

TABLE 27.11. Neighbor-joining example

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Distance matrix	$\begin{array}{c ccccc} & A & B & C & D & E \\ \hline B & 5 & & & & \\ C & 4 & 7 & & & \\ D & 7 & 10 & 7 & & \\ E & 6 & 9 & 6 & 5 & \\ F & 8 & 11 & 8 & 9 & 8 \end{array}$	$\begin{array}{c cccc} & U_1 & C & D & E \\ \hline C & 3 & & & \\ D & 6 & 7 & & \\ E & 5 & 6 & 5 & \\ F & 7 & 8 & 9 & 8 \end{array}$	$\begin{array}{c ccc} & U_1 & C & U_2 \\ \hline C & 3 & & \\ U_2 & 3 & 4 & \\ F & 7 & 8 & 6 \end{array}$	$\begin{array}{c cc} & U_2 & U_3 \\ \hline U_3 & 2 & \\ F & 6 & 6 \end{array}$	$\begin{array}{c c} & U_4 \\ \hline F & 5 \end{array}$
Step 1					
S calculations	$S_A = (5+4+7+6+8)/4 = 7.5$ $S_B = (5+7+10+9+11)/4 = 10.5$ $S_C = (4+7+7+6+8)/4 = 8$ $S_D = (7+10+7+5+9)/4 = 9.5$ $S_E = (6+9+6+5+8)/4 = 8.5$ $S_F = (8+11+8+9+8)/4 = 11$	$S_{U_1} = (3+6+5+7)/3 = 7$ $S_C = (3+7+6+8)/3 = 8$ $S_D = (6+7+5+9)/3 = 9$ $S_E = (5+6+5+8)/3 = 8$ $S_F = (7+8+9+8)/3 = 10.6$	$S_{U_1} = (3+3+7)/2 = 6.5$ $S_C = (3+4+8)/2 = 7.5$ $S_{U_2} = (3+4+6)/2 = 6.5$ $S_F = (7+8+6)/2 = 10.5$	$S_{U_2} = (2+6)/1 = 8$ $S_{U_3} = (2+6)/1 = 8$ $S_F = (6+6)/1 = 12$	Because $N - 2 = 0$, we cannot do this calculation.
$S_x = (\text{sum all } D_x)/(N - 2)$, where N is the # of OTUs in the set.					
Step 2					
Calculate pair with smallest (M), where $M_{ij} = D_{ij} - S_i - S_j$.	Smallest are $M_{AB} = 5 - 7.5 - 10.5 = -13$ $M_{DE} = 5 - 9.5 - 8.5 = -13$ Choose one of these (AB here).	Smallest is $M_{CU_1} = 3 - 7 - 8 = -12$ $M_{DE} = 5 - 9 - 8 = -12$ Choose one of these (DE here).	Smallest is $M_{CU_1} = 3 - 6.5 - 7.5 = -11$	Smallest is $M_{U_2F} = 6 - 8 - 12 = -14$ $M_{U_3F} = 6 - 8 - 12 = -14$ $M_{U_2U_3} = 2 - 8 - 8 = -14$ Choose one of these ($M_{U_2U_3}$ here).	
Step 3					
Create a node (U) that joins pair with lowest M_{ij} such that $S_{iU} = D_{ij}/2 + (S_i - S_j)/2$.	U_1 joins A and B: $S_{AU_1} = D_{AB}/2 + (S_A - S_B)/2 = 1$ $S_{BU_1} = D_{AB}/2 + (S_B - S_A)/2 = 4$	U_2 joins D and E: $S_{DU_2} = D_{DE}/2 + (S_D - S_E)/2 = 3$ $S_{EU_2} = D_{DE}/2 + (S_E - S_D)/2 = 2$	U_3 joins C and U_1 : $S_{CU_3} = D_{CU_1}/2 + (S_C - S_{U_1})/2 = 2$ $S_{U_1U_3} = D_{CU_1}/2 + (S_{U_1} - S_C)/2 = 1$	U_4 joins U_2 and U_3 : $S_{U_2U_4} = D_{U_2U_3}/2 + (S_{U_2} - S_{U_3})/2 = 1$ $S_{U_3U_4} = D_{U_2U_3}/2 + (S_{U_3} - S_{U_2})/2 = 1$	For last pair, connect U_4 and F with branch length = 5.
Step 4					
Join i and j according to S above and make all other taxa in form of a star. Branches in black are of unknown length. Branches in red are of known length.					
Step 5					
Calculate new distance matrix of all other taxa to U with $D_{xU} = D_{ix} + D_{jx} - D_{ij}$, where i and j are those selected from above.					

Comments
 Note this is the same tree we started with (drawn in unrooted form here).