

## Amino Acid Sequences and Evolutionary Relationships

One technique used to determine evolutionary relationships is to study the biochemical similarity of organisms. Though molds, aardvarks, and humans appear to have little in common physically, a study of their proteins reveals certain similarities. Biologists have perfected techniques for determining the sequence of amino acids in proteins. By comparing the amino acid sequences in homologous proteins of similar organisms and of diverse organisms, evolutionary relationships that might otherwise go undetected can be determined.

Biologists believe that the greater the similarity between the amino acid sequences of two organisms, the closer their relationship. Conversely, the greater the differences, the more distant the relationship. Further, biologists have found that such biochemical evidence compares favorably with other lines of evidence for evolutionary relationships.

In this exercise, you will compare amino acid (AA) sequences in proteins of several vertebrates. You will also study amino acid differences and infer evolutionary relationships among some diverse organisms.

### Part A: Comparing Amino Acid Sequences

1. Examine *Figure 1*, which compares corresponding portions of hemoglobin molecules in humans and five other vertebrate animals. Hemoglobin, a protein composed of several long chains of amino acids, is the oxygen-carrying molecule in red blood cells. The sequence shown is only a portion of a chain made up of 146 amino acids. The numbers in *Figure 1* indicate the position of a particular amino acid in the chain.

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101
Human	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Chimpanzee	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Gorilla	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Rhesus Monkey	GLN	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Horse	ALA	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Kangaroo	LYS	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116
Human	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Chimpanzee	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Gorilla	ASN	PHE	LYS	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Rhesus Monkey	ASN	PHE	LYS	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Horse	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	ALA	LEU	VAL	VAL	ALA	ARG
Kangaroo	ASN	PHE	LYS	LEU	LEU	GLY	ASN	ILE	ILE	VAL	ILE	CYS	LEU	ALA	GLU

*Figure 1*

2. In **Data Table 1** (next page), notice that the abbreviated names of the amino acids in human hemoglobin are printed.
3. In the appropriate spaces in **Data Table 1**, write the abbreviated name of each amino acid in chimpanzee hemoglobin that is *different from* that in human hemoglobin. If there are no differences, leave the spaces blank.
4. For the remaining organisms, write the abbreviated names of the amino acids that do *not* correspond to those in human hemoglobin. **Note:** Always be sure that you compare the amino acid sequence of each organism with that of the human and not the organism on the line above.

**Data Table 1**

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101
Human	THR	LEU	SER	GLU	LEU	HIS	CYS	ASP	LYS	LEU	HIS	VAL	ASP	PRO	GLU
Chimpanzee	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Gorilla	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Rhesus Monkey	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Horse	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Kangaroo	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116
Human	ASN	PHE	ARG	LEU	LEU	GLY	ASN	VAL	LEU	VAL	CYS	VAL	LEU	ALA	HIS
Chimpanzee	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Gorilla	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Rhesus Monkey	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Horse	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Kangaroo	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

5. Use *Figure 1* to complete **Data Table 2**.

**Data Table 2**

Organisms Being Compared	Number of Amino Acid Differences	AA Positions in Which They Vary
Human & Chimpanzee		
Human & Gorilla		
Human & Rhesus Monkey		
Human & Horse		
Human & Kangaroo		

**Analysis and Conclusions for Part A:** Use *Figure 1* to answer questions 1 and 2.

1. On the basis of the hemoglobin similarity, what organisms appear to be most closely related to humans? Justify your answer.

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2. Among the organisms that you compared, which one appears to be least closely related to humans? Justify your answer.

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**Part B: Inferring Evolutionary Relationships from Differences in Amino Acid Sequences**

- Another commonly studied protein is cytochrome *c*. This protein, consisting of 104 amino acids, is located in the mitochondria of cells. There it functions as a respiratory enzyme. Examine *Figure 2*. Using human cytochrome *c* as a standard, the amino acid differences between humans and a number of other organisms are shown.

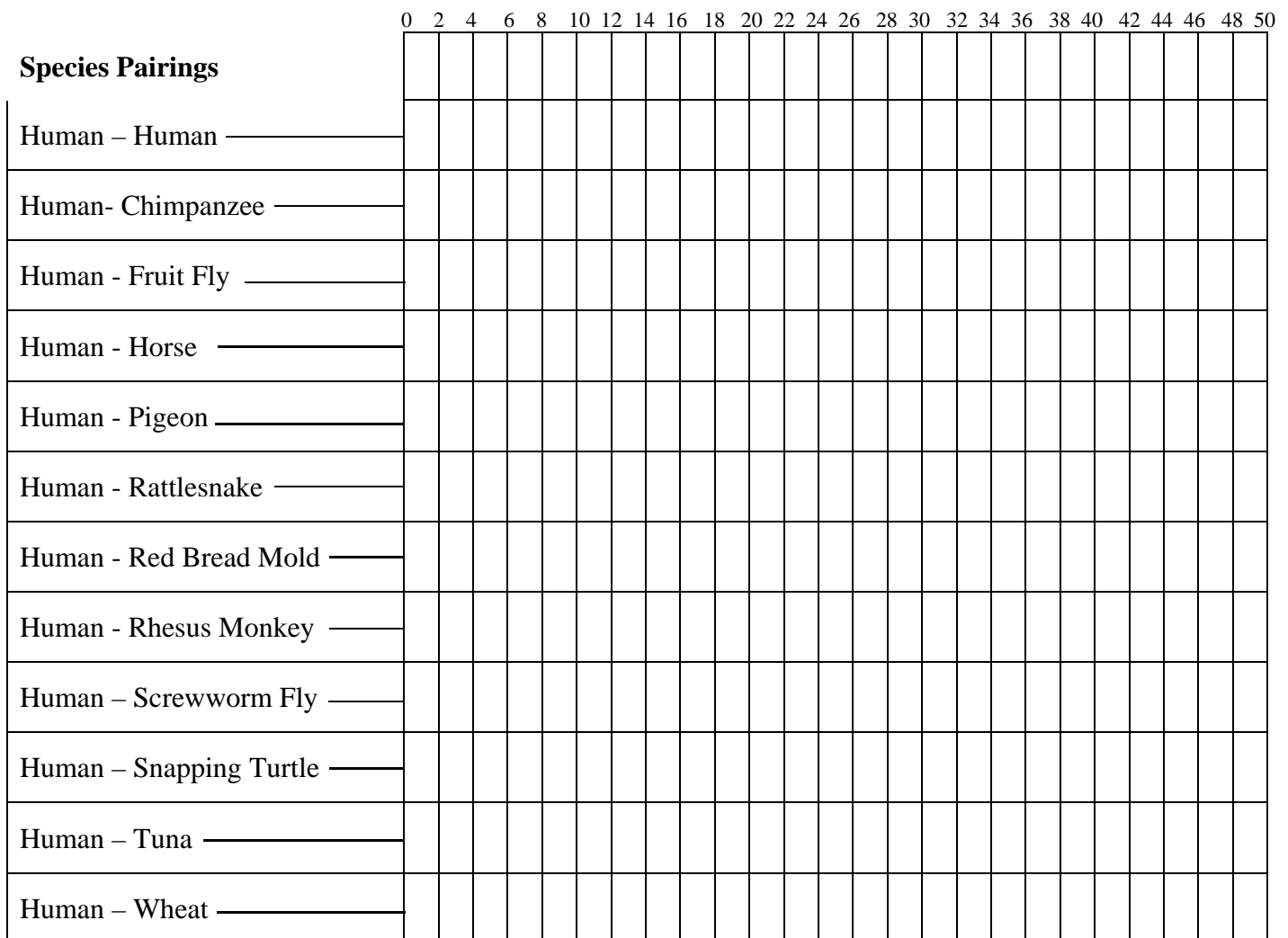
Species Parings	Number of Differences	Species Parings	Number of Differences
Human – Chimpanzee	0	Human – Rhesus Monkey	1
Human – Fruit Fly	29	Human – Screwworm Fly	27
Human – Horse	12	Human – Snapping Turtle	15
Human – Pigeon	12	Human – Tuna	21
Human – Rattlesnake	14	Human – Wheat	43
Human – Red Bread Mold	48		

*Figure 2*

- Using *Figure 2*, construct a bar graph on **Graph 1** to show the amino acid differences between human and other organisms.

**Graph 1**

**Number of Differences**



3. Now examine *Figure 3*. In this figure the cytochrome *c* of a fruit fly is used as a standard in comparing amino acid differences among several organisms. Construct a bar graph on **Graph 2** to show these differences.

Species Pairings	Number of Differences
Fruit Fly – Dogfish Shark	26
Fruit Fly – Pigeon	25
Fruit Fly – Screwworm Fly	2
Fruit Fly – Silkworm Moth	15
Fruit Fly – Tobacco Hornworm Moth	14
Fruit Fly – Wheat	47

*Figure 3*

**Graph 2**

**Number of Differences**

Species Pairings	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50
Fruit Fly – Dogfish Shark																										
Fruit Fly – Pigeon																										
Fruit Fly – Screwworm Fly																										
Fruit Fly – Silkworm Moth																										
Fruit Fly – Tobacco Hornworm Moth																										
Fruit Fly – Wheat																										

**Analysis and Conclusions for Part A:** Use *Figure 1* and *Figure 2* to answer questions 1 through 9.

1. On the basis of differences in cytochrome *c*, which organisms appear to be most closely related to humans?

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2. Which are organisms appear to be least closely related to humans?

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3. Check the pair of organisms that appears to be most closely related to each other. Justify your answer.

- \_\_\_\_\_ snapping turtle – tuna
- \_\_\_\_\_ snapping turtle – rattlesnake
- \_\_\_\_\_ snapping turtle – pigeon

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4. Agree or disagree with the following statement and justify your answer.

“Fruit flies appear to be more closely related to silkworm moths than they are to screwworm flies.”

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5. Name the pair of organisms that appears to be equally related to humans on the basis of cytochrome *c* similarity.

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6. Is it possible that the organisms identified in question 5 could be equally related to humans but not equally related to each other? Justify your answer.

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7. Agree or disagree with the following statement “Fruit flies and humans have about the same evolutionary relationship to wheat.” Justify your answer.

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8. There is a difference of only one amino acid in one chain of the hemoglobin of humans and gorillas. What might have caused this difference?

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9. If the amino acid sequences in the proteins of two organisms are similar, why will their DNA also be similar?

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