AP® Biology Laboratory 8
Population Genetics and Evolution

Objectives

• Estimate the frequency of alleles in a population using the Hardy-Weinberg equations
• Demonstrate that allele frequencies can change in a population over time

Background

In the early 1900s, many biologists attempted to explain evolution in terms of the emerging science of genetics. Because the F2 of a monohybrid cross show a 3:1 ratio of dominant to recessive phenotypes, many assumed that populations would evolve toward similar ratios of phenotypes. Two mathematicians, Godfrey Hardy and Wilhelm Weinberg, realized that the frequency of alleles in a population was independent of the alleles’ inheritance pattern from individual parents to offspring. They postulated an ideal breeding population with the following properties:

1. Population size is infinite, or very large.
2. Mating is random. Mating pairs show no preference for one phenotype over another.
3. There is no mutation.
4. There is no exchange with other populations. No immigration or emigration occurs.
5. There is no selection for one phenotype over another. All phenotypes have an equal chance of reproducing.

If these conditions are met, the population’s allele and genotype frequencies will remain statistically constant over time, a condition referred to as Hardy-Weinberg equilibrium. If we determine the frequency of a pair of alleles in a population, we can sample that population over several generations to determine if the frequency changes. If it does, we know that the population is evolving with respect to that pair of alleles.

Hardy and Weinberg developed mathematical formulas for estimating the frequencies of alleles in populations. Consider the following data on Rh blood type for a hypothetical human population of 6000:

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Number of People</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh+</td>
<td>4680</td>
</tr>
<tr>
<td>Rh−</td>
<td>1320</td>
</tr>
<tr>
<td>Total</td>
<td>6000</td>
</tr>
</tbody>
</table>
Assume that Rh blood type is inherited through two alleles: \(D\), a dominant allele for Rh+ blood type, and \(d\), a recessive allele for Rh- blood type.

Using the Hardy-Weinberg equations,
let \(p\) = frequency of the dominant allele (\(D\))
and \(q\) = frequency of the recessive allele (\(d\)).

Each person is either Rh+ or Rh-, therefore,
\(p + q = 1\) or 100% of the population.

Since humans are diploid, individuals may be homozygous dominant (\(D/D\)), heterozygous (\(D/d\)), or homozygous recessive (\(d/d\)). The basic equation must be expanded to represent all the genotype frequencies. Thus,
\[(p + q)(p + q) = 1 \text{ or} \]
\[p^2 + 2pq + q^2 = 1 \text{ where} \]
\[p^2 = \text{frequency of the homozygous dominant (\(D/D\)),} \]
\[2pq = \text{frequency of the heterozygous condition (\(D/d\)), and} \]
\[q^2 = \text{frequency of the homozygous recessive (\(d/d\)).} \]
Notice that \(p^2 + 2pq = \text{the frequency of the Rh+ phenotype in the population.} \]
Now we can calculate \(q^2\), the frequency of the homozygous recessive. Using our hypothetical population data:
\[q^2 = \text{frequency of Rh- or} \]
\[\frac{\text{Number of people Rh-}}{\text{Total number of people}} \]
\[q^2 = 1320/6000 = 0.22, \text{ therefore} \]
\[q = \sqrt{0.22} = 0.47 \text{ and} \]
\[p + q = 1 \]
\[p = 1 - q \]
\[p = 1 - 0.47 = 0.53 \]

This tells us that 53% of the population tested has the allele \(D\) and 47% has the allele \(d\). If we repeated our sample over several generations, we could tell if this population is evolving with respect to the Rh-factor alleles.

Notice that we had to calculate a value for \(q\) before we could determine \(p\). Why is this true?
Activity A: Estimating Allele Frequencies With the Hardy-Weinberg Equations

Materials
PTC paper and control paper.

Introduction
In this activity, you will collect class data on the ability to taste the chemical PTC (phenylthiocarbamide) at very low concentrations. You will assume that a bitter-taste reaction to PTC is evidence of the presence of a dominant allele in either the homozygous condition (A/A) or the heterozygous condition (A/a) and that the inability to taste PTC at all depends on the presence of homozygous recessive alleles (a/a). You will use your data and the Hardy-Weinberg equations to calculate the frequencies of the taster and non-taster alleles in your classroom population.

Procedure
1. Press a strip of control taste paper to the tip of your tongue. Wait until your saliva has completely saturated the paper, then note the taste of the paper.
2. Now, repeat Step 1 using the PTC taste paper. If you sense a bitter taste, you are a taster. If you sense little more than the taste of the paper itself (as in Step 1), you are a non-taster. If you are uncertain whether you taste PTC, you are a non-taster. Tasters will have no uncertainty. Tally your class results. Record the classroom population data in Table 1.

Analysis of Results, Activity A: Estimating Allele Frequencies with the Hardy-Weinberg Equations

1. Use your class data and the Hardy-Weinberg equations to complete Table 1.

<table>
<thead>
<tr>
<th>Class Totals</th>
<th>Tasters</th>
<th>Non-tasters</th>
<th>Tasters +</th>
<th>Frequency of the Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A (p)</td>
</tr>
</tbody>
</table>

2. What is the frequency of heterozygous tasters in your class?

3. With respect to the PTC alleles, does your class meet or at least approximate any of the requirements of an ideal Hardy-Weinberg population? Explain your answer.

4. With respect to the PTC alleles, how does your class differ from an ideal Hardy-Weinberg population? Explain your answer.
5. Would you expect the frequencies of the alleles for PTC tasting and non-tasting to remain constant for North America over the next 200 years? Support your conclusion.

Activity B: Hardy-Weinberg Equilibrium

Materials
2 cards marked A, 2 cards marked a.

Introduction
In this activity, your class will simulate a breeding population of diploid organisms. You have four cards, which represent gametes produced by meiosis. (Remember that gametes produced by meiosis are haploid.) The letter on the card represents an allele that is inherited with the gamete. You will contribute one gamete to each of your offspring. Everyone in the class begins with the same four cards, two A and two a. What are the initial frequencies of alleles A and a? Record this in Table 2.

The Hardy-Weinberg equations are used to give estimates of allele frequencies for large populations. In this simulation, there is no need to estimate allele frequencies because you will have actual counts. For example, assume that the simulation ends with the following counts for Generation 5:

\[ A/A = 6 \]
\[ A/a = 12 \]
\[ a/a = 6 \]

Total number of alleles in the population = 48

The total number of alleles in the population is equal to the number of students in the class, multiplied by 2. (For this example, \( 24 \times 2 = 48 \).) We can then calculate \( p \) and \( q \) for Generation 5 as follows:

Number of A alleles in Generation 5
Number with genotype A/A: \( 6 \times 2 = 12 \) A alleles
Number with genotype A/a: \( 12 \times 1 = 12 \) A alleles

Total = 24 A alleles

\[ p = \frac{\text{Total number of A alleles}}{\text{Total number of alleles in the population}} = \frac{24}{48} = 0.5 \]

Number of a alleles present in Generation 5
Number with genotype a/a: \( 6 \times 2 = 12 \) a alleles
Number with genotype A/a: \( 12 \times 1 = 12 \) a alleles

Total = 24 a alleles

\[ q = \frac{\text{Total number of a alleles}}{\text{Total number of alleles in the population}} = \frac{24}{48} = 0.5 \]

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Put another way, because $p + q = 1$ and we have a value for $p$, then
$q = 1 - p$
$q = 1 - 0.5 = 0.5$

Procedure
1. Shuffle your cards face-down. Pair with another student. Draw the top card from your hand and match it with your partner’s card. These two cards represent the genotype of your first offspring. One of you should record this as the Generation 1 Genotype in Table 2.

2. Retrieve your card and reshuffle your hand. Repeat Step 1 with the same partner to produce a second offspring. The individual who did not record a Generation 1 Genotype in Step 1 should record this result in Table 2 for Generation 1.

3. Now, assume the genotype of your Generation 1. For example, if you recorded the genotype $a/a$, you should have four cards, all marked $a$; if you recorded the genotype $A/A$, you should have four cards all marked $A$; and if you recorded the genotype $A/a$, keep your original hand of cards (two marked $A$ and two marked $a$). Discard and obtain cards as necessary.

4. Randomly pair with a different student and repeat this process for five generations, until you have completed Table 2.

Table 2: Hardy-Weinberg Equilibrium

<table>
<thead>
<tr>
<th>Initial Class Frequencies</th>
<th>$p =$</th>
<th>$q =$</th>
<th>Initial Genotype $A/a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>My Genotype</td>
<td></td>
<td></td>
<td>Class Totals</td>
</tr>
<tr>
<td>Generation 1</td>
<td></td>
<td></td>
<td>$A/A$</td>
</tr>
<tr>
<td>Generation 2</td>
<td></td>
<td></td>
<td>$A/a$</td>
</tr>
<tr>
<td>Generation 3</td>
<td></td>
<td></td>
<td>$a/a$</td>
</tr>
<tr>
<td>Generation 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 5 Class Frequencies</td>
<td>$p =$</td>
<td>$q =$</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Results, Activity B: Hardy-Weinberg Equilibrium

1. Are the Generation 5 values for $p$ and $q$ different from their initial values?

2. Is your answer to 1, above, consistent with the alleles being at equilibrium? If not, why not?
3. Review the five conditions that must be met for allele frequencies to remain constant. Which, if any, of these conditions might not have been met in this simulation?

Activity C: Selection

Materials
2 cards marked A, 2 cards marked a.

Introduction
A genotype can affect the reproductive success of the organism that carries it. For example, a recessive allele results in the production of an abnormal hemoglobin molecule, hemoglobin S. People who are homozygous for this recessive allele have the disease sickle-cell anemia. Historically, people with sickle-cell anemia have rarely survived childhood and thus have rarely reproduced. What happens to such an allele in a population over time? You will explore this question in the following simulation.

Procedure
In this activity, you will assume that none of the homozygous recessive individuals (a/a) reproduce and that all A/A and A/a individuals do reproduce.

As before, begin with four cards, two A and two a. Now, follow the Procedure steps in Activity B once again, but with the following exception: do not record any a/a offspring. When an a/a combination results, retrieve your card, reshuffle your hand, and try again until you get an A/A or A/a combination. Repeat this process through five generations to complete Table 3.

<table>
<thead>
<tr>
<th>Initial Class Frequencies</th>
<th>$p =$</th>
<th>$q =$</th>
<th>Initial Genotype $A/a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>My Genotype</td>
<td></td>
<td></td>
<td>Class Totals</td>
</tr>
<tr>
<td>Generation 1</td>
<td></td>
<td></td>
<td>A/A</td>
</tr>
<tr>
<td>Generation 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation 5 Class Frequencies</td>
<td>$p =$</td>
<td>$q =$</td>
<td></td>
</tr>
</tbody>
</table>
Analysis of Results, Activity C: Selection

1. Are the Generation 5 values for $p$ and $q$ different from their initial values? Explain why the values did or did not change.

2. Compare the Generation 5 values for $p$ and $q$ calculated in Activity B with the Generation 5 values for $p$ and $q$ that you just calculated for Activity C. Describe your findings.

3. What would you expect to happen to the frequencies of $p$ and $q$ if you ran the simulation for another five generations?

4. This simulation involved very high selection against the recessive allele. Did selection eliminate the allele from the population? If not, why did the allele persist?
Activity D: Heterozygotes and Selection

Materials
2 cards marked A, 2 cards marked a, coin.

Introduction
Studies indicate that individuals who are heterozygous for the hemoglobin S allele, A/a, may have an increased resistance to malaria. What happens to a recessive allele in a population when there is selection against both homozygotes (A/A and a/a)? The following simulation explores this question.

Procedure
To better reveal the results of selection, you will simulate more generations in this activity. Follow the procedure in Activity C, with this additional condition: if the offspring is A/A, flip a coin. If the result is heads, the offspring survives; if the result is tails, the individual does not survive. In this case, retrieve your card, reshuffle your hand, and try again. Remember, as in Activity C, the genotype a/a never survives. Repeat the activity until you produce two surviving offspring each generation for ten generations. Record your results in Table 4.

<table>
<thead>
<tr>
<th>Table 4: Heterozygotes and Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Class Frequencies</td>
</tr>
<tr>
<td>My Genotype</td>
</tr>
<tr>
<td>Generation 1</td>
</tr>
<tr>
<td>Generation 2</td>
</tr>
<tr>
<td>Generation 3</td>
</tr>
<tr>
<td>Generation 4</td>
</tr>
<tr>
<td>Generation 5</td>
</tr>
<tr>
<td>Generation 6</td>
</tr>
<tr>
<td>Generation 7</td>
</tr>
<tr>
<td>Generation 8</td>
</tr>
<tr>
<td>Generation 9</td>
</tr>
<tr>
<td>Generation 10</td>
</tr>
</tbody>
</table>

Generation 10 Class Frequencies | p = | q = |
Analysis of Results, Activity D: Heterozygotes and Selection

1. Account for the differences, if any, between the final frequencies of \( p \) and \( q \) in Activity C and Activity D.

2. Consider two isolated populations of people who have occupied their homelands for untold generations. One population occupies a dry, windswept plain. The other population inhabits a lowland, tropical area with many streams and swamps. In which population would you expect to find the higher frequency of the allele for hemoglobin S? Explain your reasoning.

3. Evaluate the following statement: “Malaria selects for sickle-cell anemia.”

4. “Mutations cause evolution.” On the basis of your experiences in this lab and your knowledge of population genetics, do you agree or disagree with this statement? Explain your reasoning.
5. Is the mutation for hemoglobin S harmful or beneficial? Support your answer.

6. Imagine that you are a member of a committee assigned to evaluate a report on neurofibromatosis, a disorder inherited through a dominant allele. (Neurofibromatosis is a condition in which noncancerous tumors grow along nerves.) The report concludes that, because \( \frac{3}{4} \) of the offspring of parents who are heterozygous for neurofibromatosis will have the disorder, eventually \( \frac{3}{4} \) of the population will have neurofibromatosis. Do you agree or disagree with the report’s conclusion? Explain your reasoning.