

Hardy, Weinberg, and Kuru

Introduction

A little more than a century ago, the Foré people began to eat their dead. The Foré, a stone-age culture inhabiting remote jungle valleys of Papua New Guinea's Eastern Highlands Province, consumed their deceased loved ones as a show of affection and respect. As it turned out, however, these mortuary feasts were a bad idea. By 1920, the Foré began to suffer from an illness they came to call kuru, or shivering disease. Kuru's victims first began to tremble, then stagger when they walked. Later they had difficulty speaking, chewing, and swallowing. The illness invariably ended in coma and death. At its worst, in the late 1950s, the kuru epidemic killed some 1% of the Foré population each year. After the Foré stopped practicing funereal cannibalism, the epidemic slowly faded away.



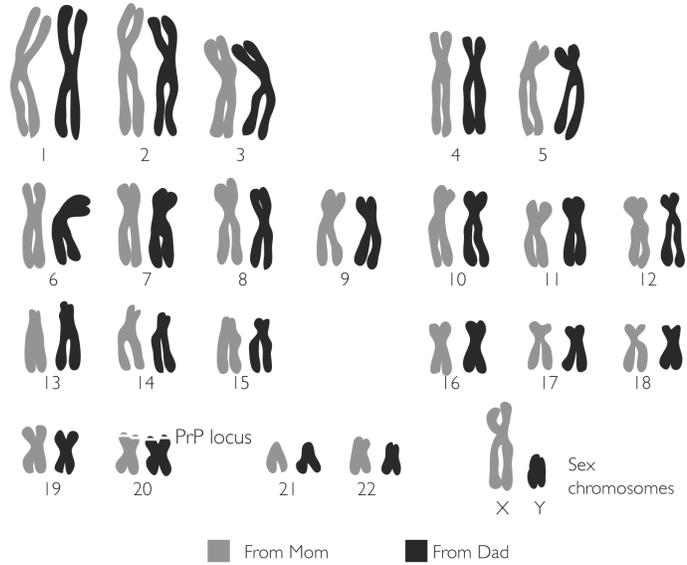
Although confined to the Foré people, shivering disease is more than just an exotic bit of medical anthropology. Kuru is but one of many spongiform encephalopathies, or spongy brain diseases. There are spongy brain diseases that affect cattle (mad cow disease), mink (transmissible mink encephalopathy), cats (feline spongiform encephalopathy), sheep and goats (scrapie), and deer and elk (chronic wasting disease). In addition to kuru, the list of human spongy brain diseases includes Creutzfeldt-Jakob disease, fatal familial insomnia, and Gerstmann-Sträussler-Scheinker disease.

In all of these diseases, normally solid tissues in the brain become riddled with holes, giving them a sponge-like appearance when viewed under a microscope. These areas of spongy degeneration are clogged with clumps of a protein called PrP. PrP is encoded in the victim's DNA and manufactured by the victim's own cells. The causal link between the accumulating clumps of PrP and the death of brain cells is unclear. It is clear, however, that PrP plays a leading role in the development of spongy brain disease. For one thing, some spongy brain diseases, including fatal familial insomnia and Gerstmann-Sträussler-Scheinker disease, are associated with specific mutations in the PrP gene. For another, genetically engineered mice that cannot make PrP don't get spongy brain diseases.

The most frightening thing about spongy brain diseases is that they are transmissible. You can get one of these diseases by eating the brain of an animal that already has it. Variant Creutzfeldt-Jakob disease is a recently recognized spongy brain disease in humans. Its victims contract it by eating meat from cattle that suffered from mad cow disease. That's why mad cow disease is particularly worrisome to disease experts. And that's where the story of kuru among the Foré becomes relevant to us all. This lab will explore recent research by Simon Mead and colleagues on the population genetics of the PrP protein in the Foré, and its implications for other people in populations around the world.

Exercise 1: Allele and Genotype Frequencies

In humans, the PrP gene is located near the top of chromosome 20. There are two common alleles, which differ by just one nucleotide. This nucleotide occupies position 1 of codon 129. In the *M* allele, codon 129 reads ATG and specifies the amino acid methionine. In the *V* allele, codon 129 reads GTG and encodes valine. Both alleles encode functional versions of the PrP protein, and are therefore normal.

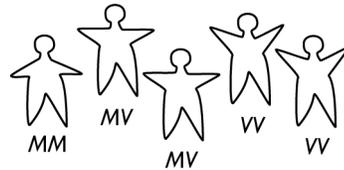


Everyone has two copies of the PrP gene as one copy is inherited from each parent. Thus, there are three possible genotypes: *MM*, *MV*, and *VV*. None of these genotypes, by itself, causes disease. Individuals with different genotypes may, however, vary in their susceptibility to disease.

The first step in investigating the genetics of PrP in a population is to determine the relative abundance of the two alleles and three genotypes. This exercise will show you how.

- [1] Launch **SimBio Virtual Labs**. Select **Hardy, Weinberg, and Kuru** from the **EvoBeaker Labs** options.
- [2] On the left side of the screen, you will see a human population. Double click (or control-click) on one of the people in the population. This will open a **Trait Editor** window in which you can see the individual's genotype. Click on the **CLOSE** button to close that individual's window.
- [3] Examine the genotypes of a few individuals. You should notice that the people in your population are color-coded by genotype, allowing you to determine an individual's genotype without opening a **Trait Editor** window.
- [4] Note that some of the individuals in your population may be hiding behind others. If you want to move a particular individual out of the way, click on the individual you want to move and drag him or her to a different place.
 - [4.1] Count the number of individuals with each genotype and use your data to fill in the middle column of the table on the following page. The total number of individuals in your population should be 100. If you find a different number, count again.

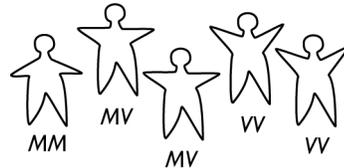
[5] The frequency of a genotype is the fraction of individuals in a population that carry it. For example, in the population at right there are 5 individuals, one of which carries genotype *MM*. Thus, the frequency of *MM* in the population is $1/5=0.2$.



[5.1] Calculate the frequencies of the three genotypes in the population on the screen and add them to the table. Check your work by making sure the three frequencies add up to 1.

GENOTYPE	NUMBER OF INDIVIDUALS	FREQUENCY
<i>MM</i>		
<i>MV</i>		
<i>VV</i>		
Total		

[6] The frequency of an allele is the fraction of gene copies in a population that are copies of that allele. In the population at right, there are a total of 10 copies of the PrP gene. Four of these are copies of the *M* allele. Thus, the frequency of the *M* allele is $4/10=0.4$. Fill in the blanks below to calculate the frequencies of the *M* and *V* alleles in the population on the screen:



[6.1] The ____ *MM* individuals carry a total of ____ copies of *M* and ____ copies of *V*.

The ____ *MV* individuals carry a total of ____ copies of *M* and ____ copies of *V*.

The ____ *VV* individuals carry a total of ____ copies of *M* and ____ copies of *V*.

Altogether there are ____ copies of *M* and ____ copies of *V*.

The frequency of allele *M* is: ____ / ____ = ____

The frequency of allele *V* is: ____ / ____ = ____

[7] Check your work by making sure the two allele frequencies add up to 1.

[8] To double check your work, close the **Trait Editor** if it is open. Then go to the **IEWS** menu at the top of the screen and select **ALLELE FREQUENCIES**. This will display the true frequencies for your population. Check to see if your calculations were accurate. If you made an error, try to figure out where you went wrong.

At this point it may feel as though we have just asked you to do the same thing twice. That is, it may seem as though there is no difference between calculating genotype frequencies versus allele frequencies. As you proceed through the lab, however, you will see that the two calculations are not redundant. If you know the genotype frequencies, you can always correctly infer the allele frequencies. If you know the allele frequencies, on the other hand, you may or may not be able to correctly infer the genotype frequencies. And when you discover that you cannot, you have learned something important about the population you are studying.

- [9] If you would like more practice calculating genotype and allele frequencies, click the **RESET** button and use the **VIEWS** menu to re-hide the **ALLELE FREQUENCIES**. This will give you a new population in which the frequencies are different.

Exercise 2: Genotype and Allele Frequencies Among the Foré

Simon Mead and colleagues in the laboratory of John Collinge, traveled to the Eastern Highlands of Papua New Guinea to determine the PrP genotypes of Foré women and girls. The researchers focused on females because females were hardest hit by the kuru epidemic. During mortuary feasts women and girls ate the internal organs of their departed kin, including the brains. Ingesting the brain of a kuru victim was the surest way to contract the illness. When men participated they tended to just eat the meat—that is, the muscles. This did not leave the men entirely immune to kuru—some did get sick, perhaps because the meat they ate had been contaminated with brain tissue during preparation.

- [1] In the **Experiment** menu, select **Foré**. You should see two windows on the screen: one with 30 adults, the other with 140 kids.
- [2] These samples replicate the Foré women Mead and colleagues examined. They determined the genotypes of 30 women who were old enough to have participated in cannibalistic feasts but who survived the kuru epidemic, and 140 women who were too young to have participated and who were therefore never exposed to kuru.
- [3] Click the **GO** button to make the individuals in the two populations move around. Compare the two populations by eye. Click the **STOP** button to stop the simulation.
 - [3.1] Does it appear to you that the genotype frequencies are different among the adults versus juveniles?
 - [3.2] What would you have to do to be sure of your answer?
- [4] Since you already know how to calculate allele and genotype frequencies, we are going to give you a short cut. Under the **VIEWS** menu, select **ADULT GENOTYPES**, then **JUVENILE GENOTYPES**.

[4.1] Record the number of juveniles and adults with each genotype in the table at the right.

[4.2] Is any of the three genotypes substantially more common—or rare—in one group than the other? Explain.

	<i>MM</i>	<i>MV</i>	<i>W</i>
Adults			
Juveniles			

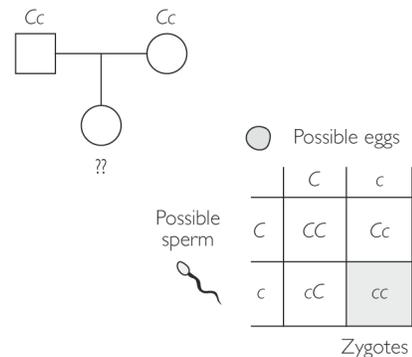
Simon Mead and colleagues thought the difference in genotype frequencies between the older versus younger Foré women was intriguing. The younger women were, after all, the descendants of the older women. Shouldn't offspring, as a group, have the same genetic composition as their parents? Instead, three-quarters of the women who survived the kuru epidemic were heterozygotes, versus only half of the women too young to have been exposed.

Before we can investigate this puzzle in more detail, we need to check our intuition about how the allele and genotype frequencies in a population are supposed to behave. That is, we need to explore some basic population genetics. Our experiments will not only shed light on what happened among the Foré, it will also give us insight into the persistence of well known genetic diseases such as sickle-cell anemia and cystic fibrosis. We will return to diseases such as these later.

Exercise 3: The Hardy-Weinberg Equilibrium Principle

Population genetics is the result of a union of Mendelian genetics with Darwinian natural selection. Mendelian genetics allows us to predict the characteristics of the next generation within particular families. Population genetics will allow us to predict the characteristics of the next generation in whole populations.

Recall that in Mendelian genetics, we predict the genotypes of offspring by tracking alleles from parents to offspring. For example, cystic fibrosis is a recessive genetic condition that renders affected individuals vulnerable to chronic and devastating lung infections. We can use C to represent the normal allele, and c to represent the disease-causing allele. If a pair of parents are both carriers for cystic fibrosis, they both have genotype Cc . We can use a Punnett square to predict that their offspring will have genotypes CC , Cc , and cc in a 1:2:1 ratio, and that the probability that any one of their offspring will have cystic fibrosis is 1 in 4.



In population genetics, alleles and genotypes are tracked through the life cycle of a population. This task is simpler if we imagine that all the individuals in the population advance through their life cycle in synchrony, and that the generations do not overlap. Eggs and sperm join to become zygotes; the zygotes develop into juveniles; the juveniles mature into adults; the adults produce gametes and die; the cycle begins anew. While this scenario is accurate for some organisms (including annual plants and many insects), for humans, it is obviously a simplification.

- [1] In the **Experiment** menu, select **Hardy-Weinberg**. On the left side of the window you should see a population of eggs and sperm. On the upper right is a set of cartoons and buttons that trace the life cycle and will allow you to escort your population around it.
- [2] Click the **GO** button. You should now be seeing the eggs and sperm swim around.
- [3] On the upper right, find the two arrow buttons that point from the eggs-and-sperm cartoon to the zygotes cartoon. We will explain these buttons in more detail shortly. Below these arrow buttons are two text fields. Next to **"Zygote pop size"** change "100" to "10." Now click on the arrow with a pair of dice next to it and watch the population on the left. As eggs and sperm find each other and fertilize, you will see zygotes appear in the population. Fertilization will continue until there are 10 zygotes, at which point all the remaining eggs and sperm disappear.
- [4] Notice that the arrow button pointing from zygotes to juveniles is now active. Click it. Your population of zygotes will instantly develop into juveniles.

- [5] Notice that the arrow button pointing from juveniles to adults is now active. Click it. Your population of juveniles will instantly mature into adults.
- [6] Finally, notice that the arrow pointing from adults to eggs and sperm is now active. Click it, and watch your population closely. You will see the adults disintegrate one by one into gametes. This is equivalent to spawning like salmon and immediately dying.
- [7] Now that you are familiar with the stages of the life cycle and how to move your population through them, let's repeat the procedure, this time paying attention to the frequencies of alleles and genotypes. First, change the zygote population size back to 100. Click on the **RESET** button to restore the simulation to its original condition. Then click the **GO** button to make the eggs and sperm swim around.
- [8] The collection of all the gametes produced by all the adults in your population can be described as a **gene pool**. Because each gamete carries exactly one copy of the PrP gene, the gene pool is a convenient place to calculate the frequencies of the two alleles. You would calculate the frequency of allele *M* by counting the eggs and sperm that carry this allele and dividing by the total number of eggs and sperm. We are not going to ask you to do it by hand. EvoBeaker has done it for you and plotted the results in the bar graph in the lower right part of the window. Note the starting frequencies: 60% of the gametes carry a copy of allele *M*; 40% carry a copy of allele *V*.
- [9] We will imagine that the gene pool is thoroughly mixed and that eggs and sperm find each other at random to make zygotes. To guide this process, stop the model by clicking on the **STOP** button. Pick an egg at random, point at it, hold the mouse button down, and drag the egg to the small "Womb" just below the main population display. Double-click the egg to see its genotype, and record it in the first line of the table below. Next, click and drag a random sperm into the womb, dropping it on top of the egg.
- [10] Click the **GO** button to resume the simulation. It may take a little time, but eventually the egg and sperm in the womb will fuse to make a zygote. The zygote will remain in the womb for a bit, then automatically move back to the main population.

[9.1] Record the sperm's genotype in the table below.

[10.1] Alongside the genotypes of the egg and sperm you chose, record the genotype of the resulting zygote in the first row of the table below.

ZYGOTE #	EGG'S GENOTYPE	SPERM'S GENOTYPE	ZYGOTE'S GENOTYPE
1			
2			
3			
4			
5			

[10.2] Why did your zygote turn out to have the genotype it did and not one of the other two possible genotypes?

[10.3] Make four more zygotes as in Step 9, recording the genotypes of the eggs, sperm, and zygotes in the table above. Note that you will need to STOP the model just after each of the zygotes is formed so that you can record the zygote genotypes and then START the model to make your next zygote.

[10.4] If you were to make five more zygotes, would you likely get the same combination of genotypes or something different? Why?

Putting eggs and sperm together may be more tedious than you might have hoped. The gene pool-to-zygotes button with the dice on it lets eggs and sperm find each other at random without your help. When we say the eggs and sperm find each other at random, we mean that eggs and sperm combine with no regard for their own or the other's genotype. Any egg can combine with any sperm, and vice versa. The gametes just swim around, blindly bump into each other, and fuse. This continues until the number of zygotes matches the number specified in the box labeled "Zygote pop size." Eggs and sperm that have not found a mate by this deadline are out of luck; they simply die and disappear.

[11] Click the **GO** button to start the simulation. Then position the mouse pointer over the **DICE** button and watch the gene pool on your screen while you click. It will take the eggs and sperm a few seconds to do their thing. You will know they are done when the Gametes bar graph is empty. Note that there is a second bar graph that reports the frequencies of the three genotypes in your population of zygotes.

[11.1] Record these frequencies here:

Frequency among zygotes of genotype *MM*: _____ *MV*: _____ *VV*: _____

★ For display on graphs, EvoBeaker rounds genotype and allele frequencies to the nearest hundredth. As a result, the frequencies may sometimes not add up to precisely 1.0.

[12] Click the arrow to advance your population to the juvenile stage.

[12.1] Record the frequencies of the three genotypes among the juveniles here:

Frequency among juveniles of genotype *MM*: _____ *MV*: _____ *VV*: _____

[13] Click the arrow to advance your population to the adult stage.

[13.1] Record the frequencies of the three genotypes among the adults here:

Frequency among adults of genotype *MM*: _____ *MV*: _____ *VV*: _____

[14] The transition from adults to eggs and sperm in the gene pool can seem mysterious, so it's worth some effort to understand how EvoBeaker does it. Before clicking the button to allow the adults to disintegrate into gametes, consider the rules EvoBeaker follows and predict what the allele frequencies will be in the new gene pool. To keep things simple, EvoBeaker takes humans to be hermaphrodites. That is, every adult makes both eggs and sperm. Each adult makes exactly four gametes. The genotypes of the four gametes are determined by the rules of Mendelian genetics. That is, an *MM* individual makes two *M* eggs and two *M* sperm. An *MV* individual makes an *M* egg and a *V* egg, plus an *M* sperm and a *V* sperm.

[14.1] Fill in the following blanks to predict what the allele frequencies will be in the gene pool.

The _____ *MM* adults will make a total of _____ *M* gametes and _____ *V* gametes.

The _____ *MV* adults will make a total of _____ *M* gametes and _____ *V* gametes.

The _____ *VV* adults will make a total of _____ *M* gametes and _____ *V* gametes.

Altogether there are _____ copies of *M* and _____ copies of *V*.

The frequency of allele *M* will be _____ / _____ = _____

The frequency of allele *V* will be _____ / _____ = _____

[15] Now watch the screen while you click the **ARROW** button to let the adults turn into gametes. This button is between the zygotes and juveniles on the right top of your screen.

[15.1] Record the allele frequencies in the new gene pool in the spaces below. If your allele frequency predictions were not correct, try to figure out why.

Frequency among gametes of allele *M*: _____ *V*: _____

[16] Recall that the starting frequency of allele *M* was 0.6, and that of allele *V* was 0.4.

[16.1] Are the allele frequencies in the new gene pool different? If so, what was the cause of the change?

[16.2] Transfer the data you recorded in Questions 11.1, 12.1, 13.1, and 14.1 to the Experiment 1 column in the table below.

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
Starting allele frequencies	M: 0.6 V: 0.4	M: 0.6 V: 0.4	M: 0.6 V: 0.4
Zygote genotype frequencies	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___
Juvenile genotype frequencies	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___
Adult genotype frequencies	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___	MM: ___ MV: ___ VV: ___
Ending allele frequencies	M: ___ V: ___	M: ___ V: ___	M: ___ V: ___

Zygote population size: 100

[17] In the experiment you have just completed, you started with a gene pool with allele frequencies of 0.6 and 0.4, let eggs and sperm find each other at random to make 100 zygotes, then allowed the zygotes to complete their life cycle and make a new gene pool.

[17.1] If you were to repeat this experiment, do you think the new gene pool would have the same ending allele frequencies? Why or why not?

[18] **RESET** the simulation to restore the initial allele frequencies to 0.6 and 0.4, then click the **GO** button to start the simulation running. Use the **DICE** button to make 100 zygotes.

[18.1] Wait until all the gametes are gone, then record the genotype frequencies among the zygotes in the Experiment 2 column in the table above. Click the population around the life cycle until you return to the zygotes, recording the genotype or allele frequencies at each stage.

[19] Repeat the experiment a third time. **RESET** the simulation to restore the initial values to 0.6 and 0.4, then click the **GO** button. Use the **DICE** button to make zygotes, then click the population around the life cycle until you return to the zygotes.

[19.1] Record the frequencies in the Experiment 3 column in the table above.

- [19.2] Was your prediction in Question 17.1 correct? Is the final frequency of allele *M* always the same? If it is not always the same, can you guess what causes it to be different every time?

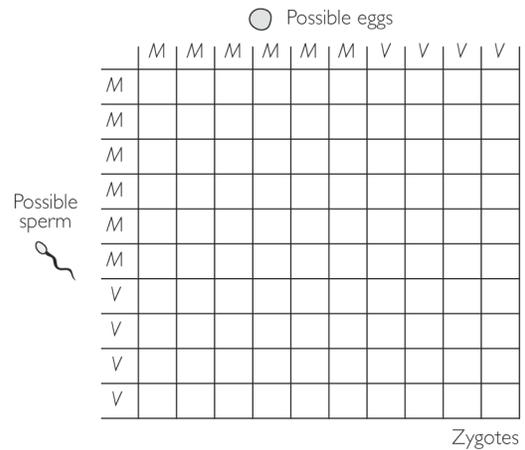
Unless you've been hitting the wrong button, your three ending frequencies will be different. The cause of this variation is the random nature of the encounters between eggs and sperm in the gene pool. Sometimes, by chance, more *M* gametes find mates before time runs out. As a result, the frequency of allele *M* is higher in the new generation than it was in the previous one. Other times, by chance, more *V* gametes find mates before time runs out. This makes the frequency of allele *M* lower in the new generation. This kind of change in allele frequencies, due to the influence of randomness in determining which gametes do and do not find a mate in the gene pool, is called **genetic drift**.

- [19.3] Do you think that genetic drift will cause more rapid change in the allele frequencies in a large population or a small one? Explain your reasoning.

- [20] [Optional] If you want to check whether you were right in Question 19.3, change the number in the "**Zygote pop size**" text box to 10. This will only make 10 zygotes when you click the **DICE** button rather than 100, so you will make a much smaller population size. You can do a similar set of experiments to those above to see whether genetic drift is more rapid in a smaller population.

Genetic drift is an important mechanism of evolution that is explored in more detail in other EvoBeaker labs. For now, we would like to know what would have happened to the allele frequencies in our population if genetic drift had not been a factor.

[21] If you changed the zygote population size, set it back to 100. **RESET** the simulation to return to a starting gene pool with allele frequencies of 0.6 and 0.4, then click the **GO** button. If chance played no part in the outcome, what proportions of *MM*, *MV*, and *VV* individuals would we expect among 100 zygotes? You can answer this question using the same technique we used to answer our question about cystic fibrosis on page 5: a Punnett square. We just have to use a bigger one. We put eggs along one side in a ratio of 6 to 4, and sperm along the other side in a ratio of 6 to 4. Then we fill in the zygotes. As in the simpler cystic fibrosis question, this gives us a general expectation under the laws of genetics and probability, not a specific prediction for a particular family or population. Fill in the boxes in the Punnett square at right to indicate the zygote genotypes. (You don't have to write a genotype in every box; just shade all the *MM* boxes one color, all the *MV* boxes another color, and so on.)



[21.1] Based on your Punnett square fill in the blanks below to indicate the predicted number, among 100 zygotes, with each genotype:

Expected number of zygotes with genotype *MM*: _____ *MV*: _____ *VV*: _____

[21.2] Divide by the total number of zygotes to get the expected genotype frequencies:

Expected frequency among zygotes of genotype *MM*: _____ *MV*: _____ *VV*: _____

[22] EvoBeaker can make Punnett squares behind the scenes. That's what the $(p + q)^2$ arrow between the gene pool and zygotes is for. When you click the $(p+q)^2$ button, it makes the requested number of zygotes in proportions predicted by a Punnett square like the one you made above. Make sure your simulation is running, and click on the $(p+q)^2$ button now.

[22.1] Record the genotype frequencies among the resulting zygotes in the space below.

[22.2] Do they match the predictions you made based on your own Punnett square? (If not, try to figure out why.)

[23] Click the appropriate buttons to develop your zygotes into juveniles and mature them into adults. Next, click the button to let the adults turn into gametes, and record the allele frequencies. Recall that the starting frequency of allele *M* was 0.6, and that of allele *V* was 0.4.

[23.1] **What are the frequencies of *M* and *V* in the new gene pool? Have they changed? Why or why not?**

[24] Unless something went wrong, you will have found that the frequencies of alleles *M* and *V* in the new gene pool are exactly what they were in the starting gene pool. The population has made a complete trip around the life cycle, but its genetic composition hasn't changed. Use the **(p+q)²** button to go from the gene pool to zygotes. Then click the buttons to move the population around its life cycle and back to a new gene pool.

[24.1] **Are the frequencies of alleles *M* and *V* still 0.6 and 0.4?**

[25] Apparently, when random chance plays no role in the gene pool, allele frequencies do not change from one generation to the next. Is this outcome special to the frequencies 0.6 and 0.4? The text box labeled **"Init freq of *M*"**, just under the **DICE** and **(p+q)²** buttons, controls the starting frequency of allele *M* in the gene pool. Try entering a different initial frequency for allele *M*, such as 0.1, or 0.3, or 0.8. **RESET** the simulation and click the **GO** button. Use the **(p+q)²** button to move from gene pool to zygotes, then move the population around its life cycle to the next generation's gene pool.

[25.1] **With a different starting allele frequency, do the allele frequencies change from one generation's gene pool to the next?**

You have discovered, by experimentation, a fundamental result in population genetics. In a randomly mating population without genetic drift, the allele frequencies do not change from one generation to the next, and the genotype frequencies can be determined by multiplying the allele frequencies (as in a Punnett square). This result was first proved algebraically in 1908 by two researchers working independently: G.H. Hardy and Wilhelm Weinberg. In their honor, the result is called the **Hardy-Weinberg equilibrium principle**. A population in which allele frequencies do not change from one generation to the next is said to be in **Hardy-Weinberg equilibrium**.

Bonus Question: Try to explain why the gametes à zygotes button has the label (p+q)²? Hint: The variables *p* and *q* represent allele frequencies. Think about your Punnett Square again, and try multiplying out $(p+q) \times (p+q)$.

The Hardy-Weinberg equilibrium principle is going to be the key to understanding Mead and colleagues' analysis of PrP genotypes among the Foré. Before we can explain why, however, we need to think about the assumptions we have built into our model population. By using the **(p+q)²** button instead of the **DICE** button, we have assumed that random chance plays no role in the gene pool. This assumption would be correct in an infinitely large population. If we had an infinite number of gametes in the gene pool making an infinite number of zygotes, the genotypes among the zygotes would have exactly the frequencies predicted with a Punnett square.

The no-drift assumption is obviously violated in any real population. You saw the result when you used the **DICE** button instead of the **(p+q)²** button. In a finite population allele frequencies can change unpredictably from one generation to the next.

Another assumption we've made is that no new individuals move into our population from elsewhere. You can violate this assumption by using the **ADD PERSON** tool to add new juveniles to the population at the juvenile stage of the life cycle.

[26] First, set the initial frequency of *M* to a fairly low value, such as 0.1, 0.2, or 0.3.

[26.1] **Record the value you chose in the first row of the table at right.**

[26.2] **RESET the simulation and click the GO button. Use the (p+q)² button to move from gene pool to zygotes.**

Starting allele frequencies	M__ V__
Zygote genotype frequencies	MM__ MV__ VV__
Juvenile frequencies before	MM__ MV__ VV__
Juvenile frequencies after	MM__ MV__ VV__
Adult genotype frequencies	MM__ MV__ VV__
Ending allele frequencies	M__ V__
Zygote Population Size: 100	

[26.3] **Record the zygote genotype frequencies.**

[26.4] **Advance the population to the juvenile stage and record the genotype frequencies in the third row of the table. Click on the STOP button.**

[27] Click on the **ADD PERSON** tool and then click inside the "**Population**" window to add an individual to your population. By default, the **ADD PERSON** tool adds a juvenile of genotype *MM*. If you want to add an individual with a different genotype, add an *MM* individual and then click on the **SELECT** tool. Double click on that individual to open a **Trait Editor** window. In the **Genotype menu**, select one of the three possible genotypes.

[28] Add several *MM* juveniles to your population, keeping an eye on the genotype frequencies as you do so.

- [28.1] When you are done adding juveniles, record the new genotype frequencies in the fourth row of the table above.
- [28.2] Click on GO to restart the simulation. Advance the population to the adult stage and record the genotype frequencies.
- [28.3] Finally, advance the population to the gene pool stage and record the ending allele frequencies.
- [28.4] Are the ending frequencies different from the starting frequencies? Why?

We have also assumed that all individuals in the population survive to reproduce. You can violate this assumption by using the **DELETE** tool to kill adults of one genotype or another.

[29] First, set the initial frequency of *M* to a fairly high value, such as 0.8 or 0.9.

[29.1] Record the value you chose in the first row of the table at right.

[29.2] RESET the simulation and click the GO button. Use the $(p+q)^2$ button to move from gene pool to zygotes. Record the zygote genotype frequencies.

[29.3] Advance the population to the juvenile stage and record the juvenile genotype frequencies.

[29.4] Advance the population to the adult stage and record the genotype frequencies in the fourth row of the table.

[30] Now STOP the simulation and select the DELETE tool from the control panel. Move the cursor into your population and click on one of your *MM* people to make it disappear from the population. Remove several more *MM* individuals, keeping an eye on the genotype frequencies as you do so.

[30.1] When you are done, record the new genotype frequencies in the fifth row of the table. Be sure to click on the SELECT tool when you are finished using the DELETE tool.

[30.2] Finally, click on the GO button to start the model and advance the population to the gene pool stage and record the ending allele frequencies. Are the ending frequencies different from the starting frequencies? Why?

Starting allele frequencies	<i>M</i> __ <i>V</i> __
Zygote genotype frequencies	<i>MM</i> __ <i>MV</i> __ <i>VV</i> __
Juvenile frequencies before	<i>MM</i> __ <i>MV</i> __ <i>VV</i> __
Adult frequencies before	<i>MM</i> __ <i>MV</i> __ <i>VV</i> __
Adult frequencies after	<i>MM</i> __ <i>MV</i> __ <i>VV</i> __
Ending allele frequencies	<i>M</i> __ <i>V</i> __
Zygote Population Size: 100	

A final assumption we have made in our model is that there are no mutations—that copies of allele M never turn into copies of allele V and vice versa. You can violate this assumption by double-clicking on an egg or sperm in the gene pool, then changing its copy of the PrP gene from one allele to the other. We will explore mutation in more detail in other EvoBeaker labs. For now, we will just tell you that because mutation rates are low, mutations from one allele to another cause only negligible changes in allele frequencies from one generation to the next.

Exercise 4: Chi-Square Tests for Hardy-Weinberg Equilibrium

Recall our definition of Hardy-Weinberg equilibrium. It is the condition of a population when all our assumptions are true: when there is no genetic drift, no immigration, no death before reproduction and no mutation. Your experiments will have illustrated that the population changes from one generation to the next—that is, the population evolves—only when one or more of our assumptions are violated. The Hardy-Weinberg equilibrium principle is thus a **null model**. It is what we expect to see if nothing interesting is happening. We can check whether a population appears to be in Hardy-Weinberg equilibrium. If it is, we shrug our shoulders and go look for another population to study. But if it is not, then we know we have found a situation worth investigating further.

- [1] The most straightforward way to tell whether a population is in Hardy-Weinberg equilibrium is to see whether its allele frequencies change over several generations. To see how this might work, go to the **Experiment** menu and select **More HW**. Notice that there are now text boxes on the screen that let you engage in the systematic violation of our various assumptions. For example, if you change the number of *MM* immigrants from 0 to 5, then every time the population passes through the juvenile stage, the computer will add five *MM* individuals.
- [2] Pick one or more of the following: immigrants, survival rates, and mutation rates. For each factor you picked, change one of the values. Click **GO** and send your population all the way around its life cycle for three generations, noting the allele frequencies in each generation's gene pool.
 - [2.1] **Do the values you have selected cause the allele frequencies to change over time? Explain.**
- [3] In the real world, we often do not have the luxury of waiting to see whether allele frequencies will change from one generation to the next. Fortunately, there is another way to check whether a population is in Hardy-Weinberg equilibrium. With a little arithmetic, we can determine whether the genotype frequencies match what you would predict from the allele frequencies using a Punnett Square. If they match, then the population is in equilibrium. If they don't match, then it isn't. Here are the steps:
 - [a] Count the number of individuals with each genotype. These are the **observed** numbers.
 - [b] From the genotype counts, calculate the frequencies of the alleles. You practiced this in Exercise 1.
 - [c] Use the allele frequencies and a Punnett square to calculate the number of individuals with each genotype you would expect to find in **an uninteresting population**. You practiced this in Exercise 3. The results are the **expected** numbers.
 - [d] Compare the expected numbers to the observed numbers to see if there is a discrepancy.

EvoBeaker will do all this for you using a window called the **Chi-Square Calculator** as follows.

[4] Make sure the number of immigrants is set to 0 for all three genotypes, that the survival rates are set to 1 for all three genotypes, and that both mutation rates are set to 0. Click the **RESET** button, then click the **GO** button to start the simulation. Using the **(p+q)²** button, move the population from gene pool to zygotes. Then click the buttons to go from zygotes to juveniles to adults.

[5] From the **TOOLS** menu at the top of the screen, select the **Chi-Square Calculator**. You should have an adult population in which there are 36 *MM* individuals, 48 *MV* individuals, and 16 *VV* individuals. Enter these numbers into the **Chi-Square Calculator** under A1A1, A1A2, and A2A2. Click the **CALCULATE** button.

[6] When you click **CALCULATE**, the **Chi-Square Calculator** compares the observed versus expected numbers for each genotype by calculating the quantity $[(\text{Observed} - \text{Expected})^2 \div \text{Expected}]$. It then sums across genotypes to get a statistic called the **chi-square**. The larger the overall difference between the observed versus expected numbers, the larger the chi-square.

[6.1] What value did you get for chi-square with the numbers you entered? Why?

[7] Move your population ahead to the gene pool, use the **(p+q)²** button to advance to zygotes, then click to advance zygotes to juveniles. Now, before you do anything else, change the survival rate for genotype *MM* to zero, the rate for genotype *MV* to 0.5, and the genotype for *VV* to 0.75.

[8] Advance the population from juveniles to adults. You now have a population in which there are 0 *MM* individuals, 24 *MV* individuals and 12 *VV* individuals, for a total population size of 36.

[8.1] Fill in the blanks below to calculate the frequencies of the *M* and *V* alleles in this population.

The ____ *MV* individuals carry a total of ____ copies of *M* and ____ copies of *V*.

The ____ *VV* individuals carry a total of ____ copies of *M* and ____ copies of *V*.

Altogether there are ____ copies of *M* and ____ copies of *V*.

The frequency of allele *M* is: ____ / ____ = ____

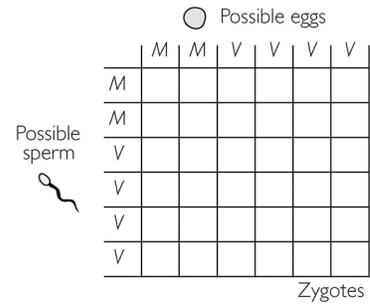
The frequency of allele *V* is: ____ / ____ = ____

[8.2] If we made 36 zygotes from a gene pool in which the 1/3 of the gametes carried allele *M* and 2/3 carried allele *V*, how many zygotes would we expect to have genotypes *MM*, *MV*, and *VV*? Fill in the zygote genotypes in the Punnett Square at right to find out.

[8.3] Expected number with genotype *MM*: ____ *MV*: ____ *VV*: ____

[9] Look back at the adult population on your computer screen. There are 0 *MM* individuals, 24 *MV* individuals and 12 *VV* individuals. Enter these numbers into the Chi-Square Calculator and click **CALCULATE**.

[9.1] Compare your expected numbers from Question 8.1 with the expected numbers in the Chi-Square Calculator. Do they match? Explain in your own words where the observed and expected numbers in the Chi-Square Calculator come from.



[9.2] What value did you get for chi-square?

[9.3] Why is it different from zero?

[9.4] Is your population in Hardy-Weinberg equilibrium? Explain.

[9.5] Review the assumptions under which a population is expected to be in Hardy-Weinberg equilibrium. Which of the assumptions has been violated in your population?

[10] Advance your population from adults to the gene pool, then from the gene pool to zygotes. Enter the number with each genotype into the Chi-Square Calculator and click **CALCULATE**.

[10.1] What value do you get for chi-square?

[10.2] Is the population in Hardy-Weinberg equilibrium now? Why?

The larger the chi-square value, the more certain we can be that there really is something interesting happening in a population. By convention, if the chi-square value we get in our test for Hardy-Weinberg equilibrium is larger than 3.841 we can conclude that the genotype frequencies are significantly different than expected under our null model.

[11] Look back at the data from the Foré populations in Exercise 2. Analyze the juveniles first.

[11.1] What value do you get for the chi-square?

[11.2] Is the juvenile Foré population in Hardy-Weinberg Equilibrium?

[11.3] Now analyze the adults. What value do you get for the chi-square?

[11.4] Is the adult population in Hardy-Weinberg Equilibrium?

[11.5] Why do you think the editors of *Science* thought Mead's data on the Foré were interesting enough to publish?

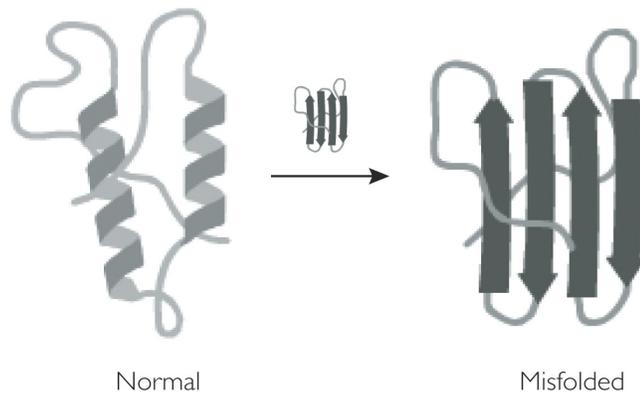
Exercise 5: What Might Have Happened Among the Foré?

- [1] If you are not there already, use the **Experiment** menu to return to **More HW**. In the **Init Freq of M** box, enter a value that matches the frequency of allele M among the Foré juveniles from Exercise 2.

- [2] Your challenge is to devise at least one plausible story in which the adult population ends up being as far from Hardy-Weinberg Equilibrium as the real Foré adults. You can change the number of immigrants for any or all genotypes, you can change the survival rates, you can change the mutation rates, you can use the **DICE** button to allow genetic drift, and you can move the population through its life cycle as many times as you like. (There is a NOTES page at the end of the workbook.)
 - [2.1] Describe a scenario you found that produces adults as far from Hardy-Weinberg Equilibrium as the Foré adults. Include in your description a plausible real-world event that might have made the immigration rates, survival rates, mutation rates, or genetic drift experienced by the Foré match the values you used in your EvoBeaker model.

 - [2.2] Did you think about the kuru epidemic among the Foré while you were devising your scenario? Which aspects of your model (survival rates, migration rates, mutation rates, genetic drift) are most strongly affected by a fatal disease? Do you think the scenario you found might plausibly have been caused by kuru?

Mead and colleagues think that the deviation from Hardy-Weinberg equilibrium in the Foré adults was a direct result of the kuru epidemic. Recall that spongy brain diseases like kuru are transmissible. You can get them by eating the brain of another victim. The leading hypothesis is that the transmissible agent is the PrP protein itself. PrP is an abbreviation for Prion Protein. Prion is a term coined by Stanley Prusiner to denote an infection particle made entirely of protein.



The idea is that PrP has two three-dimensional forms: a normal form and a misfolded form (figure adapted from Genetic Science Learning Center at the University of Utah). The misfolded form kills nerve cells, causing kuru. What flips a normal PrP protein into the misfolded conformation is contact with an already-misfolded protein. If you eat the brain of a kuru victim, you ingest misfolded copies of PrP. These flip some of your own PrP proteins into the misfolded form, which in turn flip others, and so on. As misfolded PrP accumulates, nerve cells die and your brain turns into a sponge.

Mead and colleagues believe that having two slightly different forms of PrP in your cells is somehow protective. If a group of Foré women ate a kuru victim, the *MV* heterozygotes would be less likely to get kuru themselves than *MM* women and the *VV* women.

[3] Use **EvoBeaker** to evaluate the plausibility of this hypothesis.

[3.1] **Can higher survival rates for heterozygotes produce an adult population as far from Hardy-Weinberg equilibrium as the Foré? How large does the difference in survival rates have to be? Explain below.**

Exercise 6: Kuru and the Rest of Us

- [1] There is one more twist to the kuru story. To appreciate it, we need to investigate the consequences of higher survival rates for heterozygotes. Enter a low value for the starting frequency of allele *M*, such as 0.1 or 0.2. **RESET** the EvoBeaker simulation. Enter survival rates consistent with the Mead and colleagues measurements of genotypes among the Foré adults (from Exercise 2), such as 40% for MM, 25% for VV, and 100% for MV. Make sure the number of immigrants for all genotypes, and both mutation rates, are set to zero.
- [2] Click **GO** to start the simulation and chase your population around the life cycle for at least 5 generations. (Each time you reach the gene pool phase, wait about 20 seconds to give the gametes time to become well-mixed.)
- [2.1] **What happens to the frequency of allele *M* over several generations? Why?**
- [3] Repeat the experiment, this time with a high starting frequency for *M*, such as 0.8 or 0.9. **RESET** your simulation, click **GO** the model, then chase the population around the life cycle at least five times.
- [3.1] **Now what happens to the frequency of allele *M* over several generations? Why?**
- [4] Your experiments have shown that a survival advantage for heterozygotes keeps alleles at intermediate frequency. Heterozygote advantage thus helps maintain the coexistence of two or more alleles in the population. Mead and colleagues surveyed populations from around the world. They found that in most populations, both the *M* and *V* alleles are present, usually at intermediate frequencies. The researchers speculate that heterozygotes for the PrP gene have had a survival advantage in populations everywhere. What could give heterozygotes a survival advantage? Possibly any number of things, but there is one factor we already know about: spongy brain diseases. Mead and colleagues suggest that the data may be telling us that we're all descended from cannibals.
- [4.1] **Provide your candid reaction to Mead's hypothesis. Is there another explanation for the persistence of both alleles in populations all over the world?**

Sickle-cell anemia and cystic fibrosis are severe genetic diseases that affect homozygotes carrying two mutant alleles. Until recently, most affected individuals died before reaching reproductive age. Nonetheless, the alleles that cause these diseases remain surprisingly common. The reason appears to be that heterozygotes are resistant to infectious diseases—malaria in the case of sickle-cell anemia and typhoid fever in the case of cystic fibrosis.

[5] Use EvoBeaker to show how higher survival among heterozygotes can maintain a mutant allele in a population, even when homozygotes rarely survive to adulthood. First, enter 0.1 for the initial frequency of M , which now will represent a disease allele. Enter survival rates reflecting the ideas that MM individuals suffer from a genetic disease and rarely survive to reproduce and that MV individuals are more likely to survive epidemics of infectious disease than are VV individuals. **RESET** your simulation, then chase the population around the life cycle at least five times.

[5.1] **What happens to the frequency of allele M ? Why?**

[5.2] **Do genotypes MM and VV ever disappear from the population? Why or why not?**

Exercise 7: EXTENSION

Investigating an Unknown Population

[1] Under the **Experiment** menu you will find four different **Experiments with Unknown Populations**. Select any one of them.

[2] In the window that opens, you will find a population at the adult stage of their life cycle. Collect and analyze data to determine whether this population is in Hardy-Weinberg equilibrium.

[2.1] **What do you conclude?**

[2.2] **If your population is not in Hardy-Weinberg equilibrium, suggest a possible explanation. Be as specific and detailed as possible.**

[2.3] **Based on your hypothesis, predict how the allele frequencies in the population will change from one generation to the next. Explain your reasoning.**

[3] Click **GO** to start the simulation and then escort the population through several generations around the life cycle.

[3.1] **Do the allele frequencies change in the way you predicted they would? Explain.**

[4] Finally, **STOP** the model and use the **VIEWS** menu to reveal the parameters for the model.

[4.1] **How well do these values match your hypothesis about this population?**

[5] If you have time, repeat this exercise for other **Unknown Population** scenarios.

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