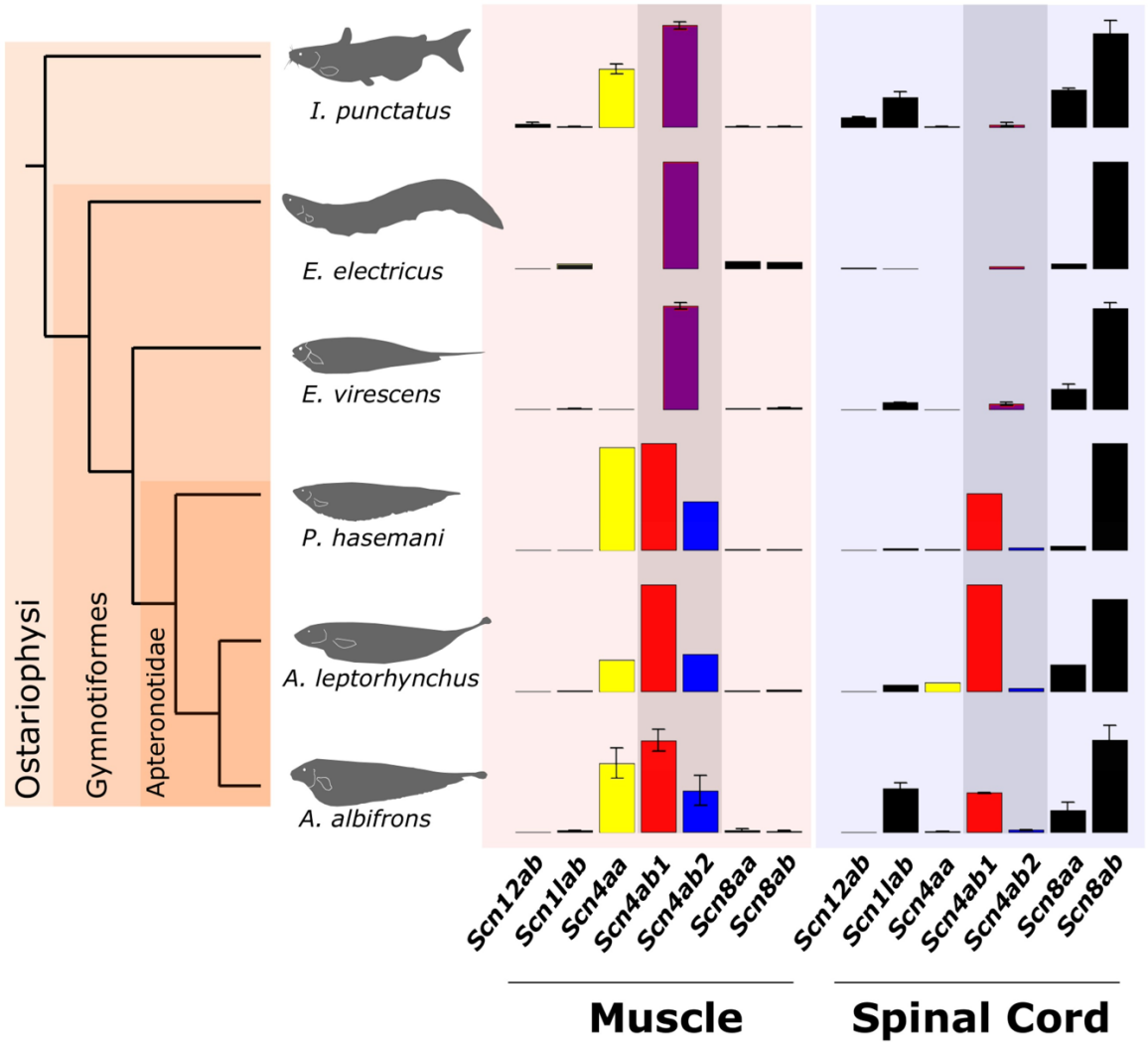
HOW TO USE THIS RESOURCE

Show the following figure and caption to your students. The accompanying Student Handout provides space below the image caption for Observations, Notes, and Questions and space next to the "Background Information" for Big Ideas, Notes, and Questions. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to prompt student thinking, increase engagement, or guide a class discussion about the characteristics of the graph and what it shows.



Caption: *The phylogenetic tree on the left shows the evolutionary relationships among three types of fish: nonelectric fish (*I. punctatus*), electric fish with electric organs derived from muscle tissue (*E. electricus, E. virescens*), and electric fish with electric organs derived from spinal neurons (*P. hasemani, A. leptorhynchus, A. albifrons*). The graphs on the right show each species' expression of different voltage-gated sodium channel genes. Gene expression was measured in both muscle tissue and the spinal cord; error bars represent one standard deviation from the mean (n = 3). The genes represented by black bars are* scn12ab, scn1lab, scn8aa, *and* scn8ab. *The genes represented by colored bars, which are all derived from a gene called* scn4a, *are* scn4aa *(yellow),* scn4ab *(purple),* scn4ab1 *(red), and* scn4ab2 *(blue).*

BACKGROUND INFORMATION

All animals, including humans, have cells that generate electrical signals called action potentials. Action potentials are used by neurons (nerve cells), muscle cells, and other cells for communication and intracellular signaling. Fast pulses or waves of action potentials can be produced by certain types of fish, called electric fish. These fish use the resulting electrical signals for a variety of functions, including navigating their environments and communicating with other electric fish. A few kinds of electric fish, such as electric eels, can even generate electricity that is powerful enough to defend against predators or stun prey.

Electric fish produce electrical signals via a specialized organ called the electric organ, which is derived from cells that can generate action potentials. In most electric fish, the electric organ is derived from muscle tissue. In one group of electric fish, the Apteronotids, or ghost knifefishes, the electric organ is derived from spinal neurons instead.

Voltage-gated sodium channels, which control the flow of sodium ions in and out of cells, are a group of proteins that play an important role in generating action potentials. Muscle cells, neurons, and electric organs all use sodium channel proteins to generate action potentials. However, electric organs can generate action potentials much faster than normal muscle cells and neurons can. The electric organs of the Apteronotids in particular can generate sustained electrical signals up to 1,800 Hz (oscillations per second), faster than those of the neurons found in any other organisms.

How do electric organs generate such fast action potentials? This ability may have evolved through the duplication and mutation of certain sodium channel genes. When genes are duplicated, one copy can maintain the function of the original gene, while the other copy can mutate freely. Over millions of years, sodium channel genes have been duplicated many times to generate a family of genes that are closely related. Electric fish have multiple sodium channel genes derived from a gene called *scn4a*, which is usually expressed in the muscles of vertebrates. Early in the evolution of fish, *scn4a* was duplicated into two different genes, *scn4aa* and *scn4ab*. In the Apteronotids, *scn4ab* was further duplicated into two more genes, *scn4ab1* and *scn4ab2*. Over time, some of these genes accumulated mutations that led to new functions.

Scientists investigated how changes in these sodium channel genes may have contributed to the evolution of electric organs in electric fish. The scientists measured the expression of several sodium channel genes, including the *scn4a*-derived genes, in three groups of fish. The first group was fish with electric organs derived from spinal neurons. This group was represented by three Apteronotid species: *P. hasemani, A. leptorhynchus,* and *A. albifrons*. The second group was fish with electric organs derived from muscle tissue. This group was represented by two species: the electric eel *E. electricus* and the glass knifefish *E. virescens*. The third and final group was nonelectric fish, which was represented by one species: the channel catfish *I. punctatus*.

INTERPRETING THE GRAPH

The phylogenetic tree on the left side of the figure shows the evolutionary relationships among the fish species in the study. The bar graphs on the right side show each species' expression of different sodium channel genes.

The genes represented by colored bars are all derived from *scn4a,* a sodium channel gene commonly expressed in vertebrate muscle tissue. Over millions of years, multiple variants of *scn4a* have arisen through gene duplication. Early in the evolution of teleosts (a group of bony fish that includes most living fish species), *scn4a* was duplicated into the genes *scn4aa* (yellow) and *scn4ab* (purple). These two genes are expressed in the muscles of most teleosts, including nonelectric fish such as *I. punctatus* and the Apteronotids. However, electric fish with muscle-derived electric organs (*E. electricus* and *E. virescens*) have a different expression pattern. In these fish, *scn4ab* (purple) is expressed in the muscles as usual, but *scn4aa* (yellow) is not. Instead, *scn4aa* is expressed in the

muscle-derived electric organ (not shown in the figure). In these particular species, *scn4aa* may have gained new functions over time that were beneficial for an electric organ but disadvantageous in the muscles. As a result, *scn4aa* eventually became specific to the muscle-derived electric organs and stopped being expressed in the muscles.

Among Apteronotids, *scn4ab* was further duplicated into the genes *scn4ab1* (red) and *scn4ab2* (blue). As shown in the figure, the *scn4ab1* gene (red) has a surprising expression pattern. Unlike the other *scn4a*-derived genes, which are typically expressed in muscles only, *scn4ab1* is also expressed in the spinal cord. This pattern suggests that *scn4ab1* may be responsible for the evolution of some of the electrical attributes of the spinal-neuron-derived electric organ found in Apteronotids. Additional experiments have shown that mutations in *scn4ab1* lead to structural and functional changes in the corresponding sodium channel. These changes likely underlie the unusually fast firing of the Apteronotid electric organ.

Teacher Tip: Prompt your students to explain the parts of the graph as applicable:

- Graph types: Phylogenetic tree/cladogram (left) and bar graphs (right)
- Phylogenetic tree: A diagram of evolutionary relationships among the fish species in the study: a nonelectric outgroup (*I. punctatus*), two electric species whose electric organs are derived from muscle tissue (*E. electricus, E. virescens*), and three species whose electric organs are derived from spinal neurons (the Apteronotids *P. hasemani*, *A. leptorhynchus*, and *A. albifrons*).
- Bar graphs, *x*-axis: Different voltage-gated sodium channel genes, expressed in either muscle tissue or the spinal cord. The genes in the middle of the axis (*scn4aa*, *scn4ab1*, and *scn4ab2*) share the ancestral gene *scn4a.* Note that *scn4ab1* and *scn4ab2* only exist in Apteronotid species. They are duplicates of the gene *scn4ab*, which is present in the other fish species but not explicitly listed on the *x*-axis.
- Bar graphs, *y*-axis: Mean expression levels for the genes listed along the *x*-axis. The error bars indicate one standard deviation from the mean. Expression levels were measured using RNA sequencing to quantify the abundances of different gene transcripts in relative units.
- Bar graphs, bar colors: Genes derived from *scn4a* are represented by colored bars: yellow for *scn4aa*, purple for *scn4ab,* red for *scn4ab1*, and blue for *scn4ab2*. Genes not derived from *scn4a* are represented by black bars.
- Bar graphs, shaded regions: The long vertical rectangles shaded in light gray highlight the relationship between the gene *scn4ab* (purple) and its two duplicates in Apteronotids, *scn4ab1* (red) and *scn4ab2* (blue).

DISCUSSION QUESTIONS

- The figure includes both a phylogenetic tree and bar graphs displaying gene expression data. Why do you think the scientists chose to show both of these components in the same figure? How might the phylogenetic tree help us interpret the bar graphs?
- What do the error bars on the bar graphs represent? Why was it important for the scientists to include these error bars?
- Why do you think the scientists included a nonelectric fish (*I. punctatus*) in their study?
- Compare the patterns of gene expression among the three different groups of fish in the study (nonelectric fish, fish with electric organs derived from muscles, and fish with electric organs derived from spinal neurons). What similarities and differences do you observe?
- The gene *scn4aa* is expressed in the muscles of all the species shown in the figure except for the two species with muscle-derived electric organs (*E. electricus* and *E. virescens*). Other research has shown that these species express *scn4aa* in their electric organs instead. Why might these species express *scn4aa* in their electric organs but not in their muscles?

- How many *scn4ab* genes do the Apteronotid species express in their muscles? How many *scn4ab* genes do the other groups of fish express? Why do these numbers differ?
- Are any of the sodium channel genes expressed in both muscles and the spinal cord in a given fish species? What might be the advantages or disadvantages of expressing these genes in both the muscles and the spinal cord?
- What is gene duplication, and how does it contribute to the evolution of new phenotypes?
- Describe some of the gene duplication events that occurred in electric fish. How might these events have contributed to the evolution of electric organs?
- Scientists believe that mutations in the sodium channel gene *scn4ab1* contributed to the evolution of the electric organ in Apteronotids. Based on what you know about the Apteronotid electric organ, how do you think these mutations affected the structure or function of the corresponding sodium channel protein?
- Scientists estimate that it took only 2 million years for *scn4ab1* to begin expressing in the spinal cord of the Apteronotids, which is relatively fast on an evolutionary timescale. What factors might have contributed to the rapid evolution of this novel adaptation in these fish?

KEY TERMS

action potential, electricity, gene duplication, ion channel, muscle, neuron, phylogenetic tree

SOURCE

Figure 1 from:

Thompson, Ammon, Daniel T. Infield, Adam R. Smith, G. Troy Smith, Christopher A. Ahern, and Harold H. Zakon. "Rapid evolution of a voltage-gated sodium channel gene in a lineage of electric fish leads to a persistent sodium current." *PLoS Biol* 16, 3 (2018): e2004892. https://doi.org/10.1371/journal.pbio.2004892.

More information about electric fish and the electrical signals they produce (electric organ discharges, or EODs) can be found at http://www.indiana.edu/~efishlab/learn.php.

AUTHOR

Bob Kuhn, Centennial High School, Roswell, Georgia
Edited by Ammon Thompson, University of California, Davis; Satoshi Amagai, Esther Shyu, and Jessica Johnson, HHMI