

# EXTINCTION AND SURVIVAL OF MUCK SWAMP FROGS

Somewhere near Xenia, Ohio lives the world's last population of Muck Swamp Frogs (nicknamed "Mucks"). Mucks come in two types, pictured below. Herpetologists are urgently trying to learn something of the ecology and population genetics of these remarkable creatures so they can devise management schemes to save the frogs from extinction. In this lab, we will model the Muck Swamp system to gain insights into the biology of Muck Swamp Frogs. It seems the least we can do.



GREEN MUCK



WHITE MUCK

In order to proceed, we must first cover some important concepts.

## POPULATION GENETICS:

The unit of inheritance is the **gene**. Genes may exist in alternative forms called **alleles**. For example, the skin of Muck Swamp frogs can be green or white. We can symbolize an allele coding for green skin with the letter "G". We'll designate the allele coding for white skin "g". Frogs, like most sexually reproducing animals, including ourselves, are diploid. This means that every allele within an individual is paired with one other allele. No matter how many types of alleles coding for skin color exist in the population as a whole, each individual carries only two. The actual combination of alleles carried by an individual is its **genotype**. So, an individual Muck Swamp frog can carry either two "G" alleles (GENOTYPE=GG), a "G" and a "g" allele (GENOTYPE=Gg), or two "g" alleles (GENOTYPE=gg). There are no other possible combinations given the simple genetic system we have outlined for this trait. If one allele masks the expression of another allele when the two are paired, the masking allele is said to be **dominant**, whereas the masked allele is **recessive**. The "G" allele in Muck Swamp frogs is **dominant** because it is always expressed when present. Thus, a Muck with a Gg genotype will be green. The "g" allele in this example is **recessive** because it is not expressed when in the presence of the "G" allele. White mucks must, therefore, have a \_\_\_\_\_ genotype. Green mucks may have either a \_\_\_\_\_ or \_\_\_\_\_ genotype.

Populations are often characterized by their **gene frequencies**, **genotype frequencies**, and **phenotype frequencies**.

**Gene frequencies** in a population indicate the frequency of alleles in a gene pool, regardless of how they are apportioned among individuals. A population of 60 Muck Swamp frogs will have a total of 120 alleles in its gene pool for skin color, since each frog carries two alleles for this trait. If there are 80 "G" alleles and 40 "g" alleles in the population, the gene frequencies are .67 (80/120) and .33 (40/120) for G and g alleles respectively.

**Genotype frequencies** in a population indicate how alleles in the gene pool are paired within individuals. Thus, if in the case above 20 frogs have a "GG" genotype, 20 have a "Gg" genotype, and 20 have a "gg" genotype, the genotype frequencies for the population is .33 GG (20/60), .33 Gg (20/60), and .33 gg (20/60).

**Phenotype frequencies** indicate what individuals in a population look like physically for a trait of interest. The actual appearance of an organism is its **phenotype**. As you discovered above, the GG and Gg genotypes in Muck Swamp frogs will be green, while the gg genotypes will be white. Thus the **phenotypic frequency** for skin color in this population is .67 green [(20 + 20)/60] and .33 white (20/60).

NOTE: ALL FREQUENCIES ARE PERCENTAGES. THEY ARE FOUND BY DIVIDING A NUMBER BY THE TOTAL THAT IS APPROPRIATE FOR THAT CALCULATION. EACH SET OF FREQUENCIES (WHETHER THEY ARE GENE, GENOTYPE OR PHENOTYPE FREQUENCIES) SHOULD ADD TO 1.

### HARDY-WEINBERG EQUATIONS

When you observe a population of Muck Swamp frogs it is a simple matter to determine phenotypic frequencies for skin color; color is obvious. But, how can you determine the population's genetic structure? You do not know, by looking, how many total G or g alleles are in the population (i.e. the gene frequencies). Nor do you know the genotype frequencies of green frogs (how many are GG vs. Gg). Start with what you do know. You know the genotype frequency of white frogs, since all white frogs must have a gg genotype. You can use this information to estimate the rest by using the following Hardy-Weinberg equations:

$$\text{Equation 1: } p^2 + 2pq + q^2 = 1$$

(GG) (Gg) (gg)

where  $p^2$  = the expected genotype frequency of GG  
 $2pq$  = the expected genotype frequency of Gg  
 $q^2$  = the expected genotype frequency of gg

$$\text{Equation 2: } p + q = 1$$

(G) (g)

Where  $p$  = the expected gene frequency of G  
 $q$  = the expected gene frequency of g

Say your population has 64 green frogs and 36 white frogs. Calculate  $q^2$  \_\_\_\_\_.  
 Calculate  $q$  \_\_\_\_\_. Use Equation 2 to calculate  $p$  \_\_\_\_\_.  
 Now that you know  $p$  and  $q$ , use Equation 1 to calculate  
 $p^2$  \_\_\_\_\_,  $2pq$  \_\_\_\_\_, and  $q^2$  \_\_\_\_\_.

Note: These equations are based on basic probability theory. Imagine a gene pool composed of .75 G ( $p$ ) and .25 g alleles ( $q$ ). If you choose one allele at random there is a 75% chance it will be G. If you choose two alleles at random the chance that you will choose GG ( $p^2$ ) is  $.75 \times .75$  (=56%). The chance that you will choose Gg ( $2pq$ ) is  $2 \times .75 \times .25$  (= .38; you multiply by 2 because there are two ways to get a Gg genotype, either by choosing G then g, or g then G). The chance of choosing gg ( $q^2$ ) is  $.25 \times .25$  (=06).

The two simple equations given above allow you to estimate the theoretically expected gene and genotype frequencies of a population.

## THE MUCK SWAMP SYSTEM

This is what is known from preliminary studies of Muck Swamp frogs:

- 1) The frogs live on three islands in the Great Muck swamp. Tadpoles that venture far from their parental population tend to be eaten by fish, so the islands can be considered genetically isolated.
- 2) The two main threats to mucks are rattlesnakes and owls. Rattlesnakes only eat green mucks; they find white mucks repulsive. Conversely, owls only eat white mucks because the green mucks are too well camouflaged for owls to find them. Snake Island has many snakes, Owl Island has many owls, Dismal Island has neither snakes or owls, but the habitat is so poor that Muck frog mortality is just as high as on the other two islands. Both green and white mucks are equally likely to die on Dismal Island.
- 3) Breeding experiments confirm that the color of mucks is determined by a simple genetic system where green is dominant to white.

### PART 1. EVOLUTION ON THREE ISLANDS

#### Methods:

##### 1. Establish a gene pool

To begin the model of Muck Swamp, the lab will be divided into six groups: two groups will model Snake Island, two will model Owl Island, and two will model Dismal Island. First, establish a gene pool by placing 50 dark beans and 50 light beans into the dish provided for your group. Dark beans represent G alleles; light beans represent g alleles. Based on this initial composition of 50% G and 50% g alleles, use the Hardy-Weinberg equations to calculate expected genotype and phenotype frequencies. Place your answers in the space indicated Worksheet 1.

##### 2. Build a Muck Frog Population

Build your own population of Muck Swamp frogs by pulling two beans from your gene pool at a time. Each pair of beans represent an individual frog. Pull out 50 individuals (pairs of alleles) and tally their genotypes (two dark beans=GG, a dark and a white = Gg, two white = gg). **REPLACE THE BEANS AFTER EACH DRAW AND MIX THE GENE POOL.** Calculate the actual genotype frequencies by dividing the number of genotypes in each category by the total number of genotypes pulled from the gene pool (50). Next calculate actual phenotype frequencies by counting the number of green frogs (GG + Gg genotypes) and white frogs (gg genotypes) and dividing each by the total number of phenotypes you pulled from the gene pool (50).

##### 3. Take your frogs to an island in Muck Swamp

Each island is represented by a deck of cards. For each of the 50 frogs you recorded the genotype for above, flip over one card and determine the muck's fate. Snakes kill green mucks only; Owls kill white mucks only; the Grim Reaper kills any muck; Crickets are good food, any muck survives. Tally the genotype frequencies only for surviving mucks. The other mucks are dead. They will not be represented in the next generation's gene pool.

4. Calculate the gene, genotype, and phenotype frequencies for your surviving mucks. Gene frequencies are calculated as follows:

$$\frac{G = 2 \times \text{the number of GG frogs surviving} + \text{the number of Gg frogs}}{\text{the total number of surviving alleles}}$$

Place your answers on Worksheet 1 (under Generation 1).

5. Establish the gene pool for the next generation

Suppose, at the end of Step three, you have 5 GG survivors, 10 Gg survivors and 10 gg survivors. The calculated gene frequencies are:  $G = [(10 + 10)/50] = .4$   $g = [(20 + 10)/50] =$

.6. Since you want your next generation's gene pool to reflect the gene frequencies of the survivors of the last generation, your new gene pool should have, in this case, 40 dark beans and 60 white beans.

6. Repeat steps 2 through 5 until you complete Worksheet 1 for your island. Make certain to establish a new gene pool at the start of each new generation. Fill in the rest of Worksheet 1 using answers calculated by the other lab groups.

It so happens that the gene, genotype, and phenotype frequencies you calculated for the final generation exactly match those found in nature in Muck Swamp.

**WORKSHEET 1: Evol. on Three Islands**

Name \_\_\_\_\_

	Snake Island			Owl Island			Dismal Island		
<b>STARTING POP.</b>									
Gene	G	g		G	g		G	g	
Freq.	.5	.5		.5	.5		.5	.5	
Expected Genotype Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Actual Genotype Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Expected Phenotype Freq.	Green	White		Green	White		Green	White	
Actual Phenotype Freq.	Green	White		Green	White		Green	White	

**GENERATION 1 (calculate using survivors of starting population)**

Gene	G	g		G	g		G	g	
Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Genotype Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Phenotype Freq.	Green	White		Green	White		Green	White	

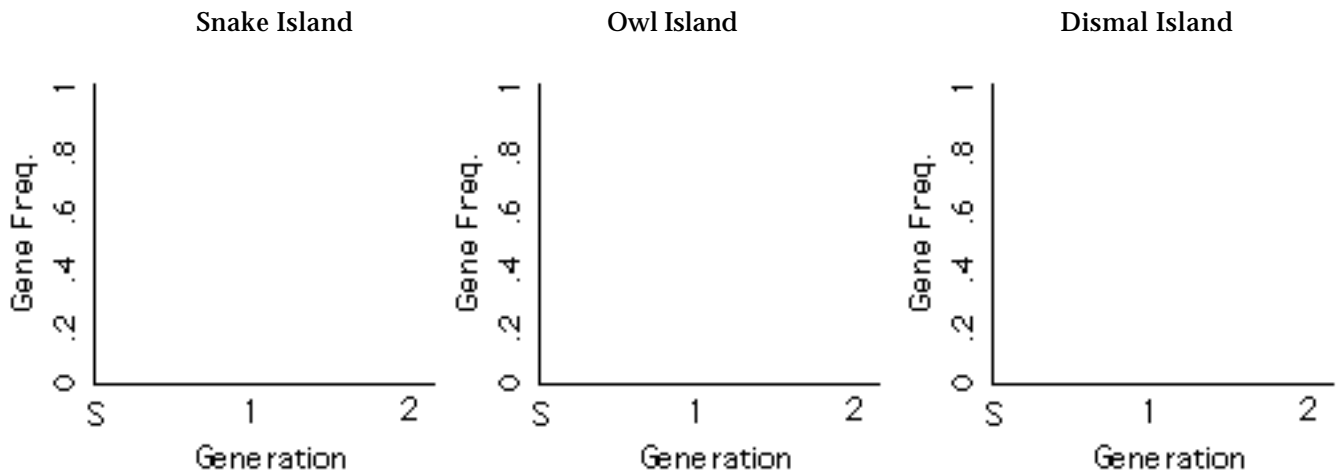
**GENERATION 2 (first make a new gene pool from survivors of Generation 1)**

Gene	G	g		G	g		G	g	
Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Genotype Freq.	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Phenotype Freq.	Green	White		Green	White		Green	White	

## Questions from Part 1: Evolution on Three Islands

1) Examine your results for the Starting Generation on Worksheet 1. It is conceivable that the actual genotype frequencies you randomly pull from the gene pool are much different than that expected from your Hardy-Weinberg equations, but probability theory suggests they will be similar (just as it is conceivable to toss a coin 50 times and get heads every time, but you will probably get closer to 25 heads and 25 tails). How well did the Hardy-Weinberg equation predict the actual genotype and phenotype frequencies you obtained by picking beans from the gene pool?

2) Plot the gene frequencies over generations for the three islands on axis below. Use solid lines to plot G, dashed lines for g. Plot for two generations



3) Evolution can be defined as a change in gene frequencies over time. So, the graphs above show the progress of evolution. The island with the greatest rate of evolution was \_\_\_\_\_ . The main agent responsible for evolution on this island was \_\_\_\_\_ . The island with the lowest rate of evolution was \_\_\_\_\_ . The main agent for evolution on this island was \_\_\_\_\_ .

4) The dominant allele, G, was selected against on Snake island, the recessive allele, g, was selected against on Owl island. Circle the type of allele that is more likely to survive in a population despite selection against it: Dominant or Recessive. Why?

5) Gene, genotype, and phenotype frequencies are not predicted to change from Hardy-Weinberg expectations if there is no migration, natural selection, or genetic drift. Compare the final gene, genotype, and phenotype frequencies for the third generation on each island with the expected frequencies calculated for the Starting Population on Worksheet 1. On which island do the final frequencies most resemble those expected from the Hardy-Weinberg equations? \_\_\_\_\_. There was the same chance of mortality on all three islands. Why, then, are the final frequencies for this one island relatively close (hopefully) to Hardy-Weinberg expectations?

## PART II. GENETIC DRIFT AND THE EXTINCTION OF FOUNDING POPULATIONS<sup>7</sup>

Wildlife biologists from Xenia, Ohio are planning to lower the water level of Muck Swamp. This, they argue, will create many small islands, or mud mounds, for the Mucks to colonize. They expect that the new mounds will quickly be inhabited by snakes and owls at random. They are experienced enough to know that this new Muck Swamp landscape will affect the genetic structure of Muck Frog populations, but they don't know how.

The founding of new populations is a common occurrence. The colonizers are often a small, random subset of a larger source population. Chance can play an important role in determining the gene frequencies of founding populations. This random process, whereby an allele is either lost or fixated in a population by sheer chance is called **genetic drift**. Note that when a population is severely reduced in size, due to habitat destruction for example, the small population that remains is also, genetically, a small random subset of the original population. Thus, genetic drift can be an important agent of evolutionary change in human-disturbed ecosystems. Below we will take a look at how genetic drift and natural selection operating together can affect the evolution of Muck Swamp frogs.

### Methods:

For each of the three source islands (Snake, Owl, Dismal), we will estimate the probability of extinction for smaller populations that leave the larger island to colonize mud mounds. These smaller populations are called **founder populations**. Not all of them will survive. We will test whether two factors affect extinction rates: 1) the source island (i.e., whether they come from Snake, Owl, or Dismal island); 2) the size of the founding population (we will compare a pop. size of 3 with a pop. size of 9). Each lab group should follow the following steps only for one source island and one pop. size.

1. Copy the gene frequencies you obtained for Generation 2, Worksheet 1, into the spaces provided on the top of Worksheet 2. These are the gene frequencies for Snake, Owl, and Dismal island at the time the mud mounds are formed. They are the frequencies you will use to establish the source gene pool.
2. Establish the gene pool for your source population  
Use 100 beans total in the proportions indicated in Step 1.
3. Establish Founding populations of Muck Swamp frogs  
Pull out 3 pairs or 9 pairs (depending on your group) from the gene pool. **REPLACE THE BEANS AFTER EACH DRAW AND MIX THE GENE POOL.** Each pair of beans represents the genotype of one frog. Make 4 populations in this way and tally the genotypes for each frog in your populations.
4. Calculate the gene and phenotype frequencies for four of your populations and record them in the space provided (Part II, Worksheet 2). This is for general comparison.
5. **Muck Swamp frogs test the swamp:** Survival of mucks on mud mounds is represented by a stack of cards. Pull out one founding population (3 or 9 pairs of beans depending on your group) from your source gene pool. Turn over *only one* card for that population. A snake means that the snakes have found the mud mound. If your founding population contains only green frogs, they're eaten. An owl card means that populations with all white frogs are extinct, other groups survive. If a founding population contains even 1 frog whose phenotype is suited for the island (white for an island with snakes, green for an island with owls), that population survives (because there is at least one survivor that may populate the mud mound). Create and test 40 populations in this way. Tally the number of populations that go extinct and record this number in part III, Worksheet 2.

**WORKSHEET 2: GENETIC DRIET AND EXTINCTION** Name \_\_\_\_\_

Snake      Owl      Dismal

I. \*\*\* Gene freq. of source islands:      G \_\_\_\_\_  
 RECORD GENE FREQ. FROM GEN. 2, WORKSHEET 1      g \_\_\_\_\_

II. Calculate gene frequencies for four founding populations. Each group should do this for only one source island and one population size. This data is for later comparisons.

Source Island	Owl	Snake	Dismal	Owl	Snake	Dismal
Founder Pop. Size	3	3	3	9	9	9
Pop.1						
G	_____	_____	_____	_____	_____	_____
g	_____	_____	_____	_____	_____	_____
%Green	_____	_____	_____	_____	_____	_____
%White	_____	_____	_____	_____	_____	_____
Pop.2						
G	_____	_____	_____	_____	_____	_____
g	_____	_____	_____	_____	_____	_____
%Green	_____	_____	_____	_____	_____	_____
%White	_____	_____	_____	_____	_____	_____
Pop.3						
G	_____	_____	_____	_____	_____	_____
g	_____	_____	_____	_____	_____	_____
%Green	_____	_____	_____	_____	_____	_____
%White	_____	_____	_____	_____	_____	_____
Pop.4						
G	_____	_____	_____	_____	_____	_____
g	_____	_____	_____	_____	_____	_____
%Green	_____	_____	_____	_____	_____	_____
%White	_____	_____	_____	_____	_____	_____

III. RECORD THE NUMBER OF FROGS THAT GO EXTINCT

Source Island	Owl	Snake	Dismal	Owl	Snake	Dismal
	3	3	3	9	9	9
#Extinct Pop.	_____	_____	_____	_____	_____	_____

**IMPORTANT: FILL IN THE REST OF WORKSHEET 2 USING DATA FROM THE OTHER LAB GROUPS. DON'T LEAVE CLASS BEFORE YOU HAVE THIS INFORMATION.**



## Questions from Part II. Genetic Drift and the Extinction of Founding Populations

1) Compare the gene frequencies you calculated for founding populations of Muck Swamp frogs with the gene frequency of their source populations (from Snake, Owl, Dismal, listed at the top of Worksheet 2). Briefly describe how the gene frequencies of your founding populations differ from the gene frequencies of the source populations. Are the differences large or small? How important is the source island and the size of the founding population in affecting the gene frequencies of founding populations?

2) For each category of source island/population size listed below, use your data to calculate the probability that a founding group of Muck Swamp frogs will go extinct for each category of source island/population size listed below (= #of extinctions/40).

Source Island	Owl Island		Snake Island		Dismal Island	
Size of Founding Pop.	3	9	3	9	3	9
Prob. of Extinction						

How much does the source island influence the chances of a founding population will go extinct? Why?

How important is the size of the founding population in determining whether that founding population will go extinct? Why?

Large climatic fluctuations are predicted in the Muck Swamp vicinity over the next few years, causing a more varied and unpredictable environment in general. Which island do you think has the best chance of having frogs survive these fluctuations? Explain your reasoning?