TCS: estimating gene genealogies
Version 1.13

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This software is distributed as it is. We are not responsible for the results obtained with it.

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 1.13)
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
Version 1.07-1.12: Fixed bug that was creating several unconnected haplotypes (when they should be connected) for some big data sets. The progress of the calculations are showed in the GUI.
Version 1.13: Fixed bug that was creating several unconnected haplotypes (when they should be connected). Maybe the same bug we thought we fixed in version 1.12.

Installation
Should be straightforward. You do not have to do nothing other than decompress the hqx or sit (Mac), or zip (Windows) distribution files . Most often, your computer will do this automatically. In Windows, do not unzip the TCS1.13.Classes.zip file.

Do not move the files from the TCS package folder. In order to run, the executable should be in the same folder with TCS1.13.Classes.jar and TCS1.13.properties in Mac, and with TCS1.13.Classes.zip in Windows

In case of problems (most often appear n Windows), repeat the full installation again: go to the TCS page. Get JRE 1.3 (or latest) (http://java.sun.com/j2se/1.3/jre/) and install it. Get TCS and let the unzipper program to unzip it. You should get a folder with everything on it.

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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Clement, M., D. Posada and K. A. Crandall 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9 (10): 1657-1660.

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 outrgroup 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.

Potential problems with missing or ambiguous data
When collapsing sequences to haplotypes, missing data may create some problems when the sequence *only differ at missing or ambiguous characters*. Missing data may create some paradoxes ins such cases, and the order of the sequences may change the results of the collapsing.

1 TGGA?AAAAAAACT
2 TGGAAAAAAAAACT
3 TGGACAAAAAAACT

It is not easy to decide whether we have 2 or three haplotypes. Moreover, in this data set, TCS will say that there is 1 haplotype ! Why is that ? Well, the way TCS works is by comparing

1-2 = 0
1-3 = 0

Therefore, there is just 1 haplotype (1+2+3)

But if we change the order of the sequences:

2 TGGAAAAAAAAACT
1 TGGA?AAAAAAACT
3 TGGACAAAAAAACT

2-1 = 0
2-3 = 1

Therefore, there are two haplotypes, 2+1 and 3.

This situation will be really uncommon. Anyway TCS will alert you in such cases.
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 parsimony 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.

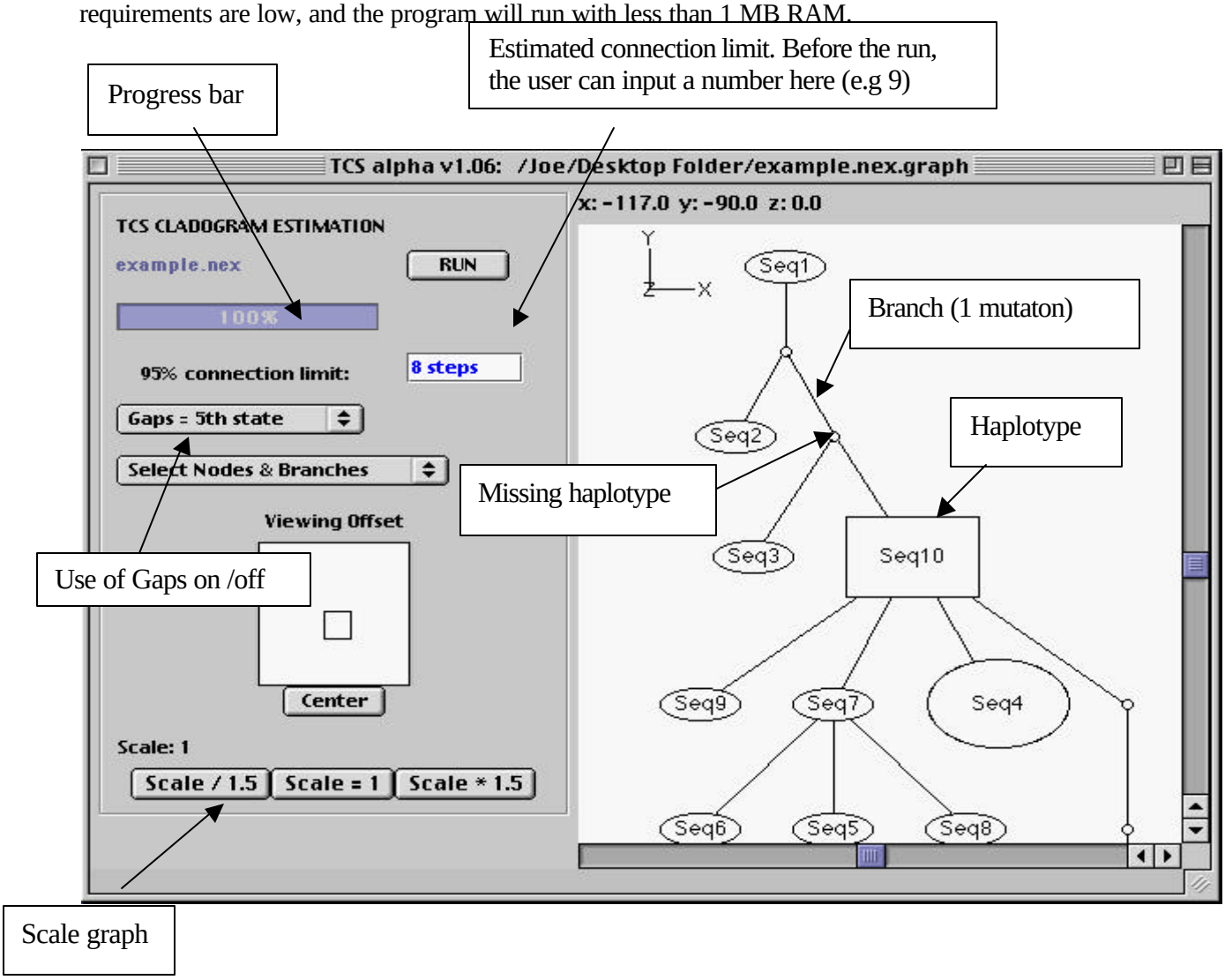
Ouput graphs

Be aware, if you have several unconnected subnetworks, TCS will not spread those automatically. If you have overlapping haplotypes, you have to move then around using the mouse. Nothing should overlap.

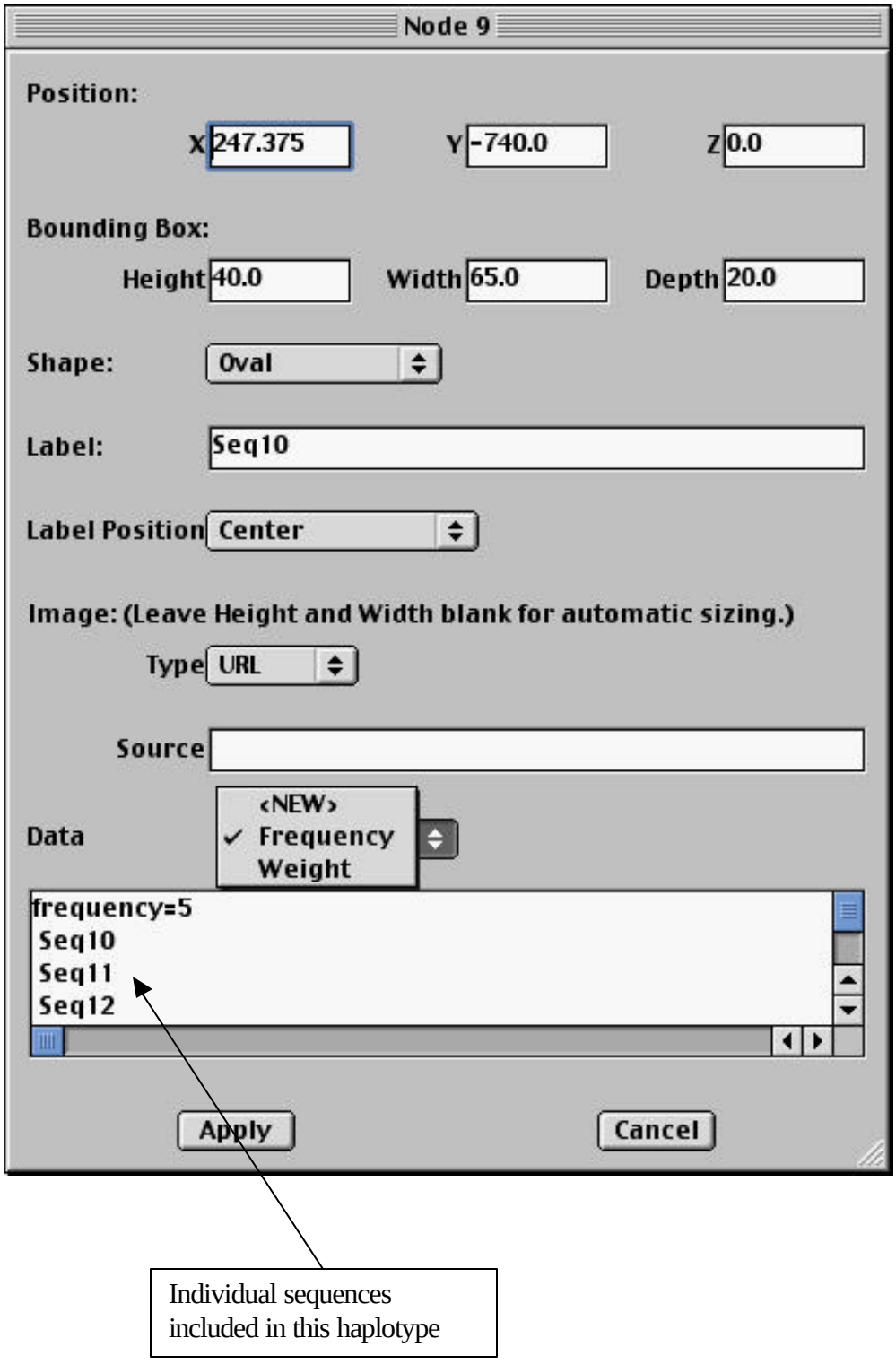
Editing the graph
You can select (by clicking), create and delete nodes (haplotypes) o branches on the graph. Automatic algorithms to order the graph are available in the menus. You can move the nodes and branches around and save the file as GML (this format will be recognized by TCs later, if you want to edit further the graph) or as postcript (and pict in Windows). 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
The graph is printed by being saved as a postcrip file and sent manually to the printer or as a PICT file.

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.



If you double click in the node “Seq10” you will display:



Useful references

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