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Practical Report

The Fern Flower Chase

HIGUCHI Shinnosuke

There are three main groups of land plants: moss plants, fern plants, and seed plants. If asked about these phylogenetic relationships, i.e., evolutionary trajectories, how would you respond? According to most individuals who have studied lower secondary school science in Japan, plants were initially divided into seed-producing and spore-producing plants from their common ancestor, followed by the division of the latter into ferns, which have a root, stem, and leaf distinction, and mosses, which have no distinction. However, molecular phylogenetic analysis has shown that the ancestor of moss plants diverged first from the common ancestor of land plants, followed by the diversification of ferns and seed plants. Therefore, the commonly held scenario is incorrect. This paper begins by acknowledging the problem of why this misconception is widespread. Next, I reported on a classroom practice in the lower secondary school science category. The practice involves creating molecular phylogenetic trees based on the comparison of genetic information to gain insight into the phylogenetic relationships of organisms. Based on my analysis, I have concluded that even if the textbooks “say nothing wrong,” the way they are written is “wrong” in a way that many people misunderstand.

Key words:　plant phylogenetic relationships, molecular phylogenetic trees, misconception

1. Introduction

1.1. “Fern Flowers”

The episode *Fern Flowers* of the five-minute animation *Hanakappa* for young children on E-television begins with the main character Hanakappa-kun entering the forest with his friends to see the “fern flowers”, based on a legend written in an old book that ferns bloom. However, Tsunenari-kun, an honours student character, says: “Ferns do not bloom. Ferns are plants consisting of leaves, stems and roots that do not flower, do not produce seeds and reproduce by spores. In other words, fern flowers do not exist in this world.” A child listening to Tsunenari-kun’s explanation would likely understand the plant classification system as follows: plants are initially classified based on the presence of roots, stems and leaves, and then based on whether they produce seeds or not. However, a parent listening next to the child would probably have a different impression: “That’s not the classification system I learnt in secondary school!” It is not that this parent lacks knowledge of biology; rather, this misconception was created because of his/her serious study of science in secondary school. In other words, the cause of this misconception can be found in the descriptions in secondary school science textbooks and the way the subject is treated in the classroom as the result. In this paper, I will first consider the relationship between biological classification and evolutionary phylogeny, and then consider a series of practical exercises, including the construction of molecular phylogenetic trees, and their implications.

What needs to be clearly stated and affirmed here is that the classification of organisms—not only in the context of modern biology, but even in the context of secondary school science education—should be a classification based on phylogenetic relationships, a phylogenetic classification. However, in the “Classification of Organisms” unit in lower secondary school science, the perspective of phylogenetic classification is carefully excluded from the description of current teaching materials, even if the main objective is not "to make students understand the phylogenetic classification of organisms" (MEXT, 2018; Higuchi, 2023). I believe that the reason why such misleading descriptions are so prevalent is a reflection of the unfortunate time that nonseed plants have spent in the previous courses of study. I will discuss the specifics of this situation at another time, but in the next section I will examine how we should clarify plant taxonomy with our students and how we should look at phylogenetic trees.

1.2. Unrooted and rooted phylogenetic trees, and taxonomic groups of the organisms

Here we have four actual groups of organisms, A, B, C, and D. Suppose that each organism is examined for its similarity—or, of course, we can say, its difference—and that the relationships shown in Figure 1a are evident. Such a phylogenetic tree is called an unrooted phylogenetic tree. What is shown here is the mutual relationship of the four groups of organisms and does not represent the process of evolution or the passage of time. Thus, Figures 1b and c can be seen as having the same meaning as Figure 1a, but Figure 1d is different from the other three.

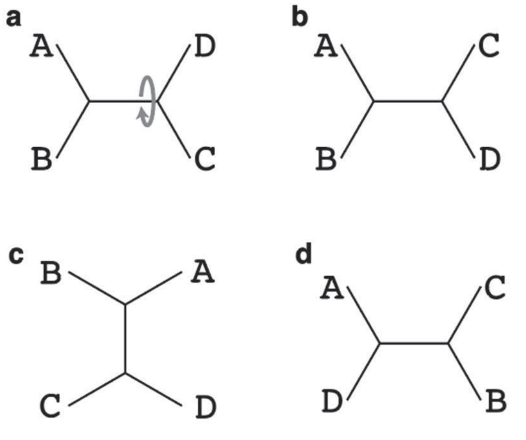

Figure 1 | How to see the unrooted phylogenetic tree.
a–c, The phylogenetic trees are essentially the same. **d,** The relationships among organisms are different from those of other phylogenetic trees.

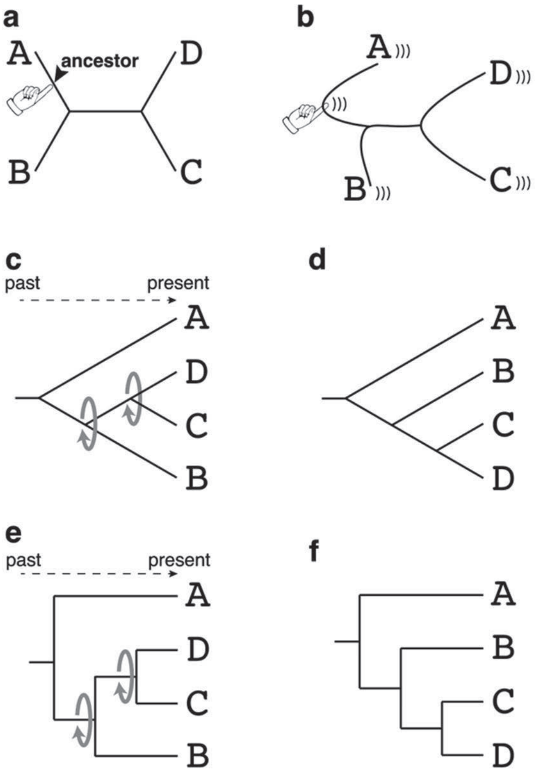

Figure 2 | Conversion between unrooted and rooted trees.
a–f, The relationships between organisms A, B, C and D are basically the same. Note that the organisms are connected by a loose line, as in **b**, and the phylogenetic tree can be reshaped by "sliding" the ancestral position to the left.

In order to transform the unrooted phylogenetic tree shown in Figure 1 into a phylogenetic tree that represents the evolutionary process and time course, i.e., a rooted phylogenetic tree, one must determine which of these organisms diverged first from the common ancestor. In other words, it is necessary to determine the position of the ancestor. Note that this paper will focus only on the way phylogenies diverge, including the rooted phylogenetic trees and specific examples that will appear later, and will not consider the length of the branches.

With the position of the ancestor determined, the unrooted tree can be rewritten as a rooted tree and the evolutionary scenario can be restored. For example, suppose that in the same unrooted phylogenetic tree as in Figure 1a, the position corresponding to the common ancestor is on the A side of the A and B branches (Figure 2a). This is transformed into a rooted phylogenetic tree as shown in Figure 2b and Figure 2c, d. That is, imagine that the four organisms (groups) shown in Figure 2a are loosely connected by a line of the same shape as the unrooted phylogenetic tree, and then “finger” the ancestral position to the “left” or “past” and reshape it. Both forms shown in Figure 2, c and d, are acceptable, but when phylogenetic relationships are complex, a phylogenetic tree with branches parallel to the time axis, as in d, is often used. It can also be organized as in Figures 2e and f, if necessary.

As mentioned earlier, when considering the classification of organisms, it is necessary to establish taxonomic groups that are consistent with their phylogenetic relationships. In attempting to classify the organisms A-D shown in Figure 2, it seems acceptable to assign organisms A and B to class α_1 and organisms C and D to class β_1 in the unrooted phylogenetic tree shown in Figure 2a, or to assign organism A to class α_2 and organisms B,Cand D to class β_2. However, if the common ancestor of the organisms A–D is known to be on the A side of the A–B split in Figure 2a, then a classification such as α_1 that does not include all organisms derived from the common ancestor of these organisms would be undesirable, although such a classification system is sometimes used in practice. Although it is sometimes done, it is not desirable. Considering the phylogenetic tree shown in Figure 2c–f above, if we want to create a taxon that includes A and B, we should also include C and D, which diverged from the last common ancestor of A and B, in that taxon. Thus, a taxon that includes all organisms that branched off from the last common ancestor is called a monophyletic group.

The shape of the phylogenetic tree is closely related to how taxonomic groups are defined. In fact, even the taxonomic groups of ferns and mosses include plants other than ferns and mosses, respectively, in the groups of organisms that diverged from their last common ancestor. Specifically, the taxa that diverged from the common ancestor of mosses include ferns and seed plants, and the taxa that diverged from ferns include seed plants (e.g., Donoghue et al., 2021). According to branching taxonomy, mosses must include all other land plants such as ferns and seed plants, but in practice, especially at the secondary level of education, too strict a focus on monophyly can lead to a loss of the essence of the classification of organisms. In reality, therefore, it is not realistic to consider all taxa as monophyletic groups, i.e., including all organisms that diverged from the last common ancestor of these animals. Seed plants are a monophyletic group (e.g., Donoghue et al., 2021).

1.3. Two Misconceptions about the Classification of Living Organisms in Secondary Education

Based on the ideas about phylogenetic trees and systematics organized above, here are two misconceptions about the classification of organisms in secondary education - which are not resolved in adulthood, but rather become more solidified after secondary education.

First, a discussion of the classification of land plants, which we discussed earlier. When most people are asked to classify mosses, ferns, and seed plants using phylogenetic trees, they first group seed plants together with mosses and ferns, which are groups that reproduce by spores. In reality, however, the ancestors of ferns and seed plants, which are “vascular plants” with differentiated roots, stems, and leaves, diverged from the ancestors of mosses.

The second is the phylogenetic relationships among the five taxonomic groups of vertebrates. Vertebrates can be divided into five major groups: fish, amphibians, reptiles, birds, and mammals. When asked about this phylogeny, many people still think of birds and mammals as sister groups. However, phylogenetic relationships based on genomic and evolutionary developmental studies indicate that birds are closely related to reptiles, a group that diverged from some crocodilians among reptiles (e.g., Tamura et al., 2011; Wang et al., 2013).

With these issues in mind, this paper reports on a class in which eighth grade students first examined their understanding of the classification and evolution of land plants and vertebrates, and then constructed a molecular phylogenetic trees among some vertebrates based on the importance of the genetic information. Then, based on the results of the examination conducted afterwards for the semester, I described the results and challenges of this practice.

2. Practice

2.1. Target Classes

This practice was conducted in the Comprehensive Science class for eighth grade students in 2023 for which I was the teacher. The textbook was *Miraie-Hirogaru Science 3* by Keirinkan (Ohya et al., 2021), and the class met for four hours a week. The two teachers in charge of this class divided the class into two hours a week, called it “Comprehensive Science α” and “Comprehensive Science β” for convenience, and assigned units based on the expertise of the teacher in charge and the perspective of curriculum management. Since this course is a school-set course under the Special Exception for Secondary Schools and the SSH (Super Science Highschool) designation, there is no textbook that fully corresponds to the content of the course. The data reported in this paper are based on the content of a five-hour class held in October 2023 and the corresponding assessments. The study was approved by the school’s ethical review committee.

2.2. Students’ Perceptions of Biological Phylogeny, Classification, and Evolution

First, as a preliminary survey, we asked students the question: “Do you believe in biological evolution?” and asked them to choose from “strongly believe,” “fairly believe,” “somewhat doubtful,” or “very doubtful” and to describe their reasons ($n = 119$). The responses were: 36 (30%) “strongly believe,” 70 (59%) “fairly believe,” 11 (9.2%) “somewhat doubtful,” 1 (0.8%) “very doubtful,” and 1 (0.8%) no response.

Then, after explaining phylogenetic trees to the students as described in section 1.2. of this paper, we asked them to construct phylogenetic trees for blanket weed, liverwort, bracken fern, and Cherry blossom. The correct phylogenetic tree was then answered by 29 (24%), a phylogenetic tree based on the misconception that mosses and ferns are sister groups was answered by 72 (61%), and an otherwise incorrect phylogenetic tree was answered by 18 (15%).

They were also asked to construct phylogenetic trees for five groups of vertebrates ($n = 119$). Seven students (5.9%) gave the correct phylogenetic tree, 71 (60%) gave a phylogenetic tree based on the misconception that mosses and ferns are sister groups, and 41 (34%) gave the other incorrect phylogenetic tree. One student (0.8%) answered both trees correctly, and 41 (34%) answered the tree based on both misconceptions.

2.3. Phylogenetic relationships of vertebrates based on molecular phylogenetic tree.

After introducing students to the molecular clock concept that the higher the degree of relatedness between species, the more similar the gene sequences are, the more similar the gene sequences of the present organisms can be compared and the order of divergence of the organisms can be reconstructed by relying on these similarities. The class members were then shown the equivalent 60 nucleotides from the globin genes of five vertebrate species, and were given a practical exercise to create a molecular phylogenetic tree. For the gene sequences used, I used the sequences registered as hemoglobin subunit beta (HBB) or these homolog in GenBank under respective accession numbers: human *Homo sapiens*, NM_000518.5; mouse *Mus musculus*, NM_001278161.1; chicken *Gallus gallus*, NM_205489.2, turtle *Chrysemys picta bellii*, XM_005290009.2; medaka *Oryzias latipes*, XM_004071342.3.

The phylogenetic trees were created using UPGMA. In the previous year’s lessons, students created a phylogenetic tree based on vertebrate characteristics such as habitat, physical appearance, respiration, and reproduction (Higuchi, 2023). Despite using different data, the students were able to apply their knowledge and work collaboratively in groups of four to create a phylogenetic tree. The supplementary material displays a modified and organized version of the worksheet utilized during this period.

2.4. Evolutionary Events and Homologous and Analogous Organs

Textbooks have developed a discussion of homologous and analogous organs, with the former being the forelimbs of mammals and the wings of birds, and the latter being the wings of insects and the wings of birds as representative examples of these organs. An extension of such a discussion should be a discussion of the relationship between the wings of birds and the wings of bats, but such a discussion is carefully avoided in lower secondary school textbooks. Although the lower secondary school textbooks carefully avoid such a discussion, as “wings” they are made differently and evolved independently. Therefore, as wings, they are analogous organs (e.g., Wagner, 2014).

This is useful material for thinking about homology and convergence, but it is also an important issue related to high school biology, so we will discuss how to handle it in another paper.

3. Verification, Achievements and Challenges

3.1. Verification based on an examination

In the relevant examination, a fall semester midterm examination conducted in early December 2023, the material text introduced the episode as described in Section 1.1. of this paper and asked questions on the conversion from an unrooted to a rooted phylogenetic tree, vertebrate phylogeny, and homology and similarity of organs. Some of the questions are shown below.

Q1 (omitted)

Q2 Rewrite the rooted phylogenetic tree for the ancestral positions (1) and (2), respectively, based on the comparison of genetic information of organisms A, B, C, and D. The unrooted phylogenetic tree is shown on the left in Figure X.

*The Figure X is omitted, but basically corre sponds to that shown in Figure 2. In (1),the group consisting of A and B is sister to the group consisting of C and D. In (2), A is the outer group of

Q3 Create a rooted phylogenetic tree that includes the following five animals: turtle (reptile), chicken (bird), human (mammal), mouse (mammal), and medaka (fish).

Q4 Explain why the wings of chickens (birds) and bats (mammals) can be viewed as both homologous and analogous organs, based on the wing construction in the figure below (figure omitted).

Scoring showed that the percentage of correct answers for each question was 83% for Q2 (1), 66% for (2), 30% for Q3, and 33% for Q4. Considering that 1.5 months had passed since the practice, the students’ understanding of how to look at phylogenetic trees has deepened, but they were not able to completely dispel misconceptions related to vertebrate phylogeny, and certain issues remain regarding the consideration based on the definition of homologous and analogous organs.

3.2. Achievements and Challenges

It is fair to say that in the current curriculum, the classification of organisms based on phylogenetic classification, i.e., is deliberately avoided at the junior high school level (Higuchi, 2023), but again, even if what is written in textbooks is not wrong, the descriptions can create so many misconceptions. The difficulty arises from the fact that students must classify organisms before learning about evolution, but again, the elementary concepts acquired in seventh grade—in this case, misconceptions—are unlikely to be overturned by learning about evolution later in eighth or nineth grade.

In this practice, because a simple phylogenetic tree based on the characteristics of organisms was constructed by UPGMA in seventh grade, the more objective content of molecular phylogenetic trees, which confronted biological evolution head-on, was easily handled in eighth grade. As indicated in the previous section, there were still some problems in the objective construction of the phylogenetic tree, but these were more advanced topics in the handling of phylogenetic trees and concepts related to evolution, and the students were able to classify organisms and use various taxonomic groups based on the viewpoint of hierarchy, after fully understanding the basic evolutionary concepts that should be understood at the lower secondary school level. They were able to use different taxonomic groups of organisms.

What was interesting about this practice was that previous studies have pointed out that, as a result of questionnaire surveys of lower secondary school students, they tend to form a false evolutionary view of evolution and common ancestry as “evolved toward humans” (Yamanoi et al., 2022), but the students who were the subjects of this practice, however, did not show such a tendency among the students in this study. The reason for such a tendency should be clarified through careful observation of students’ learning at the post-high school stage. In the “Basic Biology” and “Biology” courses for upper secondary school students starting in 2022, various issues, discussions, and results would be reported. In the future, we will be able to deepen our understanding of students’ perceptions of biological phylogeny, classification, and evolution by comparing the transformation of the students who were the subjects of this study, while keeping our eyes on these results as well.

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Competing interests

The author declares no competing interests.

Additional information

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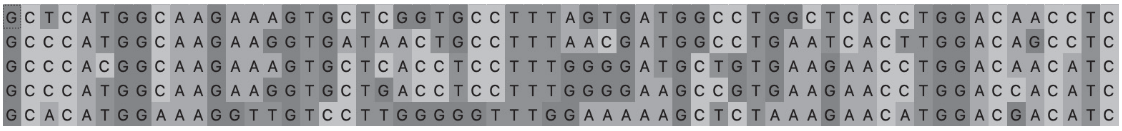
Supplementary Material: How Genes and Genomes Evolve

This is because the higher the degree of relatedness between species, the more similar their gene sequences are. The gene sequences of extant organisms can be compared to reconstruct the order of divergence of organisms. Below are the equivalent 60 nucleotides from the globin genes of five vertebrate species. Use this information to construct a phylogenetic tree.


human (mammal) GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTC
mouse (mammal) GCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTC
chicken (avian) GCCCACGGCAAGAAAGTGCTCACCTCCTTTGGGGATGCTGTGAAGAACCTGGACAACATC
turtle (reptile) GCCCATGGCAAGAAGGTGCTGACCTCCTTTGGGGAAGCCGTGAAGAACCTGGACCACATC
medaka (fish) GCACATGGAAAGGTTGTCCTTGGGGGTTTGGAAAAAGCTCTAAAGAACATGGACGACATC

①

human (mammal) GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTC
mouse (mammal) GCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTC

②

human (mammal) GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTC
chicken (avian) GCCCACGGCAAGAAAGTGCTCACCTCCTTTGGGGATGCTGTGAAGAACCTGGACAACATC

③

mouse (mammal) GCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTC
chicken (avian) GCCCACGGCAAGAAAGTGCTCACCTCCTTTGGGGATGCTGTGAAGAACCTGGACAACATC

④

human (mammal) GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTC
turtle (reptile) GCCCATGGCAAGAAGGTGCTGACCTCCTTTGGGGAAGCCGTGAAGAACCTGGACCACATC

⑤

mouse (mammal) GCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTC
turtle (reptile) GCCCATGGCAAGAAGGTGCTGACCTCCTTTGGGGAAGCCGTGAAGAACCTGGACCACATC

⑥

chicken (avian) GCCCACGGCAAGAAAGTGCTCACCTCCTTTGGGGATGCTGTGAAGAACCTGGACAACATC
turtle (reptile) GCCCATGGCAAGAAGGTGCTGACCTCCTTTGGGGAAGCCGTGAAGAACCTGGACCACATC

⑦

human (mammal) GCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTC
medaka (fish) GCACATGGAAAGGTTGTCCTTGGGGGTTTGGAAAAAGCTCTAAAGAACATGGACGACATC

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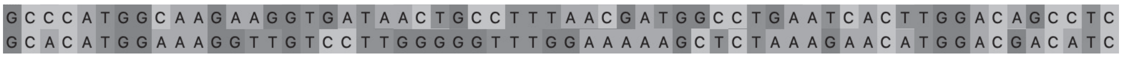
mouse (mammal) GCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTC
medaka (fish) GCACATGGAAAGGTTGTCCTTGGGGGTTTGGAAAAAGCTCTAAAGAACATGGACGACATC

⑨

chicken (avian) GCCCACGGCAAGAAAGTGCTCACCTCCTTTGGGGATGCTGTGAAGAACCTGGACAACATC
medaka (fish) GCACATGGAAAGGTTGTCCTTGGGGGTTTGGAAAAAGCTCTAAAGAACATGGACGACATC

⑩

turtle (reptile) GCCCATGGCAAGAAGGTGCTGACCTCCTTTGGGGAAGCCGTGAAGAACCTGGACCACATC
medaka (fish) GCACATGGAAAGGTTGTCCTTGGGGGTTTGGAAAAAGCTCTAAAGAACATGGACGACATC

A

	human	mouse	chicken	turtle	medaka
human					
mouse	①				
chicken	②	③			
turtle	④	⑤	⑥		
medaka	⑦	⑧	⑨	⑩	

C

B

D
