

Molecular Phylogeny

Objectives:

1. Understand how variation at the molecular level can be used to study evolution.
2. Construct cladograms and other tree diagrams to represent phylogenetic relationships.
3. Understand how cladograms and other tree diagrams represent testable hypotheses.

I. Introduction:

Hemoglobin is an oxygen-carrying molecule that is found in the red blood cells. Like all proteins, hemoglobin is constructed from building blocks called amino acids. The amino acids are bonded to each other in long chains. Thus, the amino acids are monomers and the protein is a polymer. Different proteins have different sequences of amino acids. Although all hemoglobins are similar, the amino acid sequence varies somewhat among different species. Biologists have used the similarities and differences of these sequences to test hypotheses about evolutionary relationships among these species.

Look at a drawing of the three-dimensional structure of a hemoglobin molecule. Notice that the hemoglobin molecule is made up of four parts. There are two identical α polypeptides and two identical β polypeptides. Each of the α and β polypeptides is attached to a heme group that contains an iron atom that can bind to one oxygen molecule. Thus, each hemoglobin molecule can carry a maximum of four oxygen molecules at one time.

II. Methods:

Tables on pages 4 and 5 give the amino acid sequences for the β polypeptide of hemoglobin in eight different species of mammals. Each type of amino acid is symbolized by a letter. Because there are twenty different kinds of amino acids found in living things, we need to use almost the entire alphabet for symbols. Take a few minutes to compare the sequences. Together with your group members, list three general observations or comparisons that you made from looking at the sequences.

Notice that some positions on the molecule have the same amino acid regardless of the species. List two possible explanations or hypotheses for why these positions are invariant.

Notice that some positions on the molecule are quite variable, with different species having different amino acids. List two possible explanations or hypotheses for why these positions are variable.

Table 1 summarizes the similarities and differences among the hemoglobin β polypeptides. The top number in each cell is the number of positions on the molecule where the two species have identical amino acids. The lower number is the % of positions with identical amino acids.

Complete the table by filling in the blank cells. First, count the number of positions where the two species have identical amino acids. Second, calculate the % similarity using the formula below

$$\% \text{ similarity} = 100 \times \frac{\text{number of identical positions}}{147}$$

Compare your calculations with others around you. Why do you think that computers are almost always used to construct these comparison tables?

Together with your group members study Table 1. List four comparisons that you think are particularly interesting or important.

Table 1. Sequence Similarity of Hemoglobin among Eight Species of Mammals

Opossum	Fox	Human	Bear	Rhesus Monkey	Dog	Capuchin Monkey	
106 72%	135 92%	146 99%	133 90%	142 97%	133 90%	138 94%	Gorilla
	107 73%	105 71%	107 73%		107 73%	106 72%	Opossum
		134 91%	140 95%	133 90%	145 99%	135 92%	Fox
			132 90%	141 96%	132 90%	139 95%	Human
				133 90%	139 95%	134 91%	Bear
					131 89%		Rhesus Monkey
						134 91%	Dog

The top number in each cell is the number of positions on hemoglobin where two species have identical amino acids. The bottom number is the % similarity in amino acid sequences for the two species.

III. Results:

Following your instructor's directions draw a tree diagram representing the % similarities in the hemoglobin β polypeptides among the eight species of mammals that you have studied.

This tree can be thought of as a preliminary hypothesis about phylogenetic relationships among the eight mammal species that you are studying. Following your instructor's directions redraw this tree as a cladogram. You should note that the number of possible cladograms (phylogenetic hypotheses) for eight

species is huge (there are 135,135 possibilities!). Draw one other cladogram that might plausibly be justified by the hemoglobin β data.

Using the data in Table 2 show how the various characters could have evolved on your original cladogram. (Just as you have done in previous weeks, add the characteristics at the appropriate places on your cladogram.) Remember that after characters evolve they may later be lost in some lineages. In very rare cases, similar characteristics may evolve in two separate lineages. This is referred to as convergent evolution. Be sure to identify cases of convergent evolution that appear in your cladogram.

Table 2. Character Table For Eight Species of Mammals

	Gorilla	Opossum	Fox	Human	Bear	Rhesus Monkey	Dog	Capuchin Monkey
Tail	No	Yes	Yes	No	Yes	Yes	Yes	Yes
Placenta	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Marsupial pouch	No	Yes	No	No	No	No	No	No
Opposable thumb	Yes	No	No	Yes	No	Yes	No	No
Prehensile tail	No	Yes	No	No	No	No	No	Yes
Fingernails	Yes	No	No	Yes	No	Yes	No	Yes
Habitual bipedal locomotion	No	No	No	Yes	No	No	No	No
Opposable big toe	Yes	Yes	No	No	No	Yes	No	Yes
Hair	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Menstrual cycle	No	No	No	Yes	No	No	No	No
Number of pairs of upper incisors	2	5	3	2	3	2	3	2
Carnassial teeth	No	No	Yes	No	No	No	Yes	No

Hemoglobinb		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	Rhesus Monkey	M	V	H	L	T	P	D	E	K	A	A	V	T	A	L	W	G	K	V	N	V	D	E	V	G
2	Human	M	V	H	L	T	P	E	E	K	S	A	V	T	A	L	W	G	K	V	N	V	D	E	V	G
3	Gorilla	M	V	H	L	T	P	E	E	K	S	A	V	T	A	L	W	G	K	V	N	V	D	E	V	G
4	Capuchin Monkey	M	V	H	L	T	A	E	E	K	S	A	V	T	T	L	W	G	K	V	N	V	D	E	V	G
5	Dog	-	V	H	L	T	A	E	E	K	S	L	V	S	G	L	W	G	K	V	N	V	D	E	V	G
6	Fox	-	V	H	L	T	A	E	E	K	S	L	V	T	G	L	W	G	K	V	N	V	D	E	V	G
7	Bear	-	V	H	L	T	G	E	E	K	S	L	V	T	G	L	W	G	K	V	N	V	D	E	V	G
8	Opossum	M	V	H	L	T	S	E	E	K	N	C	I	T	T	I	W	S	K	V	Q	V	D	Q	T	G

Hemoglobinb		2	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
1	Rhesus Monkey	G	E	A	L	G	R	L	L	V	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
2	Human	G	E	A	L	G	R	L	L	V	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
3	Gorilla	G	E	A	L	G	R	L	L	V	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
4	Capuchin Monkey	G	E	A	L	G	R	L	L	V	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
5	Dog	G	E	A	L	G	R	L	L	I	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
6	Fox	G	E	A	L	G	R	L	L	I	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
7	Bear	G	E	A	L	G	R	L	L	V	V	Y	P	W	T	Q	R	F	F	E	S	F	G	D	L	S	
8	Opossum	G	E	A	L	G	R	M	L	V	V	Y	P	W	T	T	R	F	F	E	S	F	G	D	L	S	

Hemoglobinb		3	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
1	Rhesus Monkey	S	P	D	A	V	M	G	N	P	K	V	K	A	H	G	K	K	V	L	G	A	F	S	D	G	
2	Human	T	P	D	A	V	M	G	N	P	K	V	K	A	H	G	K	K	V	L	G	A	F	S	D	G	
3	Gorilla	T	P	D	A	V	M	G	N	P	K	V	K	A	H	G	K	K	V	L	G	A	F	S	D	G	
4	Capuchin Monkey	T	P	D	A	V	M	N	N	P	K	V	K	A	H	G	K	K	V	L	G	A	F	S	D	G	
5	Dog	T	P	D	A	V	M	S	N	A	K	V	K	A	H	G	K	K	V	L	N	S	F	S	D	G	
6	Fox	T	P	D	A	V	M	G	N	A	K	V	K	A	H	G	K	K	V	L	N	S	F	S	D	G	
7	Bear	S	A	D	A	I	M	N	N	P	K	V	K	A	H	G	K	K	V	L	N	S	F	S	D	G	
8	Opossum	S	P	G	A	V	M	S	N	S	K	V	Q	A	H	G	A	K	V	L	T	S	F	F	G	E	A

Hemoglobin	4	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1 Rhesus Monkey	L	A	H	L	D	N	L	K	G	T	F	A	Q	L	S	E	L	H	C	D	K	L	H	V	D	
2 Human	L	A	H	L	D	N	L	K	G	T	F	A	T	L	S	E	L	H	C	D	K	L	H	V	D	
3 Gorilla	L	A	H	L	D	N	L	K	G	T	F	A	T	L	S	E	L	H	C	D	K	L	H	V	D	
4 Capuchin Monkey	L	T	H	L	D	N	L	K	G	T	F	A	Q	L	S	E	L	H	C	D	K	L	H	V	D	
5 Dog	L	K	N	L	D	N	L	K	G	T	F	A	K	L	S	E	L	H	C	D	K	L	H	V	D	
6 Fox	L	K	N	L	D	N	L	K	G	T	F	A	K	L	S	E	L	H	C	D	K	L	H	V	D	
7 Bear	L	K	N	L	D	N	L	K	G	T	F	A	K	L	S	E	L	H	C	D	K	L	H	V	D	
8 Opossum	V	K	H	L	D	N	L	K	G	T	Y	A	K	L	S	E	L	H	C	D	K	L	H	V	D	

Hemoglobin	5	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
1 Rhesus Monkey	P	E	N	F	K	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
2 Human	P	E	N	F	R	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
3 Gorilla	P	E	N	F	K	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
4 Capuchin Monkey	P	E	N	F	R	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
5 Dog	P	E	N	F	K	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
6 Fox	P	E	N	F	K	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
7 Bear	P	E	N	F	K	L	L	G	N	V	L	V	C	V	L	A	H	H	F	G	K	E	F	T	P	
8 Opossum	P	E	N	F	K	M	L	G	N	I	V	I	V	I	C	L	A	E	H	F	G	K	D	F	T	P

Hemoglobin	6	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
1 Rhesus Monkey	Q	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
2 Human	P	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
3 Gorilla	P	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
4 Capuchin Monkey	Q	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
5 Dog	Q	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
6 Fox	Q	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
7 Bear	Q	V	Q	A	A	Y	Q	K	V	V	A	G	V	A	N	A	L	A	H	K	Y	H	
8 Opossum	E	C	Q	V	A	W	Q	K	L	V	A	G	V	A	H	A	L	A	H	K	Y	H	