

Activity 1: Classifying Life

Based on video and online text content

35 minutes (30 minutes before and 5 minutes after the video)

Setup

Over time, the way that organisms have been classified into kingdoms has changed significantly. The extensive genus and species classification system developed by Linne had only two kingdoms: plants and animals. The familiar five-kingdom system was proposed by Whittaker; it classifies all organisms into plants, animals, protista, fungi, and monera (bacteria). Based in part on molecular data, Carl Woese proposed a three-domain system, in which all organisms are classified as Eubacteria, Archaea, or Eukarya.

In this activity, the group will be divided into six teams. Each team will be given characteristics for one organism. Using this information and the online text chapter, compare the three classification systems by discussing how the organism would be classified in the Linne, Whittaker, and Woese systems. Then explain your organism and its classification to the rest of the group.

After watching the video, talk about the Discussion Questions.

Materials

- One set of the Organisms to Be Classified (master copy provided, cut along dotted lines after copying)
- One copy of the Evolution and Phylogenetics online text chapter per team (available online at <http://www.learner.org/channel/courses/biology>)
- One copy of the discussion questions per person (master copy provided)
- Tips and Suggested Answers

Organisms to Be Classified

For answers, see Tips and Suggested Answers.

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 1: *Anabaena sp.*

Characteristics:

- Lacks a true nucleus
- Cytoplasm contains no organized membrane-bound organelles
- Possesses photosynthetic membranes

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 2: *Sulfolobus*

Characteristics:

- Lacks a true nucleus
- Inhabits hot sulfur springs of Yellowstone
- Obtains energy by oxidizing sulfur
- Optimal conditions for this organism are 60–80°C and pH 2–4 (thermoacidophiles)

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 3: *Laminaria* (kelp)

Characteristics:

- Possesses a true (membrane-enclosed) nucleus
 - Multicellular, marine species
 - Thallophytes (i.e., lack true vascular tissue)
 - Photosynthetic
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 4: *Physarum* (Myxomycota; "slime mold")

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Possesses hyphae
 - Obtains nutrition by engulfing bacteria and small bits of organic matter
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 5: *Jasminum sp.* (Jasmine)

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Photosynthetic
 - Multicellular
-

How would this organism be classified in each of the three systems (Linne, Whittaker, and Woese)?

Organism 6: Nematode worm (*Caenorhabditis elegans*)

Characteristics:

- Possesses true nucleus
 - Terrestrial
 - Dependent on external nutritional sources
 - Multicellular
-

Discussion Questions

1. Of the three classification schemes used in this activity, which is currently favored and why?
2. Researchers such as Carl Woese have integrated the use of molecular and phenotypic tools to re-evaluate the classification of life. Do you think one set of information is more reliable for determining relationships than the other? If so, why? What other tools might be used in the future for classification of organisms?
3. Biologists have long classified organisms according to similarities of form. They usually name them this way too. More recently, some biologists have asserted that the names/classifications that we give organisms should reflect their evolutionary histories. Why might this be a good idea? What additional data are now available to make this a reasonable standard?

Organisms To Be Classified

Classifications according to the Linne two-kingdom system of Plantae and Animalia:

- Plantae – Organisms 1–5
- Animalia – Organism 6

Classifications according to the Whittaker five-kingdom system: Monera, Protista, Plantae, Fungi, and Animalia:

- Monera – Organism 1, 2
- Protista – Organism 3
- Plantae – Organism 4
- Fungi – Organism 5
- Animalia – Organism 6

Classifications according to the Woese three-domain system: Eubacteria, Archaeobacteria, and Eukaryota:

- Eubacteria – Organism 1
- Archeobacteria – Organism 2
- Eukaryota – Organisms 3–6

Activity 2: Construction of a Phylogenetic Tree

Based on video and online text content

60 minutes

Setup

Swordtail fish in the genus *Xiphophorus* exhibit substantial variation in male body size as the result of genetic variation. Some species, however, are either uniformly large or small. In addition, males of the various species exhibit differences in the length of the sword and in mating rituals. These observed differences elicit various responses in females with regard to sexual mating. Based on these physical characteristics and molecular information (fabricated for this activity), see if you can build a phylogenetic tree for four different *Xiphophorus* species.

Work in pairs to build the best phylogenetic tree, first using physical information, and then using genetic characteristics of the related species. The Evolution and Phylogenetics online text chapter explains the logic used to generate a phylogenetic tree and provides step-by-step instructions.

Materials

- One copy of the Instructions and Physical Information per person (master copy provided)
- One copy of the Genetic (rDNA) Information and Discussion Questions per person (master copy provided)
- One copy of the Evolution and Phylogenetics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Tips and Suggested Answers

Instructions and Physical Information

Step 1:

First, construct a draft of an unrooted phylogenetic tree of swordfish, based on the provided physical information. (Unrooted means it will show only relatedness, not the direction of evolution). Use the logic of Hennig and the systemists presented in the text to construct this first-draft phylogenetic tree.

Step 2:

Now consider that parts of the 12S rDNA have been sequenced from these species. Construct a phylogenetic tree based upon the molecular data and compare it to the tree made based on physical data. Are the trees different? What would explain these differences?

Species 1 — *X. cortezi*

Physical Information: sympatric with *X. pygmaeus*; share characteristics with larger morphs of *X. nigrensis* (larger than *X. pygmaeus*, have deep bodies and swords); males perform courtship displays; loss of sword in *X. pygmaeus* may be due to avoidance of *X. cortezi*; UV component in sword to attract females

Species 2 — *X. multilineatus*

Physical Information: can be a threefold difference in mean sword length across male populations; polymorphic characteristic of swords (many shapes); sword variation may be a result of predation pressure (predator preference for males with long swords appears to be ancient); UV component in sword to attract females

Species 3 — *X. nigrensis*

Physical Information: females prefer large males with long swords; size inherited paternally; one of the most studied species because size is controlled by pituitary gene (*P*); males court females based on size; polymorphic characteristic of swords (many shapes); sword variation may be a result of predation pressure; UV component in sword to attract females

Species 4 — *X. pygmaeus*

Physical Information: Name refers to unusually small size of males (and swordless); parsimony suggests large size alleles lost in this species; could have been lost due to predatory pressure; females still prefer larger size males (retained ancestral preference); males do not court; UV component in sword to attract females

Genetic (rDNA) Information and Discussion Questions

For possible answers, see Tips and Suggested Answers.

12S rRNA sequences (shortened and fabricated for activity)

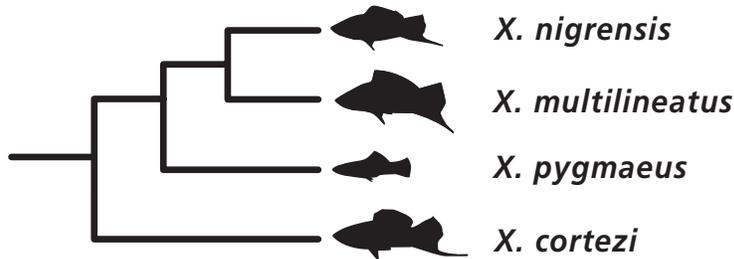
Species	12S rRNA Sequence
<i>X. multilineatus</i>	ATTAGCGCATCGGCATTTAACGGCCAATGCATTGCCCATCGTACGGCACTGTT
<i>X. nigrensis</i>	ATTAGCGCATCGGCATTGCGCTAATCGGCGTTTGCCCATCGTATTACATCATT
<i>X. cortezi</i>	ACTACCAATATGGATTACGGCTAATACGGCATAACGACGGCAATTAGGCAGAT
<i>X. pygmaeus</i>	ATTAGCGCGCGCAATATAAACGGCCTTACGCCTATGCATTGACGGCACTGTT

Discussion Questions

- The trees made in this exercise were unrooted. What additional step would be required to make a rooted tree? What additional information would be shown in a rooted tree?
- What are the major steps involved in generating a phylogenetic tree using a cladistic approach?
 - In cladistic analyses, shared, derived characteristics are more informative than shared, ancestral characteristics. Why?
 - Give an example from the text or video of homoplasy. What are two different explanations for the existence of homoplastic characteristics, and what does each say about the relatedness of the groups being compared?
- How do scientists use the genetic data to determine a cladistic tree?
- Groups of animals (nematodes, for example) are nearly identical morphologically, but have considerable genetic variation. What does this imply? Why might this occur? How might a scientist deal with this problem in making classifications?

Construction of a Phylogenetic Tree

Actual Tree:



Answers to Discussion Questions:

1. The trees made in this exercise were unrooted. What additional step would be required to make a rooted tree? What additional information would be shown in a rooted tree?
Making a rooted tree would require adding information from a distantly related outgroup. In addition to relatedness, a rooted tree would show the progression of evolution.
2. What are the major steps involved in generating a phylogenetic tree using a cladistic approach?
 - a. In cladistic analyses, shared, derived characteristics are more informative than shared, ancestral characteristics. Why?
 - b. Give an example from the text or video of homoplasy. What are two different explanations for the existence of homoplastic characteristics, and what does each say about the relatedness of the groups being compared?

Major steps in generating a tree include the assumption that evolution is a branching process, establish relationships based on taxa, taxa organized into clades based on derived character states, and so on. Cladistic analyses favor shared, derived characteristics over ancestral because primitive characters do not reveal information about which groups share more recent common ancestors (primitive character states only add noise to the system). An example of homoplasy could be wings on both birds and bats (see the online text chapter). Two different explanations of homoplasy are convergence and reversal. Convergence means different lineages do not share a common recent ancestor but evolve the same character. Reversal means mutation or selection causes the derived character state to revert back to ancestral character.

3. How do scientists use the genetic data to determine a cladistic tree?
Scientists use genetic data to back-up the similarities observed in morphological characters. (It is assumed that there is no difference between the analysis of morphological and molecular characters.)
4. Groups of animals (nematodes, for example) are nearly identical morphologically, but have considerable genetic variation. What does this imply? Why might this occur? How might a scientist deal with this problem in making classifications?

It implies that they have adapted to environmental factors in similar ways, yet reached the same goal in different ways. In other words, nematodes that evolved in different regions have had the same adaptive obstacles to overcome. So, although they are similar morphologically, genetically they did not breed to pass on traits, and relied on mutations within their species for evolutionary change. Another possibility here is that most of the genetic differences that we observe are not functionally too important, whereas similarities (strict homology) at the molecular level are still responsible for the similarities at the morphological level. To deal with problem, scientists must take DNA and phenotype information into account. not just one or the other.

Activity 3: HIV and the Dentist

Based on video and online text content

25 minutes

Setup

One of the uses of comparative evolution is in epidemiology, tracing the source of an infectious agent. In the early 1990s, a young woman in Florida died of AIDS, even though she had no known risk factors for HIV infection. Comparative genetic analysis determined that she had been infected by her dentist, who may have also infected other patients during invasive dental procedures.

To do the analysis, virus samples were isolated from each of the patients, from the dentist, and—as a control—from HIV-positive individuals from the local community. The gene sequences for the HIV-1 outer-envelope protein was determined for viruses in each sample and the degree of genetic similarity was compared. Some of the data are provided. Working in pairs, examine the data and discuss the accompanying questions.

Materials

- One copy of the Comparative Genomics Data and Questions per person (master copy provided)
- Tips and Suggested Answers

Comparative Genomics Data and Questions

For answers, see Tips and Suggested Answers.

Questions:

1. First, look at the Table of Sequence Variation. Note that *intraperson* variation, which is the genetic variation seen within HIV viruses extracted from one person, can be quite high, as in patient D.
 - a. What do you think causes the *intraperson* variation? What qualities of HIV would cause genetic variants of the virus to appear within a single individual?
 - b. What kind of factors would contribute to high *intraperson* variation? What factors about the infected individual or the environment of the virus would cause the emergence of variant viruses?
2. Now look at the Phylogenetic Tree of the different HIV quasispecies from the dentist and patients. *x* and *y* stand for two different genetic variants of HIV, called subtypes, from the same person. For example, HIV samples from the dentist had several HIV subtypes. The two used in this study are called *x* and *y*.
 - a. All the samples—except those from D and F—clustered into a single clade with the sequences from HIV taken from the dentist. On what basis were D and F excluded from the cluster of infections linked to the dentist?
 - b. From the tree, can you tell who was infected by only one of the dentist's viral subtypes and who was infected by both subtypes *x* and *y*?

The Applications:

1. Do you think it is always important to trace the origin of transmitted diseases?
2. Can the techniques used to determine the origin of HIV infection be applied to other infectious diseases? What characteristics of HIV make it more amenable to this type of analysis than other viruses—like polio or smallpox, for example?
3. What would be the advantages of tracing diseases in terms of vaccine development?

The Ramifications:

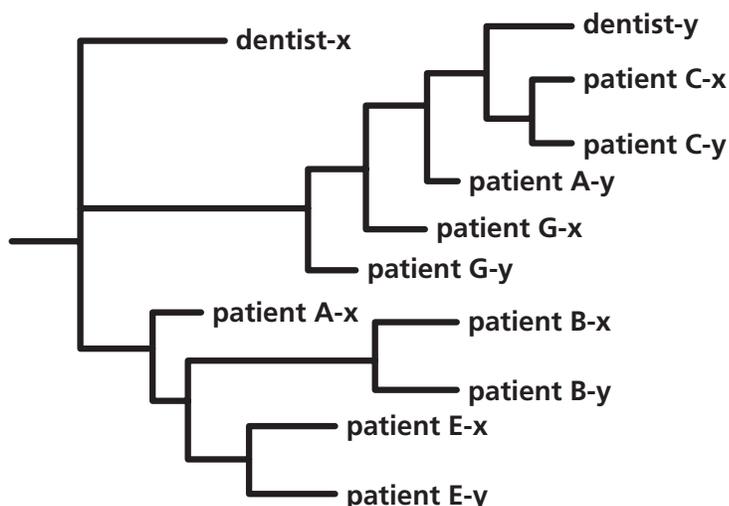
1. The dentist in this case performed invasive dental procedures for three years after he was diagnosed HIV-positive and two years after he was diagnosed with AIDS. What kind of legal ramifications should there be in this situation? What about for transmission of a disease if the person is unaware that they are infected? What if they are aware that they are infected, but believe that they have taken precautions to prevent infecting others?
2. How could information on the evolution of bacterial or viral strains be helpful in developing vaccines or drugs?

The Data:

Table of Sequence Variation. This table shows percent sequence variation for sections of the HIV *env* gene from an HIV-positive dentist, and several patients who were found to be HIV-positive after invasive dental treatments.

Sample	Intraperson Variation (%)	Intraperson Variation (compared to dentist, %)	Intraperson Variation (compared to local HIV+ persons, %)
dentist	3.3	—	11
patient A	2.0	3.4	10.9
patient B	1.9	4.4	11.2
patient C	1.2	3.4	11.1
patient D	7.5	13.6	13.1
patient E	2.1	3.4	10.8
patient F	3.0	10.7	11.9

Phylogenetic Tree. The Dentist and Patients are the same as in the Table of Sequence Variation.



Sources: Ou et al. 1992. Molecular epidemiology of HIV transmission in a dental practice. *Science* 256(5060):1165–71.

HIV and the Dentist, Suggested Answers

Questions:

- 1a. What do you think causes the *intraperson* variation? What qualities of HIV would cause genetic variants of the virus to appear within a single individual?
- 1b. What kind of factors would contribute to high *intraperson* variation? What factors about the infected individual or the environment of the virus would cause the emergence of variant viruses?
HIV has a high mutation rate. Factors that affect the number of variant (quasi-species) viruses a person has include duration of infection, selective pressures caused by the host immune system, disease stage, and therapy (for example, selection by drugs).
- 2a. All the samples—except those from D and F—clustered into a single clade with the sequences from HIV taken from the dentist. On what basis were D and F excluded from the cluster of infections linked to the dentist?
D and F were excluded because the viruses in their samples were as distantly related to the dentist's viruses as they were to viruses from the control group, which contained infections that were epidemiologically unlinked to the dental clade. D and F also had other risk factors that could explain how they were infected.
- 2b. From the tree, can you tell who was infected by only one of the dentist's viral subtypes and who was infected by both subtypes x and y?
Patient A was probably infected with both of the dentist's viral subtypes. In patient A, the subtype y shares a common ancestor with dentist y; this suggests infection with subtype y, with subsequent divergence of the virus in both patient A and the dentist. A's subtype x has a common ancestor with the dentist's other viral subtype.
B and E were infected with a single viral subtype that diverged into their different subtypes. C and G were infected with the other subtype, which diverged into their own subtypes.

The Applications:

What characteristics are required for this type of analysis?

This technique requires a virus like HIV with a high mutation rate and high genetic variability.

Notes
