

Cytochrome C Comparison Lab

PURPOSE: To compare the relatedness between organisms by examining the amino acid sequence in the protein, Cytochrome C.

BACKGROUND: Genes are made of DNA and are inherited from parent to offspring. Some DNA sequences code for mRNA which, in turn, codes for the amino acid sequence of proteins. Cytochrome C is a protein involved in using energy in the cell. Cytochrome C is found in most, if not all, known eukaryotes. Over time, random mutations in the DNA sequence occur. As a result, the amino acid sequence of Cytochrome C also changes. Cells without usable Cytochrome C are unlikely to survive.

METHOD:

A. Compare the amino acid sequence of Cytochrome C in various organisms.

1. Mark the amino acids which are different Use the example to show you how.

```

- - - - 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 V P A G D V E K 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 L F G R K T
  
```

Note that some of the amino acids are always the same in all species, these have been shaded blue or light gray.

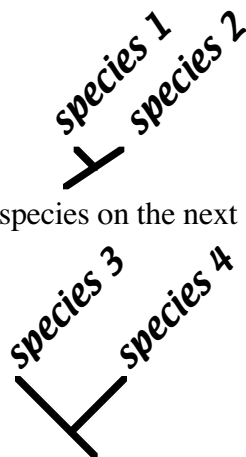
2. Count and record the total number of differences.

Tuna & Fly:	24
----------------	----

3. Note: Be sure to study the entire molecule.
Each protein sequence has 103-112 amino acids; the sequence extends onto two (2) lines.
4. Share your data with the rest of the class and complete Table 1.

B. Make a *branching tree*, or *cladogram* using the data in Table 1.

1. The two most closely related species have the fewest differences in amino acid sequence. Place the two most closely related species on the two shortest branches of the tree.



2. Place the next two closest species on the next shortest branches.

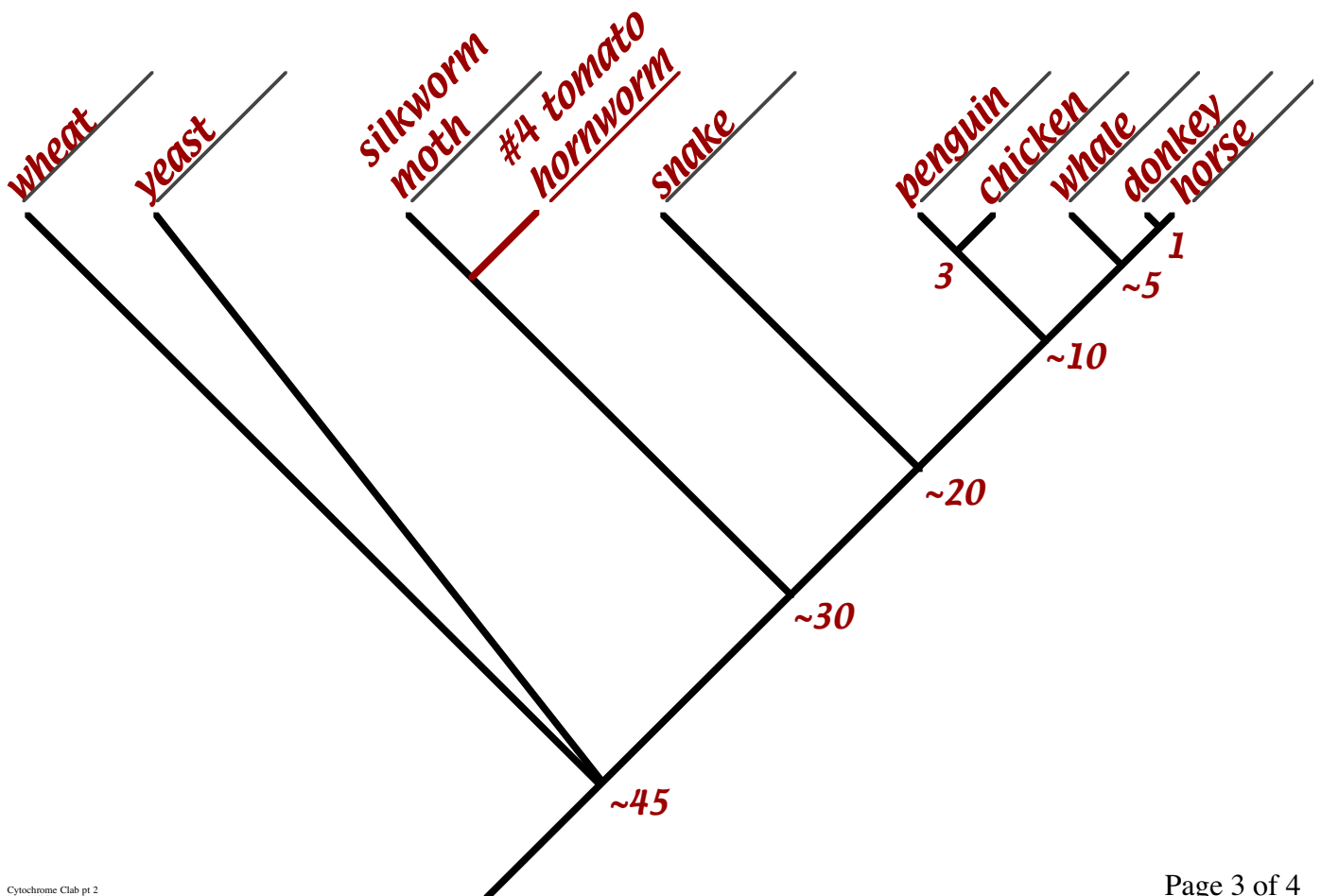
3. Place the species which is the next closest on the next longest branch. Continue until all the species have been placed.

C. Answer the *questions*.

Table 1: Differences between amino acid sequences in Cytochrome C protein for nine species.

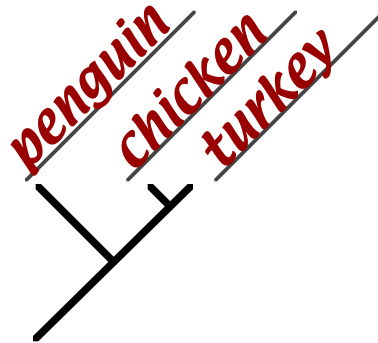
	horse	donkey	whale	chicken	penguin	snake	moth	yeast	wheat
horse	0	1	5	11	13	21	29	45	46
donkey		0	4	10	12	20	28	46	45
whale			0	9	10	18	27	45	44
chicken				0	3	18	29	46	46
penguin					0	17	27	45	46
snake						0	29	46	46
moth							0	48	45
yeast								0	47
wheat									0

**Cladogram, or Branching Tree,
Based on Cytochrome C Amino Acid Sequence**



QUESTIONS:

1. How many Cytochrome C amino acid sequence differences are there between chickens and turkeys? 0 or none
2. Make a branching tree, or cladogram for chickens, penguins, and turkeys.



3. a. Predict the number of Cytochrome C amino acid sequence differences you would expect to see between
- i. horse and zebra accept 2 or less 1
- ii. donkey and zebra 0
- b. What other information did you use to make this prediction?
Horses, donkeys and zebras have similar body structures (anatomy). They look, & act, "horsey."

4. Use this Cytochrome C sequence difference data to add a branch to the tree on page 3.

	horse	penguin	snake	tuna	silkworm moth	yeast	wheat
tomato hornworm moth	26	18	24	28	5	42	38

5. List three other things used to determine how organisms are related to each other.
1. fossils 2. anatomy (body structure)
3. embryology (development before birth)
4. direct comparison of DNA base sequence (This lab is indirect DNA analysis.)
6. Explain why more closely related organisms have more similar Cytochrome C.
- a. More closely related species have more recent common ancestor. The more recent the common ancestor, the less time for DNA mutations to occur.
b. Changes in DNA lead to changes in mRNA leads to differences in protein amino acid sequences.
7. Other data, including other genes, suggests that fungi are more closely related to animals than plants. What are some reasons that the Cytochrome C data suggests that fungi, plants, and animals are equally distantly related?
1. Limits to the total number of possible mutations for a working copy of the gene. 2. Back mutations may occur. DNA changing over time makes a "molecular clock." Here the "watch" has run down!