TCS: estimating gene genealogies
Version 1.06

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TCS is a computer program that implements the estimation of gene genealogies from DNA sequences as described by (Templeton et al. 1992). This cladogram estimation method is also known as statistical parsimony. Some useful references are indicated below.

The *TCS* software package, including executables for Mac and PC, documentation, and Java source code, is distributed freely and is available at our web site, along with a host of other programs for population genetic and phylogenetic analyses:
http://bioag.byu.edu/zoology/crandall_lab/programs.htm

Version History (Current version 1.06)
Alpha 1.00: First version of the program.
Alpha 1.01: distances file included
Alpha 1.02: outgroups weights estimation included
VERSION 1.06: several cosmetic changes and some bugs fixed

NEW features in version alpha 1.06
1. Run information and outgroup weights for each haplotype (see below) is send to the LOGFILE instead of to a window in the Macintosh version, and to both window and logfile in the Windows version.

2. A progress bar is included that displays the progress of the program. It is not linear, but will be a good indication that the program is making its calculations. For big data sets, be patient and leave the program alone for a while....

3. TCS will display in the window the maximum number of steps (substitutions) to connect parsimoniously two haplotypes with a 95% confidence.

4. Once in the graph, if you double click on a haplotype, you will be able to see which sequences belong to that haplotype and the outgroup weight for that haplotype (Castelloe and Templeton 1994)

5. A run and stop buttons are included.

Bugs report, questions, etc…
We will add new features to the next versions and try to correct potential bugs. If you are using this program, we should know that, so we can inform you about mistakes and new versions. Please feel free to make questions. Please contact David Posada at david.posada@byu.edu

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Input file (DNA / absolute distances)
The TCS software works with DNA nucleotide sequence. It opens DNA alignment files in either Nexus [Maddison, 1997 #2791] or PHYLIP (Felsenstein 1991) sequential format. Alternatively, absolute distance files in modified NEXUS or PHYLIP files can can also be used.

Aligned DNA sequences

Sequential NEXUS:
```
#NEXUS

Begin data;
      Dimensions ntax=4 nchar=6;
      Format datatype=nucleotide gap=- missing=? ;
      Matrix

Seq1      AAAAA-
Seq2      AAAAC-
Seq3      AAAAA?
Seq4      AAAAAA
      ;
end;
```

Sequential Phylip:
```
4 6
Seq1 AAAAA-
Seq2 AAAAC-
Seq3 AAAAA?
Seq4 AAAAAA
```

Sequences should not be collapsed into haplotypes as frequency data can be incorporated into the output. The program collapses sequences into haplotypes and calculates the frequencies of the haplotypes in the sample. These frequencies are used to estimate haplotype outgroup probabilities, which correlate with haplotype age (Donnelly and Tavaré 1986; Castelloe and Templeton 1994).

Distance file
We included an option to read a matrix of absolute distances AMONG HAPLOTYPES. The matrix should be LOWER DIAGONAL in NEXUS (example_dis.nex) or PHYLIP (example_dis.phy) format.

IMPORTANT: you have to add the "nchar" to these files, so the 95% connection limit can be calculated. Look a the example files:

Modified Nexus format
```
#NEXUS

Begin taxa;
        Dimensions ntax=10;
        Taxlabels
                Seq1
                Seq2
                Seq3
                Seq4
                Seq5
                Seq6
                Seq7
                Seq8
                Seq9
                Seq10
                ;
End;


Begin distances;
        Format triangle=lower labels nodiagonal;
        Matrix
Seq1
Seq2        2
Seq3        2  2
Seq4        3  3  3
Seq5        4  4  4  3
Seq6        4  4  4  3  2
Seq7        3  3  3  2  1  1
Seq8        4  4  4  3  2  2  1
Seq9        3  3  3  2  3  3  2  3
Seq10       2  2  2  1  2  2  1  2  1
        ;
End;
```


Modified Phylip format

```
10 404
Seq1
Seq2        2
Seq3        2  2
Seq4        3  3  3
Seq5        4  4  4  3
Seq6        4  4  4  3  2
Seq7        3  3  3  2  1  1
Seq8        4  4  4  3  2  2  1
Seq9        3  3  3  2  3  3  2  3
Seq10       2  2  2  1  2  2  1  2  1
```

Treatment of Gaps (5th state / missing data)

By default, gaps are counted as events (i.e. treated as a fifth state). You can turn off this option in the program interface (Figure 1) and treat gaps as missing data.

Limits of parsimony (estimated/user defined)

The probability of parsimony (as defined in Templeton *et al.* [1992], equations 6, 7, and 8) is calculated for DNA pairwise differences until the probability exceeds 0.95. The number of mutational differences associated with the probability just before this 95% cutoff is then the maximum number of mutational connections between pairs of sequences justified by the "parsimony" criterion. Alternatively, this limit can be set up by the user (see Figure 1).

Note: TCS is not for RFLPs

TCS calculations are for only for DNA sequence data. If your data is RFLPs you might think you could input absolute distances, but that would not work. The problem is that for each pair of RFLP haplotypes, the parismony connection limit could be different, depending on the number of shared sites. This is because for RFLPs the total number of characters minus the number of characters with a different state does not necessarily equal the number of shared characters (which is true for DNA sequences). The difference with DNA sequences is that ++ is a shared site, while -- is not a shared site. But you could build an RFLP network by hand. The parsimony probability for RFLP data can be calculated using the program ParsProb (http://bioag.byu.edu/zoology/crandall_lab/programs.htm) for each pair of RFLP haplotypes.

Logfile

Each time that the TCS analysis is performed, a graph file (GML format) is saved (*logfile*). This file contains information on the run: probabilities of parsimony for mutational steps, the pairwise absolute distance matrix, a test listing of connections made and missing intermediates generated, outgroup weights for each haplotype, a graph description, and the date and time ellapsed for the analysis.

Graphfile

Each time that the TCS analysis is performed, a graph file (GML format) is saved. The name of this file will be *datafilename.graph* This graph can be open posteriorly in TCS.

VGJ

For graphic purposes, the freeware VGJ 1.0.3, distributed under the terms of the GNU General Public License, Version 2), is packaged within the TCS program.
(http://www.eng.auburn.edu/department/cse/research/graph_drawing/graph_drawing.html;

Running TCS

1. Open the DNA data file in the FILE menu
2. Click on Run
3. The program reads the file and collapses sequences to haplotypes
4. An absolute distance matrix is then calculated for all pairwise comparisons of haplotypes.
5. The parsimony connection limit is calculated. Alternatively, this limit can be set up by the user (see Figure 1).
6. These justified connections are then made resulting in a 95% set of plausible networks (1 or more)
7. A graph is generated and automatically opened. In this graph, haplotytpes are drawn in a size proportional to their frequency.
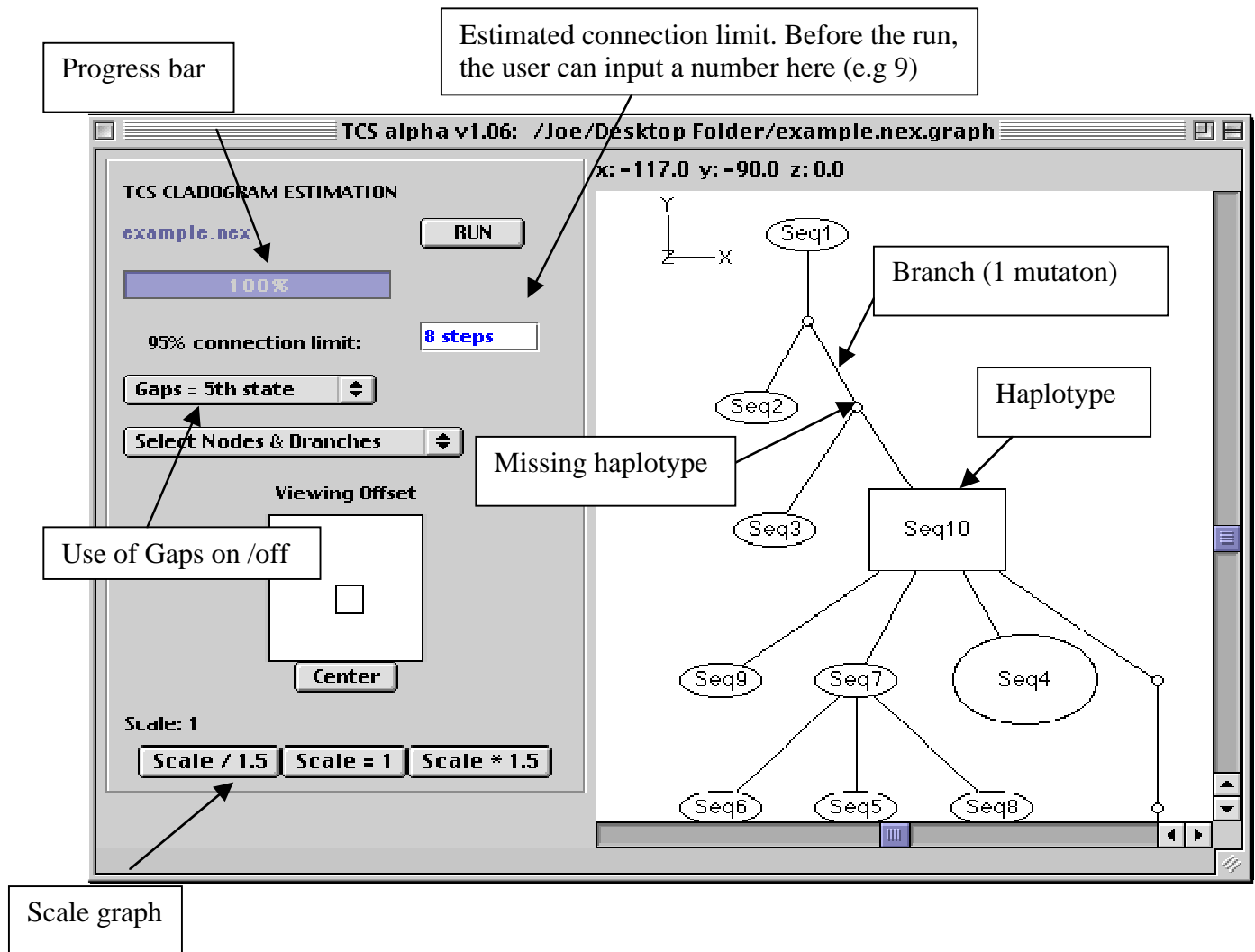
Editing the graph

You can select (by clicking), create and delete nodes (haplotypes) o branches on the graph. Automatic algorithms to order the graph are available in the menus. You can move the nodes and branches around and save the file as postcript. By double-clicking on a haplotype node, you will be able of displaying its frequency and its outgroupt weight. The haplotoype in a square has the biggest outgroup weight.

Printing

By now, the graph is printed by being saved as a postcrip file and sent manually to the printer.. Adobe Illustrator is able of reading the .ps file. We are working on exporting PICT files

Running times

The program can handle a reasonable number of sequences. For example, an HTLV data set with 69 haplotypes of length 725 bps took over one hour to run in a Macintosh G3. Memory requirements are low, and the program will run with less than 1 MB RAM.

Progress bar

Estimated connection limit. Before the run, the user can input a number here (e.g 9)

TCS alpha v1.06: /Joe/Desktop Folder/example.nex.graph

x: -117.0 y: -90.0 z: 0.0

TCS CLADOGRAM ESTIMATION

example.nex

RUN

100%

8 steps

Branch (1 mutaton)

95% connection limit:

Haplotype

Gaps = 5th state

Select Nodes & Branches

Missing haplotype

Use of Gaps on /off

Viewing Offset

Center

Scale: 1

Scale / 1.5   Scale = 1   Scale * 1.5

Seq1

Seq2

Seq3

Seq10

Seq9

Seq7

Seq4

Seq6

Seq5

Seq8

Scale graph

If you double click in the node "Seq10" you will display:

Node 9

Position:

X 247.375   Y -740.0   Z 0.0

Bounding Box:

Height 40.0   Width 65.0   Depth 20.0

Shape: Oval ⬍

Label: Seq10

Label Position Center ⬍

Image: (Leave Height and Width blank for automatic sizing.)

Type URL ⬍

Source

Data
<NEW>
✓ Frequency
Weight ⬍

frequency=5
Seq10
Seq11
Seq12

Apply   Cancel

Individual sequences
included in this haplotype

Useful references

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