

Evolution and Medicine

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This material is based on work supported by the National Institutes of Health under Contract No. HHS263200800031C. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the funding agency.

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Foreword

Evolution and Medicine is the most recent addition to the National Institutes of Health (NIH) Curriculum Supplement Series. This series brings the latest medical science and research discoveries from NIH into the K–12 classroom. NIH plays a vital role in the health of all Americans and seeks to foster interest in research, science, and medicine-related careers for future generations. The NIH Office of Science Education is dedicated to promoting scientific literacy and the knowledge and skills we need to secure a healthy future for all.

Evolution and Medicine gives students an opportunity to grapple with some of the most challenging and engaging medical issues that confront our society. We designed *Evolution and Medicine* to complement existing life science curricula and to be consistent with *National Science Education Standards*. High school science teachers, medical experts, education specialists, scientists, representatives from NIH, and curriculum-design experts from Biological Sciences Curriculum Study (BSCS) created the activities. The collaborative development process includes geographically dispersed field tests by teachers and students.

The curriculum supplements enable teachers to facilitate learning and stimulate student interest by applying scientific concepts to real-life scenarios. Design elements emphasize key biology concepts and analytic methods, cutting-edge science content, and built-in assessment tools. Activities promote active and collaborative learning to help students develop problem-solving strategies and critical-thinking skills.

Each of our curriculum supplements comes with a complete set of printed materials for teachers, including extensive background and resource information, detailed lesson plans, and masters for student worksheets. The Web site accompanying *Evolution and Medicine* has interactive materials to support the lessons.

The supplements are distributed free to educators across the United States upon request. They may be copied for classroom use and educational purposes but may not be sold.

We welcome your comments. For a complete list of curriculum supplements and ordering information, or to submit feedback, visit <http://science.education.nih.gov> or write to Curriculum Supplement Series, Office of Science Education, National Institutes of Health, 6100 Executive Boulevard, Suite 3E01, Bethesda, MD 20892-7520 or supplements@science.education.nih.gov

The development of *Evolution and Medicine* is supported by 11 NIH institutes, centers, and offices: the National Institute of General Medical Sciences; the National Cancer Institute; the National Center for Research Resources; the National Eye Institute; the National Heart, Lung, and Blood Institute; the National Institute on Aging; the National Institute of Allergy and Infectious Diseases; the National Institute on Drug Abuse; the National Institute of Dental and Craniofacial Research; the National Institute of Neurological Disorders and Stroke; and the Office of the Director.

We appreciate the valuable contributions from the talented staff at BSCS. We are also grateful to the NIH scientists, advisors, and all other participating professionals for their work and dedication. Finally, we thank the teachers and students who participated in focus groups and field tests to ensure that these supplements are both engaging and effective. I hope you find our series a valuable addition to your classroom, and I wish you a productive school year.

Bruce A. Fuchs, Ph.D.
Director
Office of Science Education
National Institutes of Health

About the National Institutes of Health

Founded in 1887, NIH is the Federal focal point for health research in the United States. Today, it is one of the agencies in the Department of Health and Human Services. Its mission is science in pursuit of fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to extend healthy life and reduce the burdens of illness and disability. NIH works toward meeting the mission by providing leadership, direction, and grant support to programs designed to improve the health of the nation through research.

NIH's education programs contribute to ensuring the continued supply of well-trained

basic research and clinical investigators, as well as the myriad professionals in many allied disciplines who support the research enterprise. These efforts also help educate people about scientific results so that they can make informed decisions about their own—and the public's—health.

This curriculum supplement is one such education effort. It is a collaboration between NIH and Biological Sciences Curriculum Study.

For more about NIH, visit <http://www.nih.gov>.

About Biological Sciences Curriculum Study

Headquartered in Colorado Springs, Colorado, BSCS was founded in 1958 as a curriculum study committed to an evidence- and inquiry-based approach to science education. BSCS instructional materials and professional development services are based on current research about teaching and learning for all science classrooms, kindergarten through college.

BSCS's materials are extensively field-tested in diverse settings across the country and

evaluated for proven effectiveness. The BSCS 5E Instructional Model and inquiry are hallmarks of its materials, placing students at the center of their learning.

The BSCS mission is to transform science teaching and learning through research and development that strengthens learning environments and inspires a global community of scientifically literate citizens. BSCS is a 501(c)(3) nonprofit organization. For more information, please visit <http://www.bsccs.org>.

Introduction to *Evolution and Medicine*

There is no question that **evolution** is the major unifying concept in biology and that “nothing in biology makes sense except in the light of evolution” (Dobzhansky, 1973). The teaching and learning of evolution, however, remains a difficult challenge. High school students need invigorating experiences that help them develop a rich understanding of evolutionary principles, and these experiences need to show the relevance of evolution to everyday life. These experiences must also be thoroughly grounded in learning about the nature of science. The *Evolution and Medicine* curriculum supplement incorporates research-based pedagogical approaches to support teachers as they help students understand the fundamental concepts of this dynamic and exciting field of science.

The questions that evolutionary biologists ask overlap with many that medical researchers ask about human health. The burgeoning field of evolution and medicine uses the models and theory of evolutionary biology to inform medical and public health problems. Though insights from evolution can provide immediate assistance in some clinical situations, the leaders in the field suggest that evolution’s greatest benefits will be to provide a theoretical framework for understanding

- why organisms are vulnerable to disease,
- how infectious agents evolve, and
- how common ancestry helps scientists use the results from animal models to understand issues related to human health.

Two primary types of questions shape the field: questions about evolutionary processes, such as natural selection, and questions

about evolutionary history, or the patterns of evolution. Students investigate case studies that help them develop explanations of medical situations that involve evolutionary processes and patterns.

What Are the Objectives of the Supplement?

Evolution and Medicine has four main objectives: to help students in grades 9–12 understand

1. the importance of evolutionary comparisons for studying biomedical problems;
2. the role of evolution in diseases, including how evolution explains many aspects of humans’ susceptibility to disease and how the principles of natural selection apply to specific diseases or health-related conditions;
3. how evolution helps researchers and health workers better understand, prevent, and treat infectious diseases; and
4. the process of scientific inquiry through studying evolution and medicine.

The lessons help students sharpen their skills in observation, critical thinking, experimental design, and data analysis. They also make connections to other disciplines such as English, mathematics, and social science.

As the supplement achieves its objectives, it helps convey to students the purpose of scientific research. Students experience how science provides evidence that we can use to understand and treat human disease. Ongoing research affects how we understand the world around us and gives us the foundation for improving choices about our personal health and the health of our community.

Table 1. Correlation of *Evolution and Medicine* to High School Biology Topics

Topics	Lesson 1	Lesson 2	Lesson 3	Lesson 4	Lesson 5
Populations evolve over time.	✓	✓	✓	✓	✓
Natural selection is a powerful mechanism of evolution.	✓	✓	✓	✓	✓
Analyses of genetic sequences provide an important line of evidence for evolution.	✓	✓	✓	✓	✓
All living things on Earth are related by descent from common ancestors.	✓	✓	✓	✓	✓
The instructions for specifying the characteristics of an organism are carried in DNA, a large polymer formed from four subunits (A, G, C, and T).	✓	✓	✓	✓	✓
The genetic information that underlies heredity is encoded in genes.	✓	✓	✓	✓	✓
Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms.	✓	✓	✓	✓	✓
Complex multicellular organisms are formed as a highly organized arrangement of differentiated cells. This differentiation is regulated through the expression of different genes.	✓		✓		✓

Why Teach the Supplement?

High school life science classes offer an ideal setting for integrating many areas of student interest. In this supplement, students participate in activities that integrate inquiry, science, human health, mathematics, and science-technology-society relationships. The real-life context of the supplement's classroom lessons is engaging for students, and they can immediately apply what they learn to their lives.

What's in It for the Teacher?

Evolution and Medicine meets many of the needs of teachers in modern classrooms:

- The supplement meets science content, teaching, and assessment standards in the *National Science Education Standards*. It pays

particular attention to the standards on **scientific inquiry**.

- It is **integrated** with other subjects, drawing most heavily from science, social science, mathematics, and health.
- It has a Web-based **technology component** that includes interactive activities, tutorials, and simulations.

In addition, the supplement provides a means for **professional development**. Teachers can engage in new and different teaching practices like those described in this supplement without completely overhauling their entire program. In *Designing Professional Development for Teachers of Science and Mathematics*, S. Loucks-Horsley and coauthors (1998) write that supplements

such as *Evolution and Medicine* can “offer a window through which teachers can get a glimpse of what new teaching strategies look like in action.” By experiencing a short-term supplement like this one, teachers can “change how they think about teaching and embrace new approaches that stimulate students to problem solve, reason, investigate, and construct their own meaning for the content.” The use of supplemental material like *Evolution and Medicine* can encourage reflection and

discussion and stimulate teachers to improve their practices by focusing on student learning through inquiry.

A correlation of the supplement’s major concepts with the biology and scientific inquiry topics often included in the high school life science curricula is shown in Tables 1 and 2. We hope this information will help teachers make decisions about incorporating this material into the curriculum.

Table 2. Correlation of *Evolution and Medicine* to High School Scientific Inquiry Topics

Topics	Lesson 1	Lesson 2	Lesson 3	Lesson 4	Lesson 5
Design and conduct scientific investigations.		✓	✓	✓	
Use technology and mathematics to improve investigations and communications.		✓	✓	✓	
Formulate and revise scientific explanations and models using logic and evidence.	✓	✓	✓	✓	✓
Recognize and analyze alternative explanations and models.		✓	✓	✓	✓

Implementing the Supplement

We designed the five lessons in this supplement to be taught in sequence for approximately 10 days, assuming class periods of about 50 minutes. The following pages offer general suggestions about using these materials in the classroom; you will find specific suggestions in the procedures of each lesson.

What Are the Goals of the Supplement?

Evolution and Medicine is designed to help students attain these major goals associated with scientific literacy:

- to understand a set of basic scientific principles related to evolution and how evolution relates to medicine,
- to experience the process of scientific inquiry and develop an enhanced understanding of the nature and methods of science, and
- to recognize the role of science in society and the relationship between basic research and human health.

What Are the Science Concepts and How Are They Connected?

The lessons are organized into a conceptual framework that allows students to start with what they already know about evolution, some of which may be incorrect. They then move to a scientific perspective on evolution and its importance to medicine and to their lives.

In Lesson 1, students begin by considering their initial thoughts about how methicillin-resistant *Staphylococcus aureus*, or MRSA, evolved antibiotic resistance. They next consider their ideas about how common ancestry helps explain the use of model systems for medical research. Students then explore the frequency of lactase persistence in different groups of people around the world and compare two alternative hypotheses for the evolution of this trait (Lesson 2). By conducting two case studies in Lesson 3, students explain how studies of both evolutionary processes (such as natural selection) and evolutionary patterns (such as changes in genetic sequences) inform medicine. In Lesson 4, students use what they learned about evolution and how it affects medicine to better understand influenza. The main question that drives the lesson is, Why is a new flu vaccine needed every few years?

Lesson 5, the final lesson, gives students an opportunity to consider what they have learned in the previous lessons. Students review an article that a fictional student prepared for the school newspaper about how humans and other species lack the ability to synthesize vitamin C. The task is to identify—and then correct—errors in the article. The lesson concludes with students writing a summary of how evolution informs medicine. The following chart (Table 3) illustrates the science content and conceptual flow of the lessons.

Table 3. Science Content and Conceptual Flow of the Lessons

Lesson	Learning Focus, from BSCS 5E Instructional Model	Major Concepts
Lesson 1— Ideas about the Role of Evolution in Medicine	Engage	Understanding mechanisms of evolution, particularly adaptation by natural selection, provides many insights that enhance medical practice and understanding. Common ancestry explains why experiments in model systems inform human health. Students may have naïve preconceptions about how organisms change over time and about common ancestry.
Lesson 2— Investigating Lactose Intolerance and Evolution	Explore	Some of the variation among humans that may affect health is distributed geographically. Natural selection helps explain some of these patterns. Scientists use data to evaluate evidence for claims about evolution.
Lesson 3— Evolutionary Processes and Patterns Inform Medicine	Explain	Human health and disease are related to our evolutionary history. Understanding evolution helps explain why some diseases are more common in certain parts of the world. Common ancestry explains why information about other organisms is useful for studying health-related issues in humans. Rates of evolutionary change in genetic sequences give clues about the role of natural selection on that genetic region. Scientists use data to evaluate evidence for claims about evolution.
Lesson 4— Using Evolution to Understand Influenza	Elaborate	We can compare genetic sequences; the rates of evolutionary change in them give clues about the role of natural selection in that genetic region, which informs medical scientists. Understanding evolution helps explain the emergence and spread of infectious diseases. Scientists use data to evaluate evidence for claims about evolution.
Lesson 5— Evaluating Evolutionary Explanations	Evaluate	Interpreting examples of evolution and medicine requires careful attention to evidence. Natural selection and common ancestry help explain why humans are susceptible to many diseases.

How Does the Supplement Correlate to the *National Science Education Standards*?

Evolution and Medicine supports teachers in their efforts to reform science education in the spirit of the National Research

Council’s 1996 *National Science Education Standards* (NSES). The content of the supplement is explicitly standards based. The following chart (Table 4) lists the specific content standards that this supplement addresses.

Table 4. Alignment of *Evolution and Medicine* Lessons with National Science Education Standards for Content, Grades 9–12

Table 4a. NSES Standard A, Science as Inquiry

As a result of activities in grades 9–12, all students should develop	Correlation to <i>Evolution and Medicine</i> Lessons
Abilities necessary to do scientific inquiry	All
<ul style="list-style-type: none"> • Identify questions and concepts that guide scientific investigations. 	1, 2, 3, 4
<ul style="list-style-type: none"> • Design and conduct scientific investigations. 	2, 3, 4
<ul style="list-style-type: none"> • Use technology and mathematics to improve investigations and communications. 	2, 3, 4
<ul style="list-style-type: none"> • Formulate and revise scientific explanations and models using logic and evidence. 	All
<ul style="list-style-type: none"> • Recognize and analyze alternative explanations and models. 	2, 3, 5
<ul style="list-style-type: none"> • Communicate and defend a scientific argument. 	2, 3, 4, 5
Understandings about scientific inquiry	All
<ul style="list-style-type: none"> • Scientists usually inquire about how physical, living, or designed systems function. Conceptual principles and knowledge guide scientific inquiries. Historical and current scientific knowledge influence the design and interpretation of investigations and the evaluation of proposed explanations made by other scientists. 	All
<ul style="list-style-type: none"> • Scientists conduct investigations for a wide variety of reasons. For example, they may wish to discover new aspects of the natural world, explain recently observed phenomena, or test the conclusions of prior investigations or the predictions of current theories. 	2, 3, 4, 5
<ul style="list-style-type: none"> • Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used. 	3, 4
<ul style="list-style-type: none"> • Mathematics is essential in scientific inquiry. Mathematical tools and models guide and improve the posing of questions, gathering data, constructing explanations and communicating results. 	3, 4
<ul style="list-style-type: none"> • Scientific explanations must adhere to criteria such as: a proposed explanation must be logically consistent; it must abide by the rules of evidence; it must be open to questions and possible modification; and it must be based on historical and current scientific knowledge. 	2, 3, 4, 5
<ul style="list-style-type: none"> • Results of scientific inquiry—new knowledge and methods—emerge from different types of investigations and public communication among scientists. In communicating and defending the results of scientific inquiry, arguments must be logical and demonstrate connections between natural phenomena, investigations, and the historical body of scientific knowledge. In addition, the methods and procedures that scientists used to obtain evidence must be clearly reported to enhance opportunities for further investigation. 	2, 3, 4, 5

Table 4b. NSES Standards C and F, Life Science and Science in Personal and Social Perspectives

As a result of activities in grades 9–12, all students should develop understanding of	Correlation to <i>Evolution and Medicine</i> Lessons
Standard C. Biological Evolution	All
<ul style="list-style-type: none"> Species evolve over time. Evolution is the consequence of the interactions of (1) the potential for a species to increase its numbers, (2) the genetic variability of offspring due to mutation and recombination of genes, (3) a finite supply of the resources required for life, and (4) the ensuing selection by the environment of those offspring better able to survive and leave offspring. 	All
<ul style="list-style-type: none"> Natural selection and its evolutionary consequences provide a scientific explanation for the fossil record of ancient life forms, as well as for the striking molecular similarities observed among the diverse species of living organisms. 	2, 3, 4, 5
<ul style="list-style-type: none"> The millions of different species of plants, animals, and microorganisms that live on earth today are related by descent from common ancestors. 	All
Standard C. The Molecular Basis of Heredity	2, 3, 4, 5
<ul style="list-style-type: none"> In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome. 	2, 3, 4, 5
<ul style="list-style-type: none"> Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism’s offspring. 	2, 3, 4, 5
Standard C. The Cell	1, 2, 3, 5
Cells can differentiate, and complex multicellular organisms are formed as a highly organized arrangement of differentiated cells. In the development of these multicellular organisms, the progeny from a single cell form an embryo in which the cells multiply and differentiate to form the many specialized cells, tissues and organs that comprise the final organism. This differentiation is regulated through the expression of different genes.	1, 2, 3, 5
Standard F. Personal and Community Health	2, 3, 4, 5
The severity of disease symptoms is dependent on many factors, such as human resistance and the virulence of the disease-producing organism. Many diseases can be prevented, controlled, or cured. Some diseases, such as cancer, result from specific body dysfunctions and cannot be transmitted.	2, 3, 4, 5

Teaching Standards

The suggested classroom strategies in all the lessons support educators as they work to meet the teaching standards outlined in the *National Science Education Standards* (National Research Council (NRC), 1996). The supplement helps science teachers plan an inquiry-based program by providing short-term objectives for students. It also includes planning tools such as the Science Content and Conceptual Flow of the Lessons chart (Table 3) and a suggested timeline for teaching the supplement (page 18). Teachers can use the supplement to update their curriculum in response to their students' interest in this topic. The focus on active, collaborative, and inquiry-based learning helps teachers support the development of student understandings and nurture a community of science learners.

The structure of the lessons enables teachers to guide and facilitate learning. All the activities encourage and support student inquiry, promote discourse among students, and challenge students to accept and share responsibility for their learning. Using the BSCS 5E Instructional Model, combined with active, collaborative learning, allows teachers to respond effectively to the diversity of student backgrounds and learning styles. The supplement is fully annotated, with suggestions for how teachers can encourage and model the skills of scientific inquiry, as well as foster the curiosity, skepticism, and openness to new ideas and data that characterize the successful study of science.

Assessment Standards

Teachers can engage in ongoing assessment of their teaching and of student learning by using the assessment components embedded in each lesson. The assessment tasks are authentic; they are similar in form to tasks that students will engage in outside the classroom or that scientists do. Annotations guide teachers to these opportunities for assessment and provide

answers to questions that can help teachers analyze students' feedback. The assessments include one or more of the following strategies:

- performance-based activities, such as developing graphs or participating in a discussion of health effects or social policies;
- oral presentations to the class, such as reporting experimental results; and
- written assignments, such as answering questions or writing about demonstrations.

How Does the BSCS 5E Instructional Model Promote Active, Collaborative, Inquiry-Based Learning?

The lessons in this supplement use a research-based pedagogical approach called the BSCS 5E Instructional Model, or the BSCS 5Es. The BSCS 5Es are based on a **constructivist** theory of learning. A key premise of this theory is that students are active thinkers who build (or construct) their own understanding of concepts out of interactions with phenomena, the environment, and other individuals. A constructivist view of science learning recognizes that students need time to

- express their current thinking;
- interact with objects, organisms, substances, and equipment to develop a range of experiences on which to base their thinking;
- reflect on their thinking by writing and expressing themselves and comparing what they think with what others think; and
- make connections between their learning experiences and the real world.

The three key findings related to student learning identified in *How People Learn* (Bransford et al., 2000), a comprehensive review of research on learning, support the pedagogical strategies promoted by implementing the BSCS 5Es:

- Students enter class with a variety of preconceptions that may later significantly interfere with learning if those preconceptions are not engaged and addressed.
- To develop competence in a given subject, students must build a strong foundation of

factual knowledge within the context of a coherent conceptual framework.

- Students benefit from a metacognitive approach to learning that emphasizes goal setting and self-monitoring.

The BSCS 5Es sequence the learning experiences so that students can construct their own understanding of a science concept over time. The model leads students through five phases of active learning that are easily described using words that begin with the letter *E*: Engage, Explore, Explain, Elaborate, and Evaluate. Rather than just listening and reading, students are also analyzing and evaluating evidence, experiencing, and talking with their peers in ways that promote the development and understanding of key science concepts. These inquiry-based experiences include both direct experimentation and development of explanations through critical and logical thinking. Students often use technology to gather evidence, and mathematics to develop models or explanations.

The BSCS 5Es emphasize student-centered teaching practices. Students participate in their learning in ways that are different from those seen in a traditional classroom. Tables 5 and 6 exemplify what teachers do and what students do in the BSCS 5E Instructional Model.

The following paragraphs illustrate how we implemented the BSCS 5Es in *Evolution and Medicine*.

Engage

Students come to learning situations with prior knowledge. The Engage lesson gives you the chance to find out what students think about evolution.

The Engage phase of this supplement (in Lesson 1) is designed to

- pique students' curiosity and generate interest in natural selection and common ancestry;
- determine students' current understandings about natural selection and common ancestry;
- encourage students to compare their own thinking about natural selection and common ancestry with that of others; and

- give you a chance to hear or read about students' current conceptions of natural selection and common ancestry, which you can address in the later lessons.

Explore

In the Explore phase of the supplement (Lesson 2), we challenge students to make sense of patterns of lactase persistence around the world. Using an interactive map that shows lactase persistence in Africa, Asia, and Europe, students explore patterns of different variables. They then use actual data from scientific research to compare two alternative hypotheses for the evolution of lactase persistence. Students will reflect and improve on their preliminary explanations after further experiences in Lesson 3. Lesson 2 allows students to express their developing understandings of evolution and medicine through analyzing and comparing data, analyzing alternative explanations, and answering questions.

Explain

The Explain phase provides opportunities for students to connect their previous experiences and formulate explanations about case studies that deal with natural selection and common ancestry. It also allows you to introduce formal language, scientific terms, and content information that might make students' previous experiences easier to describe and explain.

In the Explain phase (Lesson 3), students participate in two case studies. In the first one, they diagnose patients with a mystery disease and then develop an explanation, based on natural selection, for the frequency of the disease in certain parts of the world. In the second case study, students develop an explanation for the conservation of genetic sequences across different organisms by using a combination of natural selection and common ancestry. Students

- explain, in their own words, concepts and ideas about evolution and medicine;
- listen to and compare others' explanations of the results with their own;

Table 5. Understanding the BSCS 5E Instructional Model: What the Teacher Does

Phase	<i>Consistent with the BSCS 5E Instructional Model</i>	<i>Inconsistent with the BSCS 5E Instructional Model</i>
Engage	<ul style="list-style-type: none"> • Piques students’ curiosity and generates interest • Determines students’ current understanding (prior knowledge) of a concept or idea • Invites students to express what they think • Invites students to raise their own questions 	<ul style="list-style-type: none"> • Introduces vocabulary • Explains concepts • Provides definitions and answers • Provides closure • Discourages students’ ideas and questions
Explore	<ul style="list-style-type: none"> • Encourages student-to-student interaction • Observes and listens to the students as they interact • Asks probing questions to help students make sense of their experiences • Provides time for students to puzzle through problems 	<ul style="list-style-type: none"> • Provides answers • Proceeds too rapidly for students to make sense of their experiences • Provides closure • Tells the students that they are wrong • Gives information and facts that solve the problem • Leads the students step-by-step to a solution
Explain	<ul style="list-style-type: none"> • Encourages students to use their common experiences and data from the Engage and Explore lessons to develop explanations • Asks questions that help students express understanding and explanations • Requests justification (evidence) for students’ explanations • Provides time for students to compare their ideas with those of others and perhaps to revise their thinking • Introduces terminology and alternative explanations after students express their ideas 	<ul style="list-style-type: none"> • Neglects to solicit students’ explanations • Ignores data and information students gathered from previous lessons • Dismisses students’ ideas • Accepts explanations that are not supported by evidence • Introduces unrelated concepts or skills
Elaborate	<ul style="list-style-type: none"> • Focuses students’ attention on conceptual connections between new and previous experiences • Encourages students to use what they have learned to explain a new event or idea • Reinforces students’ use of scientific terms and descriptions previously introduced • Asks questions that help students draw reasonable conclusions from evidence and data 	<ul style="list-style-type: none"> • Neglects to help students connect new and former experiences • Provides definitive answers • Tells students that they are wrong • Leads students step-by-step to a solution
Evaluate	<ul style="list-style-type: none"> • Observes and records as students demonstrate their understanding of concept(s) and performance of skills • Provides time for students to compare their ideas with those of others and perhaps to revise their thinking • Interviews students as a means of assessing their developing understanding • Encourages students to assess their own progress 	<ul style="list-style-type: none"> • Tests vocabulary words, terms, and isolated facts • Introduces new ideas or concepts • Creates ambiguity • Promotes open-ended discussion unrelated to the concept or skill

Table 6. Understanding the BSCS 5E Instructional Model: What the Students Do

Phase	<i>Consistent with the BSCS 5E Instructional Model</i>	<i>Inconsistent with the BSCS 5E Instructional Model</i>
Engage	<ul style="list-style-type: none"> • Become interested in and curious about the concept/topic • Express current understanding of a concept or idea • Raise questions, such as, What do I already know about this? What do I want to know about this? How could I find out? 	<ul style="list-style-type: none"> • Ask for the “right” answer • Offer the “right” answer • Insist on answers or explanations • Seek closure
Explore	<ul style="list-style-type: none"> • Use materials and ideas • Conduct investigations in which they observe, describe, and record data • Try different ways to solve a problem or answer a question • Acquire a common set of experiences so they can compare results and ideas • Compare their ideas with those of others 	<ul style="list-style-type: none"> • Let others do the thinking and exploring (passive involvement) • Work quietly with little or no interaction with others (only appropriate when exploring ideas or feelings) • Stop with one solution • Demand or seek closure
Explain	<ul style="list-style-type: none"> • Explain concepts and ideas in their own words • Base their explanations on evidence acquired during previous investigations • Record their ideas and current understanding • Reflect on and perhaps revise their ideas • Express their ideas using appropriate scientific language • Compare their ideas with what scientists know and understand 	<ul style="list-style-type: none"> • Propose explanations from “thin air” with no relationship to previous experiences • Bring up irrelevant experiences and examples • Accept explanations without justification • Ignore or dismiss other plausible explanations • Propose explanations without evidence to support their ideas
Elaborate	<ul style="list-style-type: none"> • Make conceptual connections between new and former experiences • Use what they have learned to explain a new object, event, organism, or idea • Use scientific terms and descriptions • Draw reasonable conclusions from evidence and data • Communicate their understanding to others • Demonstrate what they understand about the concept(s) and how well they can implement a skill 	<ul style="list-style-type: none"> • Ignore previous information or evidence • Draw conclusions from “thin air” • Use terminology inappropriately and without understanding
Evaluate	<ul style="list-style-type: none"> • Compare their current thinking with that of others and perhaps revise their ideas • Assess their own progress by comparing their current understanding with their prior knowledge • Ask new questions that take them deeper into a concept or topic area 	<ul style="list-style-type: none"> • Disregard evidence or previously accepted explanations in drawing conclusions • Offer only yes-or-no answers or memorized definitions or explanations as answers • Fail to express satisfactory explanations in their own words • Introduce new, irrelevant topics

- become involved in student-to-student discourse in which they explain their thinking to others and debate their ideas;
- record their ideas and current understandings; and
- revise their ideas.

Elaborate

In the Elaborate lesson (Lesson 4), students make conceptual connections between new and previous experiences. They draw on their knowledge about natural selection and common ancestry to investigate why we need a new influenza vaccine every few years. In this lesson, students

- connect ideas and apply their understandings of natural selection and common ancestry to the study of influenza,
- use and understand scientific terms and descriptions accurately and in context,
- draw reasonable conclusions from evidence and data,
- add depth to their understandings of natural selection and common ancestry, and
- communicate to others how an understanding of evolution helps explain why a new influenza vaccine is needed every few years.

Evaluate

The Evaluate lesson is the final phase of the instructional model, but it only provides a “snapshot” of what students understand and how far they have come. In reality, the assessment of students’ conceptual understanding and ability to use skills begins with the Engage lesson and continues through each of the other phases. Combined with the students’ written work and performance of tasks throughout the supplement, however, the Evaluate lesson can be a summative assessment of what students know and can do.

The Evaluate lesson (Lesson 5) gives students a chance to demonstrate their understandings of natural selection and common ancestry. Students

- demonstrate what they understand about evolution and medicine by identifying and correcting misconceptions contained in a fictional article about vitamin C biosynthesis,

- share their current thinking with others, and
- assess their own progress by describing in detail one example about natural selection from the examples in the supplement.

What’s the Evidence for the Effectiveness of the BSCS 5E Instructional Model?

Support from educational research studies for teaching science as inquiry is growing (for example, Geier et al., 2008; Hickey et al., 1999; Lynch et al., 2005; and Minner et al., 2009). A 2007 study, published in the *Journal of Research in Science Teaching* (Wilson et al., 2010), is particularly relevant to the *Evolution and Medicine* supplement.

In 2007, with funding from NIH, BSCS conducted a randomized, controlled trial to assess the effectiveness of the BSCS 5Es. The study used an adaptation of the NIH supplement *Sleep, Sleep Disorders, and Biological Rhythms*, developed by BSCS in 2003 (NIH and BSCS, 2003). Sixty high school students and one teacher participated. The students were randomly assigned to the experimental or the control group. In the experimental group, the teacher used a version of the sleep supplement that was very closely aligned with the theoretical underpinnings of the BSCS 5Es. For the control group, the teacher used a set of lessons based on the science content of the sleep supplement but aligned with the most commonplace instructional strategies found in U.S. science classrooms (as documented by Weiss et al., 2003). Both groups had the same master teacher.

Students taught with the BSCS 5Es and an inquiry-based approach demonstrated significantly higher achievement for a range of important learning goals, especially when the results were adjusted for variance in pretest scores. The results were also consistent across time (both immediately after instruction and four weeks later). Improvements in student learning were particularly strong for measures of student reasoning and argumentation. The following chart (Table 7) highlights some of the study’s key findings. The results

of the experiment strongly support the effectiveness of teaching with the BSCS 5Es.

Evidence also suggests that the BSCS 5Es are effective in changing students' attitudes on important issues. In a research study conducted during the field test for the NIH curriculum supplement *The Science of Mental Illness* (NIH and BSCS, 2005), BSCS partnered with researchers at the University of Chicago and the National Institute of Mental Health. The study investigated whether a short-term educational experience would change students' attitudes about mental illness. The results showed that after completing the curriculum supplement, students stigmatized mental illness less than they had beforehand. The decrease in stigmatizing attitudes was statistically significant (Corrigan et al., 2007; Watson et al., 2004).

How Can Challenges to Teaching Evolution Be Handled in the Classroom?

Teachers sometimes feel pressure to avoid teaching evolution because some groups view the topic as controversial. These pressures can come from groups outside the school, parents, students, or even from teachers themselves. In fact, some teachers show clinically measurable levels of stress when asked to simply think about teaching evolution (Griffith and Brem, 2004). But you can make many preparations that will *help you teach evolution effectively and appropriately*.

First and foremost, it is important that you feel comfortable with your content knowledge. The Information about Evolution and Medicine section provides useful background information. Being aware of common misconceptions

Table 7. Differences in Performance of Students Receiving Inquiry-Based and Commonplace Instructional Approaches

Measure	Mean for Students Receiving Commonplace Teaching	Mean for Students Receiving Inquiry-Based Teaching	Effect Size
Total test score pretest (out of 74)	31.11	29.23	Not applicable
Total test score posttest	42.87	47.12	0.47
Reasoning pretest (fraction of responses at the highest level)	0.04	0.03	Not applicable
Reasoning posttest	0.14	0.27	0.68
Score for articulating a claim (out of 3)	1.58	1.84	0.58
Score for using evidence in an explanation (out of 3)	1.67	2.01	0.74
Score for using reasoning in an explanation (out of 3)	1.57	1.89	0.59

Source: C.D. Wilson et al. 2010. The relative effects and equity of inquiry-based and commonplace science teaching on students' knowledge, reasoning, and argumentation. *Journal of Research in Science Teaching*, 47(3), 276–301.

Note: Effect size is a convenient way of quantifying the amount of difference between two treatments. This study used the standardized mean difference (the difference in the means divided by the standard deviation, also known as Cohen's *d*). The posttest scores controlled for the variance in students' pretest scores. The reasoning posttest scores controlled for variance in students' reasoning pretest scores at the highest level.

about evolution is also important, so details on some of them are included in that section, too. For additional background on evolution and teaching evolution, consider the following resources:

- The University of California Museum of Paleontology's Web site, Understanding Evolution for Teachers, about evolution in general (<http://evolution.berkeley.edu/evosite/evohome.html>),
- *The Nature of Science and the Study of Biological Evolution* (BSCS, 2005), about evolution in general, and
- The Smithsonian's National Museum of Natural History's Web site, What Does It Mean to Be Human?, about human evolution (<http://humanorigins.si.edu/>).

It is also important to be aware of relevant state and district standards that relate to evolution. Standards are compiled by experts and reflect the concepts that the community believes are important. Standards are an important line of defense if outside pressure is applied to require teachers to avoid or dilute the teaching of evolution. As we described previously, the lessons in this supplement align directly with the *National Science Education Standards* (NRC, 1996).

To help relieve potential student fears, it is crucial to establish that, as a teacher, you are trying to help

students understand the scientific concepts related to evolution, not change their beliefs. Scientists accept evolution as the explanation for the unity and diversity of life because of the large amount of evidence that supports evolutionary theory. Science class is about understanding explanations based on evidence, not on beliefs. Some teachers find it helpful to tell students that they will not be asked to believe in evolution, but that they do need to understand concepts important in evolution and how scientists use evidence to support claims about evolution. Having students reflect on the nature of science continuously throughout their studies, not just when talking about evolution, helps reinforce that evidence and explanations are important in all aspects of science.

Many resources are available to you if specific issues or challenges to teaching evolution arise in your classroom. Two excellent resources follow:

- For dealing with roadblocks to teaching evolution, the Understanding Evolution for Teachers Web site: <http://evolution.berkeley.edu/evosite/Roadblocks/index.shtml>.
- For handling challenges to teaching evolution, The National Center for Science Education (<http://ncse.com/evolution>). This site also contains valuable information about legal decisions in the United States about teaching evolution and numerous statements from a large array of organizations supporting the teaching of evolution.

Using the Student Lessons

The heart of *Evolution and Medicine* is a set of five classroom lessons that allow students to discover important concepts related to evolution and medicine. To review these concepts in detail, refer to the Science Content and Conceptual Flow of the Lessons chart (Table 3), found on page 6.

Format of the Lessons

As you review the lessons, you will find that each contains several major features.

At a Glance summarizes the lesson with these sections:

- **Overview:** Provides a short summary of student activities.
- **Major Concepts:** Lists the central ideas the lesson is designed to convey.
- **Objectives:** Lists specific understandings or abilities students should have after completing the lesson.
- **Teacher Background:** Specifies which portions of the background section, Information about Evolution and Medicine, relate directly to the lesson. We do *not* intend for this reading material to form the basis of lectures to students, nor do we intend it to be a direct resource for students. Rather, it enhances your understanding of the content so that you can facilitate class discussions, answer student questions, and provide additional examples.

In Advance provides lists of items and other preparations needed for the activities:

- **Web-Based Activities:** Tells you which of the lesson's activities use the *Evolution and Medicine* Web site as the basis for instruction.
- **Photocopies:** Lists the paper copies and overhead transparencies that you need to make from the masters provided at the end of the supplement.

- **Materials:** Lists all the materials other than photocopies that you need for each activity in the lesson.
- **Preparation:** Outlines what you need to do to be ready to teach the activities.

Procedure outlines the steps in each activity and provides implementation hints and answers to discussion questions.

The **Lesson Organizer** briefly summarizes the lesson. It outlines procedural steps for each activity and includes icons that notify you when masters, transparencies, and the Web site are used. You should use the lesson organizer only after you become familiar with the detailed procedures for the activities. It can be a handy resource during lesson preparation as well as during classroom instruction.

The **Masters** to be photocopied (student worksheets and reference materials) are at the back of the supplement.

Icons appear throughout the lessons. They alert you to teaching aids that can help you implement the activities and enrich student learning.



Indicates steps that you can use as assessments, including informal indicators of student understanding and the final assessment at the end of each lesson.



Identifies teaching strategies that address specific science content standards as defined by the *National Science Education Standards* (NRC, 1996).



Shows when to use the Web site as part of the teaching strategy. A print-based alternative to each Web-based activity is provided for classrooms that don't have Internet access.



Identifies suggestions from field-test teachers for teaching strategies, classroom management, and supplement implementation.



Identifies a print-based alternative to a Web-based activity.

Timeline for Teaching the Supplement

The timeline below (Table 8) outlines the optimal plan for completing the five lessons. It assumes you will teach the activities on consecutive days of 50-minute class periods. If your class requires more time for discussing issues raised in this supplement or for completing activities, adjust your timeline accordingly.

Table 8. Suggested Timeline

Timeline	Activity
3 weeks ahead	Reserve computers. Check performance of Web site.
1 week ahead	Make photocopies and transparencies. Gather materials.
School day 1	Lesson 1 Activity 1: Outbreak! Activity 2: Models and Medicine
School day 2	Lesson 1 Activity 2: Models and Medicine
School day 3	Lesson 2 Activity 1: Investigating Lactose Intolerance and Evolution
School day 4	Lesson 2 Activity 1: Investigating Lactose Intolerance and Evolution
School day 5	Lesson 3 Activity 1: Investigating a Mystery Disease
School day 6	Lesson 3 Activity 1: Investigating a Mystery Disease
School day 7	Lesson 3 Activity 2: Using Evolution to Guide Research
School day 8	Lesson 4 Activity 1: Using Evolution to Understand Influenza
School day 9	Lesson 4 Activity 1: Using Evolution to Understand Influenza
School day 10	Lesson 5 Activity 1: Evaluating Evolutionary Explanations

Using the Web Site

The Web site for *Evolution and Medicine* can help you organize your use of the supplement, engage student interest in learning, and orchestrate and individualize instruction as learning is taking place. Lessons 2, 3, and 4 have activities on the Web site for classrooms with online access. To access the Web site, go to <http://science.education.nih.gov/supplements/evolution>.

Under “Web Portion of Student Activities,” click on the link to a specific lesson. (If your classes don’t have access to the site, you can use the print alternatives included with the lessons.)

Hardware and Software Requirements

The Web site can be accessed with any computer browser. To experience full functionality of the site, Adobe Flash Player must be installed on the hard drive of each computer that will access the site. Adobe Flash Player is freely available at <http://get.adobe.com/flashplayer/>.

Collaborative Groups

We designed all the activities in this supplement to be completed by groups of students working together. Although individual students working alone can complete many of the steps, this strategy will not stimulate the types of student-student interactions that are part of active, collaborative, inquiry-based learning. Therefore, we recommend that you organize collaborative groups of two to four students each, depending on the number of computers available. Students in groups larger than this will have difficulty organizing student-computer interactions equitably. This can lead to one or two students assuming the primary responsibility for the computer-based work. Although large groups can be efficient, they do not allow all students to experience the

in-depth discovery and analysis that the Web site was designed to stimulate. Group members not involved directly may become bored or lose interest.

We recommend that you keep students in the same collaborative groups for all the activities in the lessons. This will allow each group to develop a shared experience with the Web site and with the ideas and issues the activities present. A shared experience will also enhance your students’ perceptions of the lesson as a conceptual whole.

If your student-to-computer ratio is greater than four to one, you will need to change the way you teach the supplement from the instructions in the lessons.

Web Materials for People with Disabilities

The Office of Science Education (OSE) provides access to the Curriculum Supplement Series for people with disabilities. The online versions of this series comply with Section 508 of the Rehabilitation Act. If you use assistive technology (such as a Braille or screen reader) and have trouble accessing any materials on our Web site, please let us know. We will need a description of the problem, the format in which you would like to receive the material, the URL of the requested material, and your contact information.

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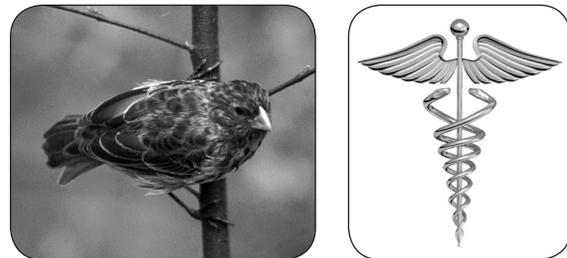
Information about Evolution and Medicine

1.0 Fundamentals of Evolution and Medicine

Biologists use the processes of scientific inquiry to try to understand two fundamental observations about living entities. The first observation centers on diversity—there are millions of species on Earth, and within each one there is diversity among individuals. The second observation seems paradoxical to the first—despite life’s incredible diversity, organisms share a number of characteristics. Biologists have proposed an explanation for both observations—evolution (National Academy of Sciences and Institute of Medicine (NAS and IOM), 2008). In the mid-19th century, Charles Darwin’s *On the Origin of Species* (1859) set the stage for the scientific studies that would provide increasingly more sophisticated and insightful evidence supporting evolution through “descent with modification” as the explanation for life’s unity and diversity. The concept of biological evolution is among the most important ideas ever developed by applying scientific inquiry to the natural world (NAS and IOM, 2008), and it offers many benefits to the field of medicine (Nesse and Stearns, 2008).

Evolution simply refers to change. In the context of biological evolution, this change refers specifically to a change in **allele** frequencies in a **population**. This change is **heritable** and occurs over time among successive generations. More simply, a population of descendants differs from an ancestral population in some characteristics (Darwin, 1859). The concept of descent with modification has tremendous explanatory power and shapes the two major types of

Figure 1. The science of evolutionary biology has important implications for medicine.



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questions that evolutionary biologists ask (Futuyma, 1998):

1. What mechanism of evolution caused a change in a **lineage** or trait?
2. What is the evolutionary history, or phylogeny, of a living lineage or a specific trait?

1.1 Processes of Evolution

“Descent” in “descent with modification” emphasizes the history of lineages, whereas “modification” refers to the fact that lineages change over time. But what are the mechanisms that cause the change? This is one of the main questions that evolutionary biologists ask. Studies involving mathematical models and organisms in nature suggest that four different mechanisms can cause changes in the genetic makeup of a population: **mutation**, **gene flow** (migration), **genetic drift**, and **natural selection** (Futuyma, 1998).

All four processes of evolution depend on and affect genetic **variation** within populations. Ultimately, all genetic variation arises from **mutation**. Genetic recombination reshuffles existing variation. Because many students struggle to consistently identify the origin

of genetic diversity when constructing explanations of natural selection, we ask them multiple times in the supplement to reflect on the role of mutations.

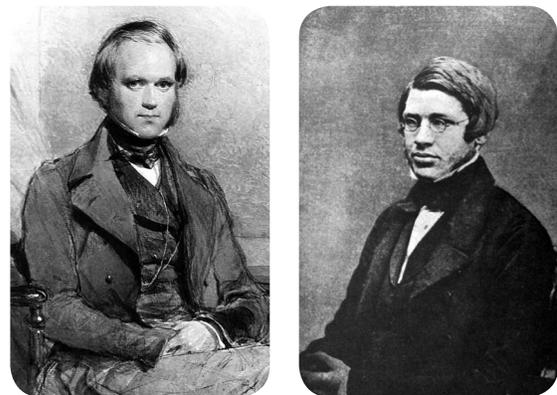
Though mutation is essential for generating genetic diversity, by itself it is not a major mechanism for changing the genetic makeup of a population from one generation to the next, because mutations happen in a single chromosome within an individual member of what is typically a large, often diploid population. **Gene flow**, also known as migration, is the movement of alleles from one population into another through immigration and emigration of individuals or through their gametes. Gene flow typically *reduces* the genetic differences among populations.

The remaining two mechanisms of evolution cause population divergence, that is, they *increase* the genetic differences among populations. **Genetic drift** refers to random changes in allele frequencies due to chance, or sampling error. Imagine a population of plants called monkeyflowers, some of which have red flowers and some have yellow ones. If a boulder rolls down a hill and flattens and kills some of the plants, this is not due to the plant's genotype for flower color, but rather to chance. One key feature of genetic drift is that it has a bigger effect on small populations than large ones. We can easily see that in a population where 75 percent of the plants have red flowers, rare events like falling boulders are more likely to drive the plants with yellow flowers to extinction if the total population size is four (that is, only one yellow flowering plant) than if the total population size is 400 (that is, 100 yellow flowering plants).

The final mechanism is **natural selection**. Alfred Russel Wallace and Darwin had jointly proposed this mechanism of evolution in 1858 through a paper delivered to the Linnean Society. Darwin described natural selection in detail in his book *On the Origin of Species* (Darwin, 1859). Darwin observed that within

a species, characteristics among individuals vary. He was also aware that, for centuries, plant and animal breeders had bred organisms to emphasize or increase certain prized characteristics. Darwin reasoned that selection in nature could also bring about change in the characteristics of a population of organisms. Some organisms survive and reproduce better than others because of the characteristics they possess. Darwin called this process “natural selection.” Natural selection provides a way to explain how new species could eventually appear from ancestral forms. Wallace developed a similar explanation around the same time.

Figure 2. Charles Darwin (left) and Alfred Russel Wallace first described the process of evolution by natural selection.



Natural selection is the only process of evolution that consistently yields adaptations. We can summarize the process of natural selection as three observations and one inescapable conclusion:

- **Observation 1:** Individuals within a population vary for many characteristics.
- **Observation 2:** Some of the differences in traits among individuals can be passed from parents to offspring. In other words, they are heritable.
- **Observation 3:** Individuals with certain variations have more offspring than others have.
- **Conclusion:** Individuals that possess heritable traits that enable them to better survive and reproduce will leave more

offspring, and these traits will increase in frequency over future generations, thus changing what the average member of the species is like.

Throughout the supplement, we ask students to explain certain observations by using natural selection. As they develop the arguments, they answer the following types of questions:

- In what ways does the population vary for an important trait?
- How did the variation arise?
- Can some of the differences in traits among individuals be passed from parents to offspring?
- Do individuals with certain traits survive and reproduce at relatively higher rates?
- How will the frequency of traits and the alleles affecting those traits change in the population over time?

The use of these types of questions across examples helps students frame the important pieces of an argument based on natural selection. A study by Bray Speth and colleagues (2009) suggests that these “concept frames” are a useful source of formative assessment data for instructors.

The activities in the supplement focus on natural selection. This is not meant to diminish the importance of the other mechanisms of evolution. In fact, the relative importance of genetic drift and natural selection is a long-standing debate among evolutionary biologists that continues to this day (Fisher, 1930; Wright, 1931; Provine, 1986; Coyne et al., 1997; Lynch, 2007). Many biologists accept that natural selection is the most powerful mechanism for phenotypic evolution, whereas genetic drift and mutation have played a pivotal role in shaping genomes and genetic architecture (Lynch, 2007).

The genome-wide approaches that researchers are using to detect positive natural selection in humans will vastly increase our understanding

of the role natural selection plays in shaping the human genome (Sabeti et al., 2006).

Positive selection occurs when variants of a gene, and the protein it produces, are continuously favored by natural selection and these “young” alleles spread rapidly in a population. One way that scientists detect positive selection in genetic sequences is by comparing the number of mutations that lead to no changes in amino acids (due to redundancy in the genetic code, so-called synonymous mutations) to the number of mutations that do lead to different amino acids (nonsynonymous mutations). In positive selection, more changes lead to different amino acids than would be expected by chance. Students get a very brief introduction to positive selection in influenza viruses in Lesson 4. Three of the examples we explore in the supplement show positive selection: MRSA (for methicillin-resistant *Streptococcus aureus*) (Harris et al., 2010), lactase in humans (Bersaglieri et al., 2004), and influenza viruses (Bush, 2001).

Understanding mechanisms of evolution, particularly adaptation by natural selection, provides many insights that enhance medical practice and understanding. A famous case involves the role of natural selection in helping researchers better understand sickle cell anemia. Sickle cell anemia affects millions of people and is a serious lifelong condition. With adequate healthcare, people with sickle cell anemia can live nearly normal lives with reasonably good health. Without adequate care, the disease can be debilitating and cause early death. This disease is caused by a recessive genetic disorder, a mutation in the *HBB* gene (which encodes β -globin). The allele that leads to sickle cell disease in homozygotes is called *HbS* (with the resulting protein hemoglobin S) and was one of the first specific genetic variants to be associated with a molecular defect (Pauling et al., 1949). *HbS* has four distinct forms, suggesting that it may have arisen independently multiple times in different locations (Kwiatkowski, 2005).

The frequency of the *HbS* allele in some regions of the world is high (about 10 percent). Biologists noticed that populations with a high frequency of this allele occurred in geographic areas with high rates of **malaria**. Malaria is thought to be the strongest selective agent known in recent human history (Kwiatkowski, 2005). Allison (1954) first hypothesized that the sickle cell allele is advantageous in certain environments because it protects carriers against malaria. Homozygotes for the typical hemoglobin allele do not have sickle cell anemia, but they are susceptible to malaria. Heterozygotes who carry one normal allele and one sickle cell allele have a 10-fold reduced risk of malaria and are only slightly anemic (Kwiatkowski, 2005). Natural selection favors the heterozygote in geographic regions with high rates of malaria and maintains both alleles in the population. The sickle cell–malaria scenario is a classic example of how selection explains why human populations vary for some genetically determined traits that affect health.

It is difficult to imagine how we would explain the high susceptibility to sickle cell anemia in some human populations without invoking evolution in the past. In other words, the frequency of the *HbS* allele is higher than we would expect if it did not influence survival in people with malaria. In fact, investigations of sickle cell were the first evidence of natural selection operating in humans (Allison, 1954). This scenario is featured in many high school biology curricula. It is important to keep in mind, however, that **heterozygote advantage** is probably relatively infrequent, and for good evolutionary reasons. The disadvantages that accrue to the homozygotes may provide a strong selection force for an alternative, superior solution. However, if the heterozygotes have a strong advantage, the polymorphisms can be maintained in populations for a very long time. Interestingly, it appears that heterozygote advantage is more common in populations exposed to a relatively recent environmental change.

Figure 3. Sickle cell anemia was one of the first diseases that was better understood by considering evolution and the impact of malaria on human populations. Upper left: microscopic view of blood cells from a person with sickle cell anemia; lower right: female mosquito taking a blood meal.



In this supplement, students explore the high prevalence of **thalassemia** in certain populations. Similar to sickle cell anemia, the high prevalence of thalassemia is partially explained by the fact that individuals with thalassemia have higher protection against severe malaria. Learning of a second disease that follows a pattern similar to sickle cell anemia's should help students generalize the main concepts of natural selection to a broader range of problems.

In general, Nesse and Williams (1994; see also Nesse, 2007) suggest several categories that help explain human vulnerability to disease, based on principles involving natural selection:

- **Mismatch to the Environment:** Modern environments in the industrialized world are radically different from those that predominated during most of human evolution. We are not yet well adapted to our current environment (Leonard, 2008), for the spread of adaptations in human populations is much slower than the rate of

cultural change. In this supplement, students explore the evolution of **lactase persistence**, which has evolved multiple times in different populations of humans since the domestication of dairy animals. Different alleles of the lactase gene are associated with persistence in different populations (Ingram et al., 2007). Selection for this trait and on lactase persistence alleles has been very strong for the last 3,000–10,000 years (Bersaglieri et al., 2004; Tishkoff et al., 2007). Lactase persistence is described in more detail on page 34.

- **Rapid Pathogen Evolution:** Pathogens usually have much shorter generation times, higher mutation rates, and vastly larger numbers of offspring than their hosts. They also face higher selection coefficients than humans and any other organism with a generation time longer than a few weeks. This results in pathogens displaying more rapid adaptation (Hillis, 2004). Human evolution occurs over longer time periods, making protection from infection a persistent challenge.
- **Constraints:** One example of a constraint relates to genetic variation. Selection can only act on the variation present within a population. Exceedingly complex structures do not evolve *de novo*; instead, they evolve

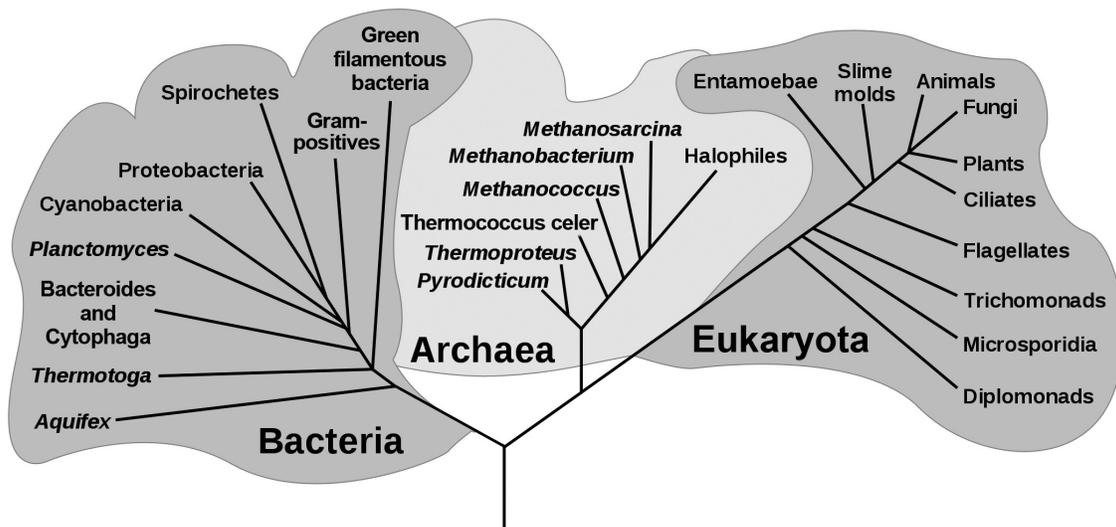
stepwise from preexisting structures that often have a different function (for example, the bacterial flagellum; Liu and Ochman, 2007). The variation present limits what selection can shape.

- **Tradeoffs:** A tradeoff occurs when an evolutionary change in one trait that increases fitness is linked to a change in another trait that decreases fitness. All organisms (including humans) must make tradeoffs, and this means that it is natural that some traits are not in an ideal state but are byproducts of selection acting on other traits. One important tradeoff found in many organisms is between reproduction and survival. Forms of genes that increase reproductive success will increase in frequency even if they negatively affect health and longevity. For example, men with high testosterone levels may compete more successfully for a mate, but they may suffer from decreased resistance to pathogens (Muehlenbein and Bribiescas, 2005). Selection for increased reproduction may result in decreased survival.

1.2 Common Ancestry

Early naturalists noticed that species can be clustered naturally into a hierarchical pattern of groups within groups—that is, species into

Figure 4. All living organisms are related in one great phylogenetic “Tree of Life.”



genera, genera into families, families into orders, and so on. But it was not obvious why this nesting pattern occurs. Darwin (1859) realized that “this natural subordination of organic beings into groups under groups” could be explained by descent with modification from common (shared) ancestors. There is no logical reason to expect species to be arranged hierarchically if they arise separately.

Although scientists today may disagree about some of the natural groupings of organisms, they all agree with the idea of descent with modification from common ancestors. Descent with modification explains two features that are characteristic of organism groupings. First, the pattern is *hierarchical*, or made of groups within groups. Second, it is *branching*, or treelike.

A branching pattern of groups results whenever an ancestral group splits into related subgroups that come to differ in some way. This pattern allows us to trace the ancestry of the subgroups back to their common ancestor. We can trace the ancestry of this ancestor back to another shared ancestor, and so on. It is like working backward along the branches of a tree from the twigs to the trunk. We can trace the growth of all twigs back through a series of branch points to the trunk. The twigs represent existing species, nearby branch points represent recent shared ancestors, and the trunk represents a distant ancestor that is common to many branches.

The similarities among organisms are evidence of their descent from a common ancestor. Scientists gather data from observable characteristics in organisms to estimate relationships. These characteristics include structural similarities (for example, skeletal features or cellular structures), patterns of embryological development, and, increasingly, molecular data. Since the development of molecular techniques in the 1980s, the use of DNA, RNA, and amino acid sequences as well as careful analyses of when

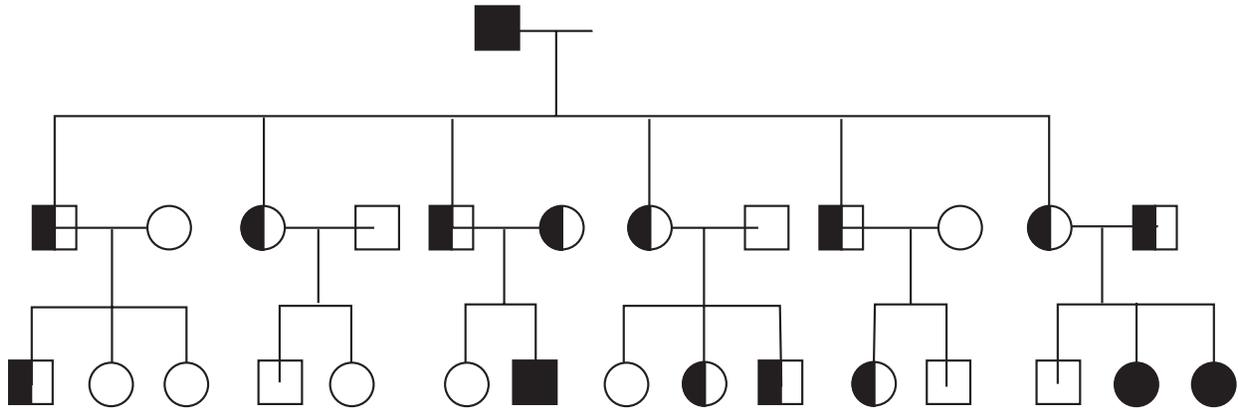
and where genes are used in the development and maintenance of living organisms has sharpened scientists’ ability to ask and answer fine-scale evolutionary questions. Many hypotheses based on morphological characteristics have gained further support. However, hypotheses of relationships can change when new data are acquired, and, in some cases, research has overturned previous ideas of relationships.

Diagrams that summarize the evolutionary history of the relationships among organisms are called “**evolutionary trees**” or “phylogenies.” The characteristics of living organisms have been shaped by their long evolutionary history. Evolutionary biologists seek to answer questions about the relationships among living and extinct species, the history of specific populations within a species, the timing and geography of diversification events, the reconstruction of **ancestral states**, and the timing and origin of specific characteristics or processes in organisms. Phylogenetic hypotheses are being rapidly developed (Hillis, 2004), and tools and models for reconstructing relationships are becoming increasingly sophisticated. Students gain experience with some of the important NIH-sponsored tools and databases through the lessons in the supplement.

The explosion of phylogenetic information afforded by the sequencing of genomes from diverse organisms across the “Tree of Life” offers many insights that may inform medicine (Nesse and Stearns, 2008). The following are just a few examples of how phylogenetics has informed medicine:

- Alleles associated with specific phenotypes are more frequent in certain human populations of different geographical origin. For example, persons of Ashkenazi Jewish ancestry living in the United States have a higher frequency of *BRCA1* and *BRCA2* mutations (Ewald, 2008; Narod and Offit, 2005), as do Icelandic, Dutch, and Polish populations (Narod and Offit, 2005).

Figure 5. Pedigrees, like the one shown below, and genetic screening help researchers understand patterns of genotypes and phenotypes.



Thus, knowing the ancestry of individuals can provide some insight into probabilities of specific genetic conditions, which may influence the genetic screening and counseling these individuals receive. Large-scale representative sampling from populations across Earth for high-risk alleles is currently under way (Crews and Gerber, 2008). Students explore allele frequencies in different human populations in two lessons in the supplement.

- The evolutionary origin of pathogens is now routinely investigated by using phylogenetic methods. Phylogenetic analysis of the human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) clearly shows that there are two major types of HIV that moved into humans from two separate hosts. Each type has become established in humans more than once (Rambaut et al., 2004; Sharp et al., 2001). The same types of analyses led to the identification of the coronavirus that causes severe acute respiratory syndrome (SARS; Ksiazek et al., 2003; Peiris et al., 2003). Phylogenetic analysis also led to the identification of bats as the reservoir for the coronavirus (Li et al., 2005). In the supplement, students use genetic sequences to explore the history of **influenza**. Scientists use these same tools and skills to identify the origin of emerging pathogens.

2.0 The Value of an Evolutionary Perspective for Medicine

The questions evolutionary biologists ask illuminate many matters that affect human health. The field of evolutionary medicine uses the models and theory of evolutionary biology to inform problems encountered in medicine and public health (Nesse, 2008). Many applications of evolutionary biology are already well established in medicine and are very useful. These include population genetics, phylogenetic analysis, and studies of antibiotic resistance. Even in these well-established areas, new evolutionary insights are leading to rapid advances.

Insights from evolution can also provide a theoretical framework for understanding why organisms are vulnerable to disease. Advocates suggest that evolutionary biology should be put on par with other fundamental basic sciences, such as biochemistry, and that teaching it will make medical education more coherent (Nesse and Stearns, 2008).

3.0 Specific Applications of Evolution in Medicine

3.1 The Relationship of Genetic Variation to Health

Variation is the raw material on which evolutionary processes operate (Futuyma, 1998). Though individuals in a population may show variation in a phenotype, only the proportion

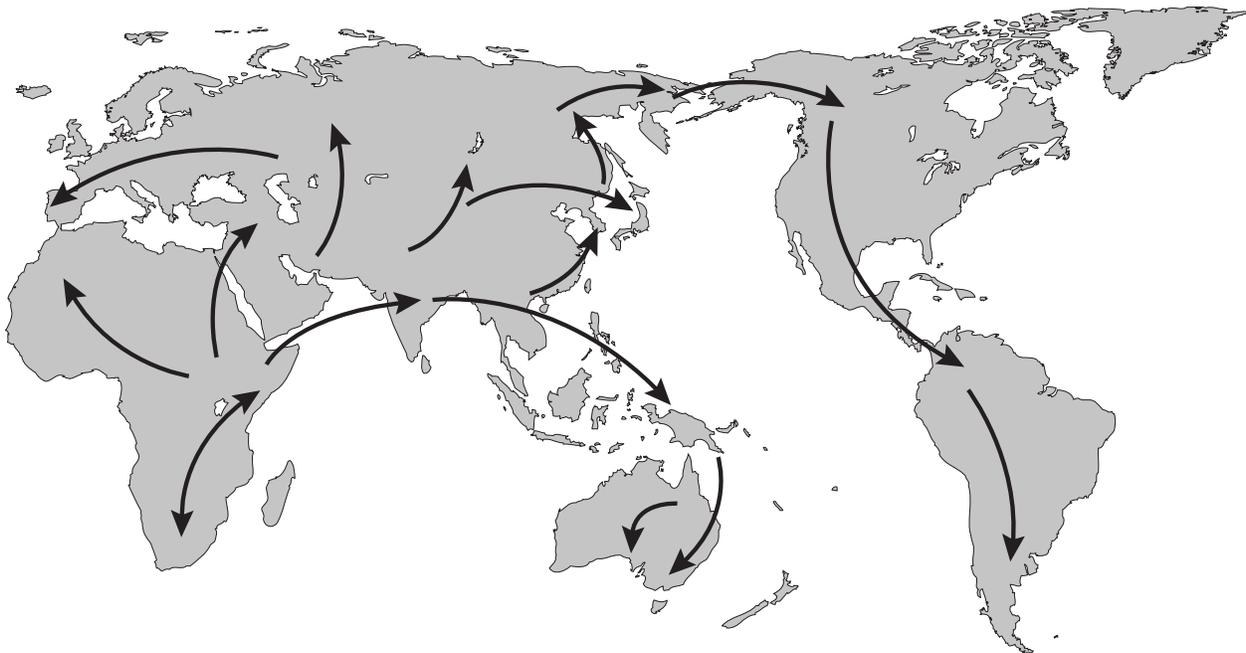
of that variation that is heritable will respond to natural selection. Evolutionary biologists try to assess the proportion of phenotypic variance attributable to genetic variance, environmental variance, and genotype \times environment interactions. Variation is often quantified within and among populations.

Humans vary across the world. Every independently conceived individual is genetically unique. This seems paradoxical in light of the fact that all humans have a high degree of genetic similarity. It is often reported that two humans are 99.9 percent similar in their DNA. However, the human genome is immense, providing multiple opportunities for genetic variation to arise; the 0.1 percent by which we differ amounts to 3.3 million nucleotides (Kidd and Kidd, 2008). Findings from the International HapMap Project confirm previous studies and show a relatively low amount of differentiation among human groups defined by ethnicity and geography (Govindaraju and Jorde, 2008). There is

much more genetic variation within (about 90 percent) than among (about 10 percent) human groups. This means that the similarities among different groups of humans far outweigh the differences. To learn more about the HapMap Project, visit <http://hapmap.ncbi.nlm.nih.gov>.

As the ability to decipher the genotypes of individuals improves and becomes more widely available, medical practitioners will be better able to give patients specific information about their health. Individual genetic profiles provide useful information about disease susceptibility and predispositions. Crews and Gerber (2008) suggest three possible medical-clinical applications of individual genetic profiling: improved screening, more-informed counseling, and individualized drug formularies. Until more individualized data are available, however, researchers continue to try to determine whether disease susceptibility is linked to specific genetic

Figure 6. Genetic data support hypotheses that humans migrated out of Africa.



(Image created from data from the National Geographic Society's Genographic Project, <https://genographic.nationalgeographic.com/genographic/lan/en/atlas.html>.)

factors and, if so, whether the genetic factors are distributed differentially among geographic groups (Kittles and Weiss, 2003). Patterns of variation among humans have been shaped by migration, genetic drift, mutation, and natural selection. These evolutionary mechanisms lead to a correlation between geographic distribution and genetic variation (Ramachandran et al., 2005; Soo-Jin Lee et al., 2008) that may be medically relevant (Kidd and Kidd, 2008).

Genetic variation in health-related traits may be simple (one gene) or complex (multiple genes). Complex traits are often described as being multifactorial, meaning that the genes interact with each other and with the environment. Decades ago, much of the focus was on human diseases that have a relatively simple genetic basis (for example, Tay-Sachs disease or Huntington's disease). Modern genetic insights show that many of the supposedly simple traits are more complex than people thought. For example, phenylketonuria (PKU) is caused by mutations to a gene that affects the production of phenylalanine hydroxylase. However, scientists have now identified multiple mutations to the gene, each of which can cause different symptoms and outcomes for people with PKU (Kidd and Kidd, 2008). NIH maintains a site that contains large amounts of up-to-date information on human genes and genetic phenotypes, Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/omim>).

Though diseases with a relatively simple genetic basis are important to study, a much larger fraction of genetic variation is likely to influence an individual's interaction with parts of the environment that influence health (Kidd and Kidd, 2008). Complex diseases that have significant genetic and environmental influences have a large impact on public health and are likely to command the attention of the biomedical community in the near future. Unraveling the causes

of complex diseases has been advanced by genome-wide association studies, which look across the entire genome for genetic influences on disease risk. To date, such studies have found many new genes that influence disease risk. But the overall amount of variation among individuals in disease risk that can be explained by genetics has remained small: on the order of 3 to 5 percent. These results suggest that much of disease risk is tied up in complex gene \times environment interactions, which means that both the particular genes a person inherits and the particular environments the person is exposed to are important. For example, the degree to which smoking and cholesterol increase the risk of heart disease depends on the particular versions of multiple genes that the person has inherited.

3.2 The Role of Evolution in Drug Response

The relationship between genetic variation and health is specifically manifested in the study of the genetic basis for variation in individuals' drug responses. The role of evolution in shaping this variation is also often relevant to these studies. We illustrate two classic cases, though numerous examples exist.

1. Various oxidant drugs can cause the destruction of red blood cells in certain people. Half a century ago, researchers showed that this response was caused by an X-linked, single-gene, recessive trait resulting in a deficiency of glucose-6-phosphate dehydrogenase (Carson et al., 1956). Alleles for this trait were common in people of African and Mediterranean ancestry. Similar to the case of sickle cell disease, researchers suspected that this allele is retained in these populations due to the protection conferred against malaria for heterozygote females (Ruwende et al., 1995), a hypothesis that has received recent support from genetic analyses (Sabeti et al., 2002; Saunders et al., 2005; Tishkoff et al., 2001). Response to selection caused

by malaria provides an explanation of the evolutionary origin of this drug response.

2. Many other drug responses are based on complex interactions rather than the action of a single gene. For example, patient response to warfarin, an anticoagulant drug, varies widely. A significant portion of this variation is attributable to genetic causes. Careful dosing and monitoring of the drug are required both to ensure an adequate patient response and to avoid excessive bleeding. Concerns about the variability in patient response and potential complications have discouraged physicians from prescribing warfarin, and an estimated 50 percent of patients who could benefit from anticoagulant therapy do not receive it (Friberg et al., 2006). Researchers have identified two genes that play significant roles in patient response to warfarin. Taken together, alleles for these two genes account for about 40 percent of the variability in the response. Selection, drift, and gene flow have shaped the patterns of genetic variation for these two genes in different groups of people, which partially explains patterns in the responses of different groups. Genetic testing of individuals aims to improve the safety of warfarin therapy. While the effect of these two genes is significant, other genetic and environmental interactions (such as diet and interactions with other drugs) also affect warfarin responses.

3.3 The Role of Evolution in Infectious Diseases

One of the most direct applications of evolution to medicine is in the well-documented evolution of antibiotic resistance in bacteria. Antibiotics exert strong selective pressures on populations of bacteria, which quickly increase the proportion of individuals that can resist the antibiotic (Bergstrom and Feldgarden, 2008). Bacterial

resistance to a new antibiotic almost always becomes prevalent just a few years after the antibiotic is introduced. The cost of antibiotic resistance in the United States is estimated to be \$80 billion annually (Bergstrom and Feldgarden, 2008). Similarly, viruses—especially retroviruses with RNA genomes—quickly evolve resistance to antiviral drugs, as in the case of HIV (Rambaut et al., 2004). This point is particularly compelling in light of the fact that the majority of emerging pathogens causing new infectious diseases are viruses, especially RNA viruses (Woolhouse and Antia, 2008).

The evolution of resistance has important implications for medicine. For example, some hospitals initiated antibiotic-cycling regimes (antibiotics are rotated on a schedule) to help reduce the evolution of resistance. However, mathematical models that incorporate principles of ecology and evolution show that this approach may be ineffective, a result supported by metaanalyses of clinical trials (Bergstrom and Feldgarden, 2008). Additionally, models of evolution help researchers formulate the vaccinations against influenza each year and design drug regimes for the treatment of AIDS (for example, highly active antiretroviral therapy; Rambaut et al., 2004).

Figure 7. The evolution of resistance in HIV requires patients to use many medications.



© Norberto Mario Lauria / Dreamstime.com

Studying the evolution of virulence provides powerful insights into infectious diseases. Before the rise of genetic engineering, researchers used the evolution of virulence to develop attenuated live viruses. Pathogens were grown in culture, and adaptations to culture conditions involved tradeoffs so that this evolved strain grew more poorly in the host (Ebert and Bull, 2008). More recently, some researchers have proposed using virulence evolution to help manage parasites. Others argue that we need better models that incorporate more details of specific biological processes before we start measures that entail risks (Ebert and Bull, 2008).

An additional role for evolution in helping us understand infectious diseases is through the use of phylogenetic methods to identify and trace the evolutionary origin of pathogens and the reservoirs of pathogens. (The phylogenetic approach for understanding HIV and SARS is described on pages 26 and 27.)

4.0 Students' Prior Conceptions about Evolution

In addition to including vivid examples of evolution and medicine, the supplement takes into account research on student preconceptions. Educational research on evolution shows that students hold several naïve preconceptions about evolution that interfere with their learning (Sinatra et al., 2008), in particular, naïve ideas about natural selection and the interpretation of evolutionary history. We list below some of the most common preconceptions that we address in the supplement. We suggest that you do not use these as a list of lecture topics for your students, but rather use them to inform your teaching as the preconceptions emerge.

4.1 Natural Selection and Processes of Change

- *Evolution is Only a Theory*: Many students do not understand the meaning of the term theory in science and equate it with meaning a hunch or a guess. This

misunderstanding stems from a large problem in evolution education; namely, a poor understanding of the nature of science. (American Association for the Advancement of Science (AAAS), 2001).

- *Acquired Characteristics Can Be Inherited*: The inheritance of acquired characteristics suggests that changes that parents make in their lifetime are passed on to the next generation, whether or not they have a genetic basis. These incorrect ideas are remarkably persistent (Crow, 2004). In contrast, biologists recognize that evolution only occurs when changes are heritable and that change occurs at the population level, not the individual level (AAAS, 2001). For students to truly grasp the idea that populations, not individuals, evolve, they must have an understanding of variation within a population. They must also recognize that a shift in an average characteristic of a population represents change. We designed the activities in this supplement to help students recognize and confront these ideas.
- *Species Have an Underlying Nature That Cannot Change*: The concept of essentialism, which is incorrect, makes it difficult for students to recognize both variation within species and that species can change over time. Many students struggle to recognize this implicit and incorrect assumption.
- *Natural Selection Leads to Perfection*: Phrases such as “more highly evolved” can be misleading. Species adapt to conditions in the present, but these conditions can and do change. Often, an adaptation that helps a species survive in one environment is a disadvantage when the environment changes. Additionally, many traits are involved in tradeoffs. The idea of a workable compromise is a much better descriptor of the state of an organism than the idea of perfect design.
- *Fitness Means Individuals Are Stronger or More Athletic*: Many student associate fitness with overall strength and the ability to fight.

However, cooperation is an essential aspect for survival in many organisms. Offering students multiple and varied examples of selection documented in real time will help them construct a more accurate view of selection. It is important that students understand that natural selection does not operate to improve survival but rather to improve reproductive success. Of course, an individual must survive long enough to reproduce. But after maturation, evolutionary changes that increase reproductive success will spread in the population if the effects on reproduction are large enough and the effects on survival are small enough, even though they decrease survival.

- *Natural Selection and Evolution Mean the Same Thing:* There are multiple mechanisms for evolution, including genetic drift and gene flow. However, natural selection is one of the most powerful and well-documented mechanisms of evolution.
- *Greatly Different Time Spans are Equivalent:* Although many people understand that evolution has taken place over large expanses of time, they seem to place events in broad categories such as “extremely ancient,” “moderately ancient,” and “least ancient” (Catley and Novick, 2009; Libarkin et al., 2005; Trend, 2001).

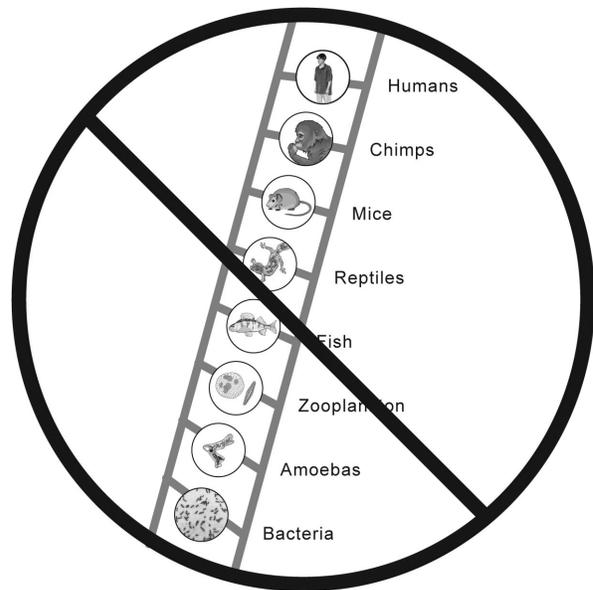
4.2 “Tree-Thinking”

- *Evolutionary Trees Are a “Ladder of Progress”:* Some people equate a progression from simple to complex as “better.” This line of reasoning may cause people to try to read evolutionary trees from left to right, with the organisms on the left evolving into the organisms on the right. Instead, evolution mostly proceeds by having one group bud off from an existing group (Meir et al., 2007). A true understanding of biodiversity shows that all species existing today are descended from ancestors that have survived since the origin of life more

than 3.5 billion years ago. Plants, worms, bacteria, and humans all descend from ancestors that survived multiple mass extinctions and many major environmental challenges. To claim that one existing group is “more highly evolved” than another ignores this basic fact.

- *Modern Species Are Ancestors:* One way this misconception is manifest is the saying “humans evolved from chimpanzees.” Instead, humans and chimpanzees evolved from a common ancestor. This common ancestor may have shared characteristics with modern chimpanzees, but both the modern chimpanzee lineage and the lineage that led to humans have been evolving for the same amount of time since splitting from the common ancestor (Baum et al., 2005).
- *The Ordering of Species at the Tips of an Evolutionary Tree Is Always Meaningful:* Many students ignore the pattern of branching in an evolutionary tree when trying to determine relatedness. They do not recognize that the most closely related species are those that share the most recent common ancestor (Baum et al., 2005).

Figure 8. Many students mistakenly think of evolutionary trees as “ladders of progress.”



To help students determine the time since common ancestry and better understand the time involved in evolution, we included a time arrow on many of the evolutionary trees in this supplement.

In addition to these prior conceptions, students have difficulty interpreting evolutionary trees that are drawn in different ways. For example, many students have difficulty interpreting evolutionary trees drawn with the nodes as a V shape. When shown such trees, students often assume that the group at the end of the longest line is the ancestor of the other groups (Novick and Catley, 2007).

The activities in this supplement give students a chance to explore the widely accepted scientific explanation for the diversity of life on Earth: evolution. As a science teacher, it is your responsibility to your role is to present the scientific evidence for evolution, while respecting students' individual beliefs. Consider reviewing the differences in ways of knowing with students. Evolution is a scientific way of explaining biological change across time. Creationism (including creation science and intelligent design) is not scientific because it invokes supernatural causes. Evolution is not inherently at odds with religion. Religion offers other ways of knowing the world.

5.0 Featured Examples of Evolution and Medicine

5.1 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The evolution of antibiotic-resistant bacteria is a classic example of evolution by natural selection that has large impacts on humans. Today, controlling the spread of multiply resistant bacterial strains is a major global health concern. MRSA stands for **methicillin-resistant *Staphylococcus aureus***. Methicillin is in a group of antibiotics that includes penicillin and amoxicillin. Unfortunately, some populations of *S. aureus* are resistant to the entire group of antibiotics, which includes methicillin.

Health specialists differentiate between hospital-acquired MRSA and community-acquired MRSA. Hospital-acquired MRSA was recognized before community-acquired MRSA. It is not yet clear if community-acquired MRSA evolved from hospital-acquired MRSA or if it evolved from different strains of *Staphylococcus aureus*. Community-acquired MRSA typically causes the outbreaks found in schools and differs from hospital-acquired MRSA in that it is not multidrug-resistant and can be treated with other antibiotics (Dominguez, 2004). *Staphylococcus aureus* affects animals other than humans; it is one of the major causes of a disease in cows called contagious bovine mastitis, for example.

Nearly all MRSA skin infections respond to treatment. Serious infections, such as pneumonia and blood and bone infections, rarely occur in healthy people.

MRSA can be spread through skin-to-skin contact or sharing items such as towels used by infected individuals. The environments that are more conducive to spreading MRSA are called the “5 C’s” by the Centers for Disease Control and Prevention (CDC): crowding, frequent skin-to-skin contact, compromised skin (cuts, for

Figure 9. Infectious diseases affect our lives in many ways. Antiseptic dispensers like this are now commonplace in many work and school environments.



© dblight / iStockphoto.com

example), contaminated items and surfaces, and lack of cleanliness. The CDC hosts a useful Web site with information about MRSA infections: <http://www.cdc.gov/Features/MRSAinfections/>.

In the first lesson, students use their prior knowledge to develop an explanation of how populations of MRSA evolve antibiotic resistance. If students are familiar with natural selection, they may suggest that the genetic variation required for the evolution of resistance came about through mutation. Indeed, mutation is the source of the requisite genetic variation in many cases. However, genes for resistance, virulence, or both can be acquired through horizontal gene transfer between closely or distantly related bacteria. Researchers at NIH used genetic sequences and an analysis tool called an “**evoprint**” to demonstrate that a strain of hospital-acquired MRSA shares multiple DNA sequence blocks with *Listeria monocytogenes* and *Staphylococcus saprophyticus* (Brody et al., 2008). Both of these bacteria can also cause bovine mastitis. These data suggest that bacterial co-infections may be an important factor in the evolution of resistance. Students gain experience analyzing an evoprint in Lesson 3.

5.2 Pax6

Pax6 is a gene that plays a crucial role in regulating the growth of eyes in a wide range of organisms, including humans, fruit flies, zebrafish, mice, squid, planarians, and even ribbon worms. In humans, the gene is involved in the early development of the eyes, brain, spinal cord, and pancreas. It is found on chromosome 11 and spans over 20,000 nucleotides. *Pax6* codes for a transcription factor in the paired box gene family (which is the origin of the name *Pax6*). By convention, *PAX6* (all capital letters) refers to the human gene, *Pax6* (just the first letter capitalized) refers to the gene in mice and rats, and *pax6* (all lowercase letters) refers to the gene in zebrafish. For simplicity, we use *Pax6* throughout the supplement.

Studies of *Pax6* caused scientists to radically reconsider the evolution of eyes in different lineages. Previously, many scientists inferred that eyes evolved independently many times, due to the large differences in anatomical structure among eyes in different groups of animals. For instance, arthropods have compound eyes, whereas vertebrates and squid have camera-type eyes (Carroll, 2006). However, the ubiquitous presence of *Pax6* as a regulator of eye development suggests that the gene was present in the common ancestor of these animals. Scientists infer that the common ancestor likely had a simple eyespot and may not have been able to form an image (Halder, 1997). Studies on *Pax6* support the claim that new, complex structures are rarely built anew throughout evolution; rather, they are often assembled from preexisting structures.

Some mutations to the *Pax6* gene cause the human disease **aniridia**. Scientists have identified over 250 such mutations in humans. Most of the mutations that cause aniridia are premature stop codons.

For more information on aniridia, visit <http://ghr.nlm.nih.gov/condition=aniridia>.

For more information on *Pax6*, visit <http://ghr.nlm.nih.gov/gene=pax6>.

5.3 Lactase Persistence

Like all mammals, infant humans have the ability to digest the sugar lactose in milk. However, in a large number of humans, the gene that codes for the enzyme lactase is “turned off” after weaning, causing these individuals to lose the ability to digest lactose. Individuals with this condition are called “**lactase nonpersistent**.” The gene for lactase remains “turned on” for individuals who are lactase persistent (**lactose tolerant**). Strictly speaking, **lactose intolerance** is not equivalent to lactase nonpersistence because intolerance may also be caused by lactose malabsorption. An NIH Consensus Development Conference

on Lactose Intolerance and Health in 2010 defined lactose intolerance as “the onset of gastrointestinal symptoms following a blinded, single-dose challenge of ingested lactose by an individual with lactose malabsorption, which are not observed when the person ingests an indistinguishable placebo.” Students use the terms “lactase persistence” and “lactase nonpersistence” in the activity. The majority of humans are lactase nonpersistent, and lactase persistence is a derived trait (Holden and Mace, 1997). This means that the common ancestor of all humans was lactase nonpersistent.

Lactase persistence has a strong genetic component and has evolved independently at least three times in human history. Scientists have found three main alleles that are associated with lactase persistence. Each allele is identified by a specific mutation to one nucleotide (called a single nucleotide polymorphism, or a SNP (pronounced “snip”). The SNPs are located thousands of nucleotides away from the coding region for the lactase gene but are all relatively close to each other. The first identified allele associated with lactase persistence is a SNP 13,910 nucleotides away from the lactase gene. The two SNPs associated with the other two other alleles are 13,915 and 14,010 nucleotides away. These nucleotides are located in an intron of a gene called *MCM6* that neighbors the lactase gene. Changes to the nucleotides in this region can affect the transcription of the lactase gene. Results of genetic analyses on each allele are consistent with positive directional selection.

Scientists conduct bioinformatic studies on the entire human genome, looking for genes that show a strong signal of positive selection. The regulatory region for the lactase gene shows one of the strongest signals for positive selection. Selection coefficients estimated in populations that have a high frequency of lactase persistence are as high as 5 to 10 percent. A selection coefficient of 5 percent is strong enough to cause an allele to increase in frequency from

1 percent to 95 percent in 300 generations, or in about 9,000 years for humans. The rapid rise in frequency of lactase persistence alleles in some populations are referred to as selective sweeps. Recent analyses of 200 human genomes suggest that selective sweeps in human evolution are the exception rather than the rule, however (Hernandez et al., 2011). Instead, human adaptation was mostly shaped by relatively small changes in many genes.

To learn more about recent findings on the evolution of lactase persistence, see Enattah et al., 2007; Enattah et al., 2008; Gerbault et al., 2009; Holden and Mace, 1997; Ingram et al., 2007; Ingram et al., 2009; Swallow, 2003; and Tishkoff et al., 2007.

A recent study examined different hypotheses for the evolution of lactase persistence in different populations (Gerbault et al., 2009). The culture-historical hypothesis was consistent with the results obtained for the evolution of lactase persistence in Africa, whereas the UV–vitamin D–calcium hypothesis was consistent with the evolution of lactase persistence in northern Europe. However, the data for Europe are complex. Alternative hypotheses for selection pressures and the effects of population history and demography are difficult to disentangle. See the masters associated with Lesson 2 for more information about these hypotheses and other recent research about them.

5.4 Thalassemia

As described in the masters associated with Lesson 3, thalassemia refers to several diseases characterized by reduced or no production of the globin proteins that form hemoglobin. Hemoglobin in adults is composed of two alpha-globin protein chains and two beta-globin protein chains. Alpha-thalassemia is a condition in which a person produces less or no alpha-globin protein. Beta-thalassemia occurs when there is a defect in the beta-globin gene, the gene for the beta-hemoglobin chain. A useful description of the history and future of research

on the thalassemias can be found in a 2004 paper by D. Weatherall.

Alpha-thalassemia is caused by mutations in the alpha-globin genes on chromosome 16. Normally, each person has four copies of the alpha-globin gene, two on each chromosome of the pair. In alpha-thalassemia, one to four of the alpha-globin genes are not functional. When people have three functional alleles of the gene, they have few if any symptoms. When two or three of the alpha-globin alleles are nonfunctional, people have more-serious symptoms. If all four alpha-globin alleles are nonfunctional, no alpha-globin protein is produced, and this state is fatal. Alpha-thalassemia is most commonly found in Africa, China, India, the Middle East, Southeast Asia, and occasionally the Mediterranean region.

The eminent scientist J.B.S. Haldane first proposed the “malaria hypothesis” for the existence of thalassemia in 1948. Haldane suggested that individuals who are heterozygous for thalassemia may be more resistant to malaria. He also noted the co-occurrence of malaria and thalassemia in certain parts of the world. Recent studies show that the high frequencies of milder forms of alpha-thalassemia are indeed related to protection from malaria (Dronamraju and Arese, 2006). A recent study by Fowkes et al. (2008) explored whether people with different numbers of nonfunctional alpha-thalassemia alleles have higher protection against severe malaria. Their results suggest that the increased number and small size of the red blood cells in children who have some nonfunctional alleles of the alpha-thalassemia gene contribute to their protection against malaria.

Beta-thalassemia is a serious health problem worldwide and causes the deaths of hundreds of thousands of children per year. Compared with alpha-thalassemia, beta-thalassemia is more common in the Mediterranean region, but it is still much more frequent in areas

where malaria is endemic. Malaria used to be endemic in the Mediterranean and is currently coming back into southern Italy. In the early 19th century, tourists often got malaria in Rome when they visited the Coliseum at night. The prevalence of beta-thalassemia in the Mediterranean is a good example of a past signature of selection that has not yet disappeared and in some areas is experiencing selection again. Beta-thalassemia is caused by different molecular defects that reduce or abolish the synthesis of the beta-globin chains.

5.5 Van der Woude Syndrome and *Irf6*

Much of the relevant information about **Van der Woude syndrome** and the *Irf6* gene is included in the masters in Lesson 3. In brief, Van der Woude syndrome is an inherited developmental disorder. Syndrome means a group of symptoms or signs that are characteristic of a specific disease. Individuals with the disorder may have pits of the lower lip and a cleft lip, a cleft palate, or both. Cleft lip and palate may or may not be a part of a syndrome. Van der Woude syndrome is the most common form of cleft palate that is associated with a syndrome.

For more information about Van der Woude syndrome, see <http://ghr.nlm.nih.gov/condition=vanderwoudesyndrome>.

Mutations to the *Irf6* gene cause Van der Woude syndrome (Kondo et al., 2002). In Lesson 3, students examine exon 3 and part of the flanking introns for the *Irf6* gene from a range of organisms. For more information about this gene, see <http://ghr.nlm.nih.gov/gene=irf6>.

The following Web site has an interesting interview with two of the authors of the paper that identified the relationship between Van der Woude syndrome and the *Irf6* gene: <http://www.nidcr.nih.gov/Research/ResearchResults/InterviewsOHR/TIS102002.htm>.

The phylogenies used in the activity are based on data from Prasad et al., 2008.

5.6 Influenza

Influenza is a disease that affects millions of people each year. Understanding the evolution of influenza viruses is critical for learning how to better avoid future pandemics and for treating people with influenza. Scientists distinguish between two main types of changes in influenza, **antigenic drift** and **antigenic shift**. Antigenic drift causes relatively small changes that occur among flu strains from year to year. Infrequent and relatively large changes in the composition of the predominant influenza virus are called antigenic shift. In the supplement, students explore antigenic drift.

Antigenic drift is not the same as genetic drift. Each year, one strain of influenza circulates widely throughout the world. People infected with influenza develop antibodies to the **hemagglutinin** antigen present on the outside of the virus. When the antibodies bind to the hemagglutinin antigen, the virus is prevented from entering the cells of the body. As a result, individuals are protected against reinfection from the same strain. Influenza vaccinations also result in the formation of protective antibodies. Because a large number of people develop immunity to a strain of influenza virus, forms of the virus that have mutations that change the

Figure 10. Outbreaks of influenza influence our lives. Wearing a mask can protect us from inhaling flu viruses.



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shape of the hemagglutinin region—and allow the virus to avoid detection by the immune system—are favored by natural selection. As a result, the influenza virus changes from year to year. This helps explain why new vaccines must be developed every few years. A fuller explanation of why the diversity in influenza differs from that in other viruses, such as measles, mumps, and rubella, is described in Lipsitch and O’Hagan (2007).

Dramatic changes to type A influenza are an example of antigenic shift. Antigenic shift occurs when a new strain of influenza A develops in which the hemagglutinin gene differs substantially from strains that have circulated in humans in recent decades. In the examples known to date, this has resulted from “reassortment” of genetic material from two or more influenza viruses. In this reassortment, the gene for the novel hemagglutinin comes from one virus and the rest of the genetic material comes from a different virus or multiple viruses. Such reassortment can occur when a host cell (in humans, pigs, birds, or other host species) is infected simultaneously by more than one genetically distinct virus. If an antigenically shifted influenza A strain is capable of being transmitted from human to human, it may cause a new pandemic of influenza. Pandemics are defined by genetically novel viruses that spread widely and infect more humans than are infected in a normal influenza season because few individuals have immunity to the novel hemagglutinin. The novel strain may also cause more severe disease than usual due to the lack of immunity, the strain’s novel genetic characteristics, or both. Pandemics have occurred most recently in 1889–1892, 1918, 1957, 1968, and 2009.

More information about antigenic shift can be found at <http://www3.niaid.nih.gov/topics/Flu/Research/basic/pages/AntigenicShiftIllustration.aspx>.

Students may be interested to learn that in 2005, scientists sequenced the entire genome

of an influenza virus from someone who died of the flu in 1918 (Taubenberger et al., 2005). The person was buried in permafrost and was remarkably well preserved.

5.7 Vitamin C Biosynthesis

All eukaryotic organisms contain nonfunctional sequences of DNA called “pseudogenes.” These genetically silent sequences of DNA are vestiges of once-functional genes inherited from ancient ancestors. One such pseudogene explains why all mammals, except humans, guinea pigs, and bats, can synthesize vitamin C. Mammals inherited a functional version of the gene for synthesizing vitamin C from an early ancestor. But in independent events in humans, guinea pigs, and some bats, the gene, even

though present, later mutated and became nonfunctional. As a result, humans, guinea pigs, and some bats have to obtain vitamin C from their food.

Scientists have found the pseudogene for an enzyme that’s required for vitamin C biosynthesis, L-gulonolactone oxidase (GULO), in the human genome (Nishikimi et al., 1994). Comparisons with the functional gene in other species show that the human pseudogene has many **substitutions** that eliminate its function. Some of these substitutions have produced stop codons within the human pseudogene.

Additional information about the evolution of vitamin C metabolism in humans is available in Lesson 5.

Glossary

alignment: Information lined up to show similarities or differences between different samples. Using complicated computer models, scientists create alignments of genetic sequences or amino acids and then use them to develop evolutionary trees.

allele: Different forms of the same gene or stretch of DNA. New alleles form through mutations. Sometimes, different forms of a gene or stretch of DNA cause different phenotypes.

ancestral state: The characteristic that was found in a common ancestor of two or more organisms. For example, suppose there are two species of plant, one with yellow flowers and one with red flowers. Scientists may use evidence to infer that the common ancestor of the two species also had a red flower. Red flowers would be the ancestral state.

aniridia: A rare genetic disease in humans caused by mutations to the *Pax6* gene. People with aniridia are missing part of the iris in the eye. People with aniridia are heterozygous for a working *Pax6* gene. They often also have other problems with their eyes.

antigenic drift: One type of evolutionary change in influenza viruses that results in relatively small changes among influenza strains from year to year.

antigenic shift: One type of evolutionary change in influenza viruses that occurs when a new strain of influenza differs substantially from strains that have recently circulated. In the examples known to date, this has resulted from “reassortment” of genetic material from two or more influenza viruses. In this reassortment, some of the genetic material comes from one virus and the rest comes from a different virus or multiple viruses.

endocytosis: A process in which a cell takes in materials from outside the cell by folding in the plasma membrane.

epidemiology: The study of diseases in a population. Scientists look for patterns in a disease to determine its cause and to find ways to prevent the disease.

evolution: Broadly, change over time. In biology, evolution is the change over time in the frequency of alleles in a population.

evolutionary tree: A diagram that summarizes the relationships among different organisms. These diagrams are based on evidence. Scientists revise them when new evidence indicates different relationships.

evoprint: A diagram that summarizes the similarities and differences in a genetic sequence for multiple species.

hemagglutinin: A protein in influenza viruses that helps the virus get inside host cells.

heritable: The degree to which a trait can be passed from parents to offspring. A trait that is highly heritable is controlled by genes.

heterozygote advantage: A situation in which individuals that have two different forms of a gene have relatively higher survival or reproductive rates compared to individuals that have one form of the gene.

homology: When two or more species have a trait that came from a common ancestor. Scientists use evidence to determine homology.

influenza: A disease caused by the influenza virus. The disease is often called the flu. Influenza is highly contagious. The influenza virus infects lung cells and causes respiratory problems. The illness caused by the influenza virus can be mild, severe, or even fatal. A wide range of influenza viruses infects birds; a smaller range infects humans.

lactase: An enzyme that breaks down the sugar lactose into two simpler sugars.

lactase nonpersistence: The condition in which individuals stop making the lactase enzyme sometime in their lives. Most human infants make lactase. After infancy, some people stop making the enzyme.

lactase persistence: The condition in which individuals make the lactase enzyme throughout their lives.

lactose intolerant: Individuals who are unable to digest the lactose sugar in milk or dairy products. When lactose intolerant individuals ingest foods with lactose, they have symptoms that can be mild or severe. Symptoms include gas, diarrhea, and pain in the abdomen. Lactose intolerant individuals are often lactase nonpersistent.

lactose tolerant: Individuals who can digest the sugar called lactose. Lactose is found in milk and other dairy products. Lactose is broken down by the enzyme lactase. People who are lactose tolerant are lactase persistent.

lineage: A line of populations or species that are connected over time. The group may contain a single species, a single population, or a group of species.

malaria: An infectious disease caused by a parasite spread to humans by mosquitoes. Symptoms of malaria include fever, chills, flulike symptoms, and anemia. Malaria causes about 1 million deaths per year, mostly in children.

methicillin-resistant *Staphylococcus aureus* (MRSA): A strain of bacteria that is not killed by methicillin, a specific type of antibiotic.

model species: Animals or other organisms that scientists often use in medical research. Information learned from experiments in these species helps researchers better understand human health and disease. This is because humans share many features with other organisms due to common ancestry. Examples of model species are mice, fruit flies, zebrafish, and the bacterium *Escherichia coli*. They are sometimes referred to as “model organisms.”

mutation: Any change to genetic information in an individual. This can include changing one nucleotide, deleting nucleotides, or adding new nucleotides. Mutations cause variation among different individuals. Mutations occur randomly. Most mutations are harmful, but some can be helpful to individuals.

natural selection: A process that can cause evolution. This process can result in adaptations. It can be summarized as five principles:

1. **variation:** Individuals within a population vary in many traits, including physical and biochemical traits.
2. **inheritance:** Some of the differences in traits among individuals can be passed from parents to offspring. (Some variation is heritable.)
3. **origin of variation:** Some of the variation in traits among individuals has a genetic basis. This variation originated, often many generations ago, as mutations—changes in the genetic information that are random with respect to the needs of the organism.
4. **fitness:** Both the environment and the traits that individuals possess affect survival and reproduction. Individuals with heritable traits that enable them to better survive and reproduce in a particular environment will leave relatively more offspring.
5. **evolutionary change in populations:** The frequency of traits and the alleles that affect those traits change in a population over time.

population: All the individuals from one species living in one area.

positive selection: A type of natural selection. In positive selection, a new form of a gene (allele), and the protein it produces, is favored. The new form of the gene spreads quickly within a population.

purifying selection: A type of natural selection. This type of selection eliminates or decreases the frequency of mutations to a gene that have a negative effect. In other words, the mutations cause serious health problems or death to individuals who have them. Natural selection then eliminates these mutations from a population.

substitution: The replacement of one nucleotide with another within a lineage of organisms. For example, the DNA nucleotide A (adenine) may be replaced with nucleotide C (cytosine) in one species of organisms.

thalassemia: A genetic disease that causes anemia. Thalassemia results from mutations in the alpha-globin gene that produces the alpha-hemoglobin protein. The disease is more severe if a person has more alleles of the alpha-globin gene that do not work correctly. A person who has one nonfunctional allele has few symptoms; someone with no functional alleles of the alpha-globin gene usually dies before or soon after birth.

Van der Woude syndrome: A genetic disease that is one cause of cleft lip and palate. Mutations to the *Irf6* gene cause the syndrome.

variation: Differences among individuals for some trait in a population. For example, some frogs in a population might have longer legs than others do. The differing leg lengths is variation.

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Lesson 1

Ideas about the Role of Evolution in Medicine



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Engage

At a Glance

Overview

Lesson 1 consists of two activities and should take two 50-minute class periods to complete. In the first activity, students offer explanations for the evolution of antibiotic-resistant bacteria. These initial explanations give you an idea of your students' ideas about evolution by natural selection. In the second activity, students examine data from the *Pax6* gene in different species used as models in medical studies. Students suggest how an understanding of common ancestry enables scientists to use model species to learn about human health.

Activity 1: *Outbreak!*

Estimated time: 30 minutes

Major Concepts

- Mutation is the source of the genetic variation that is acted on by natural selection.
- Natural selection is a powerful process of evolution and is the only mechanism to consistently yield adaptations.
- Understanding mechanisms of evolution, particularly adaptation by natural selection, provides many insights that enhance medical practice and understanding.

Objectives

After completing this lesson, students will

- have expressed their ideas about the source of genetic variation,
 - have described their thoughts about the role of evolution in antibiotic resistance, and
 - appreciate that evolution and medicine are relevant in their lives.
-

Activity 2: Models and Medicine

Estimated time: 70 minutes

Major Concepts

- “Descent with modification” suggests that modern organisms inherited their traits from ancestors and that modern species all share common ancestors at some point in time. The characteristics of living organisms are shaped by this history.
- Phylogenetic information from diverse organisms across the “Tree of Life” offers many insights that inform medicine.

Objectives

After completing this lesson, students will

- have expressed their ideas about why various organisms can serve as model species that help scientists learn about health-related issues in humans and
 - have expressed their ideas about interpreting an evolutionary tree.
-

Teacher Background

Consult the following sections in Information about Evolution and Medicine:

- 1.0 Fundamentals of Evolution and Medicine (pages 21–27)
 - 4.0 Students’ Prior Conceptions about Evolution (pages 30–32)
 - 5.0 Featured Examples of Evolution and Medicine (pages 32–37)
-

In Advance

Web-Based Activities

Activity	Web Component?
1	No
2	No

Photocopies, Transparencies, Equipment, and Materials

Photocopies and Transparencies	Equipment and Materials
Activity 1: Outbreak! 1 copy of Master 1.1 for each student	Activity 1 None
Activity 2: Models and Medicine 1 transparency each of Masters 1.2 and 1.5 1 color transparency of Master 1.4 1 copy each of Masters 1.3 and 1.5 for each student	Activity 2 Different-colored pens or pencils

Preparation

Activity 1

Each student will need to maintain a notebook or folder dedicated to this supplement for handouts and a record of their ideas. The lessons include opportunities for students to record their initial ideas and answers to questions about evolution and medicine. To help monitor their own understandings and track how their thinking has changed, students will frequently refer back to their previous work and their initial understandings. Decide what format will work best for your students. If your students normally use bound composition books, they can continue to use these and tape or staple handouts into the book. Alternatively, students can record their notes on notebook paper and keep their notes and their handouts in binders or folders.

Activity 2

No other preparations are needed except for making copies and transparencies.

Procedure

Activity 1: *Outbreak!*

Estimated time: 30 minutes

Note: During Activity 1 of this Engage lesson, students have an opportunity to express their initial thoughts about the source of genetic variation and the role of natural selection in the context of the spread of antibiotic resistance in bacteria. The activity centers on an outbreak of illness caused by a strain of bacteria that is resistant to one class of antibiotic drugs. The intent of the activity is not to teach content related to genetic variation and natural selection but rather to elicit students' prior knowledge about these concepts. We chose the antibiotic-resistance scenario because it represents an example of natural selection in action that is relevant to students' lives. Responses to the questions posed during the activity can help you assess students' relative familiarity with the

concepts and identify misconceptions. Intended to be brief, this initial assessment of preconceptions can help you adjust your teaching of the remaining lessons of the supplement.

We do not intend for the lessons in this supplement to be students' only exposure to the concepts of genetic variation, natural selection, and common ancestry. Ideally, before beginning the supplement, students already will have been introduced to

- Mendel's laws;
- the central dogma that DNA codes for mRNA, which directs the synthesis of proteins;
- types of mutations;
- natural selection and adaptation; and
- evolution and common ancestry.

1. Begin the lesson by explaining that you saw a news story about a disease outbreak at two local high schools. Read the following news story to the students:

“News flash: This just in from our News 8 Action Alert Team. Two local high schools are reporting cases of students infected with the ‘superbug’ called ‘methicillin-resistant *Staphylococcus aureus*,’ or ‘MRSA’ [pronounced mer-sah] for short. At one school, four members of the football team were diagnosed and treated for MRSA infections. Three friends at a second school are also reported to have MRSA infections. School officials are telling parents and students not to panic. Cleaning crews at both schools are sanitizing locker rooms and classrooms. Students are being told to pay better attention to hygiene. They should wash their hands frequently, keep cuts and scrapes clean and covered with bandages, and not share water bottles or towels.”

This step is designed to grab students' attention and help make the connection between their lives and evolution and medicine immediately apparent. Many communities are experiencing MRSA outbreaks in schools.



Tip from the field test: You may consider supplementing this step by using a local MRSA-related story.

2. Ask students if they know anyone who has been affected by MRSA. Ask one or two students to share their experiences.

If nobody volunteers, bring up any familiar local cases of MRSA infections. If you are unaware of any recent outbreaks, consider sharing the following story. The outbreak of MRSA among the St. Louis Rams is described in a 2005 article by Kazakova et al.

Near the end of October 2007, six high school football players from North Carolina had infections that looked like spider bites or large pimples. The infections grew larger and were identified as MRSA. All the students were eventually treated successfully with a class of antibiotics different from methicillin. The school started sanitizing lockers once a week and wrestling mats and gym towels every day. Similar cases occur across the country. One case that received a lot of attention involved a 2003 MRSA outbreak among eight players on the St. Louis Rams professional football team.

3. **Hand out one copy of Master 1.1, *Information about MRSA*, to each student. Instruct students to read the information. Provide an opportunity for students to ask about any unfamiliar terms.**

Encourage students to keep a list of unfamiliar and important terms on a separate page in their notebooks.



Tip from the field test: If your class has a large number of English language learners, consider having volunteers read Master 1.1 aloud to the class.

4. **Direct students to work independently to answer the questions at the end of Master 1.1.**

Give students a few minutes to write their best ideas at this point. The questions in this step are designed to elicit the students' initial ideas about the role of evolution in infectious diseases, including the evolution of antibiotic and antiviral resistance. You may need to reassure students that these questions are not part of a "test." Do not correct individual answers or go over the correct answers with students at this point in the learning cycle. They will revisit their answers to these questions later in the supplement.

Examine students' answers for common misconceptions such as ideas that the bacterial population purposefully changed or that it changed simply because it "needed to" or "wanted to." Expect many students to be confused about mutations and the origin of genetic variation. Section 4.0 of the Information chapter contains a further explanation of students' common misconceptions about evolution (page 31).

Optimally, answers to these questions should touch on the five principles of natural selection described in Box 1 (see also Section 1.0 of the Information chapter, page 21). However, it is likely that students won't have this level of understanding yet. For your background information, the answers in the answer key incorporate the five principles.



Students' responses to these questions will help you assess their current understandings about genetic variation and adaptation by natural selection.

Box 1. Five Major Principles of Natural Selection

Throughout this curriculum supplement, you will ask students to focus on and practice using five principles related to natural selection. Students will consider different examples and situations and how each principle is represented. As students continue building their understandings, their explanations of why each principle is important for understanding natural selection and evolution should become clearer and more complete.

1. **Variation:** Individuals within a population vary in many traits, including physical and biochemical ones.
2. **Inheritance:** Some of the differences in traits among individuals can be passed from parents to offspring. (They are heritable.)
3. **Origin of variation:** Some of the variation in traits among individuals has a genetic basis. This variation originated, often many generations ago, as mutations—changes in the genetic information that are random with respect to the needs of the organism.
4. **Fitness:** Both the environment and the traits individuals possess affect survival and reproduction. Individuals with heritable traits that enable them to better survive and reproduce in a particular environment will leave more offspring.
5. **Evolutionary change in populations:** The frequency of traits and the alleles that affect those traits change in a population over time.

Answer key for questions on Master 1.1, *Information about MRSA*

1. Researchers developed the antibiotic methicillin to treat people with infections of *S. aureus* that are resistant to penicillin. Within two years, populations of *S. aureus* that were resistant to methicillin started showing up in hospitals. How would a scientist explain how the change may have occurred in *S. aureus* populations?

Typical student responses (ranging from partially accurate to inaccurate) may include the following:

- *The genetic information in the populations mutated.*
- *The populations changed or evolved.*
- *The population got stronger.*
- *Individuals in the population changed because they needed or wanted to.*

*A complete explanation of the evolution of resistance in *S. aureus* populations includes five major principles:*

- 1) *Variation: Within a population of *S. aureus*, some individuals are resistant to methicillin and some are not.*
- 2) *Inheritance: Resistance to methicillin in *S. aureus* has a genetic basis and is passed from parent to offspring.*

- 3) *Origin of variation: Random mutations in the genetic information of S. aureus led to differences in traits among individuals in the population.*
- 4) *Fitness: When exposed to methicillin, individual S. aureus that were resistant to methicillin left more offspring than those that were not resistant.*
- 5) *Evolutionary change in populations: The frequency of the allele that causes resistance to methicillin increased in the S. aureus population over time.*

Note: A common misconception about antibiotic resistance is that the people who have the infection are resistant to antibiotics. In reality, it is the *bacteria* that are resistant, and these resistant bacteria are passed from person to person.

2. How can the study of evolution (such as the adaptation of bacterial populations to an antibiotic) help researchers improve people's health? Explain your initial ideas.

Students' answers will vary. Use this question as a formative assessment of how students view the role of evolution within medicine.

5. **Wrap up this activity by holding a brief class discussion about students' ideas.**

Do not provide the correct answers. The point of this discussion is to focus on differences of opinion among the students so that students recognize that their classmates may have different ideas.

Activity 2: Models and Medicine

Estimated time: 70 minutes

Note: This activity prompts students to begin thinking about how common ancestry enables scientists to use model organisms to learn about health conditions. Students will consider the *Pax6* gene, which is involved in eye development during embryogenesis. We chose this gene because its sequence is very similar in a wide variety of species; it was inherited from a distant common ancestor and has not changed much in any of the species. In addition, students can see examples of phenotypic changes that occur when mutations occur. This activity also introduces students to interpreting evolutionary trees, which are useful tools for understanding relationships among species.

1. **Ask students to describe what they learned about mutations and disease from the MRSA case. Then ask them to consider how mutations in people might affect health.**

This is an opportunity to further assess students' developing understandings from the previous activity. In the case of MRSA, the mutations occurred in the *S. aureus* bacteria (not in the humans who developed the disease). The mutations in the bacteria (often in previous generations) enabled some of them to be resistant to methicillin.

Step 1 helps students transition from the idea of a mutation in a pathogen causing a human disease to the idea that mutations in people can cause a disease. Students will likely say that mutations will cause disease or even death. Step 1 enables you to gauge their understandings of mutations.

- 2. Explain to the class that they will now consider a human disease. The goal is for students to express ideas about how scientists can use their knowledge of evolution to inform medical research. Project Master 1.2, *Aniridia: An Eye Disease*. Ask for a volunteer to read the information aloud to the class. Then give students a few minutes to discuss the questions with a partner before participating in a class discussion.**

In this brief vignette, students will read about a family affected by an eye disease called aniridia and deduce that it may have a genetic basis.

Answer key for questions on Master 1.2, *Aniridia: An Eye Disease*

1. Do you think the parents' concern for their baby's health is justified? Why?

Most expectant parents are concerned about the baby's health. These parents are understandably more anxious than normal because of the family history of aniridia (baby's father and grandmother).

2. From the information provided, would you expect aniridia to be caused by an infection, an environmental factor, or a genetic factor? Explain your answer.

The family history is a clue that aniridia has a genetic basis.

- 3. Confirm for students that aniridia is a genetic disease. Scientists have identified and isolated the gene that is mutated in aniridia. The gene is called "Pax6" and is important for the development of eyes in an embryo.**

Pax6 plays an important role in the development of the brain and pancreas as well as the eyes. However, for the purpose of this activity, students will focus on the gene's role in eye development.

4. **Explain that students will now examine data about aniridia and consider how an understanding of evolution can inform medicine.**
5. **Give each student one copy of Master 1.3, *Learning about Human Health from Other Organisms*. Inform students that they will work in groups of three to four to analyze the information provided and respond to the questions.**

Part 1 of the handout includes a portion of the amino acid sequence for the protein encoded by *Pax6*. This sequence uses the one-letter codes for the amino acids. Students may be more accustomed to the three-letter codes. If so, explain that this code is just a different abbreviation for the amino acids and is more commonly used by scientists. For the *Pax6* example, there are six differences in this part of the fruit fly protein compared with the other organisms on Master 1.3.

Students simply need to recognize that the sequences for the *Pax6* protein are very similar among these organisms. It is not essential that students know the specific changes in the protein sequence.

Part 2 on the handout requires looking at evidence captured in photographs. When students reach Part 2, project a color version of **Master 1.4, *Photographs of Pax6 Mutations***. As students work, circulate among groups to monitor students' discussions and ask guiding questions if necessary.

Because of the orientation of the photomicrographs, you may need to help students understand the images. The picture of the human eye will probably be understandable to students. The pictures of the mouse eye and zebrafish eye may confuse students. Instead of looking at the eye from the front (as in the picture of the human eye), the mouse and zebrafish images are cross sections of the developing eye; they show the eye from the side. The fruit fly (*Drosophila*) has compound eyes that look very different from the other organisms' eyes. The purpose of students making these observations is to look at evidence for gene function (not to learn the anatomy of the eye). The main thing that students should observe is that, for all these species, eye development is abnormal in *Pax6* mutants.

6. **Hold a brief class discussion to go over the answers that students gave to the questions on Master 1.3.**

Simply allow a few students to express their ideas. Do not go through the correct answers at this point. Students will revise their answers later.



Content Standard A:
Formulate and revise scientific explanations and models using logic and evidence.

Content Standard A:
Recognize and analyze alternative explanations and models.

Answer key for questions on Master 1.3, *Learning about Human Health from Other Organisms*

Part 1

1. What do you notice about the amino acid sequences in the different species?

In all four species, the amino acid sequences are very similar. In fact, for this part of the protein, the sequences are identical in zebrafish, mice, and humans. The fruit fly sequence differs by six amino acids.

2. What can you infer about the *Pax6* gene from the protein sequences from these four species?

Because the protein sequences are so similar, students should expect the gene sequences also to be very similar in these organisms. (If students have trouble making this connection, remind them that the DNA sequence codes for the protein sequence.)

Part 2

3. On the basis of the pictures, do you think the function of the *Pax6* gene is similar in all four species? Explain your reasoning.

*In each of the different species, individuals with mutations in the *Pax6* gene have abnormalities in the eye structure. This suggests that *Pax6* plays a role in the formation of the eye in each species.*

Part 3

4. What do these experiments suggest about the function of the *Pax6* gene? Explain your thinking.

*These experiments suggest that the *Pax6* gene is similar enough in all these species that the gene can still function in a different species.*

Note: Although students don't need to know this level of detail, they may still have questions. When either the squid *Pax6* gene or the mouse *Pax6* gene is expressed in a fruit fly, the eye that develops is a fly eye—not an eye of the original species from which the gene was taken. Although *Pax6* is critical for eye development, many other genes also play an important role in specifying eye anatomy. The combination of these genetic influences with the *Pax6* gene determines the kind of eye that forms. So in a normal mouse, the *Pax6* gene interacts with other genes involved in eye development to form a mouse eye, and in a squid, the *Pax6* gene interacts with other genes involved in eye development to form a squid eye. When the *Pax6* gene from either of these species is inserted into and expressed in a fruit fly, *Pax6* interacts with fruit fly genes involved in eye development to form a fly eye.

Summary Question

5. In the sequence data, you saw that the protein coded by the *Pax6* gene is very similar in fruit flies, zebrafish, mice, and

humans. In the other experiments, you examined evidence related to the gene's function. Why might many species have almost exactly the same gene that has a similar function?

Students may answer in a variety of ways, depending on their prior knowledge. They may express ideas about common ancestry or they may phrase the answer in terms of the gene being selected for throughout evolution.

7. **Continue the discussion by asking students, “Do the *Pax6* experiments support the idea that medical scientists can learn about gene function in humans by studying different organisms?”**

Yes, these experiments do support the idea that medical scientists can learn about human health by studying other organisms. The *Pax6* gene is a good example because

- it is conserved across a wide variety of organisms;
- when mutated, it causes abnormalities in eye development in all species; and
- the gene can be expressed in other organisms and produce the same feature that it normally would.

This leads scientists to the conclusion that the mechanisms that regulate this gene are probably very similar in a wide variety of organisms, including humans.

8. **After students express their ideas, inform them that species commonly used in scientific research are called “model species” or “model organisms.” Briefly discuss why model organisms might be valuable for scientific research. Prompt the discussion by asking, “Why would a scientist want to study a gene and its function in a mouse, a fruit fly, a zebrafish, or even a bacterium when they already know the gene is present in humans?”**

It can be difficult to study some issues in humans. Not only are the generation times very long, but some investigations would be unethical to do in humans. For example, it would never be appropriate to induce mutations intentionally in humans because the potential consequences of this manipulation are unknown.

The organisms that students considered in the *Pax6* example are very common model species, but many others exist. Which species a scientist uses for a model depends on the question being investigated. For example, scientists' extensive knowledge of the fruit fly's genetic makeup and its relatively short generation time are advantages. For some questions, a scientist would want to study a mammalian system, making the mouse a good choice.

This discussion does not need to be long, but use it as an opportunity to help students understand why medical researchers rely on model organisms to learn about human health issues.

- 9. Tell students that understanding evolution helps us explain why model species are useful for understanding human disease. Introduce students to evolutionary trees by explaining that these diagrams show relationships among species. Hand out one copy of Master 1.5, *An Evolutionary Tree*, to each student. Ask the students to use the evolutionary tree to answer the questions on the handout.**

Again, you may need to reassure students that these questions are not part of a test. Instead, the questions are designed so that they can express their initial ideas about how to interpret an evolutionary tree diagram. They will revisit their answers to these questions in Steps 11 and 12. Give students about five minutes to complete this task.

This is a chance to look for prominent misconceptions that students may have (which will inform your teaching for Step 11; see Section 4.0 in the Information chapter, page 31). Do not go over the correct answers with students at this point in the learning cycle; the correct answers are included with Step 12. The information below points out some common responses, problems, and misconceptions that students may have when interpreting trees:

1. What part of the evolutionary tree diagram represents the common ancestor of humans, mice, and zebrafish (but not fruit flies)? Why did you identify this part of the diagram?

Students' responses to this will indicate their current understandings of how a common ancestor is represented on an evolutionary tree. Students may not, at this point, understand that the line leading up to the splitting point labeled "2" represents a lineage that is the common ancestor of humans, mice, and zebrafish.

2. Does the evolutionary tree suggest that the mouse is more closely related to the zebrafish or the fruit fly?

The common incorrect response is that the mouse is more closely related to the fruit fly than to the zebrafish because the two organisms are next to each other on the evolutionary tree. This does not take into account that the mouse and the zebrafish share a more recent common ancestor than do the mouse and the fruit fly. This response may indicate that students do not understand what the splitting points signify or how time is represented on the evolutionary tree.

3. Is the fruit fly the ancestor of all the other species on the evolutionary tree? Explain your answer.

No. Many students assume that the species on the left part of the diagram or the species that branched off from the other species in the most distant past is the ancestor of the other species on the tree. Again, this may indicate a lack of understanding of the splitting points or of the representation of time on the evolutionary tree.

4. What does the vertical line beneath Point 1 represent?

The vertical line beneath Point 1 represents the root of this evolutionary tree. This line is the lineage that leads to the most recent common ancestor of the four species shown on the tree.

10. **Project Master 1.5. This evolutionary tree shows the relationships among the four species in the Pax6 case. Go over the following information with students. Ask them to record notes on their handouts.**

The information in bold below is essential for students to understand. The information in parentheses is additional information for you to use if questions arise.

The evolutionary tree in Master 1.5 shows the relationships among four different species.

- **The vertical lines represent separate lineages.** A lineage is the line of species and populations for a group of organisms over time. The group may contain a single species, a single population, or a group of species. (A common misconception is that the vertical branches, or lineages, on the tree indicate that no change occurred over time (that is, that the lines are simply connections between one ancestor species and the present-day species). In actuality, change occurs throughout time. There were certainly other organisms that went extinct on the lineage between the common ancestor and the zebrafish, for example.)
- **The vertical line below Point 1 represents the root of the tree, or the lineage leading to the common ancestor shown on this tree.**
- **The splitting points show where one lineage splits into two new lineages. The organism at the splitting point is the common ancestor for the two new lineages.**
 - **Point 1 is the most recent common ancestor of all the organisms on the tree.**
 - **Point 2 is the most recent common ancestor of zebrafish, humans, and mice.** (This ancestor can be described as “fishlike,” but it was not the same as today’s fish.)
 - **Point 3 is the most recent common ancestor of humans and mice.**



Content Standard C:
The millions of different species of plants, animals, and microorganisms that live on earth today are related by descent from common ancestors.

- **Time is represented on the vertical axis of the diagram.**
The present time is at the top. More-distant historical times are toward the bottom.
- **To answer the question, Who is more closely related to whom? compare the most recent point where the different organisms share a common ancestor.** An organism is more closely related to the organisms with which it shares more recent common ancestors. If students understand this, they should understand that the sequence of organisms from left to right on the diagram is not informative. It may help to describe the evolutionary tree as a mobile that can be picked up by the root and twirled without changing any of the relationships among the species.

- 11. Ask students to work with a partner to use the information they just learned to revise their answers to the questions on Master 1.5. Ask students to use a different-colored pen or pencil to make their revisions. Encourage students to simply put a single line through any text they wish to delete instead of erasing or scribbling it out.**

Individual students should describe why they answered the questions as they did and should change their answers based on what they learned in Step 10. Using a different color for revisions makes it easier for students to see when they changed their minds. This reinforces the importance of students monitoring their own understandings.

- 12. Conduct a class discussion to review students' revised answers to the questions on Master 1.5. Ask students to explain their answers and compare them with their initial ideas.**

Answer key for questions on Master 1.5, *An Evolutionary Tree*

1. What part of the evolutionary tree diagram represents the common ancestor of humans, mice, and zebrafish (but not fruit flies)? Why did you identify this part of the diagram?

The common ancestor for humans, mice, and zebrafish is at Point 2 on the evolutionary tree. Point 3 represents the common ancestor for mice and humans, and Point 1 is the common ancestor for all four species.

2. Does the evolutionary tree suggest that the mouse is more closely related to the zebrafish or the fruit fly?

The mouse is more closely related to the zebrafish than the fruit fly. To answer this question, students need to find the most recent common ancestor of the mouse and the zebrafish and then of the mouse and the fruit fly. Once they identify the most recent common

ancestor, they can mark the time frame for the existence of that common ancestor on the timeline. In this case, the most recent common ancestor for the mouse and the zebrafish occurred more recently than did the most recent common ancestor for the mouse and the fruit fly.

3. Is the fruit fly the ancestor of all the other species on the evolutionary tree? Explain your answer.

No. The fruit fly is a modern, living species. Fruit flies share a common ancestor with all the other species on the diagram.

4. What does the vertical line beneath Point 1 represent?

The vertical line beneath Point 1 represents the root of this evolutionary tree. It is the lineage that represents the common ancestor of the four species shown on the tree.

13. Conclude the activity by asking students to consider the following questions:

- **Do you think that the common ancestor of fruit flies, zebrafish, mice, and humans had a gene similar to Pax6? Explain your answer.**

If students understand that the Pax6 gene is very similar in these four species and that the four species all share a common ancestor, then students should respond that Pax6 was present in the common ancestor. Students should cite the highly similar amino acid sequences for the Pax6 protein in the four species and the evolutionary tree as evidence.

- **How does shared ancestry explain why scientists can use model organisms to learn about human health?**

Common (shared) ancestry allows scientists to study genes in model organisms that are similar to those in humans and make inferences to how the gene and its products function in humans. This allows scientists to conduct studies that may not be feasible in humans. If a gene had a different function in a different species or if there were a large number of significant changes in the gene in a given species, then that species might not be a good candidate as a model organism for that particular trait. Pax6 is a very strong example of the conservation of both gene sequence and gene function; not only is the sequence highly conserved in a wide variety of species, but it has a similar function in all of these species. Therefore, learning how it functions in a model organism (descended from the same common ancestor as humans) can provide valuable information for scientists about how it functions in humans.



Before discussing the answers to the questions in Step 13, you may want to ask students to write their answers first. You can then collect their responses to assess their understandings of common ancestry and its relationship to the value of model organisms. Students will revise their answers to these questions later in the supplement.

Lesson 1 Organizer

Activity 1: *Outbreak!*

Estimated time: 30 minutes

Read to the class the news story about an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA).

Page 54
Step 1

Ask one or two students who have had experiences with MRSA to share them with the class.

Page 54
Step 2

Give each student 1 copy of **Master 1.1**.

- Instruct them to read and answer the questions on the master.
- Conduct a brief discussion about students' answers.

Steps 3–5



Activity 2: *Models and Medicine*

Estimated time: 70 minutes

Ask students to describe what they learned about mutations and disease from the MRSA case. Ask how mutations in people might affect health.

Page 57
Step 1

Explain that students will learn more about how evolution informs medical research.

- Project **Master 1.2**.
- Have a volunteer read the information aloud.
- Allow time for students to discuss the questions on the handout with a partner before holding a class discussion.

Page 58
Step 2



Confirm for students that aniridia is a genetic disease. Explain that the *Pax6* gene is responsible for aniridia and it is involved in eye development.

Page 58
Step 3

Explain that students will analyze data about aniridia.

- Give each student 1 copy of **Master 1.3**. Explain that students will work in groups of three to four to analyze data and respond to questions.
- When students reach Part 2 of the master, project a color version of **Master 1.4**.

Page 59
Step 4–5



- Hold a class discussion about students' answers to questions on **Master 1.3**.

Page 59
Step 6

Ask, "Do the *Pax6* experiments support the idea that medical scientists can learn about gene function in humans by studying different organisms?" Briefly discuss this as a class.

Page 61
Step 7

Inform students that species commonly used in scientific research are called model species or model organisms.

- Ask, "Why would scientists want to study a gene and its function in a mouse, a fruit fly, or even a bacterium when they already know the gene is present in humans?"

Page 61
Step 8

<p>Introduce students to evolutionary trees by explaining that these diagrams show relationships among species.</p> <ul style="list-style-type: none"> • Give each student 1 copy of Master 1.5. • Allow time for students to answer the questions. Watch for difficulties and misconceptions but do not provide the correct answers. 	<p>Page 62 Step 9</p> <p>M</p>
<p>Project Master 1.5. Explain that the evolutionary tree shows the relationships among four different species:</p> <ul style="list-style-type: none"> • The vertical lines represent separate lineages. • The vertical line below Point 1 represents the root of the tree, or the lineage leading to the common ancestor. • The splitting points show where one lineage splits into two new lineages. The organism at the splitting point is the common ancestor for the two new lineages. <ul style="list-style-type: none"> ◦ Point 1 is the most recent common ancestor of all the organisms on the tree. ◦ Point 2 is the most recent common ancestor of zebrafish, humans, and mice. ◦ Point 3 is the most recent common ancestor of humans and mice. • Time is represented on the vertical axis, with present time at the top and more-distant historical times toward the bottom. • To answer this question, Who is more closely related to whom? compare the most recent point where the different organisms share a common ancestor. An organism is more closely related to the organisms with which it shares more recent common ancestors. 	<p>Page 63 Step 10</p> <p>T</p>
<p>Ask students to revisit the questions on Master 1.5 with a partner. Instruct them to revise their answers using a different-colored pen or pencil and not to erase their earlier answers.</p>	<p>Page 64 Step 11</p>
<p>As a class, discuss the revised answers to the questions on Master 1.5.</p>	<p>Page 64 Step 12</p>
<p>Conclude the activity by asking,</p> <ul style="list-style-type: none"> • “Do you think that the common ancestor of fruit flies, zebrafish, mice, and humans had a gene similar to <i>Pax6</i>? Explain your answer.” • “How does shared ancestry explain why scientists can use model organisms to learn about human health?” 	<p>Page 65 Step 13</p>

M = Involves copying a master. **T** = Involves making a transparency.

Lesson 2

Investigating Lactose Intolerance and Evolution



Explore

At a Glance

Overview

Lesson 2 is an Explore activity that begins with a laboratory investigation of lactase. Students examine their results and the results from a larger sample of individuals across Africa, Europe, the Middle East, and Asia, and then make initial explanations of the patterns they see for lactase persistence. Students investigate the genetic basis for lactase persistence and learn that different mutations are responsible for lactase persistence in different populations and that each mutation arose independently. Students then use data to analyze two alternative hypotheses based on natural selection that attempt to explain the global patterns in lactase persistence.

Major Concepts

- Natural selection is a powerful process of evolution and is the only mechanism to consistently yield adaptations.
- Some variation among humans is distributed geographically.
- Understanding mechanisms of evolution, particularly adaptation by natural selection, provides many insights into why humans are susceptible to disease.
- Natural selection depends on specific environmental contexts.

Objectives

- After completing this lesson, students will begin to understand
- the importance of biologists studying the genetics of a large number of humans and other organisms;
 - that evolution explains many aspects of why humans as a species are the way they are;

- that health and disease are related to our evolutionary history;
- that selection is acting at the level of the phenotype, and the phenotype is a product of genes, environment, and their interactions; and
- that natural selection only acts on traits that influence reproductive success.

Teacher Background

Consult the following sections in Information about Evolution and Medicine:

- 1.0 Fundamentals of Evolution and Medicine (pages 21–27)
- 3.0 Specific Applications of Evolution in Medicine (pages 27–31)
- 4.0 Students’ Prior Conceptions about Evolution (pages 31–33)
- 5.0 Featured Examples of Evolution and Medicine (pages 32–37)

In Advance

Web-Based Activities

Activity	Web Component?
1	Yes

Photocopies, Transparencies, Equipment, and Materials

Photocopies and Transparencies
<p>For Classes Using Web-Based Activity:</p> <ul style="list-style-type: none"> 1 copy each of Masters 2.1, 2.3, 2.6, and 2.10 for each student 1 transparency each of Masters 2.2, 2.4, 2.5, and 2.7 1 copy of Master 2.8, <i>Data from Europe, Part A</i>, for each student in half of the class 1 copy of Master 2.9, <i>Data from Europe, Part B</i>, for each student in the other half of the class
<p>For Classes Using Print-Based Activity:</p> <ul style="list-style-type: none"> 1 copy each of Masters 2.1, 2.6, 2.10, and 2.13 for each student 1 transparency each of Masters 2.2, 2.4, 2.5, 2.7, 2.11, 2.12, and 2.14 1 copy of Master 2.8, <i>Data from Europe, Part A</i>, for each student in half of the class 1 copy of Master 2.9, <i>Data from Europe, Part B</i>, for each student in the other half of the class
Equipment and Materials
<ul style="list-style-type: none"> 1 lactase tablet 100 milliliters (mL) skim milk water 72 glucose test strips 2 250-mL beakers 2 test-tube racks 70 test tubes (18 × 150-millimeters, or large enough to hold 3 mL of fluid) 4 100-mL beakers 4 graduated cylinders (5- or 10-mL, or other means of measuring 2 mL)

Preparation

In the lab-based portion of the activity, students simulate the testing of samples from people around the world. Students determine whether the sample is from someone who is lactase persistent or not. Students receive a sample that either does or does not contain lactase. The frequency of lactase persistence matches the observed frequency of lactose tolerance in different parts of the world.

Decide how many samples you want your class to examine. The activity is designed to have each student test two samples. The following description is for the full set of 70 samples. We chose to use 70 samples because this allows students to see the results for 10 individuals from 7 different geographic regions. Students can then start to visualize the differences in lactase persistence in different geographic regions.

To limit the use of supplies, you may want to reduce the number of samples. For example, you may choose to simply assign one sample per student. Students will still be able to see the full set of results from other researchers, so limiting the number of samples examined should not cause any problems.



Tip from the field test: Some field-test teachers used 25 or fewer samples, and the activity still worked well. At a minimum, you could perform a demonstration with four samples (two samples with lactase, two samples without) so students see the process.

To further reduce costs, you may cut the glucose test strips in half lengthwise. You may also use simpler materials than those included in the materials section. For example, you could replace the test tubes with small plastic cups.

Use the following steps to prepare for the lab:

- For the lactase solution, crush one lactase tablet and dissolve it in 100 mL of water in a 250-mL beaker. Some tablets include cellulose, which does not dissolve. If this is the case, crush the tablet, put it in the water, and mix thoroughly. Filter the solution to remove the cellulose. Label the filtered solution “lactase solution.”
- Label 70 test tubes from “1” to “70.” Add 1 mL of lactase solution to the following test tubes: 1, 2, 8, 9, 12, 15, 17, 18, 20, 21, 22, 27, 28, 32, 33, 34, 35, 42, 44, 47, 50, 54, 58, 62, 63, and 68.
- Add 1 mL of water to the remaining test tubes.
- Label two test-tube racks: one with the numbers of the samples that receive the lactase and the other with the numbers of the samples that receive the water.
- Put the tubes back in numerical order before class.

Once students finish the investigation, have them return the test tubes to the proper rack. This will make it easier for you to get ready for the next class.

Make the necessary photocopies and transparencies.

For classes using the Web version, verify that the computer lab is reserved for your classes or that the classroom computers are set up for the activities.



Refer to Using the Web Site for details about hardware and software requirements. Check that the Internet connection is working properly.

Set the computers to the opening screen for the activity. Log on to the “Student Activities” section of the Web site by entering the following URL:

<http://science.education.nih.gov/supplements/evolution/student>

Select “Lesson 2: Investigating Lactose Intolerance and Evolution.” This allows students to begin the activity directly.

Procedure

Activity 1: Investigating Lactose Intolerance and Evolution

Estimated time: 100 minutes

Note: This lesson is an Explore activity. We designed it to give students a common experience for building a full explanation for how natural selection affects human health and why understanding common ancestry is important for medicine. The lab experience at the beginning of the activity gives students a brief physical experience to better comprehend the role of lactase and to provide a link to other laboratory experiences they may have had with enzymes. This lab experience sets an important context for the lesson. If you find the costs and preparation prohibitive, you could skip this section and still achieve many of the student learning outcomes, but many field-test teachers commented that they felt the laboratory investigation was valuable. In the remainder of the activity, students practice important inquiry skills such as analyzing and comparing data and exploring alternative explanations. Students may still struggle with some of the basic aspects of natural selection. Research suggests that students need to practice using examples of natural selection in multiple contexts before they can generalize the concept. They will further build their understandings of evolution in later activities in the supplement.

1. Begin the lesson by reading the following short scenario to students:

Even as a young child, Chang remembered not liking milk very much. At home in China, this did not cause a problem. Her parents did not keep milk around the house, and nobody in her family

drank milk regularly. However, things changed when Chang came to the United States on a one-year high school exchange program.

At her new American school, she tried drinking milk at lunch, like many of the other students. This is when her problems started. Chang was miserable. She felt bloated and had painful cramps and diarrhea. She also seemed to be passing a lot of gas, which was embarrassing. Eventually, Chang went to the doctor. After a few tests, Chang learned that she was lactose intolerant. This means she wasn't able to digest lactose, a sugar found in milk. The doctor explained that many people cannot digest lactose, especially people from certain geographic regions. Chang wondered why she was susceptible to this condition and why it was more common in certain parts of the world.

- 2. Ask the class if they know anyone who is lactose intolerant. Then ask students for their ideas about the cause of lactose intolerance. Explain that the goal of this activity is to understand the questions that Chang asked: “Why are humans susceptible to lactose intolerance?” and “Why is it more common in certain parts of the world?”**

Many students have heard of lactose intolerance. Encourage them to discuss their best ideas and not worry about whether their initial ideas are right or wrong at this stage in the activity. They will learn more about lactose intolerance.

- 3. Explain that students will investigate patterns of lactose intolerance across the world. They will start by examining simulated samples from patients in different parts of the world. Give one copy of Master 2.1, *Lactase Investigation*, to each student. Ask students to read the introduction; then, answer any questions they have about it.**

It is helpful for students to understand that “lactase persistent” means the same thing as “lactose tolerant” and “lactase nonpersistent” means the same as “lactose intolerant.” We use the terms “lactase persistence” and “lactase nonpersistence” in this activity because the scientific literature uses them.

Note: If students ask what is in the samples they are testing, explain that the samples simulate small intestine fluid. Lactase is secreted by cells lining the small intestine, and intestinal biopsies are required to test directly for enzyme function. However, direct testing for lactase activity from intestinal samples is impractical for large-scale screening, so indirect lactose tolerance tests are typically used. A common, fairly reliable method involves testing the amount of hydrogen in the breath of individuals after



Content Standard C:

Most cell functions involve chemical reactions. Food molecules taken into cells react to provide the chemical constituents needed to synthesize other molecules. Both breakdown and synthesis are made possible by a large set of protein catalysts, called enzymes. The breakdown of some of the food molecules enables the cell to store energy in specific chemicals that are used to carry out the many functions of the cell.

they have consumed lactose. When adults who are lactase nonpersistent consume lactose, bacteria in the colon digest the lactose and produce hydrogen gas, which can appear in the breath.

4. **Write on the board the summary equation for the breakdown of lactose. Ask students how monitoring glucose provides evidence of lactase activity. Remind them that the goal is to determine whether each patient sample shows evidence for lactase persistence.**

The summary equation for the breakdown of lactose follows:



Lactose does not split into glucose and galactose at an appreciable rate without an enzyme. So the accumulation of glucose, monitored using glucose test strips, is evidence that the enzyme is working. Understanding the equation for the breakdown of lactose is important because students will measure glucose levels in the samples.

5. **Hand out the labeled sample test tubes to students. Ask them to record the sample numbers on the table on Master 2.1. Then project Master 2.2, *Lactase Results from Other Researchers*, but hide the “lactase persistence (Yes/No)” column. Ask students to record the region and country of the samples.**
6. **Perform a short demonstration to establish the amount of glucose present in the milk and to help the students understand the procedure. Pour approximately 200 mL of skim milk into a beaker. Transfer 2 mL of milk into a test tube, and then add 1 mL of water. Demonstrate to students how to use the glucose test strips to measure the amount of glucose in the diluted milk in the test tube. From this demonstration, students should record on Master 2.1 the color of the glucose test strip.**

Examine your glucose test strips to determine how long you need to wait to record the color of the strip. Some strips allow you to deduce the concentration of glucose in the milk, which you can do if students are interested.

7. **Pour 50 mL of milk into four separate 100-mL beakers, and create four workstations at which students can access materials. Each workstation should have one beaker of milk, one 5- or 10-mL graduated cylinder (or other means for measuring 2 mL), and glucose test strips. Students will record**

whether or not the amount of glucose increased in the samples by using the procedures on Master 2.1. Students should record the results on the handout. Ask them to make a claim about whether the person who provided the sample was lactase persistent.

Explain to students that they will learn later if their claims about their samples were confirmed by other researchers.

- 8. After students complete the lab investigation, ask them to clean up according to your directions.**

Have students return the test tubes to either the test-tube rack for the samples that have lactase or the rack for samples that have water. This will make it easier for you to get ready for the next class.

- 9. Explain to students that in this simulation, other researchers also analyzed the samples they investigated. Project Master 2.2. Previously, you hid the “lactase persistence (Yes/No)” column from students. Now reveal it. Hold a brief class discussion about any discrepancies in the results.**

Emphasize that many kinds of errors, including human error, can cause problems with lab results. Therefore, important medical tests are often repeated by separate researchers.

(For print version, skip to Step 10-p on page 76.)



In classrooms using the Web version of this activity:

- 10-w. Instruct students to proceed to their computers and click on “Lesson 2: Investigating Lactose Intolerance and Evolution.” Students should then click on “Fill in samples from other researchers.” Ask, “Does the percentage of people who are lactase persistent vary in different parts of the world?”**

Computers should be at <http://science.education.nih.gov/supplements/evolution/student>

Students will see differences in lactase persistence depending on geography. Map 1 plots the results that the other researchers obtained for the simulated samples students studied. The samples are organized into seven geographic regions. The frequencies of lactase persistence in the regions are based on frequencies found in the scientific literature (Enattah et al., 2007; Enattah et al., 2008; Gerbault et al., 2009; Tishkoff et al., 2007).



Tip from the field test: Students are generally very interested in the patterns shown on the map. However, they will explore these patterns in more detail in future steps. Keep the discussion on Step 10 very brief.

11-w. Explain that students will access a second map that has results from 300 individuals in the seven geographic regions found in Map 1. Then break the class into groups of four. Hand out 1 copy of Master 2.3, *Investigating Patterns in Lactase Persistence*, to each student. Ask students to work as a group to accomplish all the tasks on the handout, using Map 2 as a reference. Students access Map 2 by clicking on “View Map 2.”

The tasks described on the handout vary in difficulty. You may wish to have more-advanced students complete the tasks related to exploring geographic patterns of lactase persistence.

Students may wonder why the columns in the data table for infants (Table 3) are labeled “lactase activity” and “no lactase activity.” The answer is that the terms “lactase persistent” and “lactase nonpersistent” apply only to adults.



Tip from the field test: If you have a limited number of computers available for students, assign one computer per group and ask the students to complete all three parts of the activity together. Groups should switch the person “driving” the computer between tasks to allow wider participation.

Continue with Step 12 on page 77.

In classrooms using the print version of this activity:

10-p. Project Master 2.11, *Map of Lactase Test Results*. Ask, “Does the percentage of people who are lactase persistent vary in different parts of the world?”



Students will see differences in lactase persistence depending on geography. The map plots the simulated results that other researchers obtained for the simulated samples students studied. The samples are organized into seven different geographic regions. The frequencies of lactase persistence in the regions are based on frequencies found in the scientific literature (Enattah et al., 2007; Enattah et al., 2008; Gerbault et al., 2009; Tishkoff et al., 2007).



Tip from the field test: Students are generally very interested in the patterns shown on the map. However, they will explore these patterns in more detail in future steps. Keep the discussion on Step 10 very brief.

- 11-p. Project Master 2.12, *Expanded Map of Lactase Test Results*. This second map shows results from 300 individuals in the seven geographic regions found in the previous map. Break the class into groups of four. Hand out one copy of Master 2.13, *Exploring Patterns of Lactase Persistence*, to each student. Ask students to work as a group to accomplish all the tasks on the handout.**

The tasks described on the handout vary in difficulty. You may wish to have more-advanced students complete the tasks related to exploring geographic patterns of lactase persistence.

Students may wonder why the columns in the data table for infants (Table 3) are labeled “lactase activity” or “no lactase activity.” The answer is that the terms “lactase persistent” and “lactase nonpersistent” apply only to adults.

- 12. Hold a class discussion in which you summarize what the students learned from exploring patterns of lactase persistence.**

Students should recognize that there are dramatic differences in the frequency of lactase persistence in different geographic regions, even in areas that are relatively close geographically. East Asia, parts of the Middle East, West Africa, and Southern Europe have relatively few people who are lactase persistent. East Africa, Saudi Arabia (Middle East 2), and Northern Europe have relatively high proportions of people who are lactase persistent.

Lactase persistence is not more common in one gender than the other. Some students may focus on the fact that the numbers of males and females who show lactase persistence are not identical. This is a good opportunity to discuss reasonable expectations for the numbers in each category.

Students will recognize that the vast majority of infants have functional lactase enzymes. Infants of all mammals use lactase to digest milk. Help students understand that this means that something happens during development to “turn off” the production of lactase in many adults.

- 13. Lead a short class discussion about the three main causes of health conditions: infectious, genetic, and environmental. Ask students to use what they know and what they learned from the investigation to rule out possible causes for lactose intolerance.**

The fact that lactase nonpersistence (lactose intolerance) occurs in clusters could support the hypothesis that the cause is any of the three main causes of health conditions. If students do not mention it, ask them what would happen when people move from one region

of the world to another if lactase nonpersistence were caused by environmental or infectious agents. This would be an ideal time to bring up the fictional student Chang from the scenario in Step 1. If lactase nonpersistence had an environmental or infectious cause, we would not necessarily expect Chang to show the symptoms she did when she came to the United States. Help students understand that the evidence and their prior experiences and knowledge point to a genetic cause for lactase nonpersistence.



Tip from the field test: Many students quickly eliminated genetics as a factor in lactase persistence because nearly every infant showed evidence of lactase activity. This indicates that students do not recognize that some mutations cause changes in when and where other proteins are made. The next step helps make this point for students.

14. Explain to students that scientists have identified the gene (DNA) that codes for the lactase enzyme (which is a protein). Project Master 2.4, *Questions about the Genetic Basis for Lactase Persistence*. Go over the answers in a brief discussion.

Answer key for questions on Master 2.4, *Questions about the Genetic Basis for Lactase Persistence*

1. Do you think you would find the gene that codes for lactase in both people who are lactase persistent and people who are lactase nonpersistent? Explain your answer.

Nearly all infants can digest lactose, meaning that they must make lactase at this point in life. Therefore, individuals who are lactase persistent and those who are nonpersistent must both have the gene that codes for lactase.

2. Scientists recognize that two major types of changes in DNA sequences can affect the phenotype of organisms. One type changes the coding sequence of a gene. These changes can affect the amino acids that form the protein, which can affect the protein's shape and function. The second type of change affects when a gene gets "turned on" or "turned off." What type of change do you think causes the difference between lactase persistence and lactase nonpersistence?

Infants use lactase to digest the lactose in breast milk or infant formula, so changes to the coding sequence for lactase would likely have a detrimental effect on an individual. Because lactase is turned on in infants but turned off in people who are lactase nonpersistent, the change is more likely to be in a region that regulates other genes. In fact, scientists have identified a growing number of different mutations to a regulatory region for the lactase gene that cause lactase persistence in people from different geographic regions. Students learn about these mutations in the next steps in the activity.

15. **Explain to students that in 2002, scientists discovered a mutation in a segment of DNA that controls the production of lactase. Individuals with one copy of the mutation are lactase persistent. Individuals with two copies of the typical form of DNA are lactase nonpersistent.**

Students may recognize that this means that the allele formed from the mutation that causes lactase persistence is dominant over the other allele.

(For print version, skip to Step 16-p on page 80.)



In classrooms using the Web version of the activity:

- 16-w. Direct students to Map 2 on the Web site. Tell them to click on “Explore Lactase Persistence by Mutations,” then “Mutation 1” to see the percentage of this lactase persistence mutation in different human populations. Ask students to explain the pattern they observe.**

Students will notice that mutation 1 is prevalent in Northern Europe but is missing in other areas that also have a high amount of lactase persistence such as East Africa and Saudi Arabia (Middle East 2).

Note: The table students see on the Web site summarizes the percentage of the DNA regions with a specific mutation in each geographic region. To help visualize this amount, the same percentage of individuals in each geographic region is highlighted.

- 17-w. Ask, “What does it mean if all the people who are lactase persistent do not have mutation 1?”**

Different mutations that cause lactase persistence must be present in other populations.

- 18-w. Explain to students that scientists discovered additional mutations causing lactase persistence by studying different groups of people around the world. Ask students to use map 2 to explore the percentages of the other two mutations by clicking on “Mutation 2” and “Mutation 3.”**

Scientists have found additional mutations related to lactase persistence, but only the three mutations that are found in relatively high frequencies in different geographic regions are included in this activity. In fact, Enattah et al. (2007) suggest that the mutation called “mutation 1” in this activity was independently introduced into humans more than once. Other



Content Standard C:

Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.



Content Standard C:

Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only changes in germ cells can create the variation that changes an organism’s offspring.

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researchers suggest that the data can be explained in other ways. The independent evolution of this mutation is a hypothesis that can be further explored with new data.

Continue with Step 19 below.

For classes using the print version of this activity:



16-p. Project Master 2.14, *Lactase Persistence and Mutation*, and show students the “Mutation 1 (%)” column in the table. Hide the other two columns. Ask students to explain the pattern they observe.

Students will notice that mutation 1 is prevalent in Northern Europe but is missing in other areas that also have a high amount of lactase persistence such as East Africa and Saudi Arabia (Middle East 2).

17-p. Ask, “What does it mean if all the people who are lactase persistent do not have mutation 1?”

Different mutations that cause lactase persistence must be present in other populations.

18-p. Explain to students that scientists discovered additional mutations causing lactase persistence by studying different groups of people around the world. Show students the remaining columns in the table on Master 2.14.

Scientists have found additional mutations related to lactase persistence, but only the three mutations that are found in relatively high frequencies in different geographic regions are included in this activity. In fact, Enattah et al. (2007) suggest that the mutation called “mutation 1” in this activity was independently introduced into humans more than once. Other researchers suggest that the data can be explained in other ways. The independent evolution of this mutation is a hypothesis that can be further explored with new data.

19. Project Master 2.5, *Lactase and Human Evolution*. Ask students to answer these questions in their notebooks. Hold a class discussion on the answers.

Answer key for questions on Master 2.5, *Lactase and Human Evolution*

1. Nonhuman primates and all other mammals are lactase nonpersistent. Do you think the common ancestor of humans was lactase persistent or lactase nonpersistent? Explain your answer.



Content Standard A:
Formulate and revise scientific explanations and models using logic and evidence.

The common ancestor of all humans was very likely lactase nonpersistent.

Note: Studies of ancient DNA from Northern European skeletons 7,000–8,000 years old support the hypothesis that the mutation for lactase persistence that is common in Northern Europe today was absent or at a low frequency in the early Neolithic period (Burger et al., 2007).

2. Genetic studies show that different mutations cause lactase persistence in humans from different geographic regions. Does this evidence suggest that lactase persistence evolved once or more than once in humans? Explain your answer.

Evidence supports the conclusion that lactase persistence evolved more than once in humans.

3. Did the mutation that causes lactase persistence first come about because people needed the mutation? Explain your answer.

Mutations are random with respect to the needs of the organism. The mutations that cause lactase persistence came about in individuals through random processes. However, a very common misconception is that mutations come about because individuals want or need them. We designed this question to help students address this misconception directly. It is important to emphasize that although mutation is random, the remaining aspects of natural selection are not random. Individuals that possess genetic variants that help them survive and reproduce in a particular environment leave more offspring. This portion of the process is not random.

20. **Hand out one copy of Master 2.6, *Explaining the Evolution of Lactase*, to each student. After students complete the reading, project Master 2.7, *Data from Africa*. Lead a class discussion about their conclusions after they finish analyzing the data.**

The data in this example come from Gerbault et al. (2009) and show a pattern that is consistent with the culture-historical hypothesis in Africa. However, these data merely show a correlation. You should emphasize that correlations do not establish cause. Use this opportunity to emphasize that identifying the factors that affected populations in the past is important. In the case of the evolution of antibiotic resistance in MRSA, the factor in the environment that affects the survival and reproduction of individuals in the population is clear. However, other cases, such as the evolution of lactase persistence, are not as simple.

Scientists collect a broad range of different types of data to test hypotheses about natural selection in the past. You may wish to



Content Standard C:

The millions of different species of plants, animals, and micro-organisms that live on Earth today are related by descent from common ancestors.

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Master 2.10 can help you assess how well students understand the process of natural selection and can apply it to link evidence with explanations. As the class discusses the responses to each question, ask whether everyone agrees or anyone has anything else to add. Use student responses to inform your teaching of Lesson 3.

ask students what types of data they would like to have to further test the culture-historical hypothesis in Africa. Students may mention that it would be important to know if lactase-persistent individuals in pastoralist populations really did survive and reproduce at higher rates. They may also want to know if the increase in frequency of the lactase-persistence alleles coincided in time with dairying. In fact, estimates of the age of specific mutations in Africa are also consistent with the culture-historical hypothesis.

21. **Tell students that they will now consider data from Europe. Ask students to get back into their groups of four. Hand out**
 - **four copies per group of Master 2.8, *Data from Europe, Part A*, to half the groups;**
 - **four copies per group of Master 2.9, *Data from Europe, Part B*, to the other half the groups; and**
 - **one copy of Master 2.10, *Summing Up Lactase Persistence and Nonpersistence*, to each student.**

Ask students to follow the directions on the handouts. Again, after students analyze the data, lead a class discussion about their conclusions.

The data in Figure 2 on Master 2.8 come from Gerbault et al. (2009). Ask students who received these data to summarize their conclusions. Many students who examine these data will claim that the data support the calcium-absorption hypothesis. Some students may recognize that these data are also correlations and that correlations do not necessarily mean causation. Students who analyze the data in Master 2.9 should offer counterarguments to students with data from Master 2.8.

This exercise is important because it helps reinforce the search for alternative explanations, a critical aspect of science. The frequency of rickets from the data in Table 1 on Master 2.9 is lower than expected if rickets were responsible for driving the evolution of the lactase alleles. Therefore, these data do not support the calcium-absorption hypothesis. The data in Master 2.9 come from Simoons (2001) and Itan et al. (2009). If your students are particularly advanced, you may wish to explain that both hypotheses may, in fact, be partially correct because they do not logically exclude each other. You may also want to ask interested students to investigate other hypotheses for the evolution of lactase persistence.

22. **Conclude the lesson by having a class discussion about students' summary explanations for the evolution of lactase in human populations.**

Students should begin to recognize that there are common elements to every case of evolution by natural selection, as mentioned in Lesson 1.

Answer key for questions on Master 2.10, *Summing Up Lactase Persistence and Nonpersistence*

1. Do humans vary in their ability to digest lactose? What is the evidence for your answer?

Yes; the maps and tables of data presented to students all demonstrate that there is variation among humans for the ability to digest lactose.

2. Can the ability to digest lactose as an adult be passed from parents to offspring? What is the evidence for your answer?

Yes, the ability to digest lactose as an adult can be passed from parent to offspring. Researchers have identified mutations to DNA that keep the lactase gene turned on in some adults.

3. Describe how mutations to DNA are important in lactase persistence and nonpersistence.

Students should recognize that mutations to DNA cause the variability among different people for lactase persistence or nonpersistence. This is a good time to reemphasize that not all mutations are “bad” and that mutations provide the variation on which natural selection acts.

4. In certain environments, did digesting lactose seem to affect an individual’s ability to survive and reproduce? Explain.

Yes, it is very likely that lactose digestion affected fitness. Students should mention that scientists have different hypotheses to explain specifically how digesting lactose affected an individual’s ability to survive or reproduce in certain environments and cultural contexts.

5. What is the evidence that the frequency of the mutation that causes lactase persistence changed in certain groups of people over time?

The common ancestor of all humans was lactase nonpersistent. The fact that some groups of humans have high frequencies of the mutation that causes lactase persistence is evidence that the frequency of this mutation has changed over time.



Content Standard C:

- Species evolve over time. Evolution is the consequence of (1) the potential for a species to increase its numbers, (2) the genetic variability of the offspring due to mutation and recombination of genes, (3) a finite supply of the resources required for life, and (4) the ensuing selection by the environment of those offspring better able to survive and leave offspring.
- Recognize and analyze alternative explanations and models.
- Understandings about scientific inquiry: Scientific explanations must adhere to criteria such as: a proposed explanation must be logically consistent; it must abide by the rules of evidence; it must be open to questions and possible modification; and it must be based on historical and current scientific knowledge.

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Lesson 2 Organizer: Web Version



<p>Read to the class the scenario about a student who is lactose intolerant.</p>	<p>Page 72 Step 1</p>
<p>Ask students if they know anyone who is lactose intolerant. Explain that the activity's goal is to understand why humans are susceptible to lactose intolerance and why it is more common in certain parts of the world.</p>	<p>Page 73 Step 2</p>
<p>Explain to students that they will investigate patterns of lactose intolerance across the world.</p> <ul style="list-style-type: none"> • Give each student one copy of Master 2.1 and answer any questions they have about the introduction. • Describe the summary equation for the breakdown of lactose: $\text{lactose} + \text{water} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$	<p>Pages 73 and 74 Steps 3 and 4</p> 
<p>Hand out the labeled sample test tubes to students. Project Master 2.2 but hide the "lactase persistence (Yes/No)" column.</p>	<p>Page 74 Step 5</p> 
<p>Demonstrate the procedure for measuring glucose:</p> <ul style="list-style-type: none"> • Pour about 200 milliliters (mL) of skim milk into a beaker. • Transfer 2 mL of milk into a test tube, and then add 1 mL of water. • Demonstrate to students how to use the glucose test strips to measure the amount of glucose in the diluted milk in the test tube. <p>Students should record on Master 2.1 the color of the glucose test strip.</p>	<p>Page 74 Step 6</p>
<p>Create four workstations. Each should have</p> <ul style="list-style-type: none"> • one beaker with 50 mL of milk, • one 5- or 10-mL graduated cylinder (or other means for measuring 2 mL), and • glucose test strips. <p>After the investigation, students should make a claim about whether the person who provided the sample was lactase persistent. Then direct them to clean up.</p>	<p>Pages 74–75 Steps 7 and 8</p>
<p>Project <i>all</i> of Master 2.2. Hold a brief class discussion about any discrepancies in the results.</p>	<p>Page 75 Step 9</p> 

<p>Have students log on to the Web site and click on "Lesson 2: Investigating Lactose Intolerance and Evolution." They should then click on the "Fill in samples from other researchers" button.</p> <ul style="list-style-type: none"> Ask, "Does the percentage of people who are lactase persistent vary in different parts of the world?" 	<p>Page 75 Step 10-w</p> 
<p>Explain to students that they will access Map 2, which has additional samples, by clicking on "View Map 2."</p> <ul style="list-style-type: none"> Divide the class into groups of four. Hand out one copy of Master 2.3 to each student. <p>After groups accomplish the tasks on the handout, hold a class discussion to summarize what they learned.</p>	<p>Pages 76 and 77 Steps 11-w and 12</p> 
<p>Ask students if they can use evidence to help decide if lactase persistence has an infectious, genetic, or environmental cause.</p>	<p>Page 77 Step 13</p>
<p>Explain that scientists identified the gene that codes for lactase.</p> <ul style="list-style-type: none"> Project Master 2.4 and have students answer the questions on their own. Go over the answers in a brief discussion. 	<p>Page 78 Step 14</p> 
<p>Describe the discovery of a mutation that leads to lactase persistence.</p> <ul style="list-style-type: none"> Have students revisit the Web site and click on "Explore Lactase Persistence by Mutation," then "Mutation 1." Discuss the patterns that students observe. Ask, "What does it mean if all the people who are lactase persistent do not have mutation 1?" 	<p>Page 79 Steps 15, 16-w, and 17-w</p>
<p>Direct students to the Web site. Explain that they will explore the percentages of the other two mutations that result in lactase persistence by clicking on "Mutation 2" and "Mutation 3."</p>	<p>Page 79 Step 18-w</p>
<p>Project Master 2.5. Ask students to record their answers in their notebooks. Then lead a class discussion about the questions.</p>	<p>Page 80 Step 19</p> 
<p>Hand out one copy of Master 2.6 to each student and ask students to read it. Project Master 2.7. Discuss the data as a class.</p>	<p>Page 81 Step 20</p> 
<p>Hand out</p> <ul style="list-style-type: none"> four copies per group of Master 2.8 to half of the groups; four copies per group of Master 2.9 to the other half of the groups; and one copy of Master 2.10 to each student. <p>Discuss the data from Europe as a class.</p>	<p>Page 82 Step 21</p> 
<p>Lead a class discussion about students' summary explanations for the evolution of lactase in human populations.</p>	<p>Page 82 Step 22</p>

 = Involves copying a master.

 = Involves making a transparency.

 = Involves using the Internet.

Lesson 2 Organizer: Print Version



Activity 1: Investigating Lactose Intolerance and Evolution

Estimated time: 100 minutes

Read to the class the scenario about a student who is lactose intolerant.	Page 72 Step 1
Ask students if they know anyone who is lactose intolerant. Explain that the activity's goal is to understand why humans are susceptible to lactose intolerance and why it is more common in certain parts of the world.	Page 73 Step 2
<p>Explain to students that they will investigate patterns of lactose intolerance across the world.</p> <ul style="list-style-type: none"> • Give each student one copy of Master 2.1 and answer any questions they have about the introduction. • Describe the summary equation for the breakdown of lactose: $\text{lactose} + \text{water} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$ 	Pages 73 and 74 Steps 3 and 4 
Hand out the labeled sample test tubes to students. Project Master 2.2 but hide the "lactase persistence (Yes/No)" column.	Page 74 Step 5 
<p>Demonstrate the procedure for measuring glucose:</p> <ul style="list-style-type: none"> • Pour about 200 milliliters of skim milk into a beaker. • Transfer 2 milliliters of milk into a test tube, and then add 1 milliliter of water. • Demonstrate to students how to use the glucose test strips to measure the amount of glucose in the diluted milk in the test tube. <p>Students should record on Master 2.1 the color of the glucose test strip.</p>	Page 74 Step 6
<p>Create four workstations. Each should have</p> <ul style="list-style-type: none"> • one beaker with 50 milliliters of milk, • one 5- or 10-milliliter graduated cylinder (or other means for measuring 2 milliliters), and • glucose test strips. <p>After the investigation, students should make a claim about whether the person who provided the sample was lactase persistent. Then direct them to clean up.</p>	Pages 74–75 Steps 7 and 8
Project all of Master 2.2 . Hold a brief class discussion about any discrepancies in the results.	Page 75 Step 9 

Project Master 2.11 . Ask, “Does the percentage of people who are lactase persistent vary?”	Page 76 Step 10-p 
Project Master 2.12 , which shows a second map with additional samples. Divide the class into groups of 4. Pass out 1 copy of Master 2.13 to each student. After groups accomplish the tasks on the handout, hold a class discussion to summarize what they learned.	Page 77 Steps 11-p and 12  
Ask students if they can use evidence to help them decide if lactase persistence has an infectious, genetic, or environmental cause.	Page 77 Step 13
Explain that scientists identified the gene that codes for lactase. <ul style="list-style-type: none"> Project Master 2.4 and have students answer the questions on their own. Go over the answers in a brief discussion. 	Page 78 Step 14 
Describe the discovery of a mutation that leads to lactase persistence. <ul style="list-style-type: none"> Project Master 2.14 and show only the “Mutation 1 (%)” column in the table. Discuss the patterns that students observe. Ask, “What does it mean if all the people who are lactase persistent do not have mutation 1?” 	Pages 79 and 80 Steps 15, 16-p, and 17-p 
Show students the percentages of two additional mutations for lactase by revealing the last two columns on Master 2.14 .	Page 80 Step 18-p
Project Master 2.5 . Students should record their answers in their notebooks. Next, lead a class discussion about the questions.	Page 80 Step 19 
Hand out one copy of Master 2.6 to each student. Then project Master 2.7 . Discuss the data as a class.	Page 81 Step 20 
Hand out <ul style="list-style-type: none"> four copies per group of Master 2.8 to half of the groups, four copies per group of Master 2.9 to the other half of the groups, and one copy of Master 2.10 to each student. Discuss the data from Europe as a class.	Page 82 Step 21 
Lead a class discussion about students’ summary explanations for the evolution of lactase in human populations.	Page 82 Step 22

 = Involves copying a master.  = Involves making a transparency.

Lesson 3

Evolutionary Processes and Patterns Inform Medicine



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	Comparison
Human	TTGACTCTTCAATGTTGCA
Mouse	TTGACTCTTTCAATGTTGCA
Zebrafish	TTGGCTCTACCAATGTGGCA

↑ ↑↑ ↑

Explain

At a Glance

Overview

Lesson 3 is divided into two activities. In Activity 1, students use data to solve the mystery of a disease, and then they notice that the disease, alpha-thalassemia, occurs most frequently in the same places that malaria is a serious health issue. Students then use data and the principles of natural selection to explain the relatively high frequency of the disease in certain populations.

In Activity 2, students continue in the role of medical investigators as they learn how comparisons of genetic sequences help researchers studying cleft lip and palate. Students work in small groups to better understand the causes of cleft palate. They then learn how to compare sequences for a gene among a large number of species. Mutations in the gene they analyze cause a syndrome that includes cleft lip and palate. Through this comparison, students identify regions of the gene that have not changed over vast amounts of time. Students apply their understandings of evolution to explain how natural selection has conserved these sequences. Students also reflect on the importance of understanding common ancestry to explain the value of experiments in model species.

Activity 1: Investigating a Mystery Disease

Estimated time: 100 minutes

Major Concepts

- A genetic disease that causes health problems in humans can influence the severity of other human diseases.
- Variation in genotypes can cause differences in phenotype.

Objectives

After completing this lesson, students will

- be able to explain how different genotypes cause different phenotypes for a disease;
- understand that, in specific cases, mutations in genes that lead to human disease may also benefit the individual by influencing the severity of another disease; and
- be able to explain how natural selection can act on a human disease.

Activity 2: Using Evolution to Guide Research

Estimated time: 50 minutes

Major Concepts

- Evolutionary comparisons are important for studying biomedical problems.
- Rates of evolutionary change in genetic sequences show that natural selection can conserve some sequences in numerous lineages across vast timescales.
- Other species are used as model systems for studying health-related issues in humans.
- Descent with modification suggests that modern organisms inherited their traits from ancestors and that modern species all share common ancestors at some point in time. The characteristics of living organisms are shaped by this history.

Objectives

After completing this lesson, students will

- understand the importance of studying the genomes of a large number of humans and other organisms and
- appreciate the value of using other organisms as model systems for studying health-related issues in humans.

Teacher Background

Consult the following sections in Information about Evolution and Medicine:

- 1.0 Fundamentals of Evolution and Medicine (pages 21–27)
- 2.0 The Value of an Evolutionary Perspective for Medicine (page 27)

- 3.0 Specific Applications of Evolution in Medicine (pages 27–31)
- 4.0 Students’ Prior Conceptions about Evolution (pages 31–33)
- 5.0 Featured Examples of Evolution and Medicine (pages 33–38)

In Advance

Web-Based Activities

Activity	Web Component?
1	Yes
2	Yes

Photocopies, Transparencies, Equipment, and Materials

Photocopies and Transparencies
Activity 1: Investigating a Mystery Disease
For Classes Using Web-Based Activity: 1 transparency each of Masters 3.1, 3.5, 3.6, 3.7, and 3.9 1 copy each of Masters 3.2, 3.8, 3.12, and 3.13 (optional) for each student 1 copy of Master 3.10 for each student in half of the class 1 copy of Master 3.11 for each student in the other half of the class
For Classes Using Print-Based Activity: 1 transparency each of Masters 3.1, 3.5, 3.6, 3.7, and 3.9 1 copy each of Masters 3.2, 3.8, 3.12, and 3.13 (optional) for each student 2–3 copies of Master 3.3 (see Preparation, print version only) 1 copy of Master 3.4 for each group of 3–4 students 1 copy of Master 3.10 for each student in half of the class 1 copy of Master 3.11 for each student in the other half of the class
Activity 2: Using Evolution to Guide Research
For Classes Using Web-Based Activity: 1 copy each of Masters 3.14 and 3.17 for each student 1 transparency each of Masters 3.15, and 3.16
For Classes Using Print-Based Activity: 1 copy each of Masters 3.14 and 3.19 for each student 1 transparency each of Masters 3.15, 3.16, and 3.18 1 copy of Master 3.20 for each group of 3 students
Equipment and Materials
Different-colored pens or pencils (optional)

Preparation

Activity 1

For classes using the web version, verify that the computer lab is reserved for your classes or that the classroom computers are set up for the activities.



Refer to Using the Web Site for details about hardware and software requirements for the Web site. Check that the Internet connection is working properly. Set the computers to the opening screen for Activity 1. Log on to the “Student Activities” section of the Web site by entering the following URL:

<http://science.education.nih.gov/supplements/evolution/student>

Select “Lesson 3: Evolutionary Processes and Patterns Inform Medicine.” This allows students to begin the activity directly.

For classrooms using the print version of Activity 1, prepare two to three photocopies of **Master 3.3, Blood Test Data**. Each master includes blood test data for 10 patients. Each group will need one set of blood test data for two patients. Cut each master apart to separate the blood test results for each patient. Place the cut copies on a desk or table where students can pick up the information for their assigned patients when they are ready for that part of the investigation.



Make enough copies of the **Master 3.4, Reference Manual**, for each group to have one copy. You can print these two-sided if you wish. If you have multiple class sections, ask students to return their copies to a common area when they have completed the activity. In this way, other classes can use the same copies of the reference manual.

Make other photocopies and overhead transparencies as needed.

Activity 2

Make the necessary photocopies and overhead transparencies.

For classrooms using the Web version of Activity 2, follow the same preparation steps as described for Activity 1.



Procedure

Activity 1: Investigating a Mystery Disease

Estimated time: 100 minutes

Note: In this activity, we formally introduce students to the five major principles of natural selection (variation, inheritance, origin of variation, fitness, and evolutionary change in populations) through the context of a human disease, alpha-thalassemia. Students develop an explanation of how mutations in a human gene can lead to disease. Then they consider how variation for this trait in the human population can help them understand how a deleterious trait (mutations resulting in alpha-thalassemia) can also provide a benefit (resistance to malaria) in some environments.

- 1. Begin by explaining that you read a news story about a medical mystery on a Web site and want to share it with the class. Project Master 3.1, *A Medical Mystery*, and read the information with the class. Explain to students that exploring this scenario and figuring out what is happening is a way for them to apply and demonstrate their skills and abilities in science.**

The scenario involves a population of people in Papua New Guinea. A relatively large number of people in the population are anemic. Anemia is a condition in which a person doesn't have enough red blood cells to carry an adequate supply of oxygen to the body's cells. Many conditions cause anemia, so the students' challenge is to determine why so many people in this population have it.

- 2. Give each student one copy of Master 3.2, *Investigating a Medical Mystery*. Ask them to work in their groups of three to four students. Explain that each group will investigate two cases (patients) to determine the cause of the medical mystery. Assign two cases to each group. Tell students to write the case (patient) numbers they will investigate on Master 3.2 handout.**

Explain that the cases represent a subset of a larger number of cases in the population. You can use any of several methods to determine which cases each group explores. Groups can

- count off until all groups have 2 of the 10 patient cases,
- draw numbers out of a hat, or
- choose two numbers ranging from 1 to 10.

Be sure that each case is assigned to at least one group. Depending on the number of students in your class, it is likely that more than one group will investigate some of the cases. If necessary because of time constraints, groups could investigate only one case, but again, make sure that all cases are covered by at least one group.



Tip from the field test: Students can work in pairs for the data analysis if that works better for your class in terms of its size, availability of computers, and familiarity with group work.

(For print version, skip to Step 3-p on page 95.)



In classrooms using the Web version of this activity:

3-w. Briefly explain to groups that there are two main parts to the investigation for each case. Groups will use the virtual microscope on the Web site to look at images of blood samples from the different patients. They will also examine the laboratory results of blood tests. Point out that a reference manual on the Web site will help them determine what the likely problem is in each case.

Groups should use the online virtual microscope first and then analyze the laboratory blood test data. Students must do both parts to be able to diagnose the problem.

The laboratory blood test data include a variety of data for each patient. The units specified are typical for the measurement of each blood component. Many of these units will be unfamiliar to students, but for this activity, students don't need to know the units. They will simply compare the values for their patients with the normal range for that blood component.

4-w. Instruct students to proceed to <http://science.education.nih.gov/supplements/evolution/student>.

Students should click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” then “Activity 1: Investigating a Mystery Disease.” Allow time for groups to work through the cases.

As groups work, circulate among them to respond to questions and monitor their progress. Remind students to use the information in the Reference Manual to help them diagnose the cases. (They access it by clicking on the “Reference Manual” button.) If students have trouble determining what data to focus on, prompt them that hemoglobin and anemia relate most directly to red blood cells (RBCs). In the microscope, students may see white blood cells (WBCs) in addition to the RBCs. WBCs tend to be larger than RBCs and irregularly shaped. The nuclei of WBCs are darkly stained.

Continue with Step 5 on page 95.

In classrooms using the print version of this activity:



3-p. Briefly explain to groups that they will examine the results of blood tests to make a diagnosis. Point out where students can pick up the blood test data for their assigned patients (see the Preparation section about preparing Master 3.3, *Blood Test Data*). Explain to students that the microscopic analysis of red blood cells was completed by the lab technicians. These results are reported with the blood test data. They do not need to fill in the diameter of four different red blood cells (the first line on the Master).

4-p. Give each group one copy of Master 3.4, *Reference Manual*, and explain that they can use the information in it to help determine what the likely problem is in each case.

Students need to analyze blood test data to diagnose the problem. The blood test data include a variety of data for each patient. The units specified are typical for the measurement of each blood component. Many of these units will be unfamiliar to students, but for this activity, students don't need to know the units. They will simply compare the values for their patients with the normal range for that blood component.

5. After groups complete the investigations, conduct a class discussion to synthesize their conclusions. Project Master 3.5, *Summarizing the Mystery Disease Data*, to help guide the discussion.

Ask for volunteers from the different groups to share their conclusions for each patient. Ask students to provide the diagnosis and share the evidence that led them to decide the cause for the anemia in each case and how they eliminated the other possibilities.



Tip from the field test: Don't let this discussion drag.

It isn't necessary to go over every piece of data for each patient. Focus instead on the data that led students to distinguish the different diseases. For example, if the diagnosis is alpha-thalassemia, what are the important pieces of data that led students to this diagnosis and what data ruled out other diseases? Some teachers in the field test asked students to provide one or two pieces of evidence for the first cases of alpha-thalassemia. As the teachers proceeded through additional patients, students stated additional pieces of evidence.



Content Standard A:
Communicate and defend a scientific argument.

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In Table 3, we present some key evidence that distinguishes one disease from another for each patient. Students may also list other data (from the Reference Manual) that are characteristic of the disease but aren't included in the sample answers provided.

Table 3. Sample Answers for Master 3.5, Summarizing the Mystery Disease Data

Patient	Diagnosis	Key evidence used for diagnosis
1	Alpha-thalassemia	<ul style="list-style-type: none"> • Smaller-than-normal RBCs (microcytic; normal cells average 7–8 micrometers in diameter.) • Decreased RBC count (It could be within the normal range in mild cases.) • MCV:RBC ratio less than 13 (A ratio above 13 would indicate iron deficiency anemia.) • Hematocrit below normal • TIBC normal (It would be low in iron deficiency anemia.) • RDW normal (It would be high in iron deficiency anemia.)
2	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.
3	Iron deficiency anemia	<ul style="list-style-type: none"> • In microscope, RBC variation in size and pale in color compared with normal • High RDW (This indicates a great deal of variation in the size of the RBCs.) • Serum ferritin concentration less than 12 • Elevated TIBC • MCV:RBC ratio greater than 13 <p>(These blood test criteria distinguish iron deficiency anemia from thalassemia.)</p>
4	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.
5	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.
6	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.
7	Sickle cell disease	<ul style="list-style-type: none"> • In microscope, some blood cells sickle shaped (This is a definitive sign for sickle cell disease.) • Elevated white blood cell count • TIBC below normal
8	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.
9	Normal—no disease	<ul style="list-style-type: none"> • Red blood cells with normal appearance: normal biconcave shape and normal color • All blood test data within normal ranges
10	Alpha-thalassemia	<ul style="list-style-type: none"> • See Patient 1.

Note: Students may or may not measure a difference in size between normal RBCs and RBCs in thalassemia patients. While small RBCs (microcytic) are one characteristic of alpha-thalassemia, the degree of microcytosis depends on the severity of the disease. Individuals who are silent carriers (one nonfunctional allele of the alpha-globin gene) may not show any difference in RBC size, while individuals who have three nonfunctional copies are more likely to have measurable microcytosis. For this activity, students may not be able to detect a difference in size, but they should observe that the RBCs do not have a normal appearance.

The patients represent a sample of the individuals in Papua New Guinea who have (or are suspected of having) anemia. Make sure that students understand that this does not mean that 70 percent of the population has anemia.

6. Inform students that they will focus on thalassemia for the rest of this activity. Initiate a class discussion with the following questions:

- “Have you heard of thalassemia?”
- “Do you think thalassemia is a common disease?”

Most students have probably not heard of thalassemia and probably don't think it is very common.

7. Project Master 3.6, *The Distribution of Thalassemia Across the Eastern Hemisphere*. Ask students to draw conclusions about thalassemia from this map.

Epidemiology is the study of the incidence, distribution, and control of disease in a population. Scientists look for patterns in a disease to determine its cause and to find ways to prevent the disease.

Students should conclude that thalassemia is more common in certain parts of the world. For example, thalassemia is more common in Mediterranean and Asian countries and in certain parts of Africa than elsewhere.

Inform students that the shading on the map indicates the regions of the world where thalassemia is most common. That doesn't mean that it never occurs in the unshaded areas, just that it is much less common there. Also, it doesn't mean that everyone who lives in one of the shaded areas has thalassemia.

8. Students have now seen that thalassemia is more common in some places in the world than others. They should also recognize that most of the people who live in an area where thalassemia is more common do not have thalassemia. Ask students for a brief statement about variation for thalassemia in the human population.



Step 8 is an opportunity to assess students' current thinking about variation.

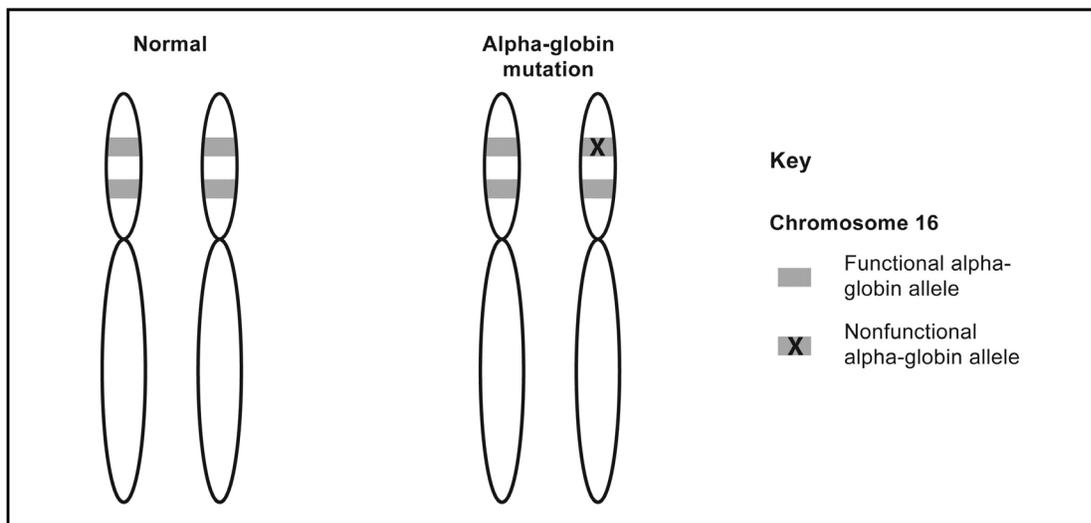
The fact that the frequency of thalassemia is higher in some places in the world suggests that there is variation for this trait. In addition, students should note that many people living in an area where thalassemia is more prevalent do not have the disease. This further suggests that there is variation in the population. In the following steps, students broaden their understandings of this variation.

If students have difficulty relating to variation among humans for thalassemia, ask, “If there were no variation in the thalassemia trait among humans, wouldn’t everyone have thalassemia?” and “If there were no variation among people, how could you explain why some people have thalassemia and others do not?”

9. **Project Master 3.7, *The Alpha-Globin Gene and Alpha-Thalassemia*.** Briefly review the information in Part 1 about what alpha-thalassemia is and how problems with the function of the alpha-globin gene cause alpha-thalassemia. Then project Part 2, which correlates the number of functional alleles of the alpha-globin gene with thalassemia symptoms. Ask students whether they can see any relationship between the number of nonfunctional alleles of the alpha-globin gene and the severity of the symptoms of alpha-thalassemia.

As you review Part 1 of Master 3.7, point out to students how the genes and alleles are represented, both in the diagrams and as a genotype. In the diagrams, a shaded area without an x represents

Figure 11. Schematic of chromosome 16 pairs showing functional and nonfunctional alpha-globin alleles.



a normal or functional allele. A shaded area with an x represents a mutated, nonfunctional allele of the gene.

The alpha-globin gene codes for the alpha-globin protein. In adults, two alpha-globin protein chains combine with two beta-globin protein chains (coded for by beta-globin genes) to form hemoglobin. Though extra detail is included, students only need a basic understanding that alpha-thalassemia is a genetic disease that results from mutations in the alpha-globin gene.

From Part 2 of Master 3.7, students do not need to know the specific symptoms for the different types of alpha-thalassemia. Students should focus on the fact that the severity of the disease depends on an individual's number of nonfunctional alleles of the alpha-globin gene. A "normal" individual has four functional alleles of the alpha-globin gene and makes normal hemoglobin. Individuals who have one, two, or three nonfunctional alleles of the alpha-globin gene produce less of the alpha-globin protein and have mild to moderate symptoms. More nonfunctional alleles increase the severity of the symptoms. Individuals with no functional alleles of the alpha-globin gene are seriously ill and almost always die around the time of birth.

Help students conclude that there is a relationship between the number of functional and nonfunctional alleles of the gene (the genotype) and the severity of the disease (the phenotype). If you wish (based on your students' knowledge of genetics), inform students that the most common types of mutation to the alpha-globin gene are deletions. (Other types of mutations, such as point mutations, also result in thalassemia, but this is beyond what students need to know.)

Note: If you wish to include the extension activity and **Master 3.13, Inheriting Thalassemia** (described on page 105), you could insert it here, after Step 9.

- 10. Give each student one copy of Master 3.8, *Alpha-Globin and Variation*. Ask students to work with their groups to answer the questions. Inform them that they need to explain their answers.**

Answer key for questions on Master 3.8, *Alpha-Globin and Variation*

1. What might cause the alpha-globin gene not to function?
Mutations in the alpha-globin gene cause it not to function.



Content Standard F:
The severity of disease symptoms is dependent on many factors, such as human resistance and the virulence of the disease-producing organism.

1

2

3

4

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2. How are differences in the thalassemia trait (phenotype) passed from parents to offspring?

Differences in the thalassemia phenotype are passed from parents to offspring through inheritance. An individual inherits half of his or her alleles from the father and half from the mother.

3. Now you know the genetic basis for alpha-thalassemia. What can you add to your description of the variation for thalassemia in humans?

From examining the map in Step 8, students know that the incidence of thalassemia is higher in certain areas of the world. With the information about the genetic basis of thalassemia, students can add the idea that different genotypes result in different forms of the disease, and each form has different symptoms and severities (phenotypes). These different forms of the disease result from variation in the genotype.

- 11. Ask students to think about why a mutant alpha-globin allele would be maintained in the human population if it causes disease.**

At this point, accept reasonable responses from students. This is another point at which you can gauge students' thinking and prior knowledge about evolution and natural selection.

- 12. Inform the class that a link in the original news story referred to another disease that has many symptoms, including anemia. This disease is malaria. Project Master 3.9, *The Epidemiology of Malaria*. Ask students to make observations about the occurrence of malaria.**

Malaria is another disease that causes anemia. It is caused by a parasite that is passed to humans through the bite of a mosquito. Malaria is usually diagnosed by using a microscope to find the parasites in a blood sample. Students should notice that malaria is most common in Africa and Asia. The cases of malaria diagnosed each year in the United States are usually in people who were infected elsewhere.

- 13. Overlay the transparencies of thalassemia and malaria (Masters 3.6 and 3.9). Ask students what conclusions they can draw from considering these maps together.**

Students should notice that the occurrence of the two diseases is similar. Areas in which malaria is most common are also more likely to have a higher incidence of alpha-thalassemia.

14. **Explain to students that in the late 1940s, a scientist hypothesized that people who have thalassemia may have an advantage in survival over people who do not have thalassemia if they contract malaria. Recent scientific investigations collected evidence to test this hypothesis.**

Students will examine data that relate to this hypothesis in Steps 17 and 18.

15. **Provide some brief information to students about malaria:**
- **Malaria is caused by a parasitic infection (a plasmodium causes the disease, which is spread to humans through the bite of a mosquito). Malaria is not a genetic disease.**
 - **The parasites invade human red blood cells, causing them to burst. These red blood cells live a much shorter time than the normal 120-day life span.**
 - **Parasitic infection leads to a reduction in the number of red blood cells in the bloodstream. The fewer the red blood cells, the more severe the anemia (and the more problems a person has getting adequate oxygen to body cells).**

Students do not need much background information about malaria for this activity, but a brief overview is helpful. Anemia can be a very serious problem for people with malaria. Some of the data that students will analyze relate to anemia being a key symptom of malaria.

Although malaria is not a health problem in the United States, about a million people die from malaria each year worldwide and many more are infected but survive.

16. **Inform students that they will look at data that relate to the hypothesis that individuals with thalassemia have an advantage over individuals without it in terms of how they are affected by malaria. Have students again work in their groups. Half the groups will use Master 3.10, *Alpha-Thalassemia and Malaria in Papua New Guinea*, and the other half will use Master 3.11, *Alpha-Thalassemia and Malaria in Kenya*. Point out the questions on the masters, which should help them analyze the data appropriately.**

Briefly review the task and then allow time for groups to analyze the data. As they work, circulate among groups to answer questions or assist if they have trouble with the data. Reinforce to students that they need to be able to support their conclusions with data.



Content Standard A:
Formulate and revise scientific explanations and models using logic and evidence.

17. After giving students time to analyze the data and write a few conclusions, hold a class discussion to review the conclusions and check understanding. Because groups analyzed different sets of data, they should begin the discussion by briefly explaining their cases to the other students.

For the Papua New Guinea investigation, the data suggest that individuals with malaria who have one or two nonfunctional alpha-globin alleles have a lower risk for severe malarial anemia than do individuals with the normal genotype for alpha-globin. Unless treated with blood transfusions, severe malarial anemia is a life-threatening condition. The odds ratio statistic used in this study will be new for students. However, the main thing they need to know is that it is a comparison. Students need to compare the statistical value for each genotype with 1 to determine whether it is more or less likely that a person with thalassemia will have severe malarial anemia compared with a normal individual.

Table 4. Alpha-Thalassemia and Severe Malarial Anemia

Risk factor	One nonfunctional allele of the alpha-globin gene	Two nonfunctional alleles of the alpha-globin gene
Risk for developing severe malarial anemia	0.74	0.52

Source: Data from F.J.I. Fowkes et al. 2008. Increased microerythrocyte count in homozygous α^+ -thalassaemia contributes to protection against severe malarial anaemia. *PLoS Medicine* 5(3): 494-501: e56. doi:10.1371/journal.pmed.0050056

The main idea for students to understand from this analysis is that individuals with thalassemia are less likely to have severe malarial anemia than are normal individuals (no thalassemia). However, students may ask about comparing the risk of severe malarial anemia in individuals who have one nonfunctional alpha-globin allele compared with individuals who have two nonfunctional alpha-globin alleles. The further away a value is from 1 indicates how much more or how much less risk the individual has. For example, the person who has two nonfunctional alleles of the alpha-globin gene has a lower risk for severe malarial anemia than does the person who has one nonfunctional allele of the alpha-globin gene because the statistical value is further away from 1 (0.52 compared with 0.74).

For the investigation of thalassemia and malaria in Kenya, the data indicate that thalassemia has a protective effect against malaria.

In this study, for each consequence of malaria (coma, severe anemia, or death), a lower percentage of individuals who have one or two nonfunctional alleles of the alpha-globin gene have the consequence than do people with the normal genotype (no thalassemia).

Table 5. Alpha-thalassemia and the Consequences of Malaria (percent of people with each genotype that had a certain consequence)

Consequence of malaria	All alleles of the alpha-globin gene function (%)	One nonfunctional allele of the alpha-globin gene (%)	Two nonfunctional alleles of the alpha-globin gene (%)
Coma	45.1	43.2	39.8
Severe anemia (hemoglobin less than 5 g/dL)	25.8	22.4	18.1
Death	12.5	10.4	8.4

Source: Data from T.N. Williams et al. 2005. Both heterozygous and homozygous α^+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood* 106(1): 368-371.

The last question on both Masters 3.10 and 3.11 asks students to summarize what they have learned about the relationship between alpha-thalassemia and malaria. The completed sentences should be similar for both sets of data.

- 18. Give each student one copy of Master 3.12, *Summing Up Thalassemia, Malaria, and Evolution*. Ask students to answer the questions. Tell them that they will return to this master during the last activity in the supplement.**

Questions 1–3 ask students to think about how and why a disease-causing mutation may persist in the human population. The remaining questions have been covered in this lesson and should be straightforward but will help students compare thalassemia with other medical conditions discussed in this supplement. (This will be important for Lesson 5.) Ask students to include the reasons for their answers. After students have answered the questions, hold a class discussion to allow them to compare their responses. Reemphasize the major principles of natural selection (see Lesson 1) and make them explicit. Students should recognize that the same principles apply to MRSA, lactase persistence, and alpha-thalassemia.



After students complete Master 3.12, you may wish to collect their papers to assess their understandings. Use this information to guide the class discussion and clarify any remaining misconceptions.

Answer key for questions on Master 3.12, *Summing Up Thalassemia, Malaria, and Evolution*

1. Do the data from the studies in Papua New Guinea and Kenya support the hypothesis that individuals who have thalassemia might have some advantage over other individuals when living in an area where malaria is common? Explain.

Both sets of data support the hypothesis. In each investigation, the data show that the symptoms of malaria are less severe in individuals who have thalassemia.

2. Depending on their genotype, individuals with nonfunctional alpha-globin alleles may have symptoms that range from mild to more serious, including anemia, fatigue, enlarged spleen, liver problems, or even death. If the alpha-globin mutations are passed from parent to child, and individuals with four nonfunctional alpha-globin alleles die, how is the mutation maintained in the population?

Even though alpha-thalassemia can cause severe health problems and death (if the individual has three or four nonfunctional alleles of the alpha-globin gene), the condition apparently provides some benefit to individuals in areas where malaria is common. Malaria is one of the leading causes of death in the world. Individuals who are affected less severely by malaria are more likely to survive and reproduce and pass their genes on to future generations.

3. The human population shows variation for alpha-thalassemia. How did the variation arise?

Some individuals in the population have alpha-thalassemia and others do not. In addition, different individuals have different forms of the disease depending on the number of nonfunctional alleles of the gene. Alpha-thalassemia has a genetic basis. Mutations in the alpha-globin gene, which occur randomly, result in variation in the population. You may want to emphasize to students that the mutations that cause alpha-thalassemia mostly occurred in the past. Many students think that the mutations that cause genetic variation are made anew each generation. The most likely explanation for why these mutations have been maintained in some groups of humans is that they have beneficial effects with respect to malaria.

4. A common misconception related to evolution is that individuals develop mutations because the mutations fulfill some “need” or the individuals gain some benefit. In this case, this reasoning would suggest that individuals develop a mutation in the alpha-globin gene because they want or need protection from malaria. On the basis of your understanding of

evolution and natural selection, explain why this reasoning is faulty.

Mutations to the alpha-globin gene arise by chance. Individuals with the mutations have alpha-thalassemia and, depending on their genotype, have symptoms and health problems because of the genotype. This is another opportunity for students to recognize that mutations don't occur because individuals want protection against malaria. Mutations are maintained in the population because the people with specific mutations had an advantage over individuals without the mutation (in terms of survival and reproduction).

5. In certain environments, did alpha-thalassemia affect an individual's ability to survive and reproduce? Explain.

In certain cultural or environmental contexts, individuals who have alpha-thalassemia had relatively higher survival rates and left relatively more offspring. Alpha-thalassemia offers some protection against malaria. In areas with high rates of malaria, individuals with alpha-thalassemia may have a lower chance of developing severe malarial anemia or dying from malaria.

- 19. Ask students to revisit their responses to the questions on Master 1.1 in Lesson 1, Activity 1, and Masters 2.5 and 2.10 in Lesson 2. Ask them to revise their answers if the new information about natural selection caused their thinking to change.**

It may be helpful for students to make their revisions with a different-colored pen or pencil so that they have evidence of how their ideas have changed. You could assign this task as homework.

Extension Activity (Optional)

Estimated time: 20 minutes

Note: This activity is related to Activity 1. Although this extension reinforces the genetic basis for alpha-thalassemia and its heritability, students who have not reviewed basic inheritance recently may find it challenging. Students can learn the most important concepts of the lesson without completing this optional activity.

If you choose to include this activity, one option is to insert it after Step 9 of Activity 1.

- 1. Project Part 1 of Master 3.7, *The Alpha-Globin Gene and Alpha-Thalassemia*. Remind students that each individual usually**

has four functional alleles of the alpha-globin gene. Introduce students to the way the alpha-globin gene is written when working with genotypes:

- **The functional allele of the gene is written as an alpha symbol (α).**
- **The nonfunctional allele of the gene (shown as an x on the chromosome picture) is written using a minus sign (-).**

2. **Continue the introduction by providing examples of how an individual's genotype for the alpha-globin gene is written:**
 - **The genotype for a normal individual is $\alpha\alpha/\alpha\alpha$.**
 - **The genotype for someone who has a mutation in one of the four alpha-globin genes would be $\alpha\alpha/\alpha-$.**

Provide other examples if helpful for your students.

3. **Explain that students will work through the problems on Master 3.13, *Inheriting Thalassemia*, to learn how mutated, nonfunctional alleles of the alpha-globin gene are passed from parent to offspring. Give each student one copy of Master 3.13.**

Decide how you want the class to work through the problems. You may want to work through the first problem as a class. To save time, each group can work on one problem and then share the solutions with the class.

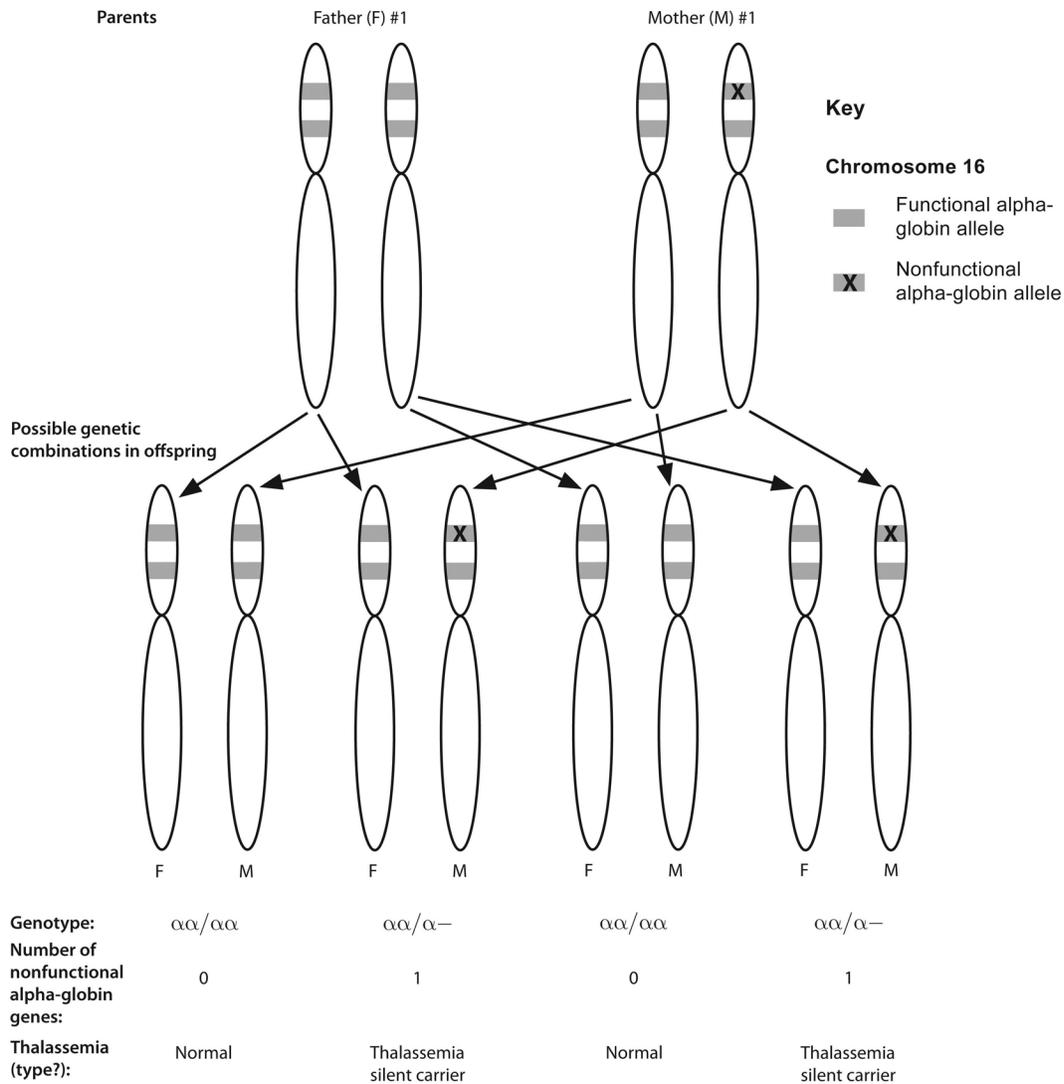
These problems should be relatively straightforward for students if they have studied basic genetics. Students should recognize that a child inherits his or her genotype from the parents, and the genotype specifies the health or disease state of the child. Students should view this as an example of probability—we can predict the probability that children of specific parents will have thalassemia. Also, this exercise helps students understand that the variation for the thalassemia phenotype has a genetic basis, which is important when considering how natural selection can act on this trait.

4. **Hold a class discussion to review the problems with the class. Allow different groups to present their results and explain their conclusions.**

Depending on the strategy you use, you may have more than one group complete each problem. If so, ask one group to present the offspring's genotypes for a problem and another group to complete the other information.

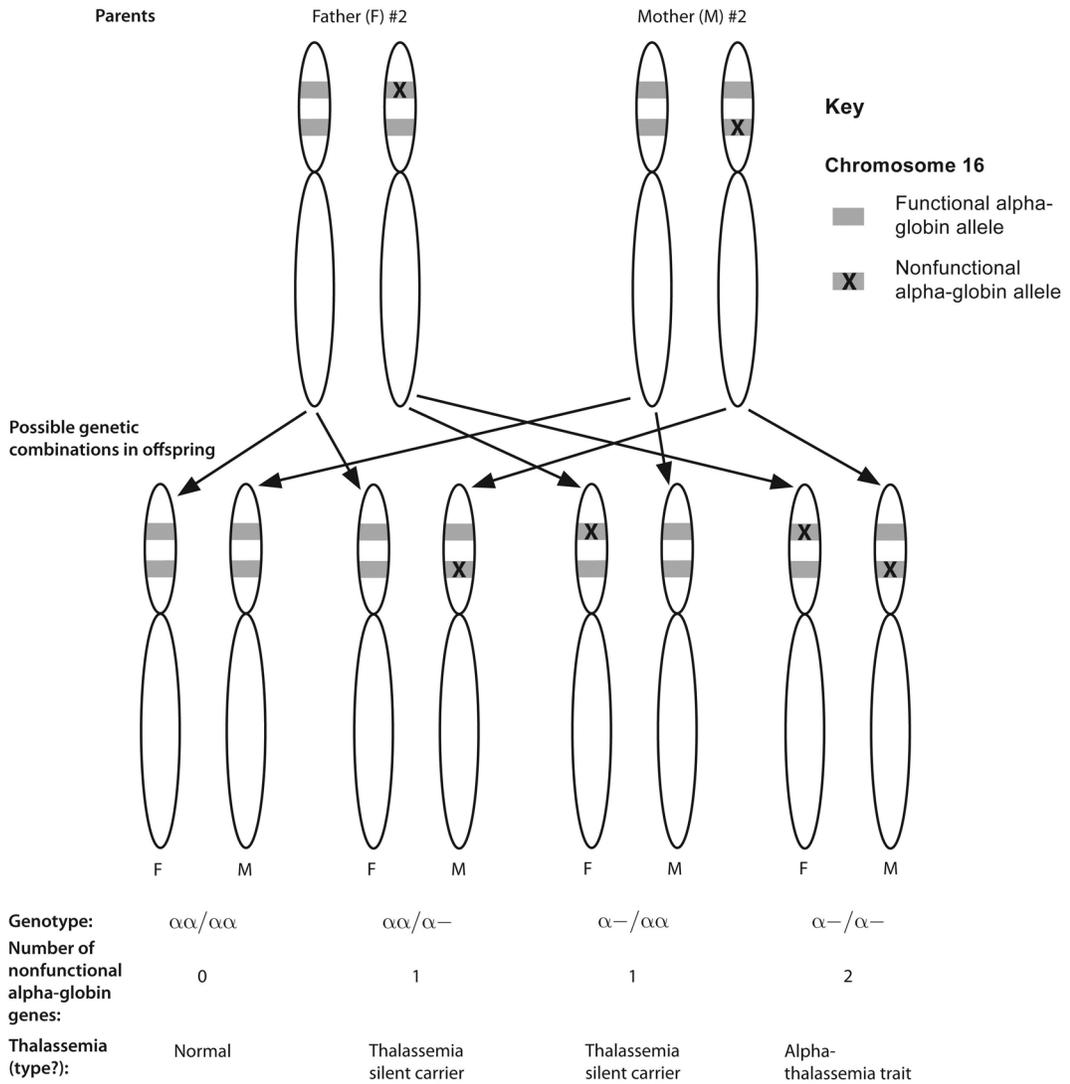
Problem 1

Figure 1. The upper part of the diagram shows a schematic of chromosome 16 pairs from Father #1 and Mother #1 with the alpha globin alleles in each parent. Each chromosome carries two copies of each allele, meaning that each person has a total of four copies of each allele. The lower part of the diagram shows the possible combinations of chromosome 16 pairs in offspring from the parents. Students fill in possible combinations of alpha-globin alleles.



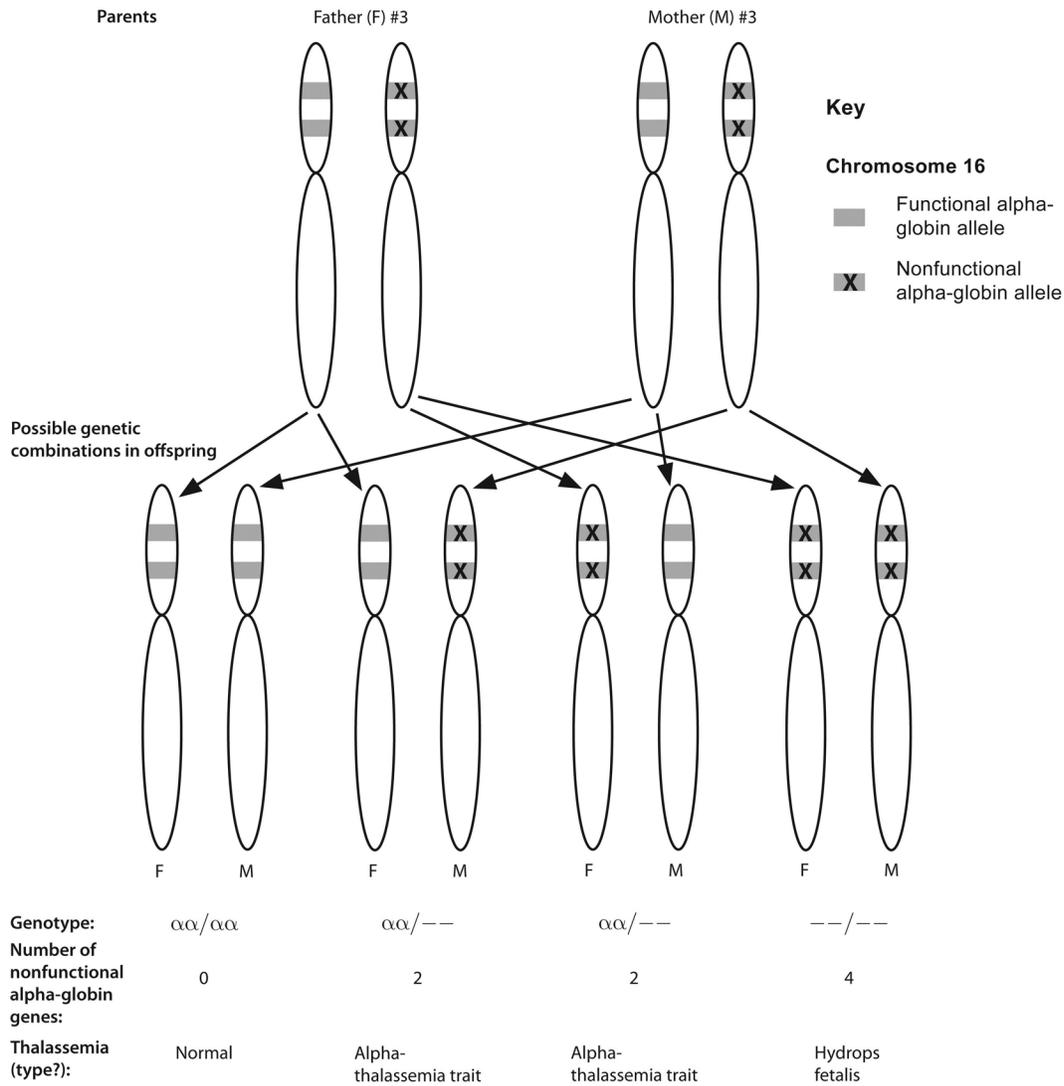
Problem 2

Figure 2. The upper part of the diagram shows a schematic of chromosome 16 pairs from Father #2 and Mother #2 with the alpha globin alleles in each parent. Each chromosome carries two copies of each allele, meaning that each person has a total of four copies of each allele. The lower part of the diagram shows the possible combinations of chromosome 16 pairs in offspring from the parents. Students fill in possible combinations of alpha-globin alleles.



Problem 3

Figure 3. The upper part of the diagram shows a schematic of chromosome 16 pairs from Father #3 and Mother #3 with the alpha globin alleles in each parent. Each chromosome carries two copies of each allele, meaning that each person has a total of four copies of each allele. The lower part of the diagram shows the possible combinations of chromosome 16 pairs in offspring from the parents. Students fill in possible combinations of alpha-globin alleles.



Procedure

Activity 2: Using Evolution to Guide Research

Estimated time: 50 minutes

Note: This activity is the second part of the Explain lesson. Its focus is to help students explain why understanding common ancestry is important for medical research. The role of common ancestry is often underemphasized in teaching about evolution, leaving students with the impression that natural selection is equivalent to evolution. However, the fact of common ancestry explains many aspects of medical research, such as the importance of using model organisms to study disease processes. The main connection between Activities 1 and 2 is that both help students build explanations for the role of evolution in medicine, though the two activities emphasize different aspects of evolution.

1. **Begin the activity by congratulating students on their work in the thalassemia case. Their work showed how the process of natural selection helps explain why some diseases are more common in certain parts of the world. Explain that now, students will undertake a new challenge. They will investigate how understanding common ancestry helps medical researchers solve problems. The students' goal in this activity is to use an understanding of common ancestry to identify sections of a gene that have not changed over vast amounts of time. Mutations to this gene cause some cases of cleft lip and palate.**
2. **Ask, “Have you heard of a condition called cleft lip and palate?” and “What do you think causes cleft lip and palate?” Ask two or three students to share their ideas in a brief class discussion.**

Some students may think that cleft lip and palate are more common in the developing world, and they may therefore conclude that environmental factors cause the condition. Be open to a range of ideas, but ask students to use logic when considering the cause. Remind students that they will have the opportunity to improve understanding throughout the activity. If students have not heard of cleft palate, tell them they will learn more about it in this lesson.



Tip from the field test: Students tend to be very interested in cleft lip and palate. Nevertheless, keep this discussion brief.

3. **Explain to students that researchers are trying to understand how clefts form and how to prevent the condition or more effectively treat it. Form student groups of three. Give each**

student one copy of Master 3.14, *Cleft Lip and Palate*. Ask students to read the questions at the end of the handout first, then read the information about cleft lip and palate, and then work together to answer the questions.

Having students read the questions they will answer before they complete the reading helps them anticipate the kinds of information they will encounter in the reading. Anticipation reading strategies help students read more successfully.

Note: The convention for three-letter code names for genes differs across species. In humans, the three-letter symbol for a gene has all the letters capitalized. In other species, only the first letter is capitalized (for example, mouse and rat), and in others, all the letters are lowercase (for example, zebrafish). To avoid confusion in this supplement, all gene symbols are in italics and have the first letter capitalized.

Answer key for questions on Master 3.14, *Cleft Lip and Palate*

1. Use the following steps to calculate the number of people expected to be born with cleft lip and palate in the United States each year.

- a. The worldwide incidence of cleft lip and palate is 14 out of 10,000 births. Calculate the frequency of cleft lip and palate by dividing the number of babies with the condition by the number of births.

The following equation shows how frequency is calculated.

$$\frac{14 \text{ babies with cleft lip or palate}}{10,000 \text{ births}} =$$

0.0014 or 1.4×10^{-3} babies with cleft lip or palate per birth

- b. Assume that there are 4,000,000 births per year in the United States. Multiply the number of births by the frequency of cleft lip and palate you calculated in Question 1a to determine the expected number of babies born with cleft palate each year in the United States.

The number of babies with cleft lip or palate can be calculated as follows.

0.0014 babies with cleft lip or palate per birth \times 4,000,000 births = 5,600 babies with cleft lip or palate born in the United States per year

2. How could a change to a gene cause cleft lip and palate? How might a change in an environmental signal cause cleft lip and palate?

Many genes play a role in the development of the head and face. Mutations to a number of these genes could disrupt the



Content Standard C:

Cells can differentiate, and complex multicellular organisms are formed as a highly organized arrangement of differentiated cells. In the development of these multicellular organisms, the progeny from a single cell form an embryo in which the cells multiply and differentiate to form the many specialized cells, tissues and organs that comprise the final organism. This differentiation is regulated through the expression of different genes.



Students' responses to Question 4 will help you assess their understanding of one of the major learning goals for this activity.

fusion of the two developing sides of the lip and palate. Similarly, a growing embryo responds to many environmental cues. Not having necessary nutrients or other environmental signals at the proper time could affect the fusion of the lip and palate.

Together, these answers imply that changes in any of many different genes and the environment may lead to the development of cleft lip and palate. This question is included to help students avoid the common misconception that many phenotypes have a simple genetic basis. In fact, most phenotypes, including disease-related ones, have a complex genetic basis and interact with the environment.

3. Assume that one parent has an allele of the *Irf6* gene with a mutation that causes cleft lip and palate and a second allele that is normal. Also assume that the second parent has two normal alleles for this gene. What is the probability that a child born to this couple will have a cleft lip and palate? Mutated *Irf6* acts in a dominant fashion.

Because Irf6 acts in a dominant fashion, there is a 50 percent chance that a child from this couple will have a cleft lip and palate.

Strictly speaking, Irf6 does not act in a simple dominant fashion. It has incomplete penetrance and variable expression. However, this is beyond what students need to know. For the purposes of this activity, students can assume that the mutated allele acts in a dominant fashion.

4. Explain how studies from mice are helpful to scientists trying to understand cleft lip and palate in humans.

Scientists learned that the gene that causes Van der Woude syndrome in humans is active in the cells that line the two sides of the forming mouth in mice. The gene is turned on and makes protein just before and during fusion of the two sides. Mice and humans inherited Irf6 from a common ancestor, and both use a similar process to develop major structures like the head. Many lines of evidence show that mice and humans share a common ancestor. Because of common ancestry, the processes that occur in the development of the face and head of mice are similar to processes in humans.

In general, scientists are able to perform experiments with mice that would not be possible or ethical in humans. What scientists learn from these experiments is helpful because humans share many chemical pathways and other physiological processes with mice due to shared ancestry.

4. **Ask one or two groups to share their ideas in a brief class discussion of the questions from Master 3.14.**



Tip from the field test: Classes that had a large number of students who struggle with math benefited from the teacher walking through the calculations in Question 1.

(For print version, skip to Step 5-p on page 120.)



In classrooms using the web version of this activity:

- 5-w. **Explain to students that they will learn a technique that scientists use to identify regions of DNA that have not changed in different organisms over long periods of time. To begin the investigation, students need to complete a tutorial on comparing genes across multiple species. They will learn a general technique that they will apply later to a gene involved in cleft palate.**

Instruct students to proceed to

<http://science.education.nih.gov/supplements/evolution/student>

Students should click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” then “Activity 2: Evoprint Tutorial.”

The tutorial lasts approximately five minutes. Ask students to take notes during the tutorial and give them time to ask questions about it after they finish. Allow students to review segments of the tutorial they found confusing.

After the tutorial, make sure that students understand that a capital letter represents a nucleotide that is identical in all the species included in the comparison. A lowercase letter represents a nucleotide that is different in at least one of the species in the comparison.

One potentially confusing section of the animation deals with calculating times in an evoprint. When comparing sequences between species, we need to consider that the sequences are evolving separately in each lineage in parallel time. So, to calculate the total amount of time, we need to add up all the times associated with each branch on the tree. It's difficult for many students to comprehend parallel time for these comparisons. For this reason, we include the following step, where students practice adding up the time on all the branches on the tree.

- 6-w. **Project Master 3.15, *Calculating Times for an Evoprint*. Ask students to calculate the amount of time that would be**



Content Standard C:

The millions of different species of plants, animals, and microorganisms that live on Earth today are related by descent from common ancestors.



Content Standard C:

In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome.

represented in evoprints that include all the species on the two different trees.

Answer key for questions on Master 3.15, *Calculating Times for an Evoprint*

1. How many years are represented in an evoprint constructed from sequences from the three species shown in the evolutionary tree below?

The time since common ancestry for each pair of species is shown below:

- Human/orangutan = 15 million years
- Human/guinea pig = 90 million years

*To calculate the total amount of time represented in an evoprint of these three species, students need to add the time from the common ancestor of humans and guinea pigs to modern humans (**90 million years**) + the time from the common ancestor of humans and guinea pigs to modern guinea pigs (**90 million years**) + the time from the common ancestor of humans and orangutans to modern orangutans (**15 million years**) = **195 million years**.*

Note: If students struggle with this calculation, consider walking through it with them. As students view the evolutionary tree in Figure 1 of Master 3.15, explain that to find the total time, they need to add up the time associated with every branch on the tree (excluding the root lineage). In other words, they need to account for every vertical line on the tree. Use a transparency pen to trace the lineage from humans to the common ancestor of humans and guinea pigs. Then write “90 million years.” Ask students what vertical lines are not accounted for. Students should note that the line leading from the common ancestor of guinea pigs and humans to modern guinea pigs is not included. Highlight this branch and ask how much time this branch represents. Write another “90 million years” on the projected master. Ask again what line or lines are not included. Students should note that the lineage leading from the common ancestor of humans and orangutans to modern orangutans is not included. Again, highlight this branch and write “15 million years.” Explain to students that to find the total number of years in the evoprint, they need to add 90 million years + 90 million years + 15 million years = **195 million years**. Ask students to use the same process to decipher the amount of time represented on the second tree.

2. If you constructed an evoprint from sequences from the four species represented in the evolutionary tree below, how many years would be represented in the evoprint?

The time since common ancestry for each pair of species is calculated as follows:

- Human/rhesus monkey = 30 million years
- Human/horse = 97 million years
- Human/platypus = 220 million years

To calculate the total amount of time represented in an evoprint of these four species, students need to add the time from the common ancestor of humans and platypuses to modern humans (**220 million years**) + the time from the common ancestor of humans and platypuses to modern platypuses (**220 million years**) + the time from the common ancestor of humans and horses to modern horses (**97 million years**) + the time from the common ancestor of humans and rhesus monkeys to modern rhesus monkeys (**30 million years**) = **567 million years**.

The times to the last common ancestor presented in this activity are estimates, but, for the sake of simplicity, they can be used here as though they were measured without error. In fact, these dates are measured with error, and they can change as we gain new information. Time estimates are from the TimeTree Web site, <http://www.timetree.org>.

7-w. To help students get a sense of the vast amount of time represented on an evolutionary tree, ask the following questions. Let students make a few guesses, and then quickly provide the answers:

- **“How long ago was 1,000 seconds?”**
Answer: About 17 minutes ago
- **“How long ago was 1 million seconds?”**
Answer: About 11½ days ago
- **“How long ago was 1 billion seconds?”**
Answer: About 32 years ago

Ask students to reflect on how 1,000 years ago seems like such a long time. Then compare that with the 567 million years they calculated in Step 6. The difference is like comparing 17 minutes (about a third of a 50-minute class period) with 18 years (longer than most of students have been alive).

This step helps students get some perspective on geologic time, which is difficult for many people to grasp. This step is meant to be brief.

8-w. Project the questions on Master 3.16, *Interpreting Evoprints*, one at a time. Ask students to use their notes and their understandings from other activities in the supplement to



Content Standard C:

Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism's offspring.

answer the questions. After giving students time to think about their answers, discuss the questions as a class.

Try to not simply provide the answers to students. Instead, use probing questions to help students make the connections necessary to answer the questions. If they are struggling, ask questions such as, “Do you think mutations occurred in the regions that did not change?” and “If so, why don't they show up in this comparison?”

Answer key for questions on Master 3.16, *Interpreting Evoprints*

1. How does an evoprint help identify regions of DNA that have not changed over large amounts of time?

Evoprints are a graphic representation that visually contrasts nucleotides that did not change against those that did change.

2. How does natural selection explain why some sequences of DNA are conserved over vast amounts of time?

Sequence conservation over vast amounts of time is an example of a type of natural selection called purifying selection. This term was introduced in the evoprint tutorial. In purifying selection, selection eliminates or decreases the frequency of mutations that have a negative effect. In other words, the mutations reduced the reproductive success of the individuals that carried them. Natural selection then eliminated these mutations.

It is important that students recognize that mutations did occur in the regions that are conserved over time. These mutations may have very briefly persisted in the gene pool. Eventually, natural selection eliminated them from the gene pool. However, new mutations are occurring all the time.

- 9-w. Explain to students that they are now ready to gather important information about a gene involved in some cases of cleft lip and palate. Their main goal is to identify sections of the *Irf6* gene that have remained the same over large amounts of time. In some cases, these regions are especially important for proper functioning. Hand out one copy of Master 3.17, *Irf6 Evoprint Comparison*, to each student. Ask students to follow the directions on the handout.**

Instruct students to proceed to <http://science.education.nih.gov/supplements/evolution/student>. They should click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” then

“Activity 2: Evoprint Comparison.” When they finish, hold a class discussion about students’ answers to the questions.

Note: In this activity, students explore 1,701 nucleotides of the *Irf6* gene. The full gene has over 18,000 nucleotides. Much of what the students see are the intron sequences flanking the third exon in the gene. The exon is near the center of the evoprint, from nucleotide 616 (starting with CTTAAAAAT, line 11) to 789 (look for GAGGGCCAT with T being nucleotide 789, line 14). The direction of transcription and translation is from the bottom to the top. The third exon is part of a DNA-binding domain, and some mutations within this exon cause Van der Woude syndrome. The bottom intron-containing conserved sequences are probably part of the gene’s regulatory machinery. If you are using the supplement with advanced classes, you may want to point out that many of the codons that span the exon show more substitutions in the third position of the codon. Substitutions to the third codon are often invisible to natural selection because they frequently do not change the amino acid in the protein due to the degeneracy of the genetic code. This is clearest on evoprints that compare multiple animals.

Answer key for questions on Master 3.17, *Irf6 Evoprint Comparison*

2. Compare the human sequence with other individual species by checking the button next to the animal of your choice in the “Comparison of two sequences” section. Compare the human sequence with at least two other species. Make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates below.

See the data in Table 1 for a summary of the actual percent similarities of all the two-species comparisons. Be open to reasonable estimates from students.

3. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species’ common ancestry with humans. Use the comparisons you completed in Step 2 and the data in Table 1 to help you with this task.

*Students should recognize that the number of changes in the *Irf6* gene increases as the time since common ancestry increases. There is an interesting exception in that rats, mice, and guinea pigs seem to show an accelerated rate of change. In other words, they have a greater number of changes than expected based on their time of divergence from humans.*

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Table 1. Time Since Common Ancestry with Humans and DNA Sequence Similarity

Species	Time since common ancestry with humans (millions of years)	Nucleotides that are the same (number)	Similarity with human sequence (%)
Chimpanzee	8	1,685	99
Orangutan	15	1,653	97
Rhesus monkey	30	1,628	96
Dog	97	1,221	72
Horse	97	1,209	71
Cat	97	1,149	68
Cow	97	891	52
Rat	91	612	36
Mouse	91	573	34
Guinea pig	91	572	34
Armadillo	105	1,039	61
Opossum	176	518	30

4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Tables 1 and 2 to complete the following tasks.

a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.

Be open to a range of reasonable answers. Many students will suggest that about half the nucleotides did not change. In fact, 688 out of 1,701 nucleotides remained the same, or 40.4 percent.

b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time represented in this comparison.

Using the method described in Step 6 of the activity, students should add the following number of years for all the branches on the tree:

$$8 + 15 + 30 + 53 + 83 + 85 + 97 + 97 = \mathbf{468 \text{ million years.}}$$

Note: Questions 4b and 5a may be difficult for some students. If you feel that students will struggle unproductively, consider answering these questions as a class.

5. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs,

horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

- a. Use the data in Tables 1, 2, and 3 and Figure 2 to calculate the amount of time represented in this evoprint.

*The total amount of time depicted on an evoprint from the species on this tree is $8 + 15 + 30 + 26 + 64 + 91 + 53 + 83 + 85 + 97 + 105 + 176 + 176 = 1,009$ million years, or **1.009 billion years**.*

- b. Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

The following sequences have not changed in the 1,009 million years represented in the evoprint:

- TTTACCTT
- TGTAGCCAGA
- TGGGCCAC
- AGCCAGGGCTT
- TGGAGGGCCATG
- CAGTTTCA
- GACTTATCA
- GATGTCAT

10-w. Explain to students that scientists recently studied the expression of the *Irf6* gene in zebrafish. They discovered that the gene is turned on during fish development in the pharyngeal arches and in cells that become the mouth, pharynx, and other structures. Ask students if this finding is consistent with how the *Irf6* gene functions in humans. Then ask how common ancestry helps explain this observation.

The discovery that zebrafish turn on the *Irf6* gene early in development and in cells that form the mouth, head, and other structures (reported by Ben et al., 2005) is consistent with how the *Irf6* gene functions in humans. This suggests that the *Irf6* gene was present in the common ancestor of humans and fish and it performed a similar function.

11-w. Students answered questions in Lesson 1, Activity 2 about the use of model organisms like mice to understand health-related issues in humans (Step 13). Give students the opportunity to revise their answers to these questions.

Encourage students to make their revisions in a different-colored pen or pencil and to merely put a line through previous



Students' answers to this question will help you assess whether they understand the importance of common ancestry for studies in model organisms.



Content Standard A:

Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used.

information they want to delete. This enables them to easily see how their thinking has changed.



Tip from the field test: It may be tempting to skip Step 11-w to save time, but asking students to revise their previous answers is an important part of the learning process.

12-w. Ask students to get back into their groups of three and write a brief report that addresses the following:

- Describe how an evoprint is a useful tool for collecting evidence to identify regions of the *Irf6* gene that did not change over large amounts of time.
- How does evolution explain why certain regions of the *Irf6* gene have not changed over large amounts of time?

Consider assigning this step for homework.

Students should mention that evoprints are a useful way to examine evidence from DNA sequence comparisons across multiple species. By comparing multiple species, it is easier to identify regions of the gene that did not change over time.

In response to the second question, students should describe that mutations did occur in the regions that are conserved over time. However, selection eliminated or decreased the frequency of mutations that had a negative effect.

End of Web-based activity.

In classrooms using the print version of this activity:



5-p. Explain to students that they will learn about a tool called an “evoprint.” Scientists can use evoprints to identify regions of DNA that have not changed in different organisms over long periods of time. Introduce students to the evoprint using the following steps.

- Project the first page of Master 3.18, *Evoprint Introduction*. Explain that this image shows 1,701 nucleotides of the *Irf6* gene in humans.**

Note: In this activity, students explore 1,701 nucleotides of the *Irf6* gene. The full gene has more than 18,000 nucleotides. Much of what the students see are intron sequences flanking the third exon in the gene. The exon is near the center of the evoprint, from nucleotide 616 (starting with CTTAAAAAT, line 11) to 789 (look for GAGGGCCAT with T being nucleotide 789, line 14). The direction of transcription and translation is from the bottom to the top. The third exon is part of a DNA-binding

domain, and some mutations within this exon cause Van der Woude syndrome. The bottom intron-containing conserved sequences are probably part of the gene's regulatory machinery. If you are using the supplement with advanced classes, you may want to point out that many of the codons that span the exon show more substitutions in the third position of the codon. Substitutions to the third codon are often invisible to natural selection because they frequently do not change the amino acid in the protein due to the degeneracy of the genetic code. This is clearest on evoprints that compare multiple animals.

- b. As you project the second page of Master 3.18, explain to students that this image is called an evoprint. It summarizes the comparison of the human and the cow sequences. Ask students what they notice about the image.**

Students will readily notice that some of the nucleotides are still represented as capital letters and some are now shown as lowercase letters. You should explain that the capital letters represent nucleotides that are identical between the two species. Lowercase letters represent nucleotides that are different in the cow compared with the human. Mention that the human sequence is used as the reference sequence, so all the nucleotides in this image are the same as those in the image showing only the human sequence. The only difference is whether or not nucleotides are capitalized.

- c. Explain to students that humans and cows last shared a common ancestor 97 million years ago. The changes they observe in the evoprint could have occurred in the lineage that led to humans or the lineage that led to cows. Each lineage has been separate for 97 million years. Thus, the evoprint represents 194 million years. Ask, “How might natural selection explain why some sequences of DNA are conserved over vast amounts of time?”**

Sequence conservation over vast amounts of time is an example of a type of natural selection called “purifying selection.” In purifying selection, selection eliminates or decreases the frequency of mutations that have a negative effect. In other words, the mutations reduced the reproductive success of the individuals that carried them. Natural selection then eliminated these mutations.

It is important that students recognize that mutations did occur in the regions that are conserved over time. These mutations may have very briefly persisted in the gene pool. Eventually, natural selection eliminated them from the gene pool. However, new mutations are occurring all the time.

- d. As you project the third page of Master 3.18, explain to students that this evoprint summarizes the comparison of**



Content Standard C: In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular “letters”) and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome.



Content Standard C: Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no differences to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism's offspring.

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the same region of DNA in four different species—humans, chimpanzees, orangutans, and rhesus monkeys. Again, ask students what they notice about the image.

Students will likely mention that there seem to be fewer changes in this evoprint compared with the human-cow evoprint. If students make this observation, ask them to try to explain the observation—why do they think there are more differences in the human-cow comparison?

- e. **Remind students that the human-cow evoprint they examined represented 194 million years. Ask students what information they think they need to figure out the amount of time for the changes on the evoprint with four species.**

Students may recognize that they need to know about the divergence times for humans and all the other species in the evoprint. If they do not recognize this, bring it to their attention.

- f. **Explain to students that examining an evolutionary tree of the species in the evoprint can help them think about the amount of time represented in the evoprint. Project the fourth page of Master 3.18, depicting an evolutionary tree of all the species in the evoprint. Point to the different lineages on the tree and emphasize that, in each lineage, substitutions could occur. Because of this, they need to add together the times for all the branches on the evolutionary tree to calculate the amount of time in the evoprint.**

To calculate the total amount of time represented in an evoprint for the four species in Master 3.18, students need to add the time from the common ancestor of humans and rhesus monkeys to modern humans (**30 million years**) + the time from the common ancestor of humans and rhesus monkeys to modern rhesus monkeys (**30 million years**) + the time from the common ancestor of humans and orangutans to modern orangutans (**15 million years**) + the time from the common ancestor of humans and chimpanzees to modern chimpanzees (**8 million years**) = **83 million years**.

Note: Calculating times in an evoprint is potentially confusing. When comparing sequences between species, we need to consider that the sequences are evolving separately in each lineage in parallel time. Thus, to calculate the total amount of time, we need to add up all the times associated with each branch on the tree. It's difficult for many students to comprehend parallel time for these comparisons.

If students struggle with this calculation, consider walking through it with them. As students view the evolutionary tree on the master, explain that to find the total time, they need to add up the time associated with every branch on the tree (excluding the root lineage). In other words, students

need to account for every vertical line on the tree. Use a transparency pen to trace the lineage from humans to the common ancestor of humans and rhesus monkeys. Then write “30 million years.” Ask students what vertical lines are not accounted for. Students should note that the line leading from the common ancestor of rhesus monkeys and humans to modern rhesus monkeys is not included. Highlight this branch and ask how much time this branch represents. Write another “30 million years” on the master. Ask again what line or lines are not included. Students should note that the lineage leading from the common ancestor of humans and orangutans to modern orangutans is not included. Again, highlight this branch and write “15 million years.” Finally, highlight the branch from the common ancestor of humans and chimpanzees leading to modern chimpanzees and write “8 million years.” Explain to students that to find the total number of years in the evoprint, they need to add 30 million years + 30 million years + 15 million years + 8 million years = **83 million years**.

The times to the last common ancestor presented in this activity are estimates, but they can be used here as though they were measured without error. In fact, these dates are measured with error, and they can change as we gain new information. Time estimates are from the TimeTree Web site, <http://www.timetree.org>.

6-p. To help students get a sense of the vast amount of time represented on the evolutionary tree, ask the following questions. Let students make a few guesses, and then quickly provide the answers:

- **“How long ago was 1,000 seconds?”**
Answer: About 17 minutes ago
- **“How long ago was 1 million seconds?”**
Answer: About 11½ days ago
- **“How long ago was 1 billion seconds?”**
Answer: About 32 years ago

Ask students to reflect on how 1,000 years ago seems like such a long time. Then compare that with the 83 million years they calculated in the previous step. The difference is like comparing 17 minutes with 2.6 years.

This step helps students get some perspective on geologic time, which is difficult for many people to grasp. This step is meant to be brief.

7-p. Explain to students that now the main goal is to identify sections of the *Irf6* gene that have remained the same over large amounts of time. In some cases, these regions are especially important for proper functioning. Divide the class into groups of three. Each student needs one copy of Master 3.19, *Investigating Irf6 Evoprints*, and each group of three needs one copy of Master 3.20, *Irf6 Evoprints*. Ask students to follow the

directions on Master 3.19. After students complete the work, hold a class discussion on the answers to the questions.

Answer key for questions in Master 3.19, *Investigating Irf6 Evoprints*

1. Work as a group to examine the evoprints in which the human sequence was compared with one other species. For at least three of the comparisons, make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates below.

See the data in Table 1 for a summary of the actual percent similarities of all the two-species comparisons. Be open to reasonable estimates from students.

2. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species' common ancestry with humans. Use the comparisons you completed in Step 1 and the data in Table 1 to help you with this task.

Students should recognize that the number of changes in the Irf6 gene increases as the time since common ancestry increases. There is an interesting exception in that rats, mice, and guinea pigs seem to show an accelerated rate of change. In other words, they have a greater number of changes than expected based on their time of divergence from humans.

Table 1. Time Since Common Ancestry with Humans and DNA Sequence Similarity

Species	Time since common ancestry with humans (millions of years)	Nucleotides that are the same (number)	Similarity with human sequence (%)
Chimpanzee	8	1,685	99
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Dog	97	1,221	72
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Cow	97	891	52
Rat	91	612	36
Mouse	91	573	34
Guinea pig	91	572	34
Armadillo	105	1,039	61
Opossum	176	518	30

3. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Tables 1 and 2 to complete the following tasks.
- a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.

Be open to a range of reasonable answers. Many students will suggest that about half the nucleotides did not change. In fact, 688 out of 1,701 nucleotides remained the same, or 40.4 percent.

- b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time represented in this evoprint.

Using the same method as described in Step 5, students should add the following number of years for all the branches on the tree: $8 + 15 + 30 + 53 + 83 + 85 + 97 + 97 = 468$ million years.

Note: Questions 3b and 4a may be difficult for some students. If you feel that students will struggle unproductively, consider answering these questions as a class.

4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

- a. Use the data in Tables 1, 2, and 3 and Figure 2 to calculate the amount of time represented in this evoprint.

*The total amount of time depicted on an evoprint from the species on this tree is $8 + 15 + 30 + 26 + 64 + 91 + 53 + 83 + 85 + 97 + 105 + 176 + 176 = 1,009$ million years, or **1.009 billion years**.*

- b. Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

The following sequences have not changed in the 1,009 million years represented in the evoprint:

- TTTACCTT
- TGTAGCCAGA
- TGGGCCAC
- AGCCAGGGCTT
- TGGAGGGCCATG
- CAGTTTCA
- GACTTATCA
- GATGTCAT



Students' answers to these questions again help you assess whether or not they understand the importance of common ancestry for studies in model organisms.



Content Standard A: Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used.

- 8-p. Explain to students that scientists recently studied the expression of the *Irf6* gene in zebrafish. They discovered that the gene is turned on during fish development in the pharyngeal arches and in cells that become the mouth, pharynx, and other structures. Ask students if this finding is consistent with how the *Irf6* gene functions in humans. Then ask how common ancestry helps explain this observation.

The discovery that zebrafish turn on the *Irf6* gene early in development and in structures that form the mouth, head, and other structures (reported by Ben et al., 2005) is consistent with how the *Irf6* gene functions in humans. This suggests that the *Irf6* gene was present in the common ancestor of humans and fish and it performed a similar function.

- 9-p. Students answered questions in Lesson 1, Activity 2 about the use of model organisms like mice to understand health-related issues in humans (Step 13). Give students the opportunity to revise their answers to these questions.

Encourage students to make their revisions in a different-colored pen or pencil and to merely put a line through previous information they want to delete. This enables them to easily see how their thinking has changed.



Tip from the field test: It may be tempting to skip Step 9 to save time, but asking students to revise their previous answers is an important part of the learning process.

- 10-p. Ask students to get back into their groups of three and write a brief report that addresses the following:
- Describe how an evoprint is a useful tool for collecting evidence to identify regions of the *Irf6* gene that did not change over large amounts of time.
 - How does evolution help explain why certain regions of the *Irf6* gene have not changed over large amounts of time?

Consider assigning this step for homework.

Students should mention that evoprints are a useful way to examine evidence from DNA sequence comparisons across multiple species. By comparing multiple species, it's easier to identify regions of the gene that did not change over time.

In response to the second question, students should describe that mutations did occur in the regions that are conserved over time. However, selection eliminated or decreased the frequency of mutations that had a negative effect.

Lesson 3 Organizer: Web Version



Activity 1: Investigating a Mystery Disease

Estimated time: 100 minutes

Project Master 3.1 and read aloud with your students. Explain that students will be exploring this scenario and finding out what is causing the health problems.	Page 93 Step 1	
Give each student one copy of Master 3.2 . Have students work in groups of three to four to investigate two cases and determine each individual's health problem.	Page 93 Step 2	
Explain to students that the case analyses include two parts: analyzing blood cell images and analyzing the laboratory results of blood tests. Point out that an online reference manual will help them figure out the problem.	Page 94 Step 3-w	
Have students work through the activity on the Web site by clicking on "Lesson 3: Evolutionary Processes and Patterns Inform Medicine," then "Activity 1: Investigating a Mystery Disease."	Page 94 Step 4-w	
Project Master 3.5 . Use this chart to guide a class discussion of the results of students' investigations.	Page 95 Step 5	
Help students focus on thalassemia by asking the following questions: <ul style="list-style-type: none"> • "Have you heard of thalassemia?" • "Do you think thalassemia is a common disease?" 	Page 96 Step 6	
Project Master 3.6 . Ask students to draw conclusions from this map.	Page 96 Step 7	
After students recognize that thalassemia is more common in some places than others, ask them for a brief statement about variation for thalassemia in the human population.	Page 97 Step 8	
Project Master 3.7 . <ul style="list-style-type: none"> • Review the information in Part 1 and then in Part 2. • Ask students if they see a relationship between the number of nonfunctional alleles of the alpha-globin gene and the severity of symptoms for alpha-thalassemia. <p>*If you want to include the optional activity, insert it after Step 9.</p>	Page 98 Step 9	
Give each student 1 copy of Master 3.8 . Have students work with their groups to answer the questions.	Page 99 Step 10	

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Ask students why a mutant alpha-globin allele would be maintained in the human population if it causes disease.	Page 100 Step 11	
Explain to the class that the original news story referenced another disease that has anemia as a symptom. <ul style="list-style-type: none"> • Project Master 3.9. • Ask students to make observations about the occurrence of malaria. 	Page 100 Step 12	
Overlay the transparencies showing thalassemia and malaria (Masters 3.6 and 3.9). Ask students to draw conclusions about the distribution of these diseases.	Page 100 Step 13	
Introduce students to the hypothesis that people who have thalassemia may have an advantage in survival over people who do not have thalassemia if they contract malaria. Provide some brief information about malaria.	Page 101 Steps 14 and 15	
Explain that students will analyze data to determine whether malaria affects people who do and who do not have thalassemia differently. <ul style="list-style-type: none"> • Give students in half the groups one copy each of Master 3.10 and students in the other groups one copy each of Master 3.11. 	Page 101 Step 16	
After students complete the analysis, hold a class discussion so groups can share their analyses and review their conclusions.	Page 102 Step 17	
Give each student one copy of Master 3.12 . Ask students to answer the questions. Inform them that they will use their answers in the last activity of this supplement.	Page 103 Step 18	
Ask students to revisit and revise their responses to the questions on Master 1.1 in Lesson 1, Activity 1, and Masters 2.5 and 2.10 in Lesson 2.	Page 105 Step 19	
Activity 2: Using Evolution to Guide Research Estimated time: 50 minutes		
Explain to students that the goal is to use an understanding of common ancestry to identify sections of a gene that have not changed over vast amounts of time. They will study a gene in which mutations cause some cases of cleft lip and palate. Ask, <ul style="list-style-type: none"> • "Have you heard of a condition called cleft lip and palate?" • "What do you think causes cleft lip and palate?" Ask two or three students to share their ideas about the cause.	Page 110 Steps 1 and 2	
Divide the class into groups of three. Hand out one copy of Master 3.14 to each student. Students should first read the questions at the end of the handout. After completing the reading, groups should work together on the answers. Have one or two groups share their answers in a class discussion.	Pages 110 and 113 Steps 3 and 4	

<p>Explain to students that they will use a technique to identify regions of DNA that have not changed in different organisms over long periods of time. Then have them log on to the Web site and click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” and then “Activity 2: Evoprint Tutorial.”</p>	<p>Page 113 Step 5-w</p> 
<p>Project Master 3.15 and ask students to calculate the amount of time represented in evoprints that include all the species on the two different trees.</p>	<p>Page 113 Step 6-w</p> 
<p>Ask students the following questions; let them make a quick guess, then quickly reveal the answers:</p> <ul style="list-style-type: none"> • How long ago was 1,000 seconds? Answer: About 17 minutes ago • How long ago was 1 million seconds? Answer: About 11½ days ago • How long ago was 1 billion seconds? Answer: About 32 years ago 	<p>Page 115 Step 7-w</p>
<p>Display the questions on Master 3.16 one at a time. After students think about their answers, lead a class discussion on the questions.</p>	<p>Page 115 Step 8-w</p> 
<p>Explain to students that they will identify sections of the <i>Irf6</i> gene that have remained the same over large amounts of time.</p> <ul style="list-style-type: none"> • Give one copy of Master 3.17 to each student. Students should follow the directions on the handout. • Have students log on to the Web site and click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” then “Activity 2: Evoprint Comparison.” <p>Hold a class discussion about students’ answers to the questions.</p>	<p>Page 116 Step 9-w</p>  
<p>Explain the discovery that zebrafish express the <i>Irf6</i> gene in cells that become the mouth, pharynx, and other structures. Ask,</p> <ul style="list-style-type: none"> • Is this consistent with how the <i>Irf6</i> gene functions in humans? • How does common ancestry help explain this observation? 	<p>Page 119 Step 10-w</p>
<p>Have students revise their answers to the questions in Step 13 from Lesson 1, Activity 2 about model organisms and health.</p>	<p>Page 119 Step 11-w</p>
<p>Ask students to get back into their groups of three and write a report on the following:</p> <ul style="list-style-type: none"> • Describe how an evoprint is a useful tool for collecting evidence to identify regions of the <i>Irf6</i> gene that did not change over large amounts of time. • How does evolution explain why certain regions of the <i>Irf6</i> gene have not changed over large amounts of time? 	<p>Page 120 Step 12-w</p>

 = Involves copying a master.

 = Involves making a transparency.

 = Involves using the Internet.

Lesson 3 Organizer: Print Version



Activity 1: Investigating a Mystery Disease		
Estimated time: 100 minutes		
Project Master 3.1 , and read the news story with your students. Explain that students will be exploring this scenario and finding out what is causing the health problems.	Page 93 Step 1	
Give each student one copy of Master 3.2 . Have students work in groups of three to four to investigate two cases and determine each individual's health problem.	Page 93 Step 2	
Explain to students that the case analyses include two parts: analyzing blood cell images and analyzing the laboratory results of blood tests. <ul style="list-style-type: none"> Point out where students can pick up the data for their assigned patients (from Master 3.3). Give each group one copy of Master 3.4. 	Page 95 Steps 3-p and 4-p	
Project Master 3.5 . Use this chart to guide a class discussion of the results of students' investigations.	Page 95 Step 5	
Help students focus on thalassemia by asking the following questions: <ul style="list-style-type: none"> "Have you heard of thalassemia?" "Do you think thalassemia is a common disease?" 	Page 96 Step 6	
Project Master 3.6 . Ask students to draw conclusions from this map.	Page 96 Step 7	
After students recognize that thalassemia is more common in some places than others, ask them for a brief statement about variation for thalassemia in the human population.	Page 97 Step 8	
Project Master 3.7 . <ul style="list-style-type: none"> Review the information in Part 1 and then in Part 2. Ask students whether they see a relationship between the number of nonfunctional alleles of the alpha-globin gene and the severity of symptoms for alpha-thalassemia. *If you want to include the optional activity, insert it after Step 9.	Page 98 Step 9	
Give each student one copy of Master 3.8 . Have students work with their groups to answer the questions.	Page 99 Step 10	
Ask students why a mutant alpha-globin allele would be maintained in the human population if it causes disease.	Page 100 Step 11	

<p>Explain to the class that the original news story referenced another disease that has anemia as a symptom.</p> <ul style="list-style-type: none"> • Project Master 3.9. • Ask students to make observations about the occurrence of malaria. 	<p>Page 100 Step 12</p> 
<p>Overlay the transparencies showing thalassemia and malaria (Masters 3.6 and 3.9). Ask students to draw conclusions about the distribution of these diseases.</p>	<p>Page 100 Step 13</p>
<p>Introduce students to the hypothesis that people who have thalassemia may have an advantage in survival over people who do not have thalassemia if they contract malaria. Provide some brief information about malaria to students.</p>	<p>Page 101 Steps 14 and 15</p>
<p>Explain that students will analyze data to determine whether malaria affects people who do and who do not have thalassemia differently.</p> <ul style="list-style-type: none"> • Give students in half the groups one copy each of Master 3.10 and students in the other groups, one copy each of Master 3.11. 	<p>Page 101 Step 16</p> 
<p>After students complete the analysis, hold a class discussion so groups can share their analyses and review their conclusions.</p>	<p>Page 101 Step 17</p>
<p>Give each student one copy of Master 3.12. Ask students to answer the questions. Inform them that they will use their answers in the last activity of this supplement.</p>	<p>Page 103 Step 18</p> 
<p>Ask students to revisit and revise their responses to the questions in on Master 1.1 in Lesson 1, Activity 1, and Masters 2.5 and 2.10 in Lesson 2.</p>	<p>Page 105 Step 19</p>
<p>Activity 2: Using Evolution to Estimated time: 50 minutes</p>	
<p>Describe to students that the goal is to use an understanding of common ancestry to identify sections of a gene that have not changed over vast amounts of time. They will study a gene in which mutations cause some cases of cleft lip and palate. Ask students,</p> <ul style="list-style-type: none"> • “Have you heard of a condition called cleft lip and palate?” • “What do you think causes cleft lip and palate?” <p>Ask two or three students to share their ideas about the cause of cleft lip and palate.</p>	<p>Page 110 Steps 1 and 2</p>
<p>Divide the class into groups of three. Give one copy of Master 3.14 to each student. Students should first read the questions at the end of the handout. After completing the reading, should work together in groups on the answers. Have one or two groups share their answers in a class discussion.</p>	<p>Pages 110–113 Steps 3 and 4</p> 

<p>Explain to students that they will learn about a tool called an “evoprint” that identifies regions of DNA that have not changed over long periods of time.</p> <ul style="list-style-type: none"> • Project the first page of Master 3.18 (the <i>Irf6</i> gene in human). • Show the human/cow evoprint comparison (page 2 of Master 3.18). Discuss student observations. • Ask, “How might natural selection explain why sequences of DNA are conserved over vast amounts of time?” • Show the human/chimpanzee/orangutan/rhesus monkey evoprint comparison (page 3 of Master 3.18). Discuss student observations. • Ask students how they think they should calculate the amount of time for the changes on this evoprint. • Describe how to calculate time on an evoprint by showing page 4 of Master 3.18. Point out that substitutions can occur in all lineages. As a result, they need to add together the times for all the branches on the evolutionary tree. 	<p>Page 120 Step 5-p</p> 
<p>Ask students the following questions; let them make a quick guess, then quickly reveal the answers.</p> <ul style="list-style-type: none"> • “How long ago was 1,000 seconds?” Answer: About 17 minutes ago • “How long ago was 1 million seconds?” Answer: About 11½ days ago • “How long ago was 1 billion seconds?” Answer: About 32 years ago 	<p>Page 123 Step 6-p</p>
<p>Explain to students that they will identify sections of the <i>Irf6</i> gene that have remained the same over large amounts of time.</p> <ul style="list-style-type: none"> • Give each student one copy of Master 3.19. • Give each group of three one copy of Master 3.20. • After students complete their work, hold a class discussion on the answers to the questions. 	<p>Page 123 Step 7-p</p> 
<p>Explain the discovery that zebrafish express the <i>Irf6</i> gene in cells that become the mouth, pharynx, and other structures. Ask students if this is consistent with how the <i>Irf6</i> gene functions in humans and how common ancestry helps explain this observation.</p>	<p>Page 126 Step 8-p</p>
<p>Have students revise their answers to the questions in Step 13 from Lesson 1, Activity 2 about model organisms and health.</p>	<p>Page 126 Step 9-p</p>
<p>Ask students to get back into their groups of three and write a report on the following:</p> <ul style="list-style-type: none"> • Describe how an evoprint is a useful tool for collecting evidence to identify regions of the <i>Irf6</i> gene that did not change over large amounts of time. • How does evolution explain why certain regions of the <i>Irf6</i> gene have not changed over large amounts of time? 	<p>Page 126 Step 10-p</p>

 = Involves copying a master.  = Involves making a transparency.

Lesson 3, Activity 1: Investigating a Mystery Disease, includes an optional activity to reinforce the inheritance of alpha-thalassemia. (Insert after Step 9 of Activity 1.)

Lesson 3 Optional Activity Organizer

Activity 1: Investigating a Mystery Disease— Extension Activity (Optional)

Estimated time: 20 minutes

Project Part 1 of Master 3.7 . Remind students that each individual usually has four functional alleles of the alpha-globin gene. Introduce students to the way the alpha-globin alleles are written when working with genotypes.	Page 105 Step 1	
Discuss examples of how an individual's genotype for the alpha-globin gene is written: <ul style="list-style-type: none"> The genotype for a normal individual is $\alpha\alpha/\alpha\alpha$. The genotype for someone who has a mutation in one of the four alpha-globin genes is $\alpha\alpha/\alpha-$. 	Page 106 Step 2	
Give each student one copy of Master 3.13 . Explain that students will work on the master with their groups to learn how mutated, nonfunctional alleles of the alpha-globin gene are passed from parent to offspring.	Page 106 Step 3	
Hold a class discussion to review the problems with the class. Allow different groups to present their results and explain their conclusions.	Page 106 Step 4	
Continue with the rest of Activity 1.		

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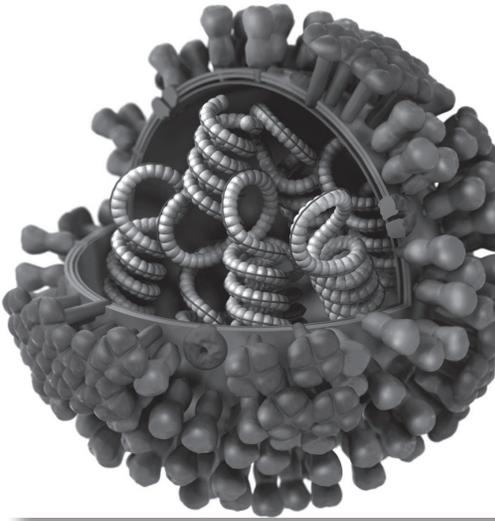
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Lesson 4

Using Evolution to Understand Influenza



Elaborate

At a Glance

Overview

In this Elaborate activity, students use what they learned about evolution and how it affects medicine to better understand influenza. The main question that drives the lesson is this: Why is a new flu vaccine needed every few years? Students answer this question and gather other information about evolution and influenza as they create an outline of a brochure for a biotechnology company. After examining introductory information about influenza, students align portions of the sequence from the hemagglutinin gene from three influenza viruses. Students then learn about genomic resources, see an alignment and an evolutionary tree for 11 influenza viruses for a larger portion of the hemagglutinin gene, and compute the number of changes that have accumulated in 35 years. After learning more about how the influenza virus interacts with the immune system, students describe how natural selection influences the evolution of influenza. The rapid rate of evolution in influenza helps explain why a new flu vaccine is needed every few years.

Major Concepts

- Natural selection is a powerful process of evolution and is the only mechanism to consistently yield adaptations.
- Understanding mechanisms of evolution, particularly adaptation by natural selection, provides many insights that enhance medical practice and understanding.
- Evolutionary comparisons are important for studying biomedical problems.
- Understanding evolution helps explain the emergence and spread of infectious diseases.

Objectives

After completing this lesson, students will

- be able to explain how comparisons of genetic sequences can help inform public health decisions,
- recognize that the rates of evolutionary change in genetic sequences give clues about the role of purifying and diversifying selection on that region,
- have used the major principles of natural selection to describe how influenza viruses evolve in response to the human immune system, and
- understand that evolutionary theory helps explain aspects of vaccines.

Teacher Background

Consult the following sections in Information about Evolution and Medicine:

4.0 Students' Prior Conceptions about Evolution (pages 31–33)

5.0 Featured Examples of Evolution and Medicine (pages 33–37)

In Advance

Web-Based Activities

Activity	Web Component?
1	Yes

Photocopies, Transparencies, Equipment, and Materials

Photocopies and Transparencies
For Classes Using Web-Based Activity: 1 transparency of Masters 4.1 and 4.5 1 copy of Masters 4.2, 4.3, 4.6, and 4.11 for each student 1 copy of Master 4.4 on two separate pages for each group of two students

Continued

Photocopies and Transparencies

For Classes Using Print-Based Activity:

1 transparency of Masters 4.1 and 4.5, 4.7, 4.8, and 4.10

1 copy of Masters 4.2, 4.3, 4.6, and 4.11 **for each student**

1 copy of Masters 4.4 and 4.9 **for each group of two students**

Equipment and Materials

Each group of two students will need one pair of scissors, one roll of tape, and one piece of blank paper.

Preparation

Provide scissors, tape, and blank paper so students can cut out and align the sequences from page 2 of **Master 4.4, Influenza Sequences**. Make the other necessary copies and overhead transparencies.

For classes using the Web version, verify that the computer lab is reserved for your classes or that the classroom computers are set up for the activities. Check that the Internet connection is working properly. Set the computers to the opening screen for the activity. Log on to the “Student Activities” section of the Web site by entering the following URL:



<http://science.education.nih.gov/supplements/evolution/student>

Select “Lesson 4: Using Evolution to Understand Influenza.”

Procedure

Activity 1: Using Evolution to Understand Influenza

Estimated time: 100 minutes

Note: Lesson 4 is an Elaborate activity, designed to have students go deeper into the major concepts they have learned and apply them to additional real-life examples. With further experience with the major concepts, students should increase their understandings of how the concepts apply more broadly. In this lesson, students explore the evolution of influenza viruses over time, using genomic resources and bioinformatic tools. With the help of a fictional genome database, students create an outline of a brochure for a fictional biotechnology company. The fictional database is a simplified version of the publicly available one housed by the National Center for Biotechnology Information (NCBI). We chose a fictional database to help students stay focused on the main learning goals of the activity. You may wish to show students the actual genome database maintained by NCBI for influenza in the “Database” link at <http://www.ncbi.nlm.nih.gov/genomes/FLU/>.

1. **Begin the lesson by asking students if they have any questions about influenza, or the “flu.”**

Ask students to record their answers to the questions in their notebooks. We included this step to help students understand that issues related to evolution and medicine are likely to affect them personally because most students will suffer from the flu at some point in their lives. Many students will recognize that improved hygiene, such as frequent hand washing, is important in preventing the flu, as is receiving a yearly vaccination.

2. **Write three to four student questions on the board. Explain to students that throughout most of human history, the cause of influenza was unknown. In temperate regions, it suddenly appeared in the fall or winter, affected and even killed people, then went away in the spring. Explain that in this activity, students will explore some of their questions about the flu.**
3. **Project Master 4.1, *E-mail from Viroformatics*, to the class. Ask for a volunteer to read the email aloud to the class. Ask students whether they have any questions. Explain that they will compile the information for Viroformatics throughout the rest of the activity.**
4. **Give each student one copy of Master 4.2, *Notes about Influenza and Evolution*. Give students a few minutes to review and start answering the “what I think before” questions on their own. Before they begin answering Question 5, explain that researchers formulated 19 different vaccines for influenza from 1975 to 2008. During the same time period, no new vaccines were needed for other diseases caused by viruses, such as polio and the measles. Question 5 asks students to write an initial explanation for why so many new influenza vaccines are needed. Reinforce that students should use what they learned about evolution to develop the initial explanation. They will fill out the “what I think after” questions as they proceed through the activity.**



The questions posed on Master 4.2 ask students to record their initial ideas as well as their ideas after completing the investigation. These responses give you an opportunity to assess students' relative understandings of some major concepts about evolution and the flu and to see how the ideas change after students complete the work.

The full explanation for why multiple vaccines are needed over time for influenza involves both natural selection by the human immune system and the transmission dynamics of the virus. Influenza is infectious for a relatively short time. Additionally, the viruses that are in circulation change relatively rapidly. This helps explain why there is limited diversity in influenza at any one time. Students will only explore the role of natural selection. One hypothesis for the usefulness of the measles, mumps, and rubella vaccination over many decades is that these viruses interact with the immune system

in multiple ways. Therefore, a virus that has a mutation that allows it to overcome one immune response is blocked from replicating because the vaccination has provided protection through additional immune responses.



Tip from the field test: Many students struggled to apply the terms “individual” and “population” to viruses. By the end of the activity, you should check to see whether students understand that mutations occur in individual viruses. If an individual with a specific mutation replicates more successfully than other viruses, the population of viruses will have a higher frequency of that mutation in future generations.

Note: If you’re interested in helping students develop a deeper understanding of vaccines, consider using Activity 4: Protecting the Herd from the NIH curriculum supplement *Emerging and Re-emerging Infectious Diseases* (available at <http://science.education.nih.gov/customers.nsf/HSDiseases.htm>).

5. Ask students to work with a partner to briefly share their answers to the questions on Master 4.2.

Allow only about five minutes for this task. As you listen to groups sharing their ideas, insist that students be explicit about how they think evolution is involved in many of the answers.

- 6. Explain to students that to complete the task for Viroformatics, they need to have a deeper understanding of both evolution and influenza. To help construct these understandings, students will use some of the tools that scientists use to study evolution in influenza viruses.**
- 7. Hand out one copy of Master 4.3, *Introduction to Influenza*, to each student. Ask students to read it individually. As they read, they should add at least five total facts from the reading to the appropriate questions on Master 4.2 in the “what I think after” sections. Students should also record in their notebooks at least one question they have about the reading.**
- 8. Students should meet with their groups again. Ask them to share and try to answer their questions within their groups. They should note questions that the group cannot answer and raise them in a brief class discussion about the reading on Master 4.3. Make sure that you address all five of the questions from Master 4.2, but do not provide full explanations at this point in the lesson.**



Content Standard F:

The severity of disease symptoms is dependent on many factors, such as human resistance and the virulence of the disease-producing organism. Many diseases can be prevented, controlled, or cured. Some diseases, such as cancer, result from specific body dysfunctions and cannot be transmitted.

The goal of the class discussion is for students to become aware of their initial ideas and the ideas of their classmates. This step helps students take control of their own learning about influenza. Students will revise and improve their answers to the questions throughout the lesson.

- 9. Explain to students that they will begin the investigation of influenza and evolution by examining a segment of the gene that codes for the hemagglutinin protein. They will examine three sequences taken from type A influenza viruses, subtype H3N2. The samples were collected from patients in 2003, 1997, and 1993. Hand out scissors, tape, a blank piece of paper, and one copy of Master 4.4, *Influenza Sequences*, to each group. Instruct students to follow the directions on the handout.**

This portion of the investigation gives students a feel for how scientists align genetic sequences. Students may notice that the sequences are recorded as DNA, but influenza is an RNA virus. The reason is that public databases of genetic sequences store information only as DNA.

- 10. Ask each group to compare their alignment to that of another group. After they discuss the alignment each group developed, project Master 4.5, *Aligned Influenza Sequences*. Ask students to compare their alignments with your alignment and to report the total number of changes they observe.**

Students should find that there are four differences among the sequences.

- 11. Explain that scientists often compare sequences from thousands of influenza viruses that are thousands of nucleotides long. Ask students if they think it would be reasonable to align the sequences by hand. Get students to agree that this task would best be completed by using a computer.**

(For print version, skip to Step 12a-p on page 144.)



In classrooms using the web version of this activity:

12-w. Explain that students will now get access to the Viral Genome Database at Viroformatics. Hand out one copy of Master 4.6, *Exploring a Genetic Database*, to each student. Have students go to <http://science.education.nih.gov/supplements/evolution/student>.

Students should click on “Lesson 4: Using Evolution to Understand Influenza.”

13-w. Ask students to work with a partner to accomplish all the tasks on Master 4.6.

Now would be a good time to mention that the National Center for Biotechnology Information (NCBI) maintains an extensive genome database that is free and available for use by the public. All federally funded researchers and most private companies submit sequences to the database. NCBI was established in 1988 as a division of the National Library of Medicine at the National Institutes of Health. NCBI’s mission is to develop information technologies to help researchers understand the molecular and genetic processes that affect health. NCBI stores sequence information for a large range of organisms across the Tree of Life, including humans. You may want to show students the actual NCBI Influenza Virus Resource Web site at <http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html>. After accessing this page, click on “Database.”

Steps 1 and 2 on Master 4.6 help students get a sense of the massive amount of genetic data available to study influenza. More broadly, students should recognize that the availability of sequence data from a range of organisms is increasing almost exponentially. Multiple career opportunities exist for students who are interested in both computation and biology. You can repeat this activity’s search at the NCBI Influenza Virus Resource Web site. Input the following selections: Type, “A”; Host, “human”; Country/Region, “any”; Protein, “HA”; Subtype, “any.” Then click on “Show results.”

14-w. Hold a class discussion about the answers to the questions on Master 4.6.

The questions on the handout ask students to perform simple calculations. To complete the tasks, students need to use division. Some students may need help setting up the problem.



Content Standard A: Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used.

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Content Standard A: Mathematics is essential in scientific inquiry. Mathematical tools and models guide and improve the posing of questions, gathering data, constructing explanations and communicating results.

Questions 6 through 8 use scientific notation. If your students are not comfortable with scientific notation, simply write out the answer using decimal places. The main concept students need to understand is that the rate of change in genetic sequences can differ by many orders of magnitude. Ultimately, students will understand the role of selection in shaping some of the differences in rates.

Answer key for questions on Master 4.6, *Exploring a Genetic Database*

1. Your first goal is to get a sense of the types and amount of data stored in the database. Click on “Viral Genome Database,” then “Full Virus Database.” Once there, explore the number of sequences available for influenza in humans for the hemagglutinin gene. Record the number available in this database for types A, B, and C. (You can explore the data for specific sequences by clicking on the accession number. By convention, the genetic sequences are recorded as DNA. Influenza viruses store their genetic information as RNA.)

There are 17,387 sequences available for type A, 2,945 sequences for type B, and none for type C.

2. Why do you think the number of sequences for each type is different?

Students may recall from their reading that type A influenza is responsible for pandemic outbreaks, which explains why researchers are very interested in this type. Types A and B cause the seasonal flu that sweeps around the globe every year. Type C causes only a mild illness and does not cause epidemics, so it's not studied as intensely. We didn't include genome data for type C in our database, although there were 948 sequence entries in the NCBI database when it was accessed in March, 2011.

5. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}} = \frac{32 \text{ changes}}{100 \text{ nucleotides}} = 0.32 \text{ changes per nucleotide}$$

6. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\begin{aligned} \frac{\text{answer from Step 5}}{\text{total number of years}} &= \frac{0.32 \text{ changes per nucleotide}}{35 \text{ years}} \\ &= 9.1 \times 10^{-3} \text{ changes per nucleotide per year} \\ &= 0.0091 \text{ changes per nucleotide per year} \end{aligned}$$

7. In Lesson 3, you investigated the sequence of a gene called *Irf6* that is involved in the development of the head and face. Use the same formulas you used in Steps 5 and 6 to calculate the expected number of changes per nucleotide per year in this sequence.

$$\frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}}$$

$$= \frac{4 \text{ changes}}{30 \text{ nucleotides}} = 0.13 \text{ changes per nucleotide}$$

$$\frac{\text{number of changes per nucleotide}}{\text{total number of years}} = \frac{0.13 \text{ changes per nucleotide}}{1,009,000,000 \text{ years}}$$

$$= 1.3 \times 10^{-10} \text{ changes per nucleotide per year}$$

$$= 0.0000000013 \text{ changes per nucleotide per year.}$$

8. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza to the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes compared to the other.

$$\frac{\text{rate of change in hemagglutinin gene}}{\text{rate of change in } Irf6 \text{ gene}} = \frac{9.1 \times 10^{-3}}{1.3 \times 10^{-10}} = 7.0 \times 10^7 = 70,000,000$$

*The rate of change in the hemagglutinin gene is 70 million times faster than the rate of change in the *Irf6* gene for the regions compared.*

9. To see a diagram that summarizes the relationships among the viruses, click on “Build a Tree.” Does this diagram show evidence that the influenza virus is changing over time?

Students should note that the relationships of the samples relate to time. For example, the sample from 2003 is more closely related to the sample from 1997, which is the closest sample in terms of date. Students may also note that there is a large distance between the sample from 1968 and the sample from 2003.

- 10a, b. How do the number of changes to the sequence relate to time? What do you think this means?

Students should see the pattern that the number of changes to the sequences increases with time. This is strong evidence that the influenza virus keeps changing over time.



Content Standard C:

Natural selection and its evolutionary consequences provide a scientific explanation for the fossil record of ancient life forms, as well as for the striking molecular similarities observed among the diverse species of living organisms.



Understanding the role of mutation in generating diversity is essential. By this stage of the learning cycle, students have had multiple opportunities to learn about the importance of mutation.

Note: If you would like students to gain additional practice graphing and interpreting results, consider having them generate a graph that shows the number of years' difference between two viruses on the x-axis and the number of changes in the hemagglutinin gene on the y-axis. Students could plot five to ten data points and describe the pattern they observe.

15-w. Ask students to revise and improve their answers to Question 3 on Master 4.2: Not all individual influenza viruses are identical. What causes viruses to differ from one another? Hold a brief class discussion about students' answers.

Students should be able to describe that the variation originally came about through mutation. You may want to directly ask students whether the variation came about because the virus “wanted” or “needed” it. Again, this is a common misconception.

Continue with Step 16 on page 147.

In classrooms using the print version of this activity:



12a-p. Explain that students will now get access to the Viroformatics Viral Genome Database to help them gain experience with the data that scientists use to learn about influenza and evolution. Then project Master 4.7, Viroformatics Virus Database. Explain that Viroformatics scientists use the database, which contains thousands of nucleotide and protein sequences from different influenza viruses collected over decades. On the site are tools to obtain and align sequences and to investigate the relationships among sequences.

Now would be a good time to mention that the National Center for Biotechnology Information (NCBI) maintains an extensive genome database that is free and available for use by the public. All federally funded researchers and most private companies submit sequences to the database. NCBI was established in 1988 as a division of the National Library of Medicine at the National Institutes of Health. NCBI's mission is to develop information technologies to help researchers understand the molecular and genetic processes that affect health. NCBI stores sequence information for a large range of organisms across the Tree of Life, including humans. You may want to show students the actual NCBI Influenza Virus Resource Web site at <http://www.ncbi.nlm.nih.gov/genomes/FLU/>. After accessing this page, click on “Database.”

12b-p. Explain to students that a search for the hemagglutinin gene in type A influenza resulted in 17,387 sequences and that the hemagglutinin gene codes for a protein in influenza that the human immune system recognizes. Then, project Master 4.8, Influenza

Hemagglutinin Sequence. Point out that the database stores information about both the RNA sequence (though it is recorded as DNA by convention) and the amino acids in the protein.

This step helps students get a sense of the massive amount of genetic data available about influenza. More broadly, you may want to tell students that the availability of sequence data from a range of organisms is increasing almost exponentially. Many career opportunities exist for students who are interested in both computation and biology. You can repeat this activity's search at the NCBI Influenza Virus Resource Web site. Input the following selections: Type, "A"; Host, "human"; Country/Region, "any"; Protein, "HA"; Subtype, "any." Then click on "Show results."

- 13-p. Explain to students that scientists at Viroformatics are studying how influenza viruses change over time. They obtained the genetic sequence for hemagglutinin from 11 viruses isolated from people around the world at different points in time. Scientists store influenza samples from the past in freezers. This way, future scientists have access to the influenza "fossil record." The scientists aligned the sequences, and the results are on Master 4.9, *Influenza Over Time Alignment*. Hand out one copy of Master 4.9 to each group. Instruct students to follow the directions on the handout.**

The questions on the handout ask students to perform simple calculations using division. Some students may need help setting up the problem. Questions 2 through 4 use scientific notation. If your students are not comfortable with scientific notation, simply write out the answer using decimal places. The main concept that students need to understand is that the rate of change in genetic sequences can differ by many orders of magnitude. Ultimately, students will understand the role of selection in shaping some of the differences in rates.

You may wish to mention to students that the sequences in this alignment were selected from samples used in actual research (Smith et al., 2004).

Answer key for questions on Master 4.9, *Influenza Over Time Alignment*

1. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}} = \frac{32 \text{ changes}}{100 \text{ nucleotides}} = 0.32 \text{ changes per nucleotide}$$



Content Standard A: Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used.



Content Standard A: Mathematics is essential in scientific inquiry. Mathematical tools and models guide and improve the posing of questions, gathering data, constructing explanations and communicating results.

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2. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\begin{aligned} \frac{\text{answer from Step 1}}{\text{total number of years}} &= \frac{0.32 \text{ changes per nucleotide}}{35 \text{ years}} \\ &= 9.1 \times 10^{-3} \text{ changes per nucleotide per year} \\ &= 0.0091 \text{ changes/nucleotide/year} \end{aligned}$$

3. In Lesson 3, you investigated a portion of the sequence of a gene called *Irf6* that is involved in the development of the head and face. Use the same formulas you used in Steps 1 and 2 to calculate the expected number of changes per nucleotide per year in this sequence.

$$\begin{aligned} \frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}} &= \frac{4 \text{ changes}}{30 \text{ nucleotides}} = 0.13 \text{ changes per nucleotide} \\ \frac{\text{number of changes per nucleotide}}{\text{total number of years}} &= \frac{0.13 \text{ changes per nucleotide}}{1,009,000,000 \text{ years}} \\ &= 1.3 \times 10^{-10} \text{ changes per nucleotide per year} \\ &= 0.0000000013 \text{ changes per nucleotide per year.} \end{aligned}$$

4. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza to the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes than the other.

$$\begin{aligned} \frac{\text{rate of changes in hemagglutinin gene}}{\text{rate of change in Irf6 gene}} &= \frac{9.1 \times 10^{-3}}{1.3 \times 10^{-10}} = 7.0 \times 10^7 \\ &= 70,000,000 \end{aligned}$$

The rate of change in the hemagglutinin gene is 70 million times faster than the rate of change in the Irf6 gene for the regions compared.

5. The number of changes in the hemagglutinin gene for six samples compared with the sample from Finland in 2003 is as follows.
- Hong Kong, 1968 = 140 changes
 - Victoria, Australia, 1975 = 128 changes
 - Philippines, 1982 = 95 changes
 - Singapore, 1989 = 74 changes
 - Madrid, Spain, 1993 = 71 changes
 - Auckland, New Zealand, 1997 = 34 changes
- a. How do the number of changes to the sequence relate to time?
b. What do you think this means?

Students should see that the number of changes to the sequences increases with time. This is strong evidence that the influenza virus keeps changing over time.

Note: If you would like students to gain additional practice graphing and interpreting results, consider having them generate a graph that shows the number of years' difference between two viruses on the x-axis and the number of changes in the hemagglutinin gene on the y-axis. Students could plot six data points and describe the pattern they observe.

14-p. Ask students to revise and improve their answers to Question 3 on Master 4.2: Not all individual influenza viruses are identical. What causes viruses to differ from one another? Hold a brief class discussion about students' answers.

Students should be able to describe that the variation originally came about through mutation. You may want to directly ask students whether the variation came about because the virus “wanted” or “needed” it. Again, this is a common misconception.

15-p. Explain to students that they can use the data in the alignment of the 11 sequences to estimate the relationships among the viruses. Project Master 4.10, Relationships among Influenza Viruses. Ask, “Does this diagram show further evidence that the influenza virus is changing over time?” Students should record their answers in their notebooks. Ask two or three students to share what they wrote.

Students should note that the relationships of the samples relate to time. For example, the sample from 2003 is more closely related to the sample from 1997, which is the closest sample in terms of date. Students may also note that there is a large distance between the sample from 1968 and the sample from 2003.

16. Hand out one copy of Master 4.11, Influenza and the Immune System to each student. Ask students to read the questions at the end of the handout first and then complete the reading. Students should work with a partner to answer the questions.

Answer key for questions on Master 4.11, *Influenza and the Immune System*

1. Assume there is a change in the gene for hemagglutinin in the influenza virus. Describe how this change could alter the ability of an animal's antibodies to bind to the virus.

A mutation in the gene for hemagglutinin could change the shape of the protein. Antibodies that previously matched up with hemagglutinin would no longer bind.



Content Standard C: Natural selection and its evolutionary consequences provide a scientific explanation for the fossil record of ancient life forms, as well as for the striking molecular similarities observed among the diverse species of living organisms.



Understanding the role of mutation in generating diversity is essential. By this stage of the learning cycle, students have had multiple opportunities to learn about the importance of mutation.

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2. Imagine an influenza virus that has a mutation that changes the shape of its hemagglutinin protein. Because of this change, antibodies no longer bind to the virus. A second virus does not have the mutation, and antibodies can bind to its hemagglutinin protein. Which virus would leave more descendants? Describe why you answered as you did.

Antibodies will not bind to the influenza virus that has the mutation. The virus will be able to infect cells and make new viruses. Thus, it will leave more descendants than the virus that the antibodies recognize.

3. How does learning about the immune system help explain the rapid rate of change in the hemagglutinin sequences you observed?

Influenza viruses adapt to the human immune system. After a strain sweeps around the globe, many people develop immunity to that strain. Viruses that have a mutation that changes the hemagglutinin protein have an advantage because antibodies made previously do not match, and therefore these viruses can gain access to human cells.

17. **Ask students to work with their partners to write the “what I think after” answers to the questions on Master 4.2, Notes about Influenza and Evolution. After students turn in the handouts, hold a class discussion about the answers.**



This discussion helps students review what they have learned as they prepare for the Evaluate activity in Lesson 5. You may want to review students' answers to find out whether they are ready for the Evaluate activity.



Tip from the field test: You may want to assign this step as homework.

Answer key for questions on Master 4.2, *Notes about Influenza and Evolution*

1. What is influenza, or the “flu”?

Students may list the following information about influenza:

- *Influenza is a respiratory disease caused by a virus.*
- *Influenza is a major world health concern.*
- *The influenza virus uses RNA to store its genetic information.*
- *Three main types of influenza exist.*
- *Influenza is considered a bird disease.*
- *Influenza is highly infectious.*

2. How do scientists use data to explore how influenza genes evolve?

Scientists are able to store influenza viruses in freezers and thus preserve the virus's “fossil record” over time. Scientists can collect

data on the genetic sequences in influenza viruses and explore how the sequences have changed over time.

3. Not all individual influenza viruses are identical. What causes viruses to differ from one another?

Students revisited their answers to this question during Step 14. Variation in viruses comes about through mutation. Reassortment among different influenza viruses is an additional source of variation among viruses, but this is beyond what we expect students to know.

4. How does natural selection help explain the evolution of influenza?

Ask students to use the major principles of natural selection as they develop answers to this question. They should note that the variation among influenza viruses arises from mutation. These mutations can be passed from one generation to the next. Some of the viruses are better able to avoid detection by the immune system, and these viruses leave more descendants. As a result, the frequency of influenza viruses with certain traits will increase over time.

5. How does evolution help explain why researchers need to make a new vaccine for influenza every few years?

Students should now be able to give a richer answer to this question, which is one of the most important questions in the lesson. Students should recognize that influenza viruses will eventually adapt to the antibody “environment” induced by human immunity (sometimes stimulated by vaccines). Individual viruses with mutations to the hemagglutinin gene that change the shape so that antibodies no longer bind will have an advantage over other viruses. Eventually, some of these new strains will become more frequent and spread throughout the population, which prompts the creation of a new vaccine. Unfortunately, this process is extremely hard to predict. Researchers use large amounts of epidemiological, genetic, and antigenic data and expertise to decide what strains to include in the influenza vaccine.

Lesson 4 Organizer: Web Version



Activity 1: Using Evolution to Understand Influenza

Estimated time: 100 minutes

<p>Ask students whether they have questions about influenza, or the “flu.” Write three to four questions on the board. Tell students they will explore some of their questions about the flu.</p>	<p>Page 138 Steps 1 and 2</p>
<p>Project Master 4.1, and ask for a volunteer to read the email on it aloud. Ask students whether they have any questions about it.</p>	<p>Page 138 Step 3</p> 
<p>Give one copy of Master 4.2 to each student.</p> <ul style="list-style-type: none"> • Have students answer the questions on their own. • Before students reach Question 5, explain that researchers formulated 19 different vaccines for influenza from 1975 to 2008. During the same time period, no new vaccines were needed for other diseases caused by viruses, such as polio and the measles. • Ask students to provide an initial explanation to the “what I think before” questions. • Have students share their answers with a partner. 	<p>Pages 138–139 Steps 4 and 5</p> 
<p>Explain to students that they will use tools that scientists use to understand influenza and evolution.</p> <ul style="list-style-type: none"> • Give each student 1 copy of Master 4.3. • Students should record on Master 4.2 at least five total facts for the “what I think after” questions and record in their notebooks one question they have. 	<p>Page 139 Steps 6 and 7</p> 
<p>Ask students to meet with their groups to share and try to answer their questions. Hold a brief class discussion about Master 4.3 and the questions the group could not answer.</p>	<p>Page 139 Step 8</p>
<p>Explain that students will examine a segment of the gene that codes for the hemagglutinin protein taken from three type A influenza viruses, subtype H3N2. Give each pair of students one copy of Master 4.4 plus scissors, tape, and a blank piece of paper to accomplish the tasks described on Master 4.4.</p>	<p>Page 140 Step 9</p> 
<p>Ask each group to compare their alignment with that of another group. Project Master 4.5, and ask students to compare this alignment with the one they created. Ask whether aligning thousands of nucleotides by hand is reasonable. Explain that computer programs help scientists align long sequences.</p>	<p>Page 140 Steps 10 and 11</p> 

<p>Explain that students will use the Viral Genome Database at Viroformatics to learn more about the data scientists use to study influenza and evolution.</p> <ul style="list-style-type: none"> • Give one copy of Master 4.6 to each student. • Ask students to work with a partner to complete Master 4.6. • Have students log on to the Web site and click on “Lesson 4: Using Evolution to Understand Influenza.” 	<p>Page 141 Steps 12-w and 13-w</p>  
<p>Hold a class discussion on the answers to Master 4.6.</p>	<p>Page 141 Step 14-w</p>
<p>Ask students to revise and improve their answers to Question 3 on Master 4.2. Lead a discussion to emphasize the importance of revisions.</p>	<p>Page 144 Step 15-w</p>
<p>Give one copy of Master 4.11 to each student and have students first read the questions at the end of the reading. Have them work with a partner to answer the questions after completing the reading.</p>	<p>Page 147 Step 16</p> 
<p>Student pairs should work together to complete Master 4.2. After they turn in their answers, lead a class discussion about the activity.</p>	<p>Page 148 Step 17</p>

 = Involves copying a master.

 = Involves making a transparency.

 = Involves using the Internet.

Lesson 4 Organizer: Print Version



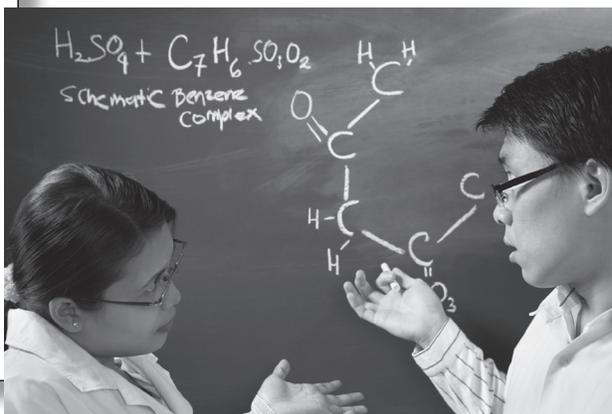
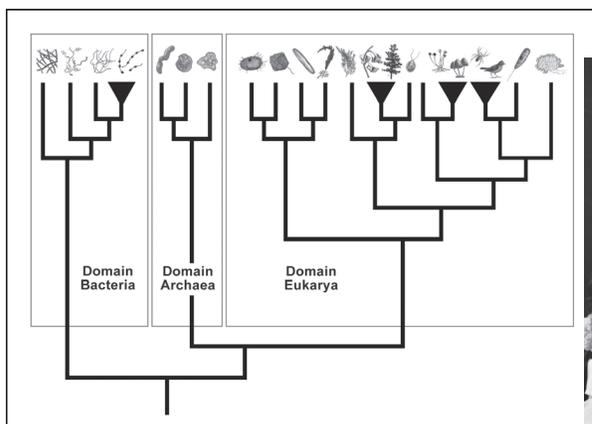
<p>Activity 1: Using Evolution to Understand Influenza</p> <p>Estimated time: 100 minutes</p>	
<p>Ask students whether they have questions about influenza, or the “flu.” Write three to four student questions on the board. Tell students they will explore some of their questions about the flu.</p>	<p>Page 138 Steps 1 and 2</p>
<p>Project Master 4.1, and ask for a volunteer to read the email on it aloud. Ask students whether they have any questions about it.</p>	<p>Page 138 Step 3</p> 
<p>Give 1 copy of Master 4.2 to each student.</p> <ul style="list-style-type: none"> • Have students answer the questions on their own. • Before students reach Question 5, explain that researchers formulated 19 different vaccines for influenza from 1975 to 2008. During the same time period, no new vaccines were needed for other diseases caused by viruses, such as polio and the measles. • Ask students to provide an initial explanation to the “what I think before” questions. • Have students share their answers with a partner. 	<p>Pages 138–139 Steps 4 and 5</p> 
<p>Explain to students that they will use tools that scientists use to understand influenza and evolution.</p> <ul style="list-style-type: none"> • Give each student one copy of Master 4.3. • Students should record on Master 4.2 at least five facts for the “what I think after” questions and record in their notebooks one question they have. 	<p>Page 139 Steps 6 and 7</p>
<p>Ask students to meet with their groups to share and try to answer their questions. Hold a brief class discussion about Master 4.3 and the questions the group could not answer.</p>	<p>Page 139 Step 8</p>
<p>Explain that students will examine a segment of the gene that codes for the hemagglutinin protein taken from three type A influenza viruses, subtype H3N2. Give each pair of students one copy of Master 4.4 plus scissors, tape, and a blank piece of paper to accomplish the tasks described on Master 4.4.</p>	<p>Page 140 Step 9</p> 
<p>Ask each group to compare their alignment with that of another group. Project Master 4.5 and ask students to compare this alignment with the one they created. Ask whether aligning thousands of nucleotides by hand is reasonable. Explain that computer programs help scientists align long sequences.</p>	<p>Page 140 Steps 10 and 11</p> 

<p>Explain that students will use the Viral Genome Database at Viroformatics to learn more about the data scientists use to study influenza and evolution. Project Master 4.7. Explain that the database has</p> <ul style="list-style-type: none"> • thousands of nucleotide and protein sequences from different influenza viruses collected over decades and • tools to obtain, align, and explore relationships among sequences. 	<p>Page 144 Step 12a-p</p> 
<p>Explain that</p> <ul style="list-style-type: none"> • a search for the hemagglutinin gene in type A influenza resulted in 17,387 sequences and • the hemagglutinin gene codes for a protein in influenza that the human immune system recognizes. <p>Project Master 4.8. Point out that the database has information about the RNA sequence (recorded as DNA by convention) and amino acids.</p>	<p>Page 144 Step 12b-p</p> 
<p>Explain that scientists at Viroformatics are studying how influenza viruses change over time. They aligned the genetic sequences for hemagglutinin from 11 viruses isolated from people around the world at different points in time.</p> <ul style="list-style-type: none"> • Give each group one copy of Master 4.9. • Ask students to use the alignment on the first page of the master to answer the questions on the second page. 	<p>Page 145 Step 13-p</p> 
<p>Ask students to revise and improve their answers to Question 3 on Master 4.2. Lead a discussion to emphasize the importance of revisions.</p>	<p>Page 147 Step 14-p</p>
<p>Explain that students can use the data in the alignment of the 11 sequences to estimate the relationships among the viruses.</p> <ul style="list-style-type: none"> • Project Master 4.10. • Ask, “Does this diagram show further evidence that the influenza virus is changing over time?” • Have two or three students share their answers. 	<p>Page 147 Step 15-p</p> 
<p>Give one copy of Master 4.11 to each student and have students first read the questions at the end of the reading. Have them work with a partner to answer the questions after completing the reading.</p>	<p>Page 147 Step 16</p> 
<p>Ask students to work with their partners to complete Master 4.2. After they turn in their answers, lead a class discussion about the activity.</p>	<p>Page 148 Step 17</p>

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Lesson 5

Evaluating Evolutionary Explanations



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Evaluate

At a Glance

Overview

Students use what they have learned about evolution and medicine to review an article written for a school publication. The task is to identify errors, explain the incorrect statements, and correct the information. They then explain the process of natural selection by creating a labeled illustration using one of the examples from an earlier lesson.

Major Concepts

Because this is an Evaluate lesson, we do not present new concepts. Students should apply what they have learned in previous lessons to this new situation.

Objectives

After completing this lesson, students will

- have evaluated explanations of evolution that are relevant for medicine,
- have identified common misconceptions about evolution and medicine, and
- have summarized their understandings of natural selection.

Teacher Background

Consult the following sections in Information about Evolution and Medicine:

- 4.0 Students' Prior Conceptions about Evolution (pages 31–33)
- 5.0 Featured Examples of Evolution and Medicine (pages 33–37)

In Advance

Web-Based Activities

Activity	Web Component?
1	No

Photocopies, Transparencies, Equipment, and Materials

Photocopies and Transparencies
1 copy of Master 5.1 for each student 1 transparency of Master 5.2
Equipment and Materials
Note or chart paper for each student Different-colored pens or pencils

Preparation

Make the copies and the transparency for this activity and gather the paper and different-colored pens or pencils.

Procedure

Activity 1: Evaluating Evolutionary Explanations

Estimated time: 50 minutes

Note: This final lesson gives you an opportunity to assess students' understandings of the major concepts the supplement addresses. It does not introduce new content.

1. **Introduce the activity by explaining that the school newspaper has decided to include a special section on evolution and medicine. The editor of the newspaper has asked for help. Students will use what they have learned about evolution and medicine to review an article written by a fictional fellow student. The task is to serve as a peer reviewer who identifies incorrect or misleading statements, corrects them, and explains why the corrections are necessary.**

In this activity, students should use what they have learned throughout this supplement to review a short article and write a short summary. This task is not a research project—students should be able to analyze the information provided and apply it to a new situation.

2. **Give each student one copy of Master 5.1, *Editing an Article about Vitamin C and Evolution*. Briefly review the instructions with the class before asking students to work in groups of four.**

Explain that all group members should read the first paragraph together. Then two group members should review and comment on Paragraph 2, and the other two should review and comment on Paragraph 3.

Students should concentrate on the author's explanations of the data rather than the accuracy of the data. For the purpose of this activity, the scientific facts are accurate. For example, L-gulonolactone oxidase (GULO) is one of the enzymes required for vitamin C biosynthesis. Vitamin C is not synthesized in humans, other primates, guinea pigs, and some bats, and the gene sequences and the evolutionary tree are scientifically accurate. The text contains some incorrect explanations of the data, based on common misconceptions. (For example, the information in the tree may be interpreted incorrectly.)

One strategy that students can use for the review is to place a number next to the piece of information they wish to rebut. Then, in their science notebooks, they can explain for each number what is incorrect, why it is incorrect, and how to correct it.

- 3. After each pair of students within the group has completed the review, pairs should discuss their comments with the other two group members (who reviewed the other paragraph).**
- 4. After groups finish, hold a class discussion to check students' understandings. Ask for volunteers to identify one thing that they felt needed to be corrected and how they changed it. Check whether other class members agree with the volunteers' ideas. If they disagree, they should be prepared to explain why and how they would (or would not) change a statement. Project Master 5.2, *Editing an Article about Vitamin C and Evolution*, Answer Key, at the end of the discussion. Point out any misconceptions or misinterpretations that students did not identify.**

It will be important to resolve any discrepancies so students do not leave the class confused about an issue or accepting common misconceptions.

Although it's beyond this supplement's content, you may wish to discuss the role of evolutionary processes in the loss of the ability of make vitamin C—if your class seems to have a good understanding of the example. If the loss of the ability to make vitamin C were driven by natural selection, then individuals with a mutation that disabled the GULO gene would have an advantage in survival or reproduction. This scenario is unlikely, however. It is more likely that individuals that had mutations in the past that disrupted the GULO gene were not at a disadvantage for survival or reproduction, and the loss of the ability to make vitamin C was due to chance.



Content Standard A: Scientific explanations must adhere to criteria such as: a proposed explanation must be logically consistent; it must abide by the rules of evidence; it must be open to questions and possible modification; and it must be based on historical and scientific knowledge.

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Content Standard A:
Communicate and defend a scientific argument.

5. Explain that the newspaper editor is concerned that readers may have misconceptions about natural selection. Along with the article about vitamin C and evolution, she wants to include some illustrations and explanatory text that describes the steps involved in the process of natural selection.
6. Instruct students to take a few minutes to create an illustration that explains the process of evolution with an example from one of the earlier lessons. Have students count off by fours.

There are four examples from the earlier lessons, so this approach ensures that approximately equal numbers of students will illustrate each example.

7. Assign students to use the examples as follows:
 - 1) Selection for methicillin-resistance in *Staphylococcus aureus* (MRSA)
 - 2) Selection for lactase persistence in some human populations
 - 3) Selection for alleles associated with alpha-thalassemia in some human populations
 - 4) Selection among influenza viruses
8. Outline on the board the elements of the task:
 - Students should create one or more simple illustrations that describe how the process of natural selection works.
 - Before making the illustrations, students should review the work in their notebooks from the previous lessons to help them reflect on natural selection and the major principles involved in the process.
 - Students should use the example they were assigned in Step 7 as the focus of the illustrations.
 - The illustrations should include labels and enough explanatory text that a reader unfamiliar with natural selection can understand how the trait of interest changed in a population over time.
9. Collect students' illustrations when they are complete. Ask for volunteers to explain the features of the illustrations. For each example, guide the discussion to bring out the five major principles of natural selection.

Box 1. Five Major Principles of Natural Selection

Throughout this curriculum supplement, students focused on five principles related to natural selection.

1. **Variation:** Individuals within a population vary for many traits, including physical and biochemical traits.
2. **Inheritance:** Some of the differences in traits among individuals can be passed from parents to offspring. (They are heritable.)
3. **Origin of variation:** Some of the variation in traits among individuals has a genetic basis. This variation originated, often many generations ago, as mutations—changes in the genetic information that are random with respect to the needs of the organism.
4. **Fitness:** Both the environment and the traits that individuals possess affect survival and reproduction. Individuals with heritable traits that enable them to better survive and reproduce in a particular environment will leave relatively more offspring.
5. **Evolutionary change in populations:** The frequency of traits and the alleles that affect those traits change in a population over time.

10. **Assess students' understanding of natural selection as depicted in the illustrations. Use the following descriptions to help you identify the five major points of natural selection for each example.**



Example 1: Selection for methicillin-resistance in *Staphylococcus aureus* (MRSA)

1. **Variation.** *Some individuals within a population of S. aureus are able to live in the presence of methicillin, whereas others cannot.*
2. **Inheritance.** *Resistance to methicillin in S. aureus is caused by specific alleles. Therefore, resistance can be inherited.*
3. **Origin of variation.** *Mutations occur randomly. Some mutations to genes enable some individual bacteria to become resistant to methicillin.*
4. **Fitness.** *In the presence of methicillin, individual bacteria that have inherited the alleles for methicillin resistance will survive and leave relatively more offspring in the next generation.*
5. **Evolutionary change in populations.** *The frequency of the alleles that cause resistance to methicillin increases in the S. aureus population over time. As a result, the frequency of methicillin resistance in the population also increases.*

Example 2: Selection for lactase persistence in some human populations

1. **Variation.** *Some individuals can digest lactose as adults, whereas others cannot.*
2. **Inheritance.** *Lactase persistence in humans is caused by specific mutations in a DNA control region. Therefore, lactase persistence or lactase nonpersistence can be inherited.*
3. **Origin of variation.** *Lactase persistence has a genetic basis. Mutations occur randomly. Mutations to a DNA control region enable some individuals to make lactase as adults.*
4. **Fitness.** *In certain cultural or environmental contexts, individuals who are lactase persistent left relatively more offspring. The culture-historical hypothesis says that individuals who are lactase persistent had a selective advantage in pastoralist populations. The calcium absorption hypothesis suggests that individuals who drank milk had a selective advantage in areas with low amounts of UV light.*
5. **Evolutionary change in populations.** *The frequency of the alleles that cause lactase persistence increased in some human populations over time. As a result, the frequency of lactase persistence in the population also increased.*

Example 3: Selection for genes associated with alpha-thalassemia in some human populations

1. **Variation.** *Some individuals have the traits associated with alpha-thalassemia, whereas other individuals do not have alpha-thalassemia.*
2. **Inheritance.** *Alpha-thalassemia in humans is caused by specific alleles. Therefore, the disease can be passed from parents to offspring.*
3. **Origin of variation.** *Alpha-thalassemia has a genetic basis. Mutations occur randomly. Mutations to a gene cause the disease.*
4. **Fitness.** *In certain cultural or environmental contexts, individuals who have alpha-thalassemia had relatively higher survival rates and left relatively more offspring. Alpha-thalassemia offers some protection against malaria. In areas with high rates of malaria, individuals with alpha-thalassemia have a lower chance of developing severe malarial anemia or dying from malaria.*
5. **Evolutionary change in populations.** *The frequency of the alleles that cause alpha-thalassemia increased in some human populations over time. As a result, the frequency of alpha-thalassemia in the population also increased.*

Example 4: Selection within the influenza virus

1. **Variation.** *Some individual viruses are able to avoid recognition by the human immune system, whereas other individual viruses are initially recognized by the immune system.*
2. **Inheritance.** *The hemagglutinin region is coded for by a gene in influenza. This gene can be passed from one generation of viruses to the next.*
3. **Origin of variation.** *Mutations occur randomly within the genome of influenza viruses. Mutations to a gene that codes for a protein that is important for immune recognition enable some individual viruses to escape detection.*
4. **Fitness.** *Individual influenza viruses that avoid initial detection by the immune system are able to enter human cells and produce relatively more offspring.*
5. **Evolutionary change in populations.** *The frequency of the alleles that allow influenza viruses to avoid initial detection by the immune system increases in the population over time. As a result, the population of influenza viruses changes rapidly.*

Lesson 5 Organizer

Activity 1: *Evaluating Evolutionary Explanations*

Estimated time: 50 minutes

Explain that that the school newspaper will include a section on evolution and medicine and that students will serve as peer reviewers for a newspaper article.	Page 156 Step 1
Give each student one copy of Master 5.1 . <ul style="list-style-type: none"> Form groups of four students. Ask all group members to read the first paragraph together. Have two group members read and comment on Paragraph 2. Have two group members read and comment on Paragraph 3. Ask student pairs to discuss their comments with the other pair in the group. 	Page 156–157 Steps 2 and 3 
Hold a class discussion to assess students' understandings. <ul style="list-style-type: none"> Project Master 5.2. Point out anything students did not identify. 	Page 157 Step 4 
Instruct students to create an illustration that explains natural selection with an example from an earlier lesson. <ul style="list-style-type: none"> Have students count off by fours. Assign examples as follows: <ol style="list-style-type: none"> selection for methicillin resistance in <i>S. aureus</i> selection for lactase persistence in some human populations selection for alleles associated with alpha-thalassemia in some human populations selection among influenza viruses 	Page 158 Steps 5–7
Outline the task on the board: <ul style="list-style-type: none"> Create one or more simple illustrations that describe how the process of natural selection works. Review the work in your notebooks to help you reflect on natural selection and the steps involved in the process. Use your assigned example as the focus of the illustrations. Create illustrations with labels and enough explanatory text that a reader unfamiliar with natural selection can understand how the trait of interest changed in a population over time. 	Page 158 Step 8
Collect the illustrations. <ul style="list-style-type: none"> Ask for volunteers to explain the features of their illustrations. Relate each example to the five major principles of natural selection. 	Page 158 Step 9
Assess students' understanding of natural selection as depicted in the illustrations.	Page 159 Step 10

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Masters

Lesson 1—Ideas about the Role of Evolution in Medicine

Activity 1: Outbreak!

Master 1.1, *Information about MRSA* student copies

Activity 2: Models and Medicine

Master 1.2, *Aniridia: An Eye Disease*..... transparency

Master 1.3, *Learning about Human Health from Other Organisms* student copies

Master 1.4, *Photographs of Normal Eyes and Eyes with*

Pax6 Mutations color transparency

Master 1.5, *An Evolutionary Tree* student copies and transparency

Lesson 2—Investigating Lactose Intolerance and Evolution

Master 2.1, *Lactase Investigation*..... student copies

Master 2.2, *Lactase Results from Other Researchers*..... transparency

Master 2.3, *Investigating Patterns in Lactase Persistence* student copies**

Master 2.4, *Questions about the Genetic Basis for*

Lactase Persistence transparency

Master 2.5, *Lactase and Human Evolution*..... transparency

Master 2.6, *Explaining the Evolution of Lactase*..... student copies

Master 2.7, *Data from Africa*..... transparency

Master 2.8, *Data from Europe, Part A*..... student copies for half of the class

Master 2.9, *Data from Europe, Part B*..... student copies for half of the class

Master 2.10, *Summing Up Lactase Persistence and Nonpersistence*..... student copies

Master 2.11, *Map of Lactase Test Results*..... transparency*

Master 2.12, *Map of Complete Lactase Test Results* transparency*

Master 2.13, *Exploring Patterns of Lactase Persistence*..... student copies*

Master 2.14, *Lactase Persistence and Mutation*..... transparency*

Lesson 3—Evolutionary Processes and Patterns Inform Medicine

Activity 1: Investigating a Mystery Disease

Master 3.1, *A Medical Mystery*..... transparency

Master 3.2, *Investigating a Medical Mystery*..... student copies

Master 3.3, *Blood Test Data*..... 2–3 copies*

Master 3.4, *Reference Manual*..... team copies*

Master 3.5, *Summarizing the Mystery Disease Data*..... transparency

Master 3.6, *Map of the Distribution of Thalassemia across*

the Eastern Hemisphere..... transparency

Master 3.7, *The Alpha-Globin Gene and Alpha-Thalassemia* transparency

Master 3.8, *Alpha-Globin and Variation*. student copies

Master 3.9, *Map of the Incidence of Malaria across*

the Eastern Hemisphere..... transparency

*Print version only **Web version only

Master 3.10, <i>Alpha-Thalassemia and Malaria in Papua New Guinea</i>	student copies for half of the class
Master 3.11, <i>Alpha-Thalassemia and Malaria in Kenya</i>	student copies for half of the class
Master 3.12, <i>Summing Up Thalassemia, Malaria, and Evolution</i>	student copies
Master 3.13, <i>Inheriting Thalassemia</i>	student copies (optional)

Activity 2: Using Evolution to Guide Research

Master 3.14, <i>Cleft Lip and Palate</i>	student copies
Master 3.15, <i>Calculating Times for an Evoprint</i>	transparency
Master 3.16, <i>Interpreting Evoprints</i>	transparency
Master 3.17, <i>Irf6 Evoprint Comparison</i>	student copies**
Master 3.18, <i>Evoprint Introduction</i>	transparency*
Master 3.19, <i>Investigating Irf6 Evoprints</i>	student copies*
Master 3.20, <i>Irf6 Evoprints</i>	team copies*

Lesson 4—Using Evolution to Understand Influenza

Master 4.1, <i>Email from Viroformatics</i>	transparency
Master 4.2, <i>Notes about Influenza and Evolution</i>	student copies
Master 4.3, <i>Introduction to Influenza</i>	student copies
Master 4.4, <i>Influenza Sequences</i>	team copies
Master 4.5, <i>Aligned Influenza Sequences</i>	transparency
Master 4.6, <i>Exploring a Genetic Database</i>	student copies**
Master 4.7, <i>Viroformatics Virus Database</i>	transparency*
Master 4.8, <i>Influenza Hemagglutinin Sequence</i>	transparency*
Master 4.9, <i>Influenza-Over-Time Alignment</i>	team copies*
Master 4.10, <i>Relationships among Influenza Viruses</i>	transparency*
Master 4.11, <i>Influenza and the Immune System</i>	student copies

Lesson 5—Evaluating Evolutionary Explanations

Master 5.1, <i>Editing an Article about Vitamin C and Evolution</i>	student copies
Master 5.2, <i>Editing an Article about Vitamin C and Evolution,</i> <i>Answer Key</i>	transparency

*Print version only **Web version only

Information about MRSA

Name: _____

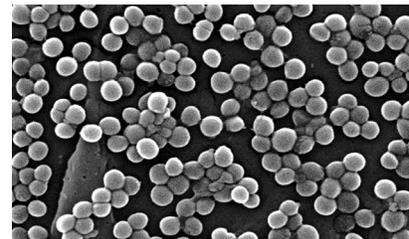
Read the following information about methicillin-resistant *Staphylococcus aureus* (MRSA). Then answer the questions at the end of this handout on your own. It's okay not to know the answers to these questions at this point. You will revise your answers later. Recording your ideas now will make it easier to learn this material. If you have questions, ask them during a class discussion.

What is *Staphylococcus aureus*?

Staphylococcus aureus is a species of bacterium. This species is often called “staph.” These bacteria are spherical in shape and sometimes form clusters that look like grapes (see Figure 1). These bacteria are commonly found on the skin or in the nose of healthy people. They are also found on other animals, such as cows, pigs, and chickens. About one in three people have populations of *S. aureus* in their nose. In most cases, this does not cause any illness. But sometimes, *S. aureus* does cause infections. While most are minor skin infections, some are serious, especially if the bacteria get into the body through a wound, such as a cut. They are frequent causes of infections after surgery, bloodstream infections, and pneumonia.

Like other bacteria, *S. aureus* reproduce by binary fission. When an *S. aureus* cell divides, it produces two daughter cells that have the same genetic instructions. However, mutation introduces genetic variation into a population of bacteria.

Figure 1. *Staphylococcus aureus*. These bacteria are about 1/1,000th millimeter in diameter. If lined up in single file, more than 25,000 of them would be 2.5 centimeters (1 inch) long.



(CDC, Janice Haney Carr; Jeff Hageman, MHS)

What is MRSA (pronounced “mer-sah”)?

Methicillin is a type of drug called an antibiotic because it can kill bacteria. Methicillin belongs to a group of antibiotics that includes others that may sound familiar to you: penicillin and amoxicillin. Unfortunately, some populations of *S. aureus* are resistant to the group of antibiotics that include methicillin. Resistant populations of bacteria are not killed by this group of drugs. So, MRSA stands for “methicillin-resistant *Staphylococcus aureus*.” Can you see why we simply call it “MRSA”?

People infected with MRSA on their skin usually don't have any health problems. Sometimes, they have red, swollen, and painful patches on their skin that look like pimples or spider bites. If the infection penetrates into the body, it can become more serious and cause pneumonia or infections of the blood or bone. Serious problems usually occur in people who have weakened immune systems.

MRSA spread through skin-to-skin contact. They also spread when a person's skin comes into contact with items or surfaces that touched the open wound of an infected person. Passing MRSA on to others can be stopped by simply washing hands, covering open wounds with bandages, and not sharing personal items (such as towels) that have been contaminated with MRSA.

MRSA populations are resistant to the group of antibiotics that are most often used to treat these infections. However, most people with MRSA infections can be treated successfully with other types of antibiotics.

Learning about Human Health from Other Organisms

Name: _____

For each part on this handout, you will analyze a different type of data about the *Pax6* gene.

Part 1: Comparing Amino Acid Sequences

Scientists have purified the protein coded by the *Pax6* gene in humans and other organisms. Look at the sequences of amino acids that make up a portion of the protein from four different species* and answer the questions that follow. Each letter in the sequence represents one amino acid in the protein.

Figure 1. Amino acids for a portion of the *Pax6* protein from humans, mice, zebrafish, and fruit flies.

Human:	LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Mouse:	LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Zebrafish:	LQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE
Fruit fly:	LQRNRTSFTNDQIDSLEKEFERTHYPDVFARERLAGKIGLPEARIQVWFSNRRAKWRREE

Questions

1. What do you notice about the amino acid sequences in the different species?

2. What can you infer about the *Pax6* gene from the protein sequences from these four species?

Part 2: What Happens If *Pax6* Does Not Function Normally?

One way that scientists study the function of genes is to find out what happens when the gene is not functioning normally. In other words, what happens if there is a mutation in the gene that affects its function? Use the pictures your teacher will show to answer the following question.

Question

3. On the basis of the pictures, do you think the function of the *Pax6* gene is similar in all four species? Explain your reasoning.

Part 3: Further Comparing Genes across Species

Scientists now have the ability to isolate DNA sequences that contain the genes they are interested in studying. In some cases, scientists can also insert the genes from one species into another species. The results tell researchers whether the gene functions similarly in the two different species.

Scientists have isolated the *Pax6* gene from a variety of animals, including squid, fruit flies, zebrafish, and mice. Scientists know that fruit flies that have two mutant copies of the *Pax6* gene do not have eyes. When scientists inserted the DNA sequence for the squid *Pax6* gene into these fruit flies, the flies developed eyes. In a second experiment, scientists inserted the DNA sequence for the mouse *Pax6* gene into the fruit flies with two mutant *Pax6* genes. The flies developed eyes in this case, too.

Question

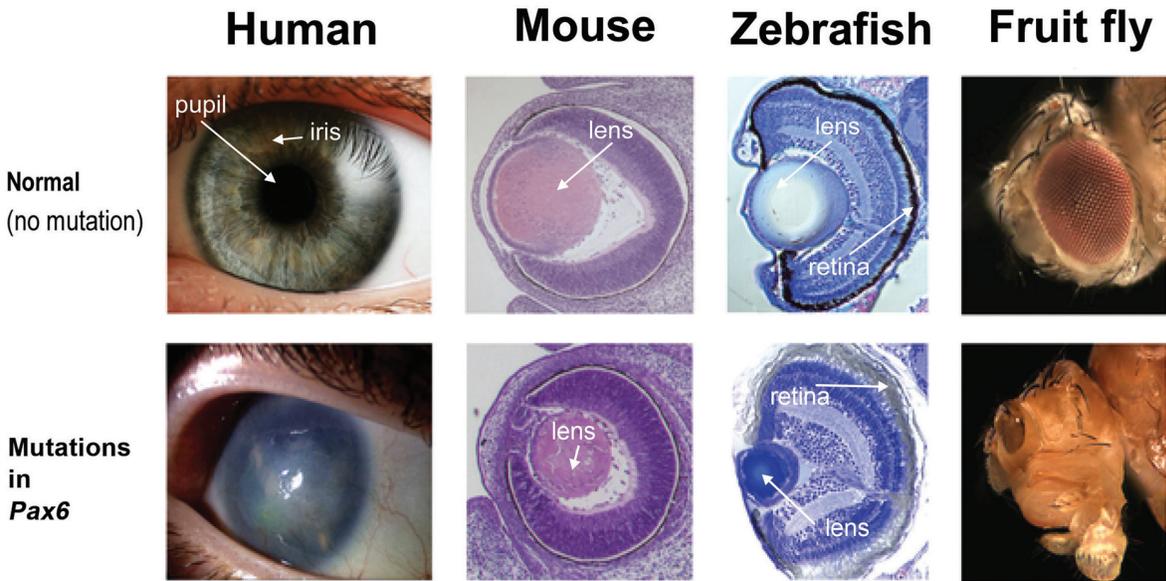
4. What do these experiments suggest about the function of the *Pax6* gene? Explain your thinking.

Summary Question

5. In the sequence data, you saw that the protein coded by the *Pax6* gene is very similar in fruit flies, zebrafish, mice, and humans. In the other experiments, you examined evidence related to the gene's function. Why might many species have almost exactly the same gene that has a similar function?

*Sequences for the fruit fly, mouse, and human are from here: S. Carroll. 2006. *The Making of the Fittest*. New York: W. W. Norton. The zebrafish sequence was downloaded from GenBank, <http://www.ncbi.nlm.nih.gov/genbank/>.

Photographs of Normal Eyes and Eyes with *Pax6* Mutations

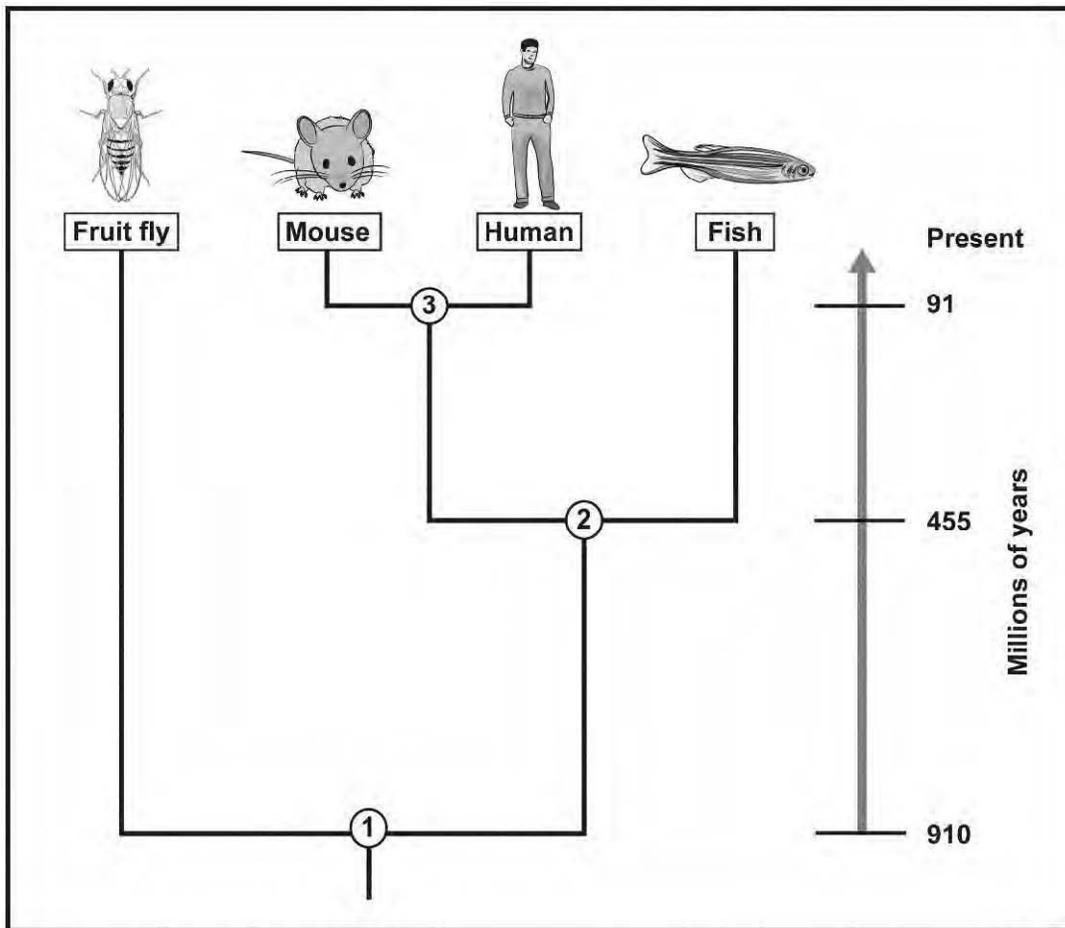


Phenotype with <i>Pax6</i> mutation	Opaque cornea Iris absent Degenerated retina Opaque lens Pressure on eyeball	Eye small Lens fused to cornea Iris anatomy changed Other eye abnormalities	Eye small Lens small Retina malformed	Eye small or absent
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Source: Adapted from N.L. Washington et al. 2009. Linking human diseases to animal models using ontology-based phenotype annotation. *PLoS Biology*, 7(11): e1000247. doi:10.1371/journal.pbio.1000247.

An Evolutionary Tree

Name: _____



Questions

1. What part of the evolutionary tree diagram represents the common ancestor of humans, mice, and zebrafish (but not fruit flies)? Why did you identify this part of the diagram?

Lactase Investigation

Name: _____

Introduction

Lactose is a type of sugar in milk. Other than milk and some dairy products made from milk, lactose is not found naturally in foods humans eat. Lactose is a type of carbohydrate made up of two simple sugars: glucose and galactose. The body digests lactose by breaking it into those simple sugars. The reaction is catalyzed by an enzyme called **lactase**. A summary of the reaction follows:



If lactase is made by the cells lining the small intestine, lactose gets broken down. The simple sugars are then absorbed into the bloodstream. Individuals who make the lactase enzyme throughout their lives (in other words, it persists) are called **lactase persistent**. People who make lactase are generally **lactose tolerant**.

If someone's small intestine does not make lactase, lactose is not broken down. Then what happens? The lactose passes into the large intestine, or colon. There, two things can happen that can cause problems. First, bacteria in the colon break down the lactose and use the galactose and glucose. In the process, they release hydrogen gas. As this gas builds up, it can cause a bloated feeling. It can also cause increased flatulence. Lactose that is not broken down by bacteria can also change the water balance in the colon. Too much lactose means that more water is drawn into the colon by osmosis, causing diarrhea. People who experience these symptoms when ingesting lactose are called **lactose intolerant**. Because these individuals have usually stopped making the enzyme lactase, they are also sometimes called **lactase nonpersistent**.

In this investigation, you will examine simulated samples from individuals in Europe, Asia, Africa, and the Middle East. You will gather evidence from your samples to determine whether or not the individuals are lactase persistent.

Procedure

1. Record the final color of the glucose test strip after your teacher placed it in the milk.
2. Your teacher will assign you a sample or two and show you information to help you determine the region and country your sample(s) came from. Record all this information in Table 1 (page 2 of this master).
3. Add 2 milliliters of milk to your sample. Hold your sample in your hand for three minutes to keep it near human body temperature.
4. Measure the amount of glucose in the sample using a glucose test strip. Record the result in Table 1.
5. Make a claim about whether or not the individual is lactase persistent. Describe how you used evidence to make that claim.

Table 1. Lactase Investigation Data

Sample number	Region	Country	Color of glucose test strip when placed in sample <i>before</i> the reaction	Color of glucose test strip when placed in sample <i>after</i> the reaction	Make a claim about whether or not the individual is lactase persistent. Describe how you used evidence to make this claim.	Were your results confirmed by other researchers?

Source: http://www.medicinenet.com/lactose_intolerance/article.htm

Lactase Results from Other Researchers

Sample number	Region	Country	Lactase persistence? (Yes/No)
1	East Africa	Kenya	Yes
2	East Africa	Tanzania	Yes
3	Southern Europe	Italy	No
4	West Africa	Nigeria	No
5	East Asia	South Korea	No
6	West Africa	Nigeria	No
7	East Asia	China	No
8	Middle East 2	Saudi Arabia	Yes
9	Northern Europe	Denmark	Yes
10	Southern Europe	Italy	No
11	Middle East 1	Pakistan	No
12	Middle East 2	Saudi Arabia	Yes
13	West Africa	Nigeria	No
14	East Asia	China	No
15	Middle East 2	Saudi Arabia	Yes
16	West Africa	Nigeria	No
17	East Africa	Sudan	Yes
18	Southern Europe	Italy	Yes
19	Middle East 1	Pakistan	No
20	Middle East 2	Saudi Arabia	Yes
21	Middle East 2	Saudi Arabia	Yes
22	Southern Europe	Spain	Yes
23	East Asia	South Korea	No
24	East Africa	Sudan	No
25	West Africa	Nigeria	No
26	Middle East 1	Iran	No
27	Northern Europe	Norway	Yes
28	Northern Europe	Ireland	Yes
29	Middle East 1	Iran	No
30	Northern Europe	United Kingdom	No
31	East Africa	Sudan	No
32	East Africa	Sudan	Yes
33	West Africa	Nigeria	Yes

(Continued)

(Continued)

Sample number	Region	Country	Lactase persistence? (Yes/No)
34	Southern Europe	Spain	Yes
35	East Africa	Tanzania	Yes
36	Southern Europe	Greece	No
37	West Africa	Nigeria	No
38	Middle East 2	Saudi Arabia	No
39	Middle East 1	Iran	No
40	East Asia	Japan	No
41	Middle East 1	Afghanistan	No
42	Northern Europe	Norway	Yes
43	East Asia	China	No
44	Northern Europe	Sweden	Yes
45	East Africa	Kenya	No
46	Southern Europe	Greece	No
47	Northern Europe	Sweden	Yes
48	Middle East 2	Saudi Arabia	No
49	East Asia	China	No
50	East Africa	Sudan	Yes
51	East Asia	China	No
52	Middle East 1	Afghanistan	No
53	Middle East 2	Saudi Arabia	No
54	Northern Europe	Sweden	Yes
55	Southern Europe	Spain	No
56	Middle East 2	Saudi Arabia	No
57	East Asia	China	No
58	Middle East 2	Saudi Arabia	Yes
59	West Africa	Nigeria	No
60	West Africa	Nigeria	No
61	Northern Europe	Norway	No
62	Northern Europe	United Kingdom	Yes
63	Middle East 1	Pakistan	Yes
64	East Africa	Tanzania	No
65	Southern Europe	Spain	No
66	West Africa	Nigeria	No
67	Southern Europe	Italy	No
68	Middle East 1	Iran	Yes
69	East Asia	Japan	No
70	Middle East 1	Afghanistan	No

Investigating Patterns of Lactase Persistence

Name: _____

Work as a group to explore patterns of lactase persistence in people from different parts of the world. Your group will explore three patterns: (1) geographic regions, (2) the role of gender, and (3) differences between adults and infants. After exploring these patterns, work as a group to summarize what you learned. To complete this activity, you will use materials on the Evolution and Medicine Web site:

<http://science.education.nih.gov/supplements/evolution/student>

Click on “Lesson 2: Investigating Lactose Intolerance and Evolution,” then “Fill in samples from other researchers,” “View map 2,” and finally, “Add samples.” After finishing a part, you can click on the “Continue” button to access the map.

Part A: Investigating Geographic Regions

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.
2. Click on “Explore Lactase Persistence by Geographic Region.” Then click on the seven geographic regions to see a summary graph for each region.
3. Estimate the percentage of people who are lactase persistent and lactase nonpersistent in each region. Write that information in Table 1 (below).

Table 1. Investigating Geographic Patterns Summary

Region	Lactase persistent (%)	Lactase nonpersistent (%)
West Africa		
East Africa		
Middle East 1		
Middle East 2		
East Asia		
Northern Europe		
Southern Europe		

4. Make a claim about whether or not lactase persistence varies geographically. Make sure your claim is linked to the evidence in the data table (Table 1).

Part B: Investigating the Role of Gender

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.
2. Click on “Explore Lactase Persistence by Gender.” Then click on the “Male” and then the “Female” buttons. Record your observations of the patterns you see.
3. Use information on the map to fill in Table 2.

Table 2. Lactase Persistence and Gender Summary

Gender	Lactase persistence	Lactase nonpersistence
Male		
Female		

4. Use the data in the table to make a claim about whether or not lactase persistence is more common in males or females.

Part C: Investigating the Differences between Adults and Infants

1. Access Map 2, which shows the simulated results from analyses of over 300 people in seven geographic regions.
2. The default map that you see shows lactase persistence or lactase nonpersistence for adults. Click on “Explore Lactase Persistence by Age,” and then the “Infants” button to see a map of the results for infants sampled for lactase activity. Describe the patterns you see in the results.
3. Use information on the map to fill in Table 3.

Table 3. Lactase Persistence and Age Summary

Age	Lactase activity (for infants) or persistence (for adults)	No lactase activity (for infants) or lactase nonpersistence (for adults)
Infants		
Adults		

4. Why do you think there is a difference between adults and infants for lactase activity?

Explaining the Evolution of Lactase

Scientists have proposed several hypotheses to explain patterns of lactase persistence. Collecting evidence helps them compare the hypotheses. In this portion of the activity, your group will first read descriptions of two of the hypotheses. You will then analyze data and decide whether the data favor one of the hypotheses.

Culture-Historical Hypothesis

Some cultures have a tradition of herding and milking cows, goats, or camels. Cultures that rely on livestock for meat and milk are called “pastoralist.” The **culture-historical hypothesis** says that individuals who are lactase persistent had a selective advantage in pastoralist populations. Two scientists separately proposed this hypothesis for the evolution of lactase persistence in humans.

According to this hypothesis, lactase persistent individuals had a higher level of nutrition because they could drink and digest milk. Milk is a high-calorie source of nutrition that is also high in protein. However, some cultures have a large number of individuals who cannot digest lactose. One way to solve this problem is to ferment milk to make cheese. Some cheeses do not have lactose. Yet, the cheese made from fresh milk has about 40 percent fewer calories than fresh milk. So, people who could digest fresh milk could get more calories in their diet than people who ate cheese made from the same amount of milk. Another possibility is that lactase persistent individuals had an advantage because they were able to take in water from the milk. Milk is an important source of liquid, especially in desert regions.

A higher level of nutrition meant that lactase persistent individuals had higher rates of survival and reproduction or had more children who survived and reproduced. Scientists collected data in Africa to test this hypothesis. They examined different groups of people and determined two things. First, they measured the level of lactase persistence in the group. Second, they estimated the level of pastoralism. The culture-historical hypothesis would be supported if populations that have high levels of lactase persistence had high levels of pastoralism historically. Populations that kept livestock but did not milk them would be expected to show low levels of lactase persistence.

Calcium-Absorption Hypothesis

The second hypothesis suggests a different selective advantage for lactase persistent individuals. Understanding this advantage of fresh milk involves knowing something about calcium. This hypothesis is called the **calcium-absorption hypothesis**. Calcium is a vital mineral that supports the structure of our bones and teeth. It's required for muscle contractions and many other functions. Vitamin D helps the body absorb calcium. Today, vitamin D is added to common foods like milk, bread, and cereal because it's so important. However, human diets do not always include foods that are high in vitamin D. Humans *can* make their own vitamin D when ultraviolet (UV) light penetrates their skin.

People who do not get enough calcium or vitamin D can develop rickets, which leads to improper bone growth (Figure 1). Women whose pelvis has been deformed by rickets may not survive the process of childbirth. *This means that women with vitamin D or calcium deficiency may have relatively fewer children.*

Figure 1. X-ray showing bone deformities associated with rickets.



Image courtesy of Michael L. Richardson, M.D.,
University of Washington
Department of Radiology

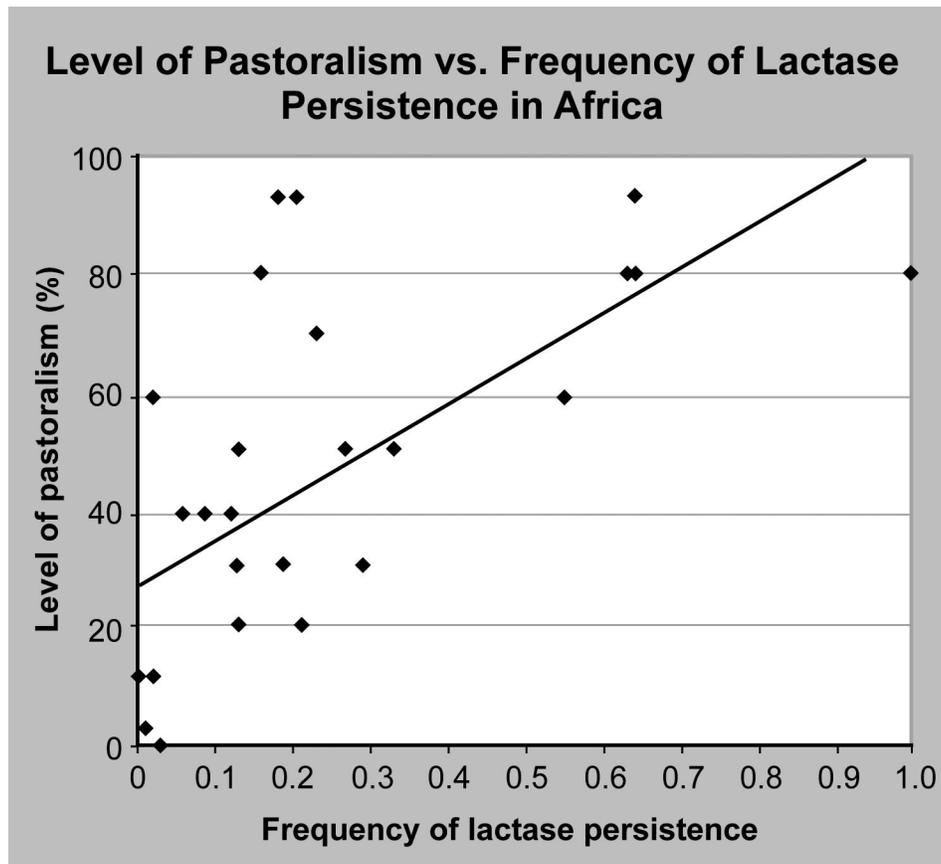
Fresh milk has a high lactose content. Some studies suggest that lactose also helps the body absorb calcium. Calcium is also present in milk. The calcium-absorption hypothesis suggests that people who could digest lactose had an advantage in environments with limited UV light. Digesting lactose also helped these individuals absorb calcium. Without the lactose, these people would have a problem absorbing calcium because of their low levels of vitamin D. Different types of evidence may support this hypothesis. For example, there should be high levels of lactase persistence in areas that have low amounts of UV at certain times of the year. There should also be evidence showing that rickets was common in the past.

Data from Africa

Question

Do the data in Figure 1 support one of the hypotheses for the evolution of lactase persistence?

Figure 1. Graph showing the relationship between the levels of pastoralism and the frequency of lactase persistence in Africa. A high level of pastoralism means that a culture used livestock for meat and milk.



Source: Data from P. Gerbault et al. 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS ONE*, 4(7): e6369. doi:10.1371/journal.pone.0006369.

Data from Europe, Part A

Work with your group to decide whether the data in Figures 1 and 2 support or refute one of the hypotheses for the evolution of lactase persistence.

Figure 1. Map of average daily ultraviolet (UV) exposure across Earth's surface. Darker shading indicates greater UV exposure.

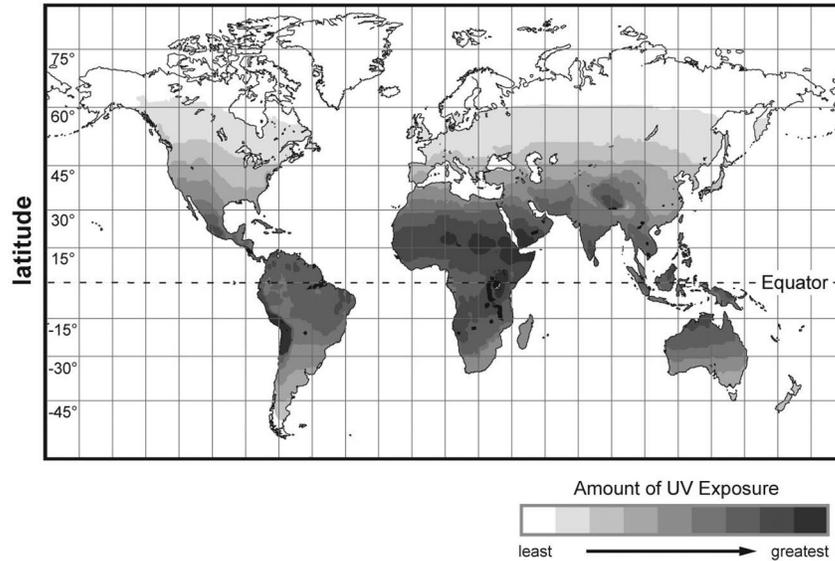
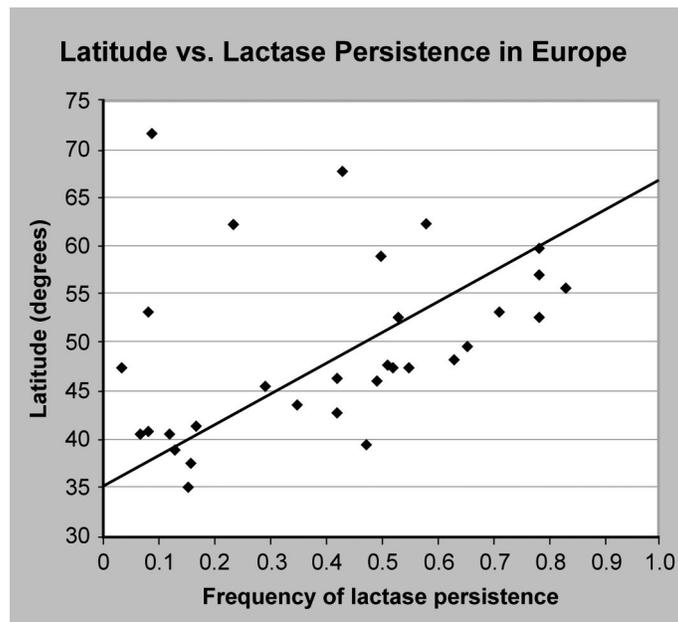


Image copyright George Chaplin. Adapted with permission. *American Journal of Physical Anthropology* 107(2): 221–224, 1998. Copyright 1998 Wiley-Liss, Inc.

Figure 2. Graph showing latitude and the frequency of lactase persistence in Europe.



Source: Data from P. Gerbault et al. 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS ONE*, 4(7): e6369. doi:10.1371/journal.pone.0006369.

Data from Europe, Part B

Work with your group to decide whether the data and conclusions described below support or refute one of the hypotheses for the evolution of lactase persistence.

Skeletal evidence

Archaeologists study human remains to infer how frequent a disease might have been in the past. Rickets causes specific changes to bones that allow researchers to determine whether a person had the disease. Use the data in Table 1 to decide whether the frequency of rickets was high in the past.

Table 1. Data about Possible Rickets Cases for Sites in Europe

Approximate time period	Number of skeletons examined	Possible cases of rickets
3000 B.C.E.	616	6*
C.E. 400–1000	635	0
C.E. 800–1100	1,055	6
C.E. 1200	364	1

*Some researchers interpret these skeletons differently and claim they did not have rickets.

Data source: F.J. Simoons. 2001. Persistence of lactase activity among Northern Europeans: A weighing of evidence for the calcium absorption hypothesis. *Ecology of Food and Nutrition* 40(5): 397–469.

Calcium absorption

Some studies suggest that simple sugars like glucose or galactose can help people absorb calcium, just as lactose does.** When people make yogurt from milk, the lactose gets broken down into simple sugars. This means that people in cultures that made dairy products like yogurt may not have had a problem obtaining enough calcium.

Computer models

Researchers developed a computer model of natural selection and the movement of individuals among populations.† Their results show that the pattern of lactase persistence in Europe could be explained by the culture-historical hypothesis.

** Summarized in F. J. Simoons. 2001. Persistence of lactase activity among Northern Europeans: A weighing of evidence for the calcium absorption hypothesis. *Ecology of Food and Nutrition*, 40(5): 397–469.

† Study by Y. Itan et al. 2009. The origins of lactase persistence in Europe. *PLoS Computational Biology*, 5(8): e1000491. doi:10.1371/journal.pcbi.1000491.

Summing Up Lactase Persistence and Nonpersistence

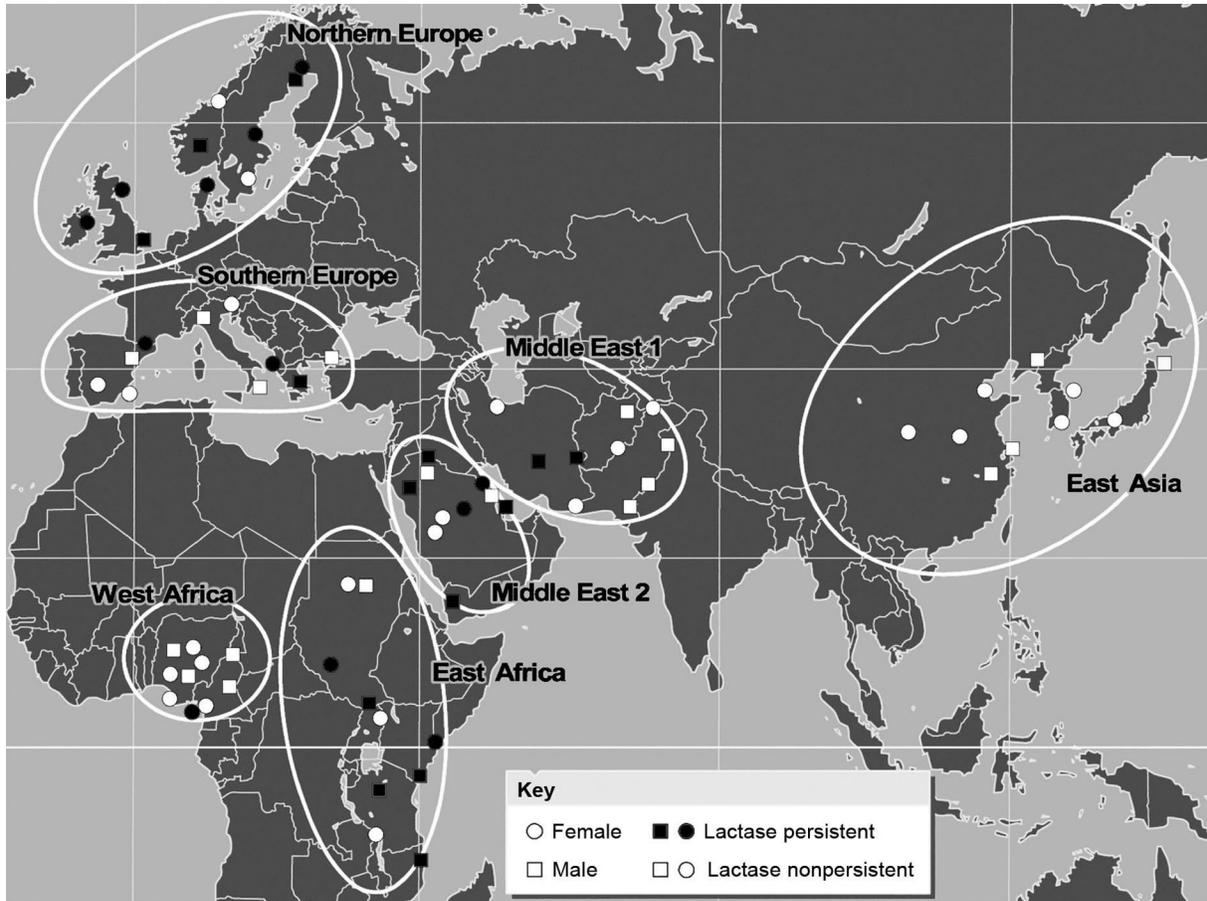
Name: _____

In this activity, you learned a lot about lactase persistence and lactase nonpersistence. To help you summarize what you learned, answer each question below.

Questions

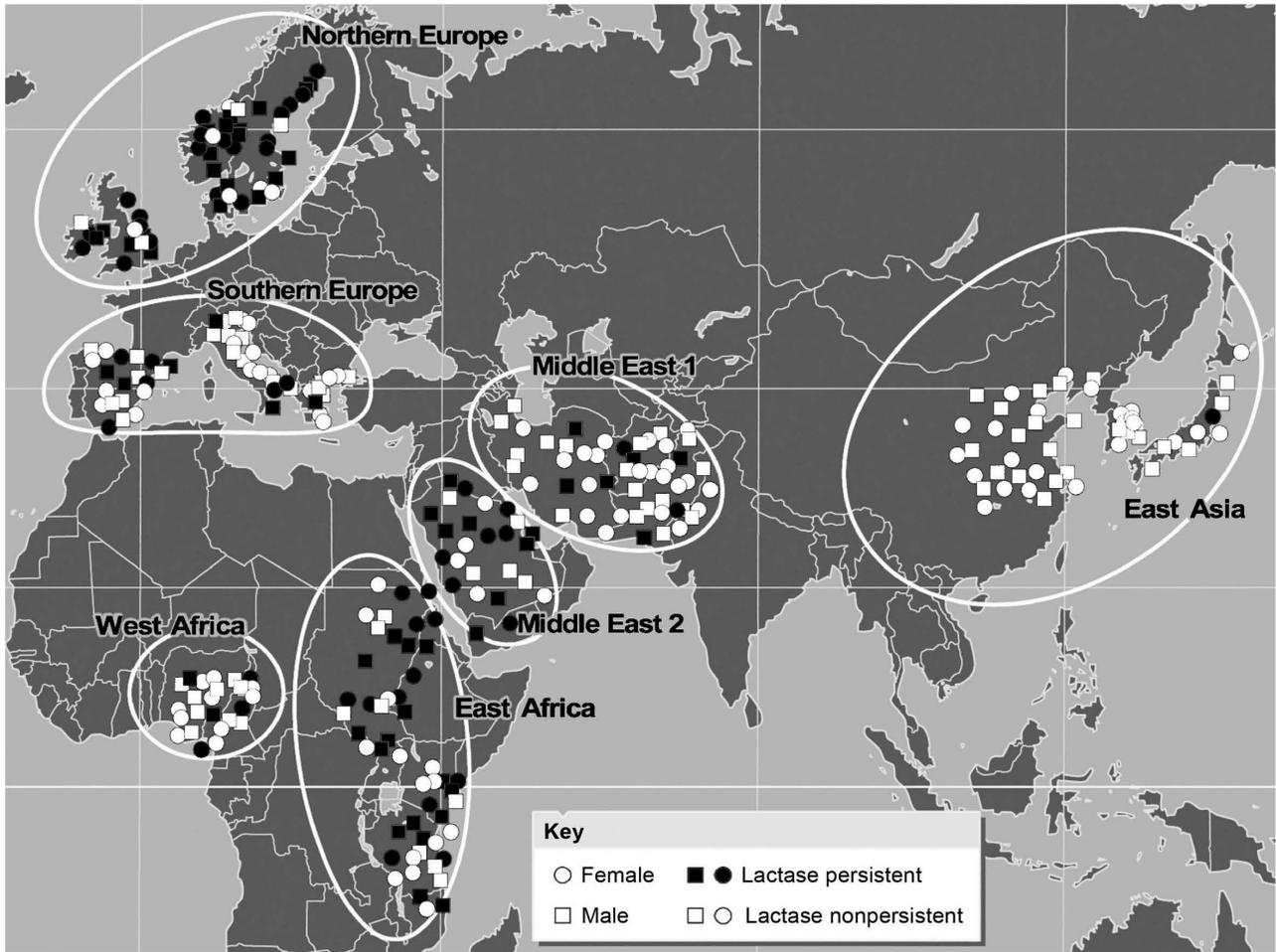
1. Do humans vary in their ability to digest lactose? What is the evidence for your answer?
2. Can the ability to digest lactose as an adult be passed from parents to offspring? What is the evidence for your answer?
3. Describe how mutations to DNA are important in lactase persistence and nonpersistence.
4. In certain environments, did digesting lactose seem to affect an individual's ability to survive and reproduce? Explain.
5. What's the evidence that the frequency of the mutation that causes lactase persistence changed in certain groups of people over time?

Map of Lactase Test Results



Use the map to answer this question: Does the proportion of people who are lactase persistent vary in different parts of the world?

Map of Complete Lactase Test Results



Exploring Patterns of Lactase Persistence

Name: _____

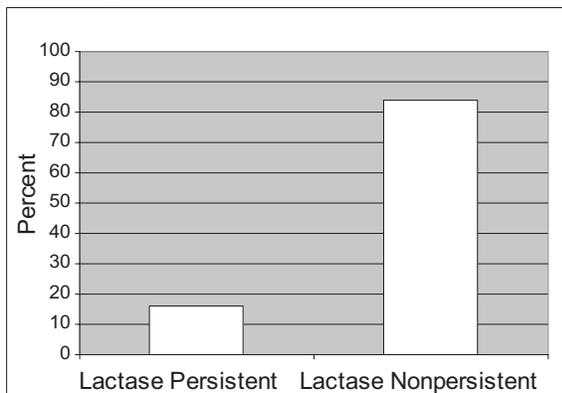
Work as a group to explore patterns of lactase persistence in people from different parts of the world.

Part A: Investigating Geographic Patterns

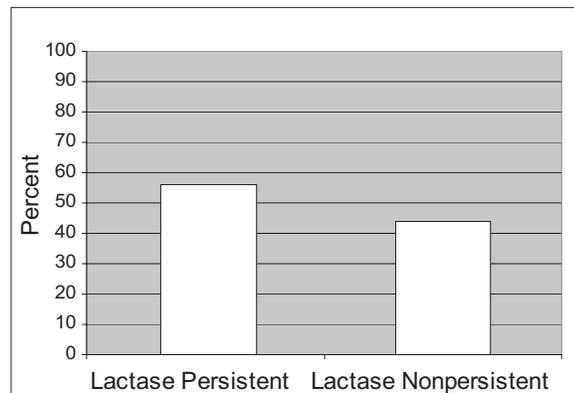
1. Graphs indicating the percentage of people who are lactase persistent or lactase nonpersistent are in Figure 1. Examine the percentage of people who are lactase nonpersistent and lactase persistent in each region. Write that information in Table 1.

Figure 1. Graphs of percentage of lactase persistence by region.

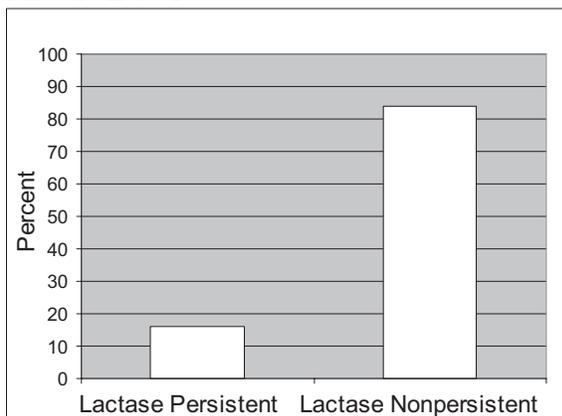
West Africa



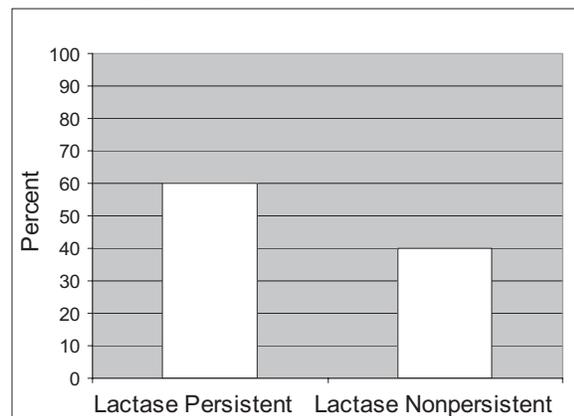
East Africa



Middle East 1



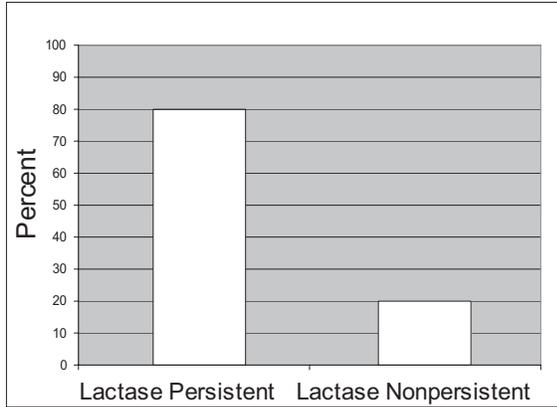
Middle East 2



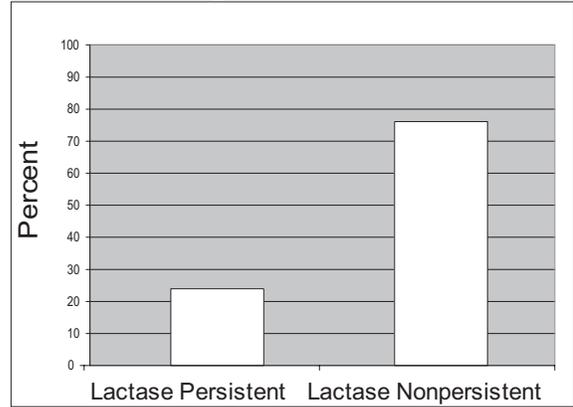
(Continued)

Figure 1. (Continued)

Northern Europe



Southern Europe



East Asia

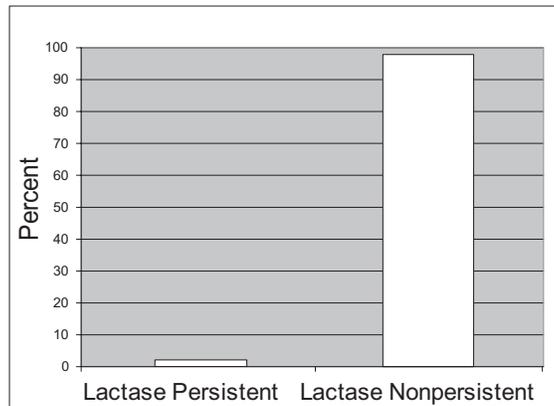


Table 1. Summary of Geographic Patterns of Lactase Persistence

Region	Lactase persistent (%)	Lactase nonpersistent (%)
West Africa		
East Africa		
Middle East 1		
Middle East 2		
Northern Europe		
Southern Europe		
East Asia		

2. Make a claim about whether or not lactase persistence varies geographically. Make sure your claim links to the evidence in Table 1.

Part B: Investigating the Role of Gender

1. Examine Table 2, which shows the number of males and females who are lactase persistent or lactase nonpersistent for the complete lactase results.

Table 2. Lactase Persistence and Gender Summary

Gender	Lactase persistent	Lactase nonpersistent
Male	56	94
Female	52	98

2. Use the data in the table to make a claim about whether or not lactase persistence is more common in males or females.

Part C: Investigating the Differences Between Adults and Infants

1. Examine the following table, which shows patterns of lactase activity in adults and infants for the complete lactase results.

Table 3. Lactase Persistence and Age Summary Table

Age	Lactase activity (infants) or lactase persistent (adults)	No lactase activity (infants) or lactase nonpersistent (adults)
Infants	299	1
Adults	108	192

2. Why do you think there's a difference between adults and infants for lactase activity?

Lactase Persistence and Mutation

Table 1. Percentage of Different Mutations for People in Seven Geographic Regions

Geographic Region	Mutation 1 (%)	Mutation 2 (%)	Mutation 3 (%)
West Africa	0	0	0
East Africa	0	26	2
Middle East 1	10	0	0
Middle East 2	0	0	56
Northern Europe	76	0	0
Southern Europe	20	0	0
East Asia	0	0	0

Data source: Enattah et al., 2007; Enattah et al., 2008; Gerbault et al., 2009; Tishkoff et al., 2007.

A Medical Mystery

High Incidence of Anemia in Papua New Guinea

Health workers are uncovering a health mystery in Papua New Guinea (Figure 1). A high percentage of the citizens on this island in the South Pacific suffer from anemia. Anemia is a medical condition in which a person does not have enough red blood cells to carry oxygen to the cells of the body. People who have anemia can have symptoms ranging from fatigue and shortness of breath to headaches to an irregular heartbeat. Doctors know that many diseases can cause anemia, and they wonder why so many people in Papua New Guinea have it. To find out, researchers are collecting blood samples from many people there who have anemia.

Figure 1. Map showing the location of Papua New Guinea, one of the countries on the world's second-largest island. Papua New Guinea is north of Australia.



Investigating a Medical Mystery

Name: _____

For each patient you investigate, complete the “Microscopic Analysis of Red Blood Cells” and the “Blood Test Data” sections of this master. The Reference Manual (Master 3.4) will help you interpret the information about each patient. Use this information to draw conclusions and then complete the “Making a Diagnosis” section.

When viewing blood samples in the virtual microscope, you will see mostly red blood cells (RBCs). You may also see white blood cells (WBCs). WBCs are usually larger than RBCs and irregular in shape. The nuclei of WBCs are darkly stained in these samples.

Patient _____

Microscopic Analysis of Red Blood Cells

1. Use the measurement tool to measure the diameter of four red blood cells in the field of view of the virtual microscope.

Cell 1 _____ Cell 2 _____ Cell 3 _____ Cell 4 _____

Average size of red blood cell _____

2. Observe the red blood cells in the microscope, noting the shape, color, and any irregularities you see. Record your observations here.

Conclusions from the Microscopic Analysis

Did you find any differences between your patient’s red blood cells and normal red blood cells that could be important clues to the patient’s condition? Explain.

Blood Test Data

Analyze the blood test data provided. Record any test results that are outside the normal range. If values are outside a normal range, be sure to record whether they are above or below normal.

Conclusions from the Blood Test Data

Do the blood test data indicate a specific disease? Explain. Use the information in the Reference Manual to help you determine the patient’s problem.

Making a Diagnosis

Now that you have observations and data to inform you, use the information from both the microscopic analysis and the blood test data to diagnose the patient's condition. Explain how both types of data support your diagnosis.

Patient _____

Microscopic Analysis of Red Blood Cells

1. Use the measurement tool to measure the diameter of four red blood cells in the field of view of the virtual microscope.

Cell 1 _____ Cell 2 _____ Cell 3 _____ Cell 4 _____

Average size of red blood cell _____

2. Observe the red blood cells in the microscope, noting the shape, color, and any irregularities you see. Record your observations here.

Conclusions from the Microscopic Analysis

Did you find any differences between your patient's red blood cells and normal red blood cells that could be important clues to the patient's condition? Explain.

Blood Test Data

Analyze the blood test data provided. Record any test results that are outside the normal range. If values are outside a normal range, be sure to record whether they are above or below normal.

Conclusions from the Blood Test Data

Do the blood test data indicate a specific disease? Explain. Use the information in the Reference Manual to help you determine the patient's problem.

Making a Diagnosis

Now that you have observations and data to inform you, use the information from both the microscopic analysis and the blood test data to diagnose the patient's condition. Explain how both types of data support your diagnosis.

Blood Test Data

Patient 1, Adult Male

Microscopic Examination of Blood

- Average red blood cell diameter: 6.4 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 1. Complete Blood Count Results for Patient 1

Blood component	Test result	Normal values
White blood cell (WBC) count	5,600 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.7 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	8.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	68 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	280 $\mu\text{g}/\text{dL}$	240–450 $\mu\text{g}/\text{dL}$
Serum ferritin concentration	75 ng/mL	15–306 ng/mL

Patient 2, Adult Female

Microscopic Examination of Blood

- Average red blood cell diameter: 6.7 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 2. Complete Blood Count Results for Patient 2

Blood component	Test result	Normal values
White blood cell (WBC) count	4,700 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.1 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	29%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	11.5 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	70 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	12	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	320 $\mu\text{g}/\text{dL}$	240–450 $\mu\text{g}/\text{dL}$
Serum ferritin concentration	62 ng/mL	15–306 ng/mL

Patient 3, Adult Female

Microscopic Examination of Blood

- Average red blood cell diameter: 6.2 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 3. Complete Blood Count Results for Patient 3

Blood component	Test result	Normal values
White blood cell (WBC) count	8,700 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	3.7 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	25%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	9.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	68 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	15	–
RDW (RBC distribution width)	High	–
Total iron-binding capacity (TIBC)	480 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	11 ng/mL	15–306 ng/mL

Patient 4, Adult Male

Microscopic Examination of Blood

- Average red blood cell diameter: 6.1 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 4. Complete Blood Count Results for Patient 4

Blood component	Test result	Normal values
White blood cell (WBC) count	6,500 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.2 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	33%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	12.5 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	12	–
RDW (RBC distribution width)	Normal	–
Total iron-binding capacity (TIBC)	350 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	81 ng/mL	15–306 ng/mL

Patient 5, Adult Female

Microscopic Examination of Blood

- Average red blood cell diameter: 6.8 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 5. Complete Blood Count Results for Patient 5

Blood component	Test result	Normal values
White blood cell (WBC) count	4,800 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	3.9 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	30%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	10.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	77 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	–
RDW (RBC distribution width)	Normal	–
Total iron-binding capacity (TIBC)	320 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	60 ng/mL	15–306 ng/mL

Patient 6, Adult Female

Microscopic Examination of Blood

- Average red blood cell diameter: 6.0 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 6. Complete Blood Count Results for Patient 6

Blood component	Test result	Normal values
White blood cell (WBC) count	6,400 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.0 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	10.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	–
RDW (RBC distribution width)	Normal	–
Total iron-binding capacity (TIBC)	380 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	61 ng/mL	15–306 ng/mL

Patient 7, Adult Male

Microscopic Examination of Blood

- Average red blood cell diameter: 7.4 μm
- Microscopic examination: Red blood cells were normal in color. Some red blood cells had an abnormal, sickle shape.

Table 7. Complete Blood Count Results for Patient 7

Blood component	Test result	Normal values
White blood cell (WBC) count	12,000 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.2 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	38%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	8.4 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	85 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	Not reported	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	220 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	148 ng/mL	15–306 ng/mL

Patient 8, Adult Male

Microscopic Examination of Blood

- Average red blood cell diameter: 6.5 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 8. Complete Blood Count Results for Patient 8

Blood component	Test result	Normal values
White blood cell (WBC) count	4,800 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.5 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	36%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	13 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	79 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	—
RDW (RBC distribution width)	Normal	—
Total iron-binding capacity (TIBC)	400 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	78 ng/mL	15–306 ng/mL

Patient 9, Adult Female

Microscopic Examination of Blood

- Average red blood cell diameter: 7.8 μm
- Microscopic examination: Red blood cells were normal in color. Shape was also normal.

Table 9. Complete Blood Count Results for Patient 9

Blood component	Test result	Normal values
White blood cell (WBC) count	6,800 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.8 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	42%	Males: 42–50% Females: 36%–45%
Hemoglobin (Hb)	13.3 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	87 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	Not reported	–
RDW (RBC distribution width)	Normal	–
Total iron-binding capacity (TIBC)	400 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	114 ng/mL	15–306 ng/mL

Patient 10, Adult Male

Microscopic Examination of Blood

- Average red blood cell diameter: 6.3 μm
- Microscopic examination: Red blood cells were lighter in color than normal. Shape was normal.

Table 10. Complete Blood Count Results for Patient 10

Blood component	Test result	Normal values
White blood cell (WBC) count	4,700 cells/ μL	4,400–11,300 cells/ μL
Red blood cell (RBC) count	4.3 million cells/ μL	Males: 4.5 million–5.9 million cells/ μL Females: 4.1 million–5.1 million cells/ μL
Hematocrit	32%	Males: 42–50% Females: 36–45%
Hemoglobin (Hb)	12.8 g/dL	Males: 14.0–17.5 g/dL Females: 12.3–15.3 g/dL
Mean corpuscular volume (MCV)	74 fL/RBC	80.0–96.6 fL/RBC
MCV:RBC ratio	11	–
RDW (RBC distribution width)	Normal	–
Total iron-binding capacity (TIBC)	400 $\mu\text{g/dL}$	240–450 $\mu\text{g/dL}$
Serum ferritin concentration	76 ng/mL	15–306 ng/mL

Source: Blood values based on data in K. Kaushansky et al. 2010. *William's Hematology* (8th ed.). New York: McGraw-Hill.

Reference Manual

The Complete Blood Count

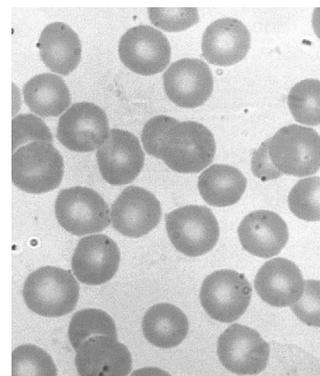
The complete blood count (CBC) is a screening test used to diagnose many diseases. The test can reveal problems with red blood cell (RBC) production and destruction, and it can help diagnose infection, allergies, and problems with blood clotting and fluid volume. It actually consists of several tests that examine different parts of the blood, including the following:

- RBC count is the number of RBCs per volume of blood.
- White blood cell (WBC) count is the number of WBCs per volume of blood.
- Hemoglobin measures the amount of oxygen-carrying protein in the blood.
- Hematocrit measures the percentage of RBCs in a given volume of whole blood.
- Mean corpuscular volume (MCV) is a measurement of the average size of RBCs. MCV increases when the RBCs are larger than normal (macrocytic) and decreases when the RBCs are smaller than normal (microcytic).
- The MCV:RBC ratio is obtained by dividing the mean corpuscular volume by the red blood cell count.
- RBC distribution width (RDW) is a measure of variation in RBC width. Normally, RBCs are fairly uniform in size and shape. In some diseases, a given blood sample may have a high RDW, meaning that there is a great deal of variation in the size of the RBCs in the sample.
- Total iron-binding capacity (TIBC) shows whether there is too much or too little iron in the blood.
- The serum ferritin concentration indicates the body's stores of iron.

Appearance of Normal RBCs

In a normal blood sample prepared for viewing under the microscope, the RBCs are 7–8 micrometers (μm) in diameter. They are very similar in size to each other and have a smooth surface. RBCs are usually dark red. (However, because of their biconcave shape, the center of the cell may look lighter when viewed in the microscope.) Mature RBCs (the kind seen in a blood sample in a microscope) do not have a nucleus. WBCs are often larger than RBCs and have nuclei that are irregularly shaped and darkly stained.

Figure 1. Normal red blood cells.



CDC, Dr. Mae Melvin

Abbreviations

The blood test data use the following abbreviations.

- Deciliter (dL) is a unit of volume equivalent to 10^{-1} liter, or 100 milliliters (one-tenth of a liter).
- Milliliter (mL) is a unit of volume equivalent to 10^{-3} liter (one-thousandth of a liter).
- Microliter (μL) is a unit of volume equivalent to 10^{-6} liter (one-millionth of a liter).
- Femtoliter is a unit of volume equivalent to 10^{-15} liter (one-quadrillionth of a liter).
- Nanogram is a unit of mass equal to 10^{-9} grams (one-billionth of a gram).
- Picogram is a unit of mass equal to 10^{-12} grams (one-trillionth of a gram).

Disease: Alpha-thalassemia

Brief Description: Alpha-thalassemia refers to a disease characterized by reduced or no production of the alpha-globin proteins that form hemoglobin.

Cause: Alpha-thalassemia is caused by changes in the alpha-globin genes on chromosome 16. Each person has four copies of this gene.

Diagnosis: Doctors usually use blood tests to diagnose alpha-thalassemia. Because it is an inherited disease, they will also check family history. In some cases, they may order DNA testing. Thalassemia affects both males and females.

Appearance of Red Blood Cells (RBCs): In patients with alpha-thalassemia, RBCs are often smaller than normal (microcytosis; normal RBCs are 7–8 micrometers (μm) in diameter). The size of the RBCs usually relates to the number of nonfunctional copies of the alpha-globin gene. If a person has one nonfunctional copy, the cells may only be slightly smaller than normal. The RBCs are significantly smaller than normal in people with two or more nonfunctional copies. RBCs in a person with thalassemia may be lighter in color.

The **mean corpuscular volume:RBC ratio (MCV:RBC ratio)** is a way to distinguish thalassemia from other kinds of anemia. In individuals with alpha-thalassemia, the MCV:RBC ratio is less than 13. In iron deficiency anemia, the ratio is above 13.

The **hematocrit** (percentage of blood taken up by RBCs) is usually decreased in people with alpha-thalassemia.

The **total iron-binding capacity (TIBC)** is usually normal in thalassemia.

The **RBC distribution width (RDW)** value is normal in thalassemia. The RBCs in a sample from a patient who has thalassemia may be somewhat smaller than normal, but they are similar in size to the other cells in the sample.

Symptoms: Symptoms of alpha-thalassemia range from no or mild symptoms to severe. One form of alpha-thalassemia is almost always fatal. In general, the symptoms are more serious in patients with more nonfunctional copies of alpha-globin genes.

Disease: Iron deficiency anemia

Brief Description: Iron deficiency anemia is a common type of anemia—a condition in which the blood lacks adequate healthy red blood cells (RBCs). These cells carry oxygen to the body's tissues.

Cause: Normally, people get iron from the food they eat. In addition, iron can be recycled from old RBCs. Iron deficiency anemia occurs if people do not consume enough iron in the diet, do not produce enough of the iron-containing hemoglobin, or lose too much iron, which occurs most commonly through blood loss.

Diagnosis: Doctors use blood tests to diagnose iron deficiency anemia. Indications of iron deficiency anemia include the following:

- **Abnormal RBCs.** The RBCs in someone with iron deficiency anemia are smaller than normal (microcytosis; normal RBCs are 7–8 μm in diameter) and paler in color than normal (hypochromic). The cells also may be irregular in size and shape. (RBCs within a sample may have different sizes and not be as smooth and round as normal RBCs.)
- **Hemoglobin levels.** Hemoglobin levels in someone with iron deficiency anemia are lower than normal.
- **Hematocrit.** In a person who has iron deficiency anemia, the hematocrit readings are below normal.

Serum Ferritin Concentration: At times, it may be hard to distinguish between iron-deficiency anemia and thalassemia. The serum ferritin concentration is one way that doctors can determine whether a person has alpha-thalassemia or iron deficiency anemia. Iron deficiency anemia is diagnosed when a person's **serum ferritin concentration** is less than 12 ng/mL.

Also, in iron deficiency anemia,

- the **mean corpuscular volume:RBC (MCV:RBC) ratio** is greater than 13,
- the **RBC distribution width (RDW)** is high, indicating a larger variation in size of the RBCs, and
- the **total iron-binding capacity (TIBC)** measurement is above normal.

Symptoms: In mild cases, a person who has iron deficiency anemia may not have any noticeable symptoms. As the deficiency becomes more serious, a person may notice symptoms including the following:

- extreme fatigue
- irregular heartbeat
- pale skin
- shortness of breath
- dizziness
- weakness
- increased number of infections
- headaches

Disease: Sickle cell disease

Brief Description: Sickle cell disease is an inherited form of anemia. As in other types of anemia, in sickle cell disease there are not enough healthy red blood cells (RBCs) to carry adequate oxygen to all the cells of the body.

Cause: Sickle cell disease is a genetic disease caused by an abnormal type of hemoglobin called hemoglobin S. Hemoglobin S distorts the shape of red blood cells, especially when oxygen levels are low.

In someone who has sickle cell disease, the RBCs are distorted. Instead of the smooth, circular, biconcave shape of normal RBCs, some of the cells are shaped like crescents. The crescent-shaped cells can clog small blood vessels.

Diagnosis: Sickle cell disease is diagnosed by examining cells under a microscope and with a blood test.

Appearance of RBCs: In sickle cell disease, some of a person's RBCs have a characteristic shape that can be observed under a microscope. Sickle cells are crescent shaped—or shaped like the tool called a “sickle.”

In an individual with sickle cell disease,

- the **total iron-binding capacity (TIBC)** measurement is sometimes below normal.
- the **white blood cell (WBC) count** is usually somewhat high.

Symptoms: Sickle cells are destroyed rapidly in the body, causing anemia, jaundice, and gallstones.

The sickle cells also block the flow of blood through vessels, resulting in lung tissue damage, pain episodes, and strokes. These cells also cause damage to most organs including the spleen, kidneys, and the liver. Patients with sickle cell disease, especially young children, with damage to the spleen can be easily overwhelmed by certain bacterial infections.

Symptoms may include the following:

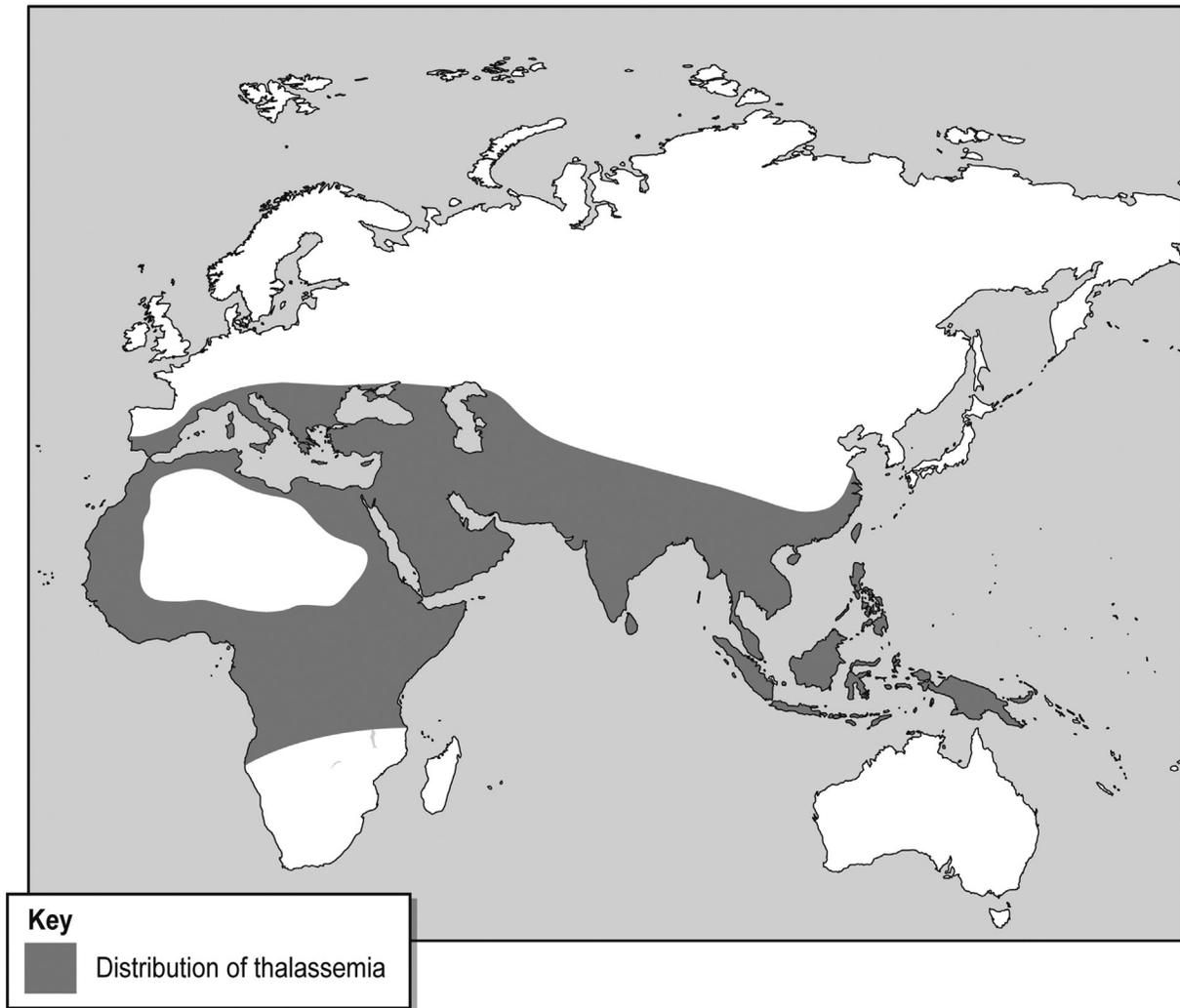
- attacks of abdominal pain
- bone pain
- delayed growth and puberty
- jaundice
- rapid heart rate
- chest pain
- poor eyesight/blindness
- strokes
- skin ulcers

Summarizing the Mystery Disease Data

Table 1. Summary of Mystery Disease Diagnoses and Evidence

Patient	Diagnosis	Key evidence used for diagnosis
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Map of the Distribution of Thalassemia across the Eastern Hemisphere



Map adapted from: <http://science.jrank.org/pages/48501/Sickle-Cell-Anemia-Thalassemia.html>

The Alpha-Globin Gene and Alpha-Thalassemia

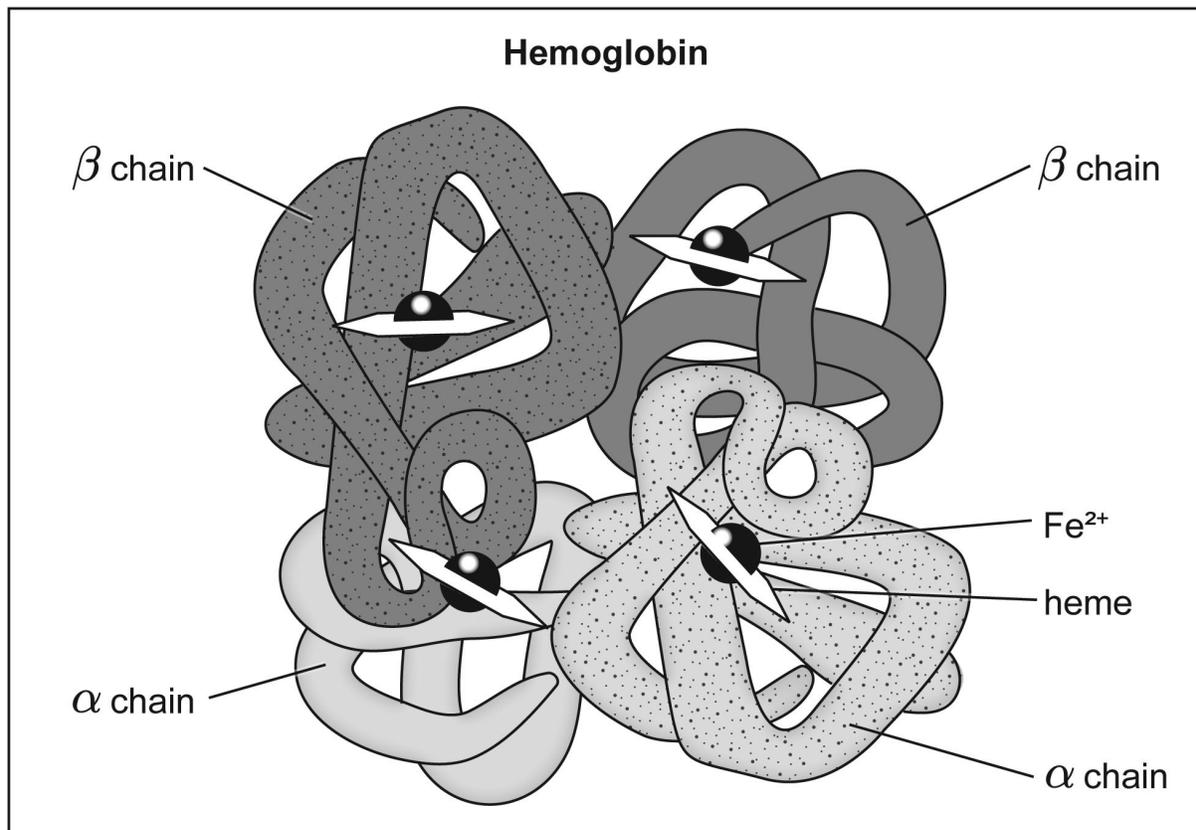
Part 1

Structure of Hemoglobin

Alpha-thalassemia is caused by problems in the production of the alpha-globin protein in hemoglobin.

Hemoglobin is normally made up of two alpha-globin protein chains and two beta-globin protein chains. Different genes code for the alpha- and beta-protein chains.

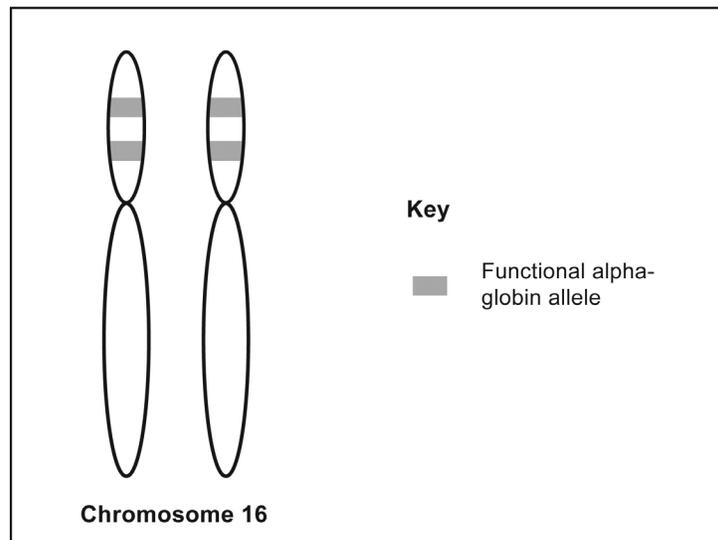
Figure 1. Schematic of hemoglobin protein complex.



Alpha-Globin Gene

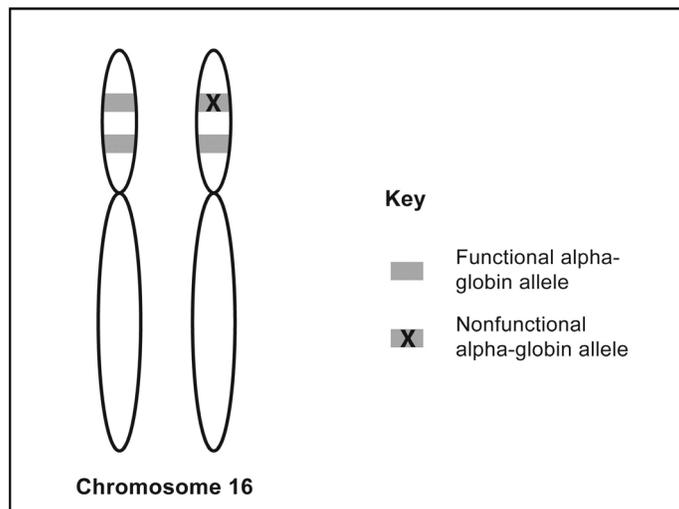
The alpha-globin gene, on chromosome 16 in humans, codes for the alpha-globin protein. Humans normally have four copies of the alpha-globin gene.

Figure 2. Schematic showing two copies of chromosome 16 and four copies of the alpha-globin gene. All the alleles of the alpha-globin gene are functional in this individual.



Individuals with alpha-thalassemia have a problem with one or more of their alpha-globin alleles.

Figure 3. Schematic showing two copies of chromosome 16 and four copies of the alpha-globin gene. One allele of the alpha-globin gene in this individual is nonfunctional.



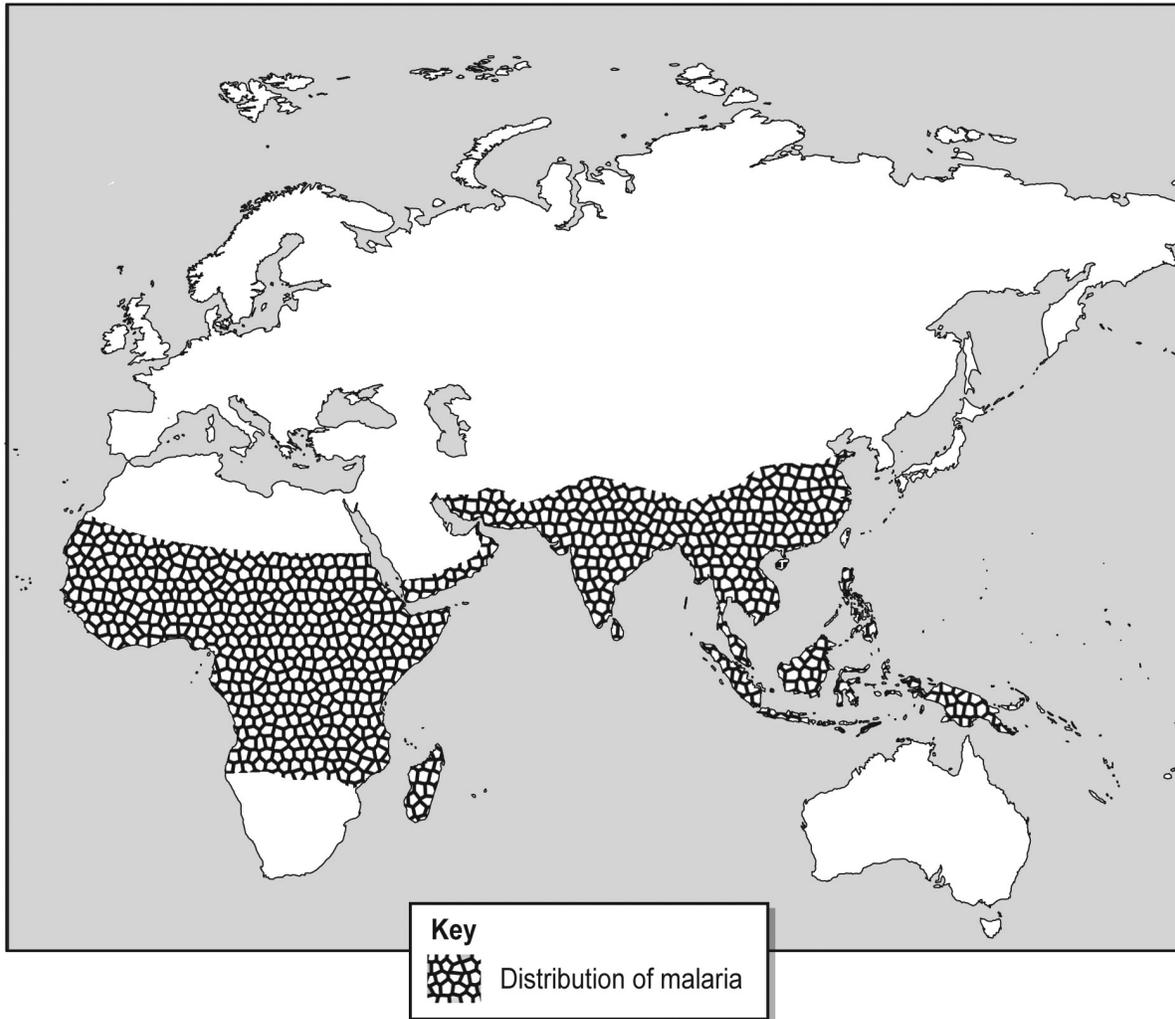
Individuals with alpha-thalassemia do not make as much alpha-globin protein as normal individuals do. The amount of protein they make depends on how many working alleles of the alpha-globin gene a person has.

Part 2

Table 1. Alpha-globin Gene Functional and Nonfunctional Alleles and Related Diseases

Number of functional alleles of the alpha-globin gene	Number of nonfunctional alleles of the alpha-globin gene	Name of disease	Symptoms
4	0	Normal condition (healthy individual)	Not applicable
3	1	Alpha-thalassemia silent carrier	<ul style="list-style-type: none"> • Usually no symptoms and no anemia • Blood tests usually normal • Hemoglobin normal • Slight changes in size of red blood cells (smaller than normal—microcytic) • Slightly lighter color of red blood cells (hypochromic)
2	2	Alpha-thalassemia trait	<ul style="list-style-type: none"> • Mild anemia • Small red blood cells (microcytic) • Light, pale color of red blood cells (hypochromic) • Blood tests usually normal • Hemoglobin normal
1	3	Hemoglobin H (HbH) disease	<ul style="list-style-type: none"> • Moderate to severe anemia • Small red blood cells (microcytic) • Light, pale color of red blood cells (hypochromic) • Fatigue • Mild jaundice • Enlarged spleen • Bone deformities (in some cases)
0	4	Alpha-thalassemia major or hemoglobin Barts hydrops fetalis (Hb Barts) syndrome	<ul style="list-style-type: none"> • Usually fatal before or shortly after birth

Map of the Incidence of Malaria across the Eastern Hemisphere



Source: Centers for Disease Control and Prevention, Division of Parasitic Diseases, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID).

Alpha-Thalassemia and Malaria in Papua New Guinea

Name: _____

Scientists studying populations in Papua New Guinea have developed a mathematical model to investigate whether people who have thalassemia have a higher risk of developing severe malarial anemia. Severe malarial anemia is often fatal unless the individual receives a blood transfusion within a short time.

The scientists use a statistic from medical studies called an “odds ratio,” which compares the level of risk for different groups. People with thalassemia have at least one nonfunctional copy of the alpha-globin gene.

For this study, an odds ratio

- **equal to 1** means that individuals with thalassemia are as likely to have severe malarial anemia as are normal individuals;
- **greater than 1** means that individuals with thalassemia are **more** likely to have severe malarial anemia than are normal individuals; and
- **less than 1** means that that the individuals with thalassemia are **less** likely to have severe malarial anemia than are normal individuals.

Table 1. Alpha-Thalassemia and Severe Malarial Anemia

Risk factor	One nonfunctional allele of the alpha-globin gene	Two nonfunctional alleles of the alpha-globin gene
Risk of developing severe malarial anemia	0.74	0.52

Source: Data from F.J.I. Fowkes et al. 2008. Increased microerythrocyte count in homozygous α^+ -thalassaemia contributes to protection against severe malarial anaemia. *PLoS Medicine*, 5(3): 494-501: e56. doi:10.1371/journal.pmed.0050056

Questions: Fill in the missing word or words for each sentence below.

1. A person who has one nonfunctional allele of the alpha-globin gene is _____ likely to develop severe malarial anemia than a normal individual (all alleles of the gene are functional).
2. A person who has two nonfunctional alleles of the alpha-globin gene is _____ likely to develop severe malarial anemia than a normal individual (all alleles of the gene are functional).
3. After analyzing the data, summarize what you have learned about the relationship between alpha-thalassemia and malaria by completing the following sentence.

The data suggest that individuals with alpha-thalassemia _____

Alpha-Thalassemia and Malaria in Kenya

Name: _____

Scientists have been studying the relationship between thalassemia and the severity of malarial anemia in a population in Kenya, a country in East Africa. Use the data in the table below to explore the relationship between thalassemia and malaria.

Consider this question: **Is each consequence of malaria more or less frequent in different genotypes?**

To understand the data in the following table, it's important that you make the appropriate comparisons. For each symptom, compare the data for each genotype with each other. In other words, for each row in the table, compare the data in the three columns.

Table 1. Consequences of Malaria by Alpha-thalassemia Genotypes

Consequence of malaria	Four functional alleles of the alpha-globin gene	One nonfunctional allele of the alpha-globin gene	Two nonfunctional alleles of the alpha-globin gene
Coma	45.1%	43.2%	39.8%
Severe anemia (hemoglobin less than 5 g/dL)	25.8%	22.4%	18.1%
Death	12.5%	10.4%	8.4%

Note: Each column shows the percentage of patients within each of three possible genotypes that showed specific symptoms when checking into a hospital due to complications from malaria.

Source: Data from T.N. Williams et al. 2005. Both heterozygous and homozygous α^+ thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood*, 106(1): 368-371.

Question

After analyzing the data, summarize what you have learned about the relationship between alpha-thalassemia and malaria by completing the following sentence.

The data suggest that individuals with alpha-thalassemia _____

Summing Up Thalassemia, Malaria, and Evolution

Name: _____

Answer the following questions to help you develop an explanation about alpha-thalassemia and its relationship to malaria in humans.

Questions

1. Do the data from the studies in Papua New Guinea and Kenya support the hypothesis that individuals who have thalassemia might have some advantage over other individuals when living in an area where malaria is common? Explain.
2. Depending on their genotype, individuals with nonfunctional alpha-globin alleles may have symptoms that range from mild to more serious, including anemia, fatigue, enlarged spleen, liver problems, or even death. If the alpha-globin mutations are passed from parent to child, and individuals with four nonfunctional alpha-globin alleles die, how is the mutation maintained in the population?
3. The human population shows variation for alpha-thalassemia. How did the variation arise?
4. A common misconception related to evolution is that individuals develop mutations because the mutations fulfill some “need” or the individuals gain some benefit. In this case, this reasoning would suggest that individuals develop a mutation in the alpha-globin gene because they want or need protection from malaria. On the basis of your understanding of evolution and natural selection, explain why this reasoning is faulty.
5. In certain environments, did alpha-thalassemia affect an individual’s ability to survive and reproduce? Explain.

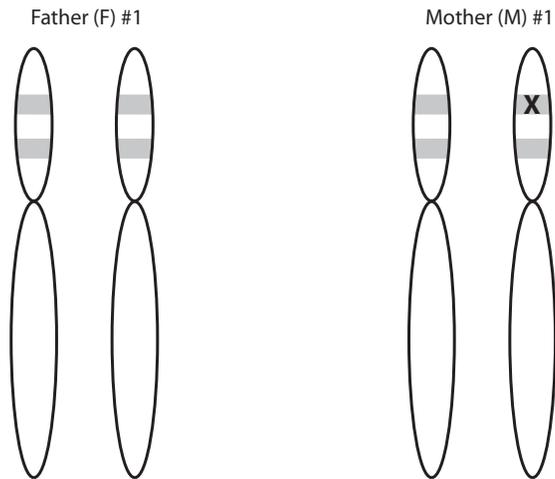
Inheriting Thalassemia

Name: _____

For each problem, use the information provided about the parents to predict the genotypes and phenotypes of their possible children.

Figure 1. The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.

Problem 1
Parents

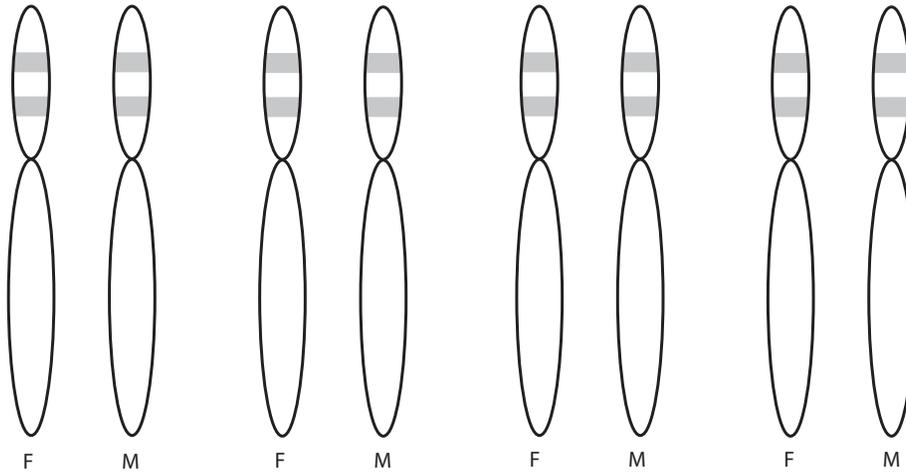


Key

Chromosome 16

- Functional alpha-globin allele
- Nonfunctional alpha-globin allele

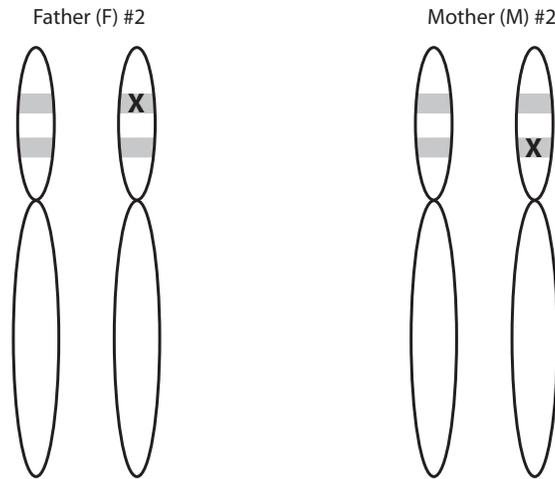
Possible genetic combinations in offspring



Genotype:	_____	_____	_____	_____
Number of nonfunctional alpha-globin genes:	_____	_____	_____	_____
Thalassemia (type?):	_____	_____	_____	_____

Figure 2. The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.

Problem 2
Parents

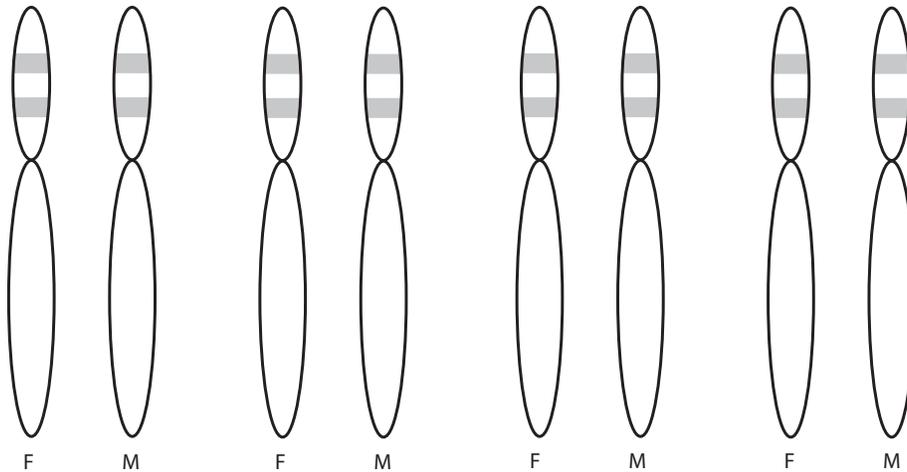


Key

Chromosome 16

- Functional alpha-globin allele
- Nonfunctional alpha-globin allele

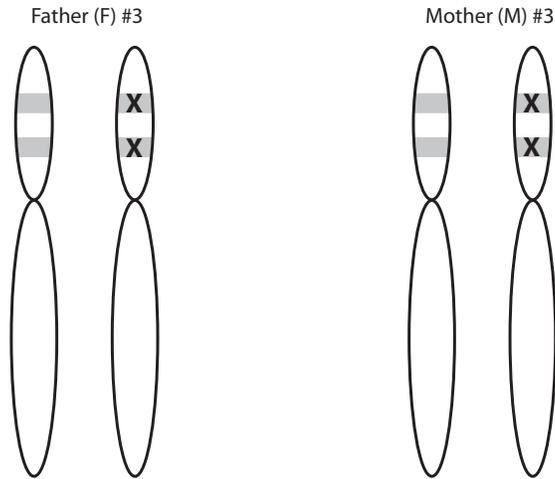
Possible genetic combinations in offspring



Genotype:	_____	_____	_____	_____
Number of nonfunctional alpha-globin genes:	_____	_____	_____	_____
Thalassemia (type?):	_____	_____	_____	_____

Figure 3. The top part of the diagram shows the genotypes of a mother and a father for the alpha-globin genes. Each chromosome carries two alleles of the gene, so each parent has four alleles of the gene. Nonfunctional alleles are shown with an "X." In the lower part of the diagram, show the different possible offspring if the parents have children. Below the diagrams are blanks to help you summarize the problem.

Problem 3
Parents

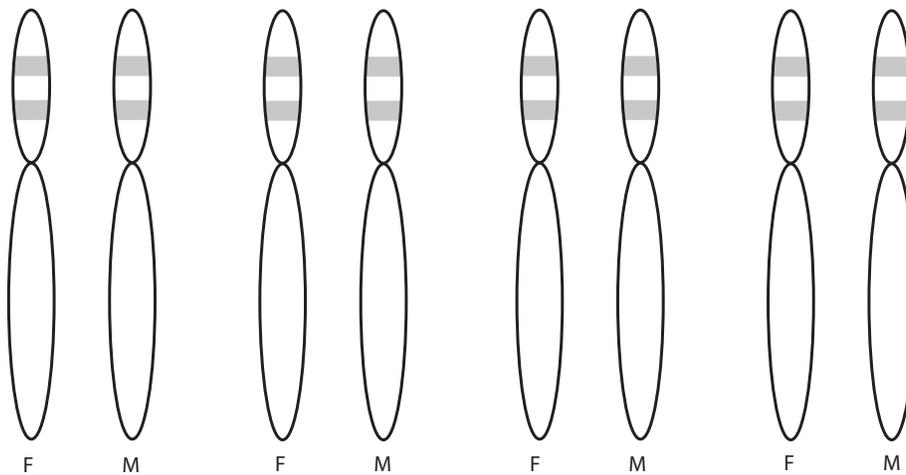


Key

Chromosome 16

- Functional alpha-globin allele
- Nonfunctional alpha-globin allele

Possible genetic combinations in offspring



Genotype:	_____	_____	_____	_____
Number of nonfunctional alpha-globin genes:	_____	_____	_____	_____
Thalassemia (type?):	_____	_____	_____	_____

Cleft Lip and Palate

Name: _____

Cleft lip and palate is a disorder caused by problems during embryonic development. The structures that form the upper lip and the roof of the mouth typically fuse during the first three months of fetal development. In cleft lip and palate, these structures do not join. The result is a gap or a split in the roof of the mouth, the upper lip, or both. The effect can be as simple as a small notch in the lip or as complicated as a large opening that runs into the roof of the mouth and nose (Figure 1). Cleft lip and palate is one of the most common birth defects in the world, occurring in about 14 out of every 10,000 births.

Figure 1. Cleft lip and palate. This infant has a cleft lip and palate. Multiple surgeries within the first year of life are often required to help children born with this condition.



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The causes of cleft lip and palate are complex. Many genes play a role in coordinating embryonic development. More than 42 genes are involved in the development of the head and face alone. Genes control the pathway of development that causes the upper lip and roof of the mouth to fuse. Mutations in these genes can cause cleft lip and palate. When genetics is part of the cause of cleft palate, sometimes mutations in many genes are involved, but other times a mutation in only one gene is the cause.

A growing embryo is also very sensitive to changes in its environment. Environmental effects, such as the lack of certain nutrients at specific times during development, can also cause cleft lip and palate. Most cases of cleft lip and palate are the result of interactions between genetics and the environment. In some cases, the exact cause is never determined.

Scientists are making headway in understanding some causes of cleft lip and palate. Some patients who develop a cleft lip and palate have a condition called Van der Woude syndrome. Evidence shows that this syndrome is caused by mutations in just one gene, called the *Irf6* gene. *Irf6* stands for “interferon regulatory factor 6,” but it is easier to just call it *Irf6*.

Irf6 is a gene that codes for a protein that controls other genes. That is, the protein from this gene turns other genes on or off. This protein is required for normal development. In mice, this gene is active in the cells that line the two sides of the forming mouth. The gene is turned on and makes protein just before and during fusion of the two sides of the mouth.

Genetic studies in humans show that some mutations to *Irf6* cause one form of cleft lip and palate (Van der Woude syndrome). Cells with these mutations don't make enough of the protein from this gene. This means that other genes don't get turned on or off at the right time. Mutations to *Irf6* act in many ways like a dominant allele. Only one of the mutated alleles is needed to cause cleft lip and palate.

To better understand this syndrome, scientists would like to know how specific changes to the gene affect the phenotype. However, the *Irf6* gene is very large, more than 18,000 nucleotides long. It would be very helpful to use evidence to identify sections of the gene that may be especially important for gene function.

Questions

Use the information you just read to answer the following questions. Work together as a group to share what you have learned.

1. Calculate the number of babies expected to be born with cleft lip and palate in the United States each year:
 - a. The worldwide incidence of cleft lip and palate is 14 out of every 10,000 births. Calculate the frequency of cleft lip and palate by dividing the number of babies with the condition by the number of births.
 - b. Assume that there are 4,000,000 births per year in the United States. Multiply the number of births by the frequency of cleft lip and palate you calculated in Question 1a to determine the expected number of babies born with cleft palate each year in the United States.
2. How could a change to a gene cause cleft lip and palate? How might a change in an environmental signal cause cleft lip and palate?
3. Assume that one parent has an allele of the *Irf6* gene with a mutation that causes cleft lip and palate and a second allele that is normal. Also assume that the second parent has two normal alleles for this gene. What is the probability that a child born to this couple will have a cleft lip and palate? Mutated *Irf6* alleles act in a dominant fashion.
4. Explain how studies from mice are helpful to scientists trying to understand cleft lip and palate in humans.

Sources:

<http://www.nlm.nih.gov/medlineplus/cleftlipandpalate.html>; <http://ghr.nlm.nih.gov/condition=vanderwoudesyndrome>

<http://ghr.nlm.nih.gov/gene=irf6>

<http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/CraniofacialBirthDefects/PrevalenceCleft+LipCleftPalate.htm>

<http://www.patient.co.uk/doctor/Cleft-Lip-and-Palate.htm>

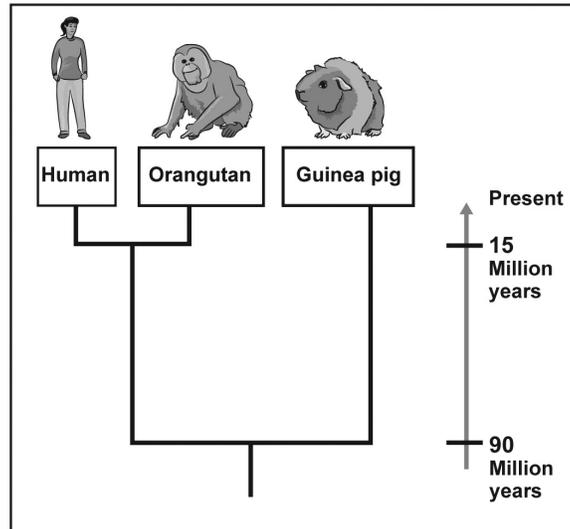
H.O. Olosoji et al. 2005. Incidence and aetiology of oral clefts: A review. *African Journal of Medicine and Medical Sciences*, 34(1): 1–7.

Calculating Times for an Evoprint

Questions

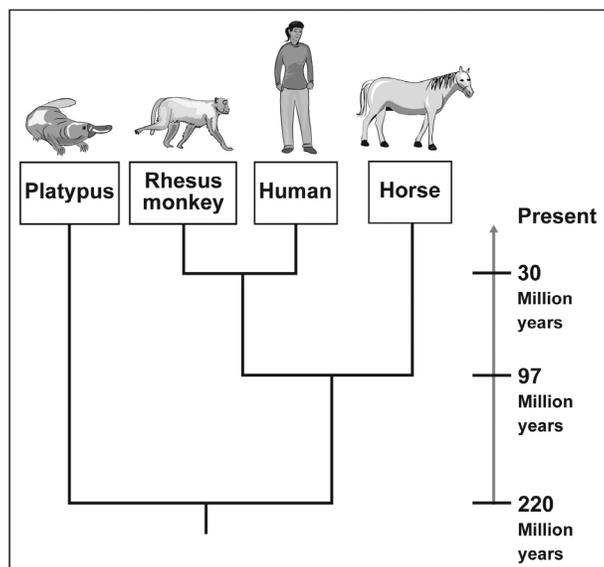
1. How many years are represented in an evoprint constructed from sequences from the three species shown in the evolutionary tree in Figure 1?

Figure 1. Evolutionary tree of humans, orangutans, and guinea pigs.



2. If you constructed an evoprint from sequences from the four species represented in the evolutionary tree in Figure 2, how many years would be represented in the evoprint?

Figure 2. Evolutionary tree of platypuses, rhesus monkeys, humans, and horses.



Irf6 Evoprint Comparison

Name: _____

In this activity, you will work to identify sections of the *Irf6* gene that may be especially important for the gene to function properly. You will compare DNA sequences for this gene across many different species. The human sequence will be the reference sequence.

Procedure

1. Access the Evolution and Medicine Web site.

Click on “Lesson 3: Evolutionary Processes and Patterns Inform Medicine,” then “Activity 2: Evoprint Comparison.” The opening page shows 1,701 nucleotides from the *Irf6* gene in humans.

2. Compare the human sequence with other individual species by checking the button next to the animal of your choice in the “Comparison of two sequences” section. Compare the human sequence with at least two other species. Make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates here.
3. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species’ common ancestry with humans. Use the comparisons you completed in Step 2 and the data in Table 1 to help you with this task.

Table 1. Time Since Common Ancestry with Humans

Species	Time since common ancestry with humans (millions of years)
Chimpanzee	8
Orangutan	15
Rhesus monkey	30
Dog	97
Horse	97
Cat	97
Cow	97
Rat	91
Mouse	91
Guinea pig	91
Armadillo	105
Opossum	176

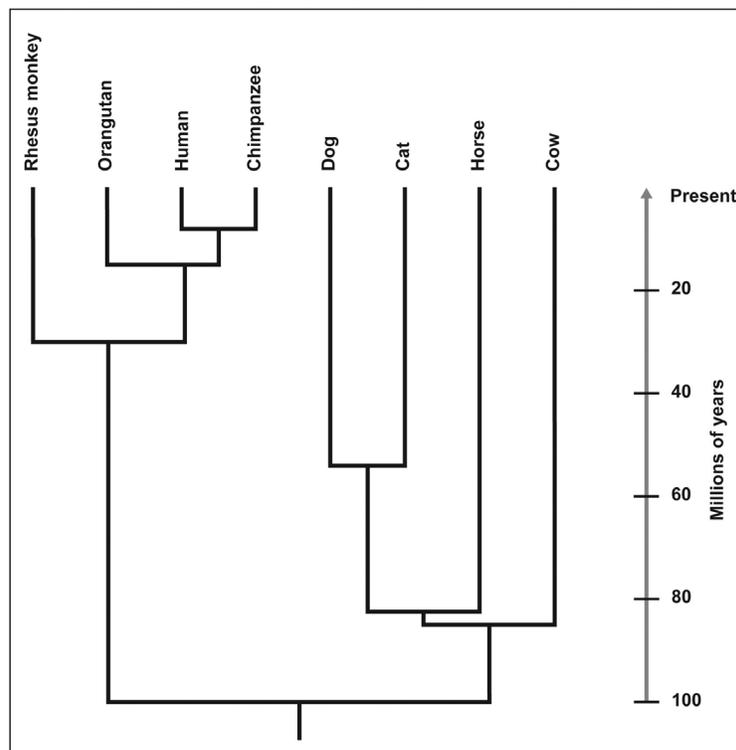
4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Tables 1 and 2 to complete the following tasks.
 - a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.
 - b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time this evoprint represents.

Part 1: Horse, Cow, Dog, and Cat

Table 2. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Horse	Cow	85
Horse	Dog	83
Horse	Cat	83
Dog	Cat	53

Figure 1. Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows.



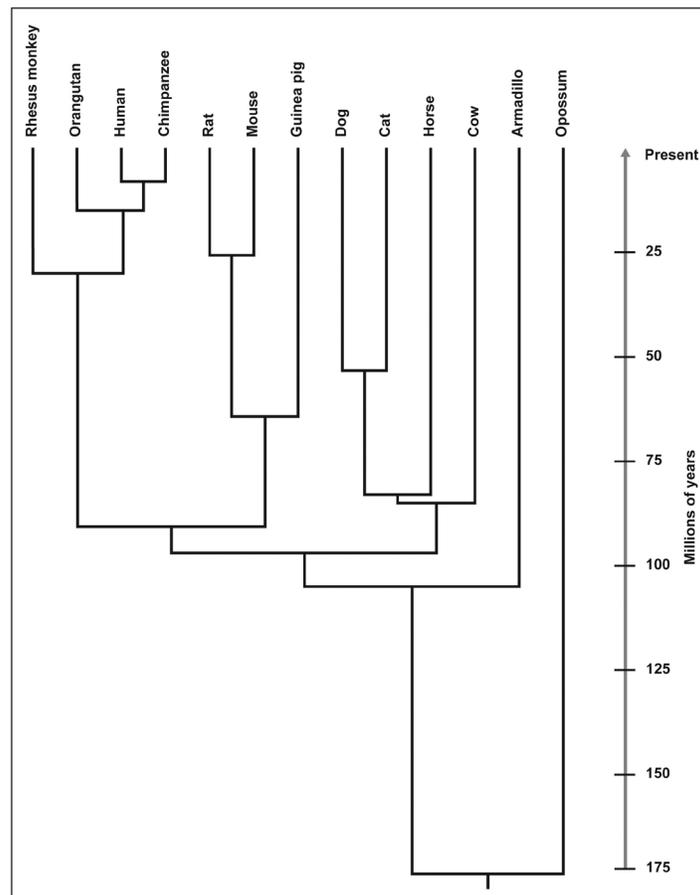
- Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

Part 2: Guinea Pig, Mouse, and Rat

Table 3. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Guinea pig	Mouse	64
Guinea pig	Rat	64
Rat	Mouse	26

Figure 2. Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.



- Use the data in Tables 1,2, and 3 and Figure 2 to calculate the amount of time represented in this evoprint.
- Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

Evoprint Introduction

Figure 1. The human reference sequence: the 1,701 nucleotides of the human sequence for the Irf6 gene.

Human Reference Sequence

```
GGTGTGTGCTTATAGTTCCAGCTACGTGGGAGGTTGAGGCGGGAGGATCGCTTGAGC
CTAGGAGGTCAGGGCTGCAGTGAGCTATGATCATGCCACTTGCCTCCAGCCTGGTT
GACAGAGCAAGACCATATCCACAAAAAAGAAAAAGAAAAAAGAAAAAAGAAAAA
CCCTCTATACCAATCACATAGGCTATACTGCGTGCCTGCTAACACCTGATAGTTCTG
TCCTGGTCTGAGTATCCATTTCCAGGCCAAGCAACTCTCTGCATAGCAAGAGCTCT
GAGTTATTGAGGAGGAAGAGTCAACTGCTGACCCTCGCTAGCTTTTGAACAACAC
ATCACTACAATCACTAATGAGAGTTTTAGCCAACCCAGCTGGTATCCTTTGGGATG
TGAGTGAGTACCTCAATGAGTGGGAAAGAATAAACGGCTTCAACCATTGCAGACAT
GCCCCAAAAGAGGAATTACTATGCCTGCTGAGTTTTGGGCACCCCATCATAAGCAT
TCTCTCTGTTTCACCAGAGTTTTAGATCTAGTGTATTCCCATGCCAAAAAAAAAAA
AAAAAAAAAATCCAGAAAGGTC TGATGGTAGAAGAAGTCCTTTACCTTAAAAATGGT
ATTTTCTCTTCTTGTGAGGGCTATGCCGGGTGGCATGTTTTCCAGGGAATCTGGAA
GCGTTTAGAGTCCCTGTGTAGCCAGATGAGCCCAGGGTAGAGGCCACTATCCACCTG
GGCCACCAGCCAGGGCTTTAGCCGGACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGTGAGAGGGAGAAATGGGAAGAGCAGAAGAATTAGGCCAGCCACTGGGAACCTT
CCCAGCCACCTTTCCCATCTACTAGAGCTACAGTTTTCACTAGATTACAGCAAAGAAA
CTAAATATGGGAATAAGCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCCTTT
TTATTCTGATTCCAAAGCCATTTACACTTCATGCTCTAGCCAAATCAGACTTATCAG
CTAAGTAGATCACATTTCTTCTATTTAAATCCTGAAGCAGAAAAATAAAAAGGTGAT
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AAATTGCTCTTTTCAATCTCATCTCAGGAGAGGGAAGAAAAAAGTTATGGAAACAGC
AACAACTATATAAGTGAGAAGGTGACACATGTTCTCTTTTTTAAACAAGACAATAAA
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AAGGGGTAGATCCTGGACTCCAGAGTCTCACTTACATGGAAGAGCAGGCAGTGTGT
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GGCTTGGGAACACTGGCGACAAAGGCTGAGAACACGTCTCCACTACTGAGTCTGGTA
AAGCTCAGAAGCCCTAGAATAACCAATACTAAAGTACTAAGAGTACTTAGAGTACT
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TTTATCTCCAGTGAAGGCTGGAGTGAACCAATTGGTTGCAGGAGCCA
```



Human

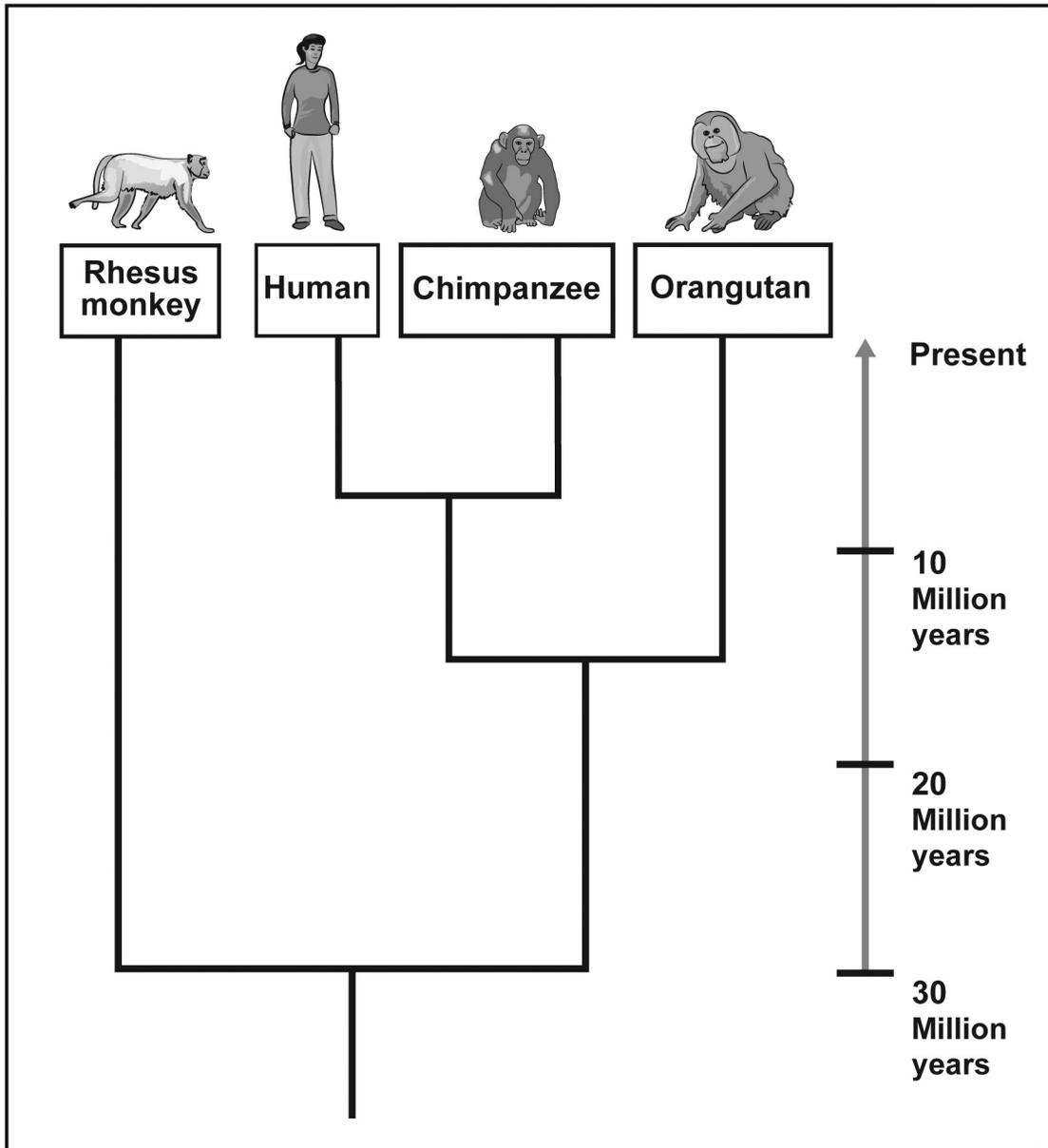
Figure 2. Evoprint for the comparison of the human and cow sequences for a portion of the Irf6 gene. Nucleotides in capital letters are the same between the two species, and nucleotides in lowercase letters differ. The human sequence is the reference sequence.



Figure 3. Evoprint for the comparison of the human, chimpanzee, orangutan, and rhesus monkey sequences for a portion of the *Irf6* gene. Nucleotides in capital letters are the same among all the species, and nucleotides in lowercase letters differ. The human sequence is the reference sequence.



Figure 4. An evolutionary tree summarizing the relationships among four primate species. How can you use the diagram to help you interpret the time represented in an evoprint for all four species?



Investigating *Irf6* Evoprints

Name: _____

In this activity, you will work to identify sections of the *Irf6* gene that may be especially important for the proper functioning of the gene. You will examine evoprints for this gene across many different species. The human sequence is the reference sequence in all cases.

Procedure

1. Work as a group to examine the evoprints in which the human sequence was compared with one other species. For at least three of the comparisons, make a rough estimate of the number of nucleotides that did *not* change. Record the comparisons you made and your estimates below.

2. Describe how the number of changes you observe in the *Irf6* gene relates to the amount of time since the species' common ancestry with humans. Use the comparisons you completed in Step 1 and the data in Table 1 to help you with this task.

Table 1. Time Since Common Ancestry with Humans

Species	Time since common ancestry with humans (millions of years)
Chimpanzee	8
Orangutan	15
Rhesus monkey	30
Dog	97
Horse	97
Cat	97
Cow	97
Rat	91
Mouse	91
Guinea pig	91
Armadillo	105
Opossum	176

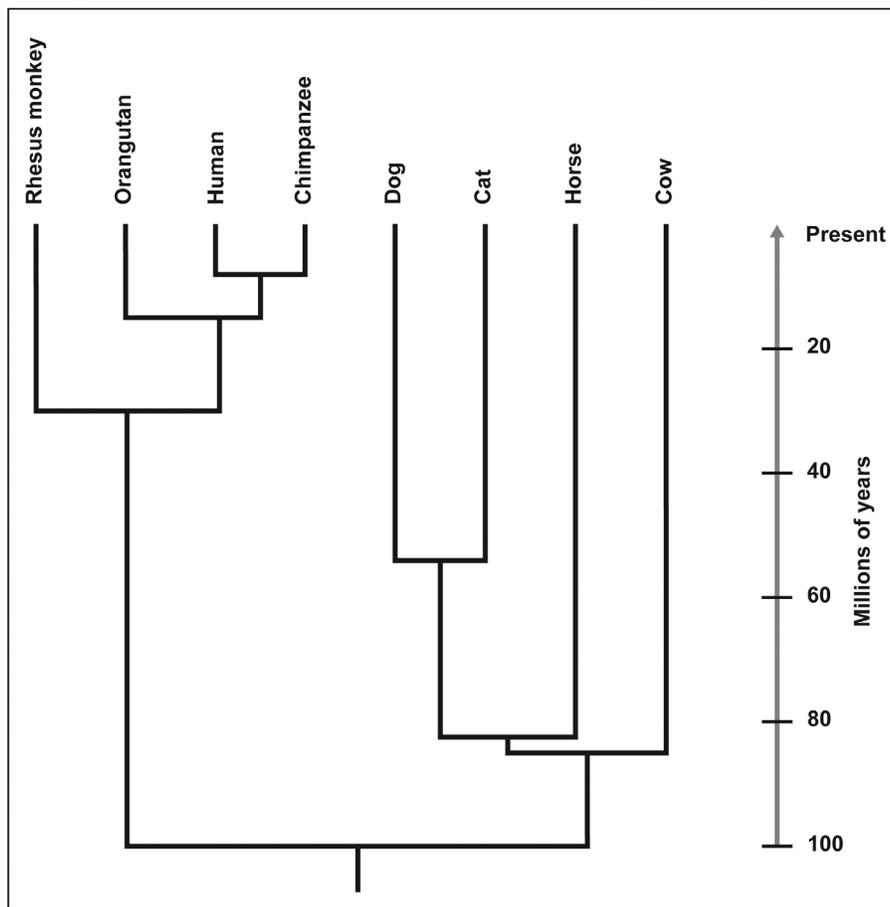
3. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows. Use your observations and data from Table 1 to answer the following questions.
 - a. Make a rough estimate of the percentage of nucleotides that did *not* change in this comparison.
 - b. Use the data in Tables 1 and 2 and Figure 1 to calculate the amount of time this evoprint represents.

Part 1: Horse, Cow, Dog, and Cat

Table 2. Time Since Common Ancestry for Additional Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Horse	Cow	85
Horse	Dog	83
Horse	Cat	83
Dog	Cat	53

Figure 1. Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, and cows.



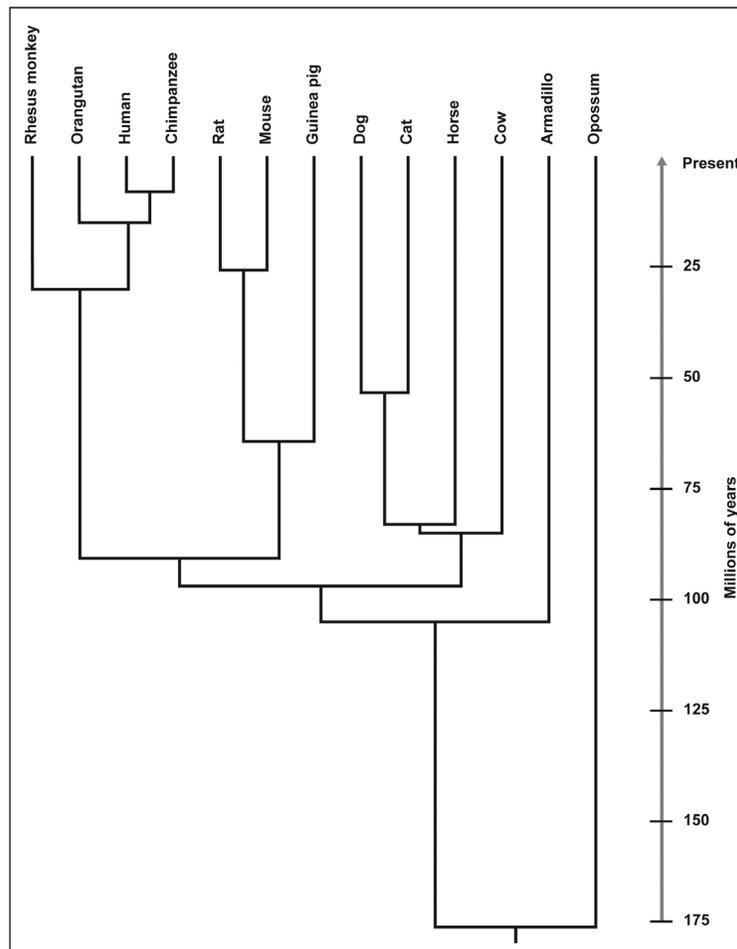
4. Explore the evoprint for the comparison of sequences from humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.

Part 2: Guinea Pig, Mouse, and Rat

Table 3. Time Since Common Ancestry for Additional Species Pairs

Species 1	Species 2	Time since common ancestry (millions of years)
Guinea pig	Mouse	64
Guinea pig	Rat	64
Rat	Mouse	26

Figure 2. Evolutionary tree summarizing the relationships among humans, chimpanzees, orangutans, rhesus monkeys, dogs, horses, cats, cows, rats, mice, guinea pigs, armadillos, and opossums.



- Use the data in Tables 1, 2, and 3 and Figure 2 to calculate the amount of time this evoprint represents.
- Identify two regions with eight or more nucleotides in a row that have not changed over the amount of time calculated in the previous step. Write out the nucleotides for these regions.

Irf6 Evoprints

Evoprint 1. Comparison of Irf6 sequences from human and chimpanzee.

Comparison: Human, Chimpanzee

```
GGTGTGTGCT+ATAGTTCAGCTACGTGGGAGGTTGAGGCGGGAGGATCGCTTGAGC
CTAGGAGGTCAGGGCTGCAGTGCAGTATGATCATGCCACTTGCACTCCAGCCTGGTT
GACAGAGCAAGACCATATCCACAAAAAAGAAAAAGAAAAAAGAAAACTTGTGAGAG
CCCTCTATACCAATCACATAGGCTATACTGCGTGCCTGCTAACACCTGATAGTTCTG
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AtCACTACAATCACTAATGAGAGTTTATAGCCAAACCCAGCTGGTATCCTTTGGGATG
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TCTCTCTGTT+ CACCAGAGTTTATAGTCTAGTGTATCCCCATGCCAAAAA
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GCGTTTATAGATCCCTGTGTAGCCAGATGAGCCAGGGTAGAGGCCATATCCACCTG
GGCCACCAGCCAGGGCTTATAGCCGACTCTGCGGGGGTGGAGGGCCATGATCTGGGG
GGGTCAGAGGGGAGAAATGGGAAGAGCAGAAGAATTAGGCCAGCCACTGGGAACCTT
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TTTATCTCCCAgTGAAGGCTGGAGTGAACCAATTGGTTGCAGGAGCCA
```



Human



Chimpanzee

Evoprint 2. Comparison of Irf6 sequences from human and dog.

Comparison: Human, Dog

```
ggtgtgtgcttatagttocagctacgtgggaggttgaggcgggaggatogcttgagc
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cTAAGTAGATCACATTTCTTCTATTTAAATCCTGAAGCAGAAAAATAAAAaggTAT
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aaaagcagtcagagacaagcttctgcatggctaggacAGCCCTCCCTGGGTCTG
TTTATCTCCCAgTGAAGGCTGGAGTGAACCAATTGGTTGCAGGAGCCA
```



Human



Dog

Evoprint 3. Comparison of *Irf6* sequences from human and guinea pig.

Comparison: Human, Guinea Pig

```

gggtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggagggtcagggtgcagtgagctatgatcatgccacttgcactccagcctgggt
gacagagcaagaccatataccocaaaaaaagaaaaagaaaaaacttgctgagag
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GGCTTTAGAGTCCTGTAGCCAGATGAGCCAGGGTAGAGCCCTATCCACCTG
GGCCACCAGCCAGGGCTTAGCCGACTCTCGGGGGTGGAGGGCCATGatCTGGGG
GggTCAGAGGGAGAAAGGGaAGAGCAgaAGAATTAGGCCAGCCaCTGGGAACcctT
CCCAGCCaCCTTTCCCATctCTAGAGCTACAGTTTACTAGATTACAGCAAAGAA
CTAAATATGGGAATAAGCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCTTT
TtATTCTGATTCCAAGCCATTTAaCTTCTGCTCTAGCCAAATCAGACTTATCAg
ctAaGTAGATCaCATTCTCTATTTAaTCTGAAGCAGaAAAAATAAAAGGTGAT
GTCATCCtgCTGGAAcacagtCCTTCTGAAGCAcAAgGGCTTGGAAAGAGAAGGa
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AAcaactatataagtgagaaggtgacacatgttctcttttaacaagacaactaaa
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CCACAGgatctgaagagtcggcttgtcttcccttgaccgctcaagattctgtat
ggagaaaaaggagggtctcagctggctatccatacaacaatccagtgctcgggagaa
ggcttgggaacactggcgacaaggtgagaacacgtctccactactgagctcggta
aagctcagaagccctagaataaccaaatacctaaagtactaagagtagcttagagtagt
aaaagcagtcagagacaagcttgcagctaggacagcctcctccttgggtctg
tttatctccagtgaggctggagtgaaaccaattgggtgcaggagcca
    
```



Human



Guinea Pig

Evoprint 4. Comparison of *Irf6* sequences from human and opossum.

Comparison: Human, Opossum

```

gggtGTGTCTTAGTAGTCCAGCTAGTGGGAGGTGAGGCAGGAGGATCGCTTGAGC
CTAGGAGGTCAGGGCTGCAGTGCGCTATGATCATGCCACTGCACTCCAGCCCTGGTt
gacagagcaagaccatataccocaaaaaaagaaaaagaaaaaacttgctgagag
ccctotataccaatcacataggotatactgctgcoctgctaacaacctgatagttctg
tcctggctgagtagtaccattcccaggcccaagcaactctctgcatagcaagagctct
gagttattgaggaggaagagtgcaactgctgacctcgctagcttttgaacaaacac
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tgagtgagtaccttcaatgagtgaggaaagaataaacggcttcaaccattgcagacat
gcccccaaaagaggaattactatgcctgctgagtttgggcacccccatcataagcat
tctctctgtttcaccagagttttagatctagtgtattcccagcccaaaaaaaaaa
aaaaaaaaaatccagaaggtctgatggtagaagaagtcTTTACCTTAAAATgGT
ATTCTCTCTCTGCTGAGGGCTATGCCGGTGCATGTTTCCAAGGAATCTGGAA
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GGCCACAGCCAGGGCTTAGCCGACTCTCGGGGTGGAGGGCCATGATCTGGgg
gGGTcAGAGGgagAAAggggAgAgCAgaAGAATTAGGCCAGcCACTGGGAaCctT
CCCAGCaaccttcccatcTACTAGAGCTaCAGTTTACTAGATTAAGcaaaAGAAA
CTAAATATGGGAATAAGCTGTGCCAGGTGGGAAAGAAAAGAGGTTAAAAGGTCTTT
TtATTCTGATTCCAAGCCATTTAaCTTCTAGCTCTAGCCAAATCAGACTTATCAg
ctAAGTAGATCACATTTCTTCTATTTAAATCCTGaaGCAGAAAAATAAAAGGTGAT
GTCATCCtgCTGGAAACACAGTCTTCTGAaGCACCaaaggcttggaaagagaagga
aaattgctcttttcaatctcatctcaggagaggggaagaaaaaagtattggaacagc
aacaactatataagtgagaaggtgacacatgttctcttttaacaagacaactaaa
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aaaagcagtcagagacaagcttgcagctaggacagcctcctccttgggtctg
tttatctccagtgaggctggagtgaaaccaattgggtgcaggagcca
    
```



Human



Opossum

Evoprint 5. Comparison of Irf6 sequences from human, chimpanzee, orangutan, Rhesus monkey, dog, horse, cat, and cow

Comparison: Human, Chimpanzee, Orangutan, Rhesus Monkey, Dog, Horse, Cat, and Cow

```

gggtgtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggaggtcagggtgcagtgagctATGATCcatgccacttgcactccagcctggtt
gacagagcaagaccatataccacaaaaaagaaaaagAAAATAAAACTTgctgagag
ccctctataccaatcacataggctatactgcgtgctgctaacacctgatagttctg
tcctggctgtagtaccatccocaggcccaagcaactctctgcatagcaagagctct
gagttattgaggaggaagagtgcaactgctgacctcgctagcttttgaacaaacac
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TGAATGAGTCCCTcaatgagTggGAAGAAATAAAcGCttcaaccatgacagAcAT
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TCTctctgtttcaccagagtttttagatctagtgtATTcccCatGCCaaaAAAAaaa
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aTTTTCTCTCTTTG+TG+GGGCT+TGCCGGGTGGCATG+TTCCAGGGAATCTGGAA
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GGCCACAGCCAGGGCTT+AG+CG+ACTCTGCGGGGTGGAGGCCATGATCT+GGG
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CCAGCCcAcctT+CCCacTAcTAGAGCTACAGTTTCA+TAGATTACAGCAAGAAA
CTAAATATGGGAATAagCTGTGCCAGGTgggAAAATAAAGAGGTTAAAGGTCTTT
TtATTCcga+TCCAAAGCCATTTacacTTCATGCTcAGCCAAATCAGACTTATCAg
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GTCATcTgCTGGAcCACAGTCTCTCTGAAgAcCAAGggctttgGAGAGAAGga
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aaaagcagtcagagacaagctttgcatggctaggacgcctccctccctgggtctg
tttatctcccagtgaggctggagtgaaaccaattggttgcaggagcca
    
```

Evoprint 6. Comparison of Irf6 sequences from human, chimpanzee, orangutan, Rhesus monkey, dog, horse, cat, cow, rat, mouse, guinea pig, armadillo, and opossum.

Comparison: Human, Chimpanzee, Orangutan, Rhesus-Monkey, Dog, Horse, Cat, Cow, Rat, Mouse, Guinea Pig, Armadillo and Opossum

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gggtgtgtgcttatagttccagctacgtgggaggttgaggcgggaggatcgcttgagc
ctaggaggtcagggtgcagtgagctatgatcatgccacttgcactccagcctggtt
gacagagcaagaccatataccacaaaaaagaaaaagaaaaaacttggctgagag
ccctctataccaatcacataggctatactgcgtgctgctaacacctgatagttctg
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tttatctcccagtgaggctggagtgaaaccaattggttgcaggagcca
    
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Image sources: PhotoDisc for human and rat; Corel for cow, chimpanzee, orangutan, rhesus monkey, guinea pig, dog, horse, opossum, and cat; Vlad Lazarenko for armadillo.

Email from Viroformatics

From: Paul Monkeyflower, President of Viroformatics

To: Biology students

Subject: Information about influenza evolution

Greetings. Our company, Viroformatics, wants your help. Viroformatics does research on viruses in order to help improve human health. We have a strong presence in the community and want to encourage all students to pursue careers in biomedicine.

We would like to create a brochure for high school students that will help them understand how we use information about evolution to better understand influenza. Your teacher has agreed to let your class help us by creating an outline of the brochure. Because you are the same age as the target audience and you are knowledgeable about evolution and medicine, your perspectives will be very helpful.

Your task is to collect information about influenza and evolution that you think is important. To help you with this, we compiled some questions and sent them to your teacher. We also want to include in the brochure some descriptions of what high school students think about the topic. To help us do that, please write down your ideas about the questions we sent before you start gathering information. Once you have gathered information, compile bulleted lists in answer to the questions. We have our own writers who will make the final brochure.

We're giving you access to our Viral Genome Database, which should help you with your task. Certain parts of the database will give you a glimpse of some of the ways scientists study influenza.

We look forward to working with you.

Sincerely,

Paul Monkeyflower
President of Viroformatics

Notes about Influenza and Evolution

Name: _____

Questions from Viroformatics

Write your answers to the following questions before the lesson and after the lesson. You may want to create a new page in your notebook for each question. This will help you with your brochure outline and with keeping track of your understandings as you proceed through the lesson. Use a bulleted-list format for your answers.

1. What is influenza, or the “flu”?

What I think before the lesson:

What I think after:

2. How do scientists use data to explore how influenza genes evolve?

What I think before the lesson:

What I think after:

- 3. Not all individual influenza viruses are identical. What causes viruses to differ from one another?**

What I think before the lesson:

What I think after:

- 4. How does natural selection help explain the evolution of influenza? (Keep in mind the major principles of natural selection.)**

What I think before the lesson:

What I think after:

- 5. How does evolution help explain why researchers need to make a new vaccine for influenza every few years?**

What I think before the lesson:

What I think after:

Introduction to Influenza

Influenza basics

Influenza, also called the flu, is an illness caused by a virus. The influenza virus infects lung cells and causes respiratory problems. The flu can be mild, severe, or even deadly, and it has a large impact on human health. For example, on average each year,

- 5 to 20 percent of Americans suffer from the flu,
- complications from the flu result in hospital stays for over 200,000 Americans, and
- flu-related effects cause the death of tens of thousands of Americans and about 500,000 people worldwide.

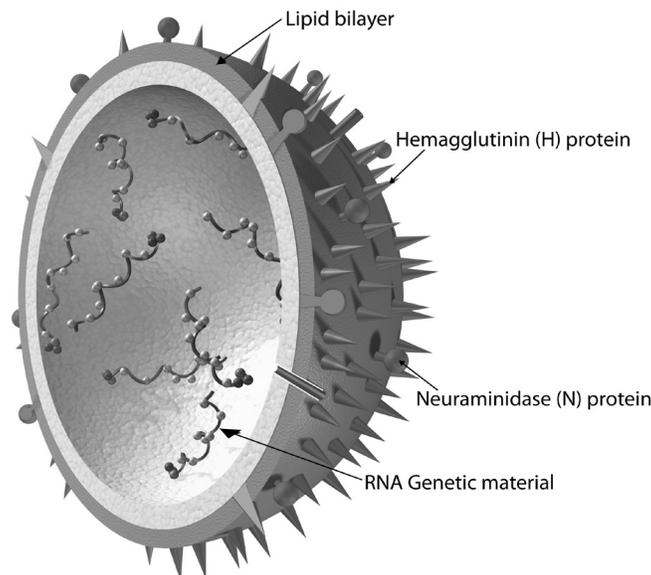
Symptoms of a flu infection include a high fever, extreme tiredness, muscle aches, a dry cough, a sore throat, and a stuffy nose. In people who are otherwise healthy, symptoms from the flu are usually gone after four to seven days, but they can last longer.

Occasionally, new strains of influenza emerge that cause global pandemics. A pandemic is a large-scale outbreak of an infectious disease that spreads throughout the world. In the “Spanish flu” pandemic of 1918–1919, hundreds of millions of people were infected and tens of millions of people died.

Structure of the virus

The influenza virus is simply genetic material surrounded by a membrane. Inserted like spikes in the membrane are two main proteins, hemagglutinin (H) and neuraminidase (N) (Figure 1). Influenza has eight segments of RNA that contain 11 genes. Importantly, the genetic material is RNA, not DNA. RNA is more prone to mutations than DNA.

Figure 1. Influenza virus.



Source: National Institute of Allergy and Infectious Diseases.

Types of influenza viruses

Three main types of influenza viruses exist: A, B, and C. The forms of specific proteins found in influenza determine the type. Types A and B cause the seasonal flu that sweeps across the globe every year. Type A causes some of the flu outbreaks that result in a larger number of deaths than normal. Type C causes only a mild illness and does not cause epidemics.

Each type of the virus can be further broken down into specific groups:

- Type A: Subtypes are based on forms of hemagglutinin (abbreviated H or HA; 16 forms, named H1–H16) and neuraminidase (abbreviated N or NA; 9 forms, named N1–N9). H3N2 and H1N1 are examples of subtypes. Within each subtype, different strains exist.
- Type B: There are no subtypes, but different strains exist.
- Type C: There are no subtypes, but different strains exist.

Influenza A (H1N1 and H3N2) and influenza B strains are included in each year's seasonal flu vaccine. The specific strains in the vaccine change over time, however.

Though influenza infects many humans and pigs each year, it's considered a bird disease. This is because many more subtypes and strains of influenza are found in birds than in other animals.

How do you get the flu?

The common way to get the flu is to inhale a virus from another infected person. Infected people release viruses when they cough and sneeze. You can also get the flu by touching your mouth or nose after touching something with flu viruses on it. You can start infecting other people one day before you show symptoms and continue to infect people five to seven days after you get sick. As you can imagine, this makes it very difficult to control the spread of the flu.

Influenza Sequences

In this exercise, you will analyze genetic sequences from three different influenza viruses that scientists have collected from people around the world over a span of 10 years. The sequences come from the gene that codes for hemagglutinin. This protein is found in the membrane of an influenza virus.

Procedure

1. Cut out the three sequences on page 2 so you can move them easily.
2. Select two of the sequences. Tape one of the sequences to a blank piece of paper. Place the second sequence beneath the first sequence. Slide the bottom sequence back and forth until many of the nucleotides line up with the upper sequence. When you are satisfied with its placement, tape the lower sequence into place.
3. Repeat Step 2 with the third sequence. The third sequence should align with the second sequence.
4. Scientists today use sophisticated computer programs to help line up sequences. This is called an “alignment.” You just performed a sequence alignment.
5. Underline the nucleotides that line up. Then draw boxes around the nucleotides that differ among the three samples. How many nucleotides differ among the three samples?

Sample 1: Virus from a patient in Finland in 2003

Sample 2: Virus from a patient in Auckland, New Zealand, in 1997

Sample 3: Virus from a patient in Madrid, Spain, in 1993

1 . CACACTGGAGTTTAACAATGAAAGCTTCAATAT

2 . CCTGGAGTTTACCAATGAAAGCTTCAATATTGG

3 . AGGCACCCTGGAGTTTACCAATGAAGACTTCAA

Aligned Influenza Sequences

Sample 1: Virus from a patient in Finland in 2003.

Sample 2: Virus from a patient in Auckland, New Zealand, in 1997.

Sample 3: Virus from a patient in Madrid, Spain, in 1993.

1.	CACACTGGAGTTTAACAATGAAAGCTTCAATAT
2.	CCTGGAGTTTACCAATGAAAGCTTCAATATTGG
3.	AGGCACCCTGGAGTTTACCAATGAAGACTTCAA

Exploring a Genetic Database

Name: _____

In this portion of the activity, you will work with a partner to explore the Viral Genome Database at Viroformatics. For security reasons, you will only have access to certain portions of it. To access the database, go to the Evolution and Medicine Web site: <http://science.education.nih.gov/supplements/evolution/student>. Click on “Lesson 4: Using Evolution to Understand Influenza” to get to the home page for Viroformatics. Once there, go through the following steps to learn how scientists at Viroformatics use evolution in their studies of influenza.

Procedure

1. Your first goal is to get a sense of the types and amount of data stored in the database. Click on “Viral Genome Database,” then “Full Virus Database.” Once there, explore the number of sequences available for influenza in humans for the hemagglutinin gene. Record the number available for types A, B, and C.

(You can explore the data for specific sequences by clicking on the accession number. By convention, the genetic sequences are recorded as DNA. Influenza viruses store their genetic information as RNA.)

2. Why do you think the number of sequences for each type is different?
3. Researchers at Viroformatics are studying how influenza viruses have changed over time. They obtained the genetic sequence for hemagglutinin from 11 viruses isolated from people around the world at different times. Scientists store influenza samples from the past in freezers. This way, future scientists have access to the influenza “fossil record.” To access the 11 sequences, return to the “Viral Genome Database” page. Then click on the “Influenza-Over-Time Project.” Note the years and the countries in which the samples were collected. (Again, you can explore specific sequences by clicking on the accession number.)
4. Click on “Align Sequences” to see a portion of the alignment for the hemagglutinin gene in all 11 viruses.
5. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}}$$

6. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\frac{\text{answer from Step 5}}{\text{total number of years}} = \text{number of changes per nucleotide per year}$$

7. In Lesson 3, you investigated a portion of the sequence of a gene called *Irf6* that is involved in the development of the head and face. You compared the sequence of this gene in many different species. Thirty nucleotides from the *Irf6* sequence are shown below:

TGGGCCAC_cAGCCAGGGCTT_tAG_cCG_gACT

The lowercase (gray) letters show nucleotides that differ among some of the species. The uppercase (black) letters did not change. The amount of time represented in this comparison is 1,009 million years (or 1.009 billion years). Use the same formulas you used in Steps 5 and 6 to calculate the expected number of changes per nucleotide per year in this sequence.

8. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza with the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes than the other.
9. To see a diagram that summarizes the relationships among the viruses, click on “Build a Tree.” Does this diagram show evidence that the influenza virus is changing over time?
10. Explore how the number of changes in the genetic sequence relates to time. Do this by selecting two sequences and then clicking on the “Compare” button. Hint: A useful comparison is to record the number of changes in all the samples compared with the most recent sample (FIN_2003). Use the data you collect to answer the following questions.
- How do the number of changes to the sequence relate to time?
 - What do you think this means?

Viroformatics Virus Database

Figure 1. Viroformatics Virus Genomic Database home page.

Viroformatics *Studying viruses to save human lives.*

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Entire Virus Genomic Database

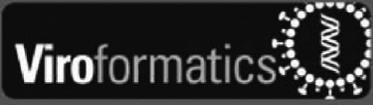
Researchers: Use this genome database to access protein or genetic sequence information for viruses studied at Viroformatics. Access to some projects requires special permissions.

Select VIRUS	Select HOST	Select GENE	Select TYPE
HIV	Avian (Bird)	Hemagglutinin (HA)	A
Influenza	Canine (Dog)	M1	B
Measles	Human	M2	C
Varicella-zoster	Swine (Pig)	Neuraminidase (NA)	
	Whale	Nucleoprotein (NP)	
	Unknown	NS1	
		NS2	
		PA	
		PB2	
		PB1	
		PB1-F2	

RETRIEVE SEQUENCES

Influenza Hemagglutinin Sequence

Figure 1. Viroformatics data for influenza A virus H3N2 hemagglutinin.



Studying viruses to save human lives.

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Data for Accession Number: 2546935698

of bases = 987
RNA sequence, recorded as DNA

Virus: Influenza A Virus H3N2
Gene / Protein: Hemagglutinin (HA)

Amino acid sequence
QKLPGNNDNSTATLCLGHHA V P N G T I V K T I T N D Q I E V T N A T E L V Q S S T G E I C D S P H Q I L D G E N C T L I D A L L G D P Q C D G F Q N K K W D L F V E R S K A Y S N C Y P Y D V P D Y A S
L R S L V A S S G T L E F N N E S F N W T G V T Q N G T S S A C K R R S N S F F S R L N W L T H L K F Y P A L N V T M P N N E K F D K L Y I W G V H H P G T D N D Q I F L Y A Q A S G R I T V S T K R S Q Q T V I
P N I G S R P R V R N I P S R I S I Y W T I V K P G D I L L I N S T G N L I A P R G Y F K M R S G K S I M R S D A P I G K C N S E C I T P N G S I P N D K P F Q N V N R I T Y G A C P R Y V K Q N T L K L A T G M R
N V P E K Q T R

Nucleotides

1	caaaaacttc	ccggaatga	caacagcacg	gcaacgctgt	gccttgggca	ccatgcagta
61	ccaaacggaa	caatagtgaa	aacaatcacg	aatgacccaa	ttgaagttac	taatgctact
121	gagctggttc	agagttcctc	aacaggtgaa	atatgcgaca	gtcctcatca	gatccttgat
181	ggagaaaact	gcacactaat	agatgctcta	ttgggagacc	ctcagtgatga	tggcttccaa
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301	gatgtgccgg	attatgcctc	ccttaggtca	ctagttgcct	catccggcac	actggagttt
361	aacaatgaaa	gcttcaattg	gactggagtc	actcaaatg	gaacaagctc	tgcttgcaaa
421	aggagatcta	ataacagt	ctttagtaga	ttgaattgg	tgaccactt	aaaattcaaa
481	taccagcat	tgaacgtgac	tatgccaaac	aatgaaaaat	tgacaaat	gtacatttgg
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661	cccagagtaa	ggaatatccc	cagcagaata	agcatctatt	ggacaatagt	aaaaccggga
721	gacatacttt	tgattaacag	cacagggaa	ctaattgctc	ctaggggta	cttcaaatg
781	cgaagtggga	aaagctcaat	aatgagatca	gatgcacca	ttggcaaatg	caattctgaa
841	tgcatcactc	caaatggaag	cattccaat	gacaaacat	ttcaaatgt	aacaggatc
901	acatatgggg	cctgtcccag	atatgttaag	caaaacactc	tgaattggc	aacagggatg
961	cgaaatgtac	cagagaaaca	aactaga			

Questions

1. Calculate the number of changes per nucleotide in this 100-nucleotide sequence by using the following formula:

$$\frac{\text{number of nucleotides that have at least one change}}{\text{total number of nucleotides}}$$

2. The viruses in this study were collected over a span of 35 years. Calculate the number of changes per nucleotide per year by using the following formula:

$$\frac{\text{answer from Step 1}}{\text{total number of years}}$$

3. In Lesson 3, you investigated a portion of the sequence of a gene called *Irf6* that is involved in the development of the head and face. You compared the sequence of this gene in many different species. Thirty nucleotides from the *Irf6* sequence are shown below:

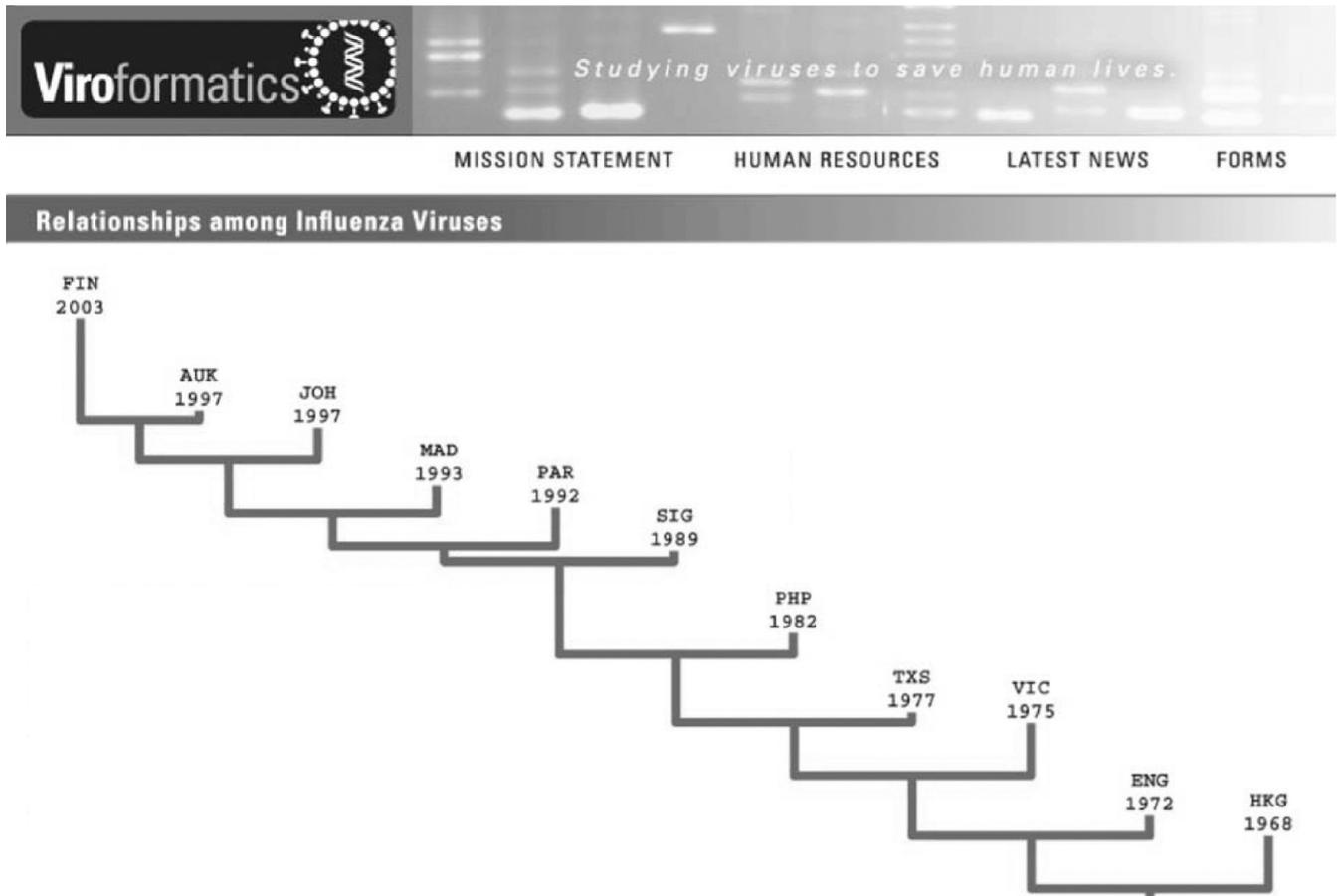
TGGGCCACcAGCCAGGGCTTtAGcCGgACT

The lowercase (gray) letters show nucleotides that differ among some of the species. The uppercase (black) letters did not change. The amount of time represented in this comparison is 1,009 million years (or 1.009 billion years). Use the same formulas you used in Steps 1 and 2 to calculate the expected number of changes per nucleotide per year in this sequence.

4. Compare the rate of change per nucleotide per year for the hemagglutinin gene in influenza with the rate for the *Irf6* gene. Do this by dividing the rate for the hemagglutinin region by the rate for the *Irf6* gene. The number you calculate will show how many times faster one region changes than the other.
5. The number of changes in the hemagglutinin gene for six samples compared with the sample from Finland in 2003 is as follows:
 - Hong Kong, 1968 = 140 changes
 - Victoria, Australia, 1975 = 128 changes
 - Philippines, 1982 = 95 changes
 - Singapore, 1989 = 74 changes
 - Madrid, Spain, 1993 = 71 changes
 - Auckland, New Zealand, 1997 = 34 changes
 - a. How do the number of changes to the sequence relate to time?
 - b. What do you think this means?

Relationships among Influenza Viruses

Figure 1. Viroformatics diagram of relationships among the 11 flu viruses sampled over 35 years.



This diagram shows the relationships among the influenza viruses sampled over 35 years. The length of the lines indicates the number of changes among the sequences. Each sample is named for the place and the year in which the virus was isolated from a patient.

FIN = Finland; AUK = Auckland, New Zealand; JOH = Johannesburg, South Africa; MAD = Madrid, Spain; PAR = Paris, France; SIG = Singapore; PHP = Philippines; TXS = Texas, USA; VIC = Victoria, Australia; ENG = England; HKG = Hong Kong.

Influenza and the Immune System

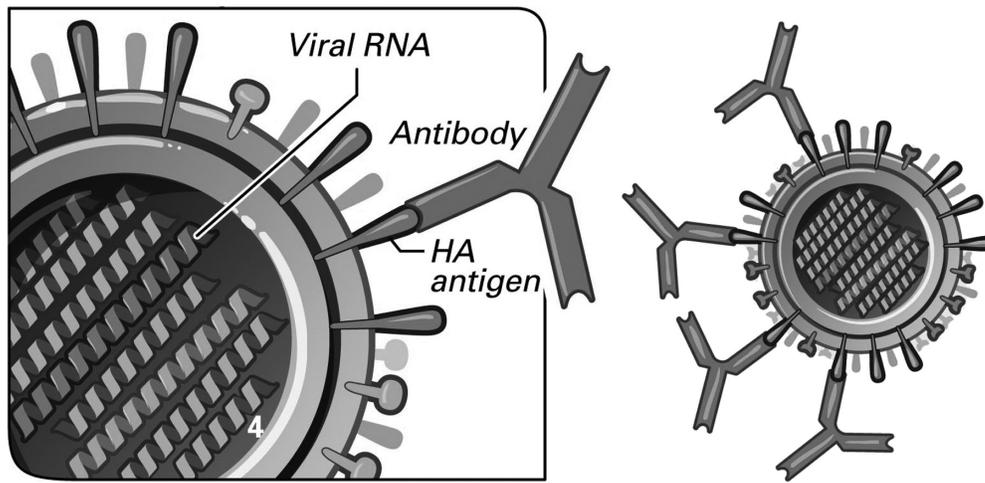
Name: _____

How your body fights influenza

Influenza is a respiratory illness caused by a virus that infects the cells lining the lungs, nose, and throat. To infect your cells, the virus has to enter them. To do that, the hemagglutinin protein on the virus binds to a receptor on the cell. The binding of the virus causes the cell to undergo endocytosis. Once inside, the virus starts making more viruses. Viruses use the “machinery” of the host cell to make new copies of their genetic material and to make proteins. These parts are assembled into a large number of new viruses.

One way your body fights influenza infections is by developing antibodies to the hemagglutinin on the virus. When antibodies attach to hemagglutinin, they keep the virus from attaching to healthy cells. This keeps the virus from infecting these cells.

Figure 1. Schematic of antibodies binding to the surface of the influenza virus.



Source: National Institute of Allergy and Infectious Diseases.

Influenza, the immune system, and natural selection

Scientists have explored changes in the hemagglutinin gene in many influenza viruses that circulated around the world. Some mutations resulted in changes in amino acids that make up hemagglutinin. These changes affected how the protein bound to cell receptors. Because these changes altered the phenotype, they affected a virus's ability to infect a cell.

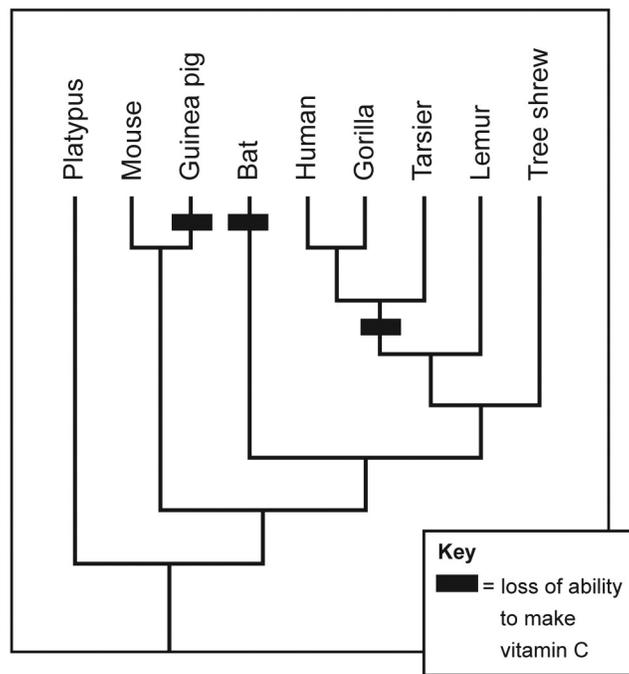
Other mutations in the hemagglutinin gene did not cause a change in any amino acids that make up hemagglutinin. These mutations did not affect a virus's ability to infect cells. As a result, these mutations are mostly “invisible” to the process of natural selection.

Editing an Article about Vitamin C and Evolution

Recently, the emergency room at the local hospital had a case that challenged the doctors. A three-year-old child was brought in by his parents because the child was pale, had unusual spots on his skin, and was weak; in addition, walking was very painful for the child. After interviewing the parents to learn more about the child's medical history and running some tests, the doctors determined that the child had scurvy. Scurvy is caused by a lack of vitamin C. The child's diet was unusual in that it did not include enough vitamin C. Most people only know of scurvy from history books. In the 1700s, sailors at sea for long periods of time developed scurvy and could be cured by eating citrus fruits. What made this child's diagnosis tough for the doctors was that scurvy is uncommon now. Most people get plenty of vitamin C from the foods in their diets. An understanding of evolution may help us understand how humans can develop scurvy.

Humans must take in vitamin C in their food—our bodies can't make it. This may be surprising to some people because plants and many other animals do synthesize vitamin C. However, humans are not the only mammals that can't make vitamin C—other primates, guinea pigs, and many bats also must get vitamin C from their diets. One question scientists want to investigate is how knowledge of evolution can help us understand when and how some species lost the ability to make vitamin C. One tool that scientists use is an evolutionary tree. Figure 1 shows a tree of the relationships among several types of mammals. Scientists know this tree is correct because it has already been published in a scientific journal. If primates, guinea pigs, and some bats all can't make vitamin C, then they must be each other's closest relatives. Reading the tree from left to right shows that the loss of the ability to

Figure 1. This evolutionary tree diagram shows the relationships among a number of species. The loss of the ability to make vitamin C is shown with bars.



make vitamin C first occurred in guinea pigs. The trait was then passed to bats, and then to primates. Reading the tree from the bottom to the top leads to a different conclusion. Read this way, the tree shows that the ability to synthesize vitamin C was lost three separate times in mammals. Because the evolutionary tree provides this evidence, it's logical to conclude that humans are susceptible to scurvy because we inherited the lack of an ability to make vitamin C from a common ancestor with gorillas and tarsiers. Bats and guinea pigs lost this ability separately.

Scientists are also comparing gene sequences from a variety of different organisms to learn more about vitamin C and evolution. Humans, other primates, some bats, and guinea pigs can't make vitamin C on their own because they lack an enzyme. The name of this enzyme is GULO, which stands for L-gulonolactone oxidase. As with other enzymes, a specific gene codes for the GULO enzyme. Scientists have found this gene in many organisms that can make vitamin C—for example, mice, dogs, cows, pigs, and horses. Figure 2 compares short segments of the gene sequences for a mouse and a cow. These animals make their own vitamin C, so individuals do not survive or reproduce well if they have mutations to the GULO gene that result in a nonfunctional protein. Eventually, these changes are weeded out by natural selection. Because of natural selection, the sequences for GULO in mice and cows have not changed much over time. Figure 3 compares the gene from a mouse and the gene from a human. Scientists found many differences between these two sequences. Some changes in the human gene stop the protein from even being made. Humans do not need the gene anymore because humans usually get vitamin C in their diets. This causes mutations to the GULO gene to occur. However, if people stop eating enough vitamin C, evolution will quickly make the gene functional again.

Figure 2. DNA Sequences from a mouse and a cow for a segment of a gene that codes for a protein that helps animals make vitamin C.

```
CTTCTGGCTGCTGTTCAA Mouse
CTTCTGGCTCCTGTTCAA Cow
```

Figure 3. DNA sequences from a mouse and a human for a segment of a gene that codes for a protein that helps animals make vitamin C.

```
CTTCTGGCTGCTGTTCAA Mouse
TTTCTGACTCCTGTTTGC Human
```

Editing an Article about Vitamin C and Evolution, Answer Key

Misconceptions or misinterpretations in the second and third paragraphs are shown in **bold**.

Paragraph 2

Humans must take in vitamin C in their food—our bodies can't make it. This may be surprising to some people because plants and many other animals do synthesize vitamin C. However, humans are not the only mammals that can't make vitamin C—other primates, guinea pigs, and many bats also must have vitamin C in their diets. One question scientists want to investigate is how knowledge of evolution can help us understand when and how some species lost the ability to make vitamin C. One tool that scientists use is an evolutionary tree. Figure 1 shows a tree that depicts the relationships among several types of mammals. **Scientists know this tree is correct because it has already been published in a scientific journal.**

Evolutionary trees are claims based on evidence. Like all claims in science, they are subject to change with new evidence.

If primates, guinea pigs, and some bats all can't make vitamin C, they must be each other's closest relatives.

Estimates of relationships are based on multiple lines of evidence, not just one.

Reading the tree from left to right shows that the loss of the ability to make vitamin C first occurred in guinea pigs. The trait was then passed to bats, and then to primates.

This evolutionary tree should not be read from left to right. The pattern of branching underneath the names should be used to determine patterns of evolution.

Reading the tree from the bottom to the top leads to a different conclusion. Read this way, the tree shows that the ability to synthesize vitamin C was lost three separate times in mammals. Because the evolutionary tree provides this evidence, it is logical to conclude that humans are susceptible to scurvy because we inherited the lack of an ability to make vitamin C from a common ancestor with gorillas and tarsiers. Bats and guinea pigs lost this ability separately.

Paragraph 3

Scientists are also comparing gene sequences from a variety of different organisms to learn more about vitamin C and evolution. Humans, other primates, some bats, and guinea pigs can't make vitamin C on their own because they lack an enzyme. The name of this enzyme is GULO, which stands for L-gulonolactone oxidase. As with other enzymes, a specific gene codes for the GULO enzyme. Scientists have found this gene in many organisms that can make vitamin C—for example,

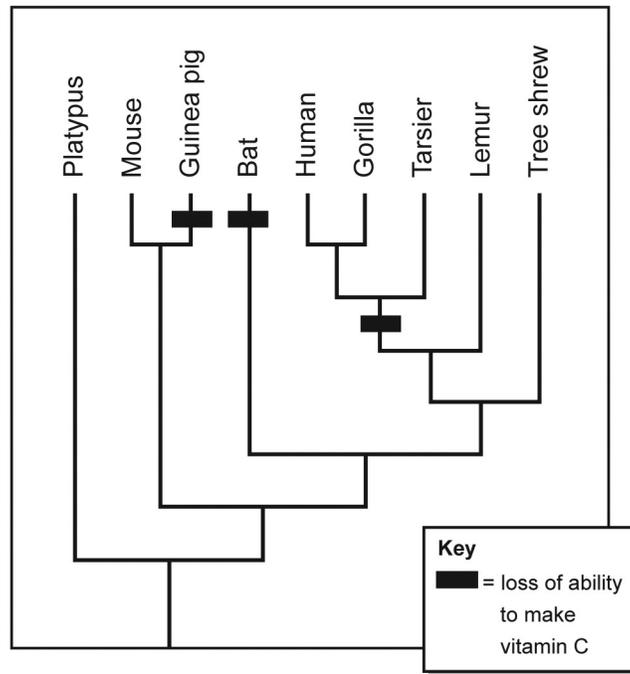
mice, dogs, cows, pigs, and horses. Figure 2 shows a comparison of a short segment of the gene sequences for a mouse and a cow. These animals make their own vitamin C, so individuals do not survive or reproduce well if they have mutations to the GULO gene that result in a nonfunctional protein. Eventually, these changes are weeded out by natural selection. Because of natural selection, the sequences for GULO in mice and cows have not changed much over time. Figure 3 shows a comparison of the gene from a mouse and the gene from a human. Scientists found many differences between these two sequences. Some changes in the human gene stop the protein from even being made. **Humans do not need the gene anymore because humans usually get vitamin C in their diets. This causes mutations to the GULO gene to occur.**

Mutations do not occur because an organism “needs” them.

Also, after students talk with other students who read Paragraph 2, they should realize that the loss of the ability to make vitamin C occurred in an ancestor to humans, gorillas, and tarsiers. The change did not occur only in the human lineage. This second error may be difficult for students identify.

However, if people stop eating enough vitamin C, evolution will quickly make the gene functional again.

Figure 1. This evolutionary tree diagram shows the relationships among a number of species. The loss of the ability to make vitamin C is shown with bars on the diagram.



This is another argument based on need. Lineages of organisms do not change simply because they need to change.

Figure 2. DNA Sequences from a mouse and a cow for a segment of a gene that codes for a protein that helps animals make vitamin C.

CTTCTGGCTGCTGTTCAA Mouse
CTTCTGGCTCCTGTTCAA Cow

Figure 3. DNA sequences from a mouse and a human for a segment of a gene that codes for a protein that helps animals make vitamin C.

CTTCTGGCTGCTGTTCAA Mouse
TTTCTGACTCCTGTTTGC Human