
Hardy-Weinberg Simulation

Name: Joe

Lab Partners: Group 1

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INTRODUCTION:

In the first part of this exercise we will demonstrate the contention of the Hardy-Weinberg Theorem that genetic recombination as a result of sexual reproduction will not by itself cause any change in allele frequencies or genotype frequencies in a population from one generation to the next. Obviously, it is difficult to demonstrate this using real organisms: depending on the organism, it could take days, weeks, months or even years to show that allele and genotype frequencies remained constant for several generations - or even for a single generation. Furthermore, the Hardy-Weinberg equilibrium depends on the absence of any forces operating which might change allele frequencies in a population. These forces - mutation, migration, selection, and chance effects - are almost never absent from natural populations; and it would be very difficult even in the laboratory to erect a set of conditions that might reliably exclude them. The Hardy-Weinberg equilibrium also assumes random mating in the population, and once again this is a situation that is rarely found in nature and difficult to achieve in the lab.

Therefore, for the purposes of our demonstration, we are going to work not with real organisms but with a model in this case, dried beans.

HYPOTHESIS:

What might be the outcome if all of the 2,000 beans were to mate and produce two children? Explain.

Given all of these traits and which are dominant. I hypothesize that the result will give 50% Speckled, 5% White, and 45% Brown. I hypothesize that this is the case because the White beans make up only 9% of the population, and Speckled representing 42%, and Brown being 49%. I think that this is not quite a very accurate demonstration of how the next generation would be. Because in most animals and humans case they only mate with those who are most like them.

MATERIALS & EQUIPMENT:

Each of the coffee cans which you see around you in the lab today contains approximately 2,000 dried beans, representing a population of 2,000 diploid organisms. The beans are of three colors-white, brown and speckled - and each color represents one of the three possible genotypes for a certain trait. Nine percent of the beans are white, and these represent individuals that are homozygous recessive for the trait (genotype aa). Forty-two percent of the beans are speckled and these represent individuals that are heterozygous for the trait (genotype Aa). Finally, forty-nine percent of the beans are brown, and these represent individuals that are homozygous dominant for the trait (genotype AA). (This starting information is summarized in Table 1.)

Table 1. Bean colors, genotypes, and genotype frequencies.

<u>Color</u>	<u>Genotype</u>	<u>Relative frequency (%)</u>
White	aa	9
Speckled	Aa	42
Brown	AA	49

PROCEDURE:

(A) Calculating allele frequencies p and q arithmetically.

The first thing we have to do is to calculate the frequency p of the dominant allele A and the frequency q of the recessive allele a. This can be done either by using simple arithmetic or by using the Hardy-Weinberg equation. To calculate p by simple arithmetic, first of all determine the *total number of alleles* for a given gene in a population of 2000 diploid individuals.

Total number of alleles = 4,000 (2 x 2,000)

Next, how many of these are dominant (A) alleles? These alleles are found in homozygous dominants and in heterozygotes, so we have to know how many of these individuals we have.

Homozygous dominant individuals = $(0.49) \times 2,000 = 980$

Heterozygous individuals = $(0.42) \times 2,000 = 840$

How many dominant (A) alleles are there in this population? Remember, each homozygous dominant individual has 2 such alleles, but each heterozygote has only 1.

Total number of A alleles = 2,800

Finally, p , the frequency of A alleles in the population, can be determined by dividing the total number of A alleles by the total number of alleles.

$p = \frac{\text{Total number of A alleles}}{\text{Total number of alleles}} = .7$ which is also equal to 70%

Now repeat this procedure to get q , the frequency of the recessive allele a in the population, by simple arithmetic.

$q = \frac{\text{Total number of a alleles}}{\text{Total number of alleles}} = .3$ which is also equal to 30%

(B) Calculating allele frequencies p and q from the Hardy-Weinberg equation

We can also derive p and q from the Hardy-Weinberg equation. To do this, we calculate p as:

p = the frequency of AA genotypes + 1/2 the frequency of Aa genotypes

In our populations, p calculated in this way would be 70%

Similarly, we can calculate q as:

q = the frequency of aa genotypes + 1/2 the frequency of Aa genotypes

Do we get the same answer for p and q by using simple arithmetic as we get by using the Hardy-Weinberg equation? Is $p + q = 1$ in both cases?

Yes, we do.

For genes that show dominance, it is often impossible to distinguish between the phenotypes of homozygous dominants and heterozygotes, because they look the same. In these cases, we might take the square root of the frequency of homozygous recessives to determine q , and then estimate the value of p by the equation $p = 1 - q$.

(C) The simulation.

We are now ready to begin our random mating simulation. Shake your can vigorously to make sure your beans are thoroughly mixed. Then, without looking, reach into the can and draw out the first two beans your fingers encounter. Use a tally mark to record the colors of the beans you have withdrawn, and the genotypes these colors represent, on Table 2. Put the beans back in the can and draw out another pair. Tally the colors and genotypes of this pair, and put them back into the can. Repeat this procedure until you have tallied a total of twenty-five matings on Table 2.

Table 2. Random mating tallies.

<u>Mating pairs (color x color)</u>	<u>Genotypes</u>	<u>M a t i n g tallies</u>
brown x brown	AA x AA	6
brown x speckled	AA x Aa	7
brown x white	AA x aa	5
speckled x speckled	Aa x Aa	3

speckled x white	Aa x aa	3
white x white	Aa x aa	1

DATA TABLES:

See Attached

(D) Using the results to calculate genotype frequencies.

Next, use Punnett squares and your knowledge of Mendelian genetics to determine the genotypic ratios that we would expect to find in the offspring that would be produced by each of the six possible mating combinations in our population. Express these genotypic ratios as fractions (1, 1/2, 1/4, or 0) and record them in Table 3. Also record the number of times you drew each of the six possible mating types. Then, determine the representation of each genotype among the offspring produced by these matings. To do this, assume that each mating produces four offspring, and multiply the number of times each mating type was drawn by four to get the total number of offspring produced by matings of that particular type. Finally, multiply that number by the fractional expected genotypic ratio for each genotype. This will give you the number of offspring of a particular genotype that we can expect to be produced from matings of each of the six possible mating types.

Table 3. Representation of various genotypes among offspring.

Mating Type	Times Drawn	Offspring per mating	Total offspring	Ratio AA	Ratio Aa	Ratio aa	# AA	# Aa	# aa
AA x AA	6	4	28	1			24		
AA x Aa	7	4	20	1/2	1/2		14	14	
AA x aa	5	4	12		1			20	
Aa x Aa	3	4	12	1/4	1/2	1/4	3	6	3
Aa x aa	3	4	12		1/2	1/2		6	6
aa x aa	1	4	4			1			4

Ratio = genotypic ratios of offspring expressed as fractions. **#** = numbers of offspring of various genotypes.

Total the number of offspring of each of the three possible genotypes, and enter these totals in Table 4. Since you drew 25 mating pairs and each mating produced four offspring, the grand total of all offspring produced is equal to 100. Therefore, the genotype frequency expressed as a percentage for each of the three possible genotypes will be numerically equal to the number of offspring for each of these genotypes. Enter these genotype frequencies in Table 4. Do they correspond to the genotype frequencies with which we began, as recorded in Table 1?

Table 4. Genotype frequencies in the new generation.

Genotype Number of offspring Relative frequency (%)

AA = 41%

Aa = 46%

aa = 13%

Now calculate the frequency **p** of the dominant allele A among the offspring produced by our simulated random matings, and the frequency **q** of the recessive allele a in the same group. You may do this either arithmetically or by using the Hardy-Weinberg equation. Has either **p** or **q** changed in the new generation? Have we demonstrated successfully that genetic recombination as a result of sexual reproduction can not by itself result in any change in allele frequencies or genotype frequencies from one generation to the next?

$$P = AA + \frac{1}{2}(Aa) \qquad Q = aa + \frac{1}{2}(Aa)$$

$$P = 41 + \frac{1}{2}(46) \qquad Q = aa + \frac{1}{2}(46)$$

$$P = 41 + 23 \qquad Q = 13 + 23$$

$$P = 64 \qquad Q = 36$$

Yes, p and q have changed from generation to generation, however not by much.

Remember that our demonstration was designed to exclude the effects of various factors that can alter allele frequencies. How successfully has this been done? Have we, for example, been entirely successful in excluding the influence of chance effects on allele and genotype frequencies? If you don't think that we have, suggest some ways in which we might change our procedure so as to eliminate or at least reduce the impact of these effects on our results.

We did not eliminate all of the chance affects, such as some people picking the beans

closer to the top, and other people picking beans lower down. We could have reduced if not eliminated the chance affects by making sure we did not drop and beans. By not looking at the beans before they came to us, and by collecting them from one specific location in the can.

(E) Demonstrating factors that skew Hardy-Weinberg predictions.

Suggest an experiment or exercise using our beans and coffee cans that would demonstrate the effect of migration on allele and genotype frequencies. How could we demonstrate the impact of selective pressures? Of non-random mating?

An example of migration would be if we took some of the beans out of the cans for a period of time and brought them back. An example of non-random mating would be setting up a table that explained that 75% of the time the white beans would mate with another white bean, and 25% of the time it would mate with a speckled bean, but never with a black one and vice-versa.

(This matter of non-random mating is particularly intriguing. What would happen if, for example, you prohibited matings between beans representing different genotypes and permitted only beans representing the same genotype to mate? Would this procedure alter genotype frequencies in the next generation, and if so how? What if you did the opposite and permitted only beans representing different genotypes to mate? Would this procedure alter genotype frequencies in the next generation, and if so how? Would either procedure have any effect on allele frequencies?)

If we were to set it up similar to the way I suggested you would probably end up with a closer amount of the same genotype. This is because if only or most of the time the beans only mate with another bean with the same genotype they would produce beans that had the same genotype as them. If you were to only allow the beans to mate with their opposites they would probably end up very different genotypes than random testing.

Design and carry out at least one of these exercises and report your results to the class. (Whatever you do, make sure that you leave the coffee cans as you found them. Put any beans that you remove from a can back in the same coffee can they came from. Do not add beans to a coffee can that were not in that can to begin with. **THIS IS VERY IMPORTANT!**)

DATA ANALYSIS:

In this lab I recorded the genotype outcomes of a random mating session. We performed the experiment by collecting a total of 2,000 beans which represented the same organism. Out of the 2,000 that we had 180 (9%) of them were white, assigned with the genotype aa. 840 (42%) of them were speckled, assigned with the genotype Aa. And the other 980 (49%) of them were brown, assigned with the genotype AA. The overall objective of this experiment known as the Hardy-Weinberg Simulation was to find out if these ratios would change if you randomly mated the beans.

Now the first calculation we did in the procedure was to figure out how many A alleles and a alleles there were in all. We calculated this to find out if it too altered or did not alter from generation to generation. Our calculation came out to 70% A alleles, and 30% a alleles. At first I thought we had made a mistake because there were only 2,000 beans, but there in fact are 2 alleles for every 1 bean. Making there a total of 4,000 alleles.

We calculated these numbers twice using two methods, and they both came out with the same answer. Once we performed both calculations we began our random mating sessions. We took two beans out of the tubber-ware 25 times (see attached). Having 25 matings gave us a total of 100 offspring (4 offspring per couple). Which was a very easy number to work with.

Once we did that we determined the outcomes of each of the six possible mating combinations, (AA x AA, Aa x AA, Aa, Aa etc). We then counted how many combinations of each we had and put tally marks, this helps us determine the genotype of the offspring. We then multiplied the number of combinations for example how many AA x AA, by the total number of offspring, we decided to use 4 for all of them. We then figured out the fraction the genotypes of the offspring were (how many were AA, Aa, aa), using our Punnet squares.

Once we figured that out we just had to multiply the fractions we got by the total number of offspring giving us the actual number of offspring were AA, Aa, aa. We then simply figured out the percentage of each genotype. These were our results:

$$AA = 41\%$$

$$Aa = 46\%$$

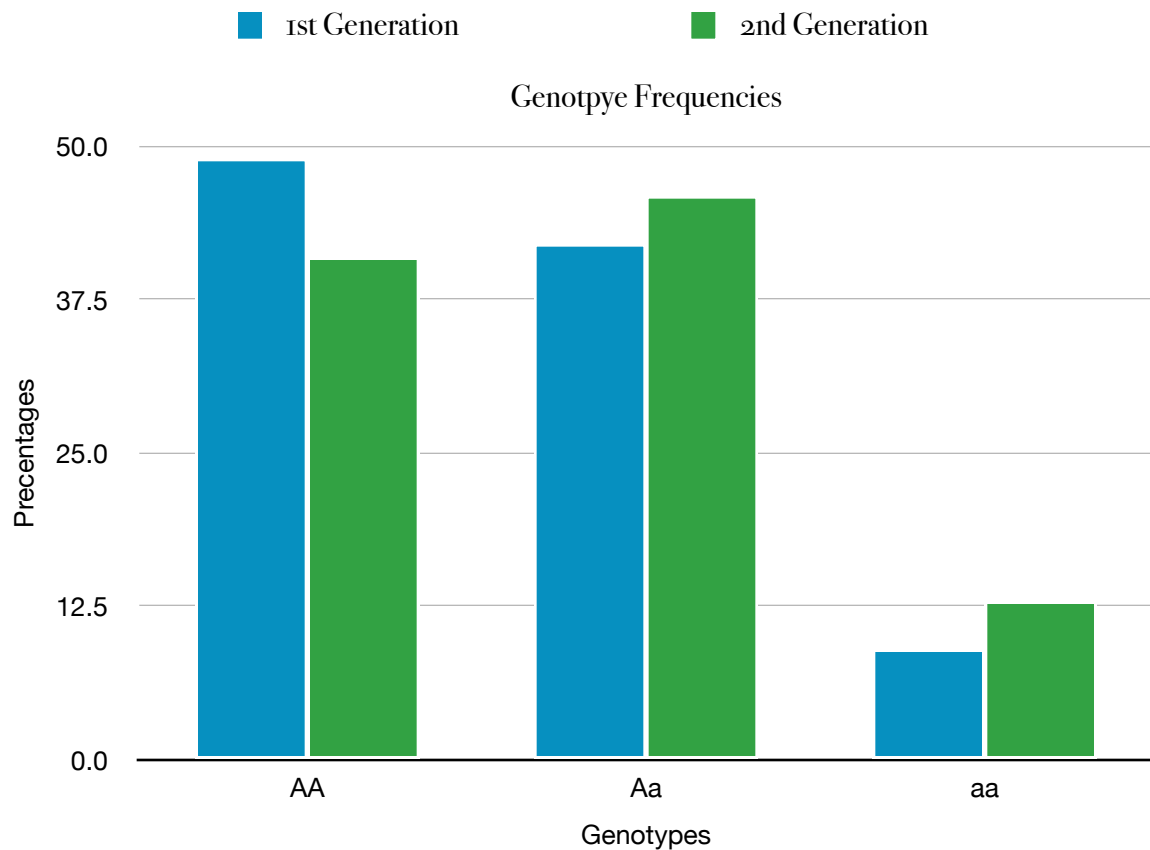
$$aa = 13\%$$

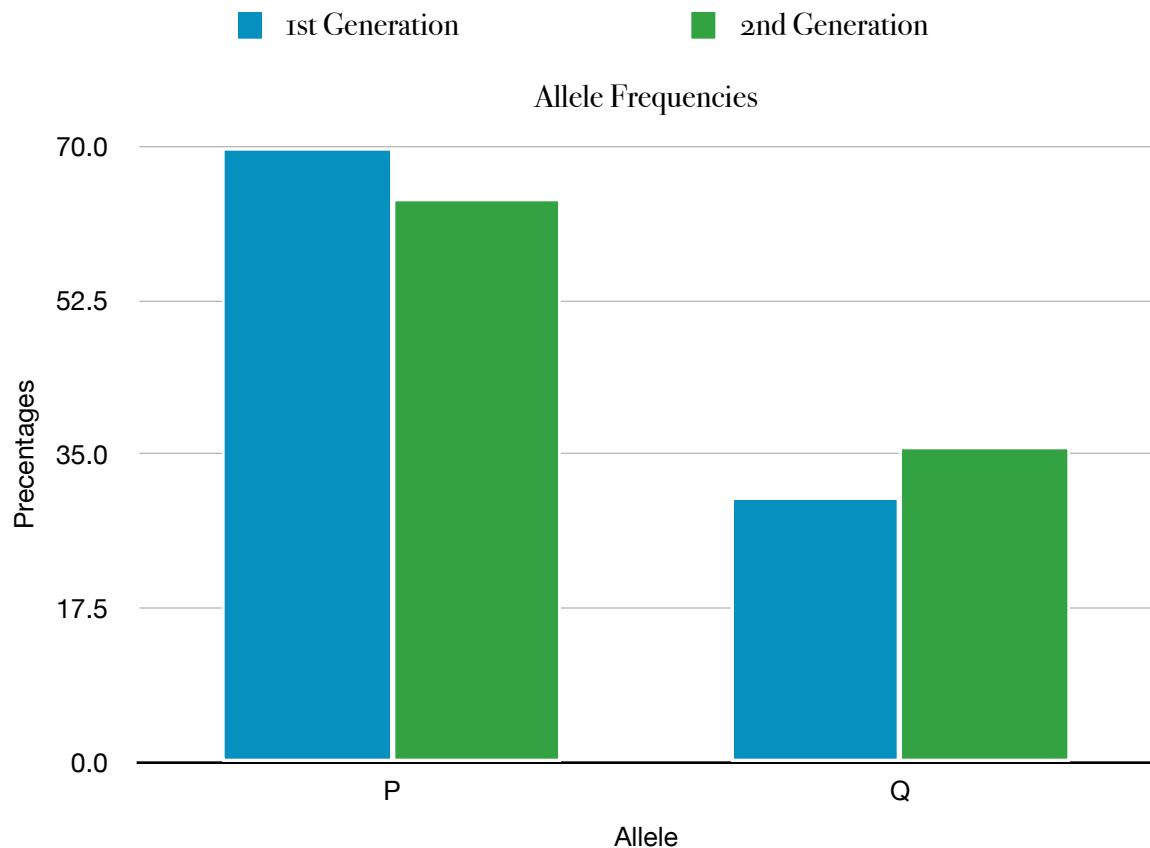
After pondering these results we preformed yet another calculation. We found out how many A alleles and a alleles there were in our offspring. We used Hardy Weinberg calculation which we believed to be much easier and straight forward. These were our results:

P = 64 %

Q= 36%

In this data table p is equal to the total number of A alleles, and q is equal to the total number of a alleles. We then compared both of our results to the previous generations statistics:





CONCLUSION:

My hypothesis was not correct, I hypothesized that the results would give 50% Speckled, 5% White, and 45% Brown. And I hypothesized that because the White beans make up only 9% of the population, and Speckled representing 42%, and Brown being 49%. The rest of my hypothesis was a believe statement, "I think that this is not quite a very accurate demonstration of how the next generation would be. Because in most animals and humans case they only mate with those who are most like them." And after preforming this experiment I still believe in that section of my hypothesis.

I learned what an Allele is, and what it means. I learned how genotypes can differ from generation to generation. Finally, I learned what the Hardy-Weinberg Theorem is, and what it tries to accomplish.

SOURCE:

http://userwww.sfsu.edu/~biol240/labs/lab_02hardyweinberg/pages/lab_01expt.html

This lab is taken from the web site for Biology 240 at San Francisco State University.