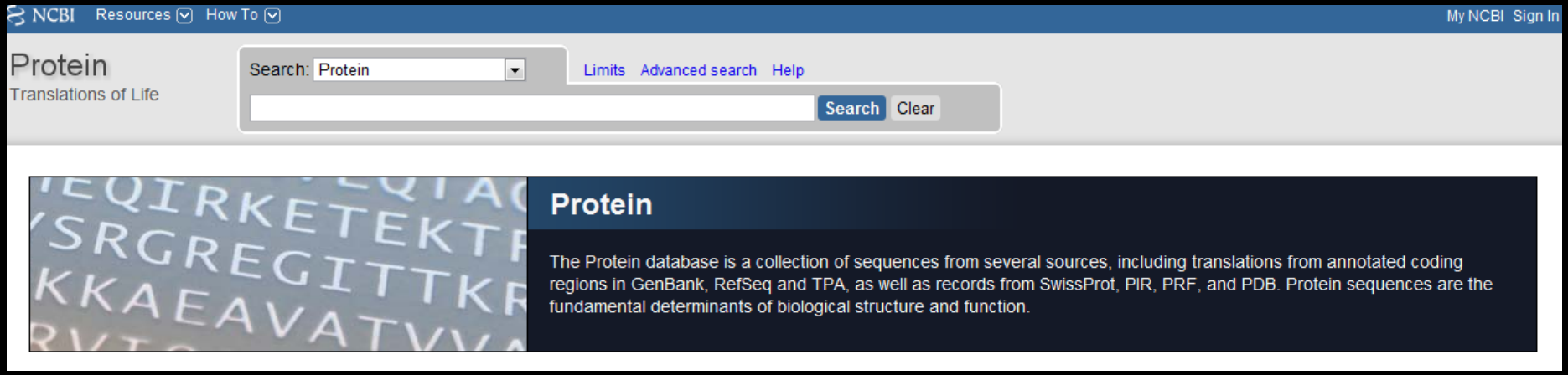
PHYLIP and phylogenies

New tools for Project 3

- The NCBI proteins database
  - Your resource for protein sequences, searchable by name and sequence.
- PHYLIP, a toolbox for generating and processing phylogenies
  - Neighbor
    - A simple phylogeny generator
  - Protpar
    - A maximum parsimony phylogeny generator
- Clustal2Matrix.py
  - A translator for ClustalW data (not ready yet)
- ClustalW

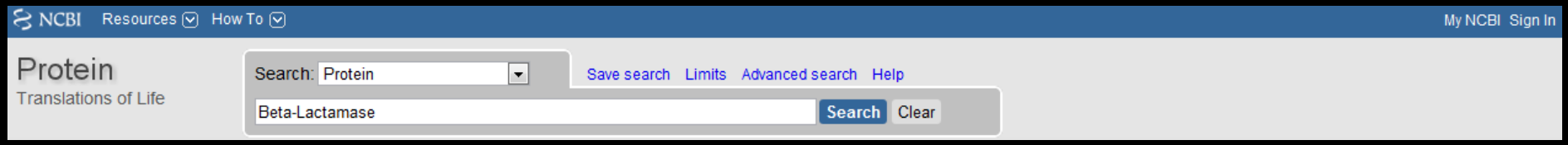
NCBI Protein database

http://www.ncbi.nlm.nih.gov/protein/

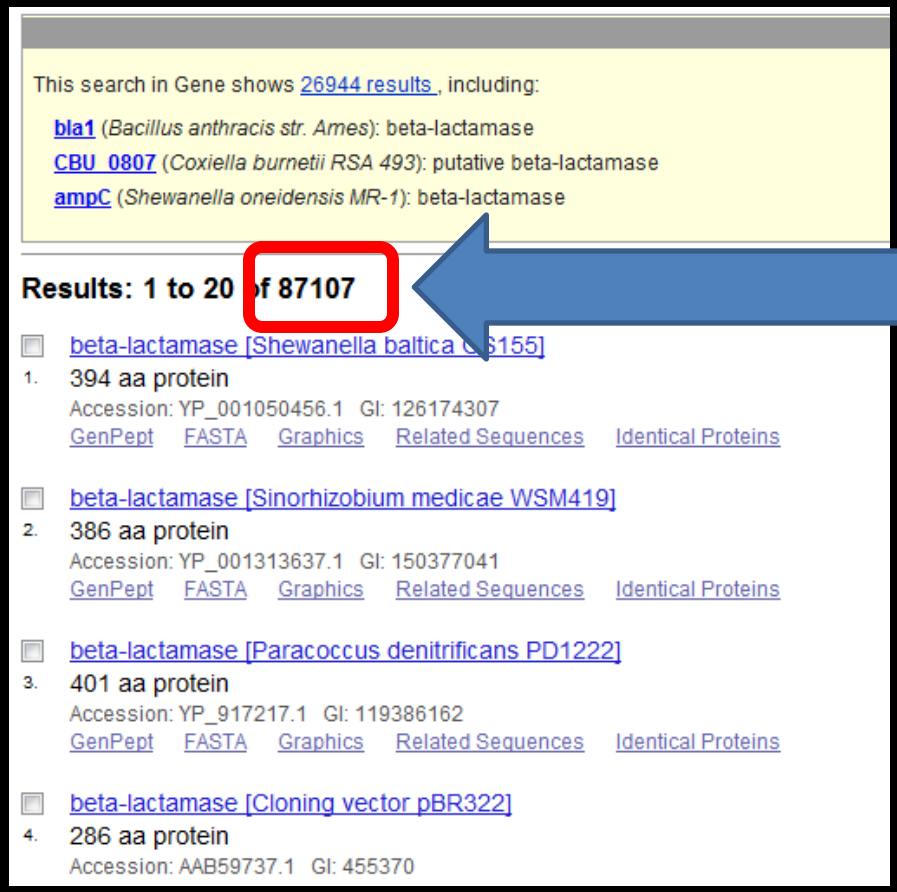


- Make sure the pulldown menu next to "Search:" says "Protein"
- Type in the search term you wish to look for

What you get from the Protein database



- Here I am searching for Beta-lactamases, a classic protein for antibiotic resistance in bacteria

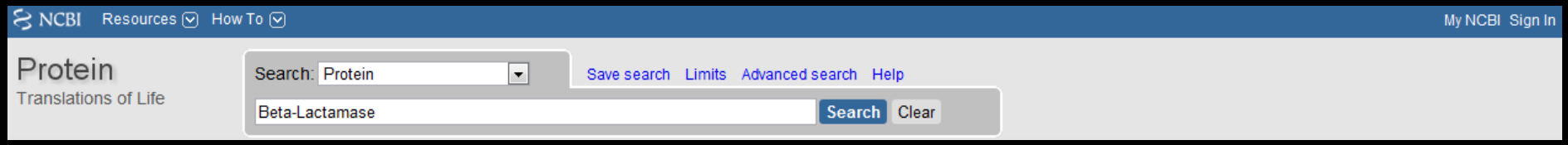


- The first thing you notice:
  - There are a huge number of matches

Expect thousands and tens of thousands of matches.

- Applications projects: you need a large database like this to get enough diversity

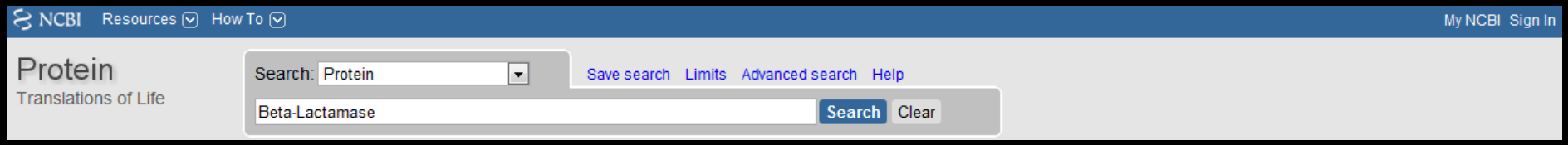
What you get from the Protein database



- Here I am searching for Beta-lactamases, a classic protein for antibiotic resistance in bacteria

- Each entry points to a single protein sequence
- Immediately next to each protein name is the source organism
- Shewanella Baltica is a bacterium that is able to alter metals so that they can be better broken down in the environment

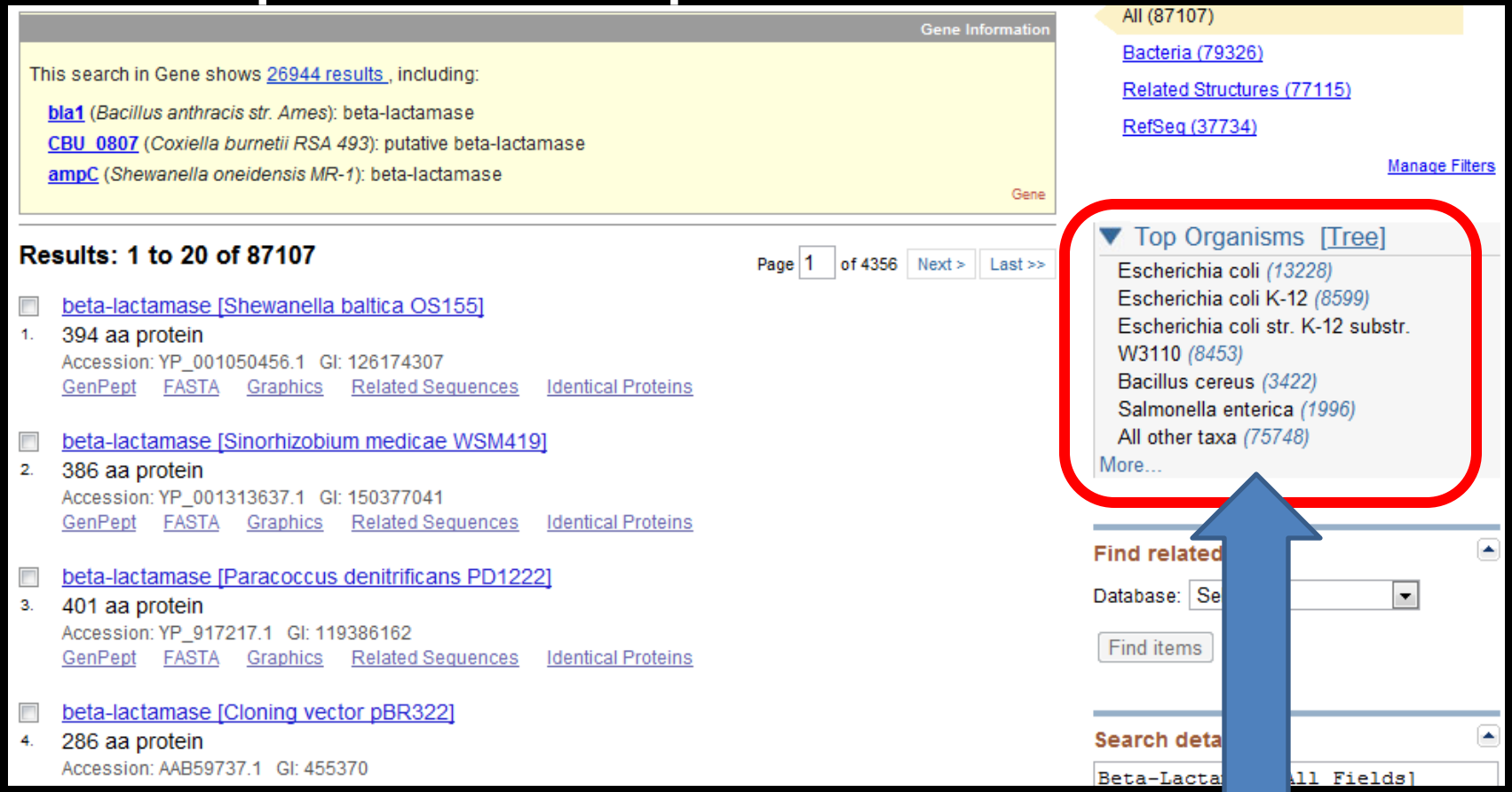
What you get from the Protein database



- Here I am searching for Beta-lactamases, a classic protein for antibiotic resistance in bacteria

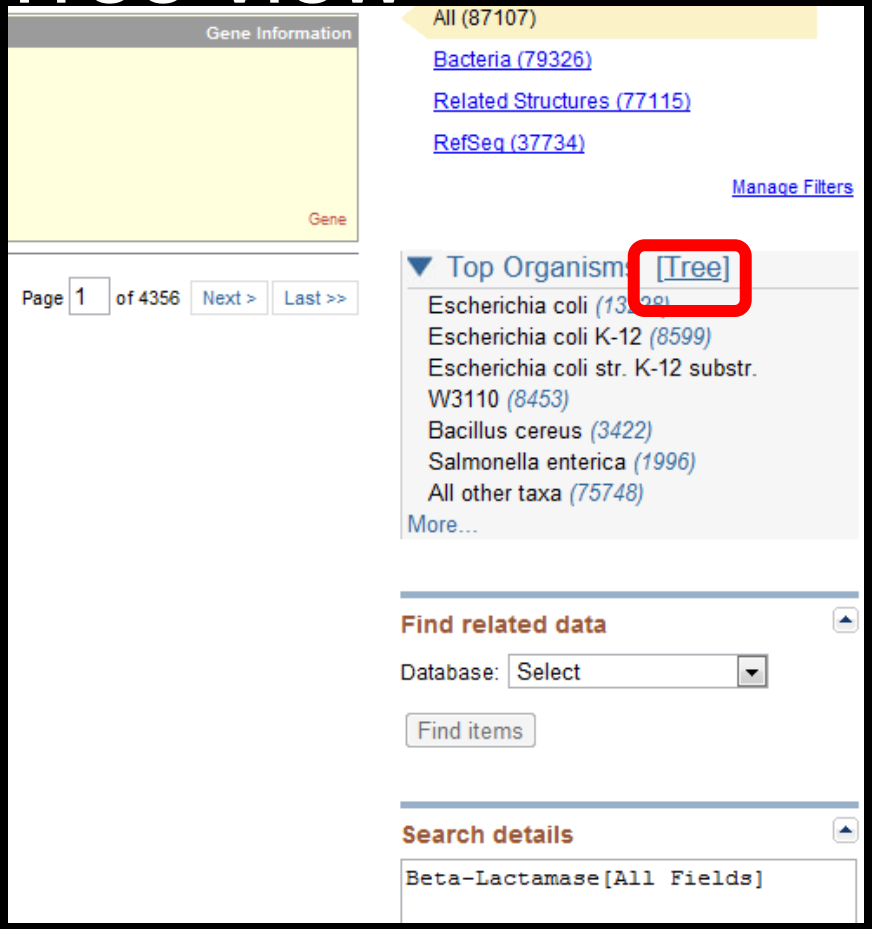
- This is not an irrelevant detail
- The number of amino acids in the protein can be a very easy indicator that you can or cannot use this sequence
  - Most sequences of the same protein have similar lengths. Something very short or very long should not be used

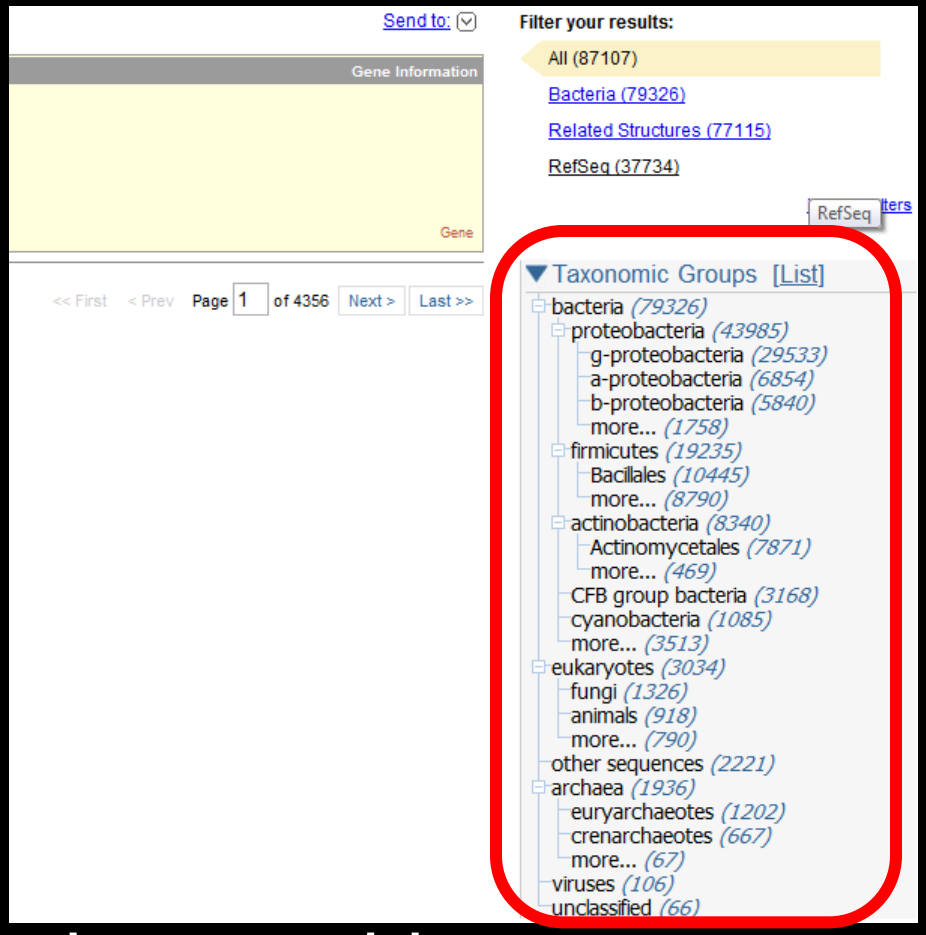
Another piece of important information



- Applications projects: you will want broad representation of many organisms. You can use this filter to get representatives from the largest groups
- Make sure to weed out similar groups, like the top two here

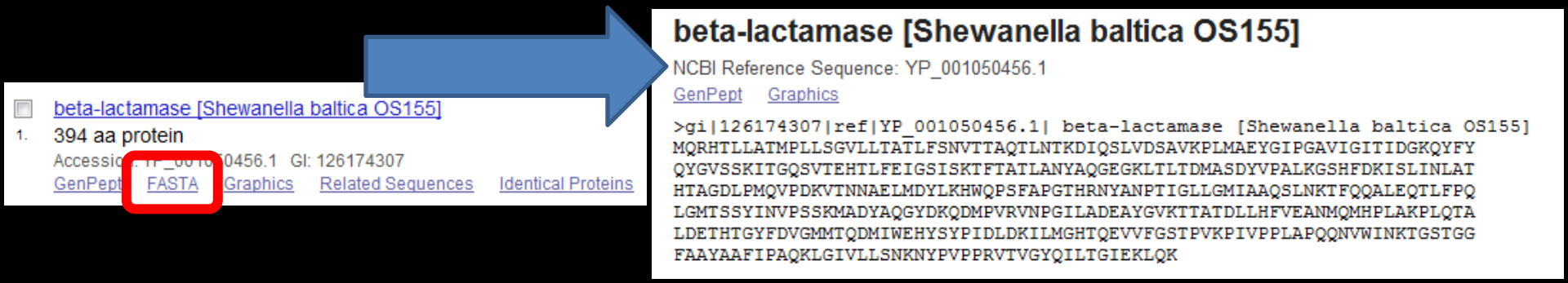
Tree view





- Applications project: This view provides a sense of how closely related the different organisms are. Selecting sequences carefully from groups will help you pick a better representative set.

Getting data out of the Protein database



- When you decide that you want to use a sequence in your project
  - Click on the FASTA link, and the Protein database provides a fasta entry
  - Cut and paste after the “>” symbol, to the end of the text.
- Note: you may want to modify the header that they give you to be something that you prefer
  - This header will help you find the same sequence again later, however

PHYLIP

- Famous and venerable phylogeny software
  - Developed for over 30 years by Joe Felsenstein (and his students) at the University of Washington
  - The baseline for phylogeny software
- Many components, but the two you will use are:
  - Neighbor
  - Protpar

Neighbor

- This software generates neighbor joining and UPGMA trees from distance matrices:

	A	B	C
A			
B	.3		
C	.5	.8	

A simple distance matrix

Implementation Projects:

- This is a useful benchmark for testing your UPGMA tree generators

How to use neighbor

- First, copy this directory to your experiment dir:

cp –r /proj/cse308/Project3 /proj/cse308-<username>

- Then enter the directory:

cd /proj/cse308-<username>/Project3/Phylip

- Enter the command:

./neighbor

- This will start neighbor, and you will see this:

```
neighbor: can't find input file "infile"
Please enter a new file name>
```

- Neighbor works much the way ClustalW works, by taking stdin

Providing input for neighbor

```
neighbor: can't find input file "infile"
Please enter a new file name>
```

- Here type the following:

neighborExample.grid

- This is an input file that contains a distance matrix. We will explain what this is and how you get this soon.

A minor error you might see:

```
neighbor: can't find input file "infile"
Please enter a new file name> neighborExample.grid

neighbor: the file "outfile" that you wanted to
     use as output file already exists.
     Do you want to Replace it, Append to it,
     write to a new File, or Quit?
     (please type R, A, F, or Q)
```

- Sometimes one of the output files will already exist.
  - This is because neighbor defaults to generating output files with the same name: outfile.
  - If you don’t care, simply press “R” to over-write it.
  - If you want to save your output file to a new name, press F to give it your own name

The main menu for neighbor

```
Neighbor-Joining/UPGMA method version 3.69

Settings for this run:
  N       Neighbor-joining or UPGMA tree?  Neighbor-joining
  O                        Outgroup root?  No, use as outgroup species  1
  L         Lower-triangular data matrix?  No
  R         Upper-triangular data matrix?  No
  S                       Subreplicates?  No
  J     Randomize input order of species?  No. Use input order
  M           Analyze multiple data sets?  No
  0   Terminal type (IBM PC, ANSI, none)?  ANSI
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                        Print out tree  Yes
  4       Write out trees onto tree file?  Yes


  Y to accept these or type the letter for one to change
```

- Here, press “N” to toggle Neighbor-joining to UPGMA
- Press “L” to toggle Lower-triangular data matrix to “On”
- Then press “Y” to accept the changes and run neighbor to generate the tree.

The menu, set correctly.

```
Neighbor-Joining/UPGMA method version 3.69

Settings for this run:
  N       Neighbor-joining or UPGMA tree?  UPGMA
  L         Lower-triangular data matrix?  Yes
  R         Upper-triangular data matrix?  No
  S                        Subreplicates?  No
  J     Randomize input order of species?  No. Use input order
  M           Analyze multiple data sets?  No
  0   Terminal type (IBM PC, ANSI, none)?  ANSI
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                         Print out tree  Yes
  4       Write out trees onto tree file?  Yes


  Y to accept these or type the letter for one to change
Y
```

- This is what your menu should look like after you've updated the settings
- (this slide is partially for reference)
- You can ignore the information that neighbor outputs to the screen; it's not terribly useful for the project

Another error you might see

```
neighbor: the file "outtree" that you wanted to
     use as output tree file already exists.
     Do you want to Replace it, Append to it,
     write to a new File, or Quit?
     (please type R, A, F, or Q)
```

- The other output file, “outtree” also defaults to this name
  - Overwrite it by pressing “R”
  - Create a new file with a custom name by pressing “F”

What neighbor generates

- Lets look at the outfile first

less ./outfile

- The outfile is a human readable file meant to give you an idea of the composition of the tree

```
 36 Populations

Neighbor-Joining/UPGMA method version 3.69


 UPGMA method

 Negative branch lengths allowed


                                                          +--1auo
                                 +----------------------------2
                        +------26                         +--1aur
                        !       !
                        !       +-------------------------------2h1i
                        !
                        !                                +--------1evq
                        !                           +---20
                        !                           !    +--------1qz3
                  +------29             +-----------22
                  !     !               !           !     +-------1u4n
                  !     !               !           +----19
                  !     !  +---------24                 +-------2hm7
                  !     !  !          !
                  !     !  !          !      +-------------------1jji
                  !     +-28          +-----23
              +-32        !                 +-------------------2c7b
              !  !        !
              !  !        !                                +---2o7r
              !  !        +--------------------------------6
              !  !                                         +---2o7v
           +-33  !
           !  !  !                                       +-----1r1d
           !  !  +----------------------------------------12
           !  !                                          +-----1tqh
           !  !
           !  +-------------------------------------------------1l7r
           !
           !                                     +----------1k4y
           !                                     !
           !                                     !      +----1mx1
           !                                     !   +-10
           !                                     !   !  +----1mx9
           !                                     !   !
           !                   +-------------------21   !             +----1mx5
           !                   !                   !   !           +-9
           !                   !                   !   !           ! ! +----1ya4
           !                   !                   !   !           ! +-7
           !                   !                   !   !           !   ! +---1yah
           !                   !                   !   !        +-11   +-4
       +--34                   !                   +--18        !  !     +---2dqy
       !   !                   !                       !        !  !
       !   !                   !                       !        !  ! +----2dqz
       !   !                   !                       !     +-13  +-8
       !   !                   !                       !     !  !    +----2dr0
       !   !                   !                       !     !  !
```

The other output file from neighbor

- This file is less useful for you initially, but will be useful for generating pretty trees

less ./outtree

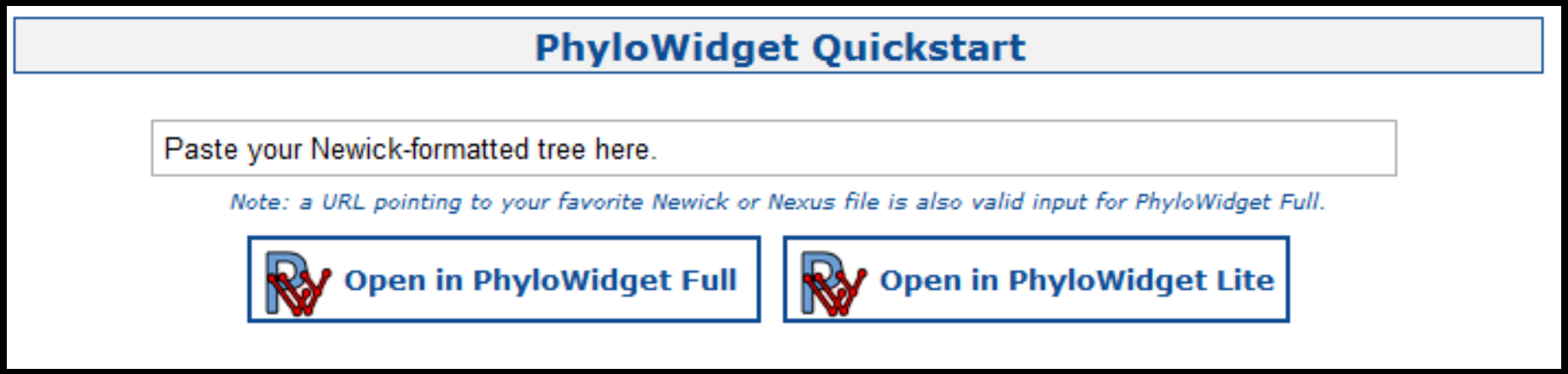
- Here, the output is not designed to be human readable. This format is called Nevvick

```
[07:55 AM][chen@titania Phylip] less outtree
((((((1auo:0.10043,1aur:0.10043):0.95379,2h1i:1.05422):0.25827,
((((1evq:0.28437,1qz3:0.28437):0.16165,(1u4n:0.26559,2hm7:0.26559):0.18044):0.42081,
(1jji:0.65050,2c7b:0.65050):0.21632):0.36467,(2o7r:0.13683,
2o7v:0.13683):1.09467):0.08099):0.25520,(1r1d:0.17612,1tqh:0.17612):1.39157):0.0424
,
117r:1.61014):0.09273,((((1k4y:0.36878,((1mx1:0.16483,1mx9:0.16483):0.08155,
(((((1mx5:0.15597,(1ya4:0.14254,(1yah:0.10982,2dqy:0.10982):0.03272):0.01342):0.012
4,
(2dqz:0.14812,2dr0:0.14812):0.02028):0.01394,(2hrq:0.13125,
2hrr:0.13125):0.05110):0.03142,2h7c:0.21376):0.01341,(1ya8:0.20241,
1yaj:0.20241):0.02476):0.01921):0.12241):0.68322,((2jey:0.08334,
2jez:0.08334):0.02550,2jf0:0.10884):0.94316):0.09190,(2ogs:0.18988,
2ogt:0.18988):0.95403):0.17810,2fj0:1.32200):0.38086):0.11228,
(1M33:1.51737,2r11:1.51737):0.29778);
outtree (END)
```

Using Nevvick files

- Nevvick is a precise description of the tree, including edge lengths.
- If you want to render a pretty tree, you can give the renderer the nevvick file.
- One such piece of software is phylowidget:

http://www.phylowidget.org



Input that Neighbor takes

- Lets open up the input file for neighbor

less ./neighborExample.grid

```
 36
1auo
1aur          0.20086
1evq          2.51542   2.53234
1jji          2.10248   2.38593 2.12385
1k4y          3.02094   3.02621 3.21465 3.10089
1l7r          2.75063   2.7837  3.25751 2.96361 3.73926
1M33          3.51014   3.32924 3.89434 3.45937 3.89971 3.85657
1mx1          3.00913   2.97639 3.32596 3.18107 0.703022        3.80591 3.65681
1mx5          2.9874    2.97568 3.31183 3.18655 0.732716        3.66989 3.99207 0.442749
1mx9          2.99461   3.03078 3.2054  3.1691  0.77225 3.85879 4.1578  0.329659        0.500686
1qz3          2.61977   2.47836 0.56875 1.99077 3.32535 3.25245 3.97636 3.42615 3.36068 3.30122
```

- The input is a lower triangular grid of distances between identifiers (left column). The file starts with a number, indicating total # of indicators
- These numbers are artificial, but in your data will reflect sequence identities between protein sequences:

(100-seqId)/100

Protpars

- Protpars attempts to compute a maximum parsimony tree based on your input sequence.
- Lets look at the input first:

less protparsExample.txt

```
     5     10
Alpha      ABCDEFGHIK
Beta       AB--EFGHIK
Gamma      ?BCDSFG*??
Delta      CIKDEFGHIK
Epsilon    DIKDEFGHIK
```

- Here the first two numbers are the number of sequences, and the number of nucleotides
- You can see that protpars can run this computation using wildcards as well: ?, *

Running protpars

- Run protpars with this simple command

./protpars

- Protpars will give you a similar error as neighbor:

```
protpars: can't find input file "infile"
Please enter a new file name>
```

- Here, typein the other sample input file:

protparsExample.txt

- This is the input example we just saw

Protpars Errors you might see

- This sort of file error can happen with protpars as well as neighbor

```
protpars: can't find input file "infile"
Please enter a new file name> protparsExample.txt

protpars: the file "outfile" that you wanted to
     use as output file already exists.
     Do you want to Replace it, Append to it,
     write to a new File, or Quit?
     (please type R, A, F, or Q)
```

- Just replace (“R”) the file with another outfile, for this example
- When you are running your own data, you will probably want to create a new file, using “F”

Options for Protpars

```
Protein parsimony algorithm, version 3.69

Setting for this run:
  U                 Search for best tree?  Yes
  J   Randomize input order of sequences?  No. Use input order
  O                        Outgroup root?  No, use as outgroup species  1
  T              Use Threshold parsimony?  No, use ordinary parsimony
  C               Use which genetic code?  Universal
  W                       Sites weighted?  No
  M           Analyze multiple data sets?  No
  I          Input sequences interleaved?  Yes
  0   Terminal type (IBM PC, ANSI, none)?  ANSI
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                        Print out tree  Yes
  4          Print out steps in each site  No
  5  Print sequences at all nodes of tree  No
  6       Write out trees onto tree file?  Yes

Are these settings correct? (type Y or the letter for one to change)
```

- Press “U” to search for the best tree.
- Press “J” to randomize the input order
- Then press “Y” to start the run
- Again you might see this error:, just press “R”

```
protpars: the file "outtree" that you wanted to
     use as output tree file already exists.
     Do you want to Replace it, Append to it,
     write to a new File, or Quit?
     (please type R, A, F, or Q)
```

Outputs for Protpars

- Protpars also generates an outfile and an outtree.
- The outtree is a Nevvick file exactly like the output from neighbor
- Lets look at the the outfile:

less ./outfile

- Here Protpars finds three possible high-parsimony trees

```
Protein parsimony algorithm, version 3.69


     3 trees in all found



     +--------Gamma
     !
  +--2     +--Epsilon
  !  !  +--4
  !  +--3  +--Delta
  1     !
  !     +-----Beta
  !
  +-----------Alpha

  remember: this is an unrooted tree!


requires a total of     16.000



          +--Epsilon
        +--4
     +--3  +--Delta
     !  !
  +--2  +-----Gamma
  !  !
  1  +--------Beta
  !
  +-----------Alpha

  remember: this is an unrooted tree!


requires a total of     16.000



          +--Epsilon
     +-----4
     !     +--Delta
  +--3
  !  !     +--Gamma
  1  +-----2
  !        +--Beta
  !
  +-----------Alpha

  remember: this is an unrooted tree!
.
```

How you can use Protpars

- Applications projects:
  - When you have assembled your multiple sequence alignments, you should feed these alignments to protpars to see how diverse your tree is
  - A diverse tree will be evenly branched, rather than having lots of nested trees in one branch.
- Implementations projects:
  - You can use Protpars as a sort of answer key, to see if your maximum parsimony algorithms are moving in the same directory
  - Computing maximum parsimony is NP-hard, so there is no right answer that can be easily computed.

Clustal2Matrix.py

- This python script will take clustalW output (pairwise sequence identities) and use it to generate the distance matrix used for running neighbor.
- This is so that you don’t have to do this processing
- I am still coding this, it will be ready this weekend.

Questions