The central claim of the theory of evolution as laid out in 1859 by Charles Darwin in *The Origin of Species* is that living species, despite their diversity in form and way of life, are the products of descent (with modification) from common ancestors. To communicate this idea, Darwin developed the metaphor of the “tree of life.” In this comparison, living species trace backward in time to common ancestors in the same way that separate twigs on a tree trace back to the same major branches. Coincident with improved methods for uncovering evolutionary relationships, evolutionary trees, or phylogenies, have become an essential element of modern biology (1). Consider the case of HIV/AIDS, where phylogenies have been used to identify the source of the virus, to date the onset of the epidemic, to detect viral recombination, to track viral evolution within a patient, and to identify modes of potential transmission (2). Phylogenetic analysis was even used to solve a murder case involving HIV (3). Yet “tree thinking” remains widely practiced only by professional evolutionary biologists. This is a particular cause for concern at a time when the teaching of evolution is being challenged, because evolutionary trees serve not only as tools for biological researchers across disciplines but also as the main framework within which evidence for evolution is evaluated (4, 5).

At the outset, it is important to clarify that tree thinking does not necessarily entail knowing how phylogenies are inferred by practicing systematists. Anyone who has looked into phylogenetics from outside the field of evolutionary biology knows that it is complex and rapidly changing, replete with a dense statistical literature, impasioned philosophical debates, and an abundance of highly technical computer programs. Fortunately, one can interpret trees and use them for organizing knowledge of biodiversity without knowing the details of phylogenetic inference. The reverse is, however, not true. One cannot really understand phylogenetics if one is not clear what an evolutionary tree is.

The preferred interpretation of a phylogenetic tree is as a depiction of lines of descent. That is, trees communicate the evolutionary relationships among elements, such as genes or species, that connect a sample of branch tips. Under this interpretation, the nodes (branching points) on a tree are taken to correspond to actual biological entities that existed in the past: ancestral populations or ancestral genes. However, tree diagrams are also used in many nonevolutionary contexts, which can cause confusion. For example, trees can depict the clustering of genes on the basis of their expression profiles from microarrays, or the clustering of ecological communities by species composition. The prevalence of such cluster diagrams may explain why phylogenetic trees are often misinterpreted as depictions of the similarity among the branch tips. Phylogenetic trees show historical relationships, not similarities. Although closely related species tend to be similar to one another, this is not necessarily the case if the rate of evolution is not uniform: Crocodiles are more closely related to birds than they are to lizards, even though crocodiles are indisputably more similar in external appearance to lizards. But what does it mean to be “more closely related”? Relatedness should be understood in terms of common ancestry— the more recently species share a common ancestor, the more closely related they are. This can be seen by reference to pedigrees: You are more closely related to your first cousin than to your second cousin because your last common ancestor with your first cousin lived two generations ago (grandparents), whereas your last common ancestor with your second cousin lived three generations ago (great-grandparents). Nonetheless, many introductory students and even professionals do not find it easy to read a tree diagram as a depiction of evolutionary relationships. For example, when presented with a particular phylogenetic tree (see the figure, left), people often erroneously conclude that a frog is more closely related to a fish than to a human. A frog is actually more closely related to a human than to a fish because the last common ancestor of a frog and a human (see the figure, label x) is a descendant of the last common ancestor of a frog and a fish (see the figure, label y), and thus lived more recently. [To evaluate your tree-thinking skills, take the quizzes (6)].

Why are trees liable to misinterpretation? Some evolutionary biologists have proposed that nonspecialists are prone to read trees along the tips (1, 7), which in this case yields an ordered sequence from fish to frogs and ultimately to humans. This incorrect way to read a phylogeny may explain the widely held but erroneous view that evolution is a linear progression from primitive to advanced species (8), even though a moment’s reflection will reveal that a living frog cannot be the ancestor of

D.A. Baum and S. D. Smith are in the Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, WI 53706, USA. E-mail: dbaum@wisc.edu; sdssmith@wisc.edu S. S. Donovan is in the Department of Instruction and Learning, University of Pittsburgh, Pittsburgh, PA 15260, USA. E-mail: sdonovan@pitt.edu

Which phylogenetic tree is accurate? On the basis of the tree on the left, is the frog more closely related to the fish or the human? Does the tree on the right change your mind? See the text for how the common ancestors (x and y) indicate relatedness.
a living human. The correct way to read a tree is as a set of hierarchically nested groups, known as clades. In this example, there are three meaningful clades: human-mouse, human-mouse-lizard, and human-mouse-lizard-frog. The difference between reading branch tips and reading clades becomes apparent if the branches are rotated so that the tip order is changed (see the figure, right). Although the order across the branch tips is different, the branching pattern of evolutionary descent and clade composition is identical. A focus on clade structure helps to emphasize that there is no single, linear narrative of evolutionary progress (1, 7).

There are other problems in reading relationships from trees (9). For example, there is a common assumption that trait evolution happens only at nodes. But nodes simply represent places where populations became genetically isolated, permitting them to accumulate differences in their subsequent evolution. Similarly, living species may be mistakenly projected backward to occupy internal nodes of a tree. But it is incorrect to read a tree as saying that humans descended from mice when all that is implied is that humans and mice shared a common ancestor. Thus, for all its importance, tree thinking is fraught with challenges.

Tree thinking belongs alongside natural selection as a major theme in evolution training. Further, trees could be used throughout biological training as an efficient way to present information on the distribution of traits among species. To this end, what is needed are more resources: computer programs (10), educational strategies (11, 12), and accessible presentations of current phylogenetic knowledge (13–15).

Phylogenetic trees are the most direct representation of the principle of common ancestry—the very core of evolutionary theory—and thus they must find a more prominent place in the general public’s understanding of evolution. As philosopher of science Robert O’Hara (16) stated, “just as beginning students in geography need to be taught how to read maps, so beginning students in biology should be taught how to read trees and to understand what trees communicate.” Among other benefits, as the concept of tree thinking becomes better understood by those in the sciences, we can hope that a wider

References
6. See the two quizzes on Science Online.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5750/979/DC1
Tree-Thinking Quizzes I and II

10.1126/science.1117727

CHEMISTRY

Following the Flow of Energy in Biomolecules

Paul M. Champion

Some biological molecules, such as those in visual or photosynthetic systems, have evolved to efficiently convert energy from one form to another. How do these molecules channel energy rapidly and efficiently so that useful work can be performed without this energy being dissipated ineffectively into the surroundings? Dissipation of molecular vibrational excitation energy typically takes place on picosecond time scales, so biological molecules must be able to channel energy rapidly and efficiently if they are to be able to direct it in a useful manner. In biological systems excited by light, the nonradiative electronic transitions can occur on time scales (< 10^-12 ps) that are even faster than vibrational energy dissipation (1–3), hinting at how nature solves the problem of directing energy flow. On page 1006 of this issue, Kukura et al. (4) take an important step forward in defining the process of directed energy flow in the visual pigment rhodopsin.

Photoexcited biological molecules offer a unique opportunity to monitor the evolution of excitation energy as it transforms a reactant molecule into its final products. With the advent of appropriate femtosecond laser techniques (5), it has become possible to examine the underlying dynamics of the elementary vibrational and electronic excitations that guide the structural changes and, ultimately, the function of a variety of biomolecules (6–8). The work presented by Kukura et al. enhances our ability to monitor rapid structural changes in such molecules by introducing the technique of femtosecond stimulated Raman spectroscopy (FSRS). In their report, Kukura et al. follow the evolution of the retinal chromophore as it is excited to photorhodopsin and decays into bathorhodopsin, all within the first picosecond of the visual process. They do this by taking advantage of the broad spectral bandwidth of their probe pulse to obtain very high quality time-resolved stimulated Raman spectra over the range of 600 to 2000 cm^-1.

How does this experiment generate ultrafast time resolution, as well as the high spectral resolution associated with Raman spectra, without violating the uncertainty principle? Although not emphasized in the report by Kukura et al., these authors are fully aware (9) that the underlying time scale for the generation of the Raman photon is dictated by the dephasing time of the coherence between the initial and final vibrational levels of the material undergoing the Raman process. A typical time scale for the vibrational dephasing time is on the order of 10^-15 s, which translates to a 10 cm^-1 Raman bandwidth. This means that the FSRS experiment reads out Raman radiation from the sample that is averaged over its vibrational dephasing time window (that is, the stimulated Raman signals continue to appear at the detector, even after the probe pulse has passed through the sample). Thus, there is no violation of the uncertainty principle. However, being able to control the “gating” of the Raman coherence by changing the time delay between the photochemical pump and the broadband probe allows the dephasing time window to be moved so that rapid structural dynamics can be monitored. Changes in the vibrational frequencies that take place within the dephasing time window affect the FSRS lineshape, and the authors have done a convincing job of simulating these lineshape changes as shown in the supporting online material of their paper.

A key conclusion of the work on rhodopsin is that low-symmetry hydrogen out-of-plane (HOOP) wagging motions
Supporting Online Material for

The Tree-Thinking Challenge

David A. Baum,* Stacey DeWitt Smith, Samuel S. Donovan

*To whom correspondence should be addressed. E-mail: dbaum@wisc.edu

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This PDF file includes:

Tree-Thinking Quizzes I and II
Basic Tree Thinking Assessment  
David A. Baum, Stacey DeWitt Smith, Samuel S. Donovan

This quiz includes a number of multiple-choice questions you can use to test yourself on your ability to accurately interpret evolutionary trees. Insofar as real biological examples have been used they are accurate based on current knowledge.

1) By reference to the tree above, which of the following is an accurate statement of relationships?
   a) A green alga is more closely related to a red alga than to a moss 
   b) A green alga is more closely related to a moss than to a red alga 
   c) A green alga is equally related to a red alga and a moss 
   d) A green alga is related to a red alga, but is not related to a moss

2) By reference to the tree above, which of the following is an accurate statement of relationships?
   a) A crocodile is more closely related to a lizard than to a bird 
   b) A crocodile is more closely related to a bird than to a lizard 
   c) A crocodile is equally related to a lizard and a bird 
   d) A crocodile is related to a lizard, but is not related to a bird
3) By reference to the tree above, which of the following is an accurate statement of relationships?
   a) A seal is more closely related to a horse than to a whale
   b) A seal is more closely related to a whale than to a horse
   c) A seal is equally related to a horse and a whale
   d) A seal is related to a whale, but is not related to a horse

4) Which of the five marks in the tree above corresponds to the most recent common ancestor of a mushroom and a sponge?
5) If you were to add a trout to the phylogeny shown above, where would its lineage attach to the rest of the tree?

6) Which of trees below is false given the larger phylogeny above?
7) Which of the four trees above depicts a different pattern of relationships than the others?

8) Which of the four trees above depicts a different pattern of relationships than the others?
9) In the above tree, assume that the ancestor had a long tail, ear flaps, external testes, and fixed claws. Based on the tree and assuming that all evolutionary changes in these traits are shown, what traits does a sea lion have?
   a) long tail, ear flaps, external testes, and fixed claws
   b) short tail, no ear flaps, external testes, and fixed claws
   c) short tail, no ear flaps, abdominal testes, and fixed claws
   d) short tail, ear flaps, abdominal testes, and fixed claws
   e) long tail, ear flaps, abdominal testes, and retractable claws

10) In the above tree, assume that the ancestor was a herb (not a tree) without leaves or seeds. Based on the tree and assuming that all evolutionary changes in these traits are shown, which of the tips has a tree habit and lacks true leaves?
   a) *Lepidodendron*
   b) Clubmoss
   c) Oak
   d) *Psilotum*
   e) Fern
Basic Tree Thinking Assessment
David A. Baum, Stacey DeWitt Smith, Samuel S. Donovan

This quiz includes a number of multiple-choice questions you can use to test yourself on your ability to accurately interpret evolutionary trees. Insofar as real biological examples have been used they are accurate based on current knowledge.

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   d) A green alga is related to a red alga, but is not related to a moss

   ‘b’ is correct. The most recent common ancestor of a green alga and a moss is at node x whereas the most recent common ancestor of a red alga and a moss is at the “deeper” node, y. If you picked ‘c’ you might be reading along the tips.

2) By reference to the tree above, which of the following is an accurate statement of relationships?
   a) A crocodile is more closely related to a lizard than to a bird
   b) A crocodile is more closely related to a bird than to a lizard
   c) A crocodile is equally related to a lizard and a bird
   d) A crocodile is related to a lizard, but is not related to a bird

   ‘b’ is correct. The most recent common ancestor of a crocodile and a bird is at node x whereas the most recent common ancestor of a crocodile and a lizard is at the “deeper” node, y. If you picked ‘a’ you might be reading along the tips.
3) By reference to the tree above, which of the following is an accurate statement of relationships?
   a) A seal is more closely related to a horse than to a whale
   b) A seal is more closely related to a whale than to a horse
   c) A seal is equally related to a horse and a whale
   d) A seal is related to a whale, but is not related to a horse

   ‘c’ is correct. The most recent common ancestor of a seal and a whale is at node y, as is the most recent common ancestor of a seal and a horse. All descendants of node x are equally related to the seal. If you picked ‘a’ you might be reading along the tips.

4) Which of the five dots in the tree above corresponds to the most recent common ancestor of a mushroom and a sponge?
   ‘d’ is the correct answer. ‘a’ is a living species and is not an ancestor. ‘e’ is an ancestor of a sponge but not of a mushroom. ‘b’ and ‘c’ are common ancestors of a sponge and a mushroom, but they are more ancient common ancestors than ‘d’.

4) Which of the five dots in the tree above corresponds to the most recent common ancestor of a mushroom and a sponge?
5) If you were to add a trout to the phylogeny shown above, where would its lineage attach to the rest of the tree?

6) Which of the trees below is false given the larger phylogeny above?

‘c’ is the correct answer. This depends on only the knowledge that a salmon and a trout are very closely related. Therefore they must share a more recent common ancestor with each other than with any other included species. Position ‘c’ is the only place such an ancestor could be.

‘d’ is the correct answer. ‘d’ shows yeast being more closely related to plants than it is to animals. The true phylogeny (right) shows that yeast is more closely related to human than to any of the plants (green algae, lily, and fern).
7) Which of the four trees above depicts a different pattern of relationships than the others?

'c' is the correct answer. In all the other trees C is more closely related to E and D than to B. In 'c,' C is more closely related to B than to E or D.

8) Which of the four trees above depicts a different pattern of relationships than the others?

'a' is the correct answer. In all the other trees B is more closely related to D and E than is C. In 'a,' C is more closely related to D and E than is B.
9) In the above tree, assume that the ancestor had a long tail, ear flaps, external testes, and fixed claws. Based on the tree and assuming that all evolutionary changes in these traits are shown, what traits does a sea lion have?

a) long tail, ear flaps, external testes, and fixed claws  
b) short tail, no ear flaps, external testes, and fixed claws  
c) short tail, no ear flaps, abdominal testes, and fixed claws  
d) short tail, ear flaps, abdominal testes, and fixed claws  
e) long tail, ear flaps, abdominal testes, and retractable claws

10) In the above tree, assume that the ancestor was an herb (not a tree) without leaves or seeds. Based on the tree and assuming that all evolutionary changes in these traits are shown, which of the tips has a tree habit and lacks true leaves?

a) *Lepidodendron*  
b) Clubmoss  
c) Oak  
d) *Psilotum*  
e) Fern

‘d’ is the correct answer. Tracing up from the ancestor to sea lions, one sees that the only changes are in tail length and testes position. For the other traits, they have retained the ancestral condition.

‘a’ is the correct answer. Clubmosses are not trees. Oak (and yew) are trees but they have leaves. *Psilotum* lacks leaves, but it is not a tree. A fern has leaves and is not a tree.

1) The figure above shows the phylogeny estimated for a sample of flowering plants (angiosperms) from *PHOTCHROME A* and *PHOTCHROME C*, a pair of genes that duplicated prior to the origin of the angiosperms. Which of the following sets of taxa constitute a clade (=monophyletic group) on one gene tree but not on the other?

- a) *Degeneria-Magnolia-Eupomatia*
- b) All angiosperms except *Amborella*
- c) *Austrobaileyana-Nymphaea-Cabombaceae*
- d) *Nelumbo-Trochodendron-Aquilegia*

2) The dendrogram on the left clusters plant species by chemical similarity; each of the four main chemical groups is indicated with a different color. This tree does not depict descent relationships, just degree of chemical similarity. On the right, the evolution of these chemical types is reconstructed on a phylogeny of the plants (this does depict inferred evolutionary relationships). The colors correspond to the chemical groups on the left, and the gray branches indicate uncertainty in character reconstruction. What does a comparison of these two figures tell us about the evolution of plant secondary chemistry?

a) The four groups of chemically similar species each constitutes a distinct evolutionary lineage
b) The group colored “black” has the most advanced chemical defenses
c) The red (3) and blue (1) chemical groups are most distantly related
d) The chemical groups have each been gained and/or lost multiple times in evolution

3) This tree depicts inferred relationships among some major frog groups with branches drawn proportional to absolute time. Error bars on internal nodes depict confidence intervals on the dates of estimated nodes. Assuming this tree and the associated ages are correct which of the following statements is true?

a) No individual living before 70 million years ago is an ancestor of Raninae

b) Raninae and Dicroglossinae shared a common ancestor about 75 million years ago

c) The divergence of Raninae and Nyctibatrachinae occurred more recently than the 85 million year old separation of India from Madagascar

d) The last common ancestor of Micrixalinae and Dicroglossinae lived before India and Madagascar separated (85 million years ago)

4) *Retia mirabilia* (sing. *rete mirabile*) are vascular bundles that allow fish to secrete O$_2$. In the above figure, red branches indicate lineages with choroid *retia*, blue branches indicate those with swimbladder *retia*, and white branches indicate absence of *retia*. Assuming the phylogeny and character evolution have been accurately inferred, we can see that:

a) Swimbladder *retia* predate choroid *retia*

b) Gains of swimbladder *retia* primarily took place in lineages that already had choroid *retia*.

c) Loss of choroid *retia* causes gain of swimbladder *retia*

d) Choroid *retia* have been gained more often than swimbladder *retia*
5) Each row in the table above lists a set of four bacterial taxa whose relationship follows the topology shown. Thus each row can be read as a four-taxon tree. Which of the four trees below is compatible with the information in the three rows of the table?
6) Lemuriformes are currently restricted to Madagascar, whereas Lorisiformes are found in Africa and Asia but not Madagascar, and Tarsius is Asian. The tree above was generated in order to assess the relationship of a fossil, Bugtilemur, found in 30 million year old deposits in Pakistan. Each branch of the tree has been annotated with two numbers, the first of which is the bootstrap percentage, a measure of support. In order to hold that Bugtilemur is more closely related to Lorisiformes than to Lemuriformes what is the minimum number of branches, with what bootstrap support, that would need to be incorrect?

a) 1: 92%
b) 2: 78%, 69%
c) 4: 78%, 45%, 30%, 69%
d) 4: 78%, 45%, 30%, 29%

7) The trees show the phylogeny estimated for the same mammalian H1 influenzas based on portions of the haemaglutinin genes. Tree A was estimated from bases 310-870 whereas tree B was estimated from bases 1070-1650. Under the assumption that these trees are correctly rooted, how does this result support the inference that the South Carolina 1918 strain (the only exemplar from the 1918 flu pandemic) arose from recombination between a human and a swine virus?

a) The fact that the South Carolina 1918 strain is near the root of both trees suggests that it is of mixed identity

b) The fact that the South Carolina 1918 strain is more closely related to the swine strains in tree A but to the human strains in tree B

c) The fact that the South Carolina 1918 strain is a direct ancestor of all the swine strains in tree A but was isolated from a human

d) The fact that the South Carolina 1918 strain is more closely related to the Iowa 30 swine strain than to the Scotland 94 human strain in both trees

8) These trees for six different groups of organisms for (A) tree frogs, (B) caecilians, (C) uropeltid snakes, (D) freshwater fishes, (E) freshwater shrimps, and (F) freshwater crabs are all colored based on whether the species come from mainland India or Sri Lanka. Assuming these trees and the reconstruction of ancestral geographic distributions are accurate, which of the trees includes a single well-supported migration from Sri Lanka back to southern India?

a) A, F
b) B, C
c) C
d) A, B

9) The three trees depict the relationships between leaf cutter ants (left), fungi cultivated by those ants (middle) and fungi parasitizing ant gardens (right). By comparing the phylogenies, we can distinguish whether fungi are usually passed on vertically (through generations of an ant lineage) or horizontally (e.g., among unrelated ants). Allowing that differences between trees could reflect error in phylogenetic inference, what does the similarity between the three phylogenies suggest?

a) Cultivars are passed horizontally and parasites vertically

b) Cultivars are passed vertically and parasites horizontally

c) Cultivars and parasites are passed vertically

d) Cultivars and parasites are passed horizontally
10) The above tree summarizes the inferred relationships among a number of hominid fossils as related to humans (*Homo*). The point where multiple lineage arise from a single node (a polytomy) is here intended to indicate uncertainty rather than evidence of a simultaneous divergence of an ancestral lineage into five descendant lineages. The fossil ages of each taxon are color-coded on the right. Note that fossils of species 7 predate those of its sister taxon, species 8. If the tree is an accurate representation of the evolutionary history of these species, what is the minimum age we could infer for lineage 8?

a) 2.25  

b) 2.5  

c) 3.0  

d) It cannot be determined from these data
Tree thinking quiz II - Key
D. A. Baum, S. D. Smith, and S. D. Donovan

1) A
2) D
3) D
4) B
5) D
6) C
7) B
8) A
9) C
10) B