Modeling Natural Selection (Genetic Drift)

SC Academic Standards: 7.L.4A; 8.E.6B; H.B.4C; H.B.5A.

NGSS DCI: MS-LS3.B; MS-LS4.B-C; HS-LS3.A-B; HS-LS4.B-C.

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.4; S.1A.5; S.1A.7

Crosscutting Concepts: Patterns; Cause and Effect: Mechanism and Explanation; Systems and Systems Models; Energy and Matter: Flows, Cycles, and Conservation; and Stability and Change.

Focus Question(s): How does evolution by natural selection occur? What might cause an allele or phenotype to be selected for / against?

Conceptual Understanding: Inheritance is the key process causing similarities between parental organisms and their offspring. Organisms that reproduce sexually transfer genetic information (DNA) to their offspring. This transfer of genetic information through inheritance leads to greater similarity among individuals within a population than between populations.

Adaptation by natural selection acting over generations is one important process by which species change in response to changes in environmental conditions. The resources of biological communities can be used within sustainable limits, but if the ecosystem becomes unbalanced in ways that prevent the sustainable use of resources, then ecosystem degradation and species extinction can occur.

Biological evolution occurs primarily when natural selection acts on the genetic variation in a population and changes the distribution of traits in that population over multiple generations.

According to the theory of biological evolution, natural selection results in populations that are adapted to a particular environment at a particular time. Changes in the physical environment have contributed to the expansion, emergence, or extinction of the Earth's species. Biodiversity is increased by the formation of new species (speciation) and decreased by the loss of species (extinction). Modern classification of Earth's biodiversity is based on the relationships of organisms to one another.

Background: There is extensive evidence that organisms tend to change very slowly over long periods of time. This change is called evolution; evolution is the process that leads to the change in prevalence of traits or alleles, the process of adaptation, and even the formation of new species.

There are three main things that are necessary for evolution to occur:

1. **Variability.** There must be variability in what traits or alleles of a gene are in a population. If there is no variability, there will be no chance for evolution to occur. Variability can be maintained many ways, including through random mating or because there is a benefit to carrying heterozygous alleles; for example, in some humans it is advantageous to have one allele for sickle cell anemia and one for normal blood at the sickle cell gene because heterozygotes are resistant to malaria. One of the most common ways variability is maintained is through mutations. Mutations are random changes in DNA. Many mutations don't alter gene expression or what proteins are made, and have no apparent effect on the organism. Most mutations that do have significant effects are bad for the organism. Very occasionally, there are mutations that produce a new or altered trait that is advantageous for an organism.

2. **Heritability.** Evolution works by changing which traits are more prevalent in succeeding generations, and this only works if the trait of interest is heritable. Traits that are not heritable can't evolve. For example, if you get a tattoo, you've changed your skin coloration, but since tattooing isn't heritable, your offspring will not have tattoos when they are born – and if you work out and get bigger muscles, these, too are not something you can pass to your offspring. Your natural hair color, on the other hand, is based on genetics and may be passed on to your children. Learned behaviors are non-heritable as well; just because your father learned how to dance the polka doesn't mean you knew how to polka when you were born.

3. **Differential Reproduction.** Some organisms in a population leave more offspring than others, and thus the heritable traits of some organisms are passed on at a higher frequency. Over time, this differential reproduction will lead to some traits or alleles being more frequent in the population, while others are less frequent or even disappear. If every organism in each generation reproduces at the same rate within a population, there will be no difference in which traits or alleles are more frequent, and therefore no evolution.

Evolution is change in allele frequencies, which occurs in every generation. However, noticeable changes in phenotype may take a long time to evolve. For example, there are 14 species of finches that live on the Galapagos, each of which differs in beak shape, beak size, and diet. All 14 species came from a common ancestor, and it took up to five million years for the 14 species to evolve. Evolution cannot occur within an individual, only within a population or species.

Evolution is not directed or predetermined. Just like you can't spontaneously grow a tail because you think it would be cool, an organism can't spontaneously obtain a trait they "want" through mutation. Mutations are random, not directed, and they don't occur because they are "needed."

The evolution of a trait can occur through two main ways: random chance (called genetic drift) or natural selection.

GENETIC DRIFT: The frequency of specific alleles in a population can change drastically due to simple chance, leading to random shifts in a population's traits. The effect of random chance on the frequency of alleles or traits in a population is called genetic drift. "Random chance" events can include everything from random mating patterns that lead to an allele being accidently removed from the population to unpredictable environmental events, such as a flood that accidently kills most or all individuals with one allele.

Genetic drift is often almost negligible in a large population. However, as population size gets smaller and smaller, genetic drift can have more and more drastic effects on allele frequencies. For example, think of flipping a quarter with a 50:50 chance of landing heads or tails up. The "heads" side can represent one allele, and the "tails" a second allele. Imagine flipping that quarter 100 times. In this case, you would expect to end your 100 flips with close to a 50:50 ratio of heads to tails tosses; it is extremely unlikely that the ratio will change much, and it's almost impossible to get all heads or all tails (without a rigged coin). If you only flip that same quarter 4 times, however, you have a much higher probability of randomly getting all four heads or all four tails. These same rules of probability apply to alleles in a population.

Population bottlenecks and founder effects are special cases of genetic drift. A **population bottleneck** occurs when a population experiences a catastrophic event that either kills almost all individuals or prevents almost all individuals from reproducing. Often, this is due to disease or natural disasters, such as volcanic eruptions, floods, ice ages, etc. In bottlenecks, even if a population began as a large population, that population will suddenly be very small. Since it is random which individuals survive a bottleneck, genetic drift will play a strong role in what alleles are present in the new, bottlenecked population.

Founder effects are another special form of genetic drift. In this case, a few random individuals from a large population leave to found a new population in a new environment. Again, since only a random subset of the larger population will make up the new population, genetic drift will play a strong role in which alleles and what proportion of alleles will be in the new population.

NATURAL SELECTION: Not all effects on allele frequencies are random or due to random events. Sometimes there are traits that give an organism a reproductive **advantage**, either through increasing survival rate or increasing fecundity. These traits will be more likely to be passed on to the next generation through natural selection, the process by which genetic traits that provide an advantage to an organism are passed on to offspring at a higher rate than non-advantageous traits. Natural selection is all about reproduction; if an organism can reproduce more or has a higher chance of survival until reproduction, their traits will be selected for.

Over many generations, an advantageous trait may become more prevalent in a population or in a species. These advantageous traits are considered adaptive, and the process of selecting for adaptive traits over many generations is termed adaptation.

There is one specific kind of natural selection that is worth mentioning: sexual selection. Sexual selection is the selection of a trait by one sex in a mating pair. Often the trait being selected for is something showy. The most commonly-cited example is in peacocks: peahens prefer to mate with males that have bigger, brighter, showier tails. Even though large tails can also attract predators and get in the way, larger tails lead to increased reproduction because the females, when given the choice, will select the male with the largest showiest tail.

In this lab we will investigate the process of genetic drift, by using "fieldmice" (beans) and their predator, "owls" (students).

Materials: 2 bags each of (same sized) dried black beans and dried white beans, a black poster board and a white posterboard (or countertop of one or other color), plastic cup, BIG eye doctor type sunglasses that have been "darkened" with colored cellophane, a dark room.

Previous Knowledge: (ecology): Evolution has led to the survival of predators that select the "right" food items – and the co-evolution of prey that can avoid the predators. Some predators employ a **generalist** strategy, and tend to have broad diets; they chase and eat many of the prey/food items with which they come into contact. There are also those with a more **specialist** strategy, having a narrow diet and ignoring many of the prey items they come across, instead searching preferentially for a few specific types of food. Usually, animals exhibit strategies ranging across a continuum between these two extremes.

Predation involves four steps: **search**, **recognition**, **capture**, and **handling**. The possibility of co-evolution of predator and prey operates at each of these steps. Predators search the environment for acceptable prey. Predator adaptations to improve foraging success include better visual acuity, development of a search image, and limiting searches to prey-rich habitats. Predators quickly learn prey types and adapt to recognize prey and to avoid inedible species. Predators must be able to capture prey. Adaptations to improve capture efficiency include improved motor skills and appendage modification. Finally, predators must handle prey by efficiently subduing them and detoxifying any defensive compounds. Adaptations promoting handling efficiency include improved foraging appendages to reduce the probability of injury and physiological specialization on otherwise poisonous prey. Predators also improve foraging efficiency by **learned avoidance**, a behavior in which predators quickly learn to recognize poisonous or distasteful species by remembering adverse reactions from attempted predation events.

Because life depends on taking life, almost all organisms on earth are potential **prey** for at least one other species. To escape this predation pressure, natural selection has favored individuals that are more difficult to find, capture, subdue, and consume. Adaptations that have **co-evolved** along with the predator's adaptations to increase foraging success, are adaptations that will decrease predation, including both warning (bright) coloration and cryptic coloration (camouflage), behavioral defenses (like the roly poly bug curling up into a ball), morphology (spines, armour), and physiology (bombardier beetle, skunk).

Previous Knowledge: **(genetics):** The terms **dominant** and **recessive** describe the inheritance patterns of certain traits. That is, they describe how likely it is for a certain phenotype to pass from parent offspring. Sexually reproducing species, including people and other animals, have two copies of each gene. The two copies, called alleles, can be slightly different from each other. The differences can cause variations in the protein that's produced, or they can change protein expression: when, where, and how much protein is made. Proteins affect traits, so variations in protein activity or expression can produce different phenotypes.

A dominant allele produces a dominant phenotype in individuals who have one copy of the allele, which can come from just one parent. For a recessive allele to produce a recessive phenotype, the individual must have two copies, one from each parent. An individual with one dominant and one recessive allele for a gene will have the dominant phenotype. They are generally considered "carriers" of the recessive allele: the recessive allele is there, but the recessive phenotype is not.

Dominant and recessive inheritance are useful concepts when it comes to predicting the probability of an individual inheriting certain phenotypes, especially genetic disorders. But the terms can be confusing when it comes to understanding how a gene specifies a trait. This confusion comes about in part because people observed dominant and recessive inheritance patterns before anyone knew anything about DNA and genes, or how genes code for proteins that specify traits. Though it may seem contradictory, recessive alleles can be present in a population higher frequencies than dominant alleles. Consider eye color. Eye color is influenced mainly by two genes, with smaller contributions from several others. People with light eyes tend to carry recessive alleles of the major genes; people with dark eyes tend to carry dominant alleles. In Scandinavia, most people have light eyes—the recessive alleles of these genes are much more common here than the dominant ones.

Further**,** mode of inheritance has nothing to do with whether an allele benefits an individual or not. Take rock pocket mice, where fur color is controlled mainly by a single gene. The gene codes for a protein that makes dark pigment. Some rock pocket mice have dark fur, and some have light fur. The dark-fur allele is dominant, and the light-fur allele is recessive. When mice live in a habitat filled with dark rocks, dark fur is "better" because it makes the mice less visible to predators. But when mice live in a habitat filled with light rocks and sand, light fur is "better." It's the environment that matters, not whether the allele is dominant or recessive.

<u>Procedure:</u> Testing NATURAL SELECTION of field mice color under predation pressure: Using black and white beans on the lab countertop, a student predator (owl) preys on the beans (field mice) for several generations. The field mice are one species, but have variation in fur color (they can be black or white).

1. Each group will select one person to be the owl "predator". This person is sent out of the room. The "predator" will also be 'blindfolded' with large extra dark sunglasses (colored cellophane taped to the lens). For safety, a "seeing" student should lead the predator into and out of the room.
2. Once the predator has been removed, each group will randomly scatter 10 white beans and 10 black beans on a BLACK poster board.
3. Turn off the lights in the lab and leave the door(s) open only enough to allow a small amount of light into the room.
4. Escort the predator come back into the room and give them 10 seconds to forage for prey in the dim light. This predator can hunt the mice with only TWO fingers of one hand as if they were claws, picking up each bean (field mouse) ONE AT A TIME.
5. After 10 seconds have the predator stop, turn on the lights and survey the carnage of mice.
6. Collect all remaining surviving beans (field mice) and reward each survivor with two new offspring that are the same color as the parent. This represents a reproductive event because those beans (field mice) survived to reproduce and pass on their phenotype to offspring.

7. Assemble this new generation (including the parent beans) and scatter the beans randomly on the table again. Repeat the above procedures of steps 3-7 for five generations. Record the percentages after each selection event in the table below.

Generation	% **Black mice**	% **White**
Start	50%	**50%**
1		
2		
3		
4		
5		

Table 1. Prey Percentages (black poster board)

8. Redo the investigation. However, during this trial have the "owl" search for "prey" that is scattered on a WHITE poster board. After completing this trial, students should compare and contrast the results from both trials. Can evolution reverse directions if the environment (selective pressure) changes?

Generation	% **Black mice**	% **White**
Start	50%	**50%**
1		
2		
3		
4		
5		

Table 2. Prey Percentages (white poster board)

Now, test the effects of GENETIC DRIFT on SMALL populations (versus LARGE). Even without natural selective pressures, changes can occur in a phenotype over time due to random death and random reproduction. As these effects are random, one might expect a more changes in a small, unstable population rather compared to a large, stable population.

1. Start with 3 beans of each color (six beans total) in a cup, mixed up.
2. Without looking, remove 3 beans from the cup. This represents random death.
3. Record the color ratio of the surviving beans (in cup) in the table below.
4. Each surviving bean gets two offspring of the same color. This is a reproductive event.
5. Repeat steps 2-4 five times, recording color ratios in the table below after each generation.

Generation	% **Black mice**	% **White**
Start	50%	**50%**
1		
2		
3		
4		
5		

Table 3. Genetic Drift in a small population.

6. Now start over, this time with 10 beans of each color in your cup (20 beans total).

7. Without looking, remove 10 beans. This represents random death.
8. Record the color ratios of surviving beans.
9. Each surviving bean gets 2 offspring of the same color as the parent bean. This is a reproductive event.
10. Repeat steps 7-9 five times, recording the colors ratios in the table below after each generation.

Generation	% **Black mice**	% **White mice**
Start	50%	**50%**
1		
2		
3		
4		
5		

Table 4. Genetic Drift in a large population.

Last, test the effects of **Founder Effect and Bottleneck Effect:** Allele frequencies can be changed over time by random chance alone. If a few individuals from a large population form a new, smaller, population (they become founders of a new population), by chance alone the new population might differ in genetic makeup (allele frequencies) compared to the parent population. Likewise, if only a few individuals of a large population survive a catastrophic event, they are the ones that will re-start their population, and only their genes will be present. In the example below, the beans you remove are the beans that survive a random death event (like a big forest fire) and thus restart the population (Bottleneck) or are the few individuals that start a new, founder, population elsewhere (a few seabirds blown to a remote island during a hurricane).

1. Start with a very large population with an equal number of both color of beans in a large beaker (30 beans of each color is good).
2. Without looking, withdraw a random sample of 5 beans.
3. Record this “founder” population’s color ratio below.
4. REPLACE the 5 beans, and repeat the steps 2-3 four more times, recording the color ration, then replacing the beans, each time.
5. Next, repeat steps 2-4 but withdraw a large population this time, a founder population of 30 beans removed.

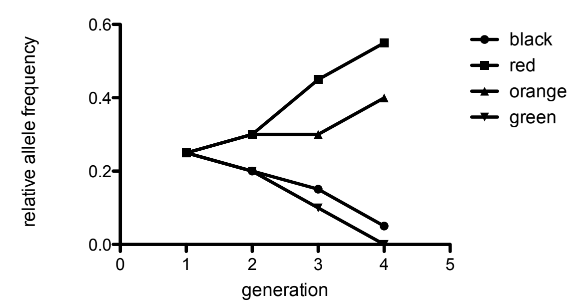
Original large population		Small Founder Population		Large Founder Population	
% black Beans	**% white beans**	**% black Beans**	**% white beans**	**% black Beans**	**% white beans**
50%	**50%**				
50%	**50%**				
50%	**50%**				
50%	**50%**				
50%	**50%**				

Table 5. Examples of Genetic Drift (Founder or Bottleneck).

Data Analysis: Basically we are comparing percentages here, looking at the original population, which was 50% black beans and 50% white beans, and seeing what the percentage is in each successive generation. If the ratio has changed from 50/50, then evolution has occurred. With the natural selection exercise particularly, it is neat to graph the % black beans (on Y) versus generation (on X). With the last exercise, founder / bottleneck, since you are replacing the withdrawn beans each time, you are NOT really looking at successive generations – just showing the differences in large versus small populations when only a few survive (bottleneck) or only a few become founders of a new population.

Extensions: 1. Modeling natural selection with jelly beans: Take 4 colors (phenotypes) and thus flavors (genotypes) of jelly beans. Try to use at least one flavor that is usually considered "yucky". Ex: Buttered popcorn flavor versus cherry and lime and orange. Or black licorice versus mint versus berry versus orange. Put 15 of each color in the center of the table. Each student takes one jellybean to eat. The remaining jelly beans reproduce (double the number of remaining beans of each color). Repeat for at least 4 trials (generations). Graph the change in relative frequency of the 4 colors under predation pressure. For the first generation, you have 15 of each of 4 colors (25% each; relative frequency = 0.25).

changes in relative allele frequency of 4 colors (phenoptypes) of jelly beans



2. Modeling natural selection with different colored macaroni: Sickel, A. and P. Friedrichsen. (2012). Using the FAR guide to teach simulations: an example of natural selection. The American Biology Teacher, Vol. 74:47-51.

3. Explore insect foraging behavior with a lab from http://www.esa.org/tiee/vol/v4/experiments/insect_predation/description.html It uses different "appendages" (forks, knives, etc) to capture prey (m&m's, candy corn, skittles), including scenarios where one prey is toxic.

4. You can also do the Project Learning Tree "Birds and Worms" exercise, which shows how camouflage has been naturally selected for to help prey avoid predation (good for younger kids, involves an outside (preferably) race).

Reflection Questions:

- **Did evolution occur in our model field mouse population? In which cases?** (Yes! In all cases. If the ratio of black beans to white beans changed, and is different from the original population, then evolution did occur. Evolution doesn't always result in the creation of a new species, though sometimes, when the new population is so different from the original that breeding between new and old no longer occurs, then speciation has also occurred).

- **What agent of evolution, genetic drift or natural selection, caused the most dramatic change in the model population?** (Drift in a small population).

- **How did the size of the original population make a difference in genetic drift?** (with a small population the likelihood of removing a subset that was identical in genetic makeup to the parent population is pretty unlikely. In a larger population, it was more likely to have numbers of black . white beans that were similar to the parent. This is like flipping a coin – if you flip it only 4 times the likelihood of getting 50% heads is unlikely, but if you flip it 100 times you are much more likely to see a 50% heads / 50% tails).

- **How could you determine if a trait in an organism evolved by natural selection?** (natural selection is more directional, moving toward a population that has a greater number of a certain allele / phenotype with each successive generation).

- **Can evolution reverse directions if the environment (selective pressure) changes?** (Yes. When beans were on the white background, the population became successively whiter – but on a black background the opposite happened. The real-life example is the English peppered moth, which, in pre-industrial England lived on light colored trees, and moths that had darker wings were more easily seen and eaten, so the lighter color was an advantage that was selected for. Once the industrial revolution hit, the smog / soot produced colored the tree bark darker, and natural selection reversed itself because now it was the light colored moths that stood out against their background and became easy prey (the light color was a disadvantage now, and was selected against).

Models and Explanations: In this lab we explored both natural selection and genetic drift as agents of evolution. **A student who demonstrates understanding** of these concepts can explain a situation in which evolution is likely to occur (a sexually reproducing species with variation in inherited alleles) and that if a particular allele confers an advantageous phenotype on an individual, that individual is more likely to survive and reproduce, so that differential reproduction will occur, and more of that allele / phenotype will be present in the next generation. This student understands that evolution is simply a change in allele / phenotype frequencies in successive generations and that both the environment can drive this (if an allele is either selected for, or against) and random chance events (called genetic drift) can drive evolution. In the case of drift, the results are usually more pronounced in a small population. **This student can explain** his/her results in a clear manner.

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Student Worksheet:

Evolution is the process by which adaptive phenotypes in a population change over time, depending on the selective pressure of the environment in which the organisms live. This results in a change in allele frequencies in a population over successive generations. This change occurs through differential reproduction, over generations of time, not to a specific individual. Populations evolve; individuals do not (their genes, and gene frequencies, are set in stone). Genes carry chromosomes which are the blueprints for construction and expression of phenotype (our appearance, both physical and physiological). If a particular phenotype provides an advantage to the organism, making it better able to survive and reproduce and pass on the beneficial genes to the next generation, then that phenotype is adaptive and that phenotype will be preserved. Two mechanisms can lead to evolution: natural selection and genetic drift. **Natural Selection** is the differential reproductive success of organisms having different phenotypes in response to interaction with natural (environmental) selective forces. **Genetic Drift** is the change in allele frequency of a small population due to chance alone, and not necessarily related to environmental forces. There are two forms of genetic drift that we will examine today: bottleneck effect and founder effect. The Bottleneck Effect is when phenotype ratios change due to random reduction in a population so that the surviving population is no longer similar to the original, larger population. Founder Effect is a change in phenotype ratio due to the colonization of a new area by a small number of founding individuals from a parent population. The main difference between drift and natural selection is that natural selection tends to be directional change in response to environmental selective pressure while drift is a change in phenotype due to random events or chance. To examine bottleneck and founder effect we will focus on the frequency of color traits under different conditions.

PROCEDURE:

1. Each group will select one person to be the owl "predator". This person is sent out of the room. The “predator” will also be ‘blindfolded’ with large extra dark sunglasses. For safety, a “seeing” student should lead the predator into and out of the room.
2. Once the predator has been removed, each group will randomly scatter 10 white beans and 10 black beans on a BLACK poster board.
3. Turn off the lights in the lab and leave the door(s) open only enough to allow a small amount of light into the room.
4. Escort the predator come back into the room and give them <u>10 seconds</u> to forage for prey in the dim light. This predator can hunt the mice with only TWO fingers of one hand as if they were claws, picking up each bean (field mouse) ONE AT A TIME.
5. After 10 seconds have the predator stop, turn on the lights and survey the carnage of mice.

6. Collect all remaining surviving beans (field mice) and reward each survivor with two new offspring that are the same color as the parent. This represents a reproductive event because those beans (field mice) survived to reproduce and pass on their phenotype to offspring.
7. Assemble this new generation (including the parent beans) and scatter the beans randomly on the table again. Repeat the above procedures of steps 3-7 for five generations. Record the percentages after each selection event in the table below.

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Table 2. Prey Percentages (white poster board)

Now, test the effects of GENETIC DRIFT on SMALL populations (versus LARGE). Even without natural selective pressures, changes can occur in a phenotype over time due to random death and random reproduction. As these effects are random, one might expect a more changes in a small, unstable population rather compared to a large, stable population.

11. Start with 3 beans of each color (six beans total) in a cup, mixed up.
12. Without looking, remove 3 beans from the cup. This represents random death.
13. Record the color ratio of the surviving beans (in cup) in the table below.

14. Each surviving bean gets two offspring of the same color. This is a reproductive event.
15. Repeat steps 2-4 five times, recording color ratios in the table below after each generation.

Generation	% Black mice	% White
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Table 3. Genetic Drift in a small population.

16. Now start over, this time with 10 beans of each color in your cup (20 beans total).
17. Without looking, remove 10 beans. This represents random death.
18. Record the color ratios of surviving beans.
19. Each surviving bean gets 2 offspring of the same color as the parent bean. This is a reproductive event.
20. Repeat steps 7-9 five times, recording the colors ratios in the table below after each generation.

Generation	% Black mice	% White mice
Start	50%	**50%**
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Table 4. Genetic Drift in a large population.

Last, test the effects of **Founder Effect and Bottleneck Effect:** Allele frequencies can be changed over time by random chance alone. If a few individuals from a large population form a new, smaller, population (they become founders of a new population), by chance alone the new population might differ in genetic makeup (allele frequencies) compared to the parent population. Likewise, if only a few individuals of a large population survive a catastrophic event, they are the ones that will re-start their population, and only their genes will be present. In the example below, the beans you remove are the beans that survive a random death event (like a big forest fire) and thus restart the population (Bottleneck) or are the few individuals that start a new, founder, population elsewhere (a few seabirds blown to a remote island during a hurricane).

6. Start with a very large population with an equal number of both color of beans in a large beaker (30 beans of each color is good).
7. Without looking, withdraw a random sample of 5 beans.
8. Record this "founder" population's color ratio below.
9. REPLACE the 5 beans, and repeat the steps 2-3 four more times, recording the color ration, then replacing the beans, each time.
10. Next, repeat steps 2-4 but withdraw a large population this time, a founder population of 30 beans removed.

Original large population		**Small Founder Population**		**Large Founder Population**	
% black Beans	**% white beans**	**% black Beans**	**% white beans**	**% black Beans**	**% white beans**
50%	**50%**				
50%	**50%**				
50%	**50%**				
50%	**50%**				
50%	**50%**				

Table 5. Examples of Genetic Drift (Founder or Bottleneck).

Reflection:

- **Did evolution occur in our model field mouse population? In which cases?**
- **What agent of evolution, genetic drift or natural selection, caused the most dramatic change in the model population?**
- **How did the size of the original population make a difference in genetic drift?**
- **How could you determine if a trait in an organism evolved by natural selection?**
- **Can evolution reverse directions if the environment (selective pressure) changes?**