NTSYSpc

Numerical Taxonomy and Multivariate Analysis System

Version 2.0

User Guide

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Preface

NTSYSpc was developed originally for use by students in a seminar course “Taxonomia númerica em microcomputadores” held in September 1985 at the Estação Agronómica Nacional, Oeiras, Portugal. Many of the programs were written on a portable computer as I worked each evening on the balcony of a hotel in Estoril—trying to develop the programs needed by the students for the next day's lab projects. The beautiful surroundings and enthusiastic students seemed to have helped. Most of the design and many of the actual programs were developed during the two-week course. It was quickly recognized that such a program on a personal microcomputer was of general interest—both for use in student laboratories and for research computations. The PC was easily able to handle most datasets.

NTSYSpc was developed originally as a mainframe system of programs (NTSYS). It was written in FORTRAN for an IBM 360/50 computer at the University of Kansas in 1966. It was developed with the help of Ron Bartcher who also converted it for use on a GE-635 computer in 1968. In 1969 John Kishpaugh and David Kirk helped with the conversion of NTSYS from the GE-635 back to an IBM 360/50 and then to the Univac 1100 computer system—both at the State University of New York at Stony Brook. In addition, many others contributed to its development over the years. But NTSYSpc is a new program. Fortunately, after all of the previous experience with conversions, most of the *computational* routines in NTSYS were by now quite system-independent and easy to convert to another language such as Pascal. At present, NTSYSpc has moved beyond NTSYS and provides many operations not available in the mainframe version of NTSYS.

NTSYSpc has gone through many revisions and has become much easier to use—especially now that it is a Windows program. The help files have been improved and contain much of the technical information that was once in the printed documentation. There is also a special spreadsheet-like editing program (NTEDIT) for the preparation of data files.

Both the program and the documentation have greatly benefited over the years by the help of many of the users (especially Drs. Richard Jensen and Leslie Marcus) who have spotted many “glitches” in the program and the documentation. Dr. Dennis Slice has also made a number of important contributions. Dean Adams and other graduate students have also been very helpful. NTSYSpc will continue to be developed. New programs and features are planned so that the system can evolve to better meet your needs. Your comments, suggestions, and criticisms are appreciated.

Setauket, New York F. James Rohlf

1. Introduction

1.1 Areas of application

NTSYSpc is a system of programs that is used to find and display structure in multivariate data. For example, one may wish to discover that a sample of data points suggests that the samples may have come from two or more distinct populations. Of equal interest is the discovery that some subsets of variables are highly inter-correlated. The program was originally developed for use in biology in the context of the field of **numerical taxonomy** (which explains why the name of the program is NTSYS—for **N**umerical **T**axonomy **SYS**tem). But the programs have also been widely used in morphometrics, ecology and in many other disciplines in the natural sciences, engineering, and the humanities. The terms **mathematical taxonomy** and **automatic classification** have also been used to describe this field of application. The techniques also represent a subset of multivariate data analysis and have close ties to some methods in the field of pattern recognition.

Within the field of systematic biology, one can distinguish two different approaches to classification. In **phenetics** one is concerned with the discovery and description of the patterns of biological diversity and forming classification based on overall similarity computed from multivariate data. These methods are commonly used in morphometric studies. In **cladistics** one is interested in inferring the evolutionary history of the organisms under study and using it as a basis for classification. Specialized methods have been developed to take into account the assumption that the underlying model is of a branching evolutionary tree. It is expected that the best biological explanation of the observed diversity of a set of organisms will come in terms of their evolutionary history. The methods are intended to make the best estimates of the evolutionary tree given a set of descriptive data on a set of organisms. The most commonly used methods are justified on the basis of the philosophical principle of parsimony (that the shortest tree that can be fitted to a set of data should be the best estimate of the true tree) but statistically more powerful methods based on the principle of maximum-likelihood are increasing in popularity.

The methods furnished in NTSYSpc are largely associated with the field of phenetics. However, they are best interpreted as simply methods for multivariate data analysis. There are programs by others that are specialized for phylogenetic methods. Some of the more well known ones are PAUP[1] and PHYLIP[2]. However, Saitou and Nei's (1987) neighbor-joining method of phylogenetic tree estimation is included in NTSYSpc.

[1] Written by David Swofford, currently distributed by the Illinois Natural History Survey.

The principal journal devoted to the general theory behind many of these techniques is the *Journal of Classification*. It is published for the Classification Society of North America by Springer-Verlag. Theoretical papers are also published in many statistical journals. Applications of these techniques are published in many scientific journals in the areas of application. For example, *Systematic Biology* (was called *Systematic Zoology*) has published many theoretical and applied papers with special emphasis to applications in biological taxonomy.

Most users of these techniques begin with a data matrix that contains information about the properties (features, characters, etc.) of a number objects (individuals, specimens, quadrats, OTUs, etc.). NTSYSpc can then be used to compute various measures of similarity or dissimilarity between all pairs of objects and then summarize this information either in terms of nested sets of similar objects (*cluster analysis*) or in terms of a spatial arrangement along one or more coordinate axes (*ordination analysis* or various types of *multidimensional scaling analysis*). This User Guide assumes that the reader has some familiarity with the methods. It does not contain much advice about *which* similarity coefficient or *which* clustering method should be used. It does, however, give many hints about the use of the methods. To keep the account general, the neutral terms "object" or "OTU" (for operational taxonomic unit) are usually used to refer to the things (specimens) being analyzed and the terms "variable" or "character" are used to refer to the properties used to describe the objects under study.

Users may find the following general references helpful (the complete references are given in the Bibliography).

- Everitt and Dunn (1992) give a good concise introduction to both cluster analysis and multidimensional scaling analysis. They furnish examples from biology.
- Gnanadesikan (1977) describes many methods for detecting patterns in multidimensional data. Applications are from many fields.
- Hartigan (1975) describes a large number of different clustering methods. Examples (with test data sets) are from a great many fields.
- Jackson (1991) is an excellent mathematical text on multivariate analysis. It is much more comprehensive than implied by its title ("A user's guide to principal components").
- Massart *et al.* (1978) gives a discussion with applications in analytical chemistry.
- Reyment (1991) gives an overview of the application of multivariate methods and features discussions of many data sets. The supplement by Marcus gives SAS procedures for the computations of many of the multivariate analyses discussed in that book.
- Romesburg (1984) gives detailed descriptions of many clustering methods.

[2] Written by Joe Felsenstein, University of Washington.

- Sneath and Sokal (1973) may be consulted for a general introduction to the field of numerical taxonomy and for definitions of most of the jargon used in this manual. Most examples are from biology but extensive references are given to applications in other fields. The older version, Sokal and Sneath (1963) is still a useful reference as it gives more complete listings of coefficients.
- Weir (1989) gives a short overview for DNA sequence data.

1.2 Program modules in NTSYSpc

Listed below are short descriptions of the computational modules included in NTSYSpc. The acronyms under which they are listed are the codes used in batch command files. Detailed technical descriptions of the modules (including equations for the operations and the various coefficients) are provided in the help file. NTSYSpc is not limited to just the analyses mentioned below. The modules can be used in sequence to build many other types of analyses (for example, Gower's principal coordinates analysis can be carried out by using the SIMINT, DCENTER, and EIGEN modules). Users experienced with earlier versions of NTSYSpc may wish to skip to Section 1.4 to see a summary of the new features.

CANPLS Performs canonical correlation and two-block partial least-squares analyses. Used to study pattern of correlations between two sets of variables.

CONSENSUS Computes a consensus tree for two of two or more trees (such as multiple tied trees from SAHN or between two different methods). Several consensus indices are also computed to measure the degree of agreement between trees.

COPH Produces a cophenetic value matrix (matrix of ultrametric values) from a tree matrix (produced, *e.g.*, by the SAHN program). This matrix can be used by the MXCOMP program to measure the goodness of fit of a cluster analysis to the similarity or dissimilarity matrix on which it was based.

CORRESP Correspondence analysis. This is a useful way to investigate the structure of 2-way contingency table.

CPCA Common principal components analysis. Attempts to fit a single set of eigenvectors to a series of variance-covariance matrices.

CVA Performs a canonical vectors analysis (a generalization of discriminant function analysis). It can also be interpreted as a single-classification multivariate analysis of variance, MANOVA

DCENTER Performs a "double-centering" of a matrix of similarities or dissimilarities among the objects. The resulting matrix can then be factored to perform a principal coordinates analysis (a method for displaying relationships among objects in terms of their positions along a set of axes based on a dissimilarity matrix).

EIGEN Computes eigenvector and eigenvalue matrices of a real symmetric similarity matrix. This program can be used to perform a principal components or a principal coordinates analysis by extracting eigenvectors (factors) from a correlation or variance-covariance matrix.

FOURIER Computes Fourier and elliptic Fourier transformations (for both 2D and 3D curves).

MDSCALE Nonmetric and linear multidimensional scaling analysis. This can be used as an alternative to principal components analysis.

MOD3D Plots a 3-way scatter diagram as a 3-D perspective view of a model with *t* "objects" at tops of wires attached to a base plane. The view can be rotated interactively. This program is often used to view the results of a principal components or principal coordinates analysis.

MST Computes a minimum-length spanning tree from a similarity or dissimilarity matrix. This is useful for showing the nearest neighbors of objects based on their positions in a multidimensional space.

MXCOMP Compares two symmetric matrices by computing their matrix correlation and then plotting a scatter diagram. The statistics for a Mantel test are also computed. It can be used to compute the goodness of fit of a cluster analysis to a dataset (by comparing a cophenetic value matrix with a dissimilarity matrix).

MXPLOT Plots 2-way scatter diagrams of rows or columns of a matrix.

NJOIN Computes Saitou and Nei's (1987) neighbor-joining method trees as estimated phylogenetic trees.

OUTPUT Formats matrices into pages for printing. The files can also be read by most word processors. This formatted output is also useful for checking to make sure that an input file has been prepared in the correct format for NTSYSpc.

POOLVCV Computes a pooled within-groups variance-covariance matrix from two or more data matrices. Can also perform a test for homogeneity.

PROJ Projects a set of objects onto one or more vectors—or onto a space orthogonal to a set of vectors. In principal components analysis one will project standardized data onto the eigenvectors of the correlation matrix in order to see the best (in a least-squares sense) low-dimensional view of a data set. The orthogonal projection option can be used to implement Burnaby's (1966) method for size adjustment.

SAHN Performs the sequential, agglomerative, hierarchical, and nested clustering methods as defined by Sneath and Sokal (1973). These include such commonly used clustering methods as UPGMA and single-link. The program can find alternative trees when there are ties in the input matrix.

SIMGEND Computes matrices of genetic distance coefficients from gene-frequency and DNA sequence data.

SIMINT Computes various similarity or dissimilarity indices for interval measure (continuous) data (*e.g.*, correlation, distance, etc. coefficients).

SIMQUAL Computes various association coefficients for qualitative data— data with unordered states (*e.g.*, simple matching, Jaccard, phi, etc. coefficients).

STAND Performs a linear transformation of a data matrix so as to eliminate the effects of different scales of measurement.

SVD Computes a singular-value decomposition of a rectangular data matrix. It allows you to compute principal axes and projections in a single step.

TPSWTS Computes projections of the 2D or 3D coordinates of objects onto the principal warps of a thin-plate spline bending energy matrix. This is done to enable a statistical analysis of the non-affine components of shape variation.

TRANSF Performs various linear and non-linear transformations of the rows or columns of a matrix. Can also be used to delete rows or columns, read Lotus 1-2-3 files, and alter the form of storage of some matrices matrix.

TREE Displays a tree (*e.g.*, from a cluster analysis) as a phenogram. Options are provided for scaling and scrolling through a tree interactively.

1.3 How to get started using NTSYSpc

Installation of NTSYSpc is quite easy since a standard type of installation program is used. Simply insert the disk and run its setup program. The only decision you will have to make during installation is to select the name of the directory to be used. A program group will be created on your startup menu. There will be icons for NTSYSpc, NTedit, help files, and the readme.txt file. A convenient program is also included to allow you to un-install NTSYSpc (*e.g.*, in case you need to move NTSYSpc to another computer). When you run NTSYSpc the first time you will be asked to provide your name, institution (optional), and a registration number.

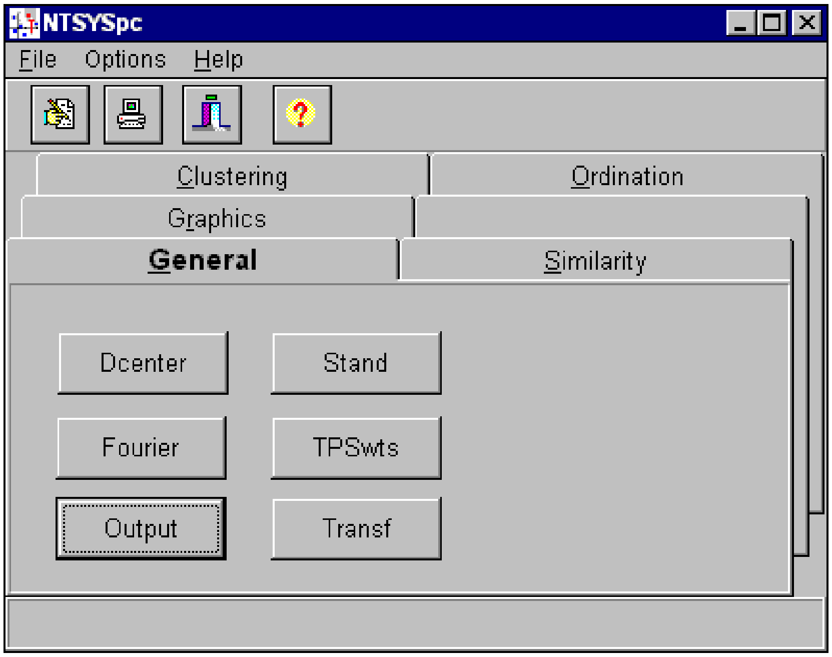
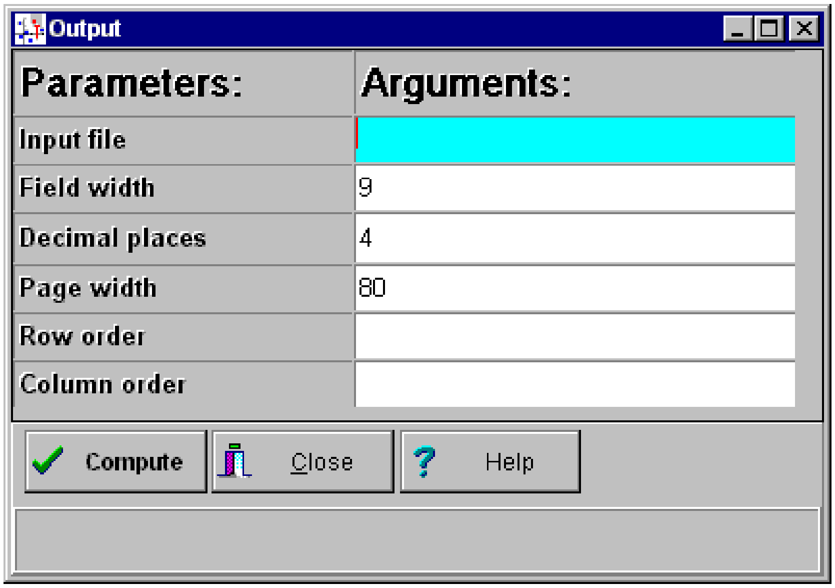


Figure 1.1. NTSYSpc main window

Once the program is installed, click on the NTSYSpc icon in the start menu to see what NTSYSpc "looks like" (see Figure 1.1). Click on a button (such as “Output”) on this main window to bring up a program module, such as the Output program. A form will be displayed in which you can specify the input file and other options for the selected module (see Figure 1.2). A test data set, TEST.NTS, is supplied so you can try a few operations right away. Click on the highlighted cell opposite “Input file” to bring up a file open dialog box. Note that by default the dialog box assumes that data file names end with the file extension .NTS. If that is not true then click on the file type window at the lower left and select “All files.” Use this dialog to locate the TEST.NTS file in C:\NTSYS (or wherever you installed NTSYSpc) and then click on the “Open” button. Then click on the “Compute” button to run the Output module. The results will be displayed in the Listing window (every time you run a module a new section is added to the listing notebook). An example is given in Figure 1.3. Press the key or use the Help menu items to open the help file.



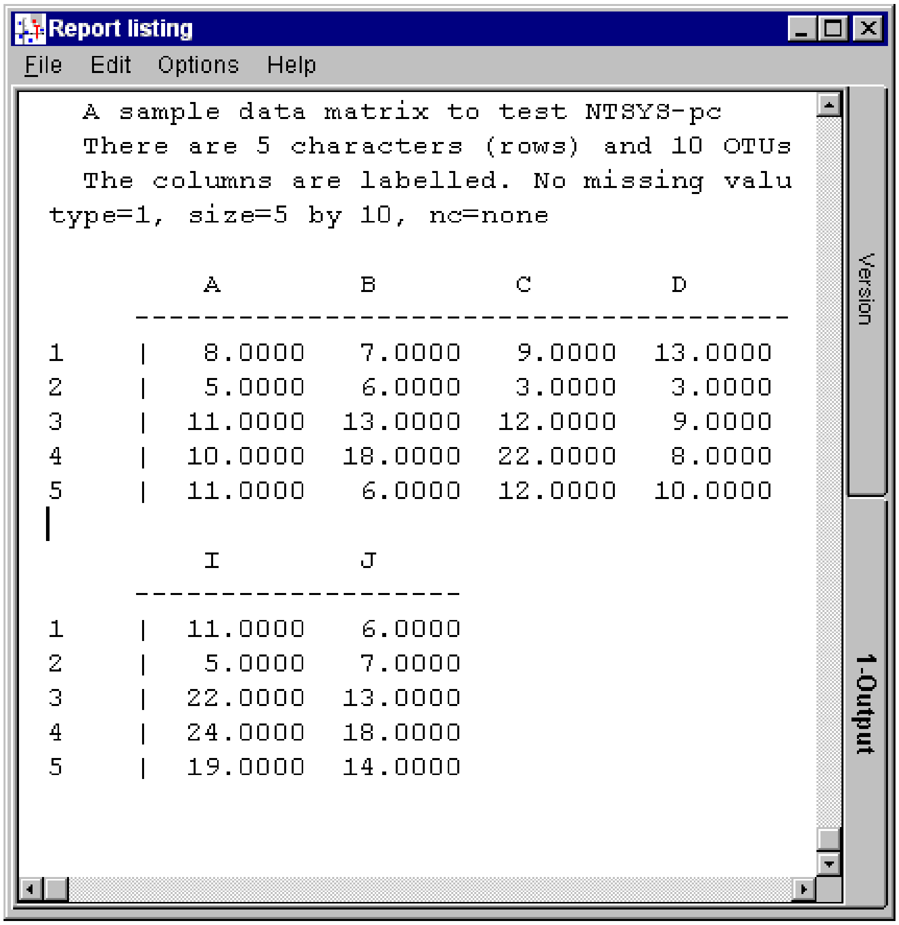
Parameters:	Arguments:
Input file	
Field width	9
Decimal places	4
Page width	80
Row order	
Column order	

Figure 1.2. Entry window for the Stand module.

You should note that the separate modules do not provide a complete analysis. Unlike many programs, you do not just run one module. You will normally use a sequence of modules in order to carry out a complete analysis. This structure makes NTSYSpc more flexible and useful in research applications. Unless batch files are used, this approach also helps one appreciate the various components making up a standard analysis. See Chaper 7 for a number of examples.

Next, you should read Chapter 2 on “**Modes of operation”** to learn how to use NTSYSpc in both interactive and batch modes. Note that This Style of type is used to indicate strings of characters that the user is expected to type into the computer, such as file names. Chapter 4 on “**Preparation of input data files”** is, of course, essential reading as it describes the format of the data files. It also describes the use of the special

Be sure to check the README.TXT file for any last-minute notes or corrections to this User Guide. The blue registration card should also be filled-out and returned since this allows us to notify you of any problems that are discovered with this version of NTSYSpc. It will also allow us to notify you of the availability of updates as new programs and features are added to NTSYSpc. Your comments, corrections, and suggestions about the program are welcomed.



```
   A sample data matrix to test NTSYS-pc
   There are 5 characters (rows) and 10 OTUs
   The columns are labelled. No missing valu
 type=1, size=5 by 10, nc=none

            A          B          C          D
       ---------------------------------------

 1     |   8.0000     7.0000     9.0000    13.0000
 2     |   5.0000     6.0000     3.0000     3.0000
 3     |  11.0000    13.0000    12.0000     9.0000
 4     |  10.0000    18.0000    22.0000     8.0000
 5     |  11.0000     6.0000    12.0000    10.0000

            I          J
       --------------------

 1     |  11.0000     6.0000
 2     |   5.0000     7.0000
 3     |  22.0000    13.0000
 4     |  24.0000    18.0000
 5     |  19.0000    14.0000
```

Figure 1.3. Listing window after running the Output module on the TEST.NTS data file.

1.4 What’s new in version 2.0

The entire user interface is new! However, much of the previous ways of using the program were preserved. One still selects a module, fills in a form, and runs the module. Some of the major differences are listed below.

- The **entry forms** have been simplified and all of the listing results are saved to a Listing notebook window where you can save, delete, or even edit the results. You can also conveniently cut and paste them into other software such as word processors.
- Input **file formats** have *not* changed — except that there is no longer a length restriction to the input lines. They were limited to just 255 characters (which seemed quite adequate just a few years ago). Long file names are now recognized. Upper and lower case letters are preserved as well as blank spaces within file names (however, batch files do not yet permit blanks within file names). Long names permits much more descriptive identifiers — often very important in more complicated analyses involving many files.
- A new **NTedit** program replaces the previous NTEDITOR program. The new program recognizes the various file formats and displays files in an appropriate spreadsheet-like format.
- **Graphics** have been greatly improved. You can now call up the appropriate graphics modules directly from many of the computational modules so that you can, for example, immediately view a phenogram after using the SAHN module to perform a cluster analysis. The entry forms for the graphics programs have also been simplified

since the graphic options are now available from option dialog boxes brought up by clicking on a plot with the right mouse button. You can now control the colors, fonts, line widths, *etc.* of most aspects of the plots.

- **Batch files** have been changed to allow both longer lines and the ability to have continuation lines for a command. To do this the program now requires that the start of each command line begins with an asterisk.
- The information in the previous **User Manual** has been divided into this **User Guide** and the very extensive **help file**. The help file contains the detailed technical descriptions of each computational module. These sections can be printed from the help file if you want the information in a printed form. This User Guide contains only general information about how to use the program.

The changes to individual modules have been relatively minor since so much effort was spent simply converting the DOS program to a Windows program. Future updates will emphasize adding new computational methods and options.

2. Modes of operation

There are two modes in which NTSYSpc can be used: *interactive* and *batch*. In interactive mode a module is selected from the main window by clicking on a button which causes a window showing the various parameters and options for that module to be displayed. After this form is filled in, click the Compute button to "run" the module and have the results appear as a new section in the Listing window. You start batch mode by selecting the "Run batch file…" item on the File menu or by using the convenient speed button on the toolbar. The batch dialog box will let you select a file containing a sequence of NTSYSpc commands, specify up to nine parameters, and run the batch file. The batch file contains commands that call up various modules, supply parameters, and execute them automatically. Batch files are convenient for the processing of large data sets or for processing a large number of data sets (perhaps from a simulation).

2.1 Interactive mode

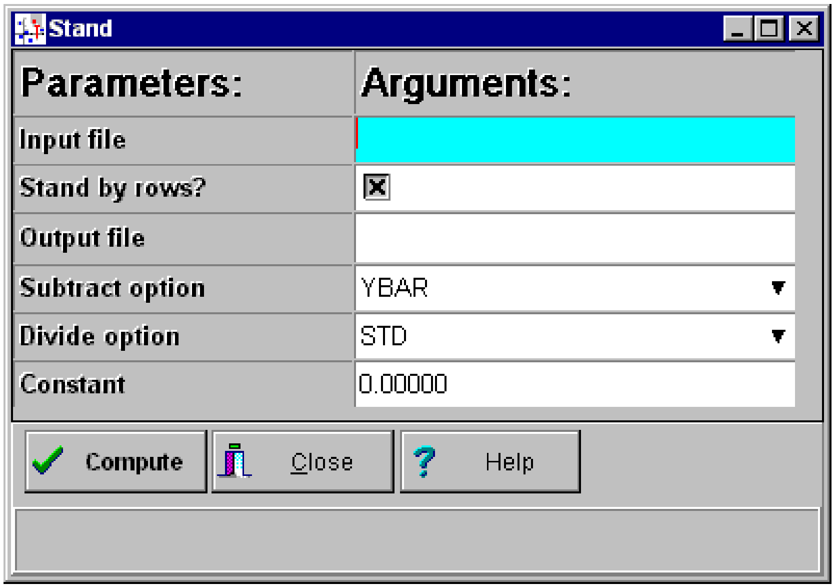
The main program window displays a "card file" with tabs labeled corresponding to sets of programs (see Figure 1.1).

Click on a tab to select a section. This displays buttons corresponding to the modules available from that section. Notice that as the mouse passes over a button, additional information is displayed in the status area at the bottom of the window. To select a module, click on the corresponding button. The selected module will then display a parameter entry window such as that for the STAND program shown in Figure 2.1. To run a program you must enter the required information in the Entry Window (you need to at least specify the name of an input file). To fill in the entry form, select the desired locations in the form using a mouse and enter the appropriate information (the method of entering the information depends on the type of field). The default choices, if there are any, will have already been entered into the form.

Input or output matrix names: names are any valid Windows file names (including long names). File names can, optionally, be preceded by a drive specification (*e.g.*, `a:test.nts`) or a path specification (*e.g.*, `d:\data\test.nts`). If the name contains either a colon or a backslash character, then the name is used as is. Otherwise the name will be appended to the current data directory. The program will remember the drive and directory from previous runs so that you do not have to enter it every time if all the files are in the same directory. It is easiest to simply double click on the cell to bring up a file open dialog box where you can select the file visually.

Numerical constants: Often numerical constants make sense only within certain limits. NTSYSpc will not permit you to enter an out-of-range value. Decimal points should not be typed when integer numbers are expected by the program.

Pick lists: Many of the programs require one to select one of several choices for a field (such as a method of standardization or a clustering method). They are indicated by the small upside down triangle at the right end of the field (there are two examples in Figure 2.1). Click on the field to display a list of the available choices. Move the cursor or the mouse to high-light the desired option and then click with the left mouse button. Sometimes there is a blank entry signifying that this option is to be ignored. The selected code will then be entered into the form.



Parameters:	Arguments:
Input file	
Stand by rows?	☒
Output file	
Subtract option	YBAR ▼
Divide option	STD ▼
Constant	0.00000

Figure 2.1. Dialog window for the STAND module.

Checkboxes: Press the space bar or click with the mouse to alternate between checked (for yes) and unchecked (for no) states. These are used to indicate, for example, whether the program should operate on the rows of the input matrix or whether additional information should be included in the output listing.

Once the fields have been filled--in correctly, click the Compute button to run the program (since the Compute button has the focus initially, you can also just press the key). The Listing window will be opened to a new section and it will show a summary of the input parameters you specified, information about the input files, and the results of the computations. If you provided names for output files then these results are stored on disk and are available as input to other modules. For some programs short-cut graphics speed buttons will appear on the small toolbar at the bottom left of the parameter entry window. Pass the mouse over the button to display the hint box describing the type of plot produced by each button. For example, Figure 2.2 shows the buttons available after running the EIGEN program.

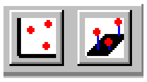


Figure 2.2. Graphic speed buttons for the EIGEN module. The first button calls MXPLOT to produce a 2D plot of the eigenvectors and the second calls MOD3D for a 3D plot.

In case of an error (such as entering the name of a non-existent file for an input data matrix), the program will "beep" and display a message in an Error Window. Click the OK button to close this window so that you may correct the problem and try again.

To close the parameter entry window for a module you may either click the Close button or simply select another module from the NTSYSpc main window.

2.2 Batch mode

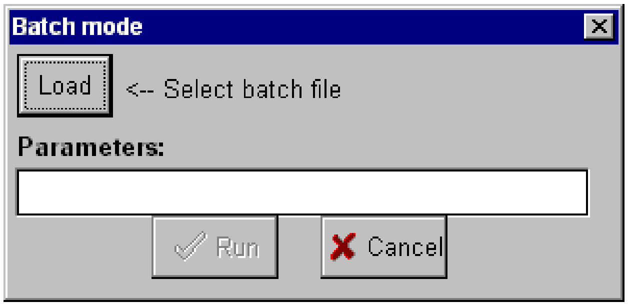


Figure 2.3. Batch mode window. Click the "Load" button to select a batch file and then click the "Run" button.

In batch mode NTSYSpc will attempt to directly execute a sequence of modules without displaying the parameter entry windows for each. Commands are entered in an ASCII file which can be prepared with an editor such as Windows notepad or wordpad (although the latter has the annoying habit of always placing the extension .txt at the end of a file name). The following is a simple example.

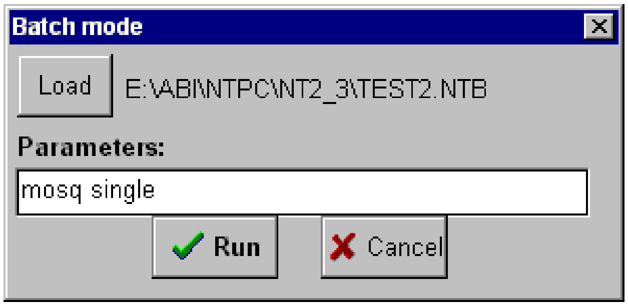
```
' standardize the rows of the data matrix
*stand o=data
       r=sdata
' compute distances among the OTUs
```

```
*simint o=sdata r=dist
' now perform a UPGMA cluster analysis
*sahn o=dist     r=tree
```

Lines that begin with a quote characters (either single or double) are treated as comments. Blank lines are ignored. Each command line begins with an asterisk followed by the name of the desired program. It is followed by parameter=value pairs that may take one or more lines (lines that do not start with either an asterisk or a quote character are considered continuation lines). Each parameter is a code for some program parameter. Value gives the value of the parameter. There must be an "=" sign (and no blanks) between the parameter and its value. Each such pair must be separated by at least one blank space. The parameter is usually a one to three letter code (they are given in the help topic for each module). They can be typed in either upper or lower case. The values can be file names, numerical constants, or option codes. The values are *identical* to what would be specified in an entry form in interactive mode. The defaults are also the same. For legibility it is convenient to keep the lines short and use more than one line for each command if convenient. The file `TEST.NTB` on the distribution disk is an example of a NTSYSpc batch file.

To execute a file containing batch commands, click on the batch speed button on the toolbar or else select the “Run batch file…” item on the File menu of the main window. This will bring up the batch mode dialog box as shown in Figure 2.3. Click on the “Load” button to bring up a file open dialog that allows you to specify which file to use. The click on the “Run” button to execute the file. While running, this window will display the currently executing line. If you change your mind you may click on the “Cancel” button and the run will be stopped at the next iteration or logical breakpoint in the currently executing module (this might take a while for a large matrix). The results will be sent automatically to the Listing window where they can be inspected when the computations are complete.

It is also possible to prepare a batch file with *replaceable parameters*. This allows batch files to be used with more than one data set. If the codes `%1, %2,…, %9` are found in a batch file they will be replaced by the values of the corresponding replaceable parameter strings given in the parameter area of the batch mode window. A maximum of 9 replaceable parameters can be specified. An example is shown below.



E:\ABI\NTPC\NT2_3\TEST2.NTB

Parameters:

mosq single

Figure 2.4. Batch mode window with the `test2.ntb` file loaded and two replaceable parameters provided.

```
*stand o=%1.nts r=sdata.nts
*simint o=sdata.nts r=dist.nts
*sahn o=dist.nts cm=%2 r=tree.nts
```

If the first replaceable parameter is "mosq" and the second is "single" (as in Figure 2.4), then this batch file will be interpreted as if it were as follows:

```
*stand o=mosq.nts r=sdata.nts
*simint o=sdata.nts r=dist.nts
*sahn o=dist.nts cm=single r=tree.nts
```

2.3 Both interactive and command modes

During execution, the programs echo the input parameters and the comment information furnished with the input matrices to the Listing window. In addition, a progress bar and a status panel given an indication of how computations are progressing within each module.

Press the Cancel button if you need to stop the execution of programs that take a long time to complete. The program should stop once it completes its next iteration or cycle of computation (it does not check constantly for a keypress since that would slow the program down). Alternatively, you can hold down the keys to bring up the Windows "Close Program" dialog box. Select NTSYSpc and then click on the "End task" button. The program should then stop abruptly — but any information that was in the listing window will be lost.

3. Menus & related windows

3.1 Main menu

Across the top of the main window is a menu bar (see Figure 1.1). The various choices are described below.

File This pulls down a submenu from which you can select "Edit data file," "View listing," "Printer setup," "Run batch file," and "Exit." The edit menu item displays a file open dialog in which you can specify the name of the NTSYSpc file you wish to edit. The separate program NTedit (included with NTSYSpc) is then run. If the file is a valid NTSYSpc file then it will be displayed in a spreadsheet like format. If there are any errors in reading the file then an alternative ASCII editor (such as the Windows notepad or some other user selectable editor) will be run. The view listing item opens the Listing window (see Section 3.3). The printer setup item opens the standard Windows printer setup dialog box. The run batch file item brings up the Batch mode dialog box so that a batch file can be run (see Section 2.2). The exit item closes the program (the program can also be closed by clicking on the Close speed button on the tool bar.

Options This pulls down a submenu from which you can select “Configuration” or “Restore defaults.” The configuration item will display a parameter entry form for various program configuration options. The restore defaults item will reset the configuration parameters back to their original states.

Help This pulls down a submenu from which you can select “Contents,” “Topic search,” or “About.” The contents item displays the table of contents for the help file. Topic search brings up the Help topics dialog box in which you can search for various terms. The about item displays the NTSYSpc about box showing copyright information, version number, and the registration number.

3.2 Configuration window and file

There are a number of aspects of how NTSYSpc operates that can be modified by a user. These are done from the Configuration Window (select “Configuration” under the Option menu on the main window). This will display a window like those used for the various computational modules. The entries here, however, are system parameters such as file formats, directory names, and other options. The information you enter will be saved in the ntsys.ini file in the Windows directory. The file also includes coded information about the position and size of various windows used by NTSYSpc.

Configuration parameters

Batch code	**Description**
FF	File format code.
LF	Listing format code.
LI	Listing indent when printing.
DD	Data directory—the directory to use as the current directory for data files.
OW	File overwrite code. ?=ask, O=overwrite, and A=append.
ED	Editor to be called from the File|Edit menu.

The “**File format**” code is used to write results to disk so they can be used as input to other modules. The default format of “e” ensures these values are saved with maximum precision. This value should not be changed except possibly when working with very large matrices and you are low on disk space. On the other hand, the “**Listing format**” code will often be changed so that numerical information displayed in the Listing window has an appropriate level of precision for a given data set. The default is “8.4f” which means that floating point numbers should be displayed with four decimal places within a field eight characters wide. You can also enter the code as “F8.4” as in FORTRAN but the NTSYSpc will always store the code with the “f” at the end.

The “**File overwrite code**” is used to determine what should happen when the program attempts to save a data file with the same name as an existing file. Ask means that a window will pop-up asking what should be done, overwrite means that the existing file should be deleted, and append means that the new file will be appended to the end of the existing file.

If you wish, another editor can be substituted for the NTedit program.

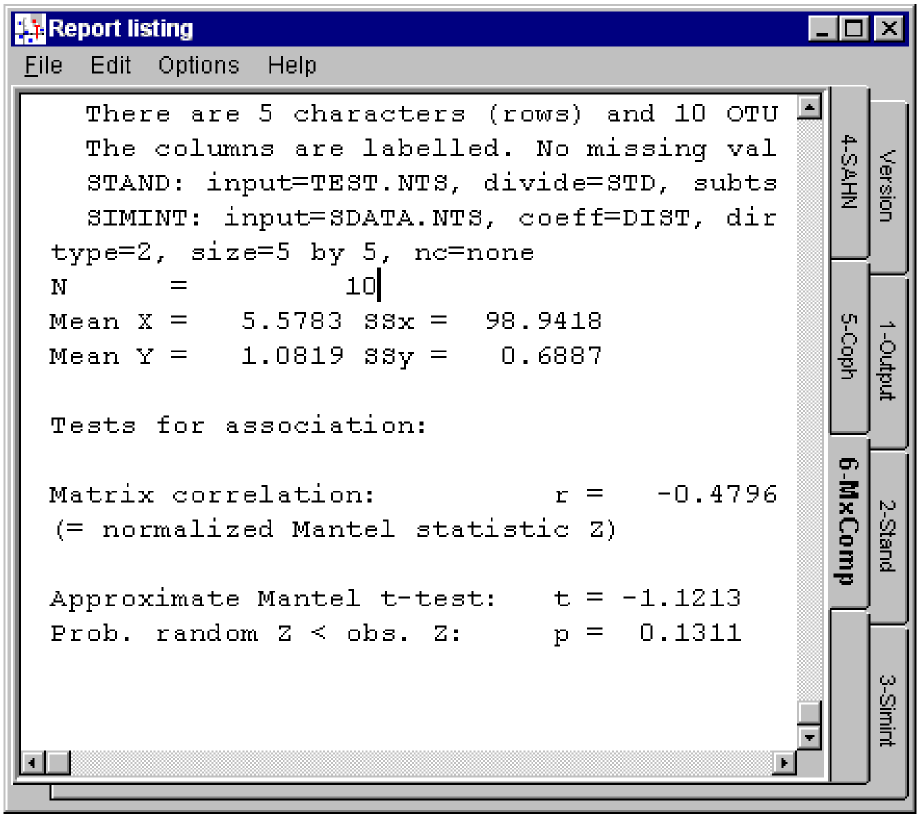
Note: the configuration parameters can also be changed through commands in a batch file. Use CONFIG as if it were a module and use the "batch codes" given above to change the values of the parameters. as the following:

```
config LF=9.6f
```

3.3 Output Listing Window

This window uses a notebook metaphor to display listing output from the computational modules. Each time a module is run a new section is created with an index tab numbered in sequence and labeled by the name of the module. An example is shown in Figure 3.1. A section can be examined by clicking on a tab and then moving the scrollbars. Note that the entire window can be resized.

The File menu provides a number of important operations. The entire “notebook” can be reloaded from a previous run, saved to an ASCII file, cleared (i.e., deleted), or printed. Alternatively, the currently displayed section of the notebook can be saved to an ASCII file, deleted, or printed.



```
   There are 5 characters (rows) and 10 OTU
   The columns are labelled. No missing val
   STAND: input=TEST.NTS, divide=STD, subts
   SIMINT: input=SDATA.NTS, coeff=DIST, dir
type=2, size=5 by 5, nc=none
N       =           10
Mean X =    5.5783 SSx =   98.9418
Mean Y =    1.0819 SSy =    0.6887

Tests for association:

Matrix correlation:            r =   -0.4796
(= normalized Mantel statistic Z)

Approximate Mantel t-test:     t = -1.1213
Prob. random Z < obs. Z:       p =  0.1311
```

Figure 3.1. Example of the listing window after running the commands in the test.ntb batch file.

The Edit menu provides commands to select all the text in the current section, to cut selected text to the Windows clipboard, to copy selected text to the clipboard, and to paste in text from the clipboard. These commands permit you to copy results into other software such as a wordprocessor. They also allow you to delete unwanted information before printing or saving to a disk file. Keeping such notebooks is a convenient way to verify which options were used to produce a certain result. The purpose of reloading a notebook is to allow the appending of new results so that a record of all the computations for a particular project can be kept together. The file format is simply an ASCII file with the form feed character separating sections.

The Options menu allows you to change the font used both for the on-screen display and for printing. The indent item controls the size of the left margin when printing.

The Help menu provides the standard contents, topic search, and about items.

4. Preparation of input data files

NTSYSpc data files are ordinary ASCII files (txt files, not binary files). A file for a data matrix may be prepared with an editor or any word processor that has a txt (non-document) mode. If you try to use a document file there may be invisible binary codes that NTSYSpc will not know how to interpret. Free-format is used for the entries in the data matrices. This means that at least one blank space or a comma is required between numbers. The NTedit program included with NTSYSpc can be used to prepare data files.

4.1 File formats

A matrix can contain 4 kinds of records. The comment and label lines are optional.

Comments These optional lines are used to include notes with the data. The first character in each line must be some type of quote character or . The information on these lines will be copied onto comment lines in any matrices based on this input matrix. In addition, each subsequent program will add an additional comment line so that the sequence of steps leading to a given matrix can be determined.

Matrix parameter line This line contains 4 integer numbers (The second and third may have a suffix letter to indicate the presence and location of row and column labels) and possibly a floating point number. They must be separated by at least one blank space.

- The first number is a code for the type of matrix.

  1 rectangular data matrix,

  2 symmetric dissimilarity matrix,

  3 symmetric similarity matrix,

4 diagonal matrix,

5 tree matrix for dissimilarity data,

6 tree matrix for similarity data,

7 graph matrix for dissimilarity data, and

8 graph matrix for similarity data.

- The second and third numbers are the numbers of rows and columns in the matrix. If labels are to be furnished for either the rows or columns (or both) then a letter must be entered right after the number (with no spaces in between). An "L" is used to indicate the presence of a list of labels in a separate record placed before the data. For example, "25L" means that there are 25 rows and labels are furnished in a separate record. A lower case "l" can also be used—but this is less desirable since it looks so similar to the number "1". The letter "B" is used to indicate that row labels are placed as the first item in each row and "E" indicates that the row labels are placed after the end of each row.
- The fourth number is 0 if there are no missing data in the matrix. If there are missing data then the fourth number should be a "1" followed by at least one blank and then the numerical code used to denote the missing values—999 is a popular choice.

Row and column labels Labels must be furnished if a "B", "E", or "L" is placed after the numbers of rows or and "L" after the number of columns in the previous line. Row labels can be placed in one of three locations: as the first element at the beginning of each row (B), as the last element at the end of each row (E), or as a separate list of row labels in front of the matrix (L). The column labels if present always consist of a list of labels with the first label beginning on a new line. Each label consists of strings of characters (up to 16 letters or digits but no blanks). They are separated by one or more blanks or by a comma, *i.e.*, the are entered free format. Examples are given below.

Matrix data lines The elements of the matrix are entered with rows in the input matrix corresponding to one or more lines in the input file (*i.e.*, matrices are always entered rowwise). Symmetric matrices are entered as rows beginning with column 1 and ending with the diagonal elements (*i.e.*, the lower half matrix with diagonals is entered rowwise). If all the elements for a row do not fit on a single line, then continue typing on as many new lines as needed. It is important that the first element of a new row starts on a new line—even if the previous line is mostly empty. The elements themselves are free format. Values must be separated by one or more blanks or a comma.

The lines can be very long (the theoretical limit is 2 GB!)—but it will be easier to work with them with most editors if you use shorter lines (80 characters or fewer). Blank lines are ignored.

More than one matrix can be stored in a single file. The records for a second matrix (starting with the optional comment lines) simply follow after those for the first. Most program modules in NTSYSpc will perform the selected set of operations on *each* of the matrices in an input file. The results for the second and subsequent matrices are simply appended to the files produced by processing the first matrix. For some programs it is necessary to put more than one matrix in a single file in order to perform a certain computation. It is required by programs such as CPCA, CVA, and POOLVCV. It is also necessary in order to compute the majority rule consensus tree for more than two trees.

Note: if you prepare the original data matrix so that the rows correspond to the characters (variables) and the columns correspond to the objects being classified (OTUs, data points, *etc.*), then you will find that default row/column direction options in most of the modules will be correct.

Since there is always the chance that there will be an error in the preparation of a data matrix, it is strongly suggested that you use the NTedit program and that you first try the OUTPUT module to display your input data matrix. It can be printed out for convenience in proofing.

4.2 Examples of data files

An example of a data matrix file with 3 comment lines and labels for the columns but not the rows is given below. This set of test data is furnished on the distribution disks for NTSYSpc and is used for many of the examples given in this manual.

```
"A sample data matrix to test NTSYSpc
"There are 5 characters (rows) and 10 OTUs (columns)
"The columns are labeled. No missing values.
1 5 10L 0
A B C D E F G H I J
  8   7   9  13   6  12   9   7  11  6
  5   6   3   3   7   10  5   7   5  7
 11  13  12   9   8  18  17  21  22  13
 10  18  22   8   7  17  18  26  24  18
 11   6  12  10  10  19  13  13  19  14
```

An example of a data matrix with column labels and with row labels placed at the beginning of each row:

```
1 4B 3L 0
c1  c2  c3
r1  1,  3,  4
r2  3,  2,  1
```

```
r3  3,  4,  2
r4  2,  1,  2
```

An example of a symmetrical correlation matrix file (note that elements past the diagonal of a symmetric matrix must not be entered). Labels can only be placed in a list in front of the data (i.e., only the “L” code is valid).

```
"A sample correlation matrix with labels
3 5L 5  0
A B C F E
1
0.4 1
0.3 0.4 1
0.6 0.3 0.4 1
0.7 0.3 0.4 0.5 1
```

In this case the “L″ can be appended to either the number of rows, the number of columns, or to both. But only one set of labels should be furnished. If a symmetric matrix is the output of some other program it may be stored as a full square matrix. In that case you should code it as a rectangular matrix and use the SYMD or SYMS options of the TRANSF program to convert it to the lower half matrix form required by NTSYSpc.

Tree matrices (matrix types 5 and 6) are usually produced by programs rather than entered by a user. The usual exception is when one wishes to enter an expected tree to compare with the observed results (using the CONSEN program). There are two styles in which a tree can be entered in NTSYSpc. The format used internally in NTSYSpc is described at the end of the description of the SAHN program.

In addition, you can describe a tree using nested parentheses as in the NEXUS format used, for example, in the program PAUP. This option is only available for tree matrices based on dissimilarities (matrix type code = 5). While complete NEXUS files cannot be read, the tree descriptions can be processed as long as the OTUs names are given as integer numbers (corresponding to their position in a data matrix). This format is provided to enable trees produced by other programs to be entered into NTSYSpc more easily. One can also enter trees by hand using this notation -- but it becomes awkward for large trees since it is easy to miscount parentheses.

In this format nesting is indicated by parentheses, branch lengths (which are optional) are given in the format ":value" after each OTU name and right parenthesis, and the end of the tree is indicated by a semicolon. If branch lengths are not provided then NTSYSpc will generate arbitrary clustering levels consistent with the set relationships given in the tree. Note that one must either provide branch lengths for all branches or else for none of them. A mixture will produce unpredictable results.

Example of a NEXUS style tree not using branch lengths:

```
" NEXUS style input with OTU labels provided.
5 5L 2 0
```

```
A B C D E
(((1,3),2),(4,5));
```

This implies a tree of the following topology:

```
1--.
3----.
2----L---.
4--.     |
5--L-----L---
```

Example of an input file using branch lengths:

```
" Example using branch lengths but no OTU labels.
5 5 2 0
(((1:2.1,3:2.5):1.6,2:3.3):0.7,(4:0.5,5:0.3):0.9);
```

This tree has the same topology as in the previous example. It should be noted, as in the above example, that the branch lengths may be inconsistent with the levels (heights) used to describe an ultrametric tree. In the above example the branch length for OTU 1 is 2.1 but the length for OTU 3 is 2.5. The program will use the average (2.4) of these values. An additional problem is that the raw average of heights of each interior node may not increase as one goes towards the root. In the above example the height at which the set {1,3,2} joins the root is 4.3 and the height at which {4,5} joins is 1.3. The average of these two values is 2.8 which is smaller than the level at which {2} joined {1,3}. The program constrains the average heights to be at least 0.0001 greater than the largest height within the sets being joined. This preserves the topology indicated by the parentheses but shows the trees graphically as looking as if there was a multifurcation.

4.3 Interface to other programs

Since the matrix files have a simple format (see the previous section), they should be usable by other programs with very few changes needed. Results from other programs should also be convertible into the format described above. The largest problems are apt to be due to different conventions for furnishing labels and for reading symmetric matrices.

Provision is included in the NTedit program for the reading of rectangular matrices from "worksheet" files compatible with Excel. These files must have an extension of “XLS.” In order import these files the Excel program itself must be present since NTSYSpc uses techniques called DDE and OLE to have Excel actually read the file and then pass the information to NTSYSpc. NTSYSpc will search the spreadsheet for the matrix by starting with cell A1. That cell and the 3 cells to its right are taken as the matrix parameter line. The first cell is the matrix type (which must be a "1"). The two cells to the right (B1 and C1) must be the number of rows and then the number of columns. Note: these must be integer numbers. Do *not* try to append a code to indicate the presence of row or column labels. Cell D1 contains the missing value code. Leave it blank if there are no missing values. The next row contains column labels beginning with cell B2. If left blank, NTSYSpc will simply number the columns. The column beginning with cell A3 contains the row labels. If blank then NTSYSpc will simply number the rows. Thus row and column labels are in their natural position—**not** as records in front of the matrix as in as in the “L” option. The matrix itself begins in cell B3. If empty cells or labels are found within the matrix, they are assumed to correspond to missing values. Regions in the spreadsheet beyond the matrix are ignored and can be used to store other information. Only one matrix can be read from each spreadsheet.

5. NTedit

The Ntedit program, included with NTSYSpc, is a data editor designed for use with NTSYSpc data files. It recognizes (actually *requires*) valid NTSYSpc data files. For each of the basic file formats (rectangular, symmetric, diagonal, tree, and graph) it displays an appropriate arrangement of the cells in the spreadsheet. Using NTedit ensures that the files are formatted correctly.

The program can be started in three ways.

1. Click on the program icon to start the program and then use the File|Open menu to load an existing data file or File|New to begin a new file.
2. Load this program from the File|Edit data file menu of NTSYSpc.
3. You can use a DOS command window and type ntedit and the name of a file and then press the key to start the program.

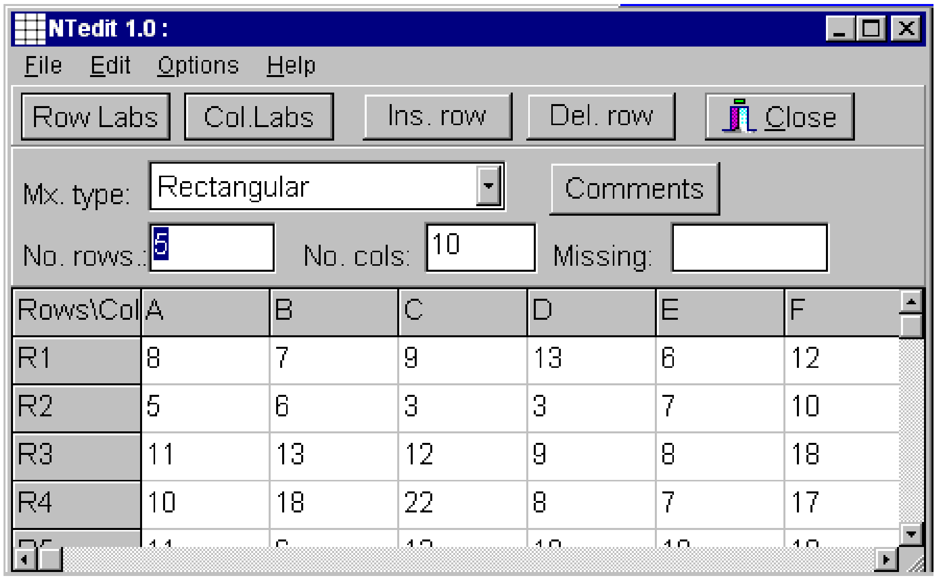
Once the program is started you can enter or correct data in any of the cells of the spreadsheet. You can insert or delete rows and columns within the table by clicking on the appropriate menu choices or the speed buttons on the tool bar. You can also add or delete rows and columns from the end of the table by entering new values in the edit boxes displaying the current numbers of rows and columns. To change the labels for the rows or columns (given in the first, protected, row or column of the data table) click on the RowLabs or Col.Labs buttons to unprotect these entries. You can then type new information in these cells. The new names should not have any blanks within them. Click these buttons again to re-protect these labels from accidental change.

To create a new file use the following steps:

1. select “New” from the file menu,
2. select the proper matrix type from the list (you may receive a warning about the possible loss of data when you change matrix types),
3. enter the correct numbers of rows and columns in the edit boxes labeled “No. rows” and “No. cols.” (note that the new values do not take effect until your cursor *leaves* the edit boxes), and then

4. start entering your data.

If there are missing data the identifying numerical code needs to be entered in the edit box labeled “Missing.” Click on the “Comments” button if you wish to add comments to the matrix. When you are done you can use the “Check matrix” item under the Edit menu to check that all data values are properly formatted numbers. It also will check to make sure there are no empty cells. This same check is made when to attempt to save the matrix to a disk file. You will be given a chance to replace all the empty cells with whatever code you specified for missing data (if that field is blank then zeroes will be used).



Mx. type: Rectangular

No. rows: 5

No. cols: 10

Missing:

Rows\Col	A	B	C	D	E	F
R1	8	7	9	13	6	12
R2	5	6	3	3	7	10
R3	11	13	12	9	8	18
R4	10	18	22	8	7	17

Figure 5.1. Example of NTedit with the test.nts file loaded.

NTedit can also be used to view and make changes in existing files. A limitation of the program is that existing files must already be in a proper format. If you try to load a file that is not formatted properly you will receive an error message and then an alternative editor (the default is the Windows notepad program) will be called to display the file. The “Alt. Editor” item under the Options menu allows you to select another editor. Note: you must select an editor capable of producing plain ASCII (txt) files.

The NTedit help can be consulted for additional information.

6. Graphics options & menu

The various plots produced by NTSYSpc can be enhanced in many ways by taking advantage of the many options available.

Begin by clicking on a plot with the right mouse button or by selecting the “Plot options” item on the Options menu above the plot. The options available depend upon the type of plot. All plots allow the user to specify a title and a subtitle and the fonts used to display them. There is also always a button labeled “General” that opens another dialog box described in the next section.

6.1 General plot options

The following options (listed by group) are available for all plots.

General: “Preserve axis aspect” means to preserve the aspect of the x and y axes with respect to the original units of measurements. This must be kept checked for the 3D and Tree plots. For 2D scatter plots it should be checked when plotting the results of analyses such as principal components analysis where the relative lengths of the axes is important. One will usually not want it checked when plotting raw data. “Center” controls whether the plot is centered in the window.

Frame: Optionally, a line can be drawn around the outside of the plot to frame it. Options are available to control its size and color.

Background color: The background color in the different regions of a plot can be set individually.

Margin size: Top, bottom, left, and right margin sizes can be set.

Legend: This group is not used at present but will be used to control how groups of points or lines are identified.

6.2 Other options

These depend upon the plot. For MXPLOT and MOD3D there are pick lists for selecting the variables to be plotted. There are also choices of whether the data points should be identified by sequential numbers or labeled using the labels in the input data. There are also options to control the various attributes of the points and lines making up a plot. There are special dialog boxes to allow you to select colors, plotting symbols, fonts, *etc.* In MOD3D the view options allow one to rotate, tilt, and change the distance of the viewing position.

6.3 Plot menu

The File menu contains the following items: “Printer setup” (which allows you to select a printer, paper size, and orientation), “Print preview” (which changes the plot to a preview of how it will look when printed), “Print” (to print the plot), and “Close” (to close the plot window). The Edit menu allows one to copy the current plot to the Windows clipboard. You can then paste it as a bitmap into a word processor or paint program. The Help menu leads to the standard Contents, Topic search, and About items.

7. Typical applications

Furnished below are some examples of typical applications of NTSYSpc. For simplicity, the required steps are shown as sequences of batch command statements. This is a compact way to describe the sequence of modules and their parameters. See the help file for more detailed information about each module.

Note lines that begin with a quote character are treated as *comment lines* and are ignored by NTSYSpc.

7.1 Cluster analysis

Perhaps the most common use of NTSYSpc is for performing various types of agglomerative cluster analysis of some type of similarity or dissimilarity matrix. The following is an example of a batch file that will standardize a data matrix, in file `data`, compute distance coefficients among the columns of the standardized data matrix (there are several other choices of coefficients), cluster the distance matrix using the single-link clustering method (there are other choices, such as UPGMA), compute a cophenetic-value (ultrametric) matrix, compute the cophenetic correlation as a measure of goodness of fit, and then plot the results in the form of a phenogram. The distance matrix is also output.

```
" Standardize the variables
*stand o=data.nts r=sdata.nts
" Compute a distance matrix
*simint o=sdata.nts r=dist.nts c=dist
" Do a single-link cluster analysis of the distance matrix
*sahn o=dist.nts r=tree.nts cm=single
" Display phenogram
*tree o=tree.nts
" Compute cophenetic values
*coph o=tree.nts r=coph.nts
" Compute the cophenetic correlation
*mxcomp x=coph.nts y=dist.nts
```

When working interactively, one can view the tree from within the SAHN module by clicking on the plot speed button. Note that the Mantel test results displayed by the MXCOMP module should be ignored since the two matrices being compared are not independently derived.

7.2 Ordination analyses and biplots

In ordination analyses the goal is to position points along coordinate axes in a low dimensional space (rather than to form sets of points as in cluster analysis). There are many different methods depending upon the criteria used to define what is meant by the "best" low-dimensional representation of the relationships among the points. Several programs in NTSYSpc can be used to perform these analyses.

When an original data matrix is available it is possible, and usually desirable, to make plots of both the variables and the points with respect to the same axes. This is called a "biplot". This allows one to not only see the patterns, trends, *etc.* among the points and of relationships (usually correlation) among the variables, but also the relationships between the points and the variables — at least to the extent that they can be summarized in a few dimensions. Unfortunately, there seems to be no strong consensus about how to scale the two ordinations relative to one another. Gabriel (1968, 1971, 1981) defines a biplot of an n×p matrix **Y** as a simultaneous bivariate plot of the *n* points in each column of a matrix **A** and of the *p* variables in each column of matrix **B**, where $\mathbf{Y}=\mathbf{AB}^t$ (it would be a bimodel if a 3-dimensional plot were made). Matrices **A** and **B** can be expressed in terms of a singular-value decomposition of matrix **Y**: $\mathbf{Y}=\mathbf{U}\Lambda\mathbf{V}^t$. One could set $\mathbf{A}=\mathbf{U}\Lambda$ and $\mathbf{B}=\mathbf{V}$ (called a JK biplot by Gabriel and Odoroff, 1986). In terms of principal components analysis, this corresponds to computing normalized eigenvectors from the correlation or variance-covariance matrix for the variables and then using the PROJ program to project the points onto these vectors. The rows of **A** are plotted as points and the rows of **B** are plotted as vectors. Note that the matrix Λ of singular values is the square root of the eigenvalue matrix obtained in a principal components analysis. This type of decomposition of a data matrix is called preference scaling or repertory grid analysis in psychology and sociology.

One can equally well set $A=\mathbf{U}$ and $B=\mathbf{V}\Lambda$ (GH biplot of Gabriel and Odoroff, 1986). One could also multiply both **U** and **V** by $\Lambda^{0.5}$ (an SQ biplot). This latter choice would seem most appropriate in correspondence analysis where both the rows and columns can be interpreted as variables The matrix Λ could also be apportioned in any other way to **U** and **V** as long as their product yields **Y**.

A problem with a JK biplot is that the vectors for the variables are of unit length and thus are not in the same scale as the vectors for the points. Since the vectors for the variables are dimensionless a plot of them does not indicate how well the variance of each variable is explained by the number of dimensions used. For this reason one can deviate from a true biplot and multiply *both* **U** and **V** by Λ. As a consequence, one cannot visually estimate an element, say y_{ij}, of the original data matrix as simply the inner product of row *i* of **A** and row *j* of **B**, one must compute the projection of row *i* of **A** onto row *j* of **B**. One compares the relationships between the ordinations of the points and of the variables by examining the angles between them. Jackson (1991) indicates that this alternative is popular "among French practitioners."

7.3 Principal components analysis

Principal components analysis, PCA, is one of the most important methods of ordination analysis. It constructs a new set of orthogonal coordinate axes such that the projection of points onto them have maximum variance in as few dimensions as possible. While defined in terms of variances and covariances, PCA is usually applied to standardized data since the results are sensitive to the choices of units of measurement and this is arbitrary in most studies.

The following batch file will standardize a data matrix by rows, compute a matrix of correlations among the variables (assumes rows), extract 3 eigenvectors from the correlation matrix, project the standardized data onto these eigenvectors, and then make a 3-dimensional plot of the objects. Various matrices are also output to files.

```
"  Standardize variables (rows)
*stand o=data.nts r=sdata.nts
"  Compute correlations among variables (rows)
*simint o=sdata.nts c=corr r=corr.nts d=row
"  Output the correlation matrix
*output o=corr.nts
"  Extract first 3 PCA axes from correlation matrix
*eigen o=corr.nts n=3 r=vect.nts val=val.nts
"  Output principal component axes
*output o=vect.nts
"  Project objects onto PCA axes
*proj o=sdata.nts d=col f=vect.nts r=proj.nts
"  Output projections
*output o=proj.nts
"  Display 3D plot of projection of objects
*mod3d o=proj.nts
"  Display 3D plot of variables defining the PCA axes
*mod3d o=vect.nts d=col
```

The last two plots together comprise a three-dimensional biplot (a “bimodel”) for these data.

An alternative procedure would be to not standardize the data and to use a variance-covariance matrix rather than a correlation matrix in the above steps. In such a case, the largest weights are given to those variables with the largest variances. This implies that the variables were measured in comparable units of measurement. This might be appropriate, for example, for a matrix of log-transformed variables in a morphometric study (perhaps with means subtracted following Darroch and Mosimann, 1985).

7.4 Principal coordinates analysis, PCOORDA

PCOORDA can be thought of as a computational alternative to PCA. The steps shown below will give results identical to PCA. One important consideration is that when there are many fewer points than variables computation time may be much less than for the usual PCA.

The batch file given below performs the following operations: the data matrix is standardized by variables (rows), a matrix of distances between the objects is computed, the distance matrix is double-centered, the double-centered matrix is then factored and a plot is made showing the objects in a 3-dimensional space.

```
"  standardize data if in different units
*stand o=data.nts r=sdata.nts
"  Compute distances among objects
*simint o=sdata.nts r=dist.nts
"  Double-center the distance matrix
*dcenter o=dist.nts r=dcent.nts
"  eigenvectors correspond to projections of objects
*eigen o=dcent.nts n=3 r=proj.nts
*output o=proj.nts
"  Display -- Note that direction is "col"
*mod3d o=proj.nts d=col
```

PCOORDA can also be viewed as a distinct ordination method since it can also be applied to various types of similarity and dissimilarity matrices - or even to experimentally determined proximity matrices where there is no original “data matrix.” The computational steps would then be as follows:

```
"  Double-center the matrix
*dcenter o=dist.nts r=dcent.nts
"  Extract eigenvectors
*eigen o=dcent.nts n=3 r=proj.nts
"  Output eigenvectors = projections
*output o=proj.nts
"  Display -- Note that direction is "col"
*mod3d o=proj.nts d=col
```

Of course, an arbitrary dissimilarity matrix may not be very compatible with a Euclidean metric. In such cases many of the eigenvalues may be negative. In performing such an analysis one hopes that such negative eigenvalues are small and can be ignored.

7.5 Nonmetric multidimensional scaling

This method is similar to PCOORDA in that it can be used to represent the relationships among a set of points in a low dimensional space. The difference is that in non-metric multidimensional scaling analysis the distances among the points in the final configuration need only have a monotone relationship to the distances implied by the original data matrix. This relaxed constraint usually makes it possible to get a much better fit in fewer dimensions than is possible in PCOORDA.

If possible, one begins with the results of a PCOORDA as an initial configuration since this usually results in many fewer iterations being necessary in the MDSCALE module.

```
"  Use PCOORDA to obtain an initial configuration
```

```
*DCENTER O=dist.nts R=dcent.nts
*EIGEN O=dcent.nts N=2 R=init.nts
"  non-metric MDSCALE using initial solution
*MDSCALE O=dist.nts N=2 I=init.nts R=final.nts
"  rotate result for ease in viewing
*SIMINT O=final.nts C=varcov R=vcv.nts
*EIGEN O=vcv.nts N=2 R=vect.nts
*PROJ O=final.nts D=row F=vect.nts R=result.nts
" plot the final rotated configuration
*MXPLOT O=result.nts
```

When viewing the plot be sure to set the option “Preserve axes aspect.”

7.6 Burnaby's method for size adjustment

The following batch file shows an example of how the ORTH option of the PROJ program can be used for Burnaby's method to remove the effect of a vector from a data set. The data are projected onto the hyperplane orthogonal to the specified vector. In the example given below the first principal component axis is used as "size". Other vectors such as the isometric vector (1,1,...,1) could also be used.

```
"  compute VCV matrix from a data matrix
*simint o=data.nts c=varcov r=vcv.nts d=row
"  compute first principal component
*eigen o=vcv.nts n=1 r=pc1.nts
"  project data onto hyperplane normal to PC1
*proj o=data.nts f=pc1.nts pt=orth r=bproj.nts
```

The resultant adjusted data matrix bproj.nts can then be used, for example, to compute a distance matrix which is then clustered by SAHN. The clusters should then not be influenced by variation in the original data set that was parallel to the first principal component—which is often mostly due to size. The first principal component points in the direction in which there is the most variation. If the organisms sampled happen to be about the same size, then this vector is apt to represent sexual differences, polymorphisms, *etc.* In many cases it may be safer to use an *a priori* defined isometric vector as a size vector (i.e., the vector 1,1,1,1…,1) or to use the first principal component based only on a carefully selected subset of variables.

The adjusted data matrix could also be used as input for a canonical variates analysis or for the computation of size-free generalized distances (see the CVA module).

7.7 Comparison of matrices

Often one wishes to test whether one set of relationships among a set of objects is independent of another. For example one may wish to test whether the degree of morphological difference between samples is related to the geographical distances between the sampled populations (see, for example, Sokal, 1979). A simple way to do this is by the use of the Mantel test (Mantel, 1967). The test assumes that the two matrices have been obtained independently—one cannot use it to test two matrices where one has been derived from the other. The steps given below assume that one already has a matrix of geographical distance, `gdist`.nts.

```
" Compute morphological dissimilarity matrix
*simint o=data.nts c=dist r=mdist.nts d=row
" Compare mdist with gdist, 250 random permutations
*mxcomp x=mdist.nts y=gdist.nts np=250
```

While less efficient than a specialized program, one can use the above steps to perform spatial autocorrelation analyses. The only modification needed to the above steps is to replace the geographical distance matrix in file `gdist`.nts with a series of matrices corresponding to a desired geographical distance class. In each matrix an entry is `1` if objects *i* and *j* are within the desired distance class and is `0` otherwise. To make a distance correlogram one simply plots the matrix correlations as a function of geographical distance. The Mantel test can be used to determine which coefficients are statistically different from zero.

Bibliography

Burnaby, T. P. 1966. Growth-invariant discriminant functions and generalized distances. Biometrics, 22:96-110.

Darroch, J. N. and J. E. Mosimann. 1985. Canonical and principal components of shape. Biometrika, 72:241-252.

Everitt, B. S. and Dunn, G. 1992. Applied multivariate data analysis. Oxford Univ. Press: New York. 304 pp.

Gabriel, K. R. 1968. The biplot graphical display of matrices with application to principal component analysis. Biometrika, 58:453-467.

Gabriel, K. R. 1971. The biplot graphical display of matrices with applicatin to principal componenet analysis. Biometrika, 58:453-467.

Gabriel, K. 1981. Biplot display of multivariate matrices for inspection of data and diagnosis. P. 147-173 in Barnett, V. (ed.) Interpreting Multivariate Data. John Wiley and Sons, New York.

Gabriel, K. and Odoroff, C. L. 1986. Illustrations of model diagnosis by means of three-dimensional biplots. Pp. 257-274 in Wegman, E.J. and DePriest, D.J. (eds.). Statistical image processing and graphics, Marcel Dekker, New York.

Gnanadesikan, R. 1977. Methods for statistical data analysis of multivariate observations. Wiley. New York. 311 pp.

Hartigan, J. A. 1975. Clustering algorithms. Wiley. New York. 351 pp.

Jackson, J. E. 1991. A user’s guide to principal components. Wiley: New York. 569 pp.

Mantel, N. A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27:209-220.

Reyment, R. A. 1991. Multidimensional paleobiology. Pergamon Press: New York, 377 pp.

Romesburg, H. C. 1984. Cluster analysis for researchers. Lifetime Learning Publications. Belmont, California. 334 pp.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4:406-425.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical Taxonomy. Freeman. San Francisco. 573 pp.

Sokal, R. R. 1979. Testing statistical significance of geographic variation patterns. Systematic Zool., 28:227-231.

Sokal, R. R. and P. H. A. Sneath. 1963. Principles of Numerical Taxonomy. Freeman. San Francisco. 359 pp.

Weir, B. S. 1989. Building trees with DNA sequences. Biometric Bulletin, 6(4):21-23.

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