MENDELIAN GENETICS, PROBABILITY, PEDIGREES, AND CHI-SQUARE STATISTICS

OVERVIEW

This classroom activity uses the information presented in the short film *The Making of the Fittest: Natural Selection in Humans* to take students through a series of questions pertaining to the genetics of sickle cell disease and its relationship to malaria resistance. The questions are divided into sections: Mendelian Genetics and Probability, Pedigrees, and Chi-Square Statistics. Within each section, the questions sequentially move from a basic level to a more advanced level in order to develop the skills of the students.

LEARNING OBJECTIVES

The student will be able to:

- explain the genetics of sickle cell disease both phenotypically and genotypically
- use Punnett squares in order to predict frequencies of genotypes in the next generation based on the genotypes of the parents
- understand the rules of probability as they relate to genetics problems
- analyze pedigrees in order to deduce genotypes, phenotypes, and probabilities
- utilize the chi-squared statistical analysis test to determine the significance of genetics data
- explain the link between the sickle cell heterozygous genotype and malaria resistance

KEY TERMS

sickle cell anemia, sickle cell disease, red blood cells, hemoglobin, malaria, Mendelian genetics, probability, pedigree, chi-squared statistical analysis, homozygous, heterozygous, genotype, phenotype, recessive, dominant, incomplete dominance, codominance, independent assortment

TIME REQUIREMENT

One to two 50-minute class periods if the chi-squared statistics section is not included; if the chi-squared statistics section is included, the additional time required will depend on the pace and background of the students.

APPROPRIATE LEVELS

high school biology (all levels including AP and IB), 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), the use of pedigrees to show family relationships, and how to analyze the pattern of inheritance of a particular trait. More advanced students should have a working knowledge of the chi-squared statistical analysis test.

TEACHING TIPS

- This activity and the storyline of the short film could be utilized as a culminating unit classroom assignment on genetics that ties together all levels of genetic analysis: Punnett squares, probability, pedigrees, and chi-square analysis.
- Teachers may discuss with the class how sickle cell disease provides an interesting example of the arbitrary nature of the terms *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, in regard to blood-cell shape, the phenotype of the sickled red blood cell is incomplete dominant because heterozygotes can display some sickled red blood cells in low-oxygen environments. Finally, in regard to hemoglobin itself, at the molecular level, there is codominance. In heterozygotes, both HbA and HbS alleles are expressed.
- The chi-square statistics portion of this activity is optional. If you teach a course in which chi-square analysis is not required, you may remove that section from this activity; it has been placed on separate pages for that reason.

ANSWER KEY

MENDELIAN GENETICS AND PROBABILITY

1. *If two people who have the sickle cell trait have children, what is the chance that a child will have normal red blood cells 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.*

	A	**S**
A	**AA**	**AS**
S	**AS**	**SS**

In high- and low-oxygen environments:

Normal Red Blood Cells: **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 red blood cells? (show work)* **1/4 x 1/4 x 1/4 = 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 work)* **1/2 x 1/2 x 1/2 = 1/8 (12.5%)**

d. *What are the chances that all three of their children will show the disease phenotype? (show work)*

1/4 x 1/4 x 1/4 = 1/64 (1.56%)

e. *What are the chances that these parents will have two children with the sickle cell trait and one with sickle cell disease? (show work)* **1/2 x 1/2 x 1/4 = 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 he/she has the sickle cell trait?* **2/3 (66.67%)**

(Note: Because you know the child does not have sickle cell disease, he/she 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.)

2. *An individual who has the 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. Show all possible genotypes of their children in a Punnett square. State the genotype and phenotype ratios of the offspring.

	A	A
A	AA	AA
S	AS	AS

Genotype Ratio:

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

Phenotype Ratio:

50% (1/2) normal hemoglobin (normal red blood cells):
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 he/she is exposed to the malaria parasite?* **1/2 (50%)**

3. *If a woman with sickle cell disease had children with a man who has the sickle cell trait:*

   a. *What are the genotypes of the parents?* **AS and SS**

   b. *What is the genetic makeup of the gametes the mother can produce?* **S**

   c. *What is the genetic makeup of the gametes the father can produce?* **A or S**

   d. Show all possible genotypes of their children in a Punnett square, and summarize the genotype and phenotype ratios of the possible offspring.

	A	S
S	AS	SS
S	AS	SS

Genotype Ratio:

50% (1/2) AS: 50% (1/2) SS

Phenotype Ratio:

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

50% (1/2) mutant hemoglobin (sickle cell disease)

   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 the sickle cell trait (AS) have two advantages: They have a greater resistance to malaria and normally do not show symptoms of sickle cell disease. Therefore, heterozygotes are more likely to survive.

4. *In humans, blood type is a result of multiple alleles: I^A, I^B, and i^O. A few simple rules of blood type genetics are: I^A is dominant over i^O. I^B is dominant over i^O. I^AI^B are codominant.*
   Two parents heterozygous for type A blood and who have the sickle cell trait have children. Answer the following questions:
   a. *What is the genotype of the parents?* **I^Ai^OAS**
   b. *What are the genetic makeups of the gametes they can produce?* **I^AA, I^AS, i^OA, or i^OS**
   c. *Complete the dihybrid Punnett square to determine the frequency of the different phenotypes in the offspring. Note: Consider blood type and normal vs. mutant hemoglobin in the various phenotypes.*

	I^AA	I^AS	i^oA	i^oS
I^AA	I^AI^AAA	I^AI^AAS	I^Ai^oAA	I^Ai^oAS
I^AS	I^AI^AAS	I^AI^ASS	I^Ai^oAS	I^Ai^oSS
i^oA	I^Ai^oAA	I^Ai^oAS	i^oi^oAA	i^oi^oAS
i^oS	I^Ai^oAS	I^Ai^oSS	i^oi^oAS	i^oi^oSS

3/16 (18.75%) **Blood type A, normal hemoglobin (normal red blood cells)**

3/8 (6/16) (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 red blood cells)**

1/8 (2/16) (12.5%) **Blood type O, normal and mutant hemoglobin (sickle cell trait)**

1/16 (6.25%) **Blood type O, mutant hemoglobin (sickle cell anemia)**

5. *Set up two monohybrid crosses with the following parents: Mom is heterozygous for type B blood and has the sickle cell trait, while Dad has type AB blood and also has the sickle cell trait.*

	I^B	i^O
I^A	I^AI^B	I^Ai^O
I^B	I^BI^B	I^Bi^O

	A	S
A	AA	AS
S	AS	SS

   a. *What are the chances that a child of this couple will have type B blood and have the sickle cell trait? (show work)*

      1/2 x 1/2 = 1/4 (25%)

   b. *What are the chances that a child will have type AB blood and will not have sickle cell disease? (show work)*

      1/4 x 3/4 = 3/16 (18.75%)

   c. *What are the chances that a child will have type B blood and have sickle cell disease? (show work)*

      1/2 x 1/4 = 1/8 (12.5%)

   d. *What are the chances that a child will have type B blood and at least some normal hemoglobin? (show work)*

      1/2 x 3/4 = 3/8 (37.5%)

PEDIGREES

6.

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 sickle cell disease? **1/2 (50%)** Have the sickle cell trait? **1/2 (50%)** Have 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 of all, they do not suffer from sickle cell disease. Secondly, they are more resistant to malaria infection due to their heterozygous genotype.

7.

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 of the children in generation II 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 their mother, because she has sickle cell anemia (SS).

d. *Regarding the answer to part c, 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 NYC confers no distinct advantage nor disadvantage. However, if these individuals mated with another heterozygous (AS) individual, they 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?

Mother: **AS** Father: **AS** Explain how you know.

Neither parent has sickle cell anemia, so they possess 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/are the possible genotype(s) of the mother in the second generation?* **AA or AS**

g. *If the couple in the third generation has another child, what are the chances that the child will have sickle cell disease?* **1/4 (25%)**; *Have the sickle cell trait?* **1/2 (50%)**; *Be homozygous for normal red blood cells?* **1/4 (25%)**; *Be resistant to malaria and not have sickle cell disease?* **1/2 (50%)**

8.

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

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

b. *If individuals 13 and 14 have another child, what are the chances that he or she will have sickle cell disease?* **1/4 (25%)**

c. *If the same couple has 3 more children, what are the chances that the 3 kids will have the sickle cell trait? (show work)*
1/2 x 1/2 x 1/2 = 1/8 (12.5%)

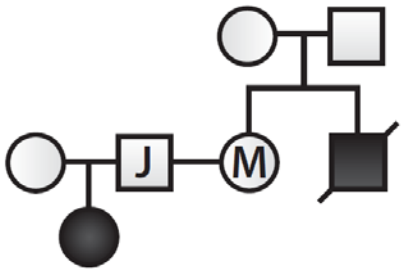
d. *Based on where this family lives, is the sickle cell trait genotype a genetic advantage? Explain.* **Since this family lives in the relatively dry highlands of eastern Africa, there is a low incidence of malaria; therefore, the heterozygous genotype confers no significant genetic advantage.**

e. *If individuals 8 and 9 have 4 more children, what are the chances that two of them will be homozygous for normal red blood cells? Explain why.*

There is 0% chance this couple will have a child who is homozygous (AA) for normal hemoglobin. This is due to the fact that their father has sickle cell disease (SS); therefore, he can only pass on the sickle cell allele (mutant hemoglobin (HbS)). Each child this father has will either have sickle cell disease (SS) or be a carrier for the sickle cell allele (AS).

9. *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 had 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.*



J = Jerome

M = Michaela

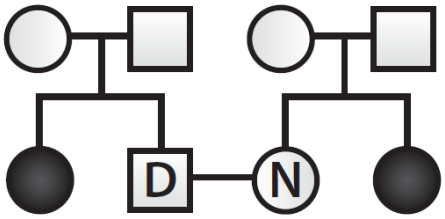
b. What is the probability that Jerome and Michaela will have a baby with sickle cell disease? (Note that neither Jerome nor Michaela have sickle cell disease.) Show your work.

2/3 x 1/4 = 2/12 = 1/6 (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 x 1/4 chance of having an SS child).

10. *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 the sickle cell trait.*

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



D = Demarcus

N = Natasha

    b. *Based on this incomplete information, calculate the probability that if this couple has a child, the child will have sickle cell disease.*

2/3 x 2/3 x 1/4 = 4/36 = 1/9 (11.11%)

Similar to #9 above, 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 in regards to the probability, so all values must be multiplied together.

CHI-SQUARE STATISTICS

11. *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-squared statistical analysis test.*

Phenotype/Genotype	Observed (o)	Expected (e)	(o-e)	$(o-e)^2/e$
Normal RBC/AA	139	125	14	1.57
Sickle cell trait/AS	279	250	29	3.36
Sickle cell disease/SS	82	125	-43	14.79

a. *What is the chi-square value (χ^2)?* **$\chi^2 = 19.72$**

b. *Calculate the degrees of freedom (df).* **$df = 3 - 1 = 2$**

c. *Using the Critical Values Table attached, 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 children (AA) and the homozygous sickle cell anemia genotype (SS).

12. *Believe it or not, there are 50 couples all 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 the sickle cell trait. The fifty couples have 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-squared statistical analysis test. (Use the table below if you need assistance.)*

Phenotype	*Observed (o)*	*Expected (e)*	*(o-e)*	*$(o-e)^2/e$*
Type A, normal RBCs	**48**	**42**	**6**	**0.86**
Type O, normal RBCs	**18**	**14**	**4**	**1.14**
Type A, sickle cell trait	**92**	**84**	**8**	**0.76**
Type O, sickle cell trait	**33**	**28**	**5**	**0.89**
Type A, sickle cell disease	**27**	**42**	**-15**	**5.36**
Type O, sickle cell disease	**6**	**14**	**-8**	**4.57**

a. *What is the chi-square value (χ^2)?* **$\chi^2 = 13.58$**

b. *Calculate the degrees of freedom (df).* **$df = 6 - 1 = 5$**

c. *Using the Critical Values Table attached, 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 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 on which very little quality healthcare 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, one hundred years from the present day, decides to complete a follow-up study and monitors another 50 couples, all of whom are heterozygous for type A blood and have the 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-squared statistical analysis test.*

Sample Data: Student predictions for the observed numbers (o) will vary. The predicted observed numbers should show lower numbers for both normal RBCs and sickle cell disease offspring than the expected and higher numbers of sickle cell trait offspring than the expected. Additionally, the 1:4 type O: type A blood ratios should be maintained. The expected numbers should be exactly as written in the data below.

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

i. *What is your predicted chi-square value (χ^2)?* **Answers will vary depending on the values the students choose. Sample data $X^2 = 24.02$ (X2 should be greater than 12c).**

ii. *Calculate the degrees of freedom (df).* **df = 6 – 1 = 5**

iii. *Using the Critical Values Table attached, 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 healthcare 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 of all, the sickle cell disease genotype (SS) is strongly selected against; secondly, 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.

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