SUGGESTED AUDIENCE
This lesson is appropriate for high school biology (all levels including AP and IB) and undergraduate introductory biology.

PRIOR KNOWLEDGE
Students should have prior knowledge of the basics of Mendelian genetics (genotype, phenotype, homozygous, heterozygous, incomplete dominance, and codominance) and the rules of probability. They should also be familiar with how to draw and interpret pedigrees (including standard symbols used therein), use pedigrees to show family relationships, and analyze the pattern of inheritance of a particular trait. More-advanced students should have a working knowledge of the chi-square statistical analysis test.

MATERIALS
critical values table

TEACHING TIPS
- You could use this lesson and the story line of the short film as a culminating unit classroom assignment on genetics that ties together all levels of genetic analysis: Punnett squares, probability, pedigrees, and chi-square analysis.
- You may discuss with the class how sickle cell disease provides an interesting example of the arbitrary nature of dominance, incomplete dominance, and codominance. Sickle cell disease, at an organismal level, is defined as an autosomal recessive disorder because one copy of HbA produces enough normal hemoglobin to prevent anemia. At the cellular level, regarding blood cell shape, the phenotype of the sickled red blood cell is incompletely dominant because heterozygotes can display some sickled red blood cells (RBCs) in low-oxygen environments. Finally, regarding hemoglobin at the molecular level, there is codominance. In heterozygotes, both HbA and HbS alleles are expressed.
- The chi-square statistics portion of this lesson is optional. If you teach a course in which chi-square analysis is not required, you may remove this section from this lesson; we have placed it on separate pages for this reason.

ANSWER KEY
MENDELIAN GENETICS AND PROBABILITY
i. If two people with sickle cell trait have children, what is the chance that a child will have normal RBCs in both high- and low-oxygen environments? What is the chance that a child will have sickle cell disease? Write the possible genotypes in the Punnett square.

<table>
<thead>
<tr>
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<th>A</th>
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<tbody>
<tr>
<td>A</td>
<td>AA</td>
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<tr>
<td>S</td>
<td>AS</td>
<td>SS</td>
</tr>
</tbody>
</table>

Normal RBCs in high- and low-oxygen environments 1/4 (25%)
Sickle cell disease 1/4 (25%)

a. What is the chance that a child will carry the HbS gene but not have sickle cell disease? 1/2 (50%)
b. What are the chances that these parents will have three children who are homozygous for normal RBCs? (Show your work.)

\[ \frac{1}{4} \times \frac{1}{4} \times \frac{1}{4} = \frac{1}{64} (1.56%)\]
c. What are the chances that these parents will have three children who have both normal and mutant hemoglobin beta chains? (Show your work.) $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8} (12.5\%)$

d. What are the chances that all three of their children will show the disease phenotype? (Show your work.)
$\frac{1}{4} \times \frac{1}{4} \times \frac{1}{4} = \frac{1}{64} (1.56\%)$

e. What are the chances that these parents will have two children with sickle cell trait and one with sickle cell disease? (Show your work.) $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{4} = \frac{1}{16} (6.25\%)$

f. In the cross above, if you know that the child does not have sickle cell disease, what is the chance that the child has sickle cell trait? 2/3 (66.67%)

(Note: Because you know the child does not have sickle cell disease, the child cannot have the SS genotype; thus, you can eliminate it from the Punnett square. The individual must be either AA or AS. There are 2 out of 3 chances that the individual will have the AS genotype.)

ii. An individual who has sickle cell trait has children with an individual who does not have the HbS allele.

a. What are the genotypes of the parents? AA and AS

b. In a Punnett square, show all the possible genotypes of their children. State the genotype and phenotype ratios of the offspring.

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<tbody>
<tr>
<td>A</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>S</td>
<td>AS</td>
<td>AS</td>
</tr>
</tbody>
</table>

Genotype Ratio
50% (1/2) AA: 50% (1/2) AS

Phenotype Ratio
50% (1/2) normal hemoglobin (normal RBCs): 50% (1/2) normal and mutant hemoglobin (sickle cell trait)

c. What are the chances that any one of this couple’s children will have sickle cell disease? 0%

d. If this couple lives in the lowlands of East Africa, what are the chances that one of their children would be resistant to malaria if exposed to the malaria parasite? 1/2 (50%)
e. What are the chances that any one of this couple’s children will have sickle cell disease? 1/2 (50%)

f. If this couple moves to the lowlands of East Africa and has children, which of their children would be more likely to survive? Explain your answer.

If this couple moves to the moist lowlands of East Africa, the family would be exposed to the Anopheles mosquito that transmits the Plasmodium parasite, which causes malaria. Children who have sickle cell disease (SS) have a lethal disease and will be less likely to survive regardless of where they live. Children with sickle cell trait (AS) have two advantages: they have a greater resistance to malaria, and they normally do not show symptoms of sickle cell disease. Therefore, heterozygotes are more likely to survive.

iv. In humans, blood type is a result of multiple alleles: I^A, I^O, and i. A few simple rules of blood type genetics are that
- I^A is dominant over I^O,
- I^O is dominant over i, and
- i^i are codominant.

Two parents who are heterozygous for type A blood and have sickle cell trait have children. Answer the following questions.

a. What is the genotype of the parents? I^A I^O AS

b. What are the genetic makeups of all the possible gametes they can produce? I^A, I^O, i^A, or i^O

c. Complete the dihybrid Punnett square to determine the frequency of the different phenotypes in the offspring.
(Note: Consider blood type and normal versus mutant hemoglobin in the various phenotypes.)

<table>
<thead>
<tr>
<th>I^A</th>
<th>I^O</th>
<th>i^A</th>
<th>i^O</th>
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<tbody>
<tr>
<td>I^A</td>
<td>I^A I^A</td>
<td>I^A I^O</td>
<td>I^A i^A</td>
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<td>I^O</td>
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<tr>
<td>i^A</td>
<td>I^A i^A</td>
<td>i^O i^A</td>
<td>i^A i^A</td>
</tr>
<tr>
<td>i^O</td>
<td>I^O i^O</td>
<td>i^O i^O</td>
<td>i^O i^O</td>
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</tbody>
</table>

3/16 (18.75%) Blood type A, normal hemoglobin (normal RBCs)
3/8 (6/16, or 37.5%) Blood type A, normal and mutant hemoglobin (sickle cell trait)
3/16 (18.75%) Blood type A, mutant hemoglobin (sickle cell anemia)
1/16 (6.25%) Blood type O, normal hemoglobin (normal RBCs)
1/8 (2/16, or 12.5%) Blood type O, normal and mutant hemoglobin (sickle cell trait)
1/16 (6.25%) Blood type O, mutant hemoglobin (sickle cell anemia)

v. Now try a different way of solving a dihybrid cross. Because of Mendel’s (second) law of independent assortment, you can work with the blood type gene and the hemoglobin gene separately. Set up two monohybrid crosses with the following parents: the mother is heterozygous for type B blood and has sickle cell trait, while the father has type AB blood and also has sickle cell trait.

<table>
<thead>
<tr>
<th>I^B</th>
<th>I^O</th>
<th>A</th>
<th>S</th>
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</thead>
<tbody>
<tr>
<td>I^B</td>
<td>I^B</td>
<td>A</td>
<td>S</td>
</tr>
<tr>
<td>I^O</td>
<td>I^O</td>
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<td>S</td>
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<td>i^A</td>
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<tr>
<td>i^O</td>
<td>i^O</td>
<td>A</td>
<td>S</td>
</tr>
</tbody>
</table>
a. What are the chances that a child of this couple will have type B blood and sickle cell trait? (Show your work.)
   \[ \frac{1}{2} \times \frac{1}{2} = \frac{1}{4} \text{ (25\%)} \]

b. What are the chances that a child will have type AB blood and will not have sickle cell disease? (Show your work.)
   \[ \frac{1}{4} \times \frac{3}{4} = \frac{3}{16} \text{ (18.75\%)} \]

c. What are the chances that a child will have type B blood and sickle cell disease? (Show your work.)
   \[ \frac{1}{2} \times \frac{1}{4} = \frac{1}{8} \text{ (12.5\%)} \]

d. What are the chances that a child will have type B blood and at least some normal hemoglobin? (Show your work.)
   \[ \frac{1}{2} \times \frac{3}{4} = \frac{3}{8} \text{ (37.5\%)} \]

PEDIGREES

vi. The following pedigree traces sickle cell disease through three generations of a family. Use the pedigree to answer the following questions.

a. What is the genotype of the father in the first generation? AS

b. What is the genotype of the daughter in the second generation? SS

c. What is the genotype of individual 3 in the second generation? How do you know?

   AS; he and his son do not have sickle cell anemia, so he has at least one normal hemoglobin gene (A). He also has a son with sickle cell disease (SS). Therefore, he must carry one mutant hemoglobin gene (S) in order to have passed it on to his son.

d. If the couple in the second generation has another child, what are the chances that the child will have the following?

   Sickle cell disease \( \frac{1}{2} \text{ (50\%)} \)
   Sickle cell trait \( \frac{1}{2} \text{ (50\%)} \)
   Completely normal hemoglobin 0%

e. If the entire family moves to the lowlands of East Africa, four of the five males in the pedigree will have two genetic advantages over the other individuals in the family. Explain the two advantages.

   Moving to the moist lowlands of East Africa exposes this family to mosquitoes carrying the *Plasmodium* parasite. Therefore, the four males who are heterozygous (AS) for the sickle cell allele have two distinct genetic advantages. First, they do not suffer from sickle cell disease. Second, they are more resistant to malaria infection due to their heterozygous genotype.

vii. The following pedigree traces sickle cell disease through four generations of a family living in New York City. Use the pedigree to answer the following questions.

a. What is the genotype of the mother in the first generation? SS

b. What are the possible genotypes of the father in the first generation? AA or AS

c. What can you say about the genotype of all the children of the couple in the first generation? Explain your answer.

   All the children in the second generation are heterozygous (AS) for the sickle cell allele. None of the children have sickle cell disease, so they possess at least one normal hemoglobin gene (A). Each child would have inherited the mutant hemoglobin gene (S) from the mother, because she has sickle cell anemia (SS).
d. Regarding the answer to Question 7c, based on where the family resides, why would this genotype be considered a disadvantage?

This family lives in New York City, which has a very low prevalence of malaria infection, so their AS genotype confers no genetic advantage. For the most part, the heterozygous genotype in New York City confers no distinct advantage or disadvantage. However, if any of these individuals mated with another heterozygous (AS) individual, they would have a 25% chance of having children with sickle cell anemia, which can be a deadly disease. The disadvantage of the AS genotype is in the possibility of future generations having sickle cell disease.

e. What are the genotypes of the parents in the third generation? Explain how you know.

Neither parent has sickle cell anemia, so each parent possesses at least one normal hemoglobin gene (A). They do have children with sickle cell anemia (SS), so each must possess at least one mutant hemoglobin gene to pass on to their children.

f. What is the possible genotype or genotypes of the mother in the second generation? AA or AS

g. If the couple in the third generation has another child, what are the child’s chances of the following?

- Having sickle cell disease: 1/4 (25%)
- Having sickle cell trait: 1/2 (50%)
- Being homozygous for normal RBCs: 1/4 (25%)
- Being resistant to malaria and not having sickle cell disease: 1/2 (50%)

viii. The following pedigree traces sickle cell disease through four generations of a family living in the highlands of eastern Africa. Use the pedigree to answer the following questions.

a. What are the genotypes of the following individuals? (If more than one genotype pertains, include all possibilities.)

- Individual 1: AS
- Individual 2: SS
- Individual 7: AS
- Individual 10: AA or AS
- Individual 13: AS
- Individual 17: AA or AS

b. If individuals 13 and 14 have another child, what are the chances that the child will have sickle cell disease?

1/4 (25%)

c. If the same couple has three more children, what are the chances that the three children will have sickle cell trait? (Show your work.)

\[
\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8} (12.5\%)
\]

d. Based on where this family lives, is the sickle cell trait genotype a genetic advantage? Explain.

Since this family lives in the relatively dry highlands of eastern Africa, there is a low incidence of malaria; therefore, the heterozygous genotype confers no significant genetic advantage.

e. If individuals 8 and 9 have four more children, what are the chances that two of the children will be homozygous for normal RBCs? Explain why.

There is a 0% chance this couple will have a child who is homozygous (AA) for normal hemoglobin. This is because the father has sickle cell disease (SS); therefore, he can only pass on the sickle cell allele (mutant hemoglobin [HbS]). Each child of this father has will either have sickle cell disease (SS) or be a carrier for the sickle cell allele (AS).
ix. Imagine that you are a genetic counselor, and a couple planning to start a family comes to you for information. Jerome was married before, and he and his first wife have a daughter with sickle cell disease. The brother of his current wife, Michaela, died of complications from sickle cell disease, but neither of her parents has the disease.

a. Draw a pedigree representing this family. Be sure to clearly label Jerome and Michaela.

b. What is the probability that Jerome and Michaela will have a baby with sickle cell disease? Note that neither Jerome nor Michaela has sickle cell disease. (Show your work.)

\[
\frac{2}{3} \times \frac{1}{4} = \frac{2}{12} = \frac{1}{6} \quad (16.67\%)
\]

Based on his child from his first marriage, Jerome is heterozygous (AS) for the sickle cell allele. In order for Michaela and Jerome to have a child with sickle cell anemia, they must both be heterozygous, which would confer a 1/4 chance of having a child with the disease. However, based on the information available, Michaela has a 1/3 chance of being homozygous (AA) normal and a 2/3 chance of being heterozygous (AS). Therefore, to calculate the probability of this “combined” event, the rule of multiplication must be applied (2/3 chance of Michaela being AS × 1/4 chance of having an SS child).

x. Natasha and Demarcus are planning on having children. Each has a sister with sickle cell disease. Neither Natasha nor Demarcus nor any of their parents have the disease, and none of them has been tested to see if they have sickle cell trait.

a. Draw a pedigree representing this family. Be sure to clearly label Natasha and Demarcus.

b. Based on this incomplete information, calculate the probability that if this couple has a child, the child will have sickle cell disease. (Show your work.)

\[
\frac{2}{3} \times \frac{2}{3} \times \frac{1}{4} = \frac{4}{36} = \frac{1}{9} \quad (11.11\%)
\]

Similar to Question 9, each parent has a 2/3 chance of being heterozygous (AS), and there is a 1/4 chance of having a child with the disease. Therefore, this is a combined event concerning its probability, so all values must be multiplied together.
CHI-SQUARE STATISTICS

xi. Multiple couples living in a small village in the eastern African lowlands, all of whom are heterozygous for the HbS allele, have 500 children among them. Of these children, 139 are homozygous for HbA, 279 are heterozygous for HbS, and 82 suffer from sickle cell disease. Are these data statistically significant? Explain using a chi-square statistical analysis test.

Chi-Square Data Table

<table>
<thead>
<tr>
<th>Phenotype/Genotype</th>
<th>Observed (o)</th>
<th>Expected (e)</th>
<th>(o – e)</th>
<th>(o – e)^2/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal RBC/AA</td>
<td>139</td>
<td>125</td>
<td>14</td>
<td>1.57</td>
</tr>
<tr>
<td>Sickle cell trait/AS</td>
<td>279</td>
<td>250</td>
<td>29</td>
<td>3.36</td>
</tr>
<tr>
<td>Sickle cell disease/SS</td>
<td>82</td>
<td>125</td>
<td>-43</td>
<td>14.79</td>
</tr>
</tbody>
</table>

a. What is the chi-square value (χ^2)? χ^2 = 19.72
b. Calculate the degrees of freedom (df). df = 3 – 1 = 2
c. Using the critical values table (see page 12), determine the P value. P < 0.01
d. Interpret the P value as it relates to these data. Explain the significance.

Since P < 0.05, the null hypothesis is rejected, which means that there is a statistically significant difference between the observed and expected data. Therefore, the difference between the observed and expected data is not solely due to chance.
e. Which of the children have the greatest chance of survival? Explain why.

Because these families live in a malaria “hot spot” in Africa, namely the moist eastern African lowlands, the children who are heterozygous for the sickle cell allele (AS) have a selective advantage over both the homozygous normal hemoglobin (AA) and the homozygous sickle cell anemia (SS) genotypes.

xii. Suppose there are 50 couples with the same blood type and hemoglobin genotypes. They live on a small, isolated Pacific island on which very few mosquitoes have been identified. All the individuals are heterozygous for both type A blood and have sickle cell trait. The 50 couples had 224 children over the years. The children were all tested for blood type and for the presence of the sickle cell allele.

Are these data significant? Explain using a chi-square statistical analysis test. (Use the table below if you need assistance.)

Chi-Square Data Table

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Observed (o)</th>
<th>Expected (e)</th>
<th>(o – e)</th>
<th>(o – e)^2/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A, normal RBCs</td>
<td>48</td>
<td>42</td>
<td>6</td>
<td>0.86</td>
</tr>
<tr>
<td>Type O, normal RBCs</td>
<td>18</td>
<td>14</td>
<td>4</td>
<td>1.14</td>
</tr>
<tr>
<td>Type A, sickle cell trait</td>
<td>92</td>
<td>84</td>
<td>8</td>
<td>0.76</td>
</tr>
<tr>
<td>Type O, sickle cell trait</td>
<td>33</td>
<td>28</td>
<td>5</td>
<td>0.89</td>
</tr>
<tr>
<td>Type A, sickle cell disease</td>
<td>27</td>
<td>42</td>
<td>-15</td>
<td>5.36</td>
</tr>
<tr>
<td>Type O, sickle cell disease</td>
<td>6</td>
<td>14</td>
<td>-8</td>
<td>4.57</td>
</tr>
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</table>
The Making of the Fittest: Natural Selection in Humans

LESSON TEACHER MATERIALS

Mendelian Genetics, Probability, Pedigrees, and Chi-Square Statistics

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a. What is $\chi^2$? $\chi^2 = 13.58$

b. Calculate df. $df = 6 - 1 = 5$

c. Using the critical values table, determine the $P$ value. $P < 0.05$ ($0.025 < P < 0.01$)

d. Interpret the $P$ value as it relates to these data. Explain the significance.

Since $P < 0.05$, the null hypothesis is rejected, which suggests that there is a statistically significant difference between the observed and expected data. Therefore, the difference between the observed and expected data is not solely due to chance.

e. From what you know about hemoglobin, sickle cell disease, and blood type, what selection pressure is acting on this population of children and causing the null hypothesis to be rejected? Explain your answer. (Hint: Look at the actual differences between the observed and expected numbers.)

This population of individuals is isolated on a small Pacific island where very little quality health care is available. Therefore, there is selection against the children with sickle cell disease. The presence of this selection pressure skews the observed numbers from the expected values, causing the null hypothesis to be rejected, which suggests that something other than chance is acting on the population. In this case, that “thing” is the selection against the SS genotype.

f. Due to the increase in global travel and the prevalence of invasive species, the Anopheles mosquito carrying the malaria parasite was inadvertently introduced to this isolated Pacific island. A researcher, 100 years from the present day, decides to complete a follow-up study and monitors another 50 couples who are all heterozygous for type A blood and have sickle cell trait. These couples had 136 children. Based on the introduction of the Anopheles mosquito carrying the malaria parasite, predict scientifically logical observed numbers of children for each genotype possibility and complete a chi-square statistical analysis test.

Chi-Square Data Table: Sample Data

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Predicted Observed (o)</th>
<th>Expected (e)</th>
<th>$(o - e)$</th>
<th>$(o - e)^2/e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A, normal RBCs</td>
<td>19</td>
<td>25.5</td>
<td>-6.5</td>
<td>1.66</td>
</tr>
<tr>
<td>Type O, normal RBCs</td>
<td>5</td>
<td>8.5</td>
<td>-3.5</td>
<td>1.44</td>
</tr>
<tr>
<td>Type A, sickle cell trait</td>
<td>70</td>
<td>51</td>
<td>21</td>
<td>8.65</td>
</tr>
<tr>
<td>Type O, sickle cell trait</td>
<td>25</td>
<td>17</td>
<td>8</td>
<td>3.76</td>
</tr>
<tr>
<td>Type A, sickle cell disease</td>
<td>13</td>
<td>25.5</td>
<td>-12.5</td>
<td>6.13</td>
</tr>
<tr>
<td>Type O, sickle cell disease</td>
<td>4</td>
<td>8.5</td>
<td>-4.5</td>
<td>2.38</td>
</tr>
</tbody>
</table>

i. What is your predicted chi-square value ($\chi^2$)? Answers will vary depending on the values the students choose. Sample data: $\chi^2 = 24.02$ ($\chi^2$ should be greater than the value in Question 12a).

ii. Calculate df. $df = 6 - 1 = 5$

iii. Using the critical values table, determine the predicted $P$ value. $P < 0.01$

iv. From your predicted numbers, do you accept or reject the null hypothesis? Reject
v. Based on what you know about hemoglobin, sickle cell disease, blood type, and malaria, what selection pressures are acting on this population of children? Explain your answer.

Answers will vary; however, the predicted answers should be along the following lines:

The isolated population sampled here is facing selection pressures caused by both limited health care as it relates to sickle cell disease and the introduction of malaria through the Anopheles mosquito, which carries the malaria-causing parasite. Therefore, the deviation of the observed numbers from the expected numbers is larger than it was from the first scenario due to the presence of three selection pressures. First, the sickle cell disease genotype (SS) is strongly selected against; second, the normal (AA) genotype is selected against due to the higher susceptibility to malaria infection. Finally, the data suggest that there is selection for the sickle cell trait (AS) genotype because these individuals do not have sickle cell disease and are more resistant to malaria.