HOW TO DO IT

Applying the Scientific Method & Phylogenetics to Understand the Transition from Kingdoms to Domains: Does One Plus One Equal Five, Six, or Three?

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Abstract

The progression of the taxonomic organization of life from Linnaeus's original two kingdoms to the traditional five-kingdom system to today's widely accepted three-domain system is explored in a group-learning activity. Working with a set of organisms, students organize them into each system. Discussion after each step focuses on viewing classification schemes as hypotheses about the relatedness of organisms and how hypotheses are altered with accumulation of new data. Finally, the connection between phylogenetic trees and the hierarchal system of biological classification is emphasized by using tree-thinking to analyze the universal phylogenetic tree as the basis of the three-domain system.

Key Words: *Phylogenetics; taxonomy; Three Domains; Five Kingdoms; nature of science; active learning.*

In recent years, there has been an emphasis in evolutionary biology education on teaching students what has been called "tree-thinking" (Meisel, 2010). The goal of tree-thinking is for students to understand and critically evaluate phylogenetic trees. Phylogentic trees are used by biologists to represent the evolutionary relationships among organisms, and the branching patterns that these trees show mirror the evolutionary process. Therefore, tree-thinking provides students with a deeper and more thorough understanding of the evolution of biodiversity.

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A logical extension of tree-thinking is its application to teaching taxonomy, the process of classification and identification of organisms. Taxonomy uses phylogenetics to construct classification systems that are essentially hypotheses about the evolutionary relationships among life forms (Case, 2008). With new molecular information being generated every day, taxonomy has become an extremely dynamic science. However, it is often taught as an exercise in memorization or as a "march through the kingdoms" that is unpopular and unsuccessful with students. Providing an evolutionary context through phylogenetics can give students a more meaningful experience and better retention of information (Smith & Cheruvelil, 2009).

One example in which the interaction between phylogenetics and taxonomy has been especially interesting is the current debate about how life should be categorized into kingdoms and domains. The five-kingdom system (Kingdoms Monera, Protista, Fungi, Plantae, Animalia) has been the dominant system taught in schools since the 1960s, when it was developed by R. H. Whittaker, and is still often presented today. With the advent of molecular technology, Woese et al. (1990) used ribosomal RNA sequences to generate a "universal phylogenetic tree" that clearly grouped living organisms into three clusters, which he used to propose his three-domain system (Domains Archaea, Bacteria, Eukarya). Although this new system has gained wide acceptance in the scientific community, its transfer to the classroom has been slower (Peirce, 1999; Offner, 2001; Blackwell, 2004; Case, 2008). Despite the inclusion of the three-domain system in newer state standards for middle school and high school (Case, 2008), at the undergraduate level we often encounter students who are unfamiliar with the domain system or are confused with how domains and kingdoms fit together. One reason may be that the three-domain system requires a re-evaluation of how many kingdoms there actually are and how they fit in the domain structure (Blackwell, 2004). For example, in the five-kingdom system, all prokaryotes are grouped into kingdom Monera. In Woese et al.'s (1990) system, prokaryotes are divided into two different domains, Archaea and Eubacteria, so kingdom Monera no longer exists in that form (Campbell & Reece, 2008). This is particularly confusing to students who have already learned the five-kingdom system and persist in trying to place it within the newer system or who equate the terms *prokaryotes* and *bacteria*. Many newer textbooks present a six-kingdom system (for example, the newest edition of *Biology, Indiana Edition* by Nowicki, 2012) in which prokaryotes are divided into Kingdom Archaea and Bacteria, so that there is a single kingdom in each of the Archaea and Eubacteria domains. Using this system, students often confound the terms *kingdom* and *domain*. Furthermore, this system ignores the debate in the scientific community about the number of kingdoms represented among the prokaryotes, minimizing the diversity represented in these groups.

Here, I present an active-learning exercise to introduce the three-domain system to students. The transition to the three-domain system from the five-kingdom system and from Linnaeus's original two-kingdom system provides students an opportunity to explore

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taxomomy as a scientific process in which hypotheses are proposed and then altered as new information is made available.

○ Learning Activity

This activity was designed for introductory-level undergraduates but could also be used for high school students. It would work best after an introduction to phylogenetics and tree-thinking concepts. Time required: one 45-minute to 1-hour class period.

Part 1

Materials required: for each group of 2–4 students, one envelope with labeled pictures (readily downloaded from the Internet) of the following organisms: cyanobacteria, Gram-positive bacteria, methanogen, halophile, thermophile, ciliate, flagellate, conifer, angiosperm, mushroom, mold, mammal, and arthropod.

1. Have each group cut a piece of paper in half crosswise. Tell them to write "Kingdom Plantae" across the top of one, and "Kingdom Animalia" across the top of the other. Have them place these side by side on their work surface.
2. Have each group work independently pulling out the photographs from the envelope and place them under the heading for the kingdom in which they think the organism belongs.
3. Have the class come together to discuss what they decided. Explain that when Linnaeus devised his system of classification, he divided all life forms into these two kingdoms. Many students will have difficulty placing some of the organisms in one of the two kingdoms. Have them discuss reasons why this was so. Finally, ask them what should be done with the organisms they felt didn't "fit" into these two groups. Most will suggest that more kingdoms are needed.

Part 2

Materials needed: each group will need an envelope with the same set of pictures as in Part 1, but with a short description of some of the characteristics of the organism below the picture (Table 1).

1. Have the students prepare three more "Kingdom" sheets for Monera, Fungi, and Protista. Most undergraduates will be familiar with these groups, but if not, or if high school students are involved, they can consult the Internet or their textbook.
2. Working in their groups, have them rearrange the new pictures under the kingdom headings.
3. Bring the class together again for a discussion of what they came up with. Ask the students why they placed some of the organisms in the categories they did: lead them to the idea that organisms within each group should be more closely related

Table 1. A list of organisms used in this exercise and a short description of each given to the students in Part 2.

Organism	Description
Cyanobacteria	Photosynthetic single-celled organisms without nuclei and with a thin layer of peptidoglycan in their cell walls. Example: *Anabaena* spp.
Gram-positive bacteria	Single-celled organisms without nuclei that have many layers of peptidoglycan in their cell walls, which causes them to be stained purple when exposed to "gram stain." Example: *Staphylococcus aureus*.
Methanogens	Single-celled organisms without a nucleus that feed off hydrogen and release methane gas and lack peptidoglycans in their cell walls. Example: *Methanopyrus kandleri*.
Halophiles	Single-celled organisms without a nucleus that live, grow, and reproduce in environments with high salt concentrations and that lack peptidoglycans in their cell walls. Example: *Halococcus dombrowskii*.
Thermophiles	Single-celled organisms without a nucleus that metabolize sulfur, thrive at temperatures >150°F, and lack peptidoglycans in their cell walls. Example: *Thermus aquaticus*.
Ciliates	Single-celled organisms with a complex internal structure that move by the beating of many short hair-like projections on the surface of the cell. Example: *Paramecium* spp.
Flagellates	Single-celled organisms with internal organelles that possess one or more long, slender flagella used for locomotion. Example: *Trypanosoma gambiense*.
Conifers	Large, multicellular photosynthetic organisms with needle-like leaves, whose reproductive structures are often in the form of cones. Example: White pine, *Pinus strobus*.
Angiosperms	Multicellular photosynthetic organisms that reproduce through structures called *flowers*. Example: Virginia rose, *Rosa virginiana*.
Mushrooms	Multicellular organisms that absorb nutrition through mostly dead organisms and reproduce by producing cells called *spores*. Example: Portobello mushroom, *Agaricus bisporus*.
Mold	Microscopic organisms that are made up of filaments called *hyphae* and that reproduce by producing cells called *spores* that are distributed by air, water, or insects. Example: *Penicillium* spp.
Mammals	Multicellular organisms that ingest their food, have hair and mammary glands, and breathe air. Example: domestic cat, *Felis catus*.
Arthropods	Multicellular organisms that ingest their food and have jointed appendages and exoskeletons made of chitin. Example: cockroach, *Periplaneta americana*.

to each other than to organisms in other groups. For example, humans are more closely related to arthropods than to flagellates. Ask the students if they could divide each group into further subdivisions, and use this to introduce again the idea of nested hierarchies and how evolution leads to this pattern.

4. Finally, ask students whether this category system is more informative than the system presented in Part 1. Ask them why they think scientists changed their minds about how to classify organisms. Someone should come up with the statement that as scientists learned more about the organisms, they were able to develop a better system of classification. Introduce the idea of classification systems as scientific hypotheses and remind students of the scientific method of constantly reviewing and revising such hypotheses on the basis of current information.

Part 3

Materials needed: each group will need a copy of Woese et al.'s (1990) universal phylogenetic tree, such as the one found in Peirce (1999), with the domain names or labels removed.

1. As students are finishing the discussion from Part 2, ask them whether, as a scientific hypothesis, they can come up with evidence that might now be available to test the five-kingdom system of classification that wasn't available in the 1960s when it was developed. Students will readily come up with DNA or molecular evidence.
2. Distribute a copy of Woese et al.'s (1990) universal phylogenetic tree. Give a brief explanation of the data used to create this tree. After students have had a couple of minutes to examine the tree, ask them to find their five kingdoms on the tree. They should realize that they are spread out along the branches. Ask whether they see any "natural" groupings in the tree and to circle them.
3. Have the students make three more labels: Domain Archaea, Domain Bacteria, and Domain Eukarya. Then have them arrange their strips of paper on which they wrote the name of the kingdoms, along with the strips on which the description of the organisms are written, underneath these new labels. When the students get to kingdom Monera, they should have a problem, as organisms in this group are in both domain Archaea and domain Bacteria. When students start to question this, make a big display by having all student groups hold up their "Kingdom Monera" label and rip it in half. This tactile experience is very effective for students to picture the relationship between Monera and the two prokaryotic domains. Then have the students place the organisms that were in this kingdom into their proper domains.
4. Introduce the idea that kingdoms are now nested within domains. Discuss the six-kingdom classification system. Ask the students whether there could be more than six kingdoms. Discuss the idea of scientific debate and tell them that the number of kingdoms is still being determined. Give some examples of different proposed prokaryotic kingdoms, such as Acidobacterium, Crenarchaeota, Proteobacteria, etc. (Campbell & Reece, 2008).
5. In the final discussion, ask students how phylogenetics has informed how we classify organisms. To emphasize how classification and the nested hierarchal system result from the evolutionary process, ask students to answer questions about the relatedness of organisms, such as "What is more closely related to a methanogen, a cyanobacteria or a human?" or "How are the kingdoms within domain Eukarya related to each other?"

○ Further Discussion Topics & Alternative Exercises

1. Discuss the meaning of the term *prokaryote*. How does this term change in the five-kingdom to the three-domain system? The five-kingdom system gives the false impression that eukaryotes encompass most of the living organisms on the planet (four-fifths!) (Peirce, 1999). How does this view of biodiversity change under the three-domain system?
2. Phylogenetic trees are hypotheses about the relatedness of living organisms, and as such are altered as new information is gathered. Students often feel that all scientific hypotheses are equal. However, there is one true phylogenetic tree that represents this relationship because these living organisms went through one evolutionary pathway.
3. Classification schemes are frequently described as human constructs, whereas phylogenetics represents "real" relationships among organisms (Doolittle, 2009). Have students debate whether the categories species, phylum, kingdom, and domain represent real biological units or are just convenient labels.
4. As an alternative, the pictures and descriptions of organisms could be laminated, with a magnet glued to the back. The instructor could then place these on a white board at the front of the class and have the students work through the exercise as one large group.
5. If more than a single class period can be used, a table of traits or DNA sequences could be constructed for the organisms that the students could then use to generate phylogenetic trees using a computer program such as MacClade.

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