

Phylogenetic Trees

In today's lab you will be constructing two phylogenetic trees using the maximum likelihood method and two mitochondrial genes. Rather than selecting a model of evolution and adjusting statistical parameters, as is typical when building a phylogeny using maximum likelihood, you will be using a simpler "one click" method.

Background

The Dodo (*Raphus cucullatus*) was a flightless bird native to the island of Mauritius. It became extinct in the 1600's and is one of the most famous examples of human-induced extinction. Having evolved in the absence of significant predators, it was fearless of humans and was extirpated by a combination of hunting and nest predation by human-introduced pigs, monkeys, cats, rats, and dogs.



It has been variously suggested that Dodo's were a type of parrot, ostrich, rail, albatross, or vulture (Owen 1846). Theodore Reinhardt suggested that it was most closely related to pigeons, which was later supported after the examination of the Oxford museum specimen (Strickland and Melville 1848). While this theory has been generally accepted, the complete evolutionary history of the Dodo remained unknown until recently. In this lab, you will use genetic data from museum specimens to reconstruct the Dodo phylogeny.

Phylogenetic Tree 1: Part 1

You have been given a data file (named “cytb”) with the species names and genetic sequences of the cytochrome b gene from the mitochondria of 35 species of birds. The data is in FASTA format, which can be seen in the following example:

Begin each entry with a 'greater than' symbol →

Give your sample a name (use letters and/or numbers without spaces) →

On the next line, enter your sequence with no spaces or line breaks →

```
>Human
GATCACAGGTATCACCTATTACCACTCACGGGAG
>Chimpanzee
GATCACTGGTATCAGGCTATTACCACTCACGGGAG
>Gorilla
GATCACTGCTATCAGGCTATTACCTCTCACGGGAG
```

Before creating your tree, your sequence data must first be aligned. To do this, you will use the program Muscle.

1. In your browser, go to the following website: <http://moyle.pasteur.fr>
2. In the “Programs” column to the left, click “alignment”.
3. Under “alignment”, click “multiple”.
4. Under “multiple”, click “muscle”.
5. Open your cytb file using Text Editor (PC) or TextEdit (Mac), copy all of the data, and paste it in the box below “Enter your data below:”. Alternatively, you may click the “upload” tab, click the “Browse...” button, and select your cytb file.
6. Click “Run”.

Mobyle @Pasteur (guest) set email | sign-in | activate | sign-out refresh workspace

Welcome Forms Data Bookmarks Jobs Tutorials

muscle x clustalw-multialign x

Muscle 3.8.31

Step 6 → Run Reset

MUSCLE is a program for creating multiple alignments of amino acid or nucleotide sequences.

advanced options

Inputs options

Sequences (-in)

paste db upload EDIT CLEAR

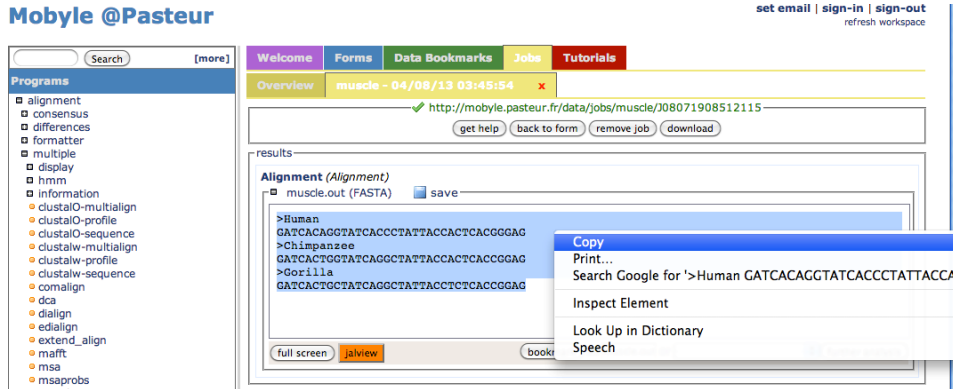
Enter your data below:

References :
Edgar, Robert C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research* 32(5), 1792-97.

Author(s): Edgar, R.C.
<http://www.drive5.com/muscle/>

Programs: alignment ← Step 2, consensus, differences, formatter ← Step 3, multiple, display, hmm, information, clustalO-multialign, clustalO-profile, clustalO-sequence, clustalw-multialign, clustalw-profile, clustalw-sequence, comalign, dca, dialign, edialign, extend_align, mafft, msa, msaprobs ← Step 4, muscle, pima, plotcon, T-Coffee@rpbs, tranalign, pairwise, structure, assembly

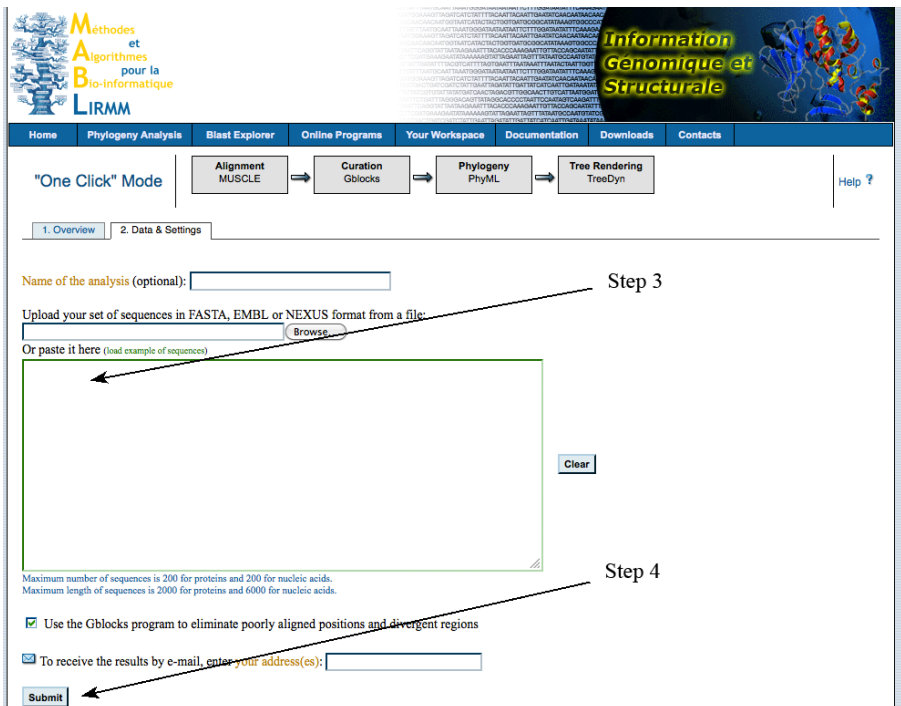
7. Enter your email address when prompted, click “Ok”.
8. Select and copy the results in the “Alignment” box.



9. Open a new file in Text Editor (PC) or TextEdit (Mac) and paste your aligned data. Save as: cytb_aligned. Alternatively, click “save” under “Alignment”; rename your file.

You will now use your aligned data to build a tree. You will use the program PhyML.

1. In your browser, go to the following website: <http://www.phylogeny.fr>
2. Under “Phylogeny analysis” (about half way down the page), click on “One Click”.
3. Open your cytb_aligned file, copy all of the data, and paste it in the box below “Or paste it here”. Alternatively, you may click the “Browse...” button and select your file.
4. Click the “Submit” button at the bottom of the page.



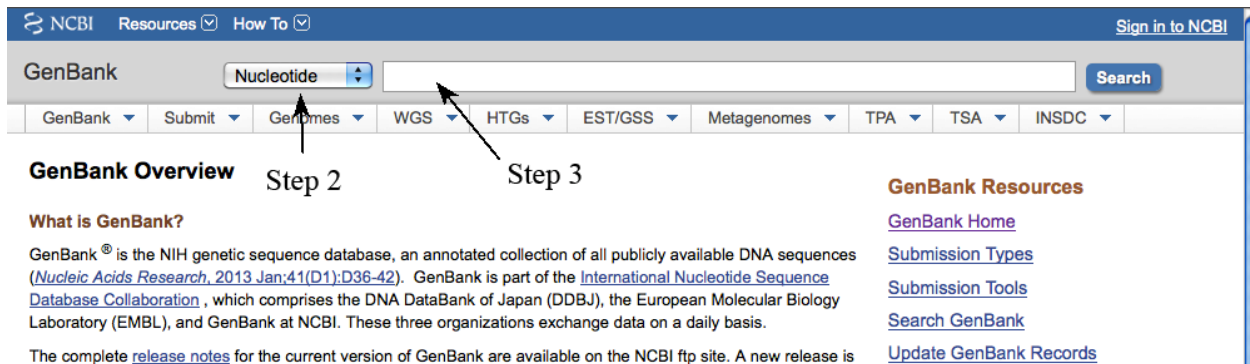
Draw your tree:

Phylogenetic Tree 1: Part 2

Next, you will add additional taxa to your tree: the Dodo (*Raphus cucullatus*) from Mauritius island and its presumed relative the Rodrigues Solitaire (*Pezophaps solitaria*) from Rodrigues island. Both of these birds have been extinct for hundreds of years but DNA was isolated from specimens originally collected for (and preserved in) natural history museums.

You will download the data from GenBank (the national repository for genetic data) and add it to your cytb file. Through GenBank, you can find genetic data by searching for the species and gene names, the name of a journal article (if there is a published article for which sequences have been deposited in GenBank), or the Accession number (each sample's unique ID) if you know it.

1. In your browser, go to the following website: <http://www.ncbi.nlm.nih.gov/genbank>
2. Verify that the pull-down menu at the top of the page is set for "Nucleotide".
3. Type the following Accession number in the search bar and click "Search": AF483338.1



What species did the data come from?

What gene is this?

What is the title of the journal article that this sequence was used for?

What journal did this article appear in?

List the authors of the article:

4. Click “FASTA” under the GenBank Accession number.

mitochondrial gene for mitochondrial product

GenBank: AF483301.1

[FASTA](#) [Graphics](#) [PopSet](#)

[Go to:](#)

Step 4

LOCUS AF483301 233 bp DNA linear VRT 18-DEC-2002

Customize view

Analyze this sequence

Run BLAST

Pick Primers

5. Copy the FASTA sequence and paste at the end of your cytb file (you may want to change the name to match the format of the rest of the file).
6. Repeat Steps 3-5 with Accession number: AF483337.1
7. Save the file as cytb-2.

What species did the data come from?

What gene is this?

Is this sequence data from the same journal article as the previous sequence?

Now you will use your new data to build another phylogeny.

1. Align your data: using your cytb-2 file, follow Steps 1-9 on Pages 2 and 3.
2. Build your tree: using your cytb-2_aligned file, follow Steps 1-5 on Pages 3 and 4.

Draw your tree:

Which species is most closely related to the Dodo?

Which species is the next most closely related to the Dodo?

Next you will compare your tree to the tree in the article for which this data was generated.

1. Go to Google Scholar (<http://scholar.google.com/>).
2. Using the information you recorded on Page 5, find and download the journal article.

Is the tree in the paper the same as yours?

Which species is most closely related to the Dodo according to the paper?

Did the authors use the same statistical method (maximum likelihood)?

How many genes did the authors use to generate their tree?

According to the paper, in which region (Australian, African/Madagascan, Asian/Southeast Asian, Mascarene Island, or New World) do the closest relatives to the Dodo and Rodrigues Solitaire reside?

Using the tree in the paper, on which continent did the ancestor of the Dodo most likely live?

How do the authors suggest the Dodo and Rodrigues Solitaire reached their islands?

Using the article, GenBank, Google, etc., fill in the empty boxes (make sure the Accession numbers are from the individual used in the article for the cytochrome b gene):

Scientific Name	Common Name	Location	cytb Accession
<i>Alectroenas madagascariensis</i>	Madagascar Blue Pigeon	African	AF483344.1
<i>Caloenas nicobarica</i>	Nicobar Pigeon	Asian	AF483336.1
<i>Chalcophaps indica</i>	Common Emerald Dove	Asian	AF483325.1
<i>Columba palumbus</i>	Common Wood Pigeon	Asian	AF483335.1
<i>Columbina cruziana</i>	Croaking Ground Dove	New World	AF483318.1
<i>Didunculus strigirostris</i>		Asian	AF483343.1
<i>Drepanoptila holosericea</i>	Cloven-feathered Dove	Asian	
<i>Ducula aenea</i>	Green Imperial Pigeon	Asian	AF483331.1
<i>Ducula pinon</i>	Pinon's Imperial Pigeon	Asian	AF483332.1
<i>Ducula zoeae</i>	Zoe's Imperial Pigeon	Asian	AF483333.1
	Passenger Pigeon	New World	AF483351.1
<i>Gallicolumba beccarii</i>	Bronze Ground Dove	Asian	AF483346.1
<i>Gallicolumba luzonica</i>	Luzon Bleeding-heart	Asian	AF483334.1
<i>Gallicolumba tristigmata</i>	Sulawesi Ground Dove	Asian	AF483319.1
<i>Geopelia cuneata</i>	Diamond Dove	Australian	AF483317.1
<i>Geotrygon versicolor</i>	Crested Quail-Dove	New World	
<i>Goura victoria</i>	Victoria Crowned Pigeon	Asian	AF483320.1
<i>Leptotila megalura</i>	Yungas Dove	New World	AF483342.1
<i>Leucosarcia melanoleuca</i>	Wonga Pigeon	Australian	AF483327.1
<i>Macropygia phasianella</i>	Slender-billed Cuckoo-Dove	Asian	AF483339.1
<i>Nesoenas mayeri</i>	Pink Pigeon	Mascarene Is.	AF483322.1
<i>Ocyphaps lophotes</i>	Crested Pigeon	Australian	AF483323.1
<i>Oena capensis</i>	Namaqua Dove		AF483353.1
<i>Otidiphaps nobilis</i>	Pheasant Pigeons	Asian	AF483352.1
	Rodrigues Solitaire	Mascarene Is.	AF483337.1
<i>Phaps chalcoptera</i>	Common Bronzewing	Australian	AF483324.1
<i>Ptilinopus melanospil</i>	Black-naped Fruit Dove	Asian	AF483328.1
<i>Ptilinopus occipitalis</i>	Yellow-breasted Fruit Dove	Asian	AF483330.1
<i>Ptilinopus superbus</i>	Superb Fruit Dove	Asian	AF483329.1
<i>Raphus cucullatus</i>		Mascarene Is.	AF483338.1
<i>Scardafella squammata</i>	Scaled Dove	New World	AF483347.1
<i>Streptopelia chinensis</i>	Spotted Dove	Asian	AF483341.1
<i>Treron australis</i>	Madagascar Green Pigeon	African	AF483349.1
<i>Treron vernans</i>	Pink-necked Green Pigeon		AF483321.1
<i>Treron waalia</i>	Bruce's Green Pigeon	African	AF483350.1
<i>Turtur afer</i>	Blue-spotted Wood Dove	African	AF483348.1
<i>Zenaida auriculata</i>	Eared Dove	New World	AF483340.1

Phylogenetic Tree 2

You will build a phylogenetic tree using the data from the 12S rRNA gene.

1. Using GenBank, find the sequence data for Accession AF483301.1
2. Copy the FASTA sequence and paste at the end of your 12S file (you may want to change the name to match the format of the rest of the file).
3. Repeat Steps 1 and 2 for Accession AF483300.1
4. Save the file as 12S-2.
5. Align the data as before, save the file as 12S-2_aligned.
6. Build a tree as before, using the 12S-2_aligned file.

Draw your tree:

Is this tree the same as your first tree?

Would you expect it to be? Why or why not?

Is it the same as the tree in the article?

What are some possible reasons that it could be different?