A Fishy Way to Discuss Multiple Genes Affecting the Same Trait

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Summary

Many genetics classroom activities focus on inheritance patterns of a single gene with two different alleles. While valuable, these activities overlook additional areas of genetics research, such as multiple genes controlling a single trait. In this activity, students are introduced to the concept of complementation (see Box 1) and then determine whether blind cave fish from different locations have mutations in the same gene or different genes. Although this activity could be taught in many ways, here it is presented as a lecture with several clicker questions (see Box 2). Student responses are shown to help instructors gauge the range of student answers.

Introduction

Although there are many examples of genetics activities involving monohybrid and dihybrid crosses, it can be difficult to find activities that focus on more than one gene and a non-simple inheritance pattern. Here, I present an activity that asks students to consider whether multiple genes are responsible for blindness in the Mexican cavefish *Astyanax mexicanus*. These cavefish live in a series of unconnected caves in northeastern Mexico, have been blind for millennia, and can breed with sighted surface fish (reviewed in [1]). In this activity, students answer questions to determine the following: are mutations in the same gene or different genes responsible for blindness in separated cavefish?

Box 1. Concepts at a Glance

Genetics/molecular biology leads into evolutionary biology

Students learn to:

- Deduce information about genes and alleles from analysis of genetic crosses and patterns of inheritance.
- Interpret complementation tests to determine whether two mutations affect the same gene, and explain the requirements and the basis for these tests.

Target age group:

The target age group is an undergraduate genetics course for majors, although this activity could also be part of a high school biology unit on mutations. The genetic alteration of DNA through mutations and the inheritance of mutations is part of the National Academy of Sciences Framework for K-12 Education [7].

Box 2. Using the Peer Instruction Method in the Classroom

During lecture, I use the peer instruction method of asking clicker questions [8]. Students vote on an answer choice, talk to their peers, and vote again. Students do not see the distribution of answers until after they talk to their peers and vote again since knowledge of individual voting results can impact peer discussion [9]. Here, I report the votes that students make both before and after discussion with peers so that teachers can follow the impact peer discussion has on student learning [10].

I have taught this concept using a variety of active learning strategies. Here, I present the information as an interactive lecture with multiple-choice clicker questions (see Box 3), but this activity could also be taught as a small group activity, assigned as homework, or changed so students would be answering only open-response questions. The advantage of presenting the activity as a...
Engagement: Introducing Complementation Using Human Deafness

I begin this unit by building on two concepts we have already covered in the course: 1) identifying autosomal inheritance patterns and 2) analyzing pedigrees. Specifically, students analyze a human pedigree on the inheritance of deafness (shaded individuals) to review these concepts (Figure 1A). Human deafness is a good example for introducing students to multi-gene traits because mutations in at least 57 genes cause deafness that is not associated with any other symptoms (http://deafnessvariationdatabase.org). I ask the class to tell me about the inheritance pattern for the pedigree in Figure 1A. Students are quick to volunteer that this pedigree is more consistent with an autosomal recessive inheritance pattern. I then ask students to tell me about the inheritance pattern in the Figure 1B pedigree. Students again say that the pedigree is more consistent with an autosomal recessive inheritance pattern.

Next, I tell students that a deaf person from the family in Figure 1A has a child with a deaf person from the family in Figure 1B, and that child can hear. This result is surprising because if we were considering a simple Mendelian inheritance pattern, two people who are deaf because of autosomal recessive mutations would have a 100% chance of having a deaf child. I then ask the class: “how can you explain the child from the mating between the two pedigrees?” Students often suggest that the sperm or egg that created the child had a spontaneous mutation that made a mutant allele normal (a possibility, but not very likely).
or that the child has the genetic mutations but for some environmental reason, s/he is not deaf (another possibility, so I respond by saying let us assume all individuals with the mutation are deaf). Sometimes a student will suggest that the parents have mutations in two different genes.

I now introduce students to the terms “complementation” and “non-complementation” using the slides in Figure 2. I state that an example of complementation occurs when two deaf parents produce hearing children. These results suggest that the parents have mutations in DIFFERENT genes. I represent the two genes using the letters A and B (Figure 2A). Then I contrast complementation with non-complementation (Figure 2B). Here, two deaf parents produce all deaf children. This result suggests that parents have recessive mutations in the SAME gene, represented by the letter B.

**Inquiry: Engaging Students in Applying Complementation to Blind Cavefish**

To apply the concept of complementation, students study eye development in the Mexican blind cavefish. These blind cavefish populations, which evolved independently from sighted surface fish at different times, are found in caves throughout northeastern Mexico (reviewed in [1,2]). The explanation for the evolution of blindness remains in dispute [3]. When students want to know more about hypotheses for why cavefish lost
You isolate 3 fish strains from different cave ponds, all the fish are blind because of autosomal recessive mutations. You mate the fish together (don’t worry about sex) and get the following results:

|   | #1 | #2 | #3 |
|---|----|----|----|
| #1 | −  | −  | +  |
| #2 | −  | −  | +  |
| #3 | +  | +  | −  |

Offspring phenotypes:
- = no complementation, blind fish
+ = complementation, fish can see

Fish strains #1 and #2 have defects:
A. In the same gene
B. In different genes

Investigation: Applying Knowledge of Complementation to Blind Fish

Next, I ask a clicker question that tests whether students can apply what they learned about complementation in human deafness to the cavefish example (Figure 3A). This question asks: if you
mate two blind fish that have recessive mutations in two different genes, what percentage of their offspring will be blind? When students answer the question before talking with their peers, 63% of the students correctly answer that none of the offspring will be blind (Figure 3B). Interestingly, the most common incorrect answers are b) 25% or c) 50%, suggesting that students are answering the question as if they were solving a simple Mendelian inheritance monohybrid cross problem. After the students talk to their peers, 86% of the students answer correctly (Figure 3B), indicating that peer discus-

Easy Question

Draw one pedigree using these four individuals (2 parents, 2 kids) in a way that illustrates complementation. Filled symbols indicate a person with a disease.

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Answer:
Medium Question

You are studying aging in fruit flies and have generated a number of homozygous long-lived fly mutants. You now wish to determine how many genes these six mutants represent and you perform pairwise crosses with all of the homozygous mutants. Results of this analysis are shown in the table below (where the intersection represents the phenotype of the offspring resulting from a particular cross):

|       | Mut 1 | Mut 2 | Mut 3 | Mut 4 | Mut 5 | Mut 6 | WT |
|-------|-------|-------|-------|-------|-------|-------|----|
| Mut 1 | -     | +     | -     | -     | +     | +     | +  |
| Mut 2 | -     | -     | +     | +     | +     | -     | +  |
| Mut 3 | -     | -     | -     | +     | +     | +     | +  |
| Mut 4 | -     | -     | +     | +     | +     | +     | +  |
| Mut 5 | -     | -     | +     | -     | +     | +     | +  |
| Mut 6 | -     | -     | -     | +     | +     | -     | +  |

+ indicates all offspring have normal lifespan
- indicates all offspring are long-lived
WT= a wild type strain of flies

a. Are these mutations dominant or recessive? How do you know?
They are all recessive to WT because they have a WT phenotype when heterozygous with the WT allele.

b. Based on these results, at least how many genes are working to affect lifespan?
Three
Complementation group 1: 1, 3, 4
Complementation group 2: 2, 6
Complementation group 3: 5

Figure 8. Medium complementation homework question.
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Difficult Question

Your lab has decided to study albino fish so you gather 10 fish from various cave ponds in Mexico. You mate the fish together (don’t worry about sex) and get the following results: (a “-” represents an albino fish and a “+” phenotype represents a fish that is wild-type in color). The “?” are for you to fill in.

|     | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-----|---|---|---|---|---|---|---|---|---|----|
| 1   | - |   |   |   |   |   |   |   |   |    |
| 2   | - | - |   |   |   |   |   |   |   |    |
| 3   | + | + | - |   |   |   |   |   |   |    |
| 4   | + | + | + | - |   |   |   |   |   |    |
| 5   | ? | - | + | + | - |   |   |   |   |    |
| 6   | + | + | - | + | + | - |   |   |   |    |
| 7   | + | + | ? | ? | ? | ? | ? | - |   |    |
| 8   | + | + | ? | + | + | - | ? | ? | - |    |
| 9   | + | + | + | + | + | + | + | - | + |    |
| 10  | + | + | + | - | + | + | ? | + | - |    |
| WT  | + | + | + | + | + | + | + | + | + |    |

You are deciding which crosses to do next. Which of the following crosses will give you new information about which mutants belong to each complementation group?
A. Mutant 7 x Mutant 5
B. Mutant 8 x Mutant 3
C. **Mutant 7 x Mutant 3**
D. Mutant 8 x Mutant 10
E. More than one of the crosses above will give you new information in the table

Figure 9. Difficult complementation homework question. The correct answer is marked in bold font.
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You carry out a screen for worm mutants that prefer to eat the bacteria *S. dysenteriae*. Normally worms prefer to eat *E. coli*.

You isolate 10 *S. dysenteriae* preferring strains (sn1 - sn10) each of which are homozygous recessive. You carry out crosses between all strains and produce the following results where a "-" phenotype indicates worms prefer *S. dysenteriae* and a "+" phenotype represents the wild type behavior (which is that worms prefer to eat *E. coli*). The "?" are for you to fill in.

|     | sn1 | sn2 | sn3 | sn4 | sn5 | sn6 | sn7 | sn8 | sn9 | sn10 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| sn1 | -   |     |     |     |     |     |     |     |     |      |
| sn2 | +   | -   |     |     |     |     |     |     |     |      |
| sn3 | +   | +   | -   |     |     |     |     |     |     |      |
| sn4 | +   | ?   | +   | -   |     |     |     |     |     |      |
| sn5 | -   | +   | +   | +   | -   |     |     |     |     |      |
| sn6 | +   | -   | ?   | ?   | +   | +   | -   |     |     |      |
| sn7 | +   | -   | +   | -   | +   | +   | +   | -   |     |      |
| sn8 | ?   | ?   | +   | +   | -   | +   | +   | +   | -   |      |
| sn9 | ?   | ?   | +   | +   | -   | +   | +   | +   | +   |      |
| sn10| +   | +   | +   | +   | +   | +   | +   | +   | +   |      |
| WT  | +   | +   | +   | +   | +   | +   | +   | +   | +   | +    |

Which statement is NOT correct:
A. sn2 X sn4 is +
B. sn3 X sn7 is +
C. sn4 X sn7 is +
D. sn1 X sn9 is -
E. sn2 X sn9 is +

**Clarification: Constructing a Complementation Table**

Next, I introduce students to the complementation table. Complementation tables track the outcomes of mating fish from different strains, and they are useful in determining whether the mutations are in the same gene or different genes (Figure 4). On the outside of the table you put a unique identifier for each fish strain collected from the different caves (for example strain #1, strain #2, etc.) and inside the table you write either a + or a –. A + means that complementation occurred (the genes are different), and a – indicates that complementation did not occur (the genes are the same). To confirm that students are following, I ask them the clicker question in Figure 4. Even before peer discussion, 94% of the class answered that mating two strains with the result that all the offspring are blind indicates that the mutations must be in the same gene.

**Interpreting Results: How Many Genes Influence a Trait?**

Geneticists can use complementation tables to determine the minimum number of genes influencing a trait. This number can be obtained by combining the mutations that do not complement and making sure all strains are accounted for. In the example in Figure 4, there are a minimum

Starting with a population of genetically identical mice, you discover two new independent mutant strains in which all of the animals have epileptic seizures. In both strains, you know that the epileptic seizures are due to a single DNA mutation. You cross a mutant mouse from one strain to a mutant mouse from the second strain and find that none of their many offspring undergo spontaneous seizures. From this experiment you would conclude that the two mutant strains of mice most likely have mutations in:

a) the same DNA base position within a particular gene.
b) the same gene, but not necessarily the same DNA base position.
c) two different genes.

**Figure 10. Exam question on complementation.** The correct answer is marked in bold font.
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**Figure 11. GCA question on complementation.** The correct answer is marked in bold font.
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of two genes controlling eye development in these fish. Strains #1 and #2 have a mutation in one gene and strain #3 has a mutation in a second gene.

Next, I expand the table in Figure 4 to five different strains and have students determine how many genes produce sight (Figure 5A). Here, strains #1 and #2 have a mutation in one gene, strains #3 and #5 have a mutation in another gene, and strain #4 complements the other strains. The results suggest that at least three genes are working to produce sight. Before peer discussion only 60% of the students answer this question correctly, but this number increases to 84% after peer discussion (Figure 5B).

Exploring: What Are the Limits of Complementation Testing?

Now I use another clicker question to have students consider a new blind fish strain, strain #6. Strain #6 is mated to a sighted surface fish and all the offspring fish are blind (Figure 6). The question asks: can the strain #6 fish be used for complementation testing? The answer is no, because the strain #6 fish has a dominant version of a gene involved in eyesight. Anytime a fish from strain #6 is mated to any blind fish, the offspring will be blind. Therefore, geneticists cannot accurately score complementation versus non-complementation using a fish that has a dominant mutation. Before discussing the question, only 54% of the students say the strain #6 fish cannot be used for complementation testing, but after peer discussion, this number increases to 77%. As with the previous questions, discussion with peers improves student performance.

Conclusion

The activity described here helps students learn how one trait, such as deafness or blindness, can be affected by mutations in more than one gene and how geneticists can determine the minimum number of genes involved. Students also learn why strains with dominant mutations cannot be used for complementation testing. The clicker question results consistently indicate that students learn about complementation by talking over questions with their peers and there is evidence that this learning serves them well on exams and a genetics concept assessment (see Box 4).

IRB Statement

Approval to evaluate student clicker, exam, and pretest and posttest responses (exempt status, Protocol No. 39014) was granted by the Institutional Review Board, University of Washington.

Supporting Information

Supporting File S1 (PPT)

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