

Section 3: Creating a phylogenetic network using a distance-based, neighbor-joining method

Phylogenetic networks like trees predict relationships between taxa; however, networks reveal more information about possible branches. Ideally, a phylogenetic network will look like a tree if the sequences cluster into distinct clades, and there is no conflicting information. Phylogenetic networks can be rapidly produced using the neighbor-joining method and bootstrapped to verify data consistency.

Below is an example of phylogenetic network created using whole genome sequences of bacteriophages using the Splitstree program.

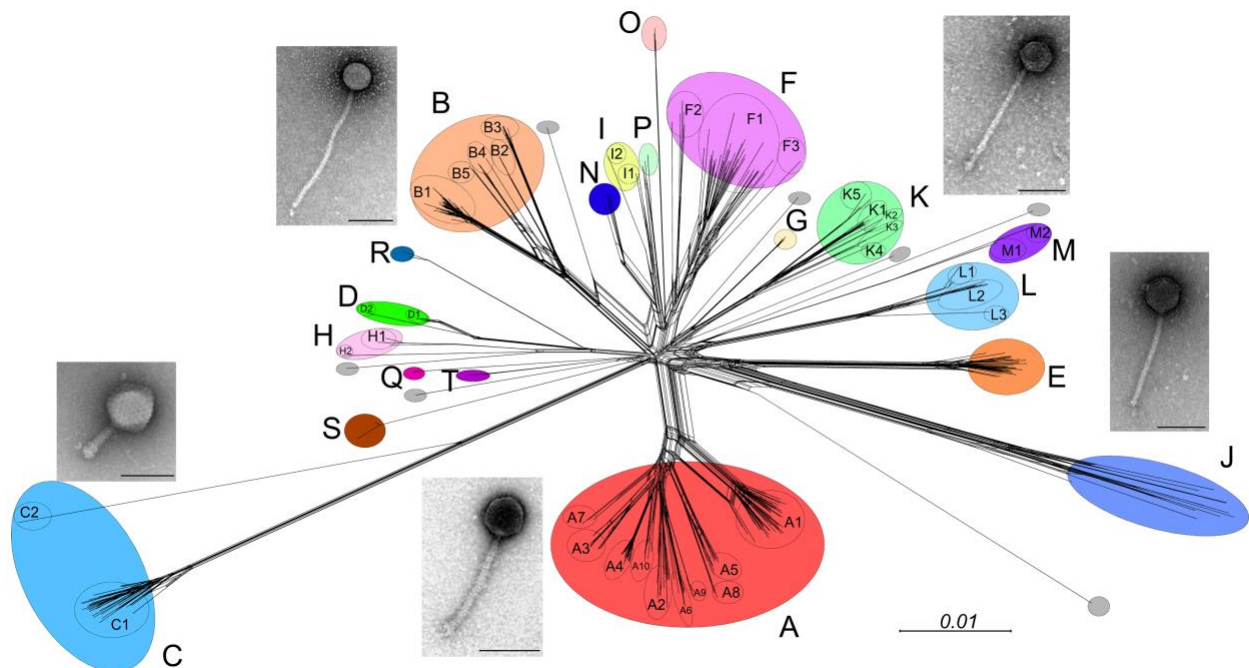


Figure 4. Splitstree diagram for bacteriophages that infect *Mycobacterium smegmatis*. (Hatfull GF (2014) Mycobacteriophages: Windows into Tuberculosis. PLoS Pathog 10(3): e1003953. <https://doi.org/10.1371/journal.ppat.1003953>)

In the following exercise you will compare the cytochrome C tree you produced in Exercise 2 to a network produced using the same sequences using neighbor-joining. As you may recall, one way to verify predictions from a tree is to produce a tree or network using the same input data but a different method. This exercise will provide you experience with this important scientific practice!

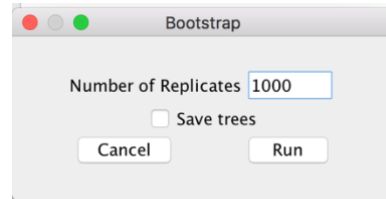
Exercise 3:

To build a network using neighbor-joining go to www.splitstree.org and download Splitstree4 onto your computer. Follow the steps below to build your network.

Step 1: Open the Splitstree4 application. Under the file tab, select 'enter data'. A new window will appear. Paste your fasta sequences from your text edit file (Exercise 2A) into the window.

Step 2: Click 'execute' in the bottom right of the window, and a phylogenetic network will be created using neighbor joining.

Step 3: To bootstrap your network: under the Analysis tab, choose 'bootstrap' and the following window will appear- select 'run'



A bootstrapped network will appear. Splitstree has many features that you may explore including the ability to zoom in on the tree or network in order to clearly read nodes and bootstrap values.

Question Set 5:

- 3.1 Submit a screen shot of your entire network on the worksheet.
- 3.2 Zoom in on the non-insect species to more clearly see the bootstrap values and submit a screen shot on the worksheet.

Splitstree4 can also be used to build a standard neighbor-joining tree. Under the tree tab, select 'NJ'. A window will appear, click 'apply'. To bootstrap this tree follow the instructions in Step 3 above.

- 3.3 In your network, which two organisms' relationship are you the least confident in based on bootstrap values?
- 3.4 Are the evolutionary relationships observed in the tree created in Exercise 2 the same as those in your Splitstree network? Which predicted relationships are consistent?
- 3.5 What differences do you observe, if any?