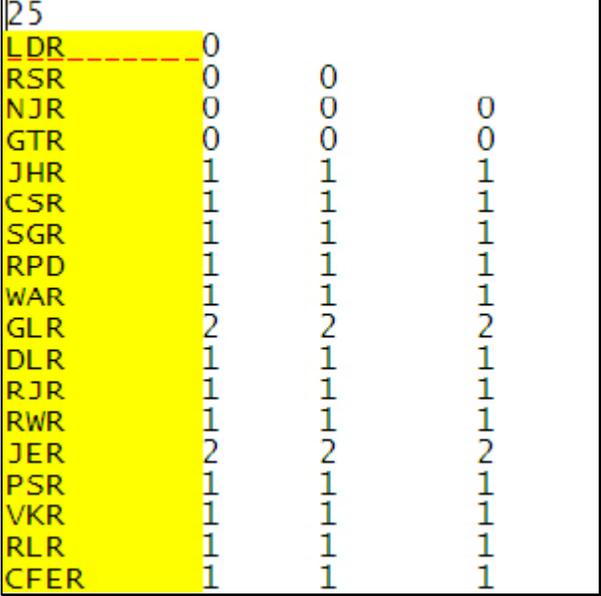
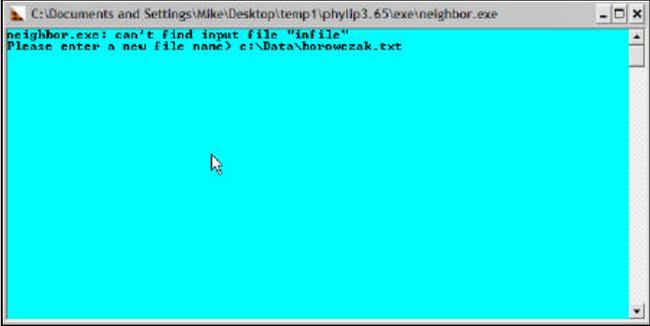
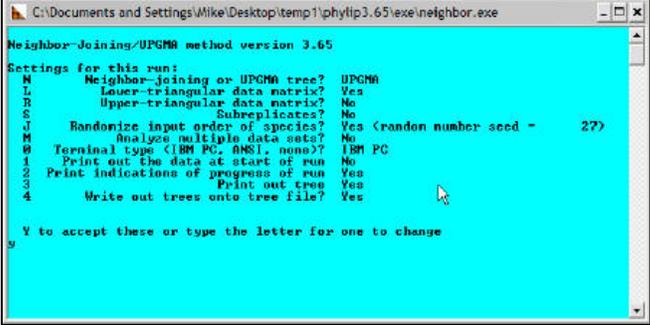
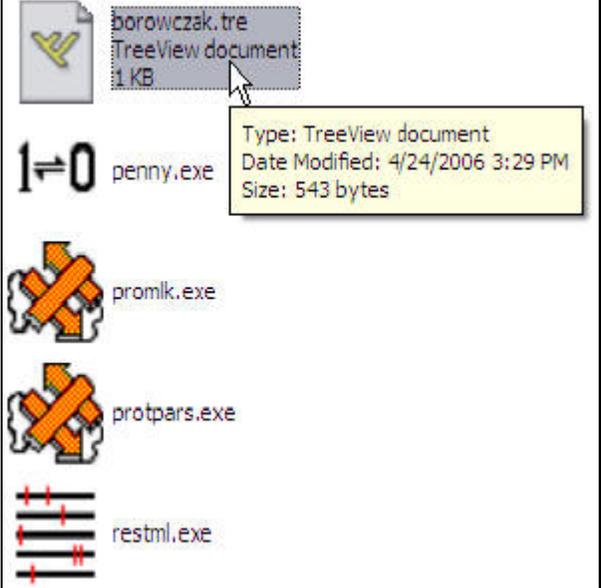
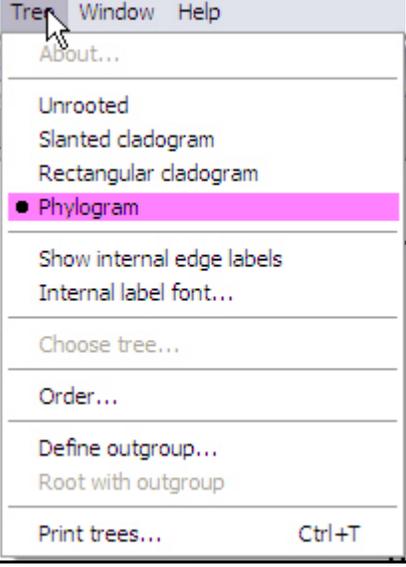


Using PHYLIP/Neighbor and TreeView to Calculate and Plot Phylograms

<u>Needed Software</u>	<p>Operating System: Windows, Apple OS or UNIX/Linux with X-Server. Spreadsheet program: Microsoft Excel, OpenOffice Workbook etc. Basic text editor: Notepad, Emacs, vi etc. PHYLIP/Neighbor Program TreeView Program</p> <p><i>Note: These instructions assume the use of Windows XP, Microsoft Excel, and Notepad. Slight modifications may be necessary depending on a user's individual system configuration.</i></p>																																																																																																																																																																		
<u>Step 1:</u>	<p>Use a spreadsheet program to create a relative-mutations matrix for the set of markers in the phylogram (See Figure 1).</p>	<table border="1" style="border-collapse: collapse; width: 100%;"> <thead> <tr> <th></th> <th>A</th> <th>B</th> <th>C</th> <th>D</th> <th>E</th> <th>F</th> </tr> </thead> <tbody> <tr><td>1</td><td>LDR</td><td>0</td><td></td><td></td><td></td><td></td></tr> <tr><td>2</td><td>RSR</td><td>0</td><td>0</td><td></td><td></td><td></td></tr> <tr><td>3</td><td>NJR</td><td>0</td><td>0</td><td>0</td><td></td><td></td></tr> <tr><td>4</td><td>GTR</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td></tr> <tr><td>5</td><td>JHR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>6</td><td>CSR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>7</td><td>SGR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>8</td><td>RPD</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>9</td><td>WAR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>10</td><td>GLR</td><td>2</td><td>2</td><td>2</td><td>2</td><td></td></tr> <tr><td>11</td><td>DLR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>12</td><td>RJR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>13</td><td>RWR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>14</td><td>JER</td><td>2</td><td>2</td><td>2</td><td>2</td><td></td></tr> <tr><td>15</td><td>PSR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>16</td><td>VKR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>17</td><td>RLR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>18</td><td>CFER</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>19</td><td>DKR</td><td>2</td><td>2</td><td>2</td><td>2</td><td></td></tr> <tr><td>20</td><td>JWR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>21</td><td>RGR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> <tr><td>22</td><td>JJR</td><td>1</td><td>1</td><td>1</td><td>1</td><td></td></tr> </tbody> </table> <p style="text-align: center;">Figure 1: Relative-Mutations Matrix</p>		A	B	C	D	E	F	1	LDR	0					2	RSR	0	0				3	NJR	0	0	0			4	GTR	0	0	0	0		5	JHR	1	1	1	1		6	CSR	1	1	1	1		7	SGR	1	1	1	1		8	RPD	1	1	1	1		9	WAR	1	1	1	1		10	GLR	2	2	2	2		11	DLR	1	1	1	1		12	RJR	1	1	1	1		13	RWR	1	1	1	1		14	JER	2	2	2	2		15	PSR	1	1	1	1		16	VKR	1	1	1	1		17	RLR	1	1	1	1		18	CFER	1	1	1	1		19	DKR	2	2	2	2		20	JWR	1	1	1	1		21	RGR	1	1	1	1		22	JJR	1	1	1	1	
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<u>Step 2:</u>	<p>Save the matrix as a tab delimited file:</p> <ol style="list-style-type: none"> a) Click on the <i>File</i> menu b) Click <i>Save As...</i> c) Change <i>Save in</i> to C:\DATA d) Change <i>Save as Type</i> to TEXT (TAB DELIMITED) (*.TXT) e) You may optionally change the <i>File name</i> to INFILE which will automate a future step. f) Click <i>Save</i>. g) Click <i>Yes</i> to any pop-up dialogs 																																																																																																																																																																		
<u>Step 3:</u>	Close the spreadsheet program.																																																																																																																																																																		
<u>Step 4:</u>	Open <YOURFILE.TXT> from Step 2 in a basic text editor.																																																																																																																																																																		
<u>Step 5:</u>	<p>Format the text file for use within PHYLIP/Neighbor Program:</p> <ol style="list-style-type: none"> a) Count the number of lines within the file. b) Enter this number on the first line of the text file. 																																																																																																																																																																		

<p><u>Step 5 (cont.):</u></p>	<p>c) Do the following to each subsequent line:</p> <ol style="list-style-type: none"> Delete the first TAB of the line Make sure that there are 10 spaces before the first number occurs <ol style="list-style-type: none"> Most lines start with a 3-letter abbreviation so simply type 7 spaces after each of these abbreviations. <p><i>Note that Figure 2 shows the final desired format of the text file.</i></p> <p><i>An example text file for 25 Y-chromosome markers can be found here!</i></p>	 <p>Figure 2: Text File Needed for Neighbor Program</p>
<p><u>Step 6:</u></p>	<p>Save <YOURFILE.TXT> to the C:\DATA directory</p>	
<p><u>Step 7:</u></p>	<p>Open the PHYLIP/Neighbor program executable from the directory you either installed it in, or use the shortcut on the Desktop. [The install directory would appear here]</p>	
<p><u>Step 8:</u></p>	<p>The pale blue command window in Figure 3 appears on the screen.</p> <p><i>Note that if you saved your text file as INFILE.TXT you may skip to Step 9.</i></p> <p>Enter your file name and directory; C:\DATA\<YOURFILE.TXT></p>	 <p>Figure 3: Phylip/Neighbor Program Command Window</p>
<p><u>Step 9:</u></p>	<p>A listing of available options will now appear in the command window (See Figure 4).</p> <p>Get the correct output file options; enter the following sequence of characters:</p> <ol style="list-style-type: none"> Type 'N' and press <enter> to enable Unweighted Pair Group Method with Arithmetic Mean. Type 'L' and press <enter> to enable Lower Triangular Data Matrix type. Otherwise Type 'R' and press <enter> to enable Upper Triangular Data Matrix Type. Type 'J' and press <enter> <ol style="list-style-type: none"> Select an odd number greater than or equal to 11 and press <enter>. 	 <p>Figure 4: Command Window with options Menu.</p>

<p>Step 10:</p>	<p>Type ‘Y’ and press <enter> to execute the Neighbor program with the given options.</p> <p>The command window, in Figure 4, will output text and close automatically.</p>
<p>Step 11:</p>	<p>The output file and associated tree file are located in the PHYLIP install directory. These files are named OUTPUT and OUTTREE.</p>
<p>Step 12:</p>	<p>Open the default PHYLIP directory [The install directory would appear here]</p>
<p>Step 13:</p>	<p>Rename the file OUTTREE to <YOURFILE.TRE> by right clicking on the file and selecting rename</p>
<p>Step 14:</p> <p>If the TreeView program is installed correctly, the icon will appear as in Figure 5.</p> <p>Double click the file <YOURFILE.TRE> and the TreeView program will now open along with the associated tree.</p>	 <p>Figure 5: The renamed TreeView file</p>
<p>Step 15:</p> <p>Change the Tree to Phylogram (Figure 6).</p> <ol style="list-style-type: none"> Select the <i>Tree</i> Menu Select <i>Phylogram</i> 	 <p>Figure 6: The Tree Menu with Phylogram Highlighted</p>

Step 16:

Save the Tree as a Graphics File

- a) Select the *File* Menu
- b) Select *Save as graphic...*
- c) Change *Save as type* to WINDOWS METAFILE (*.WMF)

You have now successfully created a Phylogram picture as seen in **Figure 7!**

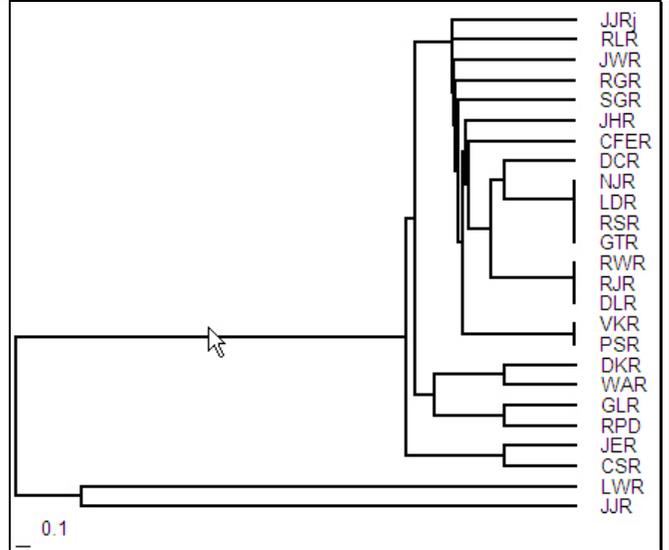


Figure 7: Final Phylogram Image!