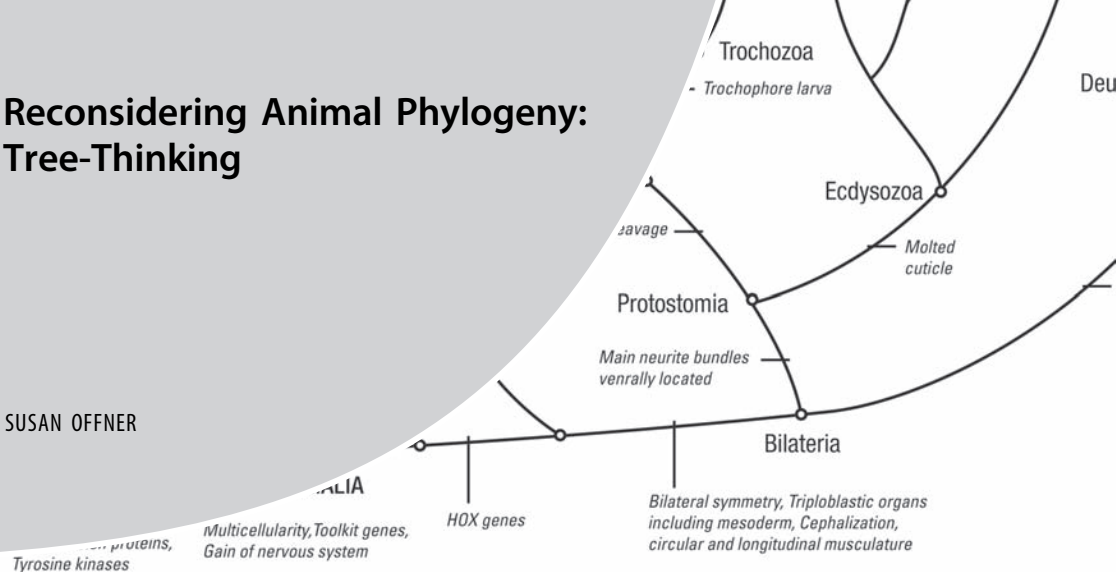


Reconsidering Animal Phylogeny: Tree-Thinking

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ABSTRACT

New phylogenomic methods have made it possible to obtain a robust phylogenetic tree of the animal kingdom. The resulting tree confirms much of what was already known but also contains some nice surprises.

Key Words: Evolution; phylogeny; animal kingdom; DNA.

Evolution is the central, unifying theme in biology. As DNA sequencing has provided easily understood evidence for evolution, I make it a personal goal to discuss evolution every day in class. I do not always succeed, but just trying has enriched many lessons.

An unintended consequence of genome sequencing is rich and irrefutable evidence for evolution. It gives us an opportunity to make evolution real for our students, something that is easily understandable within their frames of reference. When people ask me if I believe in the theory of evolution, I tell them that I “believe” in the theory of evolution about as much as I “believe” in the theory of the round Earth. The Earth is round. Evolution happened and continues to happen.

It is important that students be comfortable with evolution. Many are concerned that it will undermine their religious or philosophical beliefs. I reassure them that evolution and religion are compatible. Evolution is concerned with the natural world – with things for which there is evidence, things that can be seen and measured, things that people of good will can agree upon. Religion discusses different questions that are also very important. How do you get along with your parents? How do you get along with your brothers and sisters? How do you live in a community of people? What is the meaning of life? Once students see that evolution does not threaten their personal beliefs, they can relax and learn the material.

Phylogenetic trees make it possible to discuss evolution every day. They are visual and easy to understand. They are grounded in an objective reality and encourage students to ask scientific

questions. They show that science is not only for “geniuses,” whoever they may be, but also for ordinary people interested in the world around them. Trees show when extant organisms last had a common ancestor with each other, and when in the history of life different traits arose.

The nine major animal phyla have been known for centuries and are a standard part of any introductory biology course, yet the evolutionary relationships between these phyla have been extraordinarily difficult to tease out. Here, I present a modern animal tree that only recently has become available and that can be used in a wide variety of biology classes to teach both diversity and evolution.

Clades

A clade is a group of organisms consisting of a common ancestor and *all* of its descendants. It is the same as a monophyletic group. As you work more with trees, you will see why this is such a central concept. A clade is a natural group because all members of a clade are more closely related to each other than to any organism outside of the clade. For this reason, only clades should be used as taxonomic categories in classification. In the case of the animal tree, all animal phyla are clades. However, when you look at other trees, you find surprises. For example, in a tetrapod tree, it turns out that reptiles are not a clade because all the descendants of the common ancestor of reptiles include birds – so the clade, and the group used in taxonomy, should be reptiles and birds. In the chordate tree, there is a similar surprise. Osteichthyes, or bony fish, are actually three clades, each of which should be treated as a separate taxonomic category. There

are many excellent papers on the importance of tree-thinking in biology teaching (e.g., Baum et al., 2005; Baum & Offner, 2008) that can further enhance your approach to this topic.

Phylogenetic trees make it possible to discuss evolution every day.

○ The Cambrian Explosion

The Cambrian Explosion was a pivotal event that occurred ~541 million years ago, at the beginning of the Cambrian period. At this time, at least half of the modern animal phyla suddenly appear in the fossil record. Fossils from this time are beautifully preserved in the Burgess Shale in Canada, as discussed by Stephen Jay Gould in *Wonderful Life* and described by Briggs et al. (1995). Other Cambrian fossils very similar to those seen at the Burgess Shale are found in the Chengjiang Formation in China, as described in Xian-Guang et al. (2004). The fact that similar fossils are found at completely opposite sides of the world shows that these organisms lived all over the world. We know them today from the fossil sites where they happened, by chance, to be preserved, but they typically had global distributions. Students are fascinated by these fossils and by what they represent about the emergence of animals. It gives them a framework for thinking about the entire history of life on Earth.

Many modern animal phyla are first represented in the fossil record during the Cambrian Explosion. The different phyla diverged from each other in a brief period of 25 million years at the beginning of the Cambrian (Kouchinsky et al., 2012). They have been reproductively isolated from each other ever since, for over half a billion years. This makes it extremely difficult to tease out the few mutations that occurred during the very short time of differentiation from the more than half a billion years of subsequent mutation. For many years, people thought it might be impossible to obtain a robust animal tree.

○ The Animal Tree

In the past 10 years, new phylogenomic tools have been developed to solve these problems. These tools use large numbers of organisms and large numbers of genes, along with powerful computers and intricate computer programs. The first phylogenetic tree including a large diversity of animal phyla was published in *Nature* in April 2008 (Dunn et al., 2008). In this study, 71 taxa and 150 different genes were used. The resulting animal tree is presented in Figure 1, with slight modifications as a result of subsequent work.

The tree confirms much about what we already thought was true. Animals with bilateral symmetry (Bilateria) are a clade. The Bilateria clade itself is divided into two enormous clades, Protostomia and Deuterostomia. Yes, the starfish really is one of the closest relatives of chordates. Previously, protostomes had been distinguished from deuterostomes by their early embryological development. DNA sequencing shows that these embryological signals are evolutionarily significant. Nor is it a surprise that both bilateral symmetry (and the accompanying triploblastic tissues derived from the three layers of embryonic tissue – ectoderm, endoderm, and mesoderm) and cephalization (the presence of a head with sense organs) evolved only once, in the common ancestor of the clade Bilateria.

However, the tree also contains many surprises. It recognizes two major clades of Protostomes, the Spiralia and the Ecdysozoa (Dunn et al., 2014). The big surprise is that these two clades have nothing to do with the presence of a coelom. It turns out that the coelom, although anatomically significant, is not evolutionarily significant. Platyhelminthes (no coelom) are closely related to Mollusca and Annelida (true coelom) in the Spiralia clade, while Nematoda

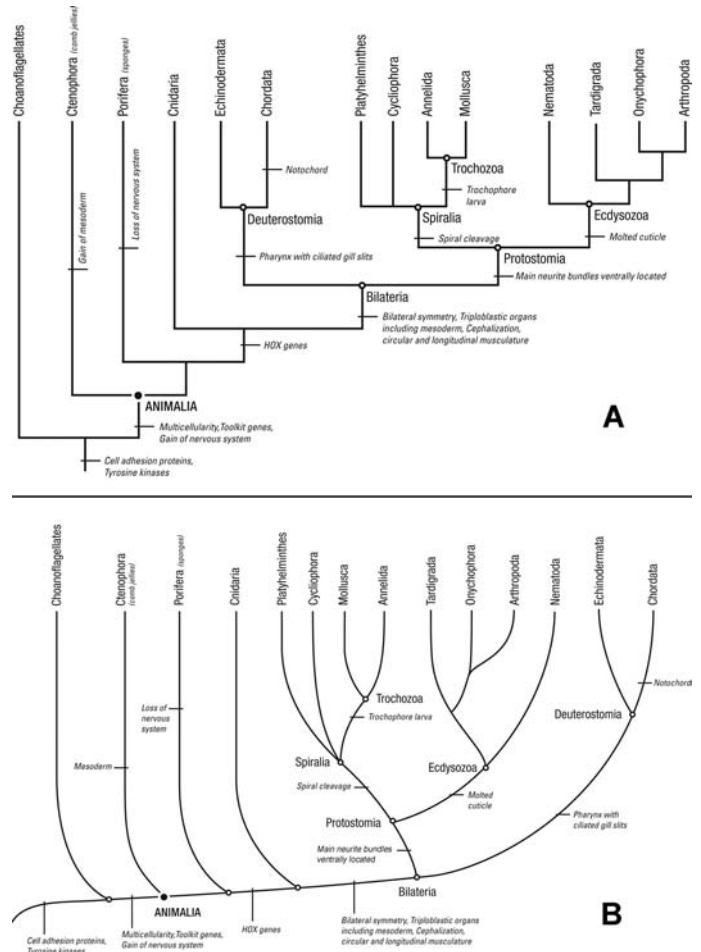


Figure 1. An animal phylogeny. This figure is presented in two different ways: panel **A** shows a tree drawn in the more traditional way; panel **B** is a more casual representation that I prefer to use in my own classes because I think it is easier for students to follow. However, the two trees are identical, and both are equally valid scientifically. Two trees are identical if all the clades represented in them are the same, as it is for these two versions of the animal tree. Use whichever one you are most comfortable with, or use them both. Having students study why these two trees are equivalent will help them become proficient at reading trees, an important skill.

(pseudocoelom) are closely related to the Arthropoda (true coelom) in the Ecdysozoa clade. The Spiralia are further divided into two clades. One of these contains the Platyhelminthes. The other one, called the Trochozoa, contains both Mollusks and Annelids and is characterized by the presence of a trochophore larva. The Ecdysozoa (Arthropoda, Nematoda) molt. As a result, we should teach more about trochophore larvae and molting since these are conserved core properties of major animal clades.

○ The Base of the Tree Suggests Some Important Questions

The base of the tree contains a surprise as well, and one that seems to be confirmed by subsequent research. Many people assumed

that sponges were the earliest branching group of animals, since they have no true tissues. But the initial April 2008 (Dunn et al., 2008) tree hands that distinction to Ctenophora, the comb jellies. This surprising finding was reinforced in 2013 with the publication of the first ctenophore genome (Ryan et al., 2013). This further refined the base of the tree, as shown in Figure 1. The implications of this are fascinating and make for excellent classroom discussions. Does this mean that diploblasty, the presence of ectoderm and endoderm, evolved twice? Or did they evolve once, in the common ancestor of all animals, and become lost in the sponges, which do not have tissues? More tantalizingly, ctenophores have what appear to be tissues derived from mesoderm, but the genes involved in ctenophore mesoderm are different from the genes involved in the mesoderm of Bilateria. The most likely implication of this is that mesoderm evolved twice: once in the common ancestor of ctenophores, and again in the common ancestor of Bilateria. And the presence of a nervous system in ctenophores implies that a nervous system was present in the common ancestor of all animals and subsequently lost in the sponges. In fact, the genes involved in the development of the nervous system are found in sponge genomes, but the cell types of the nervous system are not present. This raises yet another fascinating question. If genes have been conserved over long stretches of evolutionary time, it is likely that they serve a function. If genes do not serve a function, they decay over evolutionary time, since mutations in them are not selected against. So it is reasonable to ask what function nervous-system genes have in sponges, animals that do not have nerve cells. Finally, none of this is by any means definite, and much research is being done to further clarify the evolutionary relationships at the base of the animal tree. This, like so much science, is an ongoing story that should enrich our teaching and delight our students for years to come. They, too, can understand the questions that evolutionary biologists are wrestling with today.

○ Toolkit Genes

One of the conserved core properties of animals is the presence of what are known as “toolkit genes.” These are a set of ~300 genes that are found in all animal phyla. For perspective, the human genome contains ~20,000 genes, so the toolkit genes are a small, but significant, portion of an animal’s genome. They are expressed very early in animal development and are responsible for establishing the basic body plan of the animal. They control things like where the arms and legs or wings should be, whether they should be arms or wings, and which cells will develop into eyes or a heart. These genes are highly conserved across the animal kingdom. For example, a mouse gene for determining the presence of an eye has been inserted into fruit flies, and it functions perfectly well. So, you might ask, why do people have a different body plan from fruit flies? The answer is that these genes differ in their *regulatory* regions between the different phyla. They are expressed at different times in development, for different amounts of time, and in different quantities. It is these differences in the regulatory regions of the toolkit genes, in addition to differences in the structural genes themselves, that account for the different body plans found in the different animal phyla. Some examples of toolkit genes are the HOX, or homeotic, genes that determine the appendages on each

segment of an animal’s body; a gene whimsically called *tinman* that, when expressed, will cause a cell to develop into a heart; and a gene called *eyeless* that, when expressed, will cause a cell to develop into an eye.

○ Ctenophores

Until now, I never included ctenophores as one of the nine major animal phyla I discussed with my students. But now that it really looks like they are at the base of the animal tree, I spend some time on them. Their common name is “comb jellies,” and they are carnivores with eight rows of cilia running down their bodies. Ctenophores are magnificent animals, and a simple web search for “ctenophore images” will yield many striking pictures and videos that will delight your students.

○ Choanoflagellates

Choanoflagellates are the outgroup for the animal kingdom, as shown in Figure 1. This means they are the organisms most closely related to animals that are not themselves animals. Choanoflagellates are one-celled organisms that resemble collar cells of sponges. They are ubiquitous in both freshwater and saltwater environments (King, 2005). Because of their resemblance to collar cells in sponges, they were long assumed to be the outgroup to animals. This relationship was confirmed in 2008 when the choanoflagellate genome was published (King et al., 2008). There are choanoflagellate images on the web that are fascinating to show in class.

The choanoflagellate genome offers some insights into the origins of animals. Choanoflagellates possess genes for proteins called “cadherins” that are involved in cell adhesion. Cadherins are some of the proteins that connect cells in animals, allowing for multicellularity. Further, choanoflagellates possess genes that code for tyrosine kinases, proteins involved in cell-to-cell communication, a necessity for multicellular organisms. Yet choanoflagellates are not multicellular. But what this shows is that the origin of genes necessary for multicellularity evolved before multicellularity. The function of these genes in choanoflagellates is being studied. Multicellularity itself evolved independently at least 20 times in the history of life. Its evolution in the common ancestor of all animals is just one of these times.

I always enjoy teaching this material. I tell my students that it is at the same time the oldest and the newest unit in the course. The descriptions of the animal phyla have not really changed since I was in high school, long ago in the last millennium; what has changed is that we now know the evolutionary relationships between the phyla. As a result, we can infer when key innovations occurred in animal evolution, as shown on this tree. It makes it possible to teach about the diversity of life in an evolutionary context, rather than as a set of disconnected factoids to be memorized. For example, instead of memorizing all the phyla that have triploblastic tissues, students can see that triploblastic tissues evolved once, in the common ancestor of the Bilateria clade. As a result, triploblastic tissues are present in all Bilateria phyla. The same reasoning can be used for any trait on the tree – being careful, of course, to point out that occasionally a trait can be lost in the course of evolution. Such an approach makes it easier for us to teach evolution every day.

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The late SUSAN OFFNER was a biology teacher at Lexington High School in Lexington, Massachusetts. A lifelong member of NABT, she published dozens of articles in this journal. Susan died peacefully at home on February 13, 2016, surrounded by family.

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