IN-DEPTH FILM GUIDE

DESCRIPTION

When he was working in East Africa in the 1950s, Dr. Tony Allison became the first researcher to find a connection between the infectious parasitic disease malaria and the genetic disease sickle cell anemia. His discovery is among the first and best-understood examples of natural selection, in which the selective force, the adaptive mutation, and the critical molecules were all identified—and all in humans.

KEY CONCEPTS

A. Sickle cell disease (also known as sickle cell anemia) is a potentially deadly genetic disease, while malaria is a potentially deadly infectious disease.
B. Having two of the same alleles of a given gene means an individual is homozygous for that particular gene; if the alleles are not identical, then the individual is heterozygous for that gene.
C. In areas where the malaria parasite is present, individuals who are homozygous for the sickle cell allele (who will get sickle cell disease) and individuals who are homozygous for the normal hemoglobin allele (who can contract malaria) both have a selective disadvantage.
D. In areas where the malaria parasite is present, individuals who are heterozygous for the sickle cell allele are at a selective advantage because they are protected against malaria but do not get sickle cell disease.
E. In the absence of malaria, there is selection against the sickle cell allele.
F. Protection from malaria comes at the cost of more sickle cell disease in the population.
G. A mutation that causes a genetic disease can also protect against an infectious disease.
H. The sickle cell allele arose as a random mutation in the hemoglobin gene.

CURRICULUM AND TEXTBOOK CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	LS1.A, LS2.A, LS2.C, LS3.A, LS3.B, LS4.B, LS4.B, LS4.C
AP (2012–13)	1.A.1, 1.A.2, 3.C.1, 3.C.2, 4.C.1
IB (2009)	4.1, 4.3, 5.4, D.2

Textbook	Chapter Sections
Miller and Levine, *Biology* (2010 ed.)	13.3, 14.2, 17.1, 17.2
Reese et al., *Campbell Biology* (9th ed.)	5.4, 14.4, 17.5, 23.1, 23.3, 23.4, 42.4

PRIOR KNOWLEDGE

Students should

- have a basic understanding of natural selection, evolution, and adaptation;
- know that some traits provide organisms with a greater chance to survive and reproduce;
- be familiar with the scientific process of testing ideas with evidence; and
- have a basic understanding of genetics, including inheritance patterns, the central dogma that DNA codes for proteins, and that mutations are changes in the DNA sequence.

PAUSE POINTS

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

	Begin	End	Content description	Review Questions	
	0:00	1:30	• Sickle cell disease (also known as sickle cell anemia) is a potentially deadly genetic disease that causes red blood cells to become misshapen, preventing enough oxygen from being delivered to all parts of the body. The symptoms vary.	• What is sickle cell anemia?	NGSS (April 2013) HS.LS1.A, HS.LS3.B, AP Biology (2012–13) 1.A.1 IB Biology (2009) 4.1
1	1:31	8:22	• If natural selection eliminates harmful traits from the gene pool, then it would follow that the prevalence of sickle cell anemia should be very low. However, because the prevalence is high in some populations, there must be another reason. • There is a connection between sickle cell and malaria, a potentially deadly infectious disease. The environments in which there was a high incidence of sickle cell were where there was also a high incidence of malaria. • Dr. Allison predicted that protection against malaria is correlated with sickle cell. After collecting samples, he found that children carrying the sickle cell allele had a lower parasite count, as if they were partially protected against malaria.	• Why does it make sense that the frequency of sickle cell anemia should be very low? • What correlation did Dr. Allison discover?	NGSS (April 2013) HS.LS1.A, HS.LS2.A, HS.LS2.C, HS.LS3.B AP Biology (2012–13) 1.A.1, 1.A.2, 4.C.2 IB Biology (2009) 4.1, 5.4, D.2
2	8:23	14:03	• In areas where the malaria parasite is present, individuals who are heterozygous for the sickle cell allele are at a selective advantage because they are protected against malaria but do not get sickle cell disease. • In areas where the malaria parasite is present, individuals who are homozygous for the sickle cell allele (who will get sickle cell disease) and individuals who are homozygous for the normal hemoglobin allele (who can contract malaria) both have a selective disadvantage. • A single base substitution causes the sickle cell allele. • The mutation that causes sickle cells also compromises the ability of the malaria parasite to reproduce.	• Why does the sickle cell trait, which encodes for a deadly disease, persist in areas where malaria is found? • What type of mutation in the gene that encodes hemoglobin leads to a sickle cell allele? • How does sickle cell protect people from malaria?	NGSS (April 2013) HS.LS1.A, HS.LS2.A, HS.LS2.C, HS.LS3.A, HS.LS3.B, HS.LS4.B, HS.LS4.C AP Biology (2012–13) 1.A.1, 1.A.2, 3.C.1, 3.C.2, 4.C.1 IB Biology (2009) 4.1, 4.3, 5.4, D.2

BACKGROUND

Hemoglobin is a protein found in red blood cells; it transports oxygen to body tissues, where the oxygen is released from the hemoglobin molecule. The hemoglobin protein consists of four polypeptide chains: two alpha chains and two beta chains. The sickle cell mutation results in an amino acid substitution in the beta chain, replacing glutamic acid with valine. In individuals homozygous for the sickle cell allele, the hemoglobin tends to precipitate (or clump together) within the red blood cells when it is not bound to oxygen. This clumping causes red blood cells to assume an abnormal "sickled" shape. The sickle-shaped red blood cells block blood flow in blood vessels. Blocked blood flow can cause pain, serious infections, and organ damage.

Individuals heterozygous for the sickle cell allele produce both normal and mutant hemoglobin proteins. These individuals do not show symptoms of the disease and have mostly normal red blood cells. However, as these individuals have a mutation in one copy of the gene for the beta chain of hemoglobin, they do manifest some sickling of their red blood cells when in low-oxygen environments.

Sickle cell disease was almost always lethal, but now there are many therapeutic strategies to help individuals with the disease. Most people with sickle cell disease take medication for pain management. In addition, some patients receive blood transfusions to help them maintain a population of red blood cells that can carry oxygen throughout the body. Because infections are also common in those with sickle cell disease, some people take a daily regimen of antibiotics.

Unlike sickle cell disease, which is inherited, malaria is a mosquito-transmitted disease caused by a parasite. A person who contracts malaria usually has flu-like symptoms at first, such as fever, muscle pains, and nausea. If the disease becomes severe, patients can experience confusion, severe anemia, and difficulty breathing, and they may even become comatose. The mosquitoes that can carry the malaria parasite belong to the genus *Anopheles*. Four species in the genus *Plasmodium*, which are single-celled protists, can cause malaria.

Malaria-causing parasites need both mammals and mosquitoes to complete their life cycles. When an *Anopheles* mosquito carrying a malaria parasite bites a human, the parasite is transmitted from the salivary glands of the mosquito to the human bloodstream. The parasites travel through the bloodstream to the liver, where they reproduce asexually in liver and red blood cells. Some of the parasites in the red blood cells enter sexual reproduction to produce gametocytes, which can be picked up by the mosquitoes that happen to feed on a malaria-infected human. In the gut of the mosquito, male and female gametocytes fuse developing an oocyst. After one to two weeks, the oocyst bursts and releases thousands of *Plasmodium* parasites, which can be transmitted to another human being when the mosquito feeds again.

Contemporary strategies for dealing with malaria focus on both prevention and treatment. Highly effective prevention strategies include both avoiding mosquito bites—by sleeping under mosquito netting, keeping skin covered, and using insect repellent—and taking antimalarial drugs, such as mefloquine , which prevent the malaria parasite from establishing an infection in its human host. If a person does become infected with malaria, several drugs can effectively treat the person. However, as drug-resistant strains of malaria become more common, there is a need for novel treatments.

DISCUSSION POINTS

- With respect to sickle cell, the word "trait" is used somewhat differently than it is ordinarily used in biology. In biology, trait typically refers to one of several phenotypic variants or characters. "Sickle cell trait" specifically refers to the heterozygous state, where an individual is protected from malaria infection because the individual has one copy of the sickle cell allele.
- In the short film *The Making of the Fittest: Natural Selection in Humans*, Dr. Allison refers to the "sickle cell character." Reinforce with students that this "character" refers specifically to the sickling phenotype caused by the sickle cell allele. This character occurs mostly in individuals homozygous for the sickle cell allele but can occasionally occur in heterozygous individuals under the right conditions.
- As mentioned at the end of the film, the sickle cell allele persists in nonmalarial areas because there has not been sufficient time to eliminate the allele from the population. Eventually, however, selective pressure against the sickle cell allele will likely decrease its frequency. You may wish to have students note what other factors in a developed country, such as access to therapies that make sickle cell disease less life threatening, also contribute to the allele's persistence in modern populations. Migration of people from environments where the sickle cell allele is advantageous into areas where there is no malaria also contributes to the persistence of the allele in nonmalarial environments.
- Your students may wonder why the sickle cell allele protects individuals from malaria. Although research into how the sickle cell allele protects against *Plasmodium* infection has been ongoing for decades, scientists do not definitively know the exact mechanism for this. Factors that may be involved include structural changes to red blood cells; decreased ability of *Plasmodium* parasites to infect cells; increased sickling of cells infected with *Plasmodium* parasites; and enhanced phagocytosis of *Plasmodium*-infected cells. It is possible that some of these conditions lead to a tolerance for the parasite in the host, whereby the parasite can multiply to a certain extent, but the patient does

not have any symptoms. Researchers continue to study the mechanisms of this genetic resistance to malaria, which are an important facet of research concerning genetic resistance to infectious diseases.

- For advanced students, the example of the sickle cell allele provides a unique way to discuss differences in classical inheritance patterns, depending on whether the disease is viewed at the molecular, cellular, or organismal level. At the molecular level, the sickle cell allele is codominant with normal hemoglobin because, in a heterozygous individual, both normal and mutant hemoglobin proteins are expressed. At the cellular level, the allele is incompletely dominant because only some cells exhibit sickling in a heterozygous individual. At the organismal level, sickle cell disease is typically referred to as a recessive disease because heterozygotes don't have symptoms except in low-oxygen environments. Because the definitions of "codominance," "incomplete dominance," and "recessive" were defined before the advent of molecular genetics, dissection of these terms using the sickle cell allele allows students to compare and contrast classical and molecular genetics and their examinations of phenotypes.
- Students may wonder why Dr. Allison used children and not adults in his study. He did so because adults living in malaria-endemic areas may have some immunity against the parasite, thereby showing lower parasite levels. This effect is independent of genotype and will confound the data. Young children, however, are less likely to have been exposed to malaria and therefore have not developed immunity against the malaria parasite. Differences observed in malaria parasite counts between children who do not have the sickle cell allele and children who have one copy of the sickle cell allele would most likely be due to the genetic makeup of the children and not due to previous exposures to the parasite.

CLASSROOM RESOURCES FOR THE FILM

Testing a Hypothesis (www.biointeractive.org/testing-hypothesis)

A worksheet designed to actively engage students as they watch the film. The worksheet asks students to answer questions pertaining to the information provided in the film. Students then answer follow-up questions that probe their understanding of how Dr. Allison found the link between sickle cell disease and malaria and why this finding is important in understanding human evolution.
Appropriate for middle school life science, high school biology (all levels including AP and IB)

A Lesson on the Nature of Science (www.biointeractive.org/lesson-nature-science)

A worksheet designed to show students how scientists make their discoveries. It provides students with background information about how Dr. Allison's work built upon the contributions made by other scientists. It also probes students on their understanding of how the evidence that he collected supports the conclusions he made.
Appropriate for high school biology (all levels including AP and IB), introductory college biology

Mendelian Genetics, Probability, Pedigrees, and Chi-Square Statistics (www.biointeractive.org/mendelian-genetics-probability-pedigree-and-chi-square-statistics)

A lesson that requires students to work through a series of questions pertaining to the genetics of sickle cell disease and its relationship to malaria. These questions probe students' understanding of Mendelian genetics, probability, pedigree analysis, and chi-square statistics.
Appropriate for high school biology (honors or AP and IB), undergraduate introductory biology

How Do Fibers Form? (www.biointeractive.org/how-do-fibers-form)

A hands-on activity in which students construct models of sickle hemoglobin fibers inside red blood cells to illustrate how changes in the structure of a protein can affect cell shape. The activity then asks students to relate these changes to disease symptoms.
Appropriate for high school biology (all levels including AP and IB), introductory college biology

Population Genetics, Selection, and Evolution (www.biointeractive.org/population-genetics-selection-and-evolution)

A hands-on activity that uses different-colored beads to illustrate the concept of population genetics and evolution. This activity uses a series of questions to establish a basic understanding of the Hardy-Weinberg principle. It then takes students through an activity that illustrates how natural selection can lead to changes in allele frequencies in a population.
Appropriate for high school biology (all levels including AP and IB), introductory college biology

OTHER BIOINTERACTIVE RESOURCES

Stopping Mosquito-Borne Disease (www.biointeractive.org/stopping-mosquito-borne-disease)

In this Click and Learn activity, students learn about the nature of vector-borne diseases and the life cycle of the dengue vector mosquito. Students interactively explore how to control mosquito-borne diseases.

The Mosquito Life Cycle (www.biointeractive.org/classroom-activities-mosquito-life-cycle)

This hands-on activity has students explore the life cycle of the mosquito as it relates to mosquito-transmitted diseases.

Sickle Cell Anemia (www.biointeractive.org/sickle-cell-anemia)

This animation demonstrates that sickle cell anemia is a genetic disease that affects hemoglobin. A single nucleotide change in the hemoglobin gene causes an amino acid substitution in the hemoglobin protein from glutamic acid to valine. The resulting proteins stick together to form long fibers and distort the shape of the red blood cells.

Malaria: Human Host (www.biointeractive.org/malaria-human-host)

In this first animation, when a malaria-carrying mosquito bites a human host, malaria parasites enter the bloodstream, multiply in the liver cells, and are then released back into the bloodstream, where they infect and destroy red blood cells.

Malaria: Mosquito Host (www.biointeractive.org/malaria-mosquito-host)

In this second animation, a mosquito becomes infected with malaria when it sucks the blood from an infected human. Once inside the mosquito, the parasites reproduce in the gut and accumulate in the salivary glands, ready to infect another human host with the next bite.

The Mosquito Life Cycle (www.biointeractive.org/mosquito-life-cycle)

This video shows that, to prevent mosquitoes from spreading diseases, it's essential to understand their life cycle. Note that the specific mosquito species and virus discussed in this video are not involved in malaria transmission, but the basic mosquito life cycle is the same.

Interview with Katherine Sorber (www.biointeractive.org/interview-katherine-sorber)

In this video interview, Katherine Sorber, a graduate student in Dr. Joseph L. DeRisi's lab, describes her research on malaria.

USING THE QUIZ

The quiz is designed as a summative assessment that probes student understanding of the key concepts addressed in the film. However, some teachers use the quiz before and during the film to asses students' prior knowledge and to guide students as they watch the film Teachers are encouraged to choose the use that best fits their learning objectives and their students' needs. Moreover, because the vocabulary and concepts are complex, teachers are encouraged to modify the quiz (e.g. only ask some of the questions, explain complicated vocabulary for ELL students) as needed. The last two questions are intended for students with prior knowledge about mutations, genes expression, and development.

QUIZ QUESTIONS AND ANSWERS

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

1. (Key Concept A) *Consider the statement: "Sickle cell disease is a(n) __________ disease."*
   Which of the following terms could fill in the blank to make the statement true? Write "yes" or "no" next to each possible response. ***There may be more than one correct answer.***

Genetic	**Yes**	*Infectious*	**No**
Potentially lethal	**Yes**	*Inherited*	**Yes**

2. (Key Concept A) *Consider the statement: "Malaria is a(n) __________ disease."*
   Which of the following terms could fill in the blank to make the statement true? Write "yes" or "no" next to each possible response. ***There may be more than one correct answer.***

Genetic	**No**	*Infectious*	**Yes**
Potentially lethal	**Yes**	*Inherited*	**No**

3. (Key Concepts B, C, and D) *Consider the statement: "An individual with two normal copies of the hemoglobin gene is said to be ___________."*
   Which of the following terms could fill in the blank to make the statement true? Write "yes" or "no" next to each possible response. ***There may be more than one correct answer.*** *(Note that a "locus" is a location on a chromosome.)*

Homozygous at the hemoglobin locus	**Yes**	*Susceptible to malaria*	**Yes**
Heterozygous at the hemoglobin locus	**No**	*An identical twin*	**No**

4. (Key Concept B) *At the beginning of the film, you were introduced to Davaun and Skyy Cooper, who both have sickle cell disease. Which of the following must be true about their parents?* [Answer is in **bold**.]
   a. *One parent has at least one copy of the sickle cell allele.*
   b. Both parents have at least one copy of the sickle cell allele.
   c. *Both parents have sickle cell disease.*
   d. *One parent has sickle cell disease.*

5. (Key Concepts C, D, F, and G) *In three to five sentences, explain why sickle cell disease became so prevalent in certain East African populations.*
   The sickle cell allele is protective against malaria—even when the allele is in the heterozygous state. Malaria is prevalent in the lowlands of East Africa, and so the sickle cell allele became common because it provided protection against malaria. As a result, sickle cell disease (individuals homozygous for the sickle cell allele) also became common.

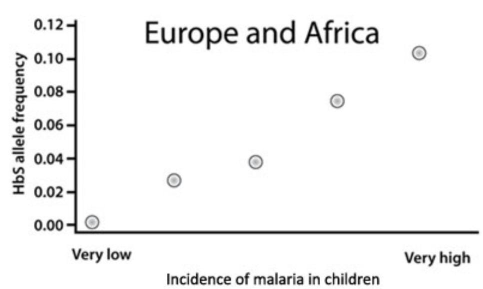
6. (Key Concept E) *There are now several effective antimalarial drugs that can treat people who have malaria or prevent them from getting the disease altogether. Predict what will happen to the frequency of the sickle cell allele as these drugs become more widely used. Support your answer with at least one piece of evidence from the film.*
   As malaria becomes less deadly, selection for a genetic mutation that protects individuals from the disease decreases. As a result, the frequency of the sickle cell allele will likely decrease.

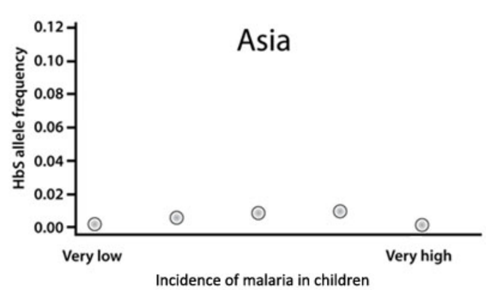
7. (Key Concepts C and D) *If sickle cell disease were caused by only one copy of the sickle cell allele, do you expect the frequency of the sickle cell allele to increase, decrease, or remain the same in places where there is a high incidence of malaria? Explain your answer in two or three sentences.*
   Sickle cell disease can be deadly; as a result, there is selection against this phenotype. If only one sickle cell allele were to cause the disease, individuals heterozygous for the sickle cell allele would have no selective advantage over individuals homozygous for the sickle cell allele. As a result, the frequency of the sickle cell allele would be lower.

8. (Key Concept D) *Due to climate change, the range of malaria is expected to spread to areas where it was previously not a problem. Given this piece of evidence, predict what will happen to the frequency of the sickle cell allele in areas where malaria is introduced.*
   Because the sickle cell allele provides some resistance to malaria, the frequency of the allele should increase if the prevalence of malaria increases.

9. (Key Concept H) *Is the following statement true or false? "Malaria caused the sickle cell allele to appear." Justify your answer in one or two sentences.*
   False; the sickle cell allele arose randomly. Malaria provided the selective pressure for the allele to increase in frequency in some populations.

10. (Key Concepts D and G) *Recently, scientists compared the frequencies of the sickle cell allele (*HbS*) with the incidence of malaria in two different geographical areas. They grouped the children in five categories based on the incidence of malaria in children. They looked at* HbS *allele frequencies in each group. Their findings are in the graphs below.*





Piel F. *et al.* Nature Communications. 2010

   a. *How does the* HbS *allele frequency relate to malaria incidence in children in European and African populations compared to children in Asian populations?*
      In Europe and Africa, when there is a high frequency of the malaria parasite, there is a high frequency of the sickle cell allele. In Asia, the frequency of the sickle cell allele is very low, regardless of the frequency of malaria parasite present.

b. *Provide an explanation, based on what you learned from the film, for the trends observed in the European and African populations.*
The scientists observed an increased frequency of the *HbS* allele in regions with a high incidence of malaria. This is because people with the sickle cell allele have a selective advantage in areas where there is a high incidence of malaria.

c. *Provide a hypothesis that explains the findings in the Asian population.*
Several hypotheses are acceptable, including these:
   i. **The sickle cell allele never arose or was never introduced to this population.**
   ii. **The sickle cell allele does not provide a protective advantage for the type of parasite that causes malaria in Asia.**
   iii. **Another gene provides a protective mechanism against malaria. For more information, see the Click and Learn activity "Recent Adaptations in Humans" (http://www.hhmi.org/biointeractive/evolution/Human_Adaptation/01.html).**

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FIELD TESTERS (QUIZ)

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