## Survival of the Fittest-Battling Beetles

## Introduction

Two important observations Charles Darwin made during his travels were (1) living things occupy a planet that is constantly changing and (2) living things change over time. These two observations led him to the concept of "descent with modification." Darwin wrote that the presence of variations within species fuels the process of change over time-evolution.

## Part One

## Phase One-Setting the Scene

Female beetles of the Ovalis glucosi species dig tunnels into the soil. When a female has dug a tunnel that is deep enough, she forms a small cavity at the end away from the entrance. This is where she will lay her eggs and raise her young.
Scientific studies have shown that an O. glucosi female will mate repeatedly. It is also known that the sperm most recently deposited is the sperm that will fertilize her eggs. Since the female beetle is in the tunnel, a male must gain access to the tunnel in order to mate.
Once they have mated, a male O. glucosi beetle attempts to control access to the female by guarding the entrance of her tunnel. The male remains inside the tunnel with the female and fights off any intruding males. A rival male could gain possession of a tunnel (and the female) if he successfully evicts the resident male. During the battle, dueling males crash into each other. The intruder tries to squeeze by the defender. The stronger male gets to stay and the weaker male is either crushed or moves on.
A research team studying mating behavior in the O. glucosi beetle species observed the appearance of a few red beetles in the population, which had previously been $100 \%$ blue. The red males appeared to be very successful in gaining access to the tunnels. Why are the red beetles so successful? Do they possess any obvious variations that would give them an advantage over blue beetles?

## Materials

- competition grid
- 1 container of 10 blue O. glucosi males
- 1 container of 10 red O. glucosi males
- small metric ruler
- 1 waste container
- red and blue markers or pencils
- Optional: electronic balance

Safety: Do NOT eat the O. glucosi beetles. They may be contaminated.

## Observations of Ovalis glucosi

1. Your container labeled "O. glucosi - red" holds 10 males. Without removing them from the bag, observe and record at least 4 traits you could use to accurately describe their appearance.
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2. Your container labeled "O. glucosi - blue" holds 10 males. Without removing them from the bag, observe and record at least 4 traits you could use to accurately describe their appearance.
3. Based on your observations, are there any obvious variations (other than color) that would distinguish a blue male from a red one? Support your answer with information recorded in observations 1 and 2.

## Phase Two-The Experiment

The research team studying mating behavior hypothesized that the color of a beetle's exoskeleton and the strength of the exoskeleton are related. To test the color and exoskeleton strength hypothesis, your team will conduct an experiment in which a blue beetle and a red beetle are crushed together.

## Procedures

1-1. Select one blue and one red beetle from your $O$. glucosi containers and place them as a pair on the appropriate circles on Chart 1: O. glucosi before and after crushing. Before starting the experiment, all 10 pairs should be in place.

1-2. To determine which beetle has the stronger exoskeleton, pick up the first pair of Red and Blue beetles. Stack one on top of the other as illustrated
 in Figure 1: Stack of beetles.

Figure 1: Stack of beetles

1-3. Hold the two beetles so that your thumbs are on the bottom surface and your index fingers are placed securely on the top. See Figure 2: Crushing technique.

1-4. Evenly apply pressure to the top and bottom of the stack. As soon as one of the exoskeletons cracks, stop. Examine the two specimens and determined which one cracked first*.
*Note: If it is impossible to determine which exoskeleton cracked first, record the one whose exoskeleton cracked the least in the Strongest exoskeleton column. The beetle with the most damage is the one that would most likely be evicted from the tunnel and would not mate with the female.

1-5. Indicate the survivor by coloring in the circle in the Strongest exoskeleton column with the appropriate colored pencil/ marker (red or blue).

1-6. Place the uncrushed and crushed beetles in the waste container.
1-7. Repeat the above procedures (1-2 through 1-6) a total of 10 times.
1-8. In Chart 2: Percent frequency of red and blue beetles before and after crushing record the number of each color present in the population before and after crushing.

1-9. In Chart 2, also record the data collected by the entire class. Use this data to calculate the percent frequency of each color present in the population before and after crushing.

Percent Frequency =(number of beetles of one color / total number of beetles) x 100

## Example:

There are 100 beetles in the population. 20 beetles are red and 80 are blue
Step 1: Percent Frequency of red beetles $=(20$ red beetles/ 100 total beetles $) \times 100$
Step 2: Percent Frequency of red beetles $=(20 / 100) \times 100$
Step 3: Percent Frequency $=.2 \times 100=20 \%$

## Questions

4. Explain why it is important to use class results and not just the results obtained by an individual team.
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5. Based on class results, are color and exoskeleton strength related? Support your answer using data from Chart 2.
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6. Based on class data, which color beetle is most likely to win the tunnel access competition and reproduce successfully? Explain why.

Chart 1: O. glucosi before and after crushing

| O. glucosi <br> (top) | O. glucosi (bottom) | Strongest exoskeleton |
| :---: | :---: | :---: |
| (R) | (B) |  |
| (B) | (R) |  |
| (R) | (B) |  |
| (B) | (R) | $\bigcirc$ |
| (R) | (B) |  |
| (B) | (R) |  |
| (R) | (B) |  |
| (B) | (R) |  |
| $R$ | (B) |  |
| (B) | (R) |  |


| Key | Red | Blue |
| :---: | :---: | :---: |
|  | R | B |

Chart 2: Percent frequency of red and blue beetles before and after crushing

| Number and percent frequency of each color in original population | Number and percent frequency of each color after crushing |
| :---: | :---: |
| Team Results |  |
| No. of Red $=10$ Freq. of Red $=\ldots \ldots$ | No. of Red $=\ldots$ Freq. of Red $=\ldots \ldots$ |
| No. of Blue $=\underline{10}$ Freq. of Blue $=\underline{\text { a }}$ ( | No. of Blue $=\ldots$ Freq. of Blue $=\ldots \ldots$ |


| Population = _---- |  | Class Results |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Population = _---- |  |  |
| No. | Freq. of Red $=\ldots \ldots$ |  | No. of Red = | Freq. of Red $=$ | \% |
| No. of Blue $=\ldots$ Freq. of Blue $=\ldots \ldots$ No. of Blue $=\ldots \ldots$ Freq. of Blue $=\ldots \ldots$ |  |  |  |  |  |

Remember: Percent Frequency = (number of beetles of one color / total number of beetles) x 100

## Phase Three-After the Experiment

Your research team has observed several generations of O. glucosi beetles. In addition to the information you have collected in Phases One and Two, you now know that when a red beetle mates with a blue one, a majority of the offspring are red. This implies that the allele for red coloration is dominant. The red color variation is due to a mutation that recently occurred. The big question is, "Does red coloration provide any selective advantage over blue and how can this be determined?
The presence of the red beetles in the population raises many additional questions. You receive funding to attend a scientific conference so that you can consult with other scientists doing similar research. Your team wants to know if others have observed the sudden appearance of a new color in an animal population and what the impact of that mutation has been.

You return from the meeting with a wealth of information. Researchers investigating the pocket mouse in southwestern United States have provided you with a short animation entitled Pocket Mouse Evolution to share with other members of your team.

## Questions

7. Explain how the mutation shown in the animation resulted in some pocket mice having a selective advantage over other pocket mice.
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8. Color does not provide O. glucosi beetles with a selective advantage. What does? Explain your answer.
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9. Over a period of time, a behavioral adaptation occurred that altered the mating behavior of some O . glucosi males. A small number of male beetles no longer challenged other males for access to the tunnel and females. Instead of dueling, these beetles secretly dug their own tunnels to intersect with and enter those dug by females. In this way, these males were able to mate and produce offspring without combat. Explain how this change in reproductive behavior could influence the evolution of the O. glucosi beetle population.
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## Part Two

## Understanding the Hardy-Weinberg Equation

A member of your research team predicts that in a few generations, the $O$. glucosi population will be made up of $50 \%$ red individuals and $50 \%$ blue individuals. This prediction can be examined mathematically using an adaptation of the Hardy-Weinberg equation ( $p^{2}+2 p q+q^{2}=1$ ). Before doing this, a basic understanding of the principles behind the Hardy-Weinberg equation is critical.

The Hardy-Weinberg theorem describes a population that is not evolving. For a population to remain in equilibrium and not undergo evolutionary change, the following must be true:

- The population is very large
- There is no migration
- There are no mutations
- Mating is random
- There is no natural selection

A population is defined as a localized group of organisms of the same species. All of the O . glucosi beetles present in your lab constitute a population.
A species is a group of populations that have the potential to breed in nature and produce fertile offspring.

The total of all of the genes in a population at any given point in time is the gene pool. For example, prior to the mutation that resulted in an allele for red exoskeleton coloration, the only allele present for color in the gene pool coded for blue.
When considering only the alleles that govern red or blue coloration, the beetles are either homozygous or heterozygous.

Homozygous means that both the alleles for a trait are the same.
Heterozygous means that an organism possesses two different alleles for a trait.
In this investigation, the letter R is used to represent the dominant allele for red. The letter r represents the recessive allele for blue. Individuals will possess one of the following three genotypes:

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\mathrm{RR}=\text { homozygous red } \quad \mathrm{Rr}=\text { heterozygous red } \quad \mathrm{rr}=\text { homozygous blue }
$$

To estimate the frequency of these two alleles in a population of O. glucosi using the Hardy-Weinberg equation, this is what you need to know:

$$
\begin{aligned}
& \mathbf{p}=\text { the frequency of the dominant allele }(\mathrm{R}) \\
& \mathbf{q}=\text { the frequency of the recessive allele }(\mathrm{r})
\end{aligned}
$$

In a population that is not evolving (in genetic equilibrium):

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\begin{aligned}
& p+q=1.0 \\
& (p+q)^{2}=1.0 \quad \text { so } \quad p^{2}+2 p q+q^{2}=1.0
\end{aligned}
$$

Referring back to the O. glucosi population:

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\begin{aligned}
& \mathbf{p}^{2}=\text { the frequency of } \mathrm{RR} \\
& \mathbf{2 p q}=\text { the frequency of } \mathrm{Rr} \\
& \mathbf{q}^{2}=\text { the frequency of } \mathrm{rr}
\end{aligned}
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## Sample Problem and Explanation

In a hypothetical population consisting of 100 O. glucosi beetles, there are 81 blue individuals. Blue is recessive so that any individuals exhibiting a blue phenotype possesses 2 alleles for blue. Their genotype is rr. Therefore, these individuals contribute a total of 162 r alleles to the gene pool.
This means that 19 out of the 100 individuals possess either one or two of the dominant R alleles for red coloration. This is because these individuals can have either the RR or Rr genotype and appear red.
It is easy to calculate $\mathrm{q}^{2}$

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q^{2}=81 / 100=0.81 \text { or } 81 \%
$$

## Calculateq.

take the square root of 0.81 .
(Answer: 0.9)
Calculatep.
Hint: remember that $p+q=1$. When added, the frequency of $p$ plus the frequency of $q$ equals $100 \%$. You know that $\mathrm{q}=0.9$
$\mathrm{p}=1-\mathrm{q}$
$\mathrm{p}=1-0.9$
(Answer: p =0.1)
Calculate 2pq.
$p=0.1$ and $q=0.9$
$2 \mathrm{pq}=2(0.1 \times 0.9)=2(0.09)$
(Answer: $2 \mathrm{pq}=0.18$ )
In the above example, $p=0.1$ and $q=0.9$ and therefore, $p+q=1$
(It can also be expressed as $\% p+\% q=100 \%$ )

## Quick Review: Hardy-Weinberg Equation

- Consider two alleles A and a
- A is a dominant allele over $a$, their relative gene frequencies are $p$ for $A$ and $q$ for a
- $p+q=1$
- The relative proportion of AA, Aa and aa genotypes are
AA $=p^{2}$
$A \mathrm{a}=2 \mathrm{pq}$
$\mathrm{aa}=\mathrm{q}^{2}$

| $\mathrm{p}^{2}$ | + | 2 pq | + | $q^{2}=$ |
| :--- | :---: | :---: | :---: | :---: |
| Frequency <br> of RR genotype | Frequency of <br> Rr plus rR genotypes | Frequency <br> of rr genotype |  |  |

## Problems

10. If there are 12 red beetles and 4 blue beetles in a population, what is the value of $q$ ? Remember that blue is recessive.
11. If the frequency of $p$ in a population is $60 \%$, what is the frequency of $q$ ?
12. In a population of 1,000 O. glucosi beetles, 360 have red exoskeletons. The others are blue. How many of the red beetles would you expect to be homozygous dominant?

## Questions

13. If a mutation occurs, one consequence is that the allele frequencies in a population change. If in a population you are studying, the allele frequencies change, does this prove that a mutation has occurred? Explain why or why not.
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14. Use your class data from Chart 2 to determine the value of $\mathrm{q}^{2}$ in the beetle population.
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15. Explain how natural selection and a color mutation associated with greater exoskeleton strength interact to result in evolutionary change in the beetle population.
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## Part Three

## Using the Hardy-Weinberg Equation to model selection

The Hardy-Weinberg Equation can be adapted to investigate what happens to the gene frequency 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. It can also be thought of 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 if one allele is $10 \%$ more likely to survive than the other allele, the selection coefficient for that allele is +0.1 . For this exercise, you will investigate what happens when the dominant allele has a selective advantage.

- The selection coefficient is represented by s.
- The fitness for an individual without any selective advantage or disadvantage is 1.
- In this activity, the R allele is completely dominant over the r allele. Both the homozygous dominant ( RR ) individuals and heterozygous ( Rr ) individuals are equally fit and have the fitness of $1+\mathrm{s}$.
- According to the Hardy-Weinberg equation, after mating, the relative proportions of $\mathrm{RR}, \mathrm{Rr}$, and rr are ${ }^{2}, 2 p q$, and $q^{2}$ respectively.
- But, after selection, the relative proportion is no longer $p^{2}, 2 p q$, and $q^{2}$. It is now $p^{2}(1+s)$, $2 \mathrm{pq}(1+\mathrm{s})$, and $\mathrm{q}^{2}$, i.e., RR and Rr individuals survive a little better and increase a little in number.
- Next you must calculate the new gene frequency p and q.
- The number of the R allele is 2 x the number of RR individuals + the number of Rr individuals. So the gene frequency for p is (the number of R allele) / 2(total number of individuals). It is 2 x the total because of diploidy.
- There is one complication. $p^{2}(1+s), 2 p q(1+s)$, and $q^{2}$, no longer adds up to 1 . The new total, represented by $T$, is equal to $p^{2}(1+s)+2 p q(1+s)+q^{2}$
The next generation of $p$ is therefore calculated by
$p_{\text {new }}=\left\{2 p^{2}(1+s)+2 p q(1+s)\right\} / 2 T$
$\mathrm{q}_{\text {new }}$ is calculated by $\mathrm{q}_{\text {new }}=1-\mathrm{p}_{\text {new }}$
- The accompanying Excel spreadsheet can perform these calculations for hundreds of generations very quickly.


## Questions

16. Use the Excel spreadsheet 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 5 generations.
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17. What might occur to change the selective advantage of a trait? Provide an example from the O. glucosi activity or the pocket mouse video.
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18. Explain how the selection coefficient and natural selection are related.
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19. Summarize how the Hardy-Weinberg equation can be used to model selection.
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20. Use the Excel spreadsheet to find out how many generations it takes for the allele with the selection advantage to be $50 \%$ of the gene pool for each of the following conditions:
a. $p=0.01, s=0.1$
b. $p=0.01, s=0.2$
c. $p=0.01, s=0.5$
d. $p=0.3, s=0.2$
e. $p=0.99 \mathrm{~s}=-0.75$
