ANSWER KEY

PART A: NATURAL SELECTION AND EVOLUTION OF ROCK POCKET MOUSE POPULATIONS

INTRODUCTION

The rock pocket mouse, *Chaetodipus intermedius*, a small, nocturnal animal, is found in the deserts of the southwestern United States. Most of these mice have a sandy, light-colored coat that enables them to blend in with the light-colored desert rocks and sand on which they live. However, populations of primarily dark-colored rock pocket mice have been found living in areas where a dark rock called basalt covers the ground. The basalt formed from cooling lava flows thousands of years ago. Scientists have collected data from a population of primarily dark-colored mice living in an area of basalt in Arizona called the Pinacate lava flow, as well as from a nearby light-colored population. Researchers analyzed the data from these two populations in search of the genetic mutation responsible for the dark color. Their analyses led to their discovery of a mutation in the *Mc1r* gene, which is involved in coat-color determination.

MATERIALS

genetic code chart (see page 4 or any biology textbook)

PROCEDURE

1. Read the following excerpt from an article published in *Smithsonian* magazine by Dr. Sean Carroll, a leading evolutionary biologist and Howard Hughes Medical Institute investigator:

> "One of the most widespread phenomena in the animal kingdom is the occurrence of darkly pigmented varieties within species. All sorts of moths, beetles, butterflies, snakes, lizards and birds have forms that are all or mostly black. . . .
>
> All of these so-called "melanic" forms result from increased production of the pigment melanin in the skin, fur, scales, or feathers. Melanic pigmentation can serve many roles. Melanin protects us and other animals from the ultraviolet rays of the sun; it can help animals in colder climates or higher altitudes warm their bodies more quickly, and . . . black pigment does conceal some animals from predators.
>
> In the deserts of the southwestern United States, for instance, there are outcrops of very dark rocks that were produced by lava flows over the past two million years. Among these rocks lives the rock pocket mouse, which occurs in dark black and a light, sandy color. Naturalists in the 1930s observed that mice found on the lava rocks were typically melanic, while those on the surrounding sand-colored granite rocks were usually light-colored. This color-matching between fur color and habitat background appears to be an adaptation against predators, particularly owls. Mice that are color-matched to their surroundings have a survival advantage over mismatched mice in each of the two habitats. . . .
>
> The gene involved in the origin of melanism in [some] rock pocket mice is called melanocortin receptor 1, or MC1R for short. That is not a very interesting nugget of information, until I tell you that the melanic forms of jaguars, snow geese, arctic fox, fairy wrens, banaquits, golden lion tamarins, arctic skua, two kinds of lizards, and of domestic cows, sheep, and chickens are caused by mutations in this very same gene. In some species, precisely the same mutations have occurred independently in the origin of their dark forms. These discoveries reveal that the evolution of melanism is not some incredibly rare accident, but a common, repeatable process. Evolution can and does repeat itself. " (Carroll, Sean B. Evolution in Black and White. Smithsonian.com, February 10, 2009).

2. Watch the short film titled *The Making of the Fittest: Natural Selection and Adaptation.*

3.

a. What specific trait did researchers study in this investigation?

Fur color, specifically melanism, is the trait they studied.

b. How does this trait affect the survival of the mice in different environments?

Depending on the color of the substrate, fur color may or may not help rock pocket mice blend in with their environment. On a light-colored substrate, mice with fur that is light in color are camouflaged and are not very obvious to predators. On a dark substrate, mice with dark-colored fur blend in and are better able to avoid predation. Predators readily spot mice with light-colored fur. Mice that survive predation are more likely to live, reproduce, and pass on their favorable trait for fur color.

c. What is the genetic basis of the trait?

Mutations in the Mc1r gene are responsible for the appearance of dark fur color in this particular population of rock pocket mice. (Note: Mutations of other genes in the pigment pathway may play a role in other mouse populations. See the lesson "The Biochemistry and Cell Signaling Pathway of the Mc1r Gene" for more information.)

4. The video describes the various mice populations having similar phenotype, but different having different genotypes. Use the information in the excerpt on page 1 and the information from the video to explain the evolutionary significance of MC1R protein variations in the different mouse populations.

This indicates that dark coat color in rock pocket mice from Arizona and New Mexico has evolved independently through changes in different genes. These discoveries reveal that the evolution of melanism (or the development of dark-colored pigment in skin or hair) is not some rare accident but a common, repeatable process. Evolution can and does repeat itself, and it has in the different dark-colored rock pocket mice studied in the southwestern United States.

5. Natural selection is just one mechanism of evolution. What are the others? Use your textbook (Chapter 23).

Gene flow: Gene flow is commonly called migration. It is the movement of alleles into and out of a population's gene pool. This is a common source of genetic variation within a population. Genetic drift: This mechanism causes a change in the gene pool of a small population and occurs strictly by chance. Genetic traits can be lost or become widespread in a population without regard to the survival or reproductive value of the alleles involved. Mutation: Mutation is a random change in the DNA sequence of an organism and the main source of new alleles in a population. In some cases, as in the rock pocket mice, a mutant allele can actually make its bearer better suited for its environment, thus enhancing reproductive success.

PART B: ALLELE AND PHENOTYPE FREQUENCIES IN ROCK POCKET MOUSE POPULATIONS

INTRODUCTION

The tiny rock pocket mouse weighs just 15 grams, about as much as a handful of paper clips. A typical rock pocket mouse is 172 millimeters long from its nose to the end of its tail, which is shorter than an average pencil. Its impact on science, however, has been enormous. What's so special about this little mouse?

Populations of rock pocket mice are found all over the Sonoran Desert in the southwestern United States. Two varieties occur widely in the area—a light-colored variety and a dark-colored variety. Similarly, there are two major colors of substrate, or surface material, that make up the rocky desert floor. Most of the desert landscape consists of light-colored sand and granite. Here and there, however, separated by several kilometers of light-colored substrate, are patches of dark volcanic rocks that formed from cooling lava. These areas of dark volcanic rock range in age from 1,000 to more than 1 million years old.

Dr. Michael Nachman of the University of Arizona and his colleagues have spent many years researching the genetics of fur color in rock pocket mice. In particular, they were interested in understanding the forces that shape genetic variation in natural populations.

Investigating the adaptive value of different coat colors in rock pocket mice is an example of how scientists are attempting to connect genotype with phenotype for fitness-related traits. In this type of research, investigators try to find the underlying gene or genes for a given adaptation. Examples of other fitness-related traits that researchers are currently investigating are resistance to the pesticide warfarin in rats, tolerance to heavy metals in plants, and antibiotic resistance in bacteria.

MATERIALS

calculator

computer and the Selection Coefficient file found under the "Survival of the Fittest—Battling Beetles" activity at http://www.hhmi.org/biointeractive/classroom-activities-battling-beetles.

PROCEDURE

PART 1: REVIEWING THE PRINCIPLES OF THE HARDY-WEINBERG THEOREM

The genetic definition of "evolution" is "a change to a population's gene pool." "Gene pool" is defined as "the total number of alleles present in a population at any given point in time." According to the Hardy-Weinberg theorem, a population is in equilibrium (and is therefore *not* evolving) when all of the following conditions are true:

1. The population is very large and well mixed.
2. There is no migration.
3. There are no mutations.
4. Mating is random.

5. There is no natural selection.

To determine whether a population's gene pool is changing, we need to be able to calculate allelic frequencies. Suppose, for example, a gene has two alleles, *A* and *a.* Each individual has one of three genotypes: *AA*, *Aa*, or *aa*. If the population is in equilibrium, the overall number of *A* alleles and *a* alleles in the gene pool will remain constant, as will the proportion of the population with each genotype. If allele frequencies or genotype frequencies change over time, then evolution is occurring.

Two equations are used to calculate the frequency of alleles in a population, where $\boldsymbol{p}$ represents the frequency of the dominant allele and $\boldsymbol{q}$ represents the frequency of the recessive allele:

$\boldsymbol{p + q = 1.0}$

and

$\boldsymbol{p^2 + 2pq + q^2 = 1.0}$.

The first equation says that if there are only two alleles for a gene, one dominant and one recessive, then 100% of the alleles are either dominant (p) or recessive (q).

The second equation says that 100% of individuals in the population will have one of these genotypes: *AA*, *Aa*, and *aa*. Let's look at each genotype one by one to understand the equation:

- If p represents the frequency of the *A* allele, then the frequency of the genotype *AA* will be $p \times p$, or p^2.
- If q represents the frequency of the *a* allele, then the frequency of the genotype *aa* will be $q \times q$, or q^2.
- For heterozygotes, we must allow for either the mother or the father to contribute the dominant and recessive alleles. You can think of it as allowing for both genotypes *Aa* and *aA*. So, we calculate the frequency of the heterozygous genotype as $2pq$.

In rock pocket mice, several genes code for fur color. Each gene has several possible alleles. That's why there is a range of fur color from very dark to light. For simplicity, we will work with two alleles at one gene. The allele for dark-colored fur (*D*) is dominant to the allele for light-colored fur (*d*). In this scenario, individual rock pocket mice can have one of three genotypes and one of two phenotypes, as summarized in the table below.

Rock Pocket Mice Genotypes and Phenotypes

Population	**Genotype**	**Phenotype**
Homozygous dominant	*DD*	Dark
Heterozygous	*Dd*	Dark
Homozygous recessive	*dd*	Light

So, applying Hardy-Weinberg, we have the following:

$\boldsymbol{p}$ = the frequency of the dominant allele (*D*)

$\boldsymbol{q}$ = the frequency of the recessive allele (*d*)

$\boldsymbol{p^2}$ = the frequency of *DD*

$\boldsymbol{2pq}$ = the frequency of *Dd*

q^2 = the frequency of *dd*

We can also express this as the frequency of the *DD* genotype + the frequency of the *Dd* genotype + the frequency of the *dd* genotype = 1.

SAMPLE PROBLEM

In a hypothetical population consisting of 100 rock pocket mice, 81 individuals have light, sandy-colored fur. Their genotype is *dd*. The other 19 individuals are dark colored and have either genotype *DD* or genotype *Dd*.

Find p and q for this population and calculate the frequency of heterozygous genotypes in the population.

It is easy to calculate q^2.

$q^2 = 81/100 = 0.81$, or 81%

Next, calculate q.

$q = \sqrt{0.81} = 0.9$

Now, calculate p using the equation $p + q = 1$.

$p + 0.9 = 1$

$p = 0.1$

Now, to calculate the frequency of heterozygous genotypes, we need to calculate $2pq$.

$2pq = 2(0.1)(0.9) = 2(0.09)$

$2pq = 0.18$

QUESTIONS

1. If there are 12 rock pocket mice with dark-colored fur and 4 with light-colored fur in a population, what is the value of q? Remember that light-colored fur is recessive.

q = 0.5
Explanation: q2 = (4/16), or 0.25; therefore, q = the √0.25, or 0.5

2. If the frequency of p in a population is 60% (0.6), what is the frequency of q?

q = .4 Explanation: If the frequency of p in a population is .6 then the frequency of q is .4 since p + q = 1

3. In a population of 1,000 rock pocket mice, 360 have dark-colored fur. The others have light-colored fur. If the population is at Hardy-Weinberg equilibrium, what percentage of mice in the population are homozygous dominant, dark-colored mice?

p2 = 0.04, or 4% Explanation: q2 = 640/1,000 = 0.64, so, q = 0.8; because p + q = 1, p = 0.2 and p2 =

(0.2)(0.2) = 0.04

PART 2: APPLYING HARDY-WEINBERG TO ROCK POCKET MOUSE FIELD DATA

Dr. Nachman and his colleagues collected rock pocket mice across 35 kilometers of the Arizona Sonoran Desert, which included both dark, rocky lava outcrops and light, rocky, granite areas. They recorded substrate color and coat-color frequencies for each location. Each site was separated from any of the others by at least eight kilometers. The researchers trapped a total of 225 mice. Their data are summarized below.

Field Data Summary

Collecting Site	Substrate Color	Number of Mice	Phenotype	
			Light	Dark
1	Light	6	6	0
2	Light	85	80	5
3	Dark	7	0	7
4	Dark	5	0	5
5	Dark	45	3	42
6	Light	77	34	43

Source of data: Hoekstra, Hopi E., Kristen E. Drumm, and Michael W. Nachman. "Ecological Genetics of Adaptive Color Polymorphism in Pocket Mice: Geographic Variation in Selected and Neutral Genes." *Evolution* 58, no. 6 (2004): 1329–1344.

QUESTIONS

1. Calculate the overall frequencies of light-colored mice and dark-colored mice caught on light-colored substrates.

frequency = number of mice of one color/total number of mice

Frequency of light-colored mice ________ Frequency of dark-colored mice ________

Frequency of light-colored mice = 120/168 = 71%; Frequency of dark-colored mice = 48/168 = 29%

2. Calculate the overall frequencies of light-colored mice and dark-colored mice caught on dark-colored substrates.

frequency = number of mice of one color/total number of mice

Frequency of light-colored mice ________ Frequency of dark-colored mice ________

Frequency of light-colored mice = 3/57 = 5%; Frequency of dark-colored mice = 54/57 = 95%

3. Using the Hardy-Weinberg equation and data from the table above, determine the number of mice with the *DD* and *Dd* genotypes on the light, rocky, granite substrate.

Frequency of mice with the *dd* genotype on light-colored substrate ________

Frequency of mice with the *DD* genotype on light-colored substrate ________

Frequency of mice with the *Dd* genotype on light-colored substrate ________

Frequency of mice with the dd genotype on light-colored substrate = 71%

Frequency of mice with the DD genotype on light-colored substrate = 3%

Frequency of mice with the Dd genotype on light-colored substrate = 26%

4. Using the Hardy-Weinberg equation and data from the table above, determine the number of mice with the *DD* and *Dd* genotypes on the dark, rocky lava substrate.

Frequency of mice with the *dd* genotype on dark-colored substrate ________

Frequency of mice with the *DD* genotype on dark-colored substrate ________

Frequency of mice with the *Dd* genotype on dark-colored substrate ________

Frequency of mice with the dd genotype on dark-colored substrate = 5%

Frequency of mice with the DD genotype on dark-colored substrate = 61%

Frequency of mice with the Dd genotype on dark-colored substrate = 34%

5. Which fur color seems to have the greatest overall selective advantage? Use data collected from both dark-colored and light-colored substrates to support your answer.

Dark fur color seems to have the greatest selective advantage. On the light-colored substrate, 29% of the mice have dark fur, while only 5% of the mice on the dark-colored substrate have light fur. Also, at collecting site no. 6, where there is a light-colored, rocky substrate, 43 out of 77 mice collected had dark-colored fur—over half of the sampled population. Dark-colored fur seems to have a selective advantage over light fur color

6. According to the film, what environmental change gave a selective advantage for one coat color over another?

The color of the landscape changed so that some members of the population were more visible to predators than other members were. That is what happened in the film. When sections of the landscape became dark, the light colored mice were at a selective disadvantage.

7. In a separate study, 76 rock pocket mice were collected from four different, widely separated areas of dark lava rock. One collecting site was in Arizona. The other three were in New Mexico. Dr. Nachman and colleagues observed no significant differences in the color of the rocks in the four locations sampled. However, the dark-colored mice from the three New Mexico locations were slightly darker than the dark-colored mice from the Arizona population. The entire *Mc1r* gene was sequenced in all 76 of the mice collected.

The mutations responsible for the dark fur color in the Arizona mice were absent from the three different populations of New Mexico mice. No *Mc1r* mutations were associated with dark fur color in the New Mexico populations. These findings suggest that adaptive dark coloration has occurred at least twice in the rock pocket mouse and that these similar phenotypic changes have different genetic bases. How does this study support the concept that natural selection is not random?

Evidence that natural selection is not random is the fact that when different genetic mutations produce the same phenotypic results in different areas, these similar adaptations are favored under similar conditions. An example provided in the film is the different populations of rock pocket mice with mutations that result in dark fur color. Dr. Carroll summed it up in the statement: “Evolution can and does repeats itself.” This is evidence that natural selection is not random.

8. To determine if the rock pocket mouse population is evolving, explain why it is necessary to collect fur color frequency data over a period of many years.

The data collected represent only one moment in time. If the population is evolving, the frequency of the two alleles for fur color will change over time. If the population is not under selective pressure, or is not evolving, the frequencies will remain approximately the same.

PART 3: HARDY-WEINBERG EXTENDED

We can adapt the Hardy-Weinberg equations to investigate what happens to gene frequencies in a population that is evolving. To do this, it is necessary to introduce a new term, **selection coefficient**. It is defined as “the relative advantage or disadvantage of a genotype with respect to survival and reproductive success.” You can also think of it as the relative selection advantage of a specific allele. For example, if there are two alleles present in a population for a particular trait and one allele is 10% more likely to survive than the other allele, then the selection coefficient for that allele is +0.1.

QUESTIONS

1. Use the spreadsheet (you will need to make a copy) to determine how the selection coefficient (*s*) influences the phenotype of future generations. Substitute increasingly large numbers for *s*. Record each new value and describe what happens to the frequencies of *p* and *q* over the next five generations.

When the value of s increases, the value of p increases while the value of q decreases in each generation. Students should record the values of s and either record or print the values for p and q. During this exercise, the frequency of the dominant phenotype increases as the frequency of the recessive phenotype decreases.

2. Explain how the selection coefficient and natural selection are related.

The selection coefficient is a numerical representation of how much advantage or disadvantage a particular variation or trait provides an organism. It provides a way to mathematically model and predict evolutionary change.

3. In areas with primarily dark-colored substrate, dark-colored mice have a selective advantage over light-colored mice. Therefore, mice with one or more copies of the dominant *Mc1r D* allele have a selective advantage over mice with two copies of the *Mc1r d* allele.

In the film, Dr. Sean Carroll says that with a 1% selection advantage, it takes 1,000 years for 95% of the mice to have the dominant phenotype. With a 10% selection advantage, it would take just 100 years. Use the spreadsheet to verify these facts.

a. Find out how many generations following the first appearance of a dark-colored mutation it would take for 95% of the mice to express the dominant dark-colored phenotype, given a 1% advantage (*s* = 0.01). Rock pocket mice have approximately one litter of pups a year, so the number of generations will be equal to the number of years. You will not be able to use the graph on the Main Page tab since it only goes up to 100 generations. So, you will need to look at column D of the worksheet called Main Worksheet. Scroll down until the value is greater than 0.95. Record your answer below.

It would take about ___________ generations.

It would take about 936 generations for 95% of the mice to express the dominant dark-colored phenotype.

b. Repeat the process for a 10% advantage (*s* = 0.1).

It would take about ___________ generations.

It would take about 100 generations.

c. What would the selection coefficient need to be for 95% of the mice to have the dominant phenotype in just 50 years? Record your answer below.

The coefficient would need to be about ____________________________________.

The coefficient would need to be about 0.22, a 22% advantage.