Using Molecular Biology to Establish Phylogeny

Characteristics like similarities in nuclear DNA, mitochondrial DNA, or protein structure, can be used to establish evolutionary relationships. If there is strong agreement between the patterns produced using anatomical similarities and those produced by using molecular biology, it provides what we call "independent confirmation" of our percieved relationships. Independent confirmation is where two or more sources of evidence that are not dependent on each other support the same conclusion. The more independent confirmation that is available, the more confidence we have that our suspected evolutionary relationships are actually correct.

A cladogram for seven animals constructed from anatomical similarities is shown below. In this activity, you will examine and compare the amino acid sequence of a protein found in a wide variety of organisms to confirm the relationships shown in the cladogram. The complete sequence of amino acids for cytochrome c, an enzyme important in the breakdown of food molecules by a cell, has been determined for many organisms and is given in Table 1. Each amino acid is represented by a unique letter in the chart. Cytochrome c is found in most, if not all, known eukaryotes. Over time, random mutations in the DNA sequence occur so the amino acid sequence of Cytochrome c also changes.We assume that if we find fewer differences in the amino acid sequences of two animals, those animals are more closely related.

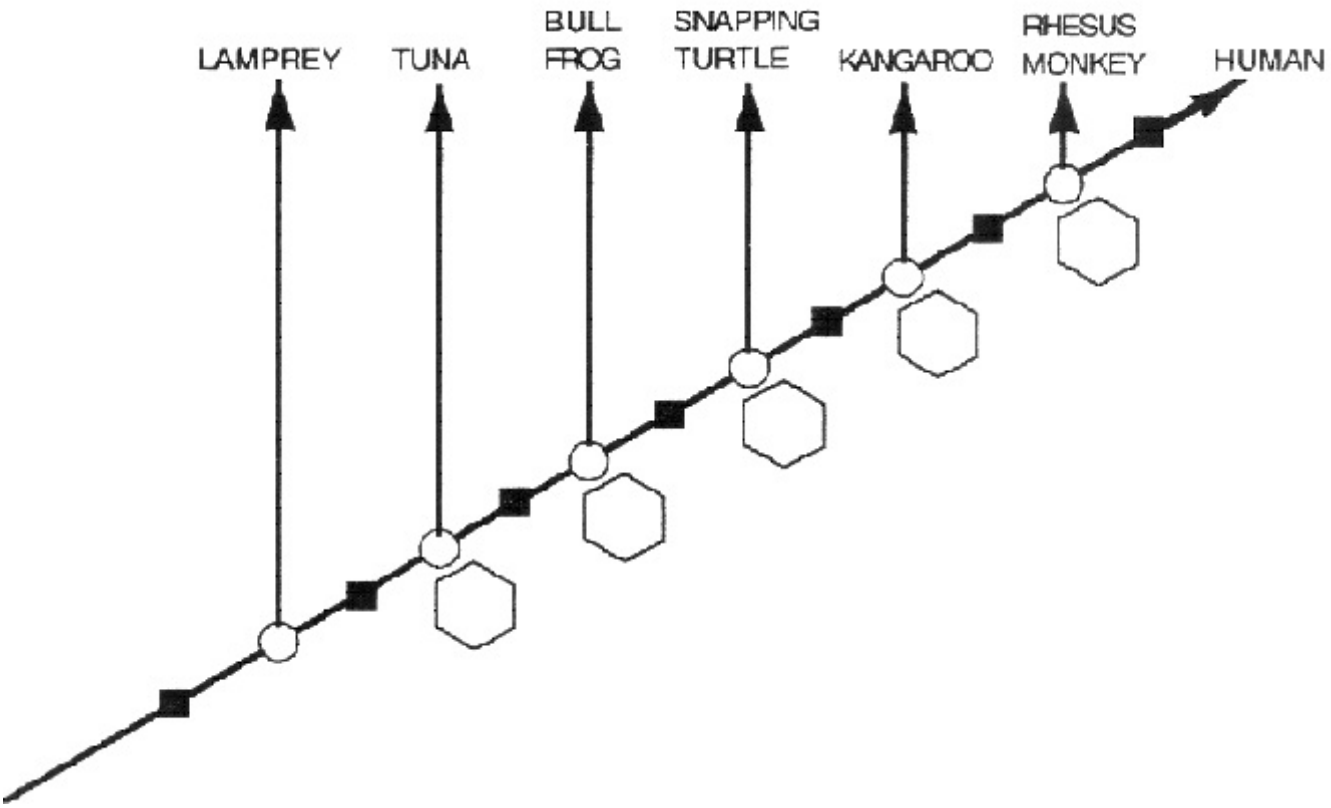

Figure 1 Cladogram for seven species

Questions

1. Compare the sequence of amino acids in human cytochrome c to the sequences for each of the five remaining animals in the cladogram in Figure 1 (the sequence for the lamprey is not given) by counting the number of differences. You might want to highlight or underline the organism being checked, then circle each amino acid which is different from the one above it in the human sequence (use pencil). Amino acids showing no differences in any of the organisms are in blue. When an organism has a "-" instead of an amino acid, that means there is no amino acid there and it should be considered as a difference. When both organisms have a "-" at that spot, it is not considered as a difference.

2. Record the number of differences next to each animal's name and then record it in the appropriate hexagon on the cladogram.

3. Do the amino acid sequence data generally agree with the anatomical data that was used to make this cladogram? (*i.e.*, do organisms with fewer shared anatomical traits also have more amino acid differences?)

4. Based on these data what can you say about the relationship between the rhesus monkey and humans? (use the "duck-chicken" relationship, which show three differences in their amino acids, as a reference).

5. If the molecular data, the structural similarities, and the fossil record all support the same pattern of relationships, can we be fairly confident that the pattern is correct?

6. Using the molecular data, make a statement that compares the "human-kangaroo" relationship to the "human-frog" relationship. Does the cladogram agree with your statement?

7. The chicken and the turkey are both birds and have the same sequence of amino acids in their cytochrome-c. Explain how two different species can have identical cytochrome-c and still be different species.

8. Neurospora and bakers yeast are both fungi. Chickens and turkeys are both birds. What can you say about the evolutionary relationships between the two birds compared to the relationship between the two fungi?

9. Complete Table 2 by counting the amino acid differences between each pair of organisms. Share your data with the class to confirm the accuracy.

10. Construct a cladogram using the data in Table 2. The two most closely related species have the fewest differences in amino acid sequence. Start by placing the two most closely related species on the two shortest branches of the tree. Place the next two closest species on the next shortest branches. Place the species which is the next closest on the next longest branch. Continue until all the species have been placed.

Table 2 Number of amino differences between species

	horse	donkey	whale	chicken	penguin	snake	moth	yeast	wheat
horse	0								
donkey		0							
whale			0						
chickenn				0					
penguin					0				
snake						0			
moth							0		
yeast								0	
wheat									0

11. Cladograms can also be constructed to show relationships between more closely related species. Build a cladogram for chickens, penguins, and turkeys.

12. Explain why more closely related organisms have more similar Cytochrome C.

Table 1: Amino Acid Sequences of Cytochrome c Protein from 17 Different Species

	10 20 30 40 50
Amino Acid #---->	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9
Human	- - - - - - - - G D V E K G K K I F I M K C S Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G Y S Y T A A
Rhesus monkey....	- - - - - - - - G D V E K G K K I F I M K C S Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G Y S Y T A A
Horse	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G F T Y T D A
Donkey..........	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G F S Y T D A
Common zebra	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G F S Y T D A
Pig, cow, sheep..	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G F S Y T D A
Dog.............	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A P G F S Y T D A
Gray whale	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A V G F S Y T D A
Rabbit..........	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A V G F S Y T D A
Kangaroo........	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L N G I F G R K T G Q A P G F T Y T D A
Chicken, Turkey..	- - - - - - - - G D I E K G K K I F V Q K C S Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A E G F S Y T D A
Penguin.........	- - - - - - - - G D I E K G K K I F V Q K C S O C H T V E K G G K H K T G P N L H G I F G R K T G Q A E G F S Y T D A
Pekin duck.......	- - - - - - - - G D V E K G K K I F V Q K C S Q C H T V E K G G K H K T G P N L H G L F G R K T G Q A E G F S Y T D A
Snapping turtle..	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T V E K G G K H K T G P N L N G L I G R K T G Q A E G F S Y T E A
Rattlesnake	- - - - - - - - G D V E K G K K I F S M K C G T C H T V E E G G K H K T G P N L H G L F G R K T G Q A V G Y S Y T A A
Bullfrog........	- - - - - - - - G D V E K G K K I F V Q K C A Q C H T C E K G G K H K V G P N L Y G L I G R K T G Q A A G F S Y T D A
Tuna............	- - - - - - - - G D V A K G K K T F V Q K C A Q C H T V E N G G K H K V G P N L W G L F G R K T G Q A E G Y S Y T D A
Silkworm moth....	- - - - G V P A G N A E N G K K I F V Q R C A Q C H T V E A G G K H K V G P N L H G F Y G R K T G Q A P G F S Y S N A
Wheat	A S F S E A P P G N P D A G A K I F K T K C A Q C H T V D A G A G H K Q G P N L H G L F G R Q S G T T A G Y S Y S A A
Baker's yeast ...	- - - T E F K A G S A K K G A T L F K T R C E L C H T V E K G G P H K V G P N L H G I F G R H S G Q A Q G Y S Y T D A
Neurospora	- - - - G F S A G D S K K G A N L F K T R C A E C H G E G G N L T Q K I G P A L H G L F G R K T G S V D G Y A Y T D A

[CONTINUED FROM ABOVE]

	60 70 80 90 100 110
Amino Acid #---->	0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2
Human	N K N K G I I W G E D T L M E Y L E N P K K Y I P G T K M I F V G I K K K E E R A D L I A Y L K K A T N E
Rhesus monkey.....	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F V G I K K K E E R A D L I A Y L K K A T N E
Horse	N K N K G I T W K E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K T E R E D L I A Y L K K A T N E
Donkey	N K N K G I T W K E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K T E R E D L I A Y L K K A T N E
Common zebra	N K N K G I T W K E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K T E R E D L I A Y L K K A T N E
Pig, cow, sheep...	N K N K G I T W G E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K G E R E D L I A Y L K K A T N E
Dog.............	N K N K G I T W G E E T L M E Y L E N P K K Y I P G T K M I F A G I K K T G E R A D L I A Y L K K A T K E
Gray whale........	N K N K G I T W G E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K G E R A D L I A Y L K K A T N E
Rabbit...........	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K D E R A D L I A Y L K K A T N E
Kangaroo.........	N K N K G I I W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K G E R A D L I A Y L K K A T N E
Chicken, Turkey...	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K S E R V D L I A Y L K D A T S K
Penguin..........	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K S E R A D L I A Y L K D A T S K
Pekin duck........	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K S E R A D L I A Y L K D A T A K
Rattlesnake......	N K N K G I I W G D D T L M E Y L E N P K K Y I P G T K M V F T G L K K K K E R T D L I A Y L K E A T A K
Snapping turtle...	N K N K G I T W G E E T L M E Y L E N P K K Y I P G T K M I F A G I K K K A E R A D L I A Y L K D A T S K
Bullfrog.........	N K N K G I T W G E D T L M E Y L E N P K K Y I P G T K M I F A G I K K K G E R Q D L I A Y L K S A C S K
Tuna.............	N K S K G I V W N N D T L M E Y L E N P K K Y I P G T K M I F A G I K K K G E R Q D L V A Y L K S A T S -
Silkworm moth.....	N K A K G I T W G D D T L F E Y L E N P K K Y I P G T K M V F A G L K K A N E R A D L I A Y L K E S T K -
Wheat	N K N K A V E W E E N T L Y D Y L L N P K K Y I P G T K M V F P G L K K P Q D R A D L I A Y L K K A T S S
Baker's yeast ...	N I K K N V L W D E N N M S E Y L T N P K K Y I P G T K M A F G G L K K E K D R N D L I T Y L K K A C E -
Neurospora	N K Q K G I T W D E N T L F E Y L E N P K K Y I P G T K M A F G G L K K D K D R N D I I T F M K E A T A -

AMINO ACID SYMBOLS

A = Alanine	F = Phenylalanine	K = Lysine	Q = Glutamine	V = Valine
C = Cysteine	G = Glycine	L = Leucine	R = Arginine	W = Tryptohpan
D = Aspartic acid	H = Histidine	M = Methionine	S = Serine	Y = Tyrosine
E = Glutamic acid	I = Isoleucine	N = Asparagine	T = Threonine	

Symbols in light blue represent amino acids which show NO differences in any organism on the list, so you can ignore them. (adapted from Strahler, Arthur, *Science & Earth History*, 1987, p. 348)