Teaching the Scientific Method: It's All in the Perspective

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An often overlooked component of scientific literacy is the understanding that science is a collaborative pursuit, and does not always follow a linear progression. I strive to make my students aware that experimental results are often surprising, even disappointing, and subsequent redirection of research can follow. Lateral exchange with researchers whose studies may be in fields other than your own can precipitate an infusion of new ideas, and lend a whole new perspective. Such exchanges have historically nudged eminent scientists past bottlenecks in their own research, with highly productive results. A nice example of this phenomenon is documented in *The Transforming Principle*, Maclyn McCarty's (1985) account of the landmark study demonstrating that DNA is the heritable genetic material. We encourage our students to read this book.

The units described in this article (targeted toward introductory undergraduate courses) have been optimized to promote learning through inquiry about the scientific method, the nature of experimental data, the necessity of comparison and replication of results in the pursuit of scientific progress, and the indirect path that such progress sometimes takes, in the context of Taxonomy and Cladistics. This approach is "grounded in the idea that learning science should be an active, engaging process that mimics the work done by actual scientists" (Lunsford & Melear, 2004).

Historical Examples

The study of evolution has benefited from the inclusion of ideas and findings in such disparate disciplines as geology, systematics, and most recently, bioinformatics. For example, Charles Darwin was greatly influenced by England's leading geologist of the time, Charles Lyell. In fact, Lyell's *Principles of Geology* accompanied Darwin during his voyage aboard the HMS Beagle, and played an instrumental role in giving Darwin a

foundation on which to build his *Theory of Evolution by Natural Selection*. Lyell's theory of extended geologic time, as contrasted to the commonly-held notion (then), that the Earth was a few thousand years old, gave Darwin a framework of millions, if not billions, of years under which his proposed gradual mechanism of evolution via natural selection could operate. Further, the application of geological principles placed the emergence of the Galapagos Islands, due to volcanic activity, as a relatively recent event (10-12 thousand years ago), suggesting a recent date for the immigration of the Blue-black Grassquit *Volatinia jacarina* from the South American mainland and its subsequent divergence into 13 distinct species unique to the Galapogos.

Scientists do not work in a bubble, nor is our work intended to be secreted away, but rather is intended to be disseminated. One of the most basic tenets of science is that findings are constantly retested and refined.

An essay by Stephen Jay Gould (1989) has been incorporated into our Introductory Biology course and illustrates the strength of this information dissemination in verifying or falsifying hypotheses. In fact, Gould asserts, an oft repeated argument against the science of evolution, employed repeatedly by proponents of "Creation Science" or "Intelligent Design," involves an incident in which this scientific method actually worked very well. The story is as follows: A worn fossil tooth was initially proposed in several published papers, to describe a novel species of New World hominid, *Hesperopithecus*. Expeditions to the fossil bed followed, to gather further physical data, but investigation of the subsequent specimens proved the *Hesperopithecus* theory false, since the teeth were now attributed to an ancestor of the modern peccary. Although the incident was embarrassing for those researchers who might have placed too much hope for a New World hominid on the characteristics of a single tooth, it is in fact a triumph of the scientific method that the misunderstanding was so quickly corrected by further evidence, which failed to corroborate the initial hypothesis.

By putting one's work before the scientific community, we invite scrutiny. In fact, this may be one of the strongest facets of the method scientists employ, since it allows data to stand the test of community evaluation, subsequent evidence to be directly compared, hypotheses to be bolstered or refuted by the

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work that follows. This, known as the peer-review process, thus strengthens our work, while it also directs funding to those projects deemed most worthwhile by experts in the field of study. The concept that research is an ongoing pursuit, subject to constant reevaluation, constitutes a strong basis for this module.

Structure of Macroevolution Module

This module (of three lab periods, each two-and-a-half hours) has been devised on macroevolution, or significant trends in evolution, to address the characteristics of scientific research discussed above. The lab sessions progress through taxonomy, cladistics, and mining bioinformatic data. This module follows a unit in which students study angiosperm (flowering plants) reproduction and development, learning such floral components as sepals, petals, pistil, carpel, ovary and ovule (and how to identify these, if present, in a mature fruit).

Part I: Taxonomy

In my most recent taxonomy lab, I incorporated "The Great Clade Race" (Goldsmith, 2003), which strengthened the module considerably. In past semesters, students have struggled with cladogram construction and the subsequent discernment of parsimony. For illustrated guidelines regarding cladogram methodologies, consult the McGraw-Hill Higher Education Web document "Taxonomic Classification and Phylogenetic Trees." "The Great Clade Race" allows us to address cladogram construction separately, and then proceed into parsimony. After completing the "Race," students are given an assortment of five fruits (tomato, pepper, orange, apple, and cacao bean) chosen for their availability at the local grocery, and an ancestral Outgroup (moss). Students then complete a numerical matrix based on five traits they observe: growth form, presence of sepals, ovary location, number of carpels, and fruit type (Figure 1A). The matrix is completed in by assigning numerical values to each of these traits, as shown in Figure 1A, with the more primitive form demonstrated by the Outgroup, represented by zero (0). While it is reasonable to argue that some of the traits (e.g., growth form) are not strong for drawing phylogenetic relationships, since they commonly show convergent evolution, these traits are purposely chosen to allow the students to obtain results that mirror other (previous) analyses, to compare results.

Including the cacao bean (cocoa) requires the use of alternate information sources to complete the matrix, since it is very difficult to locally obtain cacao beans that are still in the pod. Students utilize Internet search engines to mine this information, employing a variety of search strategies. They soon realize that the success of their search relies on the engine chosen, and the phrasing that they choose. There are usually a few students who surprise the rest by searching for images, and pull up nice photos that contain the necessary data for the matrix. This is often a highlight of the lab; both for the instructor who gets to see different strategies employed; and for the students who find the subject of cacao beans, so familiar and yet so foreign in their unprocessed state, always interesting.

Each lab group completes the matrix, then the class reviews all the findings and comes to consensus on a final version (Figure 1B). There is always some debate over the finalized version as students discover that other lab groups came to different reasonable conclusions and that their findings may differ due to variation in the specimens. This is a good time to discuss the incredible amount of variation within a population, and appreciate the obstacles faced by taxonomists (such as sexual dimorphism and mimicry between species).

Part II: Cladistics

After agreeing upon a final matrix, students start to construct cladograms using their experience from "The Great Clade Race"(Goldsmith, 2003). Looking across the matrix to elucidate patterns, the degree of relatedness between genera is assigned according the numbers of shared traits, from the matrix. A genus name is placed at each terminus of the cladogram "tree" according to where it falls in the grouping scheme. In Figure 1B, tomato and pepper are very similar (share characters) and thus lie next to one another in Figure 1C, while tomato and apple are quite different. In this construction, time is illustrated to progress from the left (earliest) to the right (latest), but no scale is implied. Students are reminded that the cladogram represents a hypothesis, and each group constructs a cladogram. All groups draw their cladograms on the board for class comparison. Many of these seem, at first glance, to be unique, until we discuss that the cladogram is like a mobile, so that having the same terminal branches A and B is not different than B and A (Goldsmith, 2003). Eventually students determine which cladograms are similar, and choose two distinct designs to test for parsimony. Two of the most common cladogram designs chosen are illustrated in Figure 1C. Once they choose the designs to test, students insert the digits from the matrix (replacing the respective genus names) to represent the present/absent form of each trait.

Figure 1. A. This table identifies each of the five morphological characters and the numerical options used to complete the matrix. B. Completed matrix, including genus and common names and numerical assignments for each of the five characters. C. Cladograms establishing relatedness between study samples. Time progresses from left (base) to right (termini), but no scale is implied. These are the two most common student-constructed cladograms, each representing a testable hypothesis.
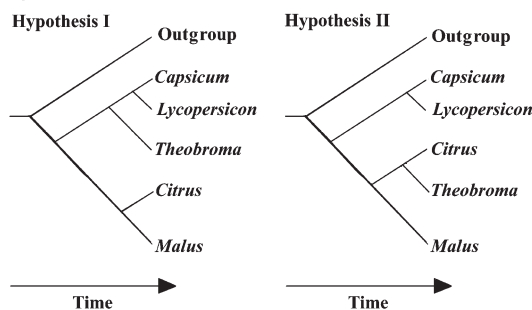
Figure 1A

Character		Numerical Options
I	Growth Form	0 = herbacious; 1 = woody
II	Sepals	0 = absent; 1 = present
III	Ovary Location	0 = N/A; 1 = superior; 2 = inferior
IV	Number of Carpels	0 = N/A; 1 = < 3; 2 $\geq$ 3
V	Fruit Type	0 = non-fruiting; 1 = dry, 2 = fleshy

Figure 1B

Organism (Genera)	Character				
	I	II	III	IV	V
Outgroup	0	0	0	0	0
Theobroma (cacao bean)	1	1	1	2	1
Lycopersicon (tomato)	0	1	1	1	2
Capsicum (pepper)	0	1	1	1	2
Malus (apple)	1	1	2	2	2
Citrus (orange)	1	1	1	2	2

Figure 1C

Hypothesis I — Outgroup, *Capsicum*, *Lycopersicon*, *Theobroma*, *Citrus*, *Malus*; Time

Hypothesis II — Outgroup, *Capsicum*, *Lycopersicon*, *Citrus*, *Theobroma*, *Malus*; Time

They will test each of the five traits against each design, evaluating ten cladograms in all.

Parsimony addresses the smallest number of evolutionary events required to have occurred in order to arrive at any particular outcome. Each trait needs to be tested against each of the distinct cladograms (i.e., hypotheses). There are a few simple rules: Numerals that were assigned to each trait in the matrix are put at their respective places at branch termini in the cladogram (Figure 2B). One then works from the terminus of the branch back to the base (left) of the cladogram, assigning numbers to each node (Figure 2B). If a node has identical numerals at each side, if is assigned that same number (Figure 2A). If the numerals on each side differ, assign a question mark. If a numeral exists on one side of the node, and a question mark on the other, assign the numeral. When the base of the cladogram has been reached, and each node has been assigned either a numeral or a question mark, we progress back along the branches to the termini again, replacing all question marks with the numeral at the prior node (closer to the base). Finally, look for discrepancies between numerals. Wherever adjacent numerals differ, the discrepancy represents an evolutionary event (represented by a perpendicular hatch in Figure 2C). Count the total number of evolutionary events for each trait, for each cladogram.

To identify the most parsimonious hypothesis, the number of evolutionary events is totaled for each cladogram, across all traits. If two cladograms have the same number of evolutionary events, parsimony is considered equal for that character. Depending upon the hypotheses chosen, the particular species that we examine usually result either in equal parsimony, or in one cladogram being more parsimonious than the other, requiring one fewer evolutionary event.

At this point, it is important for students to realize that it is best to evaluate their findings by comparing these results to the work of others. Available on the Internet, hosted by the Royal Botanic Gardens, is a phylogenetic tree summarizing the findings of gene sequence analysis conducted within angiosperms. At this point, students will realize that they have done their analysis on a finer scale (genus) than the research represented in the tree (family). The family names for each sample are as follows:

tomato and pepper:

Solanaceae

cocoa bean: Malvaceae

orange: Rutaceae

apple: Rosaceae

This is an ideal time to talk about the nature of data and how hard it can be to directly compile results from studies conducted independently, even when they are readily available. It can be as difficult (we have to say it) as comparing apples and oranges.

Part III: Mining Bioinformatic Data

Bioinformatics is the derivation of knowledge by employing computer analysis on collections of biological data. Internet access with readily available computers is crucial for the following sections. Students are now directed to the National Center for Biotechnology Information (NCBI): EntrezTaxonomy. The study of evolutionary relationships has become much easier with the accumulation of DNA, RNA, and protein sequence data from many species, as well as the development of numerous computer programs and databases facilitating comparison of data. To conserve time, students are given a handout containing lineage information available from GenBank, which is an annotated collection of all publicly available DNA sequences, maintained by the National Institutes of Health (NIH). Since it usually proves very difficult for students to deal with the breadth of the information in the gene sequence cladogram, they now reconstruct the Royal Botanic cladogram with only the taxonomic groups that we are using in the lab. With the simplified version in hand, they should see which of their hypotheses most closely mirrors their findings. In Figure 1C, Hypothesis II is the strongest match.

Because "inquiry … stands in sharp contrast to the traditional sorts of prepackaged or cookbook lab activities that have driven science classrooms over the years" (Richie & Rigano, 1996), we now take a different perspective by investigating the protein sequences of a genus of parasitic fungi that infect the plant species of study.

Coevolution

Just as predators and prey are said to participate in an evolutionary "arms race," in which changes in either side eventually cause selection to produce complementary changes in the other, parasites and their hosts have interrelated selection pressures. Over time, as host species diverge from one another, the parasites that specifically attack them are also expected to diverge, if the parasite populations are isolated from one another (the natural result of infecting hosts that are now isolated from one another). Therefore, if we can determine the phylogeny of the parasites, it may illuminate the phylogeny of the hosts. For fuller discussion of this mechanism consult pp. 539-551 of Futuyma (1998).

In the next lab session, we return to the NCBI data-

Figure 2. A. Illustration of the rules governing the assignment of numerals to the cladogram nodes. B. Termini are given numerals established in the matrix for Trait I. Nodes are labelled working from right to left following the rules in A. C. Work left to right, replacing question marks with prior numeral. Each discrepancy between numerals represents an evolutionary event, marked with a perpendicular hatch. Hypothesis II, with one fewer evolutionary event, represents the most parsimonious cladogram of the two, for this character.
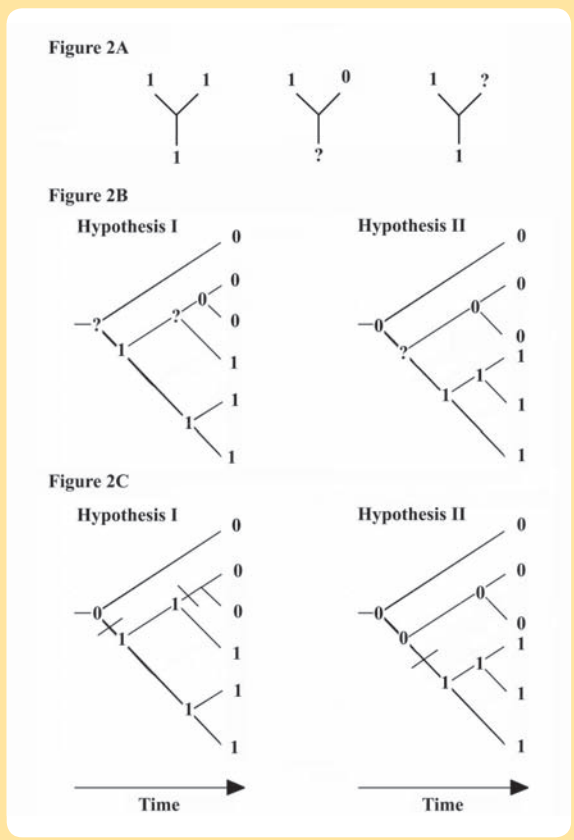
base: Entrez Protein, where we concentrate on the genus *Phytophthora*, parasitic fungi responsible for thousands of millions of dollars in crop damage every year (Nicholls, 2004), making it a huge economic concern, and therefore aggressively researched. First discovered in 2000, it has resulted in "one of the fastest-ever discovery-to-sequence stories for a complex pathogen" and there are now 60 known species that attack crops as diverse as potato, tomato, cacao, and alfalfa (Nicholls, 2004). Species of *Phytophthora* tend to attack certain species of host, in that one species infects soy, another tobacco, etc. For more information on *Phytophthora*, see the Ohio State University Extension Fact Sheet online (http://ohioline.osu.edu/ac-fact10013.html).

Students are directed to the Biology WorkBench hosted by the National Laboratory for Computational Science and Engineering at the University of California San Diego. By choosing the "Cladogram" option, students align the protein sequences of the cytochrome C oxidase Subunit II from several species of *Phytophthora* and generate a phylogenetic tree.

The species used are as follows, with host species given in parentheses:

P. megakarya (cocoa bean)

P. cryptogea (tomato)

P. capsici (pepper)

P. palmivora (orange)

P. cactorum (apple)

To align the proteins, take the accession numbers listed in Table 1, and retrieve the protein sequence for each species at NCBI. Copy the sequences and paste them into a Word (or equivalent) text file. This text file can then be taken to the Biology WorkBench (free registration required) and used to align the amino acid sequences. If you need specific step-by-step instructions on how to align, consult Maier (2001) or contact the lead author of this paper by email.

After performing this comparison, students will find that the *Phytophthora* sequence data do not exactly mirror their morphological analysis of the hosts, indicating that more explanation must be sought. Can they come up with a probable reason?

Historical Consideration

At this point we step back and approach from yet another angle. Students are now encouraged to unearth clues through researching the cultivation history of the various plants to see what they can learn about speciation patterns, to make sense of the *Phytophthora* data. They must consider the fact that the

host species have different geographical points of origin (apples from Turkey, oranges from India and eastward through the Malay archipelago, cocoa beans from east of the Andes in South America). Speciation of *Phytophthora* among these cultivars depends on their point of divergence, which has both spatial and temporal aspects. For example, introduction of host (fruit) species to the New World should also be considered.

Discussion Points

Several points can be stressed in discussions as students progress through the module, which can be tailored to suit a particular curriculum. One could focus on the collaborative nature of scientific advance, that it does not always move forward smoothly, but in fits and starts. There is the goal of research to disseminate data, to share, and the peer review process to talk about. Students may be interested in the way that data from different sources, indeed from different fields of study, are brought together to shed light on a hypothesis, as they have done. The class might discuss how choosing a system or scientific question to study is a tricky experiment in itself, and that economic considerations often play a role. Fungi that do not affect global agronomics are not so quickly sequenced, for example. More topics for discussion might lie in the proper use of the information superhighway, methods of finding and evaluating relevant information, criteria for assessing the reliability of sources they find. The nature of data, comparability and availability, is another point they might choose to explore. Extensions might also address the fact that phylogenetic methods are open to debate and, in fact are the "subject of large, sophisticated and often contentious literature" (Futuyma, 1998). See Chapter 5, "The Tree of Life: Classification and Phylogeny," in Futuyma (1998) for further expansion of these points, especially pp. 98-99.

Conclusion

The exercises we have described involve investigating the reproductive morphology of angiosperms, and using those characteristics to develop hypotheses about the taxonomy of the samples in the form of a cladogram, followed by the confirmation/rejection of those hypotheses by mining taxonomic and bioinformatic data from other sources. More important than the specific items of study, though, are the lessons learned about the nature of science and the scientific method.

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Table 1. National Center for Biotechnology Information (NCBI) accession numbers for the protein sequences of cytochrome C oxidase Subunit II, for each species of *Phytophthora,* with respective hosts.

Phytophthora Sp.	Host (genera)	NCBI Accession Number
P. megakarya	*Theobroma*	AAN01337
P. cryptogea	*Lycopersicon*	AAN01316
P. capsici	*Capsicum*	AAN01309
P. palmivora	*Citrus*	AAN01348
P. cactorum	*Malus*	AAN01307

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