Evidence of Evolution: Homologous Gene Expression in Four Mammal Species

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Homologous Genes in Four Mammal Species

🔬 Purpose

The purpose of this lesson is to provide an in depth exploration of homologies. Students will interpret phylogenetic trees, explore both structural and genetic homology, and apply what they've learned to make predictions about gene expression given phylogenetic relationships.

🔬 Audience

This lesson was designed for use in an introductory high school biology course.

🔬 Lesson Objectives

Upon completion of this lesson, students will be able to:

- 🔬 understand that a "genetic toolkit," a common set of developmental genes, is present in many species of mammals, and that differences in expression of these toolkit genes are largely responsible for differences among mammal forelimb structure.

- 🔬 explain how common genes present during limb development are evidence for evolution and common ancestry.

- 🔬 compare and contrast gene expression patterns within species across time and among species by making qualitative observations to explain how these expression patterns are evidence for evolution.

- 🔬 analyze a phylogenetic tree to interpret relationships among species and to make predictions about when, relative to the evolution of given species, homologous traits and genes arose.

🔬 Key Words

gene expression, homologous

🔬 Big Question

This lesson plan addresses the Big Question *"Where did we come from?"*

🔬 Standard Alignments

- 🔬 **Science and Engineering Practices**

  - 🔬 **SP 4.** Analyzing and Interpreting Data

  - 🔬 **SP 6.** Constructing Explanations and Designing Solutions

- 🔬 **MA Science and Technology/Engineering Standards (2016)**

  HS-LS4-1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence, including molecular, anatomical, and developmental similarities inherited from a common ancestor (homologies), seen through fossils and laboratory and field observations.

- **NGSS Standards (2013)**

   HS-LS4-1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.

- **Common Core Math/Language Arts Standards**

   - **CCSS.ELA-LITERACY.RST.9–10.7.** Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) into words.

   - **CCSS.ELA-LITERACY.WHST.9–10.1.** Write arguments focused on *discipline-specific content.*

   - **CCSS.ELA-LITERACY.WHST.11–12.1.** Write arguments focused on *discipline-specific content.*

Misconceptions Addressed

- This lesson addresses some common misconceptions about evolution. Including:

   - Evolution acts directly on genes. (Question 3a)

   - Evolution is about progress. (Question 3b)

- Further information about student misconceptions on this topic can be found here. You can find information about tree-thinking misconceptions on the Understanding Evolution website. They also have a page on general evolution misconceptions.

Primary Sources

- **Bite** "What Do a Bat, a Pig, a Mouse, and an Opossum Have in Common?" *based on:*

   Maier, Jennifer A., Marcelo Rivas-Astroza, Jenny Deng, Anna Dowling, Paige Oboikovitz, Xiaoyi Cao, Richard R. Behringer, Chris J. Cretekos, John J. Rasweiler IV, Sheng Zhong, and Karen E. Sears. "Transcriptomic Insights into the Genetic Basis of Mammalian Limb Diversity." *BMC Evolutionary Biology* 17, no. 1 (2017): 1–18. doi:10.1186/s12862-017-0902-6.

- **Blog post written by the study's primary authors for a more general audience:**

   Maier, Jennifer A., and Karen E. Sears. "From Flippers to Fingers: How Do Mammalian Limbs Display Such Diversity?" BMC Series Blog. March 23, 2017. Accessed October 26, 2018.

Materials

Copies of Student Documents and Science Bite for each student

Time

This activity should take approximately one 50-minute class period.

Student Prior Knowledge

Students should have a working knowledge of transcription and translation, the role of genes in

the development of organisms, and the role of natural selection in evolution. Students should also understand basic developmental terms like embryo.

⚛ Instructions and Teacher Tips

- ☘ **General Procedure**

  - ☘ Hand out the student documents. As students work in pairs on Questions 1–3, circulate to answer questions and clarify student understanding.

  - ☘ When students finish Question 3, you should bring your class back together to go over the Questions and for an open discussion about phylogenetic trees and homologous traits. There are many misconceptions about phylogenetic trees that you can probe in this discussion, for example:

    - ☘ Does the phylogeny tell you anything about the exact age of a living species? How do you know? No, the tree only tells us the relative ages of species. Phylogenies can be calibrated with time, but they still only show time at which a common ancestor hypothetically existed, relative to other common ancestors.

    - ☘ Was there one, single, individual common ancestor at each node on the phylogenetic tree? No. The nodes represent populations, not individuals. Although we talk about "the common ancestor" what we mean is, "population of common ancestors."

  - ☘ Have students read the Bite independently, circling any unknown words and information that is confusing. Depending on the level of your class, you may wish to pair students up to help each other or to have a class discussion about the Bite. Another option is to read the Bite aloud as a class.

  - ☘ Have students continue work on the questions in their pairs.

  - ☘ Finally, bring students together for a final discussion about the Big Question for this lesson.

- ☘ **Tips, Variations, and Extensions**

  - ☘ If you covered transcription and translation earlier in the year, consider reviewing briefly so that students can apply that information to this lesson.

  - ☘ If this lesson takes more than one class period to complete, consider having students read the Bite for homework and complete the second set of analysis questions the next day in class.

  - ☘ HHMI Biointeractive has great resources to help students understand phylogeny. If students are having trouble grasping the concepts presented in this lesson, consider screening this video, which shows students how scientists use DNA to help construct phylogenetic trees.

  - ☘ BiteScis has other lessons covering evolution. This lesson on lizard phylogeny would be a great pre-lesson to help students understand evolution by natural selection and would give them some practice in creating phylogenetic trees: https://bitescis.org/lesson-plan/trends-in-evolution-convergence-and-divergence/.

☸ If your students are curious and/or if you want to extend the lesson, consider directing exploring and comparing Hoxd13 proteins on the NCBI website. Under the "Protein Alignments" section, students can select two species to directly compare sequences. Click the "Blast" button and it will redirect you to show the two sequences aligned with one another. The similarity of the sequences is another piece of evidence that it when and where these proteins are expressed that make mammal limbs different.

⚗ Background Information and Research Details

☸ This paper compares gene expression in the forelimb and hind limb of an opossum, a mouse, a pig, and a bat at three stages of limb development. They chose each of these species for a reason: the mouse represents a well-studied lab model and a typical five-digit limb; the pig represents a mammal that has reduced digits; the bat represents a five-digit limb that has been modified for flight; and the opossum represents marsupials (the outgroup to mammals that have a placenta (eutherian mammals)) and additionally has differences in timing of fore and hind limb development (see below).

☸ The opossum, as a marsupial, completes much of development outside of the mother. The forelimbs need to be developed enough for the young opossum to climb into the mother's pouch; the hindlimbs lag behind in development. The authors are not investigating why there are differences in timing of limb development, and do not go into detailed analysis about their findings on opossum limbs.

☸ The authors found that a small set of homologous genes (most known previously to be involved in limb development) are responsible for differences in the forelimbs across species. A key component of this study was establishing homology between the genes in each organism. Only by establishing that the genes in each organism are related by common descent (homologous), can they make meaningful comparisons between the expression patterns of those genes. In other words, the researchers needed to identify "apples" in both species—they can't compare oranges in one species and apples in another.

  ☸ There are two types of homologous genes: orthologs and paralogs. Orthologs are homologous genes that are found in two different species. For example, *hoxd13* in each species in the paper are orthologs. Paralogs are two genes related by duplication (of the gene, or sometimes an entire genome). These duplicated homologous genes (paralogs) can be found in the same species, or in two different species.

  ☸ For example, beta-hemoglobin and delta-hemoglobin in humans are paralogs. Beta-hemoglobin and delta-hemoglobin in mice and humans, respectively are also paralogs. In each pair of organisms, the two genes are related because one gene in a common ancestor (or just ancestor in the case of the same organism) was duplicated. An example of orthologous genes are beta-hemoglobin in humans, and beta-hemoglobin in mice- these two genes are related because they were both inherited from an ancestor that also had beta-hemoglobin.

☸ The authors found that a small set of homologous genes differentiate the forelimb from the hindlimb in each of the four species. This set of genes is different from the set that differentiates forelimbs among species (the set referred to in bullet point #3).

☸ The authors also find that gene expression among species varies more at later

developmental stages, and that the estimated age of these genes that are expressed later are younger than those expressed earlier. This is consistent with the idea that development is a hierarchical process that builds upon previous developmental processes. Errors in early development are more likely to be lethal, and thus the early stage of limb development is the most conserved across all species.

- The *Hoxd13* mRNA codes for a protein called a transcription factor. Transcription factors bind to regions of the genome called enhancers (or repressors) and then physically bind to the promoter region of other genes. Through this binding, transcription factors affect the expression of other genes. Transcription factors can have effects on the expression of many genes. Changes in transcription factors during development can therefore have cascading effects. The cluster of genes to which *Hoxd13* belongs has been shown to have a role in limb outgrowth and patterning in mice.

- Figure 4 in the Student Document is looking at the doral (back) surface of the animal.

- Developmental biologists look at mRNA instead of DNA because mRNA expression can tell us something about what the function of the gene is. DNA of all genes in an organism is found in every cell of that organism because the genome is present in every cell. If we looked at DNA, we would not be able to tell much about how the genes function during development.

- RNAseq is a technique (used in this paper) where all the mRNAs in a given tissue, in this case, the forelimb and hindlimb are sequenced. This technique gives scientists unbiased view of what is expressed in the limb at a given time. This is in contrast to a "candidate gene approach" where scientists would attempt to understand the function of specific genes that they already know something about. RNAseq can capture novel genes and provide new routes of inquiry.

- All vertebrates share most genes in their genome. The field of "EvoDevo" suggests that it's not that different genes that make us look different, but rather that these same genes in different species are expressed in different spatial and temporal patterns (meaning transcribed in different places in the embryo and in different amounts). These different patterns are what are responsible for the big differences in physical appearances or limbs.

Big Question Discussion

This lesson should get students thinking about the Big Question "*Where did we come from?*" In particular, what can we learn from studying the genes shares among species? If you choose to delve into the Big Question, consider the following ideas:

- Ask students to write down their thoughts about Question 5. The question states, Scientists rely on all kinds of evidence to help them reconstruct evolutionary relationships. Before the discovery of DNA and molecular tools, scientists like Charles Darwin heavily relied on comparing and analyzing anatomical structures. Do you think one form of evidence is more convincing than the other? Why?"

- Then have students split into two groups depending upon their answer. Put a sign for "Molecular Evidence" and "Structural Evidence" on opposite sides of the room.

- Have students turn and talk to a neighbor or to the group that shares their opinion to

discuss their responses.

- Share out common responses to each side. Have students share evidence aloud with the class.

- Decide as a group what you believe is more convincing. Provide examples to students to clarify main points (see notes in the answer key).

Answers

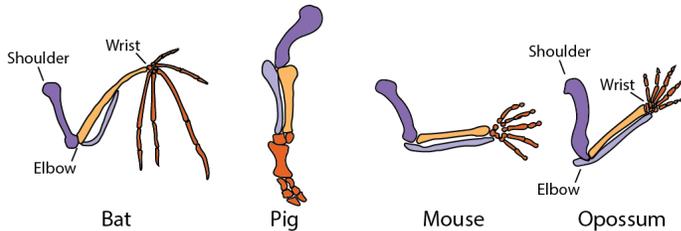The forearms of a bat, pig, mouse, and opossum are shown in **Figure 1.**

Figure 1. Forearms of Four Mammals.

1. Complete the table below by counting the number of bones in each species.

	Bat	Pig	Mouse	Opossum
Number of bones between shoulder and elbow (dark purple)	1	1	1	1
Number of bones between elbow and wrist (light purple and yellow)	2	2	2	2
Approximate number of bones in wrist and hand (orange)	many	many	many	many

A phylogenetic tree is shown in **Figure 2.** It models the evolutionary relationships among four mammals: a bat, pig, mouse, and opossum. Nodes, or branching points (shown as small circles) represent common ancestors. For example, the common ancestor of bats and pigs is represented by the node labeled C.
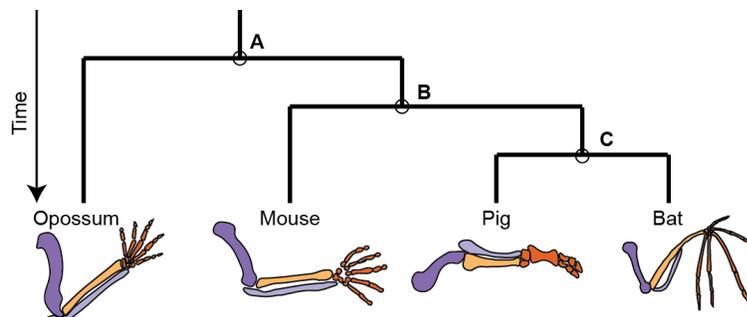
Figure 2. Phylogeny and Limbs of Four Mammals.

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2. Use **Figure 2** to answer the following questions.

    a. Which node in **Figure 2** (A, B, or C) represents the common ancestor of bats, pigs, and mice?

Node B

    b. Make a claim about how many upper arm bones (orange) and forearm bones (yellow and light purple) the ancestor represented at node A had.

1 upper arm bone, 2 forearm bones

    c. Explain, using evolutionary concepts, why you made the claim you did in Part b.?

All of the animals share a pattern even though they live in very different environments. This strongly suggests that they inherited the trait from a common ancestor.

Make a claim about when the gene, or genes, that underlie the general forearm structure you described in part b evolved. To make your claim, place an "X" on the diagram in **Figure 2** to represent when the gene(s) evolved.
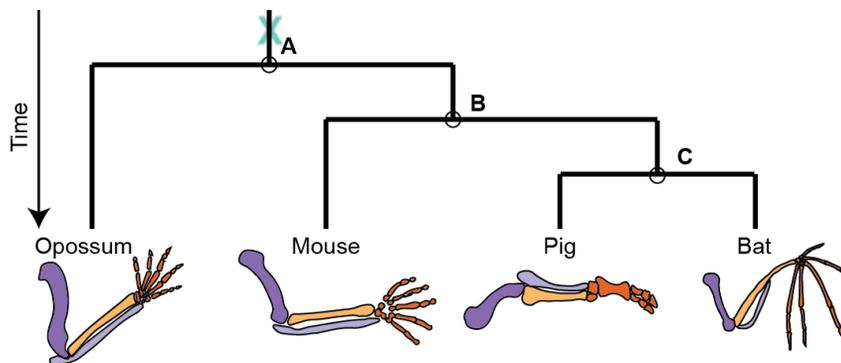
Two claims about homologous traits are below.

Claim 1: The genes underlying forearm structure in mammals are homologous.

Claim 2: The genes underlying forearm structure in mammals are not homologous.

    d. Which claim, claim 1 or claim 2, do you support? Explain your choice with evidence from Figure 2 and your answer part d.

Sample answer: I support Claim 1. The trait of one bone-two bones-many bones most likely evolved before node A, because each of the descendants of that common ancestor have the trait. Homologous traits, such as forearm structure, are inherited from a common ancestor via genes. Therefore, if a trait is homologous, the gene(s) underlying the trait are, too and the genes evolved before node A as well.

3. Although they clearly share a general overall pattern, the forearms of these animals are not identical.

    a. Propose a hypothesis for how the differences you see among the structures of the different bones in each mammal could have arisen.

Sample answer: The differences most likely arose because of natural selection. Through mutation, different tweaks to the overall similar forearm structure would have arisen in different populations of the common ancestors. Whether these variations were beneficial would have depended on the environment in which the population lived. If it were beneficial, then the variation would have spread. Over generations, these variations and others would have differentiated the populations into the species we know today.

    b. Make a claim about which of the four species (bat, mouse, pig, opossum), if any, is the most advanced. Defend your claim using evolutionary reasoning.

Sample answer*:* None of these species are more advanced.Every living species is as evolved as each other. There is no yardstick by which we measure how evolved or advanced a species is.

STOP & read **S**cience **B**ite:
What Do a Bat, a Pig, a Mouse, and an Opossum Have In Common?

Genes are transcribed into mRNA. Looking at mRNA can tell us something about the function of a gene because we assume that the mRNA will be translated into a protein. Scientists have ways of actually seeing where a particular mRNA is located in a living embryo. The researchers you read about were interested in where and when a specific "toolkit" gene, *hoxd13*, was being expressed (turned into mRNA and eventually into a protein) in a developing limb.They added a dye to the embryos that would show up as purple anywhere the *hoxd13* mRNA was found. The result is shown in **Figure 4**.
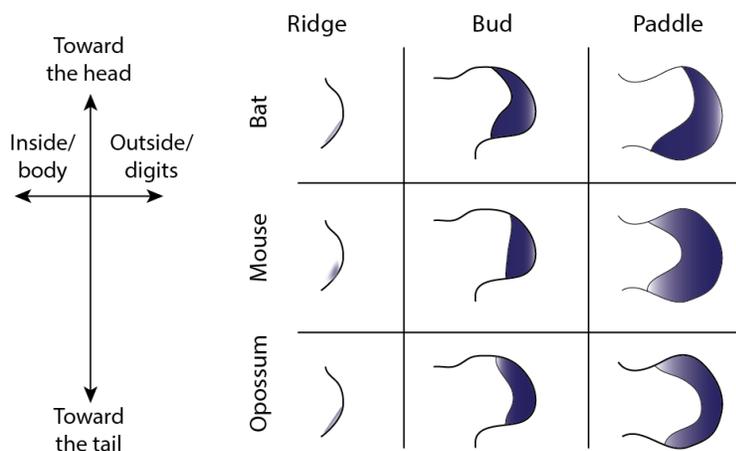

Figure 4. Comparing Expression Patterns. This figure shows where *Hoxd13* mRNA was found at three stages of limb development (ridge, bud, paddle) for three animals (bat, mouse, opossum). Purple color corresponds to gene's expression. The labeled arrows to the left of the figure help you to interpret where the mRNA is being expressed.
Source: Modified from Maier *et al.* 2017.

4. Use **Figure 4** to answer the following questions.

   a. How does the expression of *Hoxd13* change from the ridge stage to the paddle stage in the mouse? Is it a similar pattern compared to the bat and the opossum?

In the mouse, the *Hoxd13* expression increases and expands across the paddle. This is a similar pattern found in the bat and the opossum.

   b. Which developmental stage (ridge, bud, or paddle) is the expression of *Hoxd13* most similar across species? Which is the most different?

The ridge is the most similar, the paddle is the most different.

   c. Look at the forelimb structure of the bat, mouse, and opossum shown in **Figure 1** in the Bite.

   ■ Which part of the forelimb is most similar between species? The humerus

   ■ Which is the least similar? The digits (fingers)

   ■ How does this pattern of gene expression across species relate to the bone structure across species?

Because genes underlie the structures, it makes sense that the most different structures develop from the most different gene expression. Or, most different anatomical structures and gene expression is in the finger area.

   d. Do you think that the gene expression patterns and anatomical structure of the limb are two different lines of evidence for evolution? Why or why not?

Sample answer: Because homologous genes underlie homologous structures, they are really just one line of evidence for evolution.

5. **Connect to the Big Question**. Scientists rely on all kinds of evidence to help them reconstruct evolutionary relationships. Before the discovery of DNA and molecular tools, scientists like Charles Darwin heavily relied on comparing and analyzing anatomical structures. Do you think one form of evidence is more convincing than the other? Why?

Answers will vary. Some things to consider that might come up in a discussion:

● Molecular tools give us an accurate depiction of evolutionary relationships because sometimes anatomical structures can be deceiving. For example, sometimes organisms can develop similar structures due to a similarity in environment/niches (convergent evolution).

● On the other hand, analysis of DNA is not always possible! Fossils, for example, almost never contain recoverable DNA, so it cannot be used to inform us about relationships among extinct organisms. In addition, DNA only has four character states (A,C,T, & G). With only four possibilities, over millions of years of evolution, two DNA sequences could end up looking like each other by chance, especially if the DNA is coding for a important protein. In this scenario, also consider nonsynonymous and synonymous changes to DNA that would mask the true evolutionary scenario.

● Today, both types of data are used to reconstruct evolutionary relationships, and sometimes they give very different stories.