IN-DEPTH FILM GUIDE

DESCRIPTION
Icefish have a set of stunning adaptations that help them thrive in the icy but food-rich environment of the Antarctic. Both the birth and death of genes have been critical to these adaptations. Dr. Bill Detrich, Dr. Christina Cheng, Dr. Art DeVries, and other scientists have pinpointed the genetic changes that enable icefish to survive without red blood cells and use antifreeze proteins in the ice-cold ocean.

KEY CONCEPTS
A. Traits (characteristics) are determined by genes.
B. Mutations can result in both the appearance of new genes and the loss of existing genes.
C. Changes in the environment where a population lives can change which traits (and therefore genes) are favorable.
D. The frequency of an allele in a population can change depending on whether the allele is advantageous, deleterious, or neutral.
E. One way that a new gene can arise is when a gene is duplicated and one copy (or both copies) of the gene accumulates mutations, which change the function of the gene.
F. One way that a gene can be lost is when one or more mutations accumulate that destroy its function.

CURRICULUM AND TEXTBOOK CONNECTIONS

<table>
<thead>
<tr>
<th>Curriculum</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP (2012–13)</td>
<td>1.A.2, 3.C.1, 3.C.2, 4.C.1</td>
</tr>
<tr>
<td>IB (2009)</td>
<td>4.1, 5.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Textbook</th>
<th>Chapter Sections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller and Levine, Biology (2010 ed.)</td>
<td>13.3, 17.4</td>
</tr>
<tr>
<td>Reese et al., Campbell Biology (9th ed.)</td>
<td>17.5, 21.5, 23.1, 26.4</td>
</tr>
</tbody>
</table>

PRIOR KNOWLEDGE
Students should
- have a basic understanding of natural selection, evolution, and adaptation;
- know basic genetics, including understanding what a gene is and knowing that mutations, duplications, and deletions are some of the ways in which genes and DNA can change;
- understand that genetic changes can happen randomly; and
- know that genes and the traits that they produce are inherited and that some traits afford organisms a greater chance to survive and reproduce.
### PAUSE POINTS

The film may be viewed in its entirety or paused at specific points to review content with students. The table below lists suggested pause points, indicating the beginning and end times in minutes in the film.

<table>
<thead>
<tr>
<th>Begin</th>
<th>End</th>
<th>Content description</th>
<th>Review Questions</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0:00</td>
<td>Most vertebrates have red blood cells that contain hemoglobin.</td>
<td>• How does icefish blood differ from that of other vertebrates?</td>
<td>NGSS (April 2013) HS.LS1.A, HS.LS2.A, HS.LS2.C, HS.LS4.C</td>
</tr>
<tr>
<td></td>
<td>7:51</td>
<td>The icefish have thin blood that lacks hemoglobin and have anti-freeze proteins;</td>
<td>• How is the icefish adapted to live in such cold water?</td>
<td>AP Biology (2012–13) 1.A.2, 4.C.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>these adaptations enable them to thrive in freezing waters.</td>
<td>• How do antifreeze proteins give Notothenioids a selective advantage?</td>
<td>IB Biology (2009) 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Notothenioids can survive in extremely cold, nutrient-rich waters, where other fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>can't survive.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13:10</td>
<td>therefore genes) are favorable. Notothenioids' antifreeze genes evolved in the last</td>
<td>• How did the antifreeze gene evolve?</td>
<td>AP Biology (2012–13) 3.C.1, 3.C.2, 4.C.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34 million years.</td>
<td>• How did icefish eliminate hemoglobin?</td>
<td>IB Biology (2009) 4.1, 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A new gene, the antifreeze gene, arose when an ancestral gene was duplicated and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>accumulated mutations that resulted in the production of antifreeze proteins.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Icefish have a mutation in a globin gene that prevented the gene from producing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hemoglobin. Mutations can result in both the appearance of new genes and the loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of existing genes.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### BACKGROUND

In the short film *The Making of the Fittest: The Birth and Death of Genes* (http://www.hhmi.org/biointeractive/making-fittest-birth-and-death-genes), you will hear about two groups of fish: Notothenioids and icefish. Icefish are a group of fish that belong to the Notothenioidei suborder. Icefish are classified as southern cods, and they are also called white-blooded fish and crocodile fish. There are 15 different species and 11 genera of icefish. We show their general classification below:

- **Kingdom:** Animalia
- **Phylum:** Chordata
- **Class:** Actinopterygii
- **Order:** Perciformes
- **Suborder:** Notothenioidei (notothenioid fish)
- **Family:** Channichthyidae (icefish)

Scientists think that icefish evolved from a benthic fish species because, like benthic fish, all icefish lack swim bladders. (Benthic fish do not need a swim bladder because they live on the seafloor.) Benthic icefish are generalized predators. They forage nonselectively and oftentimes ambush their prey. A few species of icefish, however, have adapted to life in the water column. These pelagic (non-bottom-dwelling) icefish varieties have increased their buoyancy in novel ways. Adaptations include a less mineralized skeleton, strategically distributed fat deposits, and a high oil content. Many pelagic icefish species migrate upward in the water column to feed on krill. The feeding preferences of both benthic and...
pelagic icefish species vary with the seasonal availability of food sources. In turn, icefish are eaten by larger fish and by penguins, other birds, and seals.

Most notothenioid fish live in the icy waters near Antarctica. They survive in this environment because they have special antifreeze proteins in their blood that lower the freezing point of their blood. In addition, icefish do not make red blood cells or hemoglobin. This special adaptation lowers the viscosity of the blood and allows them to survive in extremely cold environments. To compensate for the lack of red blood cells and hemoglobin, icefish have several unique adaptations. These adaptations include scaleless skin (which allows increased diffusion of oxygen through the skin), large gills, a large heart, large-diameter blood vessels, a large blood volume, and a high concentration of mitochondria. They also have low blood pressure and a low metabolic rate.

The timing of icefish spawning in late winter or early spring coincides with the spawning season of specific krill species. At least one species of icefish eats macroalgae and diatoms during the spring and summer, and it switches to krill in the fall and winter. Most species of icefish produce large eggs, more than 4 millimeters in diameter. In part because of the energy required to produce such large eggs, icefish rarely lay more than 10,000 to 20,000 per season. In contrast, a female herring lays about 50,000 eggs per season. Icefish do not attain maturity until they are five to eight years old and typically spawn in the autumn and winter. The incubation period varies with the location of the fish, ranging from two to three months in the northernmost regions of the Southern Ocean to about six months near Antarctica. Depending on the species, icefish grow about 6 to 10 centimeters a year, reaching a size of 15 to 200 centimeters at maturity.

Because the freezing water they inhabit exhibits little variation in temperature, icefish have little tolerance for temperature changes of more than a few degrees. Scientists are concerned about the impact that global climate change may have on icefish populations.

DISCUSSION POINTS

• Be sure to reemphasize with your class that mutation is random. Selection, however, depends on the environment. As environments change, so, too, do selection pressures.

• Ask students to explain what they think Dr. Sean B. Carroll meant when he stated, “Notothenioids invented antifreeze genes.” Help them understand that icefish did not actually “invent” the antifreeze gene in the sense that they created it. Emphasize that organisms do not adapt to environmental changes through conscious design. Adaptations are the result of random mutations that are acted upon by selective forces. Icefish did not invent antifreeze so that they could live in cold waters. In fact, the antifreeze gene evolved before the ocean’s temperature dropped below the freezing point of fish blood. Discuss how the use of the word “invention” is good in terms of telling a story but not accurate in the scientific sense.

• Discuss with students what Dr. Carroll means by “birth and death” of genes. New genes are not actually born in the sense that a kitten is born. There are several mechanisms by which new genes originate. One of the most common is gene duplication. The duplication could involve individual genes, individual exons, or parts of exons. Another source of new genes is gene transfer from a different species. New genes can also originate from noncoding regions of DNA (such as regulatory regions). Yet another source is gene fusion: two genes fuse and become part of the same transcript. During adaptation to environmental changes, various icefish gene functions became superfluous. Gene death starts when a mutation or mutations occur that incapacitate the functioning of a gene. If this function is no longer critical to survival, the resulting loss is not harmful. In a sense, the gene died.

• Your students may wonder more about the origins of the icefish antifreeze gene. The gene responsible for the production of antifreeze proteins in notothenioids evolved from a trypsinogen gene through a series of mutations. First, a chance duplication resulted in the production of an extra copy of the trypsinogen gene. Over time, in addition to mutations within the original trypsinogen coding region, a small piece of the gene that would eventually code for the antifreeze protein became amplified. At the time these mutations were taking place, the ocean was warmer and the mutation neither helped nor harmed the notothenioids. As the temperature decreased, fish that happened to have the ability to produce antifreeze proteins would have survived and reproduced, thus passing the adaptation on to their offspring. Fish without the
adaptation would not have survived to reproduce. See the article by Dr. Chen, Dr. DeVries, and Dr. Cheng in the references at the end of this guide to investigate this phenomenon further.

- Emphasize to students that individuals do not evolve during their lifetimes; populations of organisms evolve over time.
- Emphasize to students how icefish provide an example of how both the birth (evolution of antifreeze proteins) and death (loss of hemoglobin) of genes can have positive effects.

CLASSROOM RESOURCES FOR THE FILM

**Icefish Adaptations** (http://www.hhmi.org/biointeractive/icefish-adaptations)
A simple hands-on activity that investigates the importance of antifreeze proteins to icefish survival. Background information and analysis questions that reinforce the main ideas of the film are also included in this lab.
*Appropriate for* middle school life science, high school biology (all levels including AP and IB)

A lesson that requires students to read detailed scientific passages and explain how an understanding of specific icefish adaptations might lead to a treatment or cure for human disorders such as osteoporosis and anemia. An optional essay framework is included in the teacher materials.
*Appropriate for* high school biology (all levels including AP and IB), introductory college biology

An advanced Click and Learn lesson that describes how mutations are an important part of both the birth and death of genes. Background information, examples, video clips, and animations are included. Questions challenge students to synthesize information and apply what they learn.
*Appropriate for* high school biology (second year, AP, IB), advanced high school genetics elective, introductory college biology, college-level genetics

A simple demonstration that uses readily available materials to simulate how blood pumps through the circulatory system of icefish and other fish. An optional extension invites students to design an experiment to test further hypotheses related to icefish circulatory system adaptations.
*Appropriate for* middle school life science, high school biology (all levels including AP and IB), introductory college biology

**Ice Crystal Formation in Icefish and Non-icefish Blood** (http://www.hhmi.org/biointeractive/ice-crystal-formation-icefish-and-non-icefish-blood)
A dramatic demonstration that simulates how tiny ice crystals would form and grow in the blood of most fish if they ventured into the icy waters of the Antarctic. Students relate their observations to what would occur in icefish and non-icefish species in freezing water.
*Appropriate for* middle school life science, high school biology (all levels AP and IB), introductory college biology

**OTHER BIOINTERACTIVE RESOURCES**

**Natural and Artificial Selection** (www.biointeractive.org/evolution/Selection/01.html)
In this Click and Learn activity, students learn about natural and artificial selection. It features multiple clips from lectures on evolution (see below).
Endless Forms Most Beautiful, by Dr. Sean B. Carroll (media.hhmi.org/hl/05Lect1.html)
In this lecture, Dr. Carroll, a leader in the field of evolutionary developmental biology (or evo devo), explores how key developmental genes, natural selection, and time fuel the evolutionary process.

Selection in Action, by Dr. David M. Kingsley (media.hhmi.org/hl/05Lect2.html)
In this lecture, Dr. Kingsley discusses the evolution of traits in vertebrates and his pioneering research with stickleback fish.

Reading Genes and Genomes, Chapter 28, by Dr. Eric S. Lander (media.hhmi.org/hl/02Lect1.html?start=ch28&end=ch29)
This lecture chapter shows how many vertebrate genes arose from gene duplication followed by variation.

Evolution of the Y Chromosome (http://www.hhmi.org/biointeractive/evolution-y-chromosome)
How did the human Y chromosome become so small relative to its X counterpart? This animation depicts the 300 million–year odyssey of the sex chromosomes that began when the proto X and Y were an identical pair.

USING THE QUIZ
The quiz is designed as a summative assessment that probes student understanding of the key concepts addressed in the film. However, some teachers use the quiz before and during the film to assess students’ prior knowledge and to guide students as they watch the film. Teachers are encouraged to choose the use that best fits their learning objectives and their students’ needs. Teachers are encouraged to modify the quiz (e.g. only ask some of the questions, explain complicated vocabulary for ELL students) as needed.

QUIZ QUESTIONS AND ANSWERS
The student version of this quiz is available as a separate file. We note the key concepts covered by each question here. You may wish to use some or all of the questions below to test your students’ knowledge, depending on the content you wish to emphasize.

1. (Key Concept A) In one sentence, explain the relationship between genes and traits.
   **Genes can encode/influence/determine traits.**

2. (Key Concept B) Which of the following processes are caused by mutations? Write “yes” or “no” next to each of the four possible responses. **There may be more than one yes answer.**

<table>
<thead>
<tr>
<th>Process</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creation of new genes</td>
<td>Yes</td>
</tr>
<tr>
<td>Changes to genes that do not affect function</td>
<td>Yes</td>
</tr>
<tr>
<td>Loss of existing genes</td>
<td>Yes</td>
</tr>
<tr>
<td>Independent assortment of genes</td>
<td>No</td>
</tr>
</tbody>
</table>

3. (Key Concept C) Explain how a change in the environment can result in a change in the frequency of a gene in a population.
   **Genes encode traits or characteristics. Because the environment determines what traits are advantageous to an organism, the prevalence of certain genes in a population can change due to changes in the environment. For example, if the environment becomes warmer, genes that help organisms survive at higher temperatures will increase in frequency in the population.**

4. (Key Concepts B, E, and F) Compare and contrast the process by which genes are born and the process by which genes die. Include at least one way in which these two processes are the same, and one way in which they are different.
   **Both the birth and death of genes can be caused by DNA mutations. The birth of genes can occur through the duplication of an existing gene followed by a mutation that results in a new function for that gene. The death of genes can occur through mutations that destroy the function of a gene.**
5. **(Key Concepts C and D)** Is the following statement true or false? Justify your answer in one or two sentences: “If the temperature of the environment where the icefish are living were to rise to 5°C, the frequency of the antifreeze gene in the population would likely increase.”

   *False; since the antifreeze gene would no longer be advantageous for the icefish, the gene would either decrease or, at most, remain at the same frequency in the population.*

6. **(Key Concept D)** If a gene increases in frequency in a population, it likely has [Answer is in bold.]
   
   a. a positive impact on survival.
   
   b. a negative impact on survival.
   
   c. no impact on survival.

7. **(Key Concept C)** In the film, you saw that icefish have evolved to thrive in extremely cold water. State two genetic changes these fish have undergone to be able to thrive in this cold environment.

   1. The loss of the hemoglobin gene; 2. the gain of an antifreeze gene

8. **(Key Concept D)** What would you expect to decrease in frequency more rapidly in a population: a gene with no impact on survival or a gene with a negative impact on survival? Why?

   A gene with a negative impact on survival should disappear in a population more rapidly. Selection against a gene with a negative impact on survival would be stronger than selection against a gene with no impact on survival (which would be very weak or nonexistent).

9. **(Key Concept C)** Scientists had hoped that the icefish antifreeze gene could be used to protect agricultural crops from early-season frosts that often kill delicate plants like tomatoes. Using genetic engineering, they inserted an icefish antifreeze gene into the genome of a certain tomato species. They then tested the cold sensitivity of this species of tomato with and without the antifreeze gene by exposing plants to freezing temperatures for different amounts of time. Scientists then checked to see what percentage of plants survived their time in cold temperatures by counting how many plants were still alive after cold treatment. In one such experiment, scientists observed the following.

   **Survival of Tomato Plants**

   ![Graph showing survival of tomato plants with and without antifreeze gene.

   a. From the results of this experiment, what can you conclude about the effect of icefish antifreeze genes on cold tolerance in this species of tomato?

   **The antifreeze gene did not change the cold tolerance of this species of tomato.**

   b. Under these conditions, does the antifreeze gene have a positive, negative, or neutral impact on the survival of this species of tomato in cold temperatures?
The impact of the gene was neutral in these conditions.

10. (All Key Concepts) Suppose you identified and collected a new species of bacteria living in frigid waters in the Arctic. You grow these bacteria in the lab at −1°C and find that they grow well at this temperature. You then take two small samples of bacteria and put each in a different flask with bacterial growth medium. You grow the bacteria in one flask at −1°C (you call this the “−1°C strain”) and you grow the bacteria in the other flask at 25°C (you call this the “25°C strain”). The bacteria in both flasks grow well. After several months, you take 2,800 bacterial cells from the −1°C strain and put them in a new flask with growth medium. You take 2,800 bacterial cells from the 25°C strain and put them into another new flask with growth medium. This time, you grow both strains of bacteria at −1°C. Every few hours, you count the number of bacteria in each flask and plot the numbers on a graph. The data are shown below. (See Figure 1 for a schematic representation of the protocol.)

**Figure 1. Schematic Representation of the Research Protocol.**

**Figure 2. Graph Showing Growth of Two Bacterial Strains at -1°C**

- The 25°C strain did not grow at all. The −1°C strain grew faster or better than the 25°C strain at the −1°C temperature.
b. Which of the following is a plausible hypothesis that explains the data above? (Put an X on any of the hypotheses below that could be plausible.)

- The 25°C strain lost a gene that helped it grow at cold temperatures.
- The 25°C strain gained a gene that made it grow less well in cold temperatures.
- The −1°C strain lost a gene that helped it grow at cold temperatures.
- The −1°C strain gained a gene that made it grow less well in cold temperatures.

Note: You may want to use this question as the basis for a group discussion. Because this question is very open ended, a wide range of creative answers is possible. Important points to be included in any answer for full credit are

- an experimental design that tests the selected hypothesis in Question 10b;
- a description of data that the students would collect in their experiments, which must be able to test the selected hypothesis; and
- a prediction of what students may find out if the selected hypothesis is supported by the observations in their experiments.

Possible complete response: “I will sequence the lab strain of bacteria and look for differences between the sequence of the lab strain and the wild strain. Any differences I find between the sequences of the wild strain and the lab strain are support for my hypothesis that there was a gain/loss [students will probably say one or the other] of a gene that resulted in decreased survival in the cold.”

REFERENCES


AUTHOR

Laura Helft, PhD, Howard Hughes Medical Institute

FIELD TESTERS (QUIZ)

Kimberly Hayen, Heritage High School; Laurie Host, Harford Community College; Tina Larson, Marian High School; Dawn Norton, Minnetonka High School; Tamara Pennington, Windsor High School; Kimberly Snook, Haslett High School