

IN-DEPTH FILM GUIDE

DESCRIPTION

The bodies of threespine stickleback fish in certain populations evolved as they adapted from living in the ocean to a life exclusively in freshwater environments. One adaptation was the loss of pelvic spines, which are homologous to the hind limbs of four-legged animals. Scientists have determined that changes to the stickleback pelvis are largely due to changes in a particular gene regulatory switch, a stretch of noncoding DNA sequence that functions to turn genes on or off. Mutations in gene switches that control developmental genes are an important evolutionary mechanism responsible for the diversity of animal forms.

KEY CONCEPTS

- Dramatic changes in traits, such as the loss of limbs, can occur through mutations in a small number of genes or even a single gene.
- Different environments can provide different selective pressures on an organism's morphology. In the threespine stickleback, pelvic spines provide a selective advantage in environments with large predatory fish, but are a liability in environments with dragonfly larvae.
- All the cells of the body contain the same genes, but only a subset of those genes is active in any particular cell type. For example, liver cells express a different set of genes than muscle cells.
- The expression of genes that control development—the process by which a fertilized egg divides, grows, and develops into an adult—is regulated by genetic elements called switches. Switches determine at which times during development and in which cells or tissues a particular gene is turned on or off.
- Mutations that alter the function of a regulatory switch can affect when and where a gene is expressed without affecting that gene's protein product. Thus, a mutation in a switch that regulates the expression of the *Pitx1* gene in the pelvis will not affect the gene's expression and protein product in other parts of the body.
- Evolutionary change can occur rapidly from a geological perspective—typically measured in millions of years—if the environment strongly favors or disfavors a particular trait. In the case of freshwater stickleback populations in North America, the loss or reduction of pelvic spines has evolved within the past 10,000 years.
- Similar evolutionary adaptations can occur independently in different populations that have similar selective pressures, and in some cases may result from the same genetic mechanism. For example, pelvic reduction occurred in separate freshwater stickleback populations due to mutations in the same regulatory switch.
- The evolution of anatomical traits can be studied by carefully measuring and comparing related populations living in different habitats, by comparing the DNA of individuals with similar and different traits, and/or by analyzing the same trait in related species found in the fossil record. It is possible to use all three approaches to study the evolution of pelvic reduction in the threespine stickleback.

CURRICULUM AND TEXTBOOK CONNECTIONS

Text/Curriculum	Chapter Sections/Curriculum Topics
Miller & Levine Biology (2010 Ed.)	2.3, 13.3, 13.4, 14.1, 16.3, 16.4, 17.1, 17.2, 17.4, 30.3
Campbell Biology (9 th Ed.) ngss	5.2, 18.2, 21.6, 22.2, 23.1, 23.3, 23.4, 25.6, 26.5, 41.1, 41.3
NGSS	MS-LS3.B, MS-LS4.B, MS-LS4.C HS-LS1.A, HS-LS3.A, HS-LS3.B, HS-LS4.B, HS-LS4.C
AP (2012-13 Standards)	1.A.1, 1.A.2, 1.C.3, 2A2, 2D2, 2E1, 3B1, 3.C.1, 3.C.2, 4A1, 4C3
IB (2009 Standards)	3.2, 3.6, 4.1, 5.4, 6.1, A.3.1, D.3.10,

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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.

	Begin	End	Content description	Review Questions
1	0:00	4:00	<ul style="list-style-type: none"> After ice sheets receded 8-10 thousand years ago, the land rose, streams and lakes were cut off from the sea, and stickleback were trapped in the fresh water. The bodies of freshwater stickleback are smaller, with different coloring, and skeletal changes. Different environments can provide different selective pressures on an organism's morphology. Ocean stickleback have bony side plates and pelvic spines as protection from predators. In Bear Paw lake, pelvic spines reduce fitness and chances for reproduction because predators grab the spines; most fish have no pelvic spines. Pelvic spines are homologous to hind limbs of four-legged vertebrates. 	<ul style="list-style-type: none"> How do oceanic stickleback differ from freshwater stickleback in Bear Paw lake? To what are pelvic spines homologous?
2	4:01	10:45	<ul style="list-style-type: none"> Changes in the physical form of animals arise from changes in development, which are due to changes in genes. Geneticists use crosses and DNA markers to determine which genetic information is linked to a trait. <i>Pitx1</i> is a developmental control gene; no differences in the protein were found in the different stickleback populations. Regulatory genetic switches control the expression of developmental control genes. Switches determine at which times during development and in which cells or tissues a particular gene is turned on or off. To find a genetic switch, scientists track the expression of reporter proteins; reporter genes are attached to DNA fragments. A mutation in a switch causes the loss of the pelvic spine in different populations of stickleback. Fish without pelvic spines deleted the pelvic switch of the <i>Pitx1</i> gene. 	<ul style="list-style-type: none"> How do scientists look determine which gene causes a mutation? Is the loss of pelvic spines in stickleback due to a mutation in the coding region of the <i>Pitx1</i> gene? What are regulatory switches? Where was the mutation that caused the loss of pelvic spines? What type of mutation caused the loss of pelvic spines? Is the mutation good or bad?
3	10:46	15:27	<ul style="list-style-type: none"> Given the same selective conditions, evolutionary adaptations can occur independently in different populations Freshwater stickleback lacking pelvic spines – from lakes in different parts of the world – have the same mutation. The same adaptation – a lack of pelvic spines in freshwater stickleback – occurred 10 million years ago. Fossils of fish with a full pelvis were found at higher frequencies at different geologic times than fossils of fish without pelvic spines. Mutations in the switches that regulate developmental control genes are a common mechanism that drives the diversity of animal bodies. 	<ul style="list-style-type: none"> What is the same about many freshwater stickleback populations worldwide? Why did scientists learn by examining stickleback fossils?

BACKGROUND

In the wild, there are three types of threespine stickleback populations:

- marine—fish that live and breed strictly in the ocean;
- sea-run—anadromous fish, like salmon, that are born in fresh water, spend most of their lives in the ocean, and migrate back to fresh water to breed; and
- freshwater—fish that live and breed entirely in fresh water.

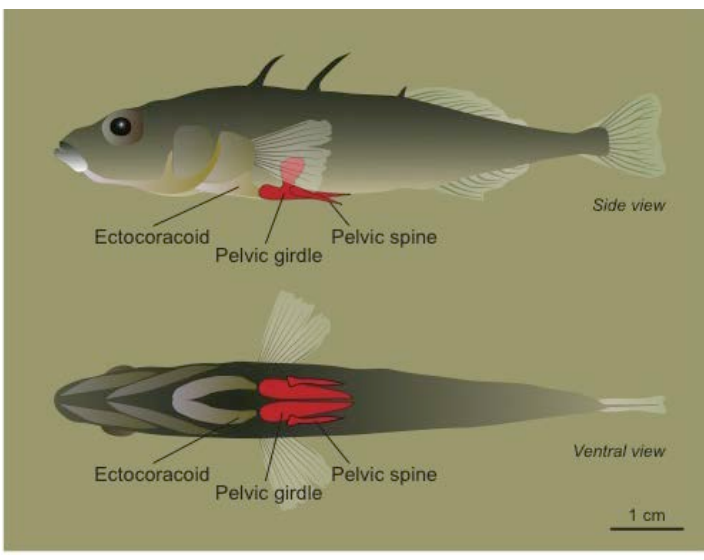
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Freshwater stickleback populations were established when some sea-run populations became trapped in lakes that formed at the end of the last ice age. These lakes were initially connected to the ocean, but were cut off as ice fields retreated.

In most cases, freshwater stickleback populations are strikingly different from sea-run and marine populations because they have adapted to life in freshwater environments. Sea-run and marine stickleback fish have strong armor defenses to protect them from large predatory fish that live in the ocean. These defenses include bony plates along the sides of their bodies and long spines projecting from their backs and pelvises. Some freshwater stickleback populations have reduced armor or none at all. In addition, freshwater stickleback fish have evolved to be slightly smaller and more streamlined than their sea-run ancestors, allowing them to swim faster and dart more easily into the many hiding places present in lakes. They also have modified gill function and an altered excretory system.

Although freshwater stickleback fish underwent many changes as they adapted to life in freshwater, researchers have focused on changes in the pelvic skeleton because this trait is highly variable and is easy to see and measure. In addition, its underlying genetic mechanisms are well studied.



The development of the pelvic girdle and spines appears to be controlled by a major gene called *Pitx1*. Evidence suggests that the function of this gene has been conserved throughout vertebrate evolution for millions of years. For example, a version of the *Pitx1* gene is involved in the formation of the pelvis and hind limbs (but not the forelimbs) of mice and possibly other four-legged vertebrates.

The pelvis is a skeletal structure that is well preserved in stickleback fossils, and there is a rich fossil record for this species. Studies of stickleback fossils have allowed researchers to observe the long-term pattern of evolution that has taken place over thousands of generations and to measure the rates of evolutionary change.

Changes in Regulatory Switches Lead to Major Changes in Body Form

The expression of many developmental genes is controlled by genetic elements called “regulatory switches.” A switch is typically located outside the protein-coding region of the gene it regulates. A single gene can be regulated by multiple switches. Each switch contains a specific DNA sequence that is recognized by a particular activator protein. The sequence-specific binding of an activator to a switch activates the expression of the gene. All cells in the body have the same genes and switches, but the kinds of activator proteins present can vary among cell types. Thus, the presence of multiple switches allows a particular gene to be on or off in different tissues and at different times, depending on which binding proteins are present.

A mutation in a switch can prevent the interaction between that switch and a binding protein. If the gene is not expressed in a particular cell, that cell may never develop into the correct cell type during development. For example, in the case of the threespine stickleback, a deletion of the switch responsible for the expression of the *Pitx1* gene in cells of the developing pelvis prevents *Pitx1* from being expressed in those cells and the pelvis does not develop normally.

A mutation in a switch can have different effects on the organism than a mutation in the coding region of the gene regulated by that switch. For example, studies in mice have shown that a mutation in the coding region of *Pitx1* is lethal to the organism. (The mice die before birth.) A mutation in the coding region of the *Pitx1* gene affects *Pitx1* expression in all the body parts where it is normally expressed, some of which are critical to survival. However, a mutation in the pelvic

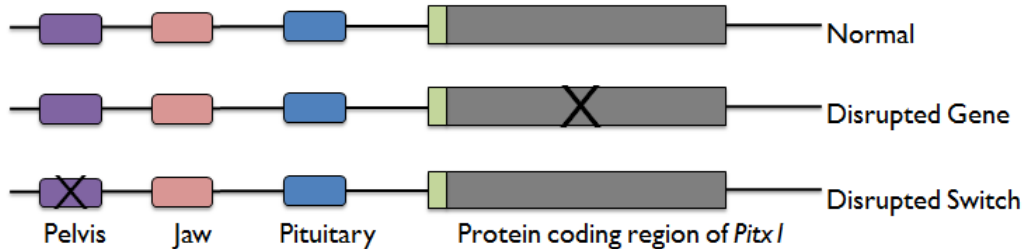
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switch of *Pitx1* has a dramatic effect on only one body part, the pelvis, which is not essential to the survival of the threespine stickleback. Mutations in regulatory switches have been found to play key roles in many traits, from the color of butterfly wings to the development of stripes in zebras.

DISCUSSION POINTS

- Emphasize that mutations occur at random, whether they occur in protein-coding regions of genes or regulatory switches. Natural selection, however, is not random; mutations that produce organisms that are more likely to survive and reproduce in a particular environment are more likely to become common in a population over many generations. The strength of selective pressure acting on a population affects how quickly a particular genetic variant, and its associated trait (phenotype), increases in frequency in a population.
- Students may believe that stickleback fish lose their spines the way some reptiles lose their tails to escape predators. In other words, if you put a marine stickleback in freshwater it will start to change, by losing its pelvic spines. This is a misconception. The loss of pelvic spines is an evolutionary adaptation that occurs after many generations. Evolution should not be confused with physiological change, which is the ability of individual organisms to adapt to a new environment during their lifetimes.
- In the film Dr. Kingsley explains that the same mutation in the *Pitx1* switch has occurred in freshwater populations all over the world. This does not mean that the exact same deletion (i.e., the same nucleotides) has occurred in all of these populations. These populations have differently sized deletions. However, all the deletions inactivate the switch responsible for *Pitx1* gene expression in the pelvis, which means that these deletions are *functionally* the same.
- The film discusses several categories of evidence that support the conclusion that stickleback fish have evolved as they adapted to freshwater environments. Dr. Bell provides evidence from field studies and fossils and Dr. Kingsley describes genetic evidence. Discuss how these categories of evidence complement one another. Ask students what evidence shows that a genetic switch has changed as populations adapted to freshwater environments and that this type of change has happened repeatedly at different times and in different geographical locations. Data obtained by analyzing living fish in lakes show the phenotypic consequences of this genetic change. Ask students what is the evidence for natural selection acting on the pelvic phenotype. The fossil data show a pattern of evolution over long stretches of time, over 100,000 years. Ask students why long-term evidence of evolutionary change is not readily accessible by studying only living populations.
- Explain how to read a gene diagram like the one depicted below. In this example, the straight line running all the way across represents a segment of the stickleback genome. The protein-coding region of a gene is symbolized by the gray rectangle. (In some diagrams, a string of rectangles represents exons, and the space between the rectangles represents introns.) The green rectangle indicates the promoter region, where transcription starts. The purple, pink, and blue rectangles represent regulatory switches. If one switch is disrupted, for example, the pelvic switch, as shown in the bottom diagram, the protein will continue to be expressed normally in the jaw and pituitary but not the pelvis. However, if the protein-coding region is disrupted, as shown in the middle diagram, the protein will not be functional in any location, even though the switches are still functional.





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CLASSROOM RESOURCES FOR THE FILM

Stickleback Evolution Lab (<http://www.hhmi.org/biointeractive/stickleback-evolution-virtual-lab>)

This virtual laboratory teaches skills of data collection and analysis to study evolutionary processes. Students work with real fish specimens as they learn how to collect, graph, and analyze data. In analyzing these data, students learn that different environments select for different traits. They also learn that changes occur over time by analyzing fossil specimens.

Modeling the Regulatory Switches of the *Pitx1* Gene in Stickleback Fish

(<http://www.hhmi.org/biointeractive/modeling-regulatory-switches-pitx1-gene-stickleback-fish>)

This hands-on activity helps students interpret molecular diagrams and build physical models of eukaryotic gene regulation. Students will review eukaryotic development and learn how those change with the appropriate selective pressure play a role in the evolution of a population.

Using Genetics Crosses to Analyze a Stickleback Trait (<http://www.hhmi.org/biointeractive/using-genetic-crosses-analyze-stickleback-trait>)

In this hands-on activity students apply the principles of Mendelian genetics to analyze the results of genetic crosses between stickleback fish with different traits.

OTHER BIOINTERACTIVE RESOURCES

CLICK AND LEARNS

Visualizing Gene-Expression Patterns

(http://www.hhmi.org/biointeractive/evolution/Visualizing_Gene_Expression/01.html)

Teaches different techniques that scientists use to visualize the expression patterns of genes. Features clips from the 2005 Holiday Lectures on Science: Evolution.

Gene Switches (http://www.hhmi.org/biointeractive/evolution/Gene_Switches/01.html)

Illustrates two examples of how a change in gene switches influences traits. Features clips from the 2005 Holiday Lectures on Science: Evolution.

ANIMATIONS

Regulation of Eukaryotic DNA Transcription

(http://www.hhmi.org/biointeractive/evolution/dna_transcription_regulation.html)

Demonstrates the mechanisms of transcriptional control in eukaryotes.

Gene Switch (http://www.hhmi.org/biointeractive/evolution/gene_switch.html)

Explains how gene switches function. Some regions of a DNA molecule, called gene switches, do not code for protein but are important for regulating when and where genes are turned on. Regulatory proteins bind these switch regions and affect the expression of these genes.

Paintbrush Gene (http://www.hhmi.org/biointeractive/evolution/paintbrush_gene.html)

Shows another great example of how mutations in regulatory regions in DNA can affect changes in body form. Dr. Carroll demonstrates how in two related *Drosophila* species, the so-called paintbrush gene is activated to paint the pigment of the body. In one species, an extra switch activates the gene in another region of the body, resulting in spotted wings.

VIDEO CLIPS

Fruit Fly Courtship (http://www.hhmi.org/biointeractive/evolution/fly_courtship.html)

Demonstrates the importance of the spotted wing in fly courtship dances.

Stickleback Fossil Primer (<http://www.hhmi.org/biointeractive/evolution/sticklebackfossil.html>)



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Demonstrates how stickleback fossils are collected and used to study evolution. It also shows the students who attended the 2005 Holiday Lectures taking part in a fossil-collecting activity.

Stickleback Environment (http://www.hhmi.org/biointeractive/evolution/stickleback_environment.html)

Dr. David Kingsley explains how Earth has changed in the 10,000 years since the last ice age and how these changes have impacted the evolution of stickleback fish. This video shows stickleback fish in their natural environment and some of the predators they may run into.

Stratigraphic Principles (<http://www.hhmi.org/biointeractive/evolution/stratigraphy.html>)

Dr. John Shea uses a clever demonstration to illustrate the principle that younger rocks are farther up in a stratigraphic rock layer than older rocks and that fossils found in the same layer are of the same relative age.

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. Moreover, because the vocabulary and concepts are complex, teachers are encouraged to modify the quiz (e.g. only ask some of the questions, explain complicated vocabulary for ELL students) as needed. The last two questions are intended for students with prior knowledge about mutations, genes expression, and development.

QUIZ QUESTIONS AND ANSWERS

1) (Key Concept B) Evaluate the following two statements as true or false.. Justify your answer in one or two sentences.

a) "Having pelvic spines is always advantageous to a stickleback."

FALSE. Pelvic spines provide a selective advantage in some environments but not others. One factor that appears to favor stickleback fish with pelvic spines is the presence of large-mouthed predatory fish, such as trout. Pelvic spines provide stickleback fish with a defense against such predators; however, pelvic spines appear to be a disadvantage in habitats without large-mouthed predatory fish and/or habitats with insects that can grab the spines, so that they can eat the fish. Students may mention other factors in the environment that could influence the presence or absence of pelvic spines.

b) "All mutations are bad."

FALSE. Whether a mutation is advantageous (favored), disadvantageous (not favored), or neutral (neither favored or not favored) depends first on whether the mutation affects a trait in the organism and next on whether that particular trait is selected for or against. The nature of the selective pressure depends on the environment the organism lives in. Some mutations that alter a trait, such as the loss of pelvic spines in freshwater stickleback fish, are advantageous in some freshwater environments but not all.

2) (Key Concept B) What specific features of the environment in Bear Paw Lake, Alaska, may have favored the survival and reproduction of stickleback fish with reduced pelvises? Answer in two or three sentences.

The lack of large-mouthed predatory fish in Bear Paw Lake meant that stickleback fish both with and without pelvic spines could survive. Insects that live in Bear Paw Lake can grab stickleback spines to catch the fish and eat them, which may have favored the survival of stickleback fish with reduced or no pelvic spines. Although this is not mentioned in the film, students may note that more vegetation in freshwater lakes could also favor fish with less armor, as they could more easily dart into hiding places.

3) (Key Concept G) In the film, Dr. Bell notes that in multiple freshwater populations and at multiple times in history, the frequency of stickleback fish with pelvic spines decreased and the frequency of stickleback fish with reduced pelvises increased. This demonstrates that:

a) only fish with smaller pelvises migrate to freshwater.

b) similar environments select for similar genetic changes.

c) the pelvis of the marine fish is destroyed by freshwater.

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4) (Key Concepts A, B, and F) Circle whether each statement below is true or false.

True/**False** Evolutionary change always takes millions of years.

True/**False** Stickleback fish lose their pelvic spines whenever they are in fresh water.

True/False Dramatic changes in traits, such as the loss of limbs, can occur through mutations affecting a single gene.

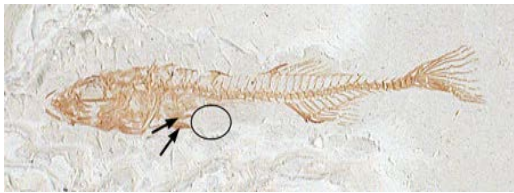
5) (Key Concept B) During a high-flood season, a lake overflows its banks and some resident freshwater stickleback fish are carried out to the ocean. For each of the following statements, circle the outcome most likely to occur.

When they reach salt water, individual fish will **will not** spontaneously generate pelvic spines during their lifetime.

More fish with **without** pelvic spines will be eaten by large-mouthed predators.

Over many generations, the frequency of stickleback fish with pelvic spines will **increase**/decrease in populations that descended from the original one that spilled out from the lake.

6) You are studying stickleback fossils from a newly discovered desert deposit site that was once the bottom of an ancient lake. Sediment, mineral, and topographical analysis indicate that when the lake first formed it was connected to the ocean by a river that eventually dried up, cutting the lake off from the ocean. As you sort through different rock layers in this desert deposit, you find two distinct types of stickleback fish fossils—those with no pelvic spines and those with full spines. You don't find any fossils of large predatory fish in this deposit.

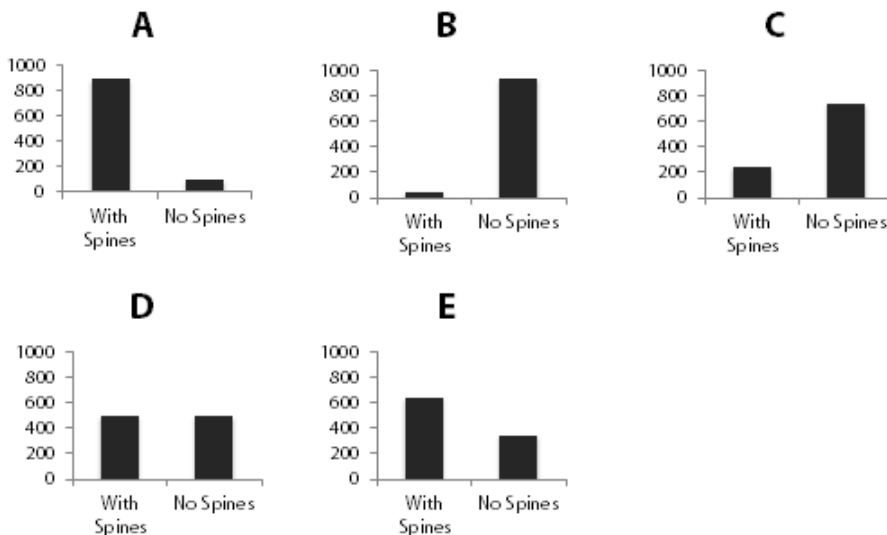


Fossil stickleback without pelvic spines. (The circle shows the region where you would expect to see the pelvis and pelvic spines; the arrows point to the ectocoracoid bone, which is not part of the pelvis.)



Fossil stickleback with a full pelvis, which includes pelvic spines.

The data below were collected from five different rock layers (labeled A to E) in the desert deposit. Each graph shows the number of fish belonging to each category. Place the rock layers in the most reasonable order from oldest to most recent. Justify your answer in two or three sentences.

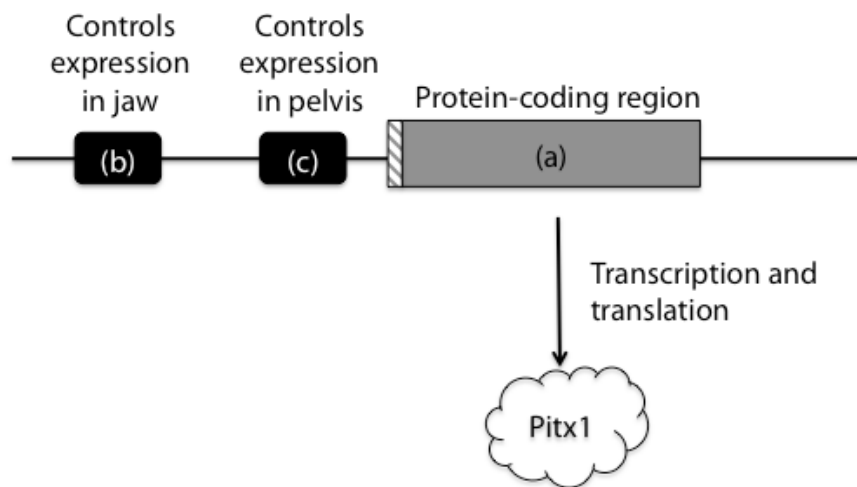


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Oldest A, E, D, C, B Most recent

Because the lake was once connected to the ocean, it is reasonable to assume that most of the oldest stickleback fish had pelvic spines, which is the trait you would find in marine stickleback fish. When the stickleback population became isolated in the freshwater lake, fish without spines had a selective advantage, whereas those with full spines were at a disadvantage. This is a reasonable assumption because the ancient lake probably had no large predatory fish. Over many generations, the number of fish without pelvic spines increased. (Because of the open-ended nature of this question, students may provide different explanations, which may be just as plausible as the one above.)

7) Below is a simplified schematic representation of some of the genetic elements involved in the regulation of Pitx1 gene expression. The large grey rectangle represents the protein-coding region of the Pitx1 gene. The smaller rectangles to the left (b and c) are regulatory switches; each one allows a specific binding protein to interact with it and turn on the Pitx1 gene in a particular tissue. When expressed, the Pitx1 gene is transcribed and then translated to generate the Pitx1 protein.



a) A deletion at location (a) causes a frameshift mutation. How likely is it that a functional Pitx1 protein would be produced in the jaw? Not likely in the pelvis? Not likely. Justify your answer in one or two sentences.

A frameshift mutation in the protein-coding region of the Pitx1 gene will affect the amino acid sequence of the protein in all cell types. This type of mutation will likely yield a nonfunctional protein in all cell types.

a) If the entire region (b) was deleted, would you see a functional Pitx1 protein in the jaw? No in the pelvis? Yes. Justify your answer in one or two sentences.

The switch at (b) will only affect Pitx1 expression in the jaw. Because the protein-coding region is unchanged, the active switch at (c) will still allow the protein to be produced in the pelvis.

8) Scientists working in a different field and in a different organism (mice) first discovered the function of the Pitx1 gene. To determine what the Pitx1 gene did, these scientists intentionally mutated the Pitx1 protein-coding region in mice so that the Pitx1 protein was no longer produced. These mice died before birth and had miniaturized hind limbs.



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- a) *What do you think these scientists concluded about the function of the Pitx1 protein after carrying out this experiment?*
Because the mice lacking a functional Pitx1 protein had miniaturized hind limbs, Pitx1 appears to play a function in hind limb development in mice. In addition, since the mice died before birth, the protein probably plays additional functions that are critical to survival.
- b) *How is the genetic change that occurred in stickleback fish with reduced pelvises in freshwater lakes different from the one that scientists produced in the mice?*
In experiments in mice, scientists changed the protein-coding region of the gene so that it was no longer functional. The fish have a mutation in a genetic switch, but the protein-coding region of the gene is still intact.
- c) *Why do you think stickleback fish with a Pitx1 mutation are able to survive but not the mice?*
By knocking out the protein-coding region of the Pitx1 gene in mice, the gene can no longer be expressed in any cells of the organism. The Pitx1 protein is critical for the normal development of the pituitary gland and jaw. Because these vital structures cannot develop in mice without a functional Pitx1 gene, the animal dies. In stickleback fish, the Pitx1 protein is only missing in pelvic tissue; the protein is still present and functional in other cell types. Because a stickleback can develop normally without a pelvis, the fish survive and grow.

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