

Lion Phylogeny: Finding connections

Review the DNA samples below. The DNA listed is a segment of a single gene for **cytochrome b**, a critical protein found in all organisms. What do you notice about the samples?

Cameroon	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT T GAAAACCGCCTCCTCAAAT
Tsavo	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT T GAAA T CGCCTCCTCAAAT
Fannie Roberts	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT C GAAAACCGCCTCCTCAAAT
Sabi Sands	TTCTCCA C TCTTCTAATCCTAATACCCATCTCAGGCATTAT T GAAAACCGCCTCCTGAAAT
Umfolozi	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT C GAAAACCGCCTCCTCAAAT
Zimbabwe	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT C GAAAACCGCCTCCTCAAAT
Zambia	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT C GAAAACCGCCTCCTCAAAT
Kalahari	TTCTCCA T TCTTCTAATCCTAATACCCATCTCAGGCATTAT C GAAAACCGCCTCCTCAAAT
Botswana	TTCTCCA C TCTTCTAATCCTAATACCCATCTCAGGCATTAT T GAAAACCGCCTCCTCAAAT
Etosha	TTCTCCA C TCTTCTAATCCTAATACCCATCTCAGGCATTAT T GAAAACCGCCTCCTCAAAT

1. Look at the DNA samples from Cytochrome b shown above. What do you notice about the sequences for each population?
2. Lion populations are found all over Sub-Saharan Africa. How do the sequences above reinforce or contradict the idea that populations of lions who are genetically similar live in the same geographic area?
3. Based only on the DNA, which populations do you think live in closer proximity to each other? What do you expect to see when comparing genes of lions that live closer together?
4. With the previous activity, multiple genes were used to create the bar microsatellite data. Now that we are zooming in on one gene, does the data support yesterday's conclusions? Cite specific evidence to support your answers.
5. We will now use the DNA sequences to analyze the DNA in order to find which lion populations are most closely related. We will construct a picture, called a phylogenetic tree. Use the directions that follow to construct your tree.

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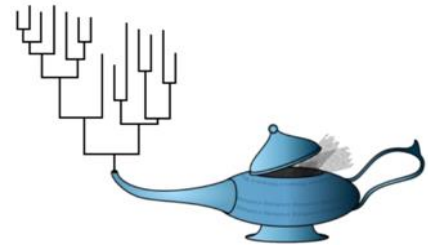
You will now copy and paste actual DNA sequences from the different lion populations into a program that will generate a phylogenetic tree, a diagram that helps illustrate the evolution of different groups of lions.

Follow this link to get the Lion DNA DATA: <http://tinyurl.com/LionDNADATA>

Go to the phylogenetic tree maker website at <http://phylogeny.lirmm.fr/phylo.cgi/index.cgi>

- Click on the "One Click" option.
- You will copy all of the DNA sequences into the large window on the website. (See below)
- IMPORTANT! Be sure there are NO spaces before the first sequence or after the last sequence. Delete only those spaces and not the spaces between the sequences of the different lion populations.
- Click Submit.
- You do NOT need to enter your email to get your results. They will simply show on the screen once the analysis is complete. This usually takes up to a minute or two.

Phylogeny.fr
Robust Phylogenetic Analysis For The Non-Specialist



Phylogeny analysis

"One Click"

Paste your set of sequences and let the software make decisions on your behalf (Each step is optimized for your data).

"Advanced"

Manually set parameters for the various steps.

"A la Carte"

Create your own phylogeny workflow using more programs available.

"One Click" Mode

Alignment MUSCLE → Curation Gblocks → Phylogeny PhyML → Tree Rendering TreeDyn

1. Overview 2. Data & Settings

Name of the analysis (optional):

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:
 No file chosen

Or paste it here (load example of sequences)

Clear

Maximum number of sequences is 200 for proteins and 200 for nucleic acids.
Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids.

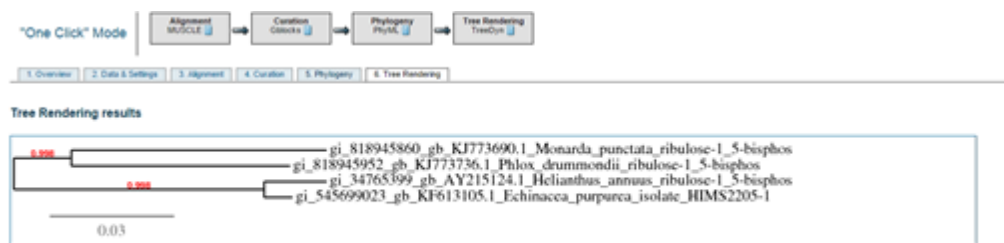
► Names association

☒ Use the Gblocks program to eliminate poorly aligned positions and divergent regions

☒ To receive the results by e-mail, enter your address(es):

☐ Do not attach result files

- Your results will look something like this:



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- Now you need to manipulate the tree so that it is easier to analyze.
 - Use the screenshot below to check and uncheck the appropriate boxes as shown.
 - Click on Reroot using mid-point rooting.
 - Under “Display,” unclick Branch annotation.
 - To make the branches easier to view, click “Ignore branch length” under “Display.”

Dynamic Tree Edition

☒ Color	☒ leaf	using color blue	☒ Reset to original tree	☒ Reroot using mid-point rooting
	☒ branch	and assign the group name	☒ Reroot (outgroup)	☒ Flip subtree
☒ Add annotations using color red			☒ Swap subtrees	☒ Change leaf name

Display:

☐ Branch annotation:	☒ Branch support values	☐ Branch length values	Use color: red
☒ Legend at position	25	120	Update Use color: dimgray

☒ Ignore branch length

Leaves font:

Tree conformation: ☒ Rectangular ☐ Radial

Image size: ☐ Small ☒ Medium ☐ Large ☐ Extra large

- You can download and save the photo or you can simply right-mouse click on it and print a copy for each of your group members. This should be included with your assignment.

Phylogeny results

Compare your tree with the sample tree provided here. Pay particular attention to pairs of populations who appear to be closely related on this tree as well as your own tree.

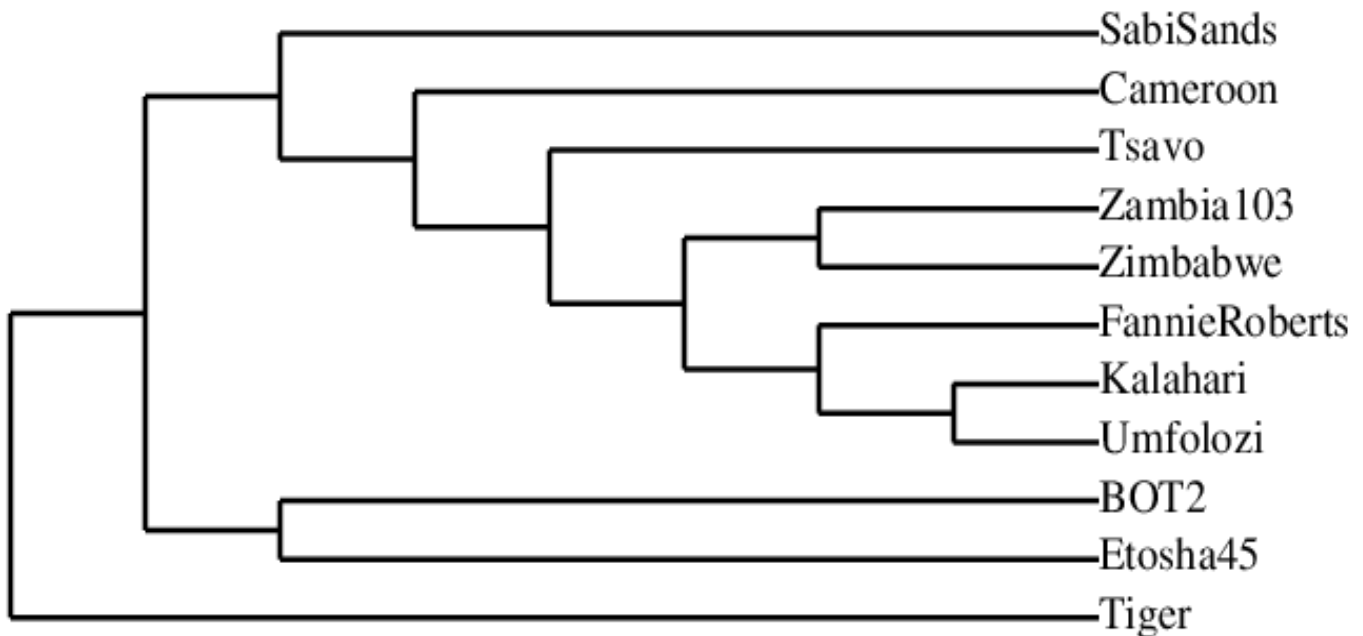


Figure 1: Phylogenetic tree.

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6. BOT2 and Etosha45 populations are on the same branch of the phylogenetic tree. What explanation can you propose from this observation? Discuss this among your pride members.

7. In the activity you did in the last class, you made a prediction about which prides lived closer together. In what way does this phylogenetic tree support or refute your prediction?

8. On the phylogenetic tree, tigers are farther from the other populations on the tree. How does this observation support what you already know about lions and tigers?

Yesterday, you located each of lion population on a geographic map using their GPS coordinates.

9. Compare the proximity of populations on the phylogenetic tree to location on the geographic map. Cite specific examples where the geographic location and genetic evidence shows relatedness.

10. What assumptions can you make about these observations?