two beads at random without looking. If you get two red beads, record a draw of **RR**. If you get a red and a white bead, record a draw of **Rr**. If you get two white beads, record a draw of **rr**. Make a total of 24 draws, **always putting the beads back into the original container before the next draw**. Tally your results in the table and calculate the new allele frequencies using formulae on page 4. Also calculate the expected genotype and allele frequencies for generation 2.

| | **Genotype counts** | | | **Allele counts** | | **Allele freq.** | |
|---|---|---|---|---|---|---|---|
| | **RR** | **Rr** | **rr** | **R** | **r** | ***f*(R)** | ***f*(r)** |
| **Original population N=24, n=48** | 8 | 8 | 8 | 2*8 + 8 = 24 | 8 + 2*8 = 24 | 24/48= 0.5 | 24/48= 0.5 |
| **Gen. 1 expected N=24, n=48** | | | | | | | |
| **Gen. 1 observed N=24, n=48** | | | | | | | |
| **Gen. 2 expected N=24, n=48** | | | | | | | |
| **** Gen. 2 observed N=24, n=48** | | | | | | | |

** Prior to doing this, adjust the beads in your container to match the new allele frequencies! For example, if in generation 1 the observed allele frequencies are f(R)=0.54

and f(r)=0.46, then you should have 26 red beads (0.54×48) and 22 white beads (0.46×48). Calculate expected genotypes and allele frequencies for generation 2 and repeat the experiment as before to create generation 2. Record your results in the table.

1. What is the expected genotype ratio (the ratio of different genotypes to one another) for generation one? And for generation two? Has there been a change?

2. What is the observed phenotype ratio for generation one (the ratio of individuals with the dominant phenotype relative to individuals with the recessive phenotype)? And for generation two? Has there been a change?

3. Did evolution occur? If so, between which generations? How do you know?

## Exercise 2. Founder effect

The effect of genetic drift is particularly large in small populations. There are at least two phenomena that can lead to a decrease in the population size: population (genetic) bottlenecks and founder effect. A **population bottleneck** is an evolutionary event in which the population's size is reduced for at least one generation. The **founder effect** is a similar phenomenon that occurs when a new population is established by a very small number of individuals from a larger population. This can occur for a number of reasons: individuals can become isolated because of geographic changes, a disaster can occur that may kill much of a population, or individuals can become stranded somewhere. For example, US ethnic groups such as Old Order Amish and Louisiana Cajun can trace their roots back to a few dozen and a couple hundred people, respectively, that immigrated into the US from Europe and Canada. A consequence of both population bottlenecks and founder effect is a loss of genetic variation; the new population may therefore be distinctively different, both genetically and phenotypically, from the parent population from which it is derived.

In this exercise, you will simulate a genetic bottleneck. To do so, place 24 red (**R**) and 24 white (**r**) beads in a plastic cup (**cup 1**). The cup represents the original population. You will also need a second cup. To create the bottleneck, perform 12 draws of two beads, placing each draw into the new cup (**cup 2**). Record your results in the table as the founder population. Next, create a new generation from the founder population as you did in the genetic drift exercise previously. Record your results in the table below.

| | **Genotype counts** | | | **Allele counts** | | **Allele freq.** | |
|---|---|---|---|---|---|---|---|
| | **RR** | **Rr** | **rr** | **R** | **r** | ***f*(R)** | ***f*(r)** |
| **Original population N=24, n=48** | 6 | 12 | 6 | 24 | 24 | 0.5 | 0.5 |
| **Founder population N=12, n=24** | | | | | | | |
| **Gen. 1 expected N=12, n=24** | | | | | | | |
| **Gen. 1 observed N=12, n=24** | | | | | | | |

1. Of the 24 beads that you randomly selected from the container, what percentage were red? What percentage were white?

2. Did evolution occur between the original and the founder population? And between the founder population and generation 1? How do you know?

3. What percentage of generation 1 observed (the population selected from container 2) has the dominant phenotype?

4. What percentage of the population selected from container 2 has the recessive phenotype?

5. How do these percentages differ from those in the original population?

**Exercise 3. Gene flow**

Up until this point, we have been assuming that each of these populations is self-contained. However, gene flow, or migration between populations, can occur and this can change the overall genotype and phenotype frequencies in a population. For example, think about the history of immigration into the Philadelphia area. Before the 17th century, this region was inhabited by Lenape Native Americans. In the 17th century many Dutch, Swedish, Finnish and English immigrants were arriving, while the Native American population was decreasing. During the 19th century many Irish and German immigrants arrived, as well as immigrants from Eastern and Southern Europe, as well as African-Americans from the American South. Over that period of time, much gene flow occurred, and allele frequencies and phenotypic and genotypic ratios present in the population changed. Today, with increasing globalization and population mobility, gene flow has become exceedingly common.

For this exercise, you will be simulating gene flow or immigration into your population in container 2 from the previous exercise (NOTE: copy the information for "Generation 1 observed" from Exercise 2 as the original population in the table below). Randomly select **six more pairs** of beads from the Mix container to add to your population in container 2. Remember to pull out all of the beads in pairs since entire individuals are immigrating! Record the number of **RR**, **Rr**, and **rr** individuals that were drawn.

| | **Genotype counts** | | | **Allele counts** | | **Allele freq.** | |
|---|---|---|---|---|---|---|---|
| | **RR** | **Rr** | **rr** | **R** | **r** | ***f*(R)** | ***f*(r)** |
| **Original population (container 2) N=12, n=24** | | | | | | | |
| **Newly drawn N= , n=** | | | | | | | |
| **Population after gene flow (row 1+ row 2) N= , n=** | | | | | | | |

1. Did gene flow lead to evolution? How do you know?

2. What do the 12 additional beads represent?

**Exercise 4. Natural selection: selection against the recessive phenotype**

In the previous exercise all of the genotypes had equal fitness, meaning that they all had an equal chance of having offspring in the next generation. In real populations, this is not always the case. Some genotypes or phenotypes are more advantageous, while others significantly decrease fitness in an individual or can be lethal. (Remember that just because a trait is recessive, it is not necessarily less common, nor are dominant traits by default more common.)

We can look at the classic example of the peppered moth. In pre-industrial England, the most common form of peppered moth was the light-colored moth (recessive phenotype). At the time, tree bark was often covered with pale lichens, so light-colored moths were better camouflaged from predators than dark-colored moths (dominant phenotype). However, as the levels of pollution dramatically increased during the Industrial Revolution, the lichens died out and the tree bark became darkened with soot. Suddenly, the lighter-colored peppered moths became much more susceptible to predation, while the darker-colored moths thrived. In this exercise, you will simulate this kind of selection against the recessive phenotype.

***REMEMBER: Natural selection acts on an individual; only populations evolve!***

Start with 24 red beads and 24 white beads in your cup. You must make 24 draws. In order that you don't discriminate on the basis of color, make each draw without looking at the beads. When you draw two beads record your result. If you get two red beads, record a draw of **RR.** If you get a red bead and a white bead, record a draw of **Rr**. If you get two white beads, record a draw of **rr**, and place all **rr** individuals in a separate cup. Make a total of 24 draws*. **DO NOT** put the beads back into the original container before the next draw, and make sure you remove beads in pairs as you draw them! Do not remove single alleles, but pairs.* After you have finished making 24 draws there should be no more beads left in your cup. This represents the first generation.

For the second generation put all of the beads that were drawn as **RR** or **Rr** back into the first cup. ***DO NOT** place the individuals that were drawn as **rr** back into the cup*, since these individuals have the disadvantageous phenotype and do not get to reproduce in the next generation (imagine these are light-colored peppered moths after the Industrial Revolution that all got eaten by predators). Repeat this process for 5 generations.

| | Genotype counts | | | Allele counts | | Allele freq. | |
|---|---|---|---|---|---|---|---|
| | **RR** | **Rr** | **rr** | **R** | **r** | ***f*(R)** | ***f*(r)** |
| **Original population N=24, n=48** | 6 | 12 | 6 | 24 | 24 | 0.5 | 0.5 |
| **Generation 1 N=24, n=48** | | | | | | | |
| **Generation 2 N= , n=** | | | | | | | |
| **Generation 3 N= , n=** | | | | | | | |
| **Generation 4 N= , n=** | | | | | | | |

1. Did evolution occur between the original population and generation 1? What about between generation 1 and subsequent generations? If evolution did occur, was it random or directional? Why?

2. Did the dominant allele become “fixed” in the population? In other words, was there a point in the experiment where there were no more recessive alleles left in the population?

3. If there is no fixation of the dominant allele, why do you think recessive alleles are kept in the population?

**Exercise 5. Natural selection: selection against the dominant phenotype**

Though there are many disadvantageous or lethal recessive conditions, there are also a few that are dominant. In this exercise you will explore what occurs when the dominant phenotype is disadvantageous. Let's look at the peppered moth again. After the Industrial Revolution and the strong selective pressure against the recessive (light-colored) phenotype, the dominant phenotype (dark-colored) was prevalent. However, as environmental conditions improved, tree bark once again became light in color, and now the dark-colored moths were once again more likely to get eaten by predators.

Repeat the previous exercise starting with 24 red and 24 white beads, only assuming that the dominant phenotype is disadvantageous/lethal. So, whenever a combination of **RR** or **Rr** is drawn, you should put those aside in a separate cup, but you should put **rr** individuals back into the first cup for the next generation.

| | **Genotypes** | | | **Allele counts** | | **Allele freq.** | |
|---|---|---|---|---|---|---|---|
| | **RR** | **Rr** | **rr** | **R** | **r** | ***f*(R)** | ***f*(r)** |
| **Original population N=24, n=48** | 6 | 12 | 6 | 24 | 24 | 0.5 | 0.5 |
| **Generation 1 N=24, n=48** | | | | | | | |
| **Generation 2 N= , n=** | | | | | | | |
| **Generation 3 N= , n=** | | | | | | | |

1. How many generations (draws) did it take for the recessive allele to become "fixed" in the population? In other words, was there a point in the experiment where there were no more dominant alleles left in the population?

2. Why did it take less time for the recessive allele to become fixed in this exercise than it did for the dominant allele to become fixed in the previous exercise?