

CHAPTER

Free Thinking



Tree Thinking

Teruko and her friend Sean were jogging around a track after work. "So, Teruko, how was your brother's wedding in Japan?" Sean asked.

"It was amazing! I couldn't believe all the ceremony," Teruko said. "The reception had some unusual food prepared by special chefs. My favorite was the kujira."

"What's kujira?" Sean asked.

"It's whale meat," Teruko replied. When Sean made a face, Teruko continued. "I know it sounds awful, but it tasted so good. In fact, my dad even tried to bring some canned whale meat back, but customs agents took it at the airport."

"Why?" Sean asked. "Isn't whaling legal in Japan?"

"Sort of. This happened at U.S. Customs. It turns out that you can't bring in any product that is made from marine mammals because so many species are endangered," Teruko explained.

"Oh, that makes sense," Sean said. "So, did your dad get into trouble at the airport?"

"No, they just took the cans away," Teruko sighed.

"I've heard there's a huge black market for whale meat in Asia, and some people pay the equivalent of \$400 a pound," Sean added.

"Yes, and they even have biotech tests now to tell if the meat is really from whales."

"How can they tell what type of meat is in the can?" Sean asked.

"Well, they extract DNA from the sample and compare its sequence to known DNA whale sequences. In fact, forensic DNA testing of 'whale meat' from Asian markets has turned up dolphin, shark, and even horse meat."

"Hm. Maybe they should run the same tests on the meat we had at lunch yesterday," Sean laughed. Now it was Teruko's turn to make a face.

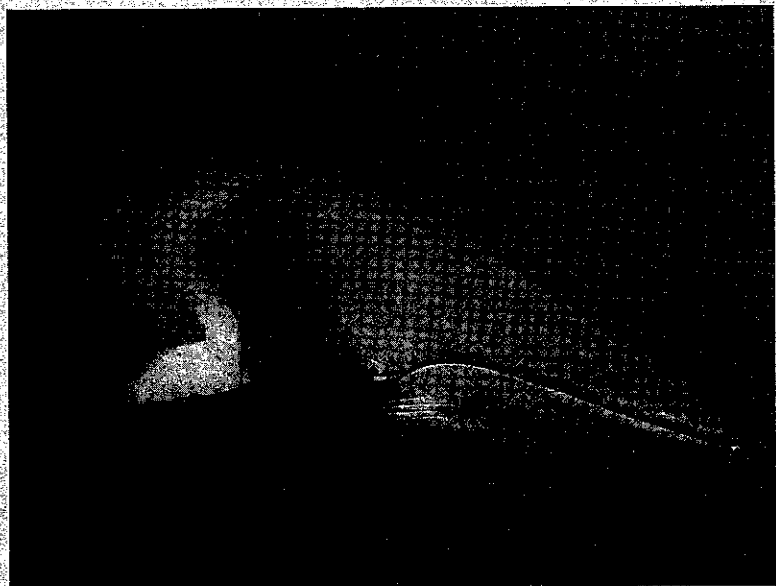


Figure 4.1 Minke whales are found in the Atlantic, Pacific, and Antarctic oceans.

CASE ANALYSIS

1. Recognize potential issues and major topics in the case. What is this case about? Underline terms or phrases that seem to be important to understanding this case. Then list three or four biology-related topics or issues in the case.

2. What specific questions do you have about these topics? By yourself, or better yet, in a group, list what you already know about this case in the “What Do I Know?” column. List questions you would like to learn more about in the “What Do I Need to Know?” column.

What Do I Know?	What Do I Need to Know?

3. Put a check mark by **one to three** questions or issues from the “What Do I Need to Know?” list that you think are most important to explore.

4. What kinds of references or resources would help you answer or explore these questions? Identify two different resources and explain what information each resource is likely to give that will help you answer the question(s). Choose specific resources.

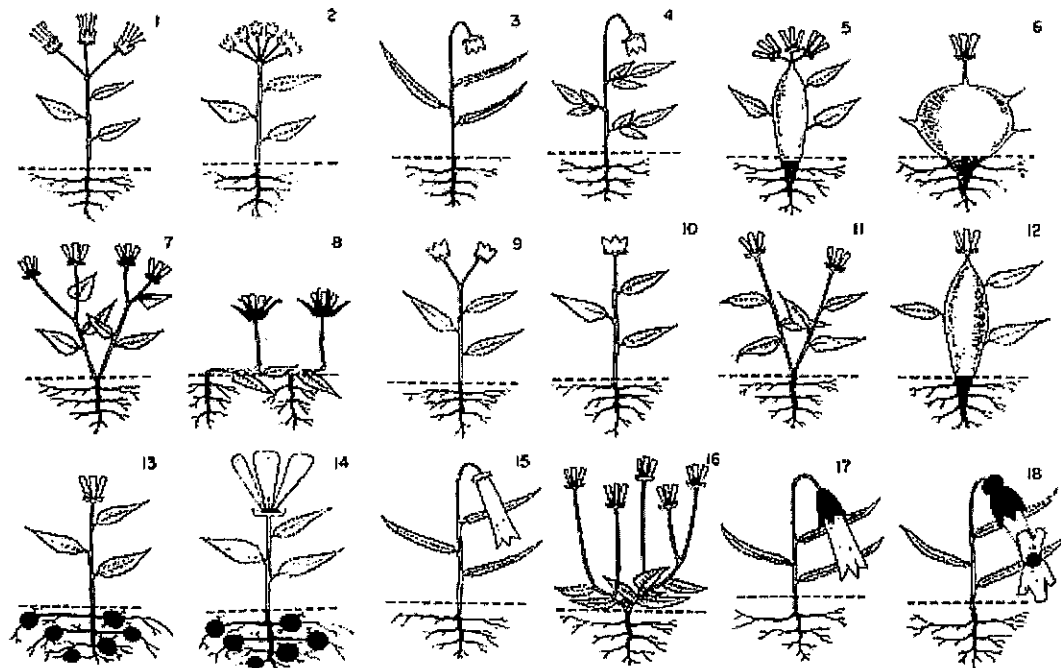
Core Investigations

I. Critical Reading

To complete this investigation, you should have already read Chapter 26: Phylogeny and Systematics.

A. Morphological Observations: Identifying Characters in the Dendrogrammaceae. In this exercise, you will observe and record morphological characters and investigate the construction of a cladogram based on five characters.

Begin by reviewing Figure 4.2 and Table 4.1. Then record the presence or absence of the five characters that are examined in Table 4.1. A "0" indicates that a taxon does not exhibit the particular character. Enter a "1" if the taxon does exhibit a particular character. For example, taxa B, C, and D have narrow leaves, so a "1" has been entered in the table.


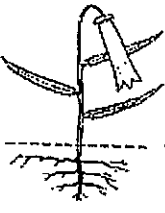
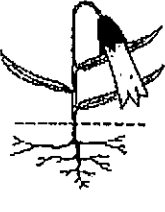
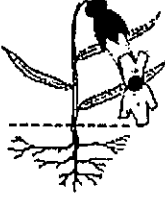



(Wagner, W.H., jr., 2001).

Figure 4.2 The Dendrogrammaceae, an imaginary family of flowering plants (Wagner, W. H., Jr., 2001).

Table 4.1 Observation and Identification of Morphological Characters

(Refer to your text for definitions of unknown terms.)

Selected Taxa	1 Leaves Narrow	2 United Petals, Not Separate	3 Tubelike Petals	4 Elongated Sepals	5 Flower with Bilateral, Not Radial, Symmetry
<p>A</p> 	0				
<p>B</p> 	1				
<p>C</p> 	1				
<p>D</p> 	1				
<p>E</p> 	0				

1. Which trait in Table 4.1 is shared by at least four taxa? Which taxa are they?
2. All five characters are present in which taxon?
3. Are any of these characters shared by all five taxa?
4. One methodology that has proven useful in developing cladograms is to include a taxon that is less related to the other taxa. This "outgroup" is assumed to have ancestral forms of the characters found in these taxa. Characters that are not shared with this "outgroup" taxon are considered derived. Which taxon is the outgroup in this plant family example?

B. Examining a Cladogram. By constructing a cladogram using the morphological characters recorded in Table 4.1, you can infer relatedness among the taxa. Cladograms can be helpful depictions of patterns in levels of relatedness for shared characters among taxa. Taxa are sorted by presence or absence of characters. However, branch distances in a cladogram do not imply chronology. (Phylogenetic trees present hypotheses about the evolution of taxa and imply chronology in diverging branch points.)

Using the characters in Table 4.1, the following cladogram indicates the relationships among the five taxa selected from the Dendrogrammaceae. Notice that there are branches in Figure 4.3 associated with each taxon. Each node is called a clade. Branches C and D are nested within the larger clade that includes B.

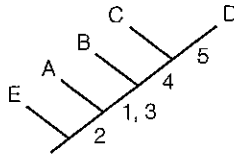


Figure 4.3 The characters are placed below the diagonal. The taxa A, B, C, D, and E are positioned in branches along the diagonal to indicate which characters they share.

1. Which character in Figure 4.3 is exclusively shared by taxa C and D?
2. Consider character 1 in the cladogram. Which taxa lack this character?
3. Can you estimate how much time passed between the development of character 2 and character 4? Explain.

4. Compare the table of characters to the cladogram. Do cladograms contain the same information as the table? Are cladograms more useful? Explain.

C. Identifying Primitive Versus Derived Characters. Simple observation helps differentiate morphological characteristics of these plant taxa, but what characters can help us understand the evolutionary relationships between taxa? To be useful for cladistics, characters must be homologous (reflect shared ancestry). Among the homologous characters, we need to identify the following:

- Shared primitive characters: homologous traits that are common to larger taxonomic groups; for example, flowers are found in all angiosperms.
- Shared derived characters: homologous traits that are limited to particular taxa; for example, flowers with united petals are found in only some angiosperms.

Depending on the taxa included in a cladogram, the same character could be considered primitive in one cladogram and derived in another. For example, consider milk production of mammals. When the taxa are all mammals, then milk production is a primitive character.

However, when the taxa include reptiles, birds, and mammals, then milk production is a derived character.

1. Look at the complete group of taxa in Figure 4.2. Select a taxon with a morphological character (other than those you used in Table 4.1) that you think is a shared derived character. What makes it likely to be derived?

D. Revising a Cladogram. Systematists use existing data or gather new data to consider carefully which characters to use in constructing a cladogram. Determination of the branch points is based on these characters. As new data are discovered, a cladogram may be reconstructed to incorporate the new information.

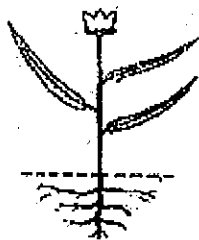
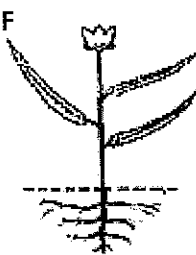



Figure 4.4 Sketch based on a new fossil of extinct Dendrogrammaceae, taxon F.

1. See Figure 4.4 to observe characteristics of a new fossil, taxon F. Enter your observations of taxon F in Table 4.2. *Note:* These are the same five characters used in Table 4.1.

Table 4.2 Character Table for Taxon F

Selected Taxa	1 Leaves Narrow	2 United Petals, Not Separate	3 Tubelike Petals	4 Elongated Sepals	5 Flower with Bilateral, Not Radial, Symmetry
F 					

2. Use the character information from taxon F to redraw the cladogram (Figure 4.3).

II. "Whale Meat Forensics"

A. Using Biotechnology and Systematics. In this investigation you will be working with DNA sequence analysis. You should be familiar with the DNA analysis methods covered in Chapter 20.

People in many regions of the world rely on animals harvested from the sea as a significant source of protein. Several cultures enjoy traditional dishes made from whale meat (Figure 4.5). With declining populations of cetaceans (the mammalian order to which whales and their close relatives belong), however, this practice has come under international scrutiny.

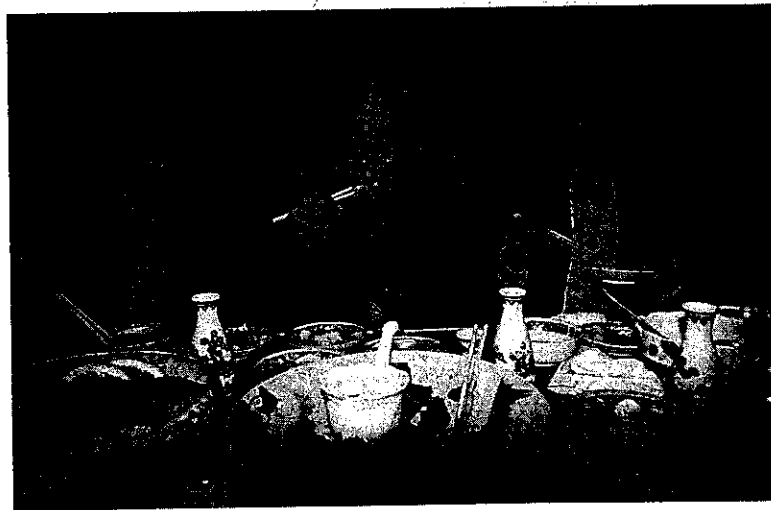


Figure 4.5 Several dishes made from different cuts of whale meat.

From 1993 to 1999, researchers from New Zealand (Baker et al., 2000) analyzed genetic variations in a defined region of cetacean DNA. One of their goals was to identify food products containing meat from protected or endangered cetacean species or noncetacean sources. Investigators began by taking tissue samples from beached and harvested cetaceans. Species identifications of the animals were done onsite by experts in cetacean systematics using multiple morphological characters. DNA from the identified cetacean tissue samples was extracted and the targeted DNA was sequenced. The researchers then sequenced the DNA of samples from whale products (“unknowns”) sold at retail markets in Japan and the Republic of (South) Korea. By comparing the genetic sequences of the unknowns with the known sequences, the researchers could infer the similarity and species identification of the meat in the “whale” products.

Analysis of 655 products revealed meat from baleen whales (eight species or subspecies), sperm whales, pygmy sperm whales, beaked whales (two species), porpoises, killer whales, dolphins (numerous species), and sharks, as well as from domestic sheep and horses (Baker et al., 2000).

1. What types of biotechnology techniques were used to investigate products sold as whale meat?

2. The researchers combined systematics with the use of biotechnology to do their forensic work. Define systematics.

3. Why was it important for a systematist to identify the “known” cetacean species from which DNA was extracted and sequenced?

4. Based on your reading in the case, why do you think vendors would substitute horse meat for whale meat in some of the Asian markets?

B. How Is the Analysis of Whale Products Done? This next investigation introduces forensic tools that were used to study whale products, as well as some of the reasoning processes used by systematists to develop hypotheses about relationships.

In our whale meat example, the task of species identification began with isolating DNA from the unknown meat and then looking for a tiny portion of the genomic DNA. A mitochondrial DNA (mtDNA) control region (shaded, Figure 4.6) consisting of only 500 base pairs (bp) was targeted.

Although much of this region is highly conserved (retained with few differences among species), known differences within a hypervariable subsection of the region were used to distinguish among cetacean species.

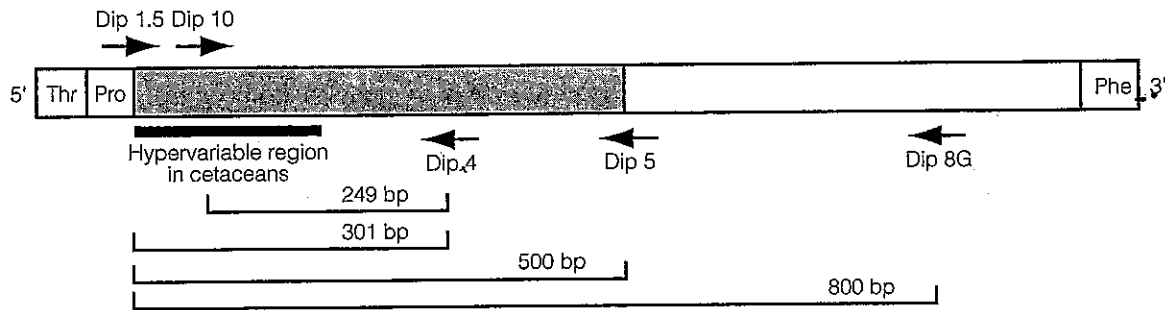


Figure 4.6 A schematic map of the mtDNA control region as well as the binding sites and orientation of the primers used in isolating cetacean DNA. The shaded region represents the portion of the control region covered by most sequences in the reference data sets.

Researchers used PCR to amplify the target mtDNA sequence in the unknown meats. The target mtDNA was then sequenced and aligned with the data set of known cetacean sequences for this segment of DNA. A computer program first compared the target sequence of the sample to known cetacean sequences. Then the program generated a model based on the overall similarities between the target sequence and known cetacean sequences (Figure 4.7).

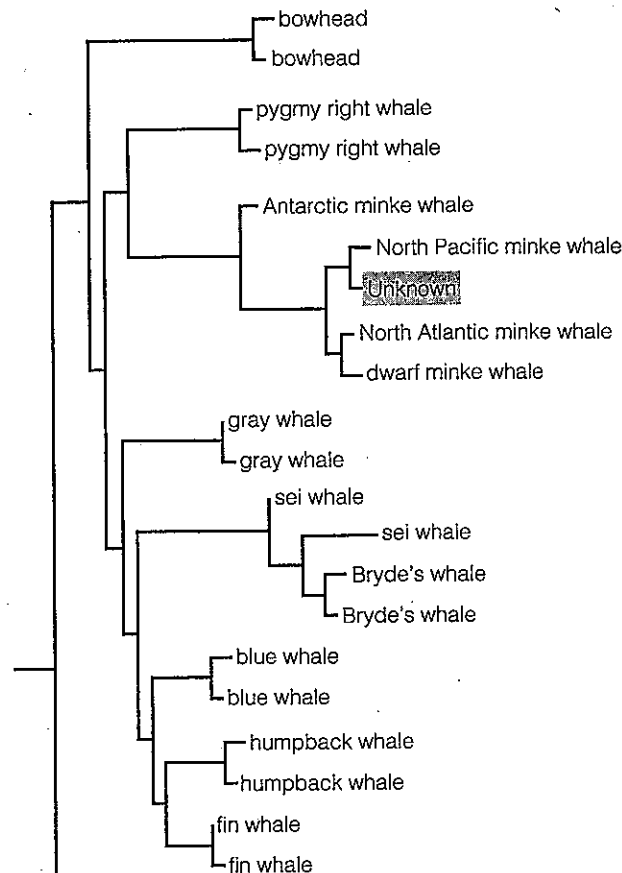


Figure 4.7 Resulting genetic similarity tree showing relative position of unknown samples. Note that the genetic similarity within species is shown by multiple samples in known species, for example, blue whale samples.

1. Which cetacean does the unknown sample most closely match?

The Convention on International Trade in Endangered Species (CITES) works to conserve endangered species by regulating and, where necessary, banning international trade. International trade is banned for those species threatened with extinction. International trade in species that are less endangered or that may become so is permitted when properly regulated. (*Note:* The cetacean identified in your answer to question 1 is found in the second group.)

2. Phylogenetic trees are hypotheses that show a pattern of evolutionary relationships, based on analyses of multiple characters, for multiple taxa. A phylogenetic tree implies a chronological sequence of divergence (branching). Explain why Figure 4.7 is not a phylogenetic tree.

3. Divergence of cetacean species occurred within the last 50 to 65 million years (O'Leary and Geisler, 1999). Explain the difference between the use of a fragment of hypervariable mitochondrial DNA as a molecular marker and the use of a gene such as the one that encodes for hemoglobin. How might you use each of these to compare DNA sequences between cetaceans and other organisms? (*Hint:* See the information in Chapter 26 on molecular clocks.)

4. An attorney defending a whale meat supplier accused of improperly labeling meat would most likely claim that the inferences drawn from the prosecution's evidence were questionable. Provide a potential argument that specifically describes a weakness in the methodology used to infer species identity of the whale meat in this investigation.

5. How might the prosecuting attorney answer this argument? (*Note:* Defend the methodology that was criticized above.)

C. Going Further: Testing Unknowns with "Witness for the Whales." At the website "Witness for the Whales," users can submit unknown mtDNA sequences to be compared against known

cetacean sequences. Genetic similarity analyses can be performed. Results are returned in tree and table format, summarizing the genetic distances between the unknown and reference sequences. (Go to the Case Book website for access to and instructions for "Witness for the Whales.")

III. Which Mammals Are Related Most Closely to Whales?

A. The Ungulates. Most scientists agree that whales are members of the ungulates, or hoofed mammals. Some evidence suggests that whales share a common ancestor that gave rise to other living ungulates such as deer, rhinoceroses, horses, camels, pigs, and hippopotamuses. The relationship between whales and other ungulate taxa is still under investigation (O'Leary and Geisler, 1999).

The ungulates are divided into two orders. Horses, zebras, tapirs, and rhinoceroses are odd-toed hoofed mammals, still known as the Perissodactyla. Even-toed ungulates such as deer, sheep, pigs, and cows are known as the Artiodactyla. Until recently, systematists considered Artiodactyla and Cetacea as two distinct mammalian orders. However, several sources of scientific data support the idea that whales are closely related to the members of Artiodactyla. Artiodactyls and cetaceans are now placed in the same order—Cetartiodactyla. The idea of whales sharing an ancestor with these ungulates would seem unlikely if we compared anatomy in living species only.

1. List three characters that you could observe in living whales that white-tailed deer or other even-toed ungulates do not seem to share.
2. Molecular data can also be used to examine relationships between organisms, but the inaccessibility of comparative DNA samples for extinct taxa limits the usefulness of these data. Evidence for shared characters between whales and ungulates based on protein or nucleic acid sequences involves sampling of extant taxa only. However, whale skeletal data incorporate extensive paleontological data from fossils as well as data from extant species. Scientists use fossil data to help reconstruct the hypothetical relationships among whales and other even-toed ungulate taxa. This is a valuable source of data, because like many other taxa, most of the even-toed ungulates that have existed are now extinct.

Name a character you would expect to find in fossils of early whales that would provide evidence that whales share a common ancestor with other even-toed ungulates.

B. Explore Whale Evolution with the Whippo Problem Space. A good place to begin exploring the relationship between whales and other mammals is the Whippo Problem Space at the BEDROCK bioinformatics education site (see the Case Book website for access information). This Whippo site organizes diverse resources including data and tools to support inquiry.

Consider Figure 4.8. Examine the two trees carefully. Each represents different hypotheses about the evolutionary relationships among the whales and various ungulates. Note that although there are differences in branching patterns (tree shape), both trees include the same outgroup.

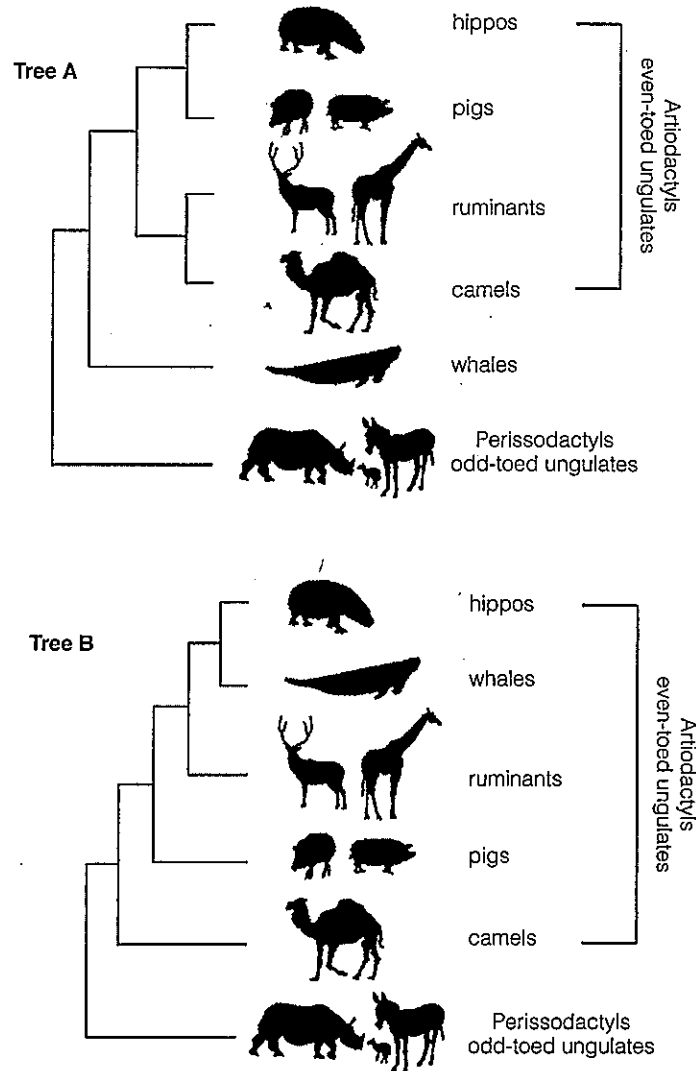


Figure 4.8 Phylogenetic tree representing different hypotheses about the relationships between artiodactyls, the even-toed ungulates. Note the position of whales in each.

1. What is the hypothesized outgroup for both trees?
2. Which tree shows whales and hippos sharing the closest relationship?
3. *Tree thinking* is a term biologists use to describe the process of approaching biological problem solving by considering the role of descent with modification based on phylogenetic evidence. This can result in controversies such as that surrounding the evolution of birds. Did the clade that includes birds diverge from a particular group of dinosaurs? Or did it diverge from a line of reptiles that did not include dinosaurs? These types of questions have fueled much debate and extensive research efforts for decades. Because multiple sources of data exist, both the conclusions reached and the trees drawn by independent researchers may not agree. Not only the selection but also the interpretation of characters

can support different phylogenies. Biologists try to resolve these questions by carefully weighing evidence from multiple sources.

Look closely at the tree shape in Figure 4.9. When you investigate the reasonableness of a tree, you should examine all the hypotheses it contains (Donovan and Hornack, 2004). Each branch point in the tree represents a hypothesis about the relationships among members of the ungulates. The order in which groups diverged is also an explicit hypothesis.

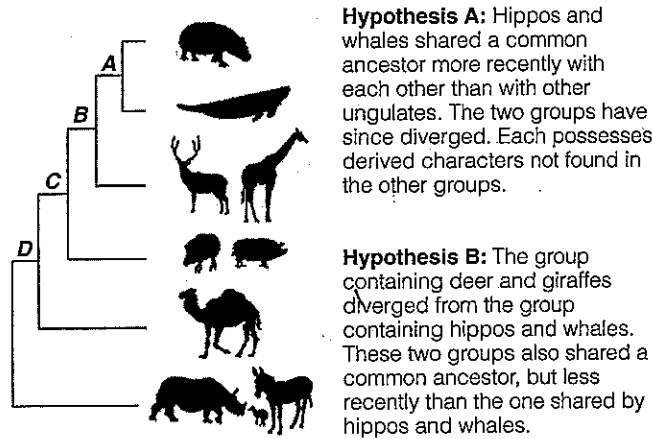


Figure 4.9 Hypotheses in tree B (Figure 4.8) represented by the letters A, B, C, and D.

After you have reviewed the descriptions of hypothesis A and hypothesis B, describe hypothesis D.

4. What does hypothesis D tell us about the relationship between perissodactyls and artiodactyls?

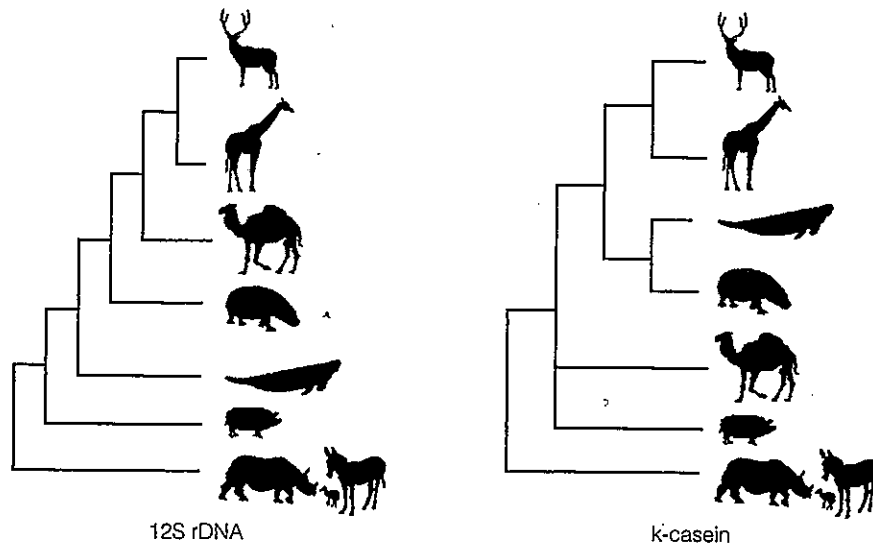


Figure 4.10 Different phylogenetic trees based on select genetic sequences for different molecules. (Note: You can read more about tree interpretation on the Whippo site.)

5. Which of the trees based on molecular sequence data shown in Figure 4.10 supports hypothesis A in tree B? Explain.

6. Does either tree in Figure 4.10 support hypothesis B in tree B? Explain.

7. Do you think molecular sequence data are helpful characters to use to infer phylogenetic relationships? What concerns might you have if a tree were based on a single molecule?

Additional Investigation

IV. Position Paper on Whaling

Explore the management of whaling in the modern world. Explain the role of biotechnology and systematics in increasing the global potential for biologically sustainable management of whale populations. Introduce two or more historical, cultural, economic, political, or ethical issues that should be addressed by policies governing whaling practices. Include reliable resources of data on whale population biology.

Reason from information and data to prepare a three- to five-page position paper that specifically addresses your findings about modern whaling policy.

Examples of issues that your paper might address include:

- How are whale populations sampled?
- What ethical issues are raised by the use of biotechnology to police the whale market?
- Should anyone be allowed to whale? Why or why not?
- Which cetaceans should or could be harvested?
- How much might they be harvested?
- Are cetacean sanctuaries feasible?
- Is the U.S. ban on all marine mammal products reasonable?
- What are relevant cultural issues that might need to be considered?

Resources and links to some websites can be found on the Case Book website.

V. Open-Ended Investigations

A. Four new species of additional Dendrogrammaceae have been discovered. How might this change your phylogram? Evolution can include both acquisition and loss of traits, so more than one cladogram may be possible without further information.

B. Consider using Biology Workbench (<http://workbench.sdsu.edu>) to explore the relationships between cetaceans and taxa other than artiodactyles using genes from the same mitochondrial control region.

References

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