OVERVIEW
In this activity, students analyze aspects of a scientific paper that investigates genes involved in the colors and patterns of butterfly wings. This paper shows how evolutionary biologist Robert Reed and his colleagues used the biotechnology tool CRISPR-Cas9 to inactivate a number of butterfly genes and determine their functions. Students’ analyses of the paper are scaffolded by having students review the abstract and introduction, make a concept map of important terms in the paper, and interpret a subset of the results.

Additional information related to pedagogy and implementation can be found on this resource’s webpage, including suggested audience, estimated time, and curriculum connections.

KEY CONCEPTS
- Scientists communicate their findings, including the importance and relevance of their work, in scientific papers.
- CRISPR-Cas9 is a biotechnology tool that can be used to explore the function of specific genes in a variety of organisms.

STUDENT LEARNING TARGETS
- Analyze the abstract and introduction of a scientific paper to identify the authors’ argument structure.
- Describe how CRISPR-Cas9 was used to explore the genetic basis of coloration and spot formation in butterfly wings.
- Interpret the results of CRISPR-Cas9 experiments by annotating figures from the results section of a scientific paper.
- Interpret the experimental design used in a CRISPR-Cas9 experiment.

PRIOR KNOWLEDGE
Students should be familiar with:
- the connection between genotype and phenotype
- the process of gene expression
- the role of gene inactivation, or “knockouts,” in genetic analyses
- CRISPR-Cas9 technology

MATERIALS
- the scientific paper Zhang et al. 2017 (“Single master regulatory gene coordinates the evolution and development of butterfly color and iridescence”), which can be viewed or downloaded from the link
- copies of the “Student Handout,” which includes Figure 1 from the paper
- The Atlantic article “Scientists Can Now Repaint Butterfly Wings,” which can be downloaded from this activity’s webpage or viewed online

BACKGROUND
CRISPR-Cas9 (often shortened to “CRISPR”) is a technology that allows scientists to edit a cell’s DNA, which can be used to inactivate (“knock out”) genes. Since it was first described in 2012, CRISPR has generated much interest for its exciting potential in both research and medicine. The CRISPR system was first discovered in bacteria, where it functions as a type of immune system. Scientists have modified the bacterial system to produce a biotechnology tool for editing DNA.
CRISPR-based technologies have become widely used in research and have been applied in a broad range of biological studies. The technology is relatively cheap, easy to use, and allows researchers to ask new questions and get results faster than previously possible.

TEACHING TIPS

- Before students start reading the scientific paper for this activity, you may want them to do one or both of the following BioInteractive resources for background information on CRISPR-Cas9:
  - The [CRISPR-Cas9 Mechanism & Applications](#) Click & Learn provides information on how CRISPR works and how it is used by scientists. Encourage students to view the videos and profile of Robert Reed, one of the authors of the paper, which can be found in the “How It’s Used” section of the Click & Learn.
  - The “[Using CRISPR to Identify the Functions of Butterfly Genes](#)” activity introduces some of the research and techniques described in the paper. Figures 2–4 in the “Student Handout” are directly from the paper.

- This activity can be shortened with the following strategies:
  - Students could complete most of the activity as homework, then share their work in class.
  - Question 6 could be done using a “jigsaw” approach. Students could complete 6b and 6c individually, each focusing on only one part of the figure (A, B, C, etc.). They could then form groups with all the parts represented to debrief the entire figure.

- Before students start reading the paper, you may want to discuss some of the scientific terms used by the authors. Explain to students that even scientists often have to look up terms as they read a scientific paper. Unfamiliar terms in this paper may include:
  - **ommochromes**: chemical pigments that produce yellow, red, and brown colors in insect tissues
  - **blue structural iridescence**: a way of creating a blue color that uses microscopic structures instead of pigments
  - **wing conjugation scales**: a specialized type of scale found in the wings of butterflies and moths
  - **genetic mosaicism**: a condition in which different cells in a single individual have different numbers or arrangements of chromosomes

- You may also want to introduce students to some of the experimental techniques mentioned in the paper, such as **in situ hybridization** (used to identify the location of specific genes in chromosomes or tissues) or **RNA sequencing** (used to determine the sequence and expression levels of RNA present in cells in specific tissues).

- Question 3 asks students to make a concept map of several terms. Consider having students download and use concept mapping software, such as the free tool [Cmap](#). This will allow students to more easily revise their maps later — for example, after viewing an expert-created map or discussing their maps in a group.

- For Question 4, students read an article written by a science journalist that summarizes the Zhang et al. (2017) research article for a more general audience. Consider having a discussion to compare and contrast how the same information is presented in these two different articles. Students could consider how the articles differ in their target audiences, their descriptions of species, the images they show, etc.

**ANSWER KEY**

1. The goal of the “Abstract” is to provide a brief summary of the paper. Most abstracts present the research question, the methods used, the results, and the significance of the findings. In your own words, describe the research question for this study.

   **What is the role of the optix gene in determining the colors and patterns of butterfly wings?**

2. The goal of the “Introduction” is to help readers understand why the research question is important. To do this, scientists often briefly summarize what is and is not known about the research question, as well as what they did to address the question. They may also describe the structure of the rest of the paper.
a. What argument do the authors make for why their research is important?

The authors argue that the genes controlling colors and patterns of butterfly wings are important for explaining adaptation in many butterfly species, and for understanding the development of complex traits. They believe that scientists need to learn more about the functions of these genes and the extent to which these functions have been conserved over evolutionary time.

b. What do the authors say is known and not known about the genetics of butterfly wing patterns?

Based on earlier studies, it was known that the functions of a small number of genes may explain wing patterns in butterflies. It was also known that the optix gene affects wing traits, including red coloration, and is the target of adaptation in certain butterfly species. The authors say that, before this study, scientists did not know the specific developmental role of optix or the extent to which this gene affects wing patterns, colors, and other structural features of wings in other, more distantly related species.

c. How do the authors prepare readers for reading the rest of the paper? Consider the entire “Introduction” in your response.

The authors first make the case for why their work is important. They briefly describe what they did overall (“a comparative functional analysis of the optix gene in butterflies”) and the methods they used (“We used Cas9-mediated targeted deletion of optix to test its color patterning function in four species of nymphalid butterflies.”). They then summarize their results (“Not only did we confirm deeply conserved roles for optix in coordinating pigmentation and scale morphology in all species surveyed, but we were surprised to find that this gene simultaneously regulates blue structural iridescence in some butterflies. Importantly, this coordinated regulation of pigmentation and iridescence strongly phenocopies wing patterns seen in other distantly related species.”).

3. Create a concept map that uses the following terms as your concepts. Put lines between related terms, labeling each line with a brief phrase describing the relationship between the terms.

There are many possible maps. A sample map created with Cmap is shown below.

![Concept Map Image]

4. Create a diagram or cartoon to illustrate the experimental design described in this article. Your illustration can be fairly general but should show how the scientists did the overall experiment.

Student illustrations are likely to be very simple, but they should highlight that scientists inactivated specific genes (optix and WntA) in several butterfly species using CRISPR. The scientists then carefully documented the phenotypes of individuals with and without the functioning target genes.
5. What were the controls and experimental manipulations in this experiment? Give specific examples of each from Figure 1.

The controls were the unmodified, wild-type individuals or cells with active optix, which are labeled “normal,” “wild type,” or “wt” in Figure 1. The experimental manipulations were the mutant individuals or cells in which the optix gene was inactivated/knocked out, which are labeled “mKO” or “mutant” in Figure 1. (Note some lineages of cells in the mutant butterflies still had active optix, allowing the scientists to compare both wild-type and mutant cells within the wings of these individuals.)

6. Annotate the copy of Figure 1 at the end of your handout by following the instructions below. Write everything in your own words and in a way that a general audience would understand.

   a. Add a title for the overall figure.
      A possible title is “The effect of knocking out the optix gene in two butterfly species.”

   b. Write a simple driving question for each part of the figure labeled with a letter (A, B, C, etc.). In other words, what question did the scientists try to answer in each part?

      Possible questions for each part are as follows:

      A. What happens to wing colors and patterns when optix is deleted in H. erato?
      B. What do the scales of the wing look like when optix is active in H. erato and when it is deleted?
      C. Does the type of scales produced in a wing differ if optix is deleted in H. erato?
      D. What happens to wing colors and patterns when optix is deleted in A. vanillae?
      E. What do the scales of the wing look like when optix is active and when it is deleted in A. vanillae?
      F. What happens to wing colors and patterns when optix is deleted in A. vanillae?
      G. Does the type of scales produced in a wing differ if optix is deleted in A. vanillae?

   c. Write a simple summary of what is shown by each part of the figure labeled with a letter (A, B, C, etc.). Your goal is to annotate the entire figure so that a reader could understand it without reading the caption.

      Students should use information in the figure legend to annotate the figure, using their own words.

7. Create a new diagram or cartoon to illustrate the experimental designs of the experiments from Figure 1 only. Make sure to include how the scientists used controls. Refer to the “Materials and Methods” section of the paper as needed to clarify the experiment. (Note you do not need to read this section in-depth. Many scientists don’t either, unless they have a specific question about the procedure or want to do a similar experiment.)

      Student illustrations should demonstrate how the authors compared individuals in which the optix gene had been inactivated with individuals with functional optix.

REFERENCES


CREDITS

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