

## Investigating Evolutionary Questions using Online Databases

### PART I: “Are Bats Birds?”

Calvin, from the “Calvin and Hobbes” cartoon series, failed his first school report "Bats are Bugs". He is trying to repair his report and has changed the title to "Bats are Birds". Help convince Calvin that just because bats have wings, they are not really birds, but in fact, bats share more features with mammals than with birds.

**Procedure Part I:** (Answer questions in spaces provided on Data Table sheets)

1. Evaluate the data table below to determine if bats are more closely related to birds or to mammals. Include the morphological features that are shared in your explanation.

TABLE 1: Morphological comparison of birds, bats, and mammals

Feature	Bird	Bat	Other Mammals
Presence of hair	No	Yes	Yes
Presence of feathers	Yes	No	No
Presence of mammary glands	No	Yes	Yes
Presence of wings	Yes	Yes	No
Homeothermy	Yes	Yes	Yes
Four chambers in heart	Yes	Yes	Yes

A. Answer question 1.1 and 1.2 on Data Sheet.

2. Generate a **distance matrix** for the beta-hemoglobin chain for two bird species, two bat species, and two non-bat mammal species. Follow the steps below to do this:

*Step 1:* Begin by going to the UniProt database at <http://www.uniprot.org/>.

*Step 2:* Find the “**Search**” box at the top of the page and make sure **UniProtKB** (Protein Knowledgebase) is selected in the blue box in the search box.

*Step 3:* Type the phrase “**hemoglobin beta**” and click on “**search.**” The computer will retrieve many entries and display them.

*Step 4:* Use the right-hand scroll bar to scroll through the names of the many entries and the black arrows to navigate to the next screens. Find a bird, a bat, or some other mammal. When you find one, check to make sure that it has the hemoglobin *beta* chain (without a number after it) and *not* the alpha or gamma or other hemoglobin subunit. **Click on the box next to the species** to place a check in the box and then click on “**Add to basket**”. Finally, **write** both the common name, scientific name, and the black HBB code of this species in Table 2. The HBB code will help you to identify the species later.

*Step 5:* Repeat Step #4 until you have selected six different species: **two bird species, two bat species, and two non-bat mammal species**. Be sure to save them to the basket each time you are about to leave a screen. You will have to scroll through multiple screens before the first bat appears.

*Step 6:* Click on the **basket** in the upper right hand part of the screen. Click on the **two bat species** and then click the blue **“Align”** to do a pairwise comparison between the two species. This will take you to a new screen, which will run the alignment of DNA sequences and display the results. (You may need to wait a short time initially for this to be done.)

*Step 7:* The protein sequence is located under the “alignment” heading in the middle of the page. The amino acids are indicated with their single-letter symbols. This shows you the actual alignment of the two sequences. Identical amino acids are marked with two dots between them (:). If there is one dot, the change in amino acid is conservative (both amino acids have similar properties and charge), and if there are asterisks (\*) then the two amino acids have different biochemical properties.

*Step 8:* Move farther down the page to the **“Result Information”** heading and find the **percent Identity**. Record that piece of information in the Table 3 grid. This value is essentially the percent of amino acids that are similar. If all the amino acids were the same, the percent would be 100%. The different amino acids result from mutations that have accumulated in the DNA over time. More differences indicate that a longer amount of time has passed since the species diverged.

*Step 9:* A **distance matrix** is a table that shows all the pairwise comparisons between species. Continue to make all pairwise comparisons until Table 3 is filled. For each comparison, use the percent identity for the overlap of all the 146 amino acids.

*Step 10:* Use Table 3 to answer the questions listed below that table. For the final question, select all six species in your basket and click **“Align”**. Find the section that says **“Tree”**. Use the recorded HBB codes to determine the difference between the species in the phylogenetic tree.

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### PART II: “Whale Feet?”

Help a graduate student figure out what the feet of the ancestors of whales were like! She knows that whales are mammals and that, based on earlier studies, they very likely evolved from terrestrial four-legged hooved mammals (ungulates) which, over many generations and millions of years, gave rise to animals fully adapted to life in the sea: whales. She heard that whales could have evolved either from perissodactyl (odd-toed) or from artiodactyl (even-toed) ungulates, and that these two ungulate groups have distinct differences in their feet. Unfortunately, modern whales do not have feet so she can not examine the morphology of those whales to figure out which is the more likely scenario. Help her use hemoglobin beta chain sequence data to evaluate these two hypotheses.

**Procedure Part II:** (Answer questions in spaces provided on data table sheets)

1. Research and examine some specimens or pictures of whales, fish, and perissodactyl (odd-toed) and artiodactyl (even-toed) mammals, and take note of the morphological differences of these on your data sheet and answer questions 2.1 – 2.3.
2. Generate a **distance matrix** for the beta-hemoglobin chain for one whale, one fish, two perissodactyl mammals, and two artiodactyl mammals. Follow the steps below to do this:

*Step 1:* Begin by going to the UniProt database at <http://www.uniprot.org/>.

*Step 2:* Find the “**Search**” box at the top of the page and make sure **UniProtKB** (Protein Knowledgebase) is selected in the blue box in the search box.

*Step 3:* Type the phrase “**hemoglobin beta**” and click on “**search.**” The computer will retrieve many entries and display them.

*Step 4:* Use the right-hand scroll bar to scroll through the names of the many entries and the black arrows to navigate to the next screens. Find a whale, fish, perissodactyl mammal, or artiodactyl mammal. When you find one, check to make sure that it has the hemoglobin *beta* chain (without a number after it) and *not* the alpha or gamma or other hemoglobin subunit. **Click on the box next to the species** to place a check in the box and then click on “**Add to basket**”. Finally, **write** both the common name, scientific name, and the black HBB code of this species in Table 4. The HBB code will help you to identify the species later.

*Step 5:* Repeat Step #4 until you have selected six different species: **one whale, one fish, two perissodactyl mammals, and two artiodactyl mammals**. Be sure to save them to the basket each time you are about to leave a screen. You will have to scroll through multiple screens before the first bat appears.

*Step 6:* Click on the **basket** in the upper right hand part of the screen. Click on the **whale and fish species** and then click the blue “**Align**” to do a pairwise comparison between the two organisms. This will take you to a new screen, which will run the alignment of DNA sequences and display the results. (You may need to wait a short time initially for this to be done.)

*Step 7:* The protein sequence is located under the “alignment” heading in the middle of the page. The amino acids are indicated with their single-letter symbols. This shows you the actual alignment of the two sequences. Identical amino acids are marked with two dots between them (:). If there is one dot, the change in amino acid is conservative (both amino acids have similar properties and charge), and if there are asterisks (\*) then the two amino acids have different biochemical properties.

*Step 8:* Move farther down the page to the “**Result Information**” heading and find the **percent Identity**. Record that piece of information in the Table 5 grid. This value is essentially the percent of amino acids that are similar. If all the amino acids were the same, the percent would be 100%. The different amino acids result from mutations that have accumulated in the DNA over time. More differences indicate that a longer amount of time has passed since the species diverged.

*Step 9:* A **distance matrix** is a table that shows all the pairwise comparisons between species. Continue to make all pairwise comparisons until Table 5 is filled. For each comparison, use the percent identity for the overlap of all the 146 amino acids.

*Step 10:* Use Table 5 to answer the questions listed below that table. For the final question, select all six species in your basket and click “**Align**”. Find the section that says “**Tree**”. Use the recorded HBB codes to determine the difference between the species in the phylogenetic tree.

### Part III: “Reptiles With Feathers?”

Some phylogenetic systematists (scientists who work to make the classification of organisms match their evolutionary history) complain that the vertebrate class Reptilia is improper because it should include birds. In technical terms, the vertebrate class Reptilia is *paraphyletic* because it contains some but not all of the species that arose from the most recent common ancestor to this group. Just how similar are reptiles and birds in terms of the beta-hemoglobin chain? Should birds be considered a type of reptile? You will evaluate this question in this exercise using a BLAST (Best Local Alignment Search Tool) search.

**Procedure Part III:** (Answer questions in the spaces provided on the Data Table Sheets)

*Step 1:* As in the previous two exercises, begin by going to the UniProt database at <http://www.uniprot.org/>.

*Step 2:* Find a beta-hemoglobin chain for any type of crocodile. You can do this just as in the previous two exercises or you can type in hemoglobin beta for the search keywords OR you can type “crocodile” into the query box instead. Make sure to select the hemoglobin *beta* chain (preferably without a number after it)

*Step 3:* A BLAST (Best Local Alignment Search Tool) search takes a particular sequence and then locates the most similar sequences in the entire database. A BLAST search will result in a list of sequences with the first sequence being most close to the one entered and the last sequences being least similar. The easiest way is for us to do a BLAST search is using links within UNIPROT as follows. Once you have found and clicked on a crocodile entry for the beta-hemoglobin chain, click on the blue “Blast” button at the top of your screen. It may take a few minutes for the results to appear.

*Step 4:* The next screen will have a list of sequences in order of similarity. List those species in Table 6 beginning with the first most similar species that is not a crocodile, then answer the 5 questions (3.1-3.6) asked following the data table. You may need to Google the scientific name in order to determine which organism it is.

## Articles

Science. 1990 Jul 13;249(4965):154-7.

### **Hind limbs of eocene basilosaurus: evidence of feet in whales.**

Gingerich PD, Smith BH, Simons EL.

#### **Modified Abstract**

New specimens of middle Eocene (geologic timescale epoch from 56 to 33.9 million years ago) **Basilosaurus isis** from Egypt include the first functional pelvic limb and foot bones known in Cetacea. Cetacea is a clade of carnivorous, finned, aquatic marine mammals. These are important in corroborating the intermediate evolutionary position of **archaeocetes** between generalized Paleocene (geologic timescale epoch from 66 to 56 million years ago) land mammals that used hind limbs in locomotion and Oligocene-to- Recent (geologic timescale epoch from 33.9-23 million years ago) whales that lack functional pelvic limbs. The foot is paraxonic, consistent with derivation from **mesonychid** Condylarthra. Hind limbs of Basilosaurus are interpreted as copulatory guides.

\*\*Artiodactyls are paraxonic meaning their bodyweight is separated evenly on two parallel axes on their feet. Perissodactyls are mesaxonic meaning weight tends to be distributed to the middle digit of the foot.

Nature. 1997 Aug 14;388(6643):666-70.

### **Molecular evidence from retroposons that whales form a clade within even-toed ungulates.**

Shimamura M, Yasue H, Ohshima K, Abe H, Kato H, Kishiro T, Goto M, Munechika I, Okada N.

#### **Abstract**

The origin of whales and their transition from terrestrial life to a fully aquatic existence has been studied in depth. Palaeontological, morphological and molecular studies suggest that the order Cetacea (whales, dolphins and porpoises) is more closely related to the order Artiodactyla (even-toed ungulates, including cows, camels and pigs) than to other ungulate orders. The traditional view that the order Artiodactyla is monophyletic has been challenged by molecular analyses of variations in mitochondrial and nuclear DNA. We have characterized two families of short interspersed elements (SINEs) that were present exclusively in the genomes of whales, ruminants and hippopotamuses, but not in those of camels and pigs. We made an extensive survey of retropositional events that might have occurred during the divergence of whales and even-toed ungulates. We have characterized nine retropositional events of a SINE unit, each of which provides phylogenetic resolution of the relationships among whales, ruminants, hippopotamuses and pigs. Our data provide evidence that whales, ruminants and hippopotamuses form a monophyletic group.

## Teachers Notes on Lesson

- Students will work in pairs to split up the work of running the % identity differences
- For a faster search, type in beta hemoglobin AND the name of the organism they are searching for instead of scrolling through pages and pages and scanning for what they need. Works great for bats, but might not be as good for birds. They can search for a specific bird.
- Students can duplicate the pages and run multiple alignments at one time
- Will work on the ipad as well
- Students can click on the blue names of the species to see a picture of the organism