

PHYLIP (PHYlogeny Inference Programs)

biol4230

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PHYLIP via EMBOSS

- Tree building:
 - distance: (f)fitch,(f)kitsch, needs (f)dndist or (f)protdist first
 - parsimony: (f)dnapars, (f)protpars
 - Likelihood: (f)dnaml, (f)dnamlk, (f)protml
- Tree drawing:
 - (f)drawtree – unrooted
 - (f)drawgram – draws a tree
- Utilities:
 - (f)consense – show consensus tree
 - rtreer – reroot trees (use interactively)

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PHYLIP (PHYlogeny Inference Programs)

- A package of programs developed by Joe Felsenstein; available since 1980
- Written in 'C' for a command line interface
- Available for most popular computers
- Provides a diverse variety of methods for sequence and other data

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Phylib 3.69

Advantages

- Free (GNU license)
- Runs on all major platforms
- Good documentation
- Well known/widely used
- Possible to automate
- File formats supported by other packages

Disadvantages

- Much slower than PAUP
- Search strategy less comprehensive
- Primitive command-line interface (user hostile)
- Much file renaming required
- Cannot read NEXUS files

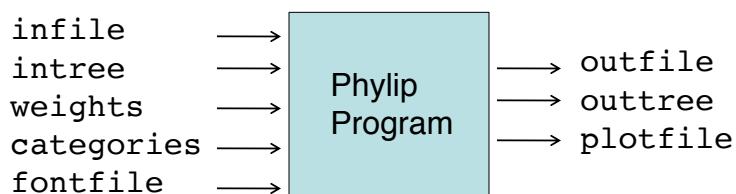
PHYLIP Tree-building programs

- Parsimony:
 - dnparse - parsimony (protparse)
 - No branch lengths on trees
- Distance Methods
 - dnadist, protdist – produce corrected distance matrices
 - fitch, kitsch – Fitch-Margoliash distance (clock, kitsch) trees from distances
 - Neighbor – Neighbor-joining trees (no explicit optimization criterion)

PHYLIP Tree-building programs

- Maximum Likelihood
 - dnaml, dnamlk - DNA maximum likelihood
 - proml, promlk - protein maximum likelihood
 - *mlk methods assume evolutionary clock (all branches end at same level (time))

PHYLIP Program Data/Output



- The phylip programs re-use the same file names: "infile", "outfile", every time a program is used. In current versions, if the input file is not present, it is prompted for, and if the output file is present, one is warned before over-writing it.
- However, it is easy to analyse the wrong data (old "infile") and over write (or mis-name) the output file.
- Develop a protocol for ensuring that file names make sense. NEVER use `infile` and `outfile`, `outtree`. This can be difficult. Scripts help.

PHYLIP via EMBOSS

- EMBOSS (European Molecular Biology lab Open Software Suite)
 - command line options
 - interactive when needed (sometimes annoying)
 - use –help
- EMBOSS PHYLIP:
 - f+PHYLIP name: fdnadist, fconsense, ffitch, fkitsch, etc

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PHYLIP sequence format (interleaved)

Number of taxa	Length of alignment
7 112	
Bovine	CCAAACCTGT CCCCACCATC TAACACCAAC CCACATATAC AAGCTAAACC AAAAATACCA
Mouse	CCAAAAAAAC ATCCAAACAC CAACCCCAGC CCTTACGCAA TAGGCCATACA AAGAATATTA
Gibbon	CTATACCCAC CCAACTGCAC CTACACCAAT CCCACATAGCACACAGACC AACACCTCC
Orang	CCCCACCCGT CTACACCAGC CAACACCAAC CCCCACCTAC TATACCAACC AATAACCTCC
Gorilla	CCCCATTATAT CCATAAAAAC CAACACCAAC CCCCATCTAA CACACAAACT AATGACCCCC
Chimp	CCCCATCCAC CCATACAAAC CAACATTACC CTCCATCCAA TATACAAACT AACAAACCTCC
Human	CCCCACTCAC CCATACAAAC CAACACCACT CTCCACCTAA TATACAAATT AATAACCTCC
	CCCCAGCCCA ACACCCCTTC ACAAACTCTT AATATACGCA CCATAAATAA CA
	TCCCACCAAA TCACCCCTCCA TCAAATCCAC AAATTACACA ACCATTAACC CA
	GCACGCCAAG CTCTCTACCA TCAAACGCAC AACTTACACA TACAGAACCA CA
	ACACCTTAAG CCACCTTCTT CAAATCCAA AACCCACACA ACCGAAACAA CA
	ACACCTCAAT CCACCTCCCC CCAAATACAC AATTACACACA AACAAATACCA CA
	ACATCTTGAC TCGCCTCTCT CCAAACACAC AATTACGCA AACAAACGCCA CA
	ACACCTTAAC TCACCTCTCT CCAAACGCCAC AATTCCGACA CACAAACGCCA CA

use EMBOSS seqret to convert to PHYLIP format (-osformat2 phylip)

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PHYLIP Tree representation (NEWICK)

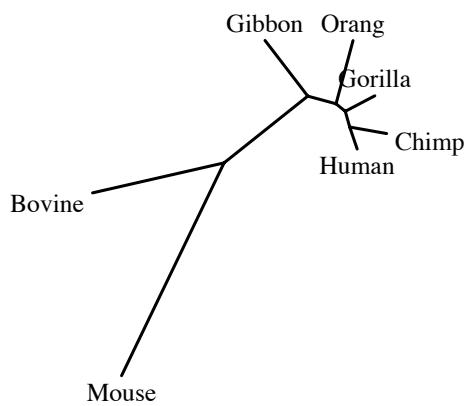
Taxa
label Branch
Length

```
(Mouse:0.87231,Bovine:0.49807,(Gibbon:0.25930,(Orang:0.24
166, (Gorilla:0.12322,(Chimp:0.13846,
Human:0.08571):0.06026):0.04405):0.10815):0.39538);
(Mouse:0.87558,Bovine:0.49718,(Gibbon:0.25698,(Orang:0.24
477, ((Gorilla:0.16328,Chimp:0.13802):0.01842,
Human:0.08495):0.06610):0.10637):0.39287);
(Mouse:0.87819,Bovine:0.49461,(Gibbon:0.25837,(Orang:0.24
161, (Chimp:0.13941,(Gorilla:0.16639,
Human:0.09533):0.00616):0.06709):10938):0.39630);
```

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PHYLIP Tree representation (NEWICK)



```
(Mouse:0.87231,Bovine:0.49807,(Gibbon:0.25930,(Orang:0.24
166, (Gorilla:0.12322,(Chimp:0.13846,
Human:0.08571):0.06026):0.04405):0.10815):0.39538);
```

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Tree-analysis/display

- Tree comparison:
 - (f) `consense` – Calculate consensus tree from bootstraps
 - (f) `treedist` – compare trees by "partition distance"
 - Manipulation
 - `retree` – flip nodes, re-root, re-arrange – run interactively
 - Display
 - (f) `drawgram` – draw "tree-like" tree
 - (f) `drawtree` – draw unrooted tree

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Running PHYLIP

```

15 675
----- --ATGCCCAT GATACTGGG TACTGGGACA TCCGGGGCT
GTM1_HUMAN ----- --ATGCCCAT GACACTGGG TACTGGAACA TCCGGGGCT
GTM2_HUMAN ----- --ATGCCCAT GACTCTCTAT GTTCTTCGGG TACTGGGATA TTCTGTGGGCT
GTM3_HUMAN ATGTCGTCGCA ACTCTCTCTAT GTTCTTCGGG TACTGGGATA TTCTGTGGGCT
GTM4_HUMAN ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTM5_HUMAN ----- --ATGCCCAT GACTCTGGG TACTGGGACA TCCGGGGCT
GTM1_MOUSE ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTM2_MOUSE ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTM3_MOUSE ----- --ATGCCCAT GACACTGGG TATTGGAAAC CCCCGGGACT
GTM5_MOUSE ATGTCATCCA ACTCTT--AT GTTCTTCGGG TACTGGGATA TCCGGGGCT
GTM1_RAT ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTM2_RAT ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTM3_RAT ----- --ATGCCCAT GACACTGGG TACTGGGACA TCCGGGGCT
GTMU_CIRLO ----- --ATGCCCAT GACACTGGG TACTGGGAAAT TCCGGGGCT
GTMU_MESAU ----- --ATGCCCTGT GACACTGGG TACTGGGACA TCCGGGGCT
GTM2_CHICK ----- --ATGGTGTGT CACGTGGGT TATTGGGACA TCCGGGGCT

GGCCCACGCC ATCCGCTGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GGCCCATTCG ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTACGAGG
GGCCGACCGC ATCCGGCTGC TCCTGGAAGTT CACGGATAC TCTTATGAGG
GGCCCACGCC ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTACGAGG
GGCCCACGCC ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GACACACCCC ATCCGGCATGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GGCTCACGCC ATCCGGCTGC TCCTGGAATA CACAGACACA AGCTATGAGG
GACTCACTC ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GGCTCATGCG ATCCGGCATGC TTCTGGAGTT TACTGATACCA AGCTATGAGG
GACACACCCC ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GGCTCACGCC ATCCGGCTGC TCCTGGAATA CACAGACACA AGCTATGAGG
AGGGCATGCC ATCCGGCTGC TCCTGGAATA CACAGACTCG AGCTATGAGG
GACAAACCCC ATCCGGCTGC TCCTGGAATA CACAGACTCA AGCTATGAGG
GGCTCATGCC ATCCGGCTGC TTCTGGAGTA CACAGACACA AGCTATGAGG
GGCCCACGCC ATCCGGCTGC TCCTGGAATA CACCGAGACCA CCTCTTACAGG

```

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Running PHYLIP - dnaml

```
$ fdnaml -help
  Standard (Mandatory) qualifiers:
    [-sequence]      seqsetall  File containing one or more sequence
                      alignments
    [-intreefile]     tree       Phylib tree file (optional)
    [-outfile]        outfile   [*.fdnaml] Phylib dnaml program output file

  Additional (Optional) qualifiers (* if not always prompted):
    -ncategories      integer    [1] Number of substitution rate categories
                                (Integer from 1 to 9)
    -weights          properties  Weights file
  *  -njumble         integer    [0] Number of times to randomise (Integer 0
                                or more)
  *  -seed            integer    [1] Random number seed between 1 and 32767
                                (must be odd) (Integer from 1 to 32767)
  *  -global          boolean   [N] Global rearrangements
  -outgrno          integer    [0] Species number to use as outgroup (Integer
                                optional)
  •  -outtreefile     outfile   [*.fdnaml] Phylib tree output file (optional)

  General qualifiers:
    -help              boolean   Report command line options. More
                                information on associated and general
                                qualifiers can be found with -help -verbose
```

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Running PHYLIP – (f)dnaml

```
Nucleic acid sequence Maximum Likelihood method, version 3.63
Settings for this run:
  U      Search for best tree? Yes
  T      Transition/transversion ratio: 2.0000
  F      Use empirical base frequencies? Yes
  C      One category of sites? Yes
  R      Rate variation among sites? constant rate
  W      Sites weighted? No
  S      Speedier but rougher analysis? Yes
  G      Global rearrangements? No
  J      Randomize input order of sequences? No. Use input order
  O      Outgroup root? Yes, at sequence number 15
  M      Analyze multiple data sets? No
  I      Input sequences interleaved? Yes
  O      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
  5      Reconstruct hypothetical sequences? No
Y to accept these or type the letter for one to change
j
Random number seed (must be odd)?
123
Number of times to jumble?
5
```

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Running PHYLIP – (f)dnaml

Nucleic acid sequence Maximum Likelihood method, version 3.63
 Empirical Base Frequencies:
 A 0.25824
 C 0.25662
 G 0.25997
 T(U) 0.22516
 Transition/transversion ratio = 2.000000

	Betwn	And	Length	Approx.	Confid.	Limits
	----	---	-----	-----	-----	-----
+---GTM3_MOUSE	11	GTM2_CHICK	0.31594	(0.25746,	0.37441)	**
+--8	11	3	0.08672	(0.05406,	0.11939)	**
+--GTMU_CRIL0	3	4	0.02793	(0.01168,	0.04422)	**
+--9	4	2	0.02634	(0.01094,	0.04173)	**
+--GTM1_MOUSE	2	8	0.03562	(0.01808,	0.05315)	**
+--2 +--12	8	GTM3_MOUSE	0.08136	(0.05732,	0.10551)	**
+--GTM1_RAT	8	9	0.01699	(0.00496,	0.02902)	**
+---GTMU_MESAU	9	12	0.01836	(0.00619,	0.03053)	**
+--4 +--5	12	GTM1_MOUSE	0.03072	(0.01641,	0.04505)	**
+--GTM2_RAT	12	GTM1_RAT	0.03335	(0.01837,	0.04833)	**
+--1	2	5	0.04458	(0.02524,	0.06391)	**
+GTM2_MOUSE	5	GTMU_MESAU	0.07139	(0.04896,	0.09383)	**
+---GTM3_RAT	5	1	0.02084	(0.00814,	0.03354)	**
+---3 +---GTM3_RAT	1	GTM2_RAT	0.04346	(0.02629,	0.06055)	**
+---GTM2_HUMAN	1	GTM2_MOUSE	0.01543	(0.00448,	0.02638)	**
+--6 +--GTM4_HUMAN	4	GTM3_RAT	0.08214	(0.05771,	0.10667)	**
+--10	3	6	0.02335	(0.00714,	0.03966)	**
+--7 +--GTM1_HUMAN	6	GTM2_HUMAN	0.07147	(0.04929,	0.09368)	**
+---GTM5_HUMAN	6	7	0.00694	(zero,	0.01543)	*
+---GTM5_MOUSE	7	10	0.01316	(0.00296,	0.02336)	**
11-----13	10	GTM4_HUMAN	0.05475	(0.03560,	0.07398)	**
+---GTM3_HUMAN	10	GTM1_HUMAN	0.03047	(0.01614,	0.04490)	**
+-----GTM2_CHICK	7	GTM5_HUMAN	0.06349	(0.04281,	0.08419)	**
	11	13	0.13085	(0.09317,	0.16853)	**
	13	GTM5_MOUSE	0.07982	(0.05403,	0.10560)	**
	13	GTM3_HUMAN	0.06202	(0.03845,	0.08568)	**

* = significantly positive, P < 0.05
 ** = significantly positive, P < 0.01

remember: (although rooted by outgroup) fasta.bioch.virginia.edu/bioc4230

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Running PHYLIP – (f)dnapars

DNA parsimony algorithm, version 3.63

Setting for this run:

U	Search for best tree?	Yes
S	Search option?	More thorough search
V	Number of trees to save?	10000
J	Randomize input order of sequences?	No. Use input order
O	Outgroup root?	Yes, at sequence number 15
T	Use Threshold parsimony?	No, use ordinary parsimony
N	Use Transversion parsimony?	No, count all steps
W	Sites weighted?	No
M	Analyze multiple data sets?	No
I	Input sequences interleaved?	Yes
O	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

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

Y

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Running PHYLIP – (f)dnapars

```
DNA parsimony algorithm, version 3.63 requires a total of 913.000
      3 trees in all found
      -----
      |-----GTM2_CHICK
      |   |
      |   +---GTM5_MOUSE
      |   |
      |   +---GTM3_HUMAN
      |   |
      |   +---GTM3_RAT
      |   |
      |   |   +--GTMU_MESAU
      |   |   |
      |   |   |   +-GTM2_RAT
      |   |   |   +-10
      |   |   |   +GTM2_MOUSE
      |   |   |
      |   |   |   +-GTM3_MOUSE
      |   |   |
      |   |   |   +-7
      |   |   |   +GTMU_CRILO
      |   |   |
      |   |   |   +-12
      |   |   |   |   +GTM1_RAT
      |   |   |   |   +-9
      |   |   |   |   +GTM1_MOUSE
      |   |   |
      |   |   |   +-GTM5_HUMAN
      |   |   |
      |   |   |   +-4
      |   |   |   +GTM2_HUMAN
      |   |   |
      |   |   |   +-1
      |   |   |   |   +-GTM4_HUMAN
      |   |   |   |   +-3
      |   |   |   +GTM1_HUMAN
      |
      |-----GTM2_CHICK
      |   |
      |   |   2   GTM2_CHICK 0.203366
      |   |   2   8   0.131035
      |   |   8   GTM5_MOUSE 0.075672
      |   |   8   GTM3_HUMAN 0.061172
      |   |   2   5   0.085438
      |   |   5   11  0.026262
      |   |   11  GTM3_RAT 0.067351
      |   |   11  6   0.027000
      |   |   6   13  0.038716
      |   |   13  GTMU_MESAU 0.062522
      |   |   13  10  0.020370
      |   |   10  GTM2_RAT 0.037725
      |   |   10  GTM2_MOUSE 0.017584
      |   |   6   7   0.032519
      |   |   7   GTM3_MOUSE 0.067937
      |   |   7   12  0.020952
      |   |   12  GTMU_CRILO 0.049136
      |   |   12  9   0.018272
      |   |   9   GTM1_RAT 0.031111
      |   |   9   GTM1_MOUSE 0.028148
      |   |   5   1   0.030898
      |   |   1   4   0.009778
      |   |   4   GTM5_HUMAN 0.056824
      |   |   4   GTM2_HUMAN 0.061695
      |   |   1   3   0.013210
      |   |   3   GTM4_HUMAN 0.047152
      |   |   3   GTM1_HUMAN 0.030750
```

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(f)dnapars – three alternate trees

```
(GTM2_CHICK:0.20337,(GTM5_MOUSE:0.07567,GTM3_HUMAN:0.06117):0.13103,
((GTM3_RAT:0.06735,((GTMU_MESAU:0.06252,(GTM2_RAT:0.03772,GTM2_MOUSE:0.01758):0.02037):0.03872,
(GTM3_MOUSE:0.06794,(GTMU_CRILO:0.04914,(GTM1_RAT:0.03111,GTM1_MOUSE:0.02815):0.01827):0.02095):0.03252):0.02700):0.02626,
((GTM5_HUMAN:0.05682,GTM2_HUMAN:0.06169):0.00978,(GTM4_HUMAN:0.04715,
GTM1_HUMAN:0.03075):0.01321):0.03090):0.08544)[0.3333];
(GTM2_CHICK:0.19762,(GTM5_MOUSE:0.07698,GTM3_HUMAN:0.05942):0.13647,
(((GTMU_MESAU:0.06103,(GTM2_RAT:0.03807,GTM2_MOUSE:0.01723):0.02135):0.03741,
(GTM3_MOUSE:0.06916,(GTMU_CRILO:0.04806,(GTM1_RAT:0.03111,GTM1_MOUSE:0.02815):0.01935):0.02106):0.03236):0.02522,
(GTM3_RAT:0.06150,(GTM2_HUMAN:0.05333,(GTM5_HUMAN:0.05213,(GTM4_HUMAN:0.04975,
GTM1_HUMAN:0.02815):0.01713):0.01605):0.04058):0.02860):0.08532)[0.3333];
(GTM2_CHICK:0.20335,(GTM5_MOUSE:0.07591,GTM3_HUMAN:0.06098):0.13099,
((GTM3_RAT:0.06487,((GTMU_MESAU:0.06237,(GTM2_RAT:0.03787,GTM2_MOUSE:0.01744):0.02037):0.03904,
(GTM3_MOUSE:0.06806,(GTMU_CRILO:0.04899,(GTM1_RAT:0.03111,GTM1_MOUSE:0.02815):0.01842):0.02098):0.03254):0.02944):0.02617,
(GTM2_HUMAN:0.05754,(GTM5_HUMAN:0.05427,(GTM4_HUMAN:0.05030,GTM1_HUMAN:0.02760):0.01481):0.01128):0.03306):0.08668)[0.3333];
```

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Running PHYLIP – distance methods

- Distance methods do not work on alignments, they work on distances
 - take alignment and build (corrected) distance matrix `fdnadist`, `fprotodist`
 - take distance matrix, build tree using `ffitch` (no evolutionary clock), or `fkitsch` (clock-like tree)
 - `fneighbor` for speed

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Running PHYLIP – (f)dnadist

Nucleic acid sequence Distance Matrix program, version 3.63

Settings for this run:
D Distance (F84, Kimura, Jukes-Cantor, LogDet)? F84
G Gamma distributed rates across sites? No
T Transition/transversion ratio? 2.0
C One category of substitution rates? Yes
W Use weights for sites? No
F Use empirical base frequencies? Yes
L Form of distance matrix? Square
M Analyze multiple data sets? No
I Input sequences interleaved? Yes
O 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

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

Distances calculated for species
GTM1_HUMAN
GTM2_HUMAN
GTM3_HUMAN
GTM4_HUMAN
GTM5_HUMAN
GTM1_MOUSE
GTM2_MOUSE
GTM3_MOUSE

Distances written to file "gstm_n.ddist"
Done.

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Running PHYLIP – (f)dnadist

```
15
GTM1_HUMAN 0.000000 0.111515 0.328043 0.084938 0.098515 0.202847
0.160670 0.222157 0.323212 0.195992 0.188005 0.176254 0.169073
0.202499 0.472135
GTM2_HUMAN 0.111515 0.000000 0.370425 0.122881 0.135281 0.234489
0.198432 0.246131 0.367307 0.220479 0.235718 0.162609 0.200569
0.245624 0.499002
GTM3_HUMAN 0.328043 0.370425 0.000000 0.330864 0.337744 0.395844
0.350801 0.407140 0.141206 0.397266 0.389013 0.385259 0.364146
0.386434 0.489052
GTM4_HUMAN 0.084938 0.122881 0.330864 0.000000 0.131796 0.233678
0.187505 0.236442 0.337068 0.235722 0.213963 0.182756 0.204816
0.204302 0.452330
GTM5_HUMAN 0.098515 0.135281 0.337744 0.131796 0.000000 0.230120
0.186003 0.230817 0.353029 0.215696 0.218532 0.174287 0.201916
0.216947 0.470660
GTM1_MOUSE 0.202847 0.234489 0.395844 0.233678 0.230120 0.000000
0.160969 0.116636 0.395293 0.062703 0.200109 0.200296 0.105091
0.202873 0.486157
GTM2_MOUSE 0.160670 0.198432 0.350801 0.187505 0.186003 0.160969
0.000000 0.172174 0.370651 0.159042 0.058864 0.178584 0.146716
0.103994 0.474313
. . .
```

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Running PHYLIP – (f)fitch

```
Fitch-Margoliash method version 3.63

Settings for this run:
D      Method (F-M, Minimum Evolution)? Fitch-Margoliash
U      Search for best tree? Yes
P      Power? 2.00000
-      Negative branch lengths allowed? No
O      Outgroup root? Yes, at species number 15
L      Lower-triangular data matrix? No
R      Upper-triangular data matrix? No
S      Subreplicates? No
G      Global rearrangements? Yes
J      Randomize input order of species? No. Use input order
M      Analyze multiple data sets? No
O      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
```

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Running PHYLIP – (f)fitch

```
+---GTM5_MOUSE          15 Populations
+-----7                  Fitch-Margoliash method version 3.63
!       +---GTM3_HUMAN           \   \
!       ! +---GTM5_HUMAN           /   / (Obs - Exp)
!       +---GTM5_HUMAN           Sum of squares = /_/_ -----
!       +_2                           2
!       ! ! +---GTM2_HUMAN
!       ! +_3
!       ! ! +---GTM4_HUMAN
!       ! +_1
13---4      +-GTM1_HUMAN
!       !
!       ! +---GTM3_RAT          Average percent standard deviation = 4.78966
!       !
!       ! ! +---GTMU_MESAU
!       +_10 +_12          Between      And      Length
!       ! ! ! +---GTM2_RAT          -----  ---  -----
!       ! ! +_9             13        7      0.13286
!       +_5      +GTM2_MOUSE         7        GTM5_MOUSE    0.07381
!       !             13        4      0.05956
!       ! +---GTMU_CRILO          4        2      0.02688
!       +_11             2        GTM5_HUMAN    0.06200
!       ! +---GTM3_MOUSE          2        3      0.00263
!       +_6             3        GTM2_HUMAN    0.06785
!       ! +---GTM1_RAT            3        1      0.00736
!       +_8             1        GTM4_HUMAN    0.05312
!       +---GTM1_MOUSE          . . .
!
+-----GTM2_CHICK
remember: (although rooted by outgroup) this is an unrooted tree!
Sum of squares = 0.47717
```

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Drawing trees- (f)drawtree

```
wrpmbp 29% drawtree
DRAWTREE from PHYLIP version 3.67
Drawtree: can't find input tree file "intree"
Please enter a new file name> gstm_n.fdd_tree
Reading tree ...
Tree has been read.
Loading the font ...
Font loaded.
```

Most common problem missing fontfile:
cp /seqprg/data/font1 fontfile

2nd most common problem:
overwriting/renameing plotfile

Unrooted tree plotting program version 3.67
Here are the settings:

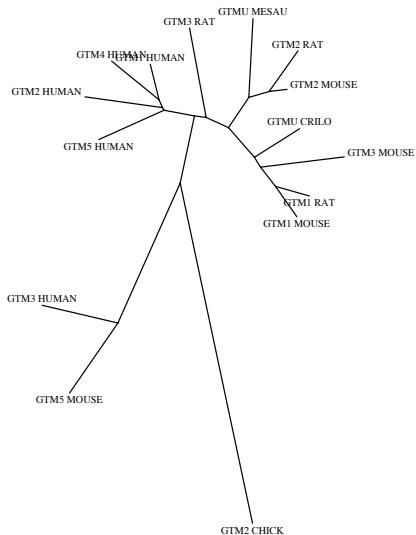
```
O Screen type (IBM PC, ANSI)? (none)
P     Final plotting device: Postscript printer
V     Previewing device: Macintosh graphics screen
B     Use branch lengths: Yes
L     Angle of labels: branch points to Middle of label
R     Rotation of tree: 90.0
I     Iterate to improve tree: Equal-Daylight algorithm
D     Try to avoid label overlap? No
S     Scale of branch length: Automatically rescaled
C     Relative character height: 0.3333
F     Font: Times-Roman
M     Horizontal margins: 1.65 cm
M     Vertical margins: 2.16 cm
#     Page size submenu: one page per tree
```

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

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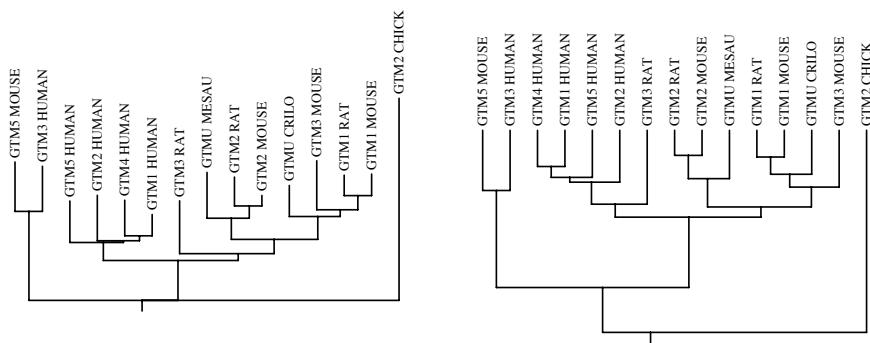
Drawing trees- (f)drawtree



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Drawing trees- (f)drawgram



fitch

kitcsh -
(evolutionary clock)

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Evaluating trees- (f)consense

```
Consensus tree program, version 3.63

Settings for this run:
C      Consensus type (MRe, strict, MR, Ml): Majority rule (extended)
O          Outgroup root: Yes, at species number 15
R      Trees to be treated as Rooted: No
T      Terminal type (IBM PC, ANSI, none): ANSI
1      Print out the sets of species: Yes
2      Print indications of progress of run: Yes
3          Print out tree: Yes
4      Write out trees onto tree file: Yes

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

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Evaluating trees- (f)consense

```
Consensus tree program, version 3.63          Sets included in the consensus tree

Species in order:                         Set (species in order)      How many times out of 3.00
                                              ..... ****.           3.00
1. GTM5 MOUSE                            ..... . ....           3.00
2. GTM3 HUMAN                            ..****. ....           3.00
3. GTM5 HUMAN                            ..... . **.           3.00
4. GTM2 HUMAN                            ..... . * . ....       3.00
5. GTM4 HUMAN                            ..... . * . ....       3.00
6. GTM1 HUMAN                            ..... . *** ****.     3.00
7. GTM3 RAT                             ..... *****. ****.     3.00
8. GTMU MESAU                           ..... *.*. ....       3.00
9. GTM2 RAT                             ..... . ***. ....       3.00
10. GTM2 MOUSE                           **. ....           3.00
                                         ..... ****. ****.     2.67
11. GTMU CRILO                           ..... . ....           2.00
12. GTM3 MOUSE                           ..****. ....           2.00
13. GTM1 RAT                            ..... . ....           2.00
14. GTM1 MOUSE                           ..... . ....           2.00
15. GTM2 CHICK                           Sets NOT included in consensus tree:

                                              Set (species in order)      How many times out of 3.00
                                              ..... *.*.           1.00
..... *.*. ....           0.67
..... *.... ....           0.33
..... ****. ....           0.33
```

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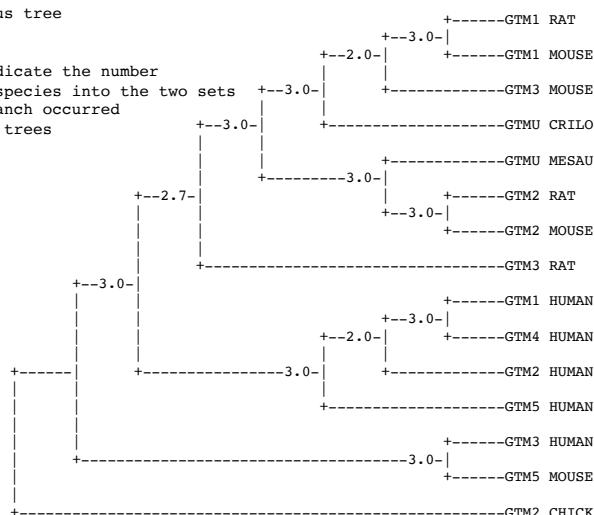
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Evaluating trees- (f) consense

Extended majority rule consensus tree

CONSENSUS TREE:

the numbers on the branches indicate the number of times the partition of the species into the which are separated by that branch occurred among the trees, out of 3.00 trees



remember: (though rerooted by outgroup) this is an unrooted tree!
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Putting it all together, the User tree

- The problem:
 - the (f)consense program produces the best consensus tree, but the branches reflect the consensus frequencies, not the evolutionary branch lengths
 - The solution:
 - give consensus tree to fdnaml or fffitch using the 'U' user tree option – calculates branches for a single tree, does not do a search (fast)

User tree – (f)dnaml

```
Nucleic acid sequence Maximum Likelihood method, version 3.63

Settings for this run:
U          Search for best tree? No, use user trees in input file
L          Use lengths from user trees? No
T          Transition/transversion ratio: 2.0000
F          Use empirical base frequencies? Yes
C          One category of sites? Yes
R          Rate variation among sites? constant rate
W          Sites weighted? No
O          Outgroup root? No, use as outgroup species 1
M          Analyze multiple data sets? No
I          Input sequences interleaved? Yes
O          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
5          Reconstruct hypothetical sequences? No

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

Asks for infile (alignment) and intree (consensus tree)

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User tree – dnaml

```
User-defined tree:
               +-GTM1_RAT
               +-7
               +-6   +-GTM1_MOUSE
               |
               |
               +-5   +---GTM3_MOUSE
               |
               |
               +-4   +-GTMU_CRILO
               |
               |
               |
               +-8
               |
               |   +-GTMU_MESAU
               |
               +-3   |   +-GTM2_RAT
               |   |
               |   +-9
               |   |
               |   +---GTM2_MOUSE
               |
               |   +---GTM3_RAT
               |
               +---2
               |
               |   +-GTM1_HUMAN
               |
               |   +-12
               |
               |   +-11   +-GTM4_HUMAN
               |
               |   |
               |   +-10   +-GTM2_HUMAN
               |
               |   +---GTM5_HUMAN
               |
               |   +---GTM3_HUMAN
               |
               |   +---GTM5_MOUSE
               |
               +-----GTM2_CHICK
remember: (although rooted by outgroup) this is an unrooted tree!
```

Consensus tree DNAML:
Ln Likelihood = -4977.65455

Original best DNAML:
Ln Likelihood = -4967.04025

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Phylip for dummies

- Programs for Parsimony, Distance, and Maximum Likelihood
- `infile/outfile/outtree/intree`
 - either always change, or never use
 - Use EMBOS (f) programs
- `(f)consense` to build consensus tree (but invalid branch lengths)
- User tree to calculate branch lengths for consensus tree
- `(f)drawtree` for non-trees, `(f)drawgram` for trees