

Name: _____

Clams - Reviewing Hardy-Weinberg Equilibrium (Activity 1)

1. Define allele, gene, and genotype.

- Allele:

- Gene:

- Genotype:

2. Describe our gamete pool.

a. Allele counts: Red (A) allele = _____ Yellow (a) allele = _____

b. Allele frequencies: f_A = _____ f_a = _____

c. What is the probability of pulling...

- two consecutive red (A) alleles? _____
- two consecutive yellow (a) alleles? _____
- One red (A) allele and one yellow (a) allele in either order?

○ Note: when using the “AND rule” you should multiply the probabilities, and when using the “OR rule” you should add probabilities together.

3. What is the Hardy-Weinberg equation: _____

- How does this relate to the probabilities we calculated above?

4. What are the assumptions of Hardy-Weinberg Equilibrium (HWE)?

- Can we use HWE to make predictions if the assumptions are not met?
 - Why do we use it?

5. Predict genotype frequencies given our allele frequencies (assuming HWE).

a. Define p and q: p= _____ q = _____ (remember: p+q = 1)

b. What is...

○ $p^2 =$ _____

○ $q^2 =$ _____

○ $2pq =$ _____

○ How do the above values relate to genotype frequency?

6. Predict how many of each genotype you expect in a population of 30 individuals, given the allele frequencies above.

Genotype	Genotype frequency	Count expected (Genotype freq. X population size)
AA		
Aa		
aa		

7. Testing our predictions.

a. Describe our population after spawning.

○ Genotype counts: AA= _____ Aa= _____ aa= _____

○ Genotype frequencies: $f_{AA} =$ _____ $f_{Aa} =$ _____ $f_{aa} =$ _____

○ Allele frequencies: $f_A =$ _____ $f_a =$ _____

b. Do our results differ from expected?

c. Are they significantly different?

d. Do a Chi-square test to determine if our observed genotype counts statistically significantly deviate from our expected genotype counts.

- Generate a Chi-square test statistic value:
 - The equation to the right is the Chi-square calculation.
 - Use the table below to organize and complete the Chi-square calculation.
 - Sum the last column to get your value.
- Find your degrees of freedom:
 - $df = k - 1 - m$
 - k : number of classes
 - m : number of independent allele frequencies estimated

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

O = the frequencies observed

E = the frequencies expected

\sum = the 'sum of'

Genotype	Observed	Expected	O-E	(O-E) ²	((O-E) ²)/E
AA					
Aa					
aa					

Sum: _____

- Record your Chi-square value and degrees of freedom on the next page.
- Compare your Chi-square value to the cut-off value at $p=0.05$.
 - If your Chi-square value is less than the cut-off value at $p=0.05$, then there is not a significant difference between the expectations and observed genotype counts. Therefore, our population is considered in HWE with respect to that locus and we could then assume that evolution is not occurring at that locus.
 - If your Chi-square value is greater than or equal to the cut-off value at $p=0.05$, then there is a significant difference between the expectations and observed genotype counts. Therefore, our population is not considered to be in HWE with respect to that locus and we could then assume that evolution is likely occurring at that locus.
- Record your results below:
 - Did your expectations match your observed values?
 - Is this a surprise? Why or why not?

Chi-square value: _____

Degrees of freedom: _____

Degree of Freedom	Probability of Exceeding the Critical Value								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72
12	3.571	5.226	6.304	8.438	11.340	14.85	18.55	21.03	26.22
13	4.107	5.892	7.042	9.299	12.340	15.98	19.81	22.36	27.69
14	4.660	6.571	7.790	10.165	13.339	17.12	21.06	23.68	29.14
15	5.229	7.261	8.547	11.037	14.339	18.25	22.31	25.00	30.58
16	5.812	7.962	9.312	11.912	15.338	19.37	23.54	26.30	32.00
17	6.408	8.672	10.085	12.792	16.338	20.49	24.77	27.59	33.41
18	7.015	9.390	10.865	13.675	17.338	21.60	25.99	28.87	34.80
19	7.633	10.117	11.651	14.562	18.338	22.72	27.20	30.14	36.19
20	8.260	10.851	12.443	15.452	19.337	23.83	28.41	31.41	37.57
22	9.542	12.338	14.041	17.240	21.337	26.04	30.81	33.92	40.29
24	10.856	13.848	15.659	19.037	23.337	28.24	33.20	36.42	42.98
26	12.198	15.379	17.292	20.843	25.336	30.43	35.56	38.89	45.64
28	13.565	16.928	18.939	22.657	27.336	32.62	37.92	41.34	48.28
30	14.953	18.493	20.599	24.478	29.336	34.80	40.26	43.77	50.89
40	22.164	26.509	29.051	33.660	39.335	45.62	51.80	55.76	63.69
50	27.707	34.764	37.689	42.942	49.335	56.33	63.17	67.50	76.15
60	37.485	43.188	46.459	52.294	59.335	66.98	74.40	79.08	88.38
Not Significant								Significant	

Group Names: _____

Clams – Simulating evolution and real data (activity 2)

Materials: Each group should have...

- a bag of red and yellow marbles, 100 of each color
- a population tray (ice cube tray) with 24 labelled wells
- a data sheet to record genotype and allele count and frequency data

Procedure:

1. Your group is a single population of 24 breeding clams. Your bag is the collective gametes produced by your population.

- Red marbles represent the dominant allele (A)
- Yellow marbles represent the recessive allele (a)

If you have 100 red and 100 yellow marbles in your bag, what are the allele frequencies in your gamete pool?

$$f_A = f_{(\text{red allele})} =$$

$$f_a = f_{(\text{yellow allele})} =$$

2. Enter the frequencies into "Generation 0" row on your datasheet.

If you randomly created 24 individuals (by pulling two alleles at a time), how many individuals would you expect of each genotype?

$$AA =$$

$$Aa =$$

$$aa =$$

3. Now that you have your allele frequencies and predictions, go ahead and create your population. Create individuals by pulling 2 marbles (alleles) at a time out of your gamete bag at random (no peeking!). Place the two marbles (alleles) in the first compartment in your population tray. Continue this process until all 24 compartments are filled with two alleles each.

4. This population (in the tray) represents the individuals that have survived and settled after gametes have fused. Record data about this population in row "Generation 1 - *before* event".

- Record the total number of each genotype.
- Calculate the allele frequencies.

5. Before your individuals get a chance to produce gametes an **EVENT** happens. Draw one event card and follow the instructions on the card. After you have followed your event card instructions and altered your population, record data about this altered population in row - "Generation 1 - *after* event".

- Record the total number of each genotype.
- Calculate the allele frequencies.

6. The individuals left in your population tray represent the breeding population that will contribute gametes to the next generation.

Compare the allele frequencies between your two time points (*before* and *after* the event card). Are the allele frequencies the same or different?

Is this surprising? Why or why not?

Do you think the allele frequencies will change from Generation 1 to Generation 2 as a result of the event?

7. Create your new gamete pool based on individuals remaining in the population tray after the event. Your gamete pool should always be 200 alleles.

- Make sure that you empty your gamete bag of leftover gametes from the previous generation before creating your new gamete pool contributing to the next generation in the bag.
- Use the allele frequencies to generate your gamete pool.
 - For example: If your allele frequencies are $f_A = 0.7$, $f_a = 0.3$, then your gamete pool should have...
 - Red marbles (A) = $0.7 * 200 = 140$ red marbles
 - Yellow marbles (a) = $0.3 * 200 = 60$ yellow marbles
- Record your gamete counts on your data sheet and make your new gamete pool.

8. Repeat steps 3-7 until you have completed your entire datasheet. A brief overview is below.

- Pull 24 individuals (2 alleles at a time from the gamete bag) and place individuals into each well of the population.
- Record genotype counts and calculate allele frequencies in the "*before* event" row for that generation.
- Draw one event card and follow the instructions.
- Record genotype counts and calculate allele frequencies in the "*after* event" row for that generation.
- The individuals remaining will contribute gametes into the next generation. Calculate allele counts to be used to create a gamete pool of 200.
- Empty the gamete bag of all gametes from the previous generation and make your new gamete pool.
- Repeat until you reach Generation 10.

Record your final allele frequencies: $f_A = f_{(\text{red allele})} = \underline{\hspace{2cm}}$ $f_a = f_{(\text{yellow allele})} = \underline{\hspace{2cm}}$

9. Describe how/if your allele frequencies changed over time.

Graph your changes in allele frequencies over time using the allele frequency data from the “before event” rows.

- Label your axes
- Write a brief figure caption below:



What contributed to the changes you observed? What kinds of events did your population deal with?

Did all individuals in the population (*before* event) contribute equally to the gamete pool in the every generation?

What affected whether an individual contributed to the gamete pool? Were these the result of random events or non-random events? Explain.

10. Evaluate whether your population experienced evolution.

Calculate a chi-square test statistic to evaluate support your statement above.

- Recall that if we are assuming the population is in HWE (no evolution), then you expect your final allele frequencies at the end of the simulation to be the same as the initial allele frequencies.
- Your expected values are the expected genotype counts of a population of 24 individuals if the allele frequency is $f_A = 0.5$, $f_a = 0.5$. Your observed values are the genotype counts you observed in Generation 11
 - Remember: You need to use whole numbers when calculating a chi-square test.

Chi-square test statistic = $\sum((O-E)^2/E)$

d.f. = n - 1

Chi-square value =

d.f. =

p =

What is the null hypothesis? Does your test statistic fail to reject or reject the null hypothesis?

Is your population in Hardy-Weinberg Equilibrium with respect to the locus we were observing? Do you think evolution was occurring at that locus?

If you did observe evolution in your population, speculate as to the evolutionary force(s) was (were) driving the changes in allele frequencies over time.

11. Keep this sheet, you will need it for the next group activity. Each group had a different population, you will now regroup with someone from each of the other populations to discuss similar or different trends in allele frequency changes and environmental conditions the populations experienced.

12. Compare allele frequency changes over time and the environment (*event cards*) each population.

Population	Stochasticity and trends in allele frequencies over time	Final allele frequencies	Summary of environmental conditions the population experienced
Havre- Aubert			
Lawrencetown			
Lepreau Basin			
Essex			
Orleans			

Likely all of the populations had different allele frequencies at the end of the game.

Where there any populations that were more similar than others, in terms of final allele frequencies?

Speculate on why they had similar frequencies.

Did all populations undergo evolution? What force(s) caused the allele frequency changes observed in each population? Explain your rationale.

13. Some populations experienced outbreaks of *Alexandrium spp.*, a dinoflagellate that produces a saxitoxin. These outbreaks are often referred to as Red Tide. Saxitoxin is a toxic compound that alters the function of the voltage-gated sodium channel, and is known to cause Paralytic Shellfish Poisoning (PSP). Some soft shell clam populations are polymorphic for a mutation that confers resistance to the toxin. All of our populations started out with genotypes associated with resistance and susceptibility to PSP.

Would you expect populations that experience frequent outbreaks of *Alexandrium spp.* to have a higher or lower frequencies of resistant individuals? Why?

Based on your above table, predict the allele associated with resistance and predict which populations you would expect to have a high frequency of resistant individuals given the environmental conditions.

13. Compare your simulation results (Table 1) to actual research results from Connell *et al.* (2008) (Figure 1).

Were your results similar to frequencies found in natural populations of *Mya arenaria*?

Do populations that...

...experience PSP toxins in the environment typically have greater or fewer resistant individuals? Speculate as to why.

...do not experience PSP toxins in the environment typically have greater or fewer resistant individuals? Speculate as to why.

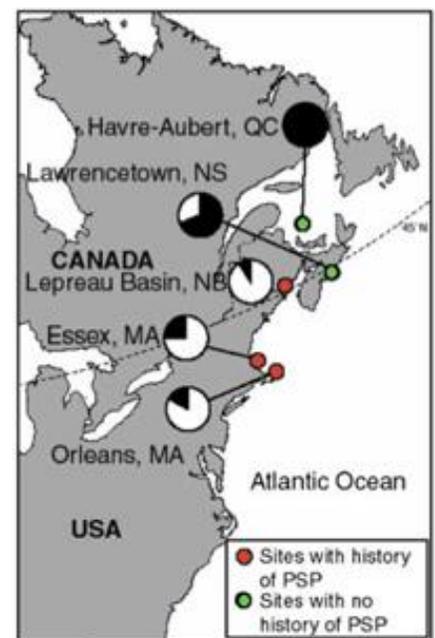


Figure 1. Map showing *Mya arenaria* collection sites on the Atlantic coast of North America in relation to their history of paralytic shellfish poisoning toxin exposure (PSP). Pie charts show the proportion of individual clams that are sensitive (filled) or resistant (open) to PSP toxins based on results from an in vitro nerve assay (Connell *et al.* 2008)

14. Watch the video of burrowing resistant and susceptible *Mya arenaria*. **In the absence of saxitoxin in the environment, do you think there is a cost of having the resistant phenotype?**

15. Using what you have already learned and Figure 2, answer the following.

When exposed to saxitoxin, are resistant or susceptible individuals most fit? Justify your answer using relevant data.

Looking specifically at Figure 2c, are resistant or susceptible individuals more likely to sequester toxins?

How might the toxin sequestration affect the intertidal foodweb and the economic value of *Mya arenaria*?

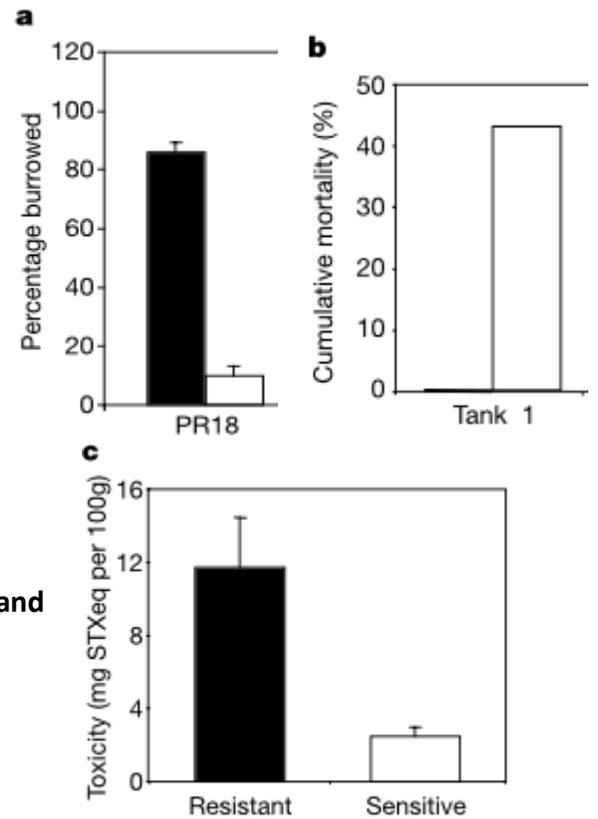


Figure 2. Comparing burrowing, mortality, and toxin sequestration of exposed/resistant populations (black bars) and naïve/susceptible populations (white bars) populations of *Mya arenaria* to PSP (Paralytic Shellfish Poisoning) toxin. (Bricelj *et al*, 2005). 2a: Burrowing ability, 2b: mortality, 2c: toxin sequestration