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RH: NEXUS FILE FORMAT

NEXUS: AN EXTENSIBLE FILE FORMAT FOR SYSTEMATIC INFORMATION

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³ Department of Ecology and Evolutionary Biology University of Arizona, Tucson, Arizona 85721, USA Proofs to: David Maddison Department of Entomology University of Arizona Tucson, AZ 85721 (520) 621-9781 beetle@ag.arizona.edu [email address can be published] *ABSTRACT.*— NEXUS is a file format designed to contain systematic data for use by computer programs. The goals of the format are to allow future expansion, to include diverse kinds of information, to be independent of particular computer operating systems, and to be easily processed by a program. To this end, the format is modular, with a file consisting of separate blocks, each containing one particular kind of information, and consisting of standardized commands. Public blocks (those containing information utilized by several programs) house information about taxa, morphological and molecular characters, distances, genetic codes, assumptions, sets, trees, etc.; private blocks contain information of relevance to single programs. A detailed description of commands in public blocks is given. Guidelines are provided for reading and writing NEXUS files and for extending the format. [File format; systematics; computer program; NEXUS.] NEXUS is a file format designed to house systematic data. Although it is currently in use by several computer programs (including MacClade 3 [Maddison and Maddison, 1992], PAUP 3 [Swofford, 1989] and the forthcoming PAUP* 4, COMPONENT 2 [Page, 1993], SplitsTree [Huson and Wetzel, 1994], and Genetic Data Analysis [Lewis and Zaykin, 1996]), the file format, and the principles underlying its design and governing future expansion, have never been formally described. This document provides the description of the file format.

This paper serves several purposes. First, we seek to promote our goal of a common file format to be shared by phylogenetic computer programs. Virtually every new computer program in systematic biology has used a unique file format. Unique file formats are easier for the programmer, as they contain no information extraneous to the particular program, but they often transfer to users the task of reformatting their files for use with different programs. Second, we hope to convey the general principles of NEXUS files, so that programmers and users may better understand them. Third, we will describe the technical details of the file format, and give guidelines for extending it, so that those writing computer programs that read and write NEXUS files can abide by the standard. If our goal of a shared file format is to be reached, it is important that the standard be followed faithfully, and that those inventing extensions to the format do so in such a way as to maintain ease of shared use.

Modularity is the primary design feature of a NEXUS file. A NEXUS file is composed of a number of blocks, such as TAXA, CHARACTERS, and TREES blocks. Following is a NEXUS file containing a short DNA sequence for each of four taxa, as well as a single tree:

```
#NEXUS
BEGIN TAXA;
   DIMENSIONS NTAX=4;
   TAXLABELS fish frog snake mouse;
END;
BEGIN CHARACTERS;
   DIMENSIONS NCHAR=20;
   FORMAT DATATYPE=DNA;
   MATRIX
      fish ACATA GAGGG TACCT CTAAG
      frog ACTTA GAGGC TACCT CTACG
      snake ACTCA CTGGG TACCT TTGCG
      mouse ACTCA GACGG TACCT TTGCG;
END;
BEGIN TREES;
   TREE best=(fish, (frog, (snake, mouse)));
END;
```

Other blocks can be added to the file to house various kinds of data, including discrete

and continuous morphological characters, distance data, frequency data, as well as information about protein coding regions, genetic codes, assumptions about weights, trees, and so on. Images can be included. This modular format allows a computer program reading the file to ignore safely the unfamiliar parts of the file, permits sharing of the file by various programs, as well as future expansion to encompass new information.

HISTORY OF NEXUS FILE FORMAT

The development of NEXUS began in 1987 for joint use by MacClade 3 (Maddison and Maddison, 1992) and PAUP 3 (Swofford, 1989). MacClade is a program for editing systematic data, and for conducting phylogenetic analysis of character evolution. PAUP is a program primarily for inference of phylogenies. During the long gestation period of the two programs, the file format evolved. Most of these changes took place before PAUP 3 was released, although some significant modifications were made in the early 1990s.

Roderic Page adopted NEXUS for version 2 of COMPONENT (Page, 1993). COMPO-NENT is a program for comparing trees, and includes commands for reconciling associated phylogenies (e.g., host and parasite trees). COMPONENT's requirements forced some rethinking of the format, and were the major reason the TAXA block was invented. The general structure of a NEXUS command was improved to allow easy avoidance of foreign commands.

A number of other projects have chosen to house data using the NEXUS file format. Huson and Wetzel (1994) have adopted the NEXUS format for use in SplitsTree, a program that conducts a "split decomposition" analysis (Bandelt and Dress, 1992; Wetzel, 1994). The NEXUS format is used in Genetic Data Analysis (Lewis and Zaykin, 1996), and portions of the TreeBASE system (Donoghue et al., 1996).

The NEXUS format will continue to coevolve with systematics computer programs. Commands will be added to accommodate additional kinds of data and assumptions; within this document, some commands are described that are not yet used by any existing computer program.

GOALS

In designing the NEXUS format, we had four goals:

- Expandability. New elements can be added to the file format with minimum disruption. Programs that were not designed to read these new elements should still be able to acquire as much other information as possible from the file. This goal is achieved by commands of standardized structure organized into labeled blocks, allowing programs to recognize relevant elements and disregard irrelevant ones.
- 2. Inclusivity. The file format ideally encompasses all information a systematist or phylogenetic

biologist might wish to use, including character and distance data, assumptions, trees, and so on.

- 3. Portability. The file format can be used by programs running on many different computer operating systems and hardware. The use of simple text files and the insensitivity to varied newline text characters allow for broad portability.
- 4. Processibility. Programs can easily read the information they desire, and can easily detect and skip other information. A program should be able to tell, if it detects an element in the file it doesn't understand, whether or not that lack of knowledge will be fatal to its reading the file. The uniform definition of words, punctuation, and whitespace and the use of a modular structure enhance processibility.

BASIC ELEMENTS OF A NEXUS FILE

NEXUS files consist of a string of standard text characters. The text characters form words bounded by whitespace and punctuation, and comments. Words in a NEXUS file are equivalent to words in a sentence, whitespace to blank spaces, and punctuation to commas, periods, and the like. (To avoid confusion between the text characters of which a text file is composed and the characters of an organism, we will always use the adjective "text" when referring to the former, or, in the special case of a text character indicating the end of a line, "newline character.") For example, in the following line

BEGIN TREES;

there are two words (BEGIN and TREES), separated by whitespace, and followed by a punctuation mark, and then by whitespace (in this case a newline character, which isn't evident, but which is present in the file). Punctuation marks and words are also called tokens. Comments can be added by enclosing text with brackets:

BEGIN TREES; [some trees follow]

Comments can contain information that might be processed, including commands, but also can safely be skipped.

The first token in a standard NEXUS file must be:

#NEXUS

A program reading a NEXUS file seeks to process, in sequence, the tokens it encounters. For the most part, whitespace, including newline characters, is ignored, with two exceptions: (1) whitespace indicates boundaries between words, (2) in interleaved matrices, newline characters indicate the boundary between data of different taxa (for nontransposed matrices) or characters (for transposed matrices) (see descriptions in the CHARACTERS and DISTANCES blocks). For example, a program reading the following file:

```
#NEXUS
BEGIN TREES;
    TREE best=(fish, (frog, (snake, mouse)));
END;
```

would first encounter the token #NEXUS, then the words BEGIN and TREES, then the semicolon, then the word TREE, and so on, until the last semicolon was found. In total, the program would read 23 tokens.

The tokens in a NEXUS file are organized into commands, which are in turn organized into blocks. Commands follow a simple format: the first token in the command is the command name, which is followed by a series of tokens and whitespace; the command is terminated by a semicolon. Thus, each command is of the form:

```
command-name token1 token2 ...;
```

All elements of a NEXUS file are organized into such commands, except for comments (see Appendix 1) and the #NEXUS token at the start of the file.

Blocks are a series of commands, beginning with a BEGIN command, and ending with an END command. Typically a block holds a particular sort of infomation, such the definition of taxa, or the specification of assumptions. A block has the following basic form:

```
BEGIN block-name;
    command-name token1 ...;
    command-name token1 ...;
    ...
END;
```

In the above example, as in the rest of this article, predefined NEXUS words are shown in small caps, and words to be replaced by relevant tokens are shown in italics.

PUBLIC BLOCKS

Public blocks will be used by a number of current and future programs. All of the blocks defined in this document are considered public. In contrast, private blocks might be designed to contain information that would be relevant only for a single program. The MACCLADE block and PAUP block are two examples of private blocks.

The eight primary public blocks are TAXA, CHARACTERS, UNALIGNED, DISTANCES, SETS, ASSUMPTIONS, CODONS, TREES, and NOTES. The TAXA block defines taxa, and gives them names. The block also establishes the order (numbering) of the taxa. The CHARACTERS block contains information about discrete and continuous data, including that for morphological structure and molecular sequences. Polymorphisms and frequency data can be accommodated. Names can be given to the characters and their states. The UNALIGNED block is similar to a CHARACTERS block, but contains unaligned molecular sequence data. The SETS block contains descriptions of collections of objects. These objects include characters (so that a collection of characters can be referred to and manipulated easily), taxa (perhaps to allow enforcing monophyly of a group of taxa, or to remove them quickly from the tree), trees (perhaps to select quickly a set of trees for study), states, and kinds of changes (perhaps to allow study of hydrophobic to hydrophobic changes in a protein). In addition, partitions of characters, taxa, and trees can be formed. The ASSUMPTIONS block contains assumptions about the data. This can include assignment of weights to various characters, specification of the nature of character changes (for example, the costs of state to state changes for parsimony analysis), exclusion of particular characters, and designation of ancestral states. The CODONS block designates the sites in nucleotide sequence data that are protein coding, the position within a codon of each site, as well as assigning a genetic code to molecular sequence data. Custom genetic codes can also be defined within this block. The NOTES block allows one to attach additional information (text, pictures, etc.) to various objects (trees, taxa, characters, etc.) in the file. Full details of the commands available in each public block are provided in the Technical Description.

BLOCKS IN TYPICAL FILES

The blocks encountered in a typical NEXUS files will vary, depending upon the purpose of the file. Three objects commonly defined in NEXUS files are taxa, characters, and trees, defined in blocks of the same name. Taxa consist of the entities (biological species, haplotypes, manuscripts, etc.) whose attributes might be recorded in characters, and whose relationships are described in trees. Most programs will therefore require blocks defining or describing one or more of these classes of objects. A simple data file may contain TAXA and CHARACTERS blocks; a tree file TAXA and TREES blocks. Additional public blocks (e.g., ASSUMPTIONS, SETS) and private blocks (e.g., MACCLADE, PAUP) will often be present.

READING AND WRITING NEXUS FILES

Although the NEXUS format contains many elements, some of them complex, it is easier to use than it may at first appear, largely due to its consistent structure and modular approach. Here we give some suggestions for reading and writing NEXUS files. If a program adopts the NEXUS format, it not only gains compatibility with other computer programs, but also the modular format allows easier future expansion of the program's own capabilities.

Reading NEXUS Files

Because NEXUS files are organized into tokens (words and punctuation), along with whitespace and comments, the most basic component of the code to read a file is a routine that returns the next punctuation or word in a file. This routine needs to recognize boundaries between NEXUS tokens and to be able to distinguish words from punctuation (Appendix 2).

The first token after the #NEXUS word or after the semicolon that terminates a command is the name of a NEXUS command. This command will indicate either the start or end of a block, or a command within a block. Routines need to be written to handle the commands and blocks of relevance to the program, and to skip those irrelevant to the program. A routine to handle a command needs to recognize its first token, which indicates what sort of command it is, and subsequent tokens, which specify details of the command. If the first token indicates it is a command that is not relevant to the program, the program skips it by using its token-reading routine to move to the next semicolon. A routine to handle a block needs to recognize the sort of block it is, then process the commands one after another. If the block is not relevant to the program, it is ignored by skipping command after command until the END or ENDBLOCK command is reached.

Programs that read NEXUS files do not have to be able to understand all aspects of the file format; in fact, no program at this time can understand more than perhaps 60% of the elements described in this document. It is thus critical for a program reading a NEXUS file to be able to deal well with elements that are foreign to the program. For example, if MacClade were to encounter a DISTANCES block, it must be able to skip successfully over this foreign block. Individual foreign commands within a block must also be gracefully ignored.

Sample code for reading a NEXUS file is presented on the NEXUS web site, at http://phy-logeny.arizona.edu/nexus/nexus.html.

Writing NEXUS Files

Many programs using the NEXUS format will be designed to read the format, but not to write automatically files of NEXUS format. However, if a program generates NEXUS format files, then these files should conform to the rules of the NEXUS format. For example, elements of the file need to be written as NEXUS words, punctuation, and whitespace: if the user types in the taxon name "Bembidion velox" then the program should write the blank space as an underscore (Bembidion_velox) to form a standard NEXUS token. In addition, the program should not discard information that had been in the file when read, such as unfamiliar blocks or commands. In rewriting the file the program should attempt to place the comments, blocks, and commands intact back into the file, and preferably in the same order in which they were found. If the program cannot restore foreign blocks, commands, or comments, then it should attempt to leave untouched the

original copy of the file.

EXTENDING THE NEXUS FORMAT

Extensions to the NEXUS format should be made with due consideration for the goals of the format (as described under "Goals", above).

- 1. Always think about the future. New blocks and new commands for housing a new class of information should be designed with enough flexibility to contain not just the information needed for one version of one particular program, but additional information that might be needed by future versions or other programs. For example, the CODESET command in the CODONS block was designed to allow one to assign different genetic codes to different taxa in the matrix. No program currently accepts files using this feature, but one can easily imagine some future program, that analyzes evolution of the genetic code, requiring this capability. As the CODESET command requires that genetic codes be given names, the design of a command (GENETICCODE) that is used by current programs to create custom genetic codes was altered to accommodate names, even though these names are not considered by existing programs.
- 2. Information should be placed in public blocks whenever possible. In general, private blocks should be used to store information that probably will not be used by other programs; other information, that may be more generally useful, should be placed in public (e.g., CHARACTERS, ASSUMPTIONS, TREES) blocks. This applies to extensions to the format as well as use of the current format.
- 3. Information contained within a public block should be as uniform as possible. Thus, CHARAC-TERS blocks should contain information about organismal characteristics, not assumptions, and so on. Blocks containing data should contain just one class of data (do not, for instance, invent a new type of block that contains allozyme frequency data, sequence data and geographic data). A future program might want to deal with one of the sorts of information but not the other, and separating them into different blocks will allow the program more easily to find what it needs. This is the rationale behind separating taxon definition and character information into two separate blocks.
- 4. All similar information should reside in one type of block. For example, all tree descriptions should be housed in a TREES block.
- 5. New commands in public blocks can be added, but they must be clearly distinguished from the existing ones. Some programs (e.g., PAUP) allow use of abbreviated command names. Abbreviations for new command names should not duplicate abbreviations for existing command names. The formats of any new commands should follow as closely as possible to those described in this document. New command or subcommand names that are no more than sin-

gular versions of previously existing plural names are not allowed as new command or subcommand names, nor plural versions of existing singular names. Commands or subcommands that differ only in case are homonymous.

- 6. All commands defined in this document are reserved command names, in the sense that they cannot be used with another meaning. (However, subcommands can be added to change the format of those commands.) In addition, the following tokens are reserved for future use for names of blocks: INTERNATIONAL, NETWORK, NODES, COORDINATES, ASSOCIATION, CLASSIFICA-TION, and FORMAT. The following command names are reserved for future use: INCLUDEFILE (anywhere in the file); TITLE and ENTITLE (in any block); SOUND and VIDEO (in the NOTES block); GRAPH (in the TREES block).
- 7. New commands should be designed to follow the format of an existing command, whenever possible. For example, to name and describe some object, the format of an object definition command should be used.
- 8. If a new MATRIX format is devised for a block containing data, this should be indicated by a subcommand in the block's FORMAT command. All of the information necessary to read the matrix should be contained in the DIMENSIONS command and the FORMAT command, and any commands referenced in the FORMAT command (for example, the CHARSTATELABELS command if the FORMAT subcommand TOKENS is used with DATATYPE=STANDARD).
- 9. New command comments should be invented with the same care as normal NEXUS commands. Except in TREE and CSTREE commands, command comments should not contain information that is critical to an analysis.
- 10. All dark characters in a file must be either: (1) #NEXUS at the start of the file, (2) a comment, or(3) a command consisting of NEXUS tokens, terminated by a semicolon.
- 11. Whenever possible, newline characters should have no special meaning in addition to the standard meaning of whitespace.
- 12. Additions to the format should be made in consultation with the authors of this document.

TECHNICAL DESCRIPTION OF NEXUS FORMAT

This description is divided into several parts. First, general issues having to do with computer operating system, file components and structure, objects, and text display are covered. Second, the public blocks are described.

Any particular implementation of the NEXUS format, even by authors of this initial description, should not necessarily be considered as complying exactly with the NEXUS standard.

NEXUS Files and Computer Operating Systems

All NEXUS-compatible programs should be able to read standard text files. On the many computers that use the ASCII character set (American National Standards Institute, 1986), standard ASCII files should be supported (files of text characters composed of ASCII 0-127). Ability to read extended ASCII (text characters 128-255) files is optional. Programs on computers that use character sets other than ASCII should support the native character set.

On operating systems that allow designation of file types (for example, the MacOS[™]), NEXUS files should be designated as type TEXT.

Programs that read NEXUS files should consider the variation in text characters that specify a new line. For example, a program should interpret a single carriage return (ASCII 13), the newline character used on Macintosh computers, as indicating a new line, even if the program runs under the UNIX operating system (which expects new lines to be indicated by a linefeed (ASCII 10)). A NEXUS-compatible program reading a NEXUS file should treat all newline characters (as defined in Appendix 1) as equivalent.

Tokens, words, punctuation, and whitespace

Words, punctuation, and whitespace together form a NEXUS file. Tokens are the words and punctuation of a NEXUS command. Words are separated by either whitespace (blank spaces, tabs, and the like) or by punctuation. Appendix 1 contains formal definitions of these terms.

The following general rules apply to NEXUS tokens. Case is insignificant in NEXUS files except where explicitly stated otherwise (e.g., the EQUATE subcommand in the CHARACTERS block). Abbreviations of command, subcommand, and option words are not allowed (although some programs, such as PAUP, may be lenient in this regard).

File Structure

```
A NEXUS file has the following basic form:
```

```
#NEXUS
BEGIN block-name;
    command-name token1 ...;
    command-name token1 ...;
    ...
END;
BEGIN block-name;
    command-name token1 ...;
    command-name token1 ...;
    command-name token1 ...;
    END;
```

All currently defined commands in a NEXUS file are included within blocks. Any particu-

lar block may contain data, trees, assumptions, and so on. Blocks cannot be nested. Blocks start with the BEGIN command, and end with the END or ENDBLOCK commands. The BEGIN command consists of only two words: BEGIN, and a word designating the type of block. Example block names include CHARACTERS, TAXA, and TREES.

The order of blocks is predetermined for some pairs of blocks, but not others. For example, most programs will require a CHARACTERS or DATA block to precede the ASSUMPTIONS block, so that the characters to which the ASSUMPTIONS block refers will be defined when the ASSUMPTIONS block is encountered. Information must be defined before it is needed (not after).

Although the NEXUS standard does not impose constraints on the number of blocks, particular programs will. For example, MacClade 3.07 does not allow more than one TAXA block in a file.

NEXUS Objects

Many of the commands in a NEXUS file define objects or specify characteristics about them. Currently defined objects include taxa, characters, states, trees, genetic codes, sets (of taxa, characters, states, classes of changes between states, trees), partitions (of taxa, trees, and characters), weight sets, types, type sets, character exclusion sets, ancestral states, and codon position sets.

All objects can be labeled (given names). Duplicate names should be avoided if this might cause ambiguity. For example, two taxa should not have the same name, nor should a taxon and taxon-set, nor a character and a character-set. Names that differ only in case (e.g., Beetles and beetles) should also be avoided, as they are considered homonyms. Names are single NEXUS words; they cannot consist of entirely digits (e.g., a taxon called 123 is illegal).

Taxa, characters, and trees possess an inherent order, determined by their order of definition. This allows them to be referred to by name (if they have been given names) or by number. Thus, in the following matrix:

MATRIX	
taxon_A	001020101
taxon_B	101001022
taxon_C	001010201;

the second taxon could be referred to as taxon_B or as 2. Characters, taxa, and trees can be referred to by their name or number; discrete states can be referred by name or state symbol. All objects other than taxa, characters, states, and trees must be referred to by name, not number.

Although the manner of definition of an object varies among different types of objects, several of the object definitions follow a particular format. Here are two examples of object defini-

tion commands:

```
CHARSET larval=1-15;
WTSET myweights (VECTOR)=2 1 2 0 2;
```

The objects that are defined in this way are: GENETICCODE, CODONPOSSET, CODESET, CHARSET, STATESET, CHANGESET, TAXSET, TREESET, CHARPARTITION, TAXPARTITION, TREEPARTI-TION, USERTYPE, WTSET, TYPESET, EXSET, ANCSTATES, and TREE.

Taxa can be defined in a TAXA and DATA blocks, as well as CHARACTERS, UNALIGNED, and DISTANCES blocks (if the NEWTAXA token is included in the DIMENSIONS command).

Display of Text

In general, programs should present text to users as it appears in the NEXUS file. If the NEXUS file contains the token Beetle, then this should be displayed as Beetle, not BEETLE. If the program presents the text outside of a display of the NEXUS file itself, however, there are cases in which the program should first alter the text. Underscores should be converted to blanks for display purposes. For example, "Big_Beetle" should appear in a printed tree as "Big Beetle." Two adjacent single quotes within quoted tokens should be converted to one single quote.

Some programs will display text in various styles (italics, bold, underlined) if the appropriate formatting information is included in the NEXUS file. This is done by way of special set of comments:

[\i]: subsequent text is to be displayed in italics
[\b]: subsequent text is to be displayed in **bold** face
[\u]: subsequent text is to be displayed <u>underlined</u>
[\p]: subsequent text is to be displayed in a plain style
 (that is, not italics, boldface, or underlined)

For example, [\i]Homo sapiens[\p] Linneaus would tell a program to display the text as *Homo sapiens* Linnaeus; [\i]Homo [\b]sapiens[\p] Linneaus would tell a program to display the text as *Homo sapiens* Linnaeus.

Descriptions of Public Blocks

The following conventions are used in syntax descriptions of public blocks that follow. Upper-case items should not be abbreviated. They can be entered in upper or lower case. Italicized items (e.g., x) represent items to be substituted with the relevant text characters. Items inside of square brackets (e.g., [X]) are optional. Items inside of curly braces and separated by vertical bars (e.g., {X | Y | Z}) are mutually exclusive options. Underlined options are the default values. An item followed by an ellipsis (...) means that it may be repeated one or more times.

TAXA Block

The TAXA block specifies information about taxa.

```
BEGIN TAXA;
DIMENSIONS NTAX=number-of-taxa;
TAXLABELS taxon-name taxon-name ...;
END;
```

DIMENSIONS must appear before TAXLABELS. Only one of each command is allowed per block.

DIMENSIONS.---The NTAX subcommand of the DIMENSIONS command indicates the number of taxa. The NEXUS standard does not impose limits on number of taxa; a limit may be imposed by particular computer programs.

TAXLABELS.--- This command defines taxa, specifies their names, and determines their order.

TAXLABELS Fungus Insect Mammal;

Taxon names are single NEXUS words. They must not correspond to another taxon name or number; thus, 1 is not a valid name for the second taxon listed. The standard defines no limit to their length, although individual programs might impose restrictions.

Taxa may also be defined in the CHARACTERS, UNALIGNED, and DISTANCES blocks, if the NEWTAXA token is included in the DIMENSIONS command; see the descriptions of those blocks for details.

CHARACTERS Block

A CHARACTERS block defines characters and includes character data. Taxa are usually not defined in a CHARACTERS block; if they are not defined here, this block must be preceded by a block which defines taxon labels and ordering (e.g., TAXA). Details about different types of character data are given at the end of this section.

Syntax of the CHARACTERS block is as follows:

```
BegIN CHARACTERS;
DIMENSIONS [NewTaxa NTax=number-of-taxa] NCHAR=number-of-
characters;
[ForMat
    [DataType={Standard|DNA|RNA|Nucleotide|Protein|Continuous}]
    [RESPECTCASE]
    [MISSING=symbol]
    [GAP=symbol]
    [GAP=symbol]
    [SYMBOLS="symbol symbol..."]
    [EQUATE="<symbol=entry> [<symbol=entry>...]"]
```

```
[MATCHCHAR=symbol]
      [[No]<u>Labels</u>]
      [TRANSPOSE]
      [INTERLEAVE]
      [ITEMS=([MIN][MAX][MEDIAN][AVERAGE][VAR][SAMPLESIZE][STATES]])]
      [STATESFORMAT={STATESPRESENT | INDIVIDUALS | COUNT | FREQUENCY}]
      [[NO]TOKENS]
      ;]
   [ELIMINATE character-set;]
   [TAXLABELS taxon-name taxon-name...;]
   [ CHARSTATELABELS
      character-number [character-name]
          [/state-name [state-name...]],
      ...;]
   [CHARLABELS character-name [character-name...];]
   [STATELABELS
      character-number [state-name [state-name...]],...;]
   MATRIX data-matrix;
END;
```

DIMENSIONS, FORMAT, and ELIMINATE must all precede CHARLABELS, CHARSTATELABELS, STATELABELS, and MATRIX. DIMENSIONS must precede ELIMINATE. Only one of each command is allowed per block.

DIMENSIONS.--- The DIMENSIONS command specifies the number of characters. The number following NCHAR is the number of characters in the data matrix. The NEXUS standard does not impose limits on the number of characters; a limit may be imposed by particular computer programs.

It is strongly advised that new taxa not be defined in a CHARACTERS block, for the reasons discussed in the description of the DATA block. If new taxa are to be defined, this must be indicated by the NEWTAXA subcommand, specifying that new taxa are to be defined (this allows the computer program to prepare for creation of new taxa). NEWTAXA, if present, must appear before the NTAX subcommand.

The NTAX subcommand, indicating the number of taxa in the MATRIX command in the block, is optional, unless NEWTAXA is specified, in which case it is required.

FORMAT.--- The FORMAT command specifies the format of the data MATRIX. This is a crucial command, as misinterpreting the format of the data matrix could lead to anything from incorrect results to spectacular crashes. The DATATYPE subcommand must appear first in the command. The RESPECTCASE subcommand must appear before the MISSING, GAP, SYMBOLS, and MATCHCHAR subcommands.

The following are possible formatting subcommands:

DATATYPE={Standard|DNA|RNA|Nucleotide|Protein|Continuous}. This subcommand specifies the class of data. If present, it must be the first subcommand in the FORMAT command. Standard data consist of any general sort of discrete character data, and is typically used for morphological data, restriction site data, and so on. DNA, RNA, NUCLEOTIDE, and PRO-TEIN designate molecular sequence data. Meristic morphometric data and other information with continuous values can be housed in matrices of DATATYPE=CONTINUOUS. These DATATYPEs are described in detail, with examples, at the end of the description of the CHARACTERS block.

RESPECTCASE. By default, information in a MATRIX may be entered in upper case, lower case, or a mixture of upper and lower case. If RESPECTCASE is requested, case is considered significant in SYMBOLS, MISSING, GAP, MATCHCHAR subcommands and in subsequent references to states. For example, if RESPECTCASE is invoked, then SYMBOLS="A a B b" designates four states whose symbols are A, a, B, and b, which can then each be used in the MATRIX command and elsewhere. If RESPECTCASE is not invoked, then A and a are considered homonymous state symbols. This subcommand must appear before the SYMBOLS subcommand. This subcommand is not applicable to DATATYPE=DNA, RNA, NUCLEOTIDE, PROTEIN, and CONTINUOUS.

MISSING. This subcommand declares the symbol that designates missing data. The default is '?'. For example, MISSING=X defines an X to represent missing data. Whitespace is illegal as a missing data symbol, as are the following: () [] {} / $\$, ; := * ' "` <>^

GAP. This subcommand declares the symbol that designates a data gap (e.g., base absent in DNA sequence due to deletion, or an inapplicable character in morphological data). There is no default gap symbol; a gap symbol must be defined by the GAP subcommand before any gaps can be entered into the matrix. For example, GAP=- defines a hyphen to represent a gap. Whitespace is illegal as a gap symbol, as are the following: () [] {} / \, ; := * ' " <>^

SYMBOLS. Specifies the symbols and their order for character states used in the file (including in the MATRIX command). For example, SYMBOLS="0 1 2 3 4 5 6 7" designates numbers 0 through 7 as acceptable symbols in a matrix. The SYMBOLS subcommand is not allowed for DATATYPE=CONTINUOUS. The default symbols list varies from one DATATYPE to another, as described under "state symbol" in Appendix 1. Whitespace is not needed between elements: SYM-BOLS="012" is equivalent to SYMBOLS="0 1 2".

For STANDARD DATATYPES, a SYMBOLS subcommand will replace the default symbols list of "0 1". For DNA, RNA, NUCLEOTIDE, and PROTEIN DATATYPES, a SYMBOLS subcommand will not replace the default symbols list, but will add character-state symbols to the SYMBOLS list. The NEXUS standard does not define the position of these additional symbols within the SYMBOLS list. (These additional symbols will be inserted at the beginning of the SYMBOLS list in PAUP, at the end in MacClade. MacClade will accept additional symbols for protein but not DNA, RNA, and NUCLEOTIDE matrices.)

EQUATE. This allows one to define symbols to represent one matrix entry. For example, EQUATE="E=(012)" means that each occurence of E in the MATRIX command will be interpreted as meaning states 0, 1, and 2. The equate symbols cannot be () [] {} / \, ; := * ' "`<>~^^, or any of the currently defined MISSING, GAP, MATCHCHAR, or state SYMBOLS. Case is significant in equate symbols. That is, MISSING=? EQUATE="E=(012) e=?" means that E will be interpreted as 0, 1, and 2, e as missing data.

MATCHCHAR. This subcommand defines a matching character symbol. If this subcommand is included, then a matching character symbol in the MATRIX indicates that the states are equivalent to the states possessed by the first taxon listed in the matrix for that character. In the following matrix, the sequence for taxon 2 is GACTTTC:

```
BEGIN DATA;
DIMENSION NCHAR=7;
FORMAT DATATYPE=DNA MATCHCHAR=.;
MATRIX
taxon_1 GACCTTA
taxon_2 ...T..C
taxon_3 ..T.C..;
END;
```

Whitespace is illegal as a matching character symbol, as are the following: () [] {} , ; = * ' "` / \<>^

[No]LABELS. This subcommand declares whether taxon or character labels are to appear on the left hand side of the matrix. By default, they should appear. If NOLABELS is used, then no labels appear, but then all currently defined taxa must be included in the MATRIX, in the order in which they were originally defined.

TRANSPOSE. This indicates that the MATRIX is in transposed format, with each row of the matrix representing the information from one character, and each column the information from one taxon.

Following is an example of a TRANSPOSEd MATRIX:

MATRIX						
character_1	1	0	1	1	0	1
character_2	0	1	1	1	0	0
character_3	0	1	1	1	1	0;

INTERLEAVE. This indicates that the MATRIX is in interleaved format, that is, that the data matrix is broken up into sections. If the data is not transposed, then each section contains the infor-

mation for some of the characters, for all taxa. For example, the first section might contain data for characters 1–50 for all taxa, the second section data for characters 51–100, and so on. Taxa in each section must occur in the same order. This format is especially useful for molecular sequence data, where the number of characters can be large. A small interleaved matrix follows:

MATRIX								
taxon_1	А	С	С	Т	С	G	G	С
taxon_2	А	С	С	Т	С	G	G	С
taxon_3	А	С	G	Т	С	G	С	Т
taxon_4	А	С	G	Т	С	G	С	Т
taxon_1	Т	Т	А	А	С	G	А	
taxon_2	Т	Т	А	А	С	С	А	
taxon_3	С	Т	С	А	С	С	А	
taxon_4	Т	Т	С	А	С	С	А	
;								

The interleaved sections need not all be of the same length. In an interleaved matrix, newline characters are significant: they indicate that the next character information encountered applies to a different taxon (for nontransposed matrices).

ITEMS. Each *entry* in the matrix gives information about a character's condition in a taxon. The ITEMS subcommand indicates what items of information are listed at each entry of the matrix. With discrete character data, the entry typically consists of the states observed in the taxon (either the single state observed, or several states if the taxon is polymorphic or of uncertain state). This can be specified by the statement ITEMS=STATES, but since it is the default and the only option allowed by most current programs for discrete data, an ITEMS statement is usually unnecessary. For continuous data, however, the wealth of alternatives (average, median, variance, minimum, maximum, sample size) often requires an explicit ITEMS statement to indicate what information is represented in each data matrix entry. Some ITEMS (such as VARIANCE) would be appropriate to only some DATATYPES; other ITEMS such as SAMPLESIZE and STATES would be appropriate to most or all DATATYPES. If more than one item is indicated, parentheses must be used to surround the list of items (e.g., "ITEMS=(AVERAGE VARIANCE)"); otherwise the parentheses are unnecessary (e.g., "ITEMS=AVERAGE"). More information about ITEMS options can be found in the discussion of the different DATATYPES under MATRIX, while information specifically about the STATES option is given below under STATESFORMAT.

STATESFORMAT. As noted under ITEMS, the entry in a matrix usually lists (for discrete data) or may list (for continuous data) the states observed in the taxon. The STATESFORMAT subcommand specifies what information is conveyed in that list of STATES. In most current programs for discrete data, when a taxon is polymorphic, the entry of the matrix lists only what distinct states

were observed, without any indication of the number or frequency of individuals sampled with each of the states. Thus, if all individuals sampled within the taxon have state A, the matrix entry would be "A", while if some have state A and others state B, the entry would be "(AB)". This corresponds to the option STATESFORMAT=STATESPRESENT. Because it is the default for discrete data, this statement is typically unnecessary with current programs. The other STATESFORMAT options can be illustrated with an example, in which two individuals of a taxon were observed to have state A and three were observed to have state B. When STATESFORMAT=INDIVIDUALS, the state of each of the individuals (or other appropriate sampling subunit) is listed exhaustively, "(A A B B B)"; when STATESFORMAT=COUNT, the number of individuals with each observed states is indicated, "(A:2 B:3)"; when STATESFORMAT=FREQUENCY the frequencies of various observed states are indicated, "(A:0.40 B:0.60)". The STATESFORMAT command may also be used for continuous data, for which the default is STATESFORMAT=INDIVIDUALS.

[NO] TOKENS. This subcommand specifies whether or not data matrix entries are single symbols, or can be tokens. If TOKENS, then the data values must be full NEXUS tokens, separated by whitespace or punctuation as appropriate, as in the following example:

```
Begin Characters;
DIMENSIONS NCHAR=3;
CHARSTATELABELS 1 hair/absent present, 2 color/red blue,
        3 size/small big;
FORMAT TOKENS;
MATRIX
        taxon_1 absent red big
        taxon_2 absent blue small
        taxon_3 present blue small;
END;
```

TOKENS is the default (and the only allowed option) for DATATYPE=CONTINUOUS; NOTO-KENS is the default for all other DATATYPES. TOKENS is not allowed for DATATYPES DNA, RNA, and NUCLEOTIDE. If TOKENS is invoked, the standard 3-letter amino acid abbreviations can be used with DATATYPE=PROTEIN, and defined state names for DATATYPE=STANDARD.

ELIMINATE.--- This command allows one to specify a list of characters that are to be excluded from consideration. Programs are expected to ignore ELIMINATEd characters completely during reading. In avoiding allocation of memory to store character information, the programs can save a considerable amount of computer memory. (This is similar to the ZAP subcommand in version 3.1 of PAUP.) For example,

```
ELIMINATE 4-100;
```

tells the program to skip over characters 4 through 100 in reading the matrix. Character-

set names are not allowed in the character list. This command does not affect character numbers.

<u>**TAXLABELS.---**</u> This command allows one to specify the names of the taxa. It serves to define taxa, and is only allowed in a CHARACTERS block if the NEWTAXA token is included in the DIMENSIONS statement.

<u>CHARSTATELABELS</u>.--- CHARSTATELABELS allows one to specify both the names of the characters and names of the states. This command was developed as an alternative to the older commands CHARLABELS and STATELABELS.

Here is an example:

CHARSTATELABELS 1 eye_color/red blue green, 3 head_shape/round square, 5 pronotum_size/small medium large ;

A forward slash (/) separates the character name and the state names, with a comma separating the information for different characters. If no state names are to be specified, the slash may be omitted; if no character names are to be specified, the slash must be included, but no token needs to be included between the character number and the slash. If state x is the last state to be named, then subsequent states need not be named but states 1 through x must be. If no name is to be applied to a state, enter a single underscore for its name. Character and state names are single NEXUS words. Character names must not correspond to another character name or number; thus, 1 is not a valid name for the second character listed. There is no restriction on the length of a character or state name imposed by the NEXUS standard; however, particular programs may limit the length. State names cannot be applied if DATATYPE=CONTINUOUS.

<u>CHARLABELS</u>.--- This command allows one to specify names of characters:

```
CHARLABELS
flange microsculpture body_length hind_angles #_spines
spine_size ____ head_size pubescent_intervals head_color
clypeal_margin;
```

Character labels are listed consecutively. If character x is the last character to be named, then subsequent characters need not be named, but characters 1 through x need to be. If no name is to be applied to a character, a single underscore can be used for its name. Character names are single NEXUS words. They must not correspond to another character name or number; thus, 1 is not a valid name for the second character listed.

There is no restriction on the length of a character name imposed by the NEXUS standard;

however, particular programs may limit the length. The command should be used only for nontransposed matrices (in transposed matrices, the character labels are defined in the MATRIX command).

We recommend that programs abandon this command in place of the more flexible CHARSTATELABELS command when writing NEXUS files, although programs should continue to read CHARLABELS as many existing NEXUS files use CHARLABELS.

<u>STATELABELS</u>.--- This command allows one to specify the names of states:

```
STATELABELS
1 absent present,
2 isodiametric transverse,
3 '4.5-6.2mm' '6.3-7.0mm' '7.7-11.0mm' '>12.0mm',
4 rounded subangulate angulate,
10 0 '1-4' '6-9' '7-9' '8-9' 7 8 9,
11 black rufous metallic flavous,
12 straight concave,
;
```

State labels need not be specified for all characters. A comma must separate state labels for each character. State labels are listed consecutively within a character. If state x is the last state to be named, then subsequent states need not be named, but states 1 through x must be. If no name is to be applied to a state, enter a single underscore for its name. State names are single NEXUS words. The standard defines no limit to their length, although individual programs might impose restrictions. This command is not valid for DATATYPE=CONTINUOUS.

We recommend that programs abandon this command in place of the more flexible CHARSTATELABELS command when writing NEXUS files, although programs should continue to read STATELABELS as many existing NEXUS files use STATELABELS.

MATRIX.--- In its standard format, the MATRIX command contains a sequence of taxon names and state information for that taxon.

The MATRIX itself is of the form:

```
MATRIX
taxon-name entry entry...entry
taxon-name entry entry...entry
...
taxon-name entry entry...entry;
```

Each entry in the matrix is the information about a particular character for a particular taxon. For example, it might be the assignment of state 0 to taxon 1 for character 1. Thus, the entry

would consist of one state symbol, "0". If the taxon were polymorphic, the entry would consiste of multiple state symbols (e.g., "(0 1)", indicating the taxon has both states 0 and 1). More details about the nature of each entry of the matrix are given above under ITEMS and below under each DATATYPE.

Each entry needs to be enclosed in parentheses or braces whenever more than one state symbol is given (e.g. "(01)" with standard data and the default NOTOKENS option) or if the information is conveyed by more than one NEXUS token (e.g., "(0:100)", or "(2.3 4.5 6.7)"). Otherwise, the parentheses or braces are optional. No whitespace is needed between entries in the matrix unless the TOKENS subcommand of the FORMAT command is invoked or implied and parentheses or braces do not surround an entry.

Taxa need not be in same order as in the TAXA block, and the matrix need not contain all taxa. For interleaved matrices, all sections must have the same taxa in the same order.

Examples of matrices of different DATATYPES are described below.

STANDARD data. For DATATYPE=STANDARD, each entry of the matrix consists of a single state-set. Under the defaults (ITEMS=STATES and STATESFORMAT=STATESPRESENT), each entry of the matrix consists of a single state-set; if there are multiple states, then the entry must be enclosed in parentheses (indicating polymorphism) or braces (indicating uncertainty in state). For example, in the following matrix,

```
Begin Characters;
DIMENSIONS NCHAR=9;
FORMAT SYMBOLS="- + x";
MATRIX
    taxon_1 (-+){-+}+---+--
    taxon_2 +x-++--+x;
taxon_3 -++++--+x;
END;
```

taxon_1 is polymorphic for the first character, and has either state - or + for the second character. If STATESFORMAT=COUNT or FREQUENCY, then each entry must be enclosed in parentheses, because more than one token is required to convey information for even one state:

```
Begin Characters;
DIMENSIONS NCHAR=3;
Format StatesFormat=Frequency Symbols="0 1 2";
Matrix
    taxon_1 (0:0.25 1:0.75) (0:0.3 1:0.7) (0:0.5 1:0.3 2:0.2)
    taxon_2 (0:0.4 1:0.6) (0:0.8 1:0.2) (1:0.15 2:0.85)
    taxon_3 (0:0.0 1:1.0) (0:0.55 1:0.45) (0:0.1 1:0.9);
END;
```

DNA, RNA, NUCLEOTIDE, and PROTEIN data. For DATATYPE=DNA, RNA, NUCLEOTIDE, or PROTEIN, each entry of the matrix consists of one or more state symbols describing the state(s) at one site in a molecular sequence. If STATESFORMAT= STATESPRESENT, and if an entry represents a single state, then it is represented as a single state symbol (or, if DATATYPE=PROTEIN and TOKENS, as a three-letter amino acid name). If an entry represents multiple states, then it must be enclosed in parentheses (indicating polymorphism) or braces (indicating uncertainty in state). Following is a matrix of DATATYPE=DNA:

```
BEGIN CHARACTERS;
DIMENSIONS NCHAR=12;
FORMAT DATATYPE=DNA;
MATRIX
taxon_1 ACCATGGTACGT
taxon_2 TCCATGCTACCC
taxon_3 TCCATGGAACCC;
END;
```

CONTINUOUS data. For DATATYPE=CONTINUOUS, each entry in the matrix must be enclosed by parentheses if more than one item is specified in the ITEMS subcommand. Parentheses must also be used whenever multiple tokens are needed for an entry in the matrix. If an entry consists of a single token (e.g., 0.231), it may be written without parentheses, but must then be separated from other entries by whitespace.

```
MATRIX
A 0.453 1.43 78.6
B 0.34 1.02 55.7
C 0.22 1.79 69.1;
```

A matrix entry can include average, minimum, maximum, variance, sample size, and a listing of states observed in the taxon, as specified in the ITEMS subcommand. The sample size, if included, must be in the form of an integer; the other numbers can either in English decimal (e.g., 0.00452) or in exponential form (e.g., 4.52E-3).

The information listed for each taxon for a continuous character is specified in the ITEMS subcommand of the FORMAT command. For example, if the matrix contains only information about the minimum and maximum value for each taxon, the ITEMS subcommand would be

```
ITEMS=(MIN MAX)
```

and a small matrix might look something like this:

```
MATRIX
taxon_1 (0.21 0.45) (0.34 0.36)
taxon_2 (0.13 0.22) (0.45 0.55);
```

If the ITEMS include the raw measurements (states), for instance to list a sample of measurements from individuals, then the other items must precede the listing of states. There is no restriction on the number of elements in the listing of states. This example has only one continuous character:

```
FORMAT DATATYPE=CONTINUOUS ITEMS=(AVERAGE STATES)
    STATESFORMAT=INDIVIDUALS;
MATRIX
taxon_1 (1.2 2.1 1.6 0.8 1.8 0.3 0.6)
taxon_2 (1.6 2.2 1.7 1.0 2.0 1.6 1.9 0.8);
```

in which the first value is the sample average, and the subsequent values comprise the sample of observed states.

Possible ITEMS to be included are MIN (minimum), MAX (maximum), AVERAGE (sample average), VAR (sample variance), MEDIAN (sample median), SAMPLESIZE, and STATES. The manner of presentations of states can be indicated using the STATESFORMAT command. The default ITEMS for continuous data is AVERAGE.

UNALIGNED Block

The UNALIGNED block includes data that is not aligned. Its primary intent is to house unaligned DNA, RNA, NUCLEOTIDE, and PROTEIN sequence data. Taxa are usually not defined in an UNALIGNED block; if not, this block must be preceded by a block that defines taxon labels and ordering (e.g., TAXA).

Syntax of the UNALIGNED block is as follows:

```
Begin Unaligned;
[Dimensions NewTaxa NTax=number-of-taxa;]
[Format
    [DataType={Standard|DNA|RNA|Nucleotide|Protein}]
    [RespectCase]
    [Missing=symbol]
    [SymBoLs="symbol symbol..."]
    [Equate="<symbol=entry> [<symbol=entry>...]"]
    [[No]LaBeLs];]
[TaxLaBeLs taxon-name taxon-name...;]
Matrix data-matrix;
END;
```

Commands must appear in the order listed. Only one of each command is allowed per block.

The DIMENSIONS command should only be included if new taxa are being defined in this block, which is discouraged (see discussion under DATA block, below). The format for the DIMEN-

SIONS command is as in the CHARACTERS block, except NCHAR is not allowed.

Subcommands of the FORMAT command are described in the CHARACTERS block.

The TAXLABELS command serves to define taxa, and is only allowed if the NEWTAXA token is included in the DIMENSIONS statement. It follows the same form as described in the TAXA block.

Here is an example:

```
BegIN UNALIGNED;
FORMAT DATATYPE=DNA;
MATRIX
taxon_1 ACTAGGACTAGATCAAGTTCAGAGGATCC,
taxon_2 ACCAGGACTAGCGGATCAAGTTCAGAGGATCC,
taxon_3 ACCAGGACTAGATCAAGTTCAGAGGGATCC,
taxon_4 AGCCAGGACTAGTTCAAGTTCAGAGGATCC,
taxon_5 ATCAGGACTAGATCAAGTTCAGGATCC;
END;
```

A comma must be placed at the end of each sequence (except the last, which requires a semicolon). Each sequence can occupy more than one line.

DISTANCES block

This block contains distance matrices. Taxa are usually not defined in a DISTANCES block; if they are not, this block must be preceded by a block that defines taxon labels and ordering (e.g., TAXA).

The syntax of the block is as follows:

```
BEGIN DISTANCES;
[DIMENSIONS [NEWTAXA] NTAX=number-of-taxa
NCHAR=number-of-characters;]
[FORMAT
[TRIANGLE={Lower|Upper|Both}]
[[N0]Diagonal]
[[N0]Labels]
[[N0]Labels]
[MISSING=SYMBOL]
[INTERLEAVE]
;]
[TAXLABELS taxon-name taxon-name...;]
MATRIX distance-matrix ;
END;
```

Commands must appear in the order listed. Only one of each command is allowed per block.

<u>DIMENSIONS</u>.--- The NTAX subcommand of this command is needed to process the matrix when some defined taxa are omitted from the distance matrix. The NCHAR subcommand is

optional, and can be used to indicate the number of characters for those analyses that need to know how many characters (if any) where used to calculate the distances. NCHAR is not required for successful reading of the matrix.

As for the CHARACTERS and UNALIGNED block, taxa can be defined in a DISTANCES block if NEWTAXA precedes the NTAXA subcommand in the DIMENSIONS command. It is advised that new taxa not be defined in a DISTANCES block, for the reasons discussed in the description of the DATA block. NEWTAXA, if present, must be appear before the NTAX subcommand.

FORMAT.--- This command specifies the formatting of the MATRIX. The [NO]LABELS and MISSING subcommands are as described in the CHARACTERS block.

 $Triangle = \{ \underline{Lower} | Upper | Both \}.$ This subcommand specifies whether only the lower left half of the matrix is present, or only the upper right, or both halves. Below is one example of an upper triangular matrix and one of a matrix with both halves included.

```
BEGIN DISTANCES;
  FORMAT TRIANGLE=UPPER;
  MATRIX
     taxon_1 0.0 1.0 2.0 4.0 7.0
     taxon 2 0.0 3.0 5.0 8.0
     taxon_3
taxon_4
taxon_5
                  0.0 6.0 9.0
                      0.0 10.0
                             0.0;
End;
BEGIN DISTANCES;
  FORMAT TRIANGLE=BOTH;
  MATRIX
     taxon_1
               0 1.0 2.0 4.0
                                  7.0
     taxon_2 1.0 0 3.0 5.0 8.0
     taxon_3 2.0 3.0 0 6.0 9.0
     taxon 4 4.0 5.0 6.0 0 10.0
     taxon_5 7.0 8.0 9.0 10.0
                                    0;
END;
```

DIAGONAL. If DIAGONAL is turned off, the diagonal elements are not included

```
FORMAT NODIAGONAL;
MATRIX
taxon_1
taxon_2 1.0
taxon_3 2.0 3.0
taxon_4 4.0 5.0 6.0
taxon_5 7.0 8.0 9.0 10.0;
```

If TRIANGLE is not BOTH, then there will be one row that contains only the name of a taxon.

This row is required. If TRIANGLE=BOTH, then the diagonal must be included.

INTERLEAVE. As in CHARACTERS block, although interleaved matrices take a slightly different form for distance matrices:

taxon_1	0		
taxon_2	1	0	
taxon_3	2	3	0
taxon_4	4	5	6
taxon_5	7	8	9
taxon_6	11	12	13
taxon_4	0		
taxon_5	10	0	
taxon_6	14	15	0;

As in the CHARACTERS block, newline characters in interleaved matrices are significant, in that they indicate a switch to a new taxon.

<u>**TAXLABELS</u></u>.--- TAXLABELS allows one to specify the names and ordering of the taxa. This command serves to define taxa, and is allowed only if the NEWTAXA token is included in the DIMENSIONS statement.</u>**

MATRIX.--- The MATRIX command contains the distance data.

DATA Block

This block is equivalent to a CHARACTERS block in which the NEWTAXA subcommand is included in the DIMENSIONS command. That is, it is a CHARACTERS block that includes not only the definition of characters, but also the definition of taxa.

In versions 3.0-3.07 of MacClade and 3.0-3.1.1 of PAUP data were generally saved in a DATA block, not in TAXA and CHARACTERS blocks. However, as the NEXUS format developed, it became apparent that a number of programs would be written that would not use any of the information in the DATA block except the taxon ordering and labels. For example, a program might be interested only in trees, and need only the taxon labels and TREES block (this is the case for COM-PONENT [Page, 1993]). Forcing such a program to know enough about the many formats of the MATRIX command of the DATA block to extract information about taxon labels is excessive, when the information can be stored in a separate, easy-to-read TAXA block. Another program might only be interested in a distance matrix, making a file with DNA sequence data and distance data much easier to process if the taxon labels were not buried in a DATA block of DNA data.

The DATA block and the ability to define new taxa in CHARACTERS, UNALIGNED, and DIS-TANCES blocks have been retained in the NEXUS format as there are circumstances in which it will be much easier to manually edit and maintain files with taxa definitions and data housed in the same matrix. One disadvantage of the placement of taxon labels into a separate block is the increased chance that manual editing of the file will lead to conflicts between the taxon labels defined in the TAXA block and those used in other blocks (see TAXA block).

We strongly recommend against use of a DATA block, or definition of taxa in a CHARAC-TERS, UNALIGNED, or DISTANCES block, as this will prevent some programs from extracting taxon information from the file.

The following DATA block serves to define and order five taxa, as well as to contain descriptions of sequence data for the taxa:

```
BEGIN DATA;
DIMENSIONS NTAX=5 NCHAR=20;
FORMAT DATATYPE=DNA GAP=-;
MATRIX
taxon_1 A-CTAGGACTA---GATCAA
taxon_2 A-CCAGGACTAGCGGATCAA
taxon_3 A-CCAGGACTA---GATCAA
taxon_4 AGCCAGGACTA---GTTCAA
taxon_5 ATC-AGGACTA---GATCAA;
END;
```

CODONS Block

The CODONS block contains information about the genetic code, the regions of DNA and RNA sequences that are protein coding, and the location of triplets coding for amino acids in nucleotide sequences.

```
BEGIN CODONS;
   [CODONPOSSET [*] name [({STANDARD | VECTOR})]=
      N: character-set,
      1: character-set,
      2: character-set,
      3: character-set;]
   [GENETICCODE code-name
      [([CODEORDER=<u>132</u>|other]
      [NUCORDER=<u>TCAG</u>|other]
      [[No]Tokens]
      [EXTENSIONS="symbols-list"])]
          =genetic code description];]
   [CODESET [*] codeset-name {(CHARACTERS|Unaligned|Taxa)}=
      code-name:character-set or taxon-set,
      code-name:character-set or taxon-set,...;]
END;
```

GENETICCODE must precede any CODESET that refers to it.

There are several predefined genetic codes:

UNIVERSAL	[universal]
UNIVERSAL.EXT	[universal, extended]
MTDNA.DROS	[Drosophila mtDNA]
MTDNA.DROS.EXT	[Drosophila mtDNA, extended]
MTDNA.MAM	[Mammalian mtDNA]
MTDNA.MAM.EXT	[Mammalian mtDNA, extended]
MTDNA.YEAST	[Yeast mtDNA]

For a summary of the genetic codes, see Osawa et al. (1992). Extended codes are those in which "extra" amino acids have been added to avoid disjunct amino acids (see the EXTENSIONS subcommand under GENETICCODE).

<u>**CODONPOSSET</u></u>.--- This command stores information about protein coding regions and the codon positions of nucleotide bases in protein-coding regions, and follows the format of a standard object definition command.</u>**

Those characters designated as 1, 2, or 3 are coding bases which are specified as being of positions 1, 2, and 3, respectively. Those characters designated as N are considered non-proteincoding. Those characters designated as ? are of unknown nature. Any unspecified bases are considered of unknown nature (equivalent to ?). If no CODONPOSSET statement is present, all bases are presumed of unknown nature.

For example, the following command

```
CODONPOSSET * coding=
N:1-10,
1:11-.\3,
2:12-.\3,
3:13-.\3;
```

designates bases 1-10 as noncoding, and positions of the remaining bases in the order 123123123....

<u>GENETICCODE</u>.--- GENETICCODE stores information about a user-defined genetic code. Multiple GENETICCODES may be defined in the block. This is a standard object definition command except that the default genetic code is not indicated by an asterisk after GENETICCODE.

The genetic code description is a listing of amino acids. By default, the first amino acid listed is that coded for by the triplet TTT, the last amino acid listed that coded for by GGG. In between, the order of triplets follows a pattern controlled by the subcommands NUCORDER and CODEORDER. By default, the amino acids are listed in the following order: TTT, TCT, TAT, TGT,

GENETICCODE UNTITLED=

FSYC FSYC LS** LS*W LPHR LPHR LPQR LPQR ITNS ITNS ITKR МТКR VADG VADG VAEG VAEG ;

This assigns TTT to phenylalanine, TCT to serine, TAT to tyrosine, TGT to cysteine, and so

The subcommands are:

CODEORDER. The default CODEORDER is 231, indicating that the second codon nucleotides change most quickly (i.e., codons represented by adjacent amino acids in the listing always differ at second positions), the third nucleotide changes next most quickly, and the first nucleotide most slowly in the list (such that the codons representing the first 16 listed amino acids all have the same first nucleotide).

NUCORDER. The default NUCORDER is TCAG, indicating that the codons with T at a given position are listed first, C next, and so on.

For example, if CODEORDER is 123 and the NUCORDER is ACGT, then the amino acids would be listed in order to correspond to codons in the order AAA CAA GAA TAA ACA CCA GCA TCA and so on.

[<u>No</u>]Tokens. If TOKENS, then amino acids are to be listed by their standard three-letter abbreviations for amino acids (e.g., Leu, Glu, etc.). A termination codon is designated by Ter or Stp. If NOTOKENS (the default), then the IUPAC symbols are used in the listing. A termination codon is designated by an asterisk.

on.

EXTENSIONS. This command lists the symbols for "extra" amino acids that are added to avoid disjunct amino acids. For example, serines in the universal code are coded for by two distinct groups of codons; one cannot change between these groups without going through a different amino acid. Serine is therefore disjunct. In the UNIVERSAL code, serine is kept disjunct, with all serines symbolized by S. In the UNIVERSAL.EXT code, however, one group of serine is symbolized by the extra amino acid symbol 1, the other group by 2. To indicate this the EXTENSIONS subcommand would read EXTENSIONS="S S", indicating that "extra" amino acids 1 and 2 are both serines. In the genetic code description, the symbols 1 and 2 would then both stand for serine. For example, the universal extended genetic code could be represented by:

```
GENETICCODE * universal (NucOrder=TCAG CodeOrder=213

EXTENSIONS="S S")=

F 1 Y C L P H R I T N 2 V A D G

F 1 Y C L P H R I T N 2 V A D G

L 1 * * L P Q R I T K R V A E G

L 1 * W L P Q R M T K R V A E G;
```

Note that the CODEORDER has been changed in this example.

<u>**CODESET</u></u>.--- This object definition assigns genetic codes to various characters and taxa. All nucleotide sites designated as coding, and all amino acid sites have a genetic code assigned to them. If the CHARACTERS format is used, then character-sets are to be used in the description, and the genetic code is thus applied to the characters listed. For example,</u>**

CODESET oddcodeset=customcode:4-99;

designates the genetic code "custom code" as applying to characters 4–99, for all taxa.

If UNALIGNED, then genetic code is presumed to apply to all sites in an UNALIGNED block. Such a CODESET command might look like this:

CODESET mycodeset (UNALIGNED)=universal:ALL;

For UNALIGNED, the only character-set that can be used is "ALL".

If the TAXA format is used, then taxon-sets are used in the description. This allows one to assign different genetic codes to different taxa, for all characters. Current programs do not accept any character-set other than "ALL", nor any taxon-set other than "ALL".

SETS Block

This block stores sets of objects (characters, states, taxa, etc.). The general structure of the SETS block is as follows:

BEGIN SETS;

```
[CHARSET charset-name [({STANDARD | VECTOR})]=character-set;]
   [STATESET stateset-name [({STANDARD|VECTOR})]=state-set;]
   [CHANGESET changeset-name=
      state-set<->state-set state-set<->state-set...;]
   [TAXSET taxset-name [({STANDARD|VECTOR})]=taxon-set;]
   [TREESET treeset-name [({Standard | Vector})]=tree-set;]
   [CHARPARTITION partition-name
       [([{[N0]<u>Tokens</u>}]
      [{<u>Standard</u>|Vector}])]
          = subset-name:character-set,...;]
   [TAXPARTITION partition-name
       [([{[NO]TOKENS}]]
       [\{\underline{Standard} | Vector\}])]
          = subset-name:taxon-set,...;]
   [TREEPARTITION partition-name
       [([{[NO]<u>Tokens</u>}]
       [{<u>Standard</u>|Vector}])]
          = subset-name:tree-set,...;]
END;
```

See object definition command in Appendix 1 for information about STANDARD versus VECTOR formats.

An example SETS block follows:

```
BegIN SETS;
CHARSET larval=1-3 5-8;
STATESET eyeless=0;
STATESET eyed=1 2 3;
CHANGESET eyeloss=eyed -> eyeless;
TAXSET outgroup=1-4;
TREESET AfrNZVicariance=3 5 9-12;
CHARPARTITION bodyparts=head:1-4 7, body:5 6, legs:8-10;
END;
```

CHARSET.--- CHARSET specifies and names a set of characters; this name can then be used in subsequent CHARSET definitions or wherever a character-set is required. The VECTOR format consists of 0's and 1's: a 1 indicates that the character is to be included in the CHARSET; whitespace is not necessary between 0's and 1's. The name of a CHARSET cannot be equivalent to a character name or character number.

The character-set CONSTANT is predefined for all DATATYPES; it specifies all invariant characters. The character-set REMAINDER is predefined for all DATATYPES; it specifies all characters not previously referenced in the command. The character-set GAPPED is predefined for all DATATYPES; it specifies all characters with a gap for at least one taxon. There are four additional predefined character-sets for characters of DATATYPE=DNA, RNA, and NUCLEOTIDE:

POS1: all characters defined by current CODONPOSSET as first positions.

POS2: all characters defined by current CODONPOSSET as second positions.

POS3: all characters defined by current CODONPOSSET as third positions.

NONCODING: all characters defined by current CODONPOSSET as non-protein-coding sites.

<u>STATESET</u>.--- This command allows one to name a set of states; it is not currently supported by any program. It is not available for DATATYPE=CONTINUOUS.

For STANDARD format, the state-set is described by a list of state symbols, except that it should not be enclosed in parentheses or braces. Any current state-set symbols are valid in the state-set description. The following STATESET

```
STATESET theSet=2 3 4 5;
```

defines the set composed of states 2, 3, 4, and 5.

The VECTOR format consists of 0's and 1's: a 1 indicates that the state is to be included in the STATESET; whitespace is not necessary between 0's and 1's. For example, the state-set

STATESET theSet (VECTOR)=1001000;

designates the Set to be the set containing first and fourth states.

<u>**CHANGESET</u></u>.--- This command allows one to name a set of state changes; it is not currently supported by any program. It is not available for DATATYPE=CONTINUOUS.</u>**

The description of the CHANGESET consists of pairs of state-sets joined by an operator. State-sets that consist of more than one token must be contained in parentheses. There are two allowed operators: -> and <-> (<- is not allowed). These operators can best be explained by example:

```
CHANGESET changes1=(1 2 3)->(4 6);
CHANGESET changes2=1<->4;
CHANGESET transversions=(A G)<->(C T);
```

The first CHANGESET represents any change from 1 to 4, 1 to 6, 2 to 4, 2 to 6, 3 to 4, or 3 to 6, and the second set represents changes from 1 to 4 and 4 to 1. The CHANGESET "transversions" defines the set of all changes between purines and pyrimidines as transversions.

TAXSET.--- This command defines a set of taxa. A TAXSET name can be used in subsequent

TAXSET definitions or wherever a taxon-set is required. The name of a TAXSET cannot be equivalent to a taxon name or taxon number. The taxa to be included are described in a taxon-set. For example, the following command

```
TAXSET beetles=Omma-.;
```

defines the TAXSET "beetles" to include all taxa from taxon Omma to the last defined taxon.

The VECTOR format consists of 0's and 1's: a 1 indicates that the taxon is to be included in the TAXSET; whitespace is not necessary between 0's and 1's.

<u>**TREESET</u></u>.--- This command defines a set of trees. A TREESET name can be used in subsequent TREESET definitions or wherever a tree-set is required. It is not currently supported by any program. It follows the same general format as a TAXSET command.</u>**

<u>CHARPARTITION, TAXPARTITION, TREEPARTITION</u>.--- These commands define partitions of characters, taxa, and trees, respectively. The partition divides the objects into several (mutually exclusive) subsets. They follow the same format, and will be described together.

There are several formatting options. The VECTOR format consists of a list of partition names. By default, the name of each subset is a NEXUS word (this is the TOKENS option). The NOTOKENS option is only available in the VECTOR format; this allows one to use single symbols for the subset names. Each value in a definition in VECTOR format must be separated by whitespace if the names are tokens, but not if they are NOTOKENS.

The following two examples are equivalent:

```
TAXPARTITION populations=1:1-3, 2:4-6, 3:7 8;
TAXPARTITION populations (Vector NoTokens)=11122233;
```

The following two examples are equivalent:

TaxPartition mypartition=Chiricahua: 1-3, Huachuca: 4-6, Galiuro: 7 8; TaxPartition mypartition (VECTOR)=Chiricahua Chiricahua Chiricahua Huachuca Huachuca Huachuca Galiuro;

ASSUMPTIONS Block

The ASSUMPTIONS block houses assumptions about the data, or gives general directions as to how to treat it (e.g., which characters are to be excluded from consideration). The commands currently placed in this block were primarily designed for parsimony analysis. More commands, embodying assumptions useful in distance, maximum likelihood, and other sorts of analyses, will be developed in the future. For example, matrices specifying relative rates of character state change, useful for both distance and likelihood analyses, will eventually be included here.

The general structure of the assumptions block is

```
BEGIN ASSUMPTIONS;
[OPTIONS [DEFTYPE=type-name]
    [POLYTCOUNT={MINSTEPS|MAXSTEPS}]
    [GAPMODE={Missing|NewState}];]
[USERTYPE type-name
    [({STEPMATRIX|CSTREE})]=USERTYPE-description;]
[TYPESET [*] typeset-name [({STANDARD|Vector})]=
    TYPESET-definition;]
[WTSET [*] wtset-name [({STANDARD|Vector} {Tokens|NoTokens})]=
    WTSET-definition;]
[EXSET [*] exset-name [({STANDARD|Vector})]=character-set;]
[ANCSTATES [*] ancstates-name [({STANDARD|Vector}]
    {Tokens|NoTokens})]=ANCSTATES-definition;]
END;
```

An example Assumptions block follows:

```
BEGIN ASSUMPTIONS;
  OPTIONS DEFTYPE=ORD;
  USERTYPE myOrd=4
         0 1 2 3
         . 1 2 3
         1 . 1 2
         21.
                  1
         3 2 1
                 .;
  USERTYPE myTree (CSTREE) = ((0,1)a,(2,3)b)c;
  TYPESET * mixed=IRREV: 1 3 10, UNORD 5-7;
  WTSET * one=2: 1-3 6 11-15, 3: 7 8;
  WTSET two=2:4 9, 3:1-3 5;
  ExSET nolarval=1-9;
  ANCSTATES mixed=0: 1 3 5-8 11, 1: 2 4 9-15;
END;
```

USERTYPES must be defined before they are referred to in any TYPESET.

In earlier versions of MacClade and PAUP, TAXSETS and CHARSETS also appeared in the ASSUMPTIONS block. These now appear in the SETS block. There are a number of other commands in the ASSUMPTIONS block which also have the SET in their name (e.g., WTSET, EXSET), but these commands assign of values to objects, not define sets of objects, and therefore do not belong in the SETS block. (Commands like WTSET and EXSET are so named for historical reasons; while they might ideally be renamed CHARACTERWEIGHTS and EXCLUDEDCHARACTERS, doing so would cause

existing programs to be incompatible with the file format.) It is recommended that programs also accept them in the ASSUMPTIONS block, so that older files can be read. In addition, the GAPMODE subcommand of the OPTIONS command of this block was originally housed in an OPTIONS command in the DATA block. As this subcommand dictates how data are to be treated, rather than provide details about the data themselves, it was moved into the ASSUMPTIONS block.

<u>OPTIONS</u>.--- This command houses a number of disparate subcommands. They are all of the form subcommand=option.

DEFTYPE: This specifies the default character type for parsimony analyses. Whenever a character's type is not explicitly stated, its type is taken to be the default type. Default DEFTYPE is UNORD (see Appendix 1, character transformation type, for a definition of UNORD).

POLYTCOUNT: Setting POLYTCOUNT to MINSTEPS specifies that trees with polytomies are to be counted (in parsimony analyses) in such a way that the number of steps for each character is the minimum number of steps for that character over any resolution of the polytomy. A treelength that is the sum of these minimum number of steps may be below the treelength of the most parsimonious dichotomous resolution. Setting POLYTCOUNT to MAXSTEPS specifies that trees with polytomies are to be counted in such a way that occurrence of derived states on elements of a polytomy are to be counted as independent derivations. Such a treelength may be above the treelength of any fully dichotomous resolution. The NEXUS format does not specify a default value for POLYTCOUNT; the default value may vary from program to program.

GAPMODE: Specifies how gaps are to be treated. GAPMODE=MISSING designates gaps to be treated in the same way as missing data; GAPMODE=NEWSTATE specifies that gaps are to be treated as an additional state (for DNA/RNA/NUCLEOTIDE data, as a fifth base).

<u>USERTYPE</u>.--- The USERTYPE command defines a character transformation type, as used in parsimony analysis to designate the cost of changes between states. There are several predefined character types (see character transformation type in Appendix 1); USERTYPE allows additional character types to be created.

USERTYPE is an object definition command with the exception that an asterisk cannot be used to indicate the default type (default type is stated in the OPTIONS command of the ASSUMP-TIONS block).

The standard defines no limit to the length of the type name, although individual programs might impose restrictions.

STEPMATRIX format is as follows:

USERTYPE myMatrix (STEPMATRIX)=n

S	S	S	S
	k	k	k
k		k	k
k	k		k
k	k	k	.;

where n is the number of rows and columns in the step matrix, the s's are state symbols, and the k's are the cost for going between states. n can take any value _ 2. Diagonal elements may be listed as periods. If a change is to be prohibited, then one enters an "i" for infinity. Typically, the state symbols will be in sequence, but they need not be. The following matrices assign values identically:

```
USERTYPE myMatrix (STEPMATRIX)=4
  0 1 2 3
  . 1 5 1
    . 5 1
  1
          5
  55.
  1 1 5
          .;
USERTYPE myMatrix2 (STEPMATRIX)=4
  2 0 3 1
     5 5 5
  .
  5.11
  5 1 . 1
  5 1 1 .;
```

The number of steps may be either integers or real numbers. The range of possible values will vary from program to program. Versions 3.0 to 3.04 of MacClade use the format name "REAL-MATRIX" rather than "STEPMATRIX" if the matrix contained real numbers. Future programs should treat REALMATRIX as a synonym of STEPMATRIX.

CSTREE format is very similar to the TREE format in a TREES block (see below). That is, character state trees are described in the parenthesis notation following the rules given for TREES of taxa. Instead of taxon labels, character state symbols are used. Thus

```
USERTYPE cstree-name (CSTREE)=
[(list of subtrees)][state-symbol]];
```

where each subtree has the same format as the overall tree and the subtrees are separated by commas. Two examples are shown in Figure 1.

TYPESET.--- A TYPESET specifies the type assigned to each character as used in parsimony analysis. This is a standard object definition command. Any characters not listed in the character-set have the default character type. See "object definition command" in Appendix 1 for informa-

tion about STANDARD versus VECTOR formats. The type names to be used are either the predefined ones or those defined in a USERTYPE command. Each value in a definition in VECTOR format must be separated by whitespace.

The following are equivalent type sets:

TYPESET mytypes=ORD: 1 4 6, UNORD: 2 3 5; TYPESET mytypes (Vector)=ORD UNORD UNORD ORD;

WTSET.--- A WTSET specifies the weights of each character. This is a standard object definition command. Any characters not listed in the character-set have weight 1. The weights may be either integers or real numbers. The minimum and maximum weight value will vary from program to program. Each value in a definition in VECTOR format must be separated by whitespace, unless the NOTOKENS option is invoked, in which case no whitespace is needed, and all weights must be integers in the range 0-9.

The following are equivalent weight sets:

WTSET mywts=3: 1 4 6, 1: 2 3 5; WTSET mywts (VECTOR)=3 1 1 3 1 3;

In earlier versions of MacClade, the formatting subcommand REAL was used to indicate that real-valued weights were included in the WTSET. This subcommand is no longer in use; programs are expected to detect the presence of integral or real-valued weights while reading the WTSET command.

EXSET.--- EXSET specifies which characters are to be excluded from consideration. This is a standard object definition command. Any characters not listed in the character-set are included. The VECTOR format consists of 0's and 1's - a 1 indicates that the character is to be excluded; whitespace is not necessary between 0's and 1's. The following commands are equivalent, and serve to exclude characters 5, 6, 7, 8, and 12.

```
EXSET * toExclude=5-8 12;
EXSET * toExclude (VECTOR)=000011110001;
```

ANCSTATES.--- The ANCSTATES command allows one to specify ancestral states. This is a standard object definition command. Any valid state symbol can be used in the description for discrete data, and any valid value for continuous data. TOKENS is the default for DATATYPE=CONTIN-UOUS; NOTOKENS is the default for all other DATATYPES. TOKENS is not allowed for DATATYPES DNA, RNA, and NUCLEOTIDE. If TOKENS is invoked, the standard 3-letter amino acid abbreviations can be used with DATATYPE=PROTEIN, and defined state names for DATATYPE=STANDARD. NOTOKENS is not allowed for DATATYPE=CONTINUOUS.

The following commands are equivalent:

```
ANCSTATES ancestor=0:1-3 5-7 12, 1:4 8-10, 2:11;
ANCSTATES ancestor (VECTOR)=000100011120;
```

TREES Block

This block stores information about trees.

The syntax for the TREES block follows:

```
BEGIN TREES;
[TRANSLATE
arbitrary-token-used-in-tree-description valid-taxon-name
[, arbitrary-token-used-in-tree-description
valid-taxon-name...];]
[TREE [*] tree-name=tree-specification;]
END;
```

TRANSLATE command, if present, must precede any TREE command.

TRANSLATE.--- The tree description requires references to the taxa defined in a TAXA, DATA, CHARACTERS, UNALIGNED, or DISTANCES block. These references can be made using the label assigned to them in the TAXA or data block, their numbers, or using a token specified in the TRANSLATE command. The TRANSLATE statement maps arbitrary labels in the tree specification to valid taxon names. If the arbitrary labels are integers, they are mapped onto the valid taxon names as dictated by the TRANSLATE command, without any consideration of the order of the taxa in the matrix. Thus, if an integer is encountered in the tree description, a program first checks to see if it matches one of the arbitrary labels defined in the TRANSLATE command; only if no matching label is found will the integer be presumed to refer to the taxon in that position in the matrix (e.g., if the label in the description is 15, but this is not a label defined in the TRANSLATE command, a program should take this to refer to the fifteenth taxon).

In the following example,

```
BEGIN TAXA;
TAXLABELS Scarabaeus Drosophila Aranaeus;
END;
BEGIN TREES;
TRANSLATE beetle Scarabaeus, fly Drosophila, spider Aranaeus;
TREE tree1=((1,2),3);
TREE tree2=((beetle,fly),spider);
TREE tree3=((Scarabaeus,Drosophila),Aranaeus);
END;
```

the TRANSLATE command specifies that the label beetle can be used in the tree description to refer to Scarabaeus, fly to Drosophila, and spider to Aranaeus. This means that Scarabaeus can be referred to in a tree description as 1, Scarabaeus, or beetle. Thus, the three trees are identical.

TREE.--- This command describes a tree. Tree descriptions are standard object definition commands. They use the familiar parenthesis notation, with node names, branch lengths and comments following the established Newick tree standard (see, for instance, Felsenstein, 1993).

A tree description consists of nested descriptions of clades. Thus, ((A,B), (C,D)) indicates that the tree includes two clades (or subtrees), (A,B) and (C,D), each of which consists of two terminal taxa. Formally, a description of a clade consists of a label for the node that is its most recent common ancestor (if that node has a label) *preceded* by a list of descriptions of the clade's subclades (if the clade has subclades) and *followed* by the length of the branch below the node (optional) and any extra information about the node. Thus, a clade is described as follows:

[(list of subclades)][label of node][:length of branch below node][other node information]

The <u>list of subclades</u> is enclosed in parentheses, and the subclades separated by commas:

(description of first subclade [, ..., description of last subclade])

Since the descriptions of subclades would include lists of <u>their</u> subclades, the familar nested parenthesis notation results. (Note that an alternative way to describe this notation is to say that the parentheses and label describe a node, and within the parentheses are all the nodes immediately descendant from it.) Terminal nodes, of course, do not have subclade lists. Thus the description of a terminal node consists only of its label and any additional information such as the length of the branch below the node.

The <u>label of the node</u> is a NEXUS token that is either a taxon's defined name, a taxon's number, a taxon's label from the translation table, or a clade's defined name. The label is optional for internal nodes that are not observed taxa; it is not optional for terminal nodes. Internal nodes that have no label are represented implicitly by the parentheses containing the list of subclades. If the name of a TAXSET is used, it is interpreted as a list of the terminal taxa defined to be in the TAX-SET (with commas implicitly inserted between the taxa).

The <u>length of the branch</u> below the node is a number, positive or negative.

Rooted and unrooted trees can be specified using the [&R] and [&U] comments at the start of the tree description. For example,

```
TREE mytree=[&R] ((1,2),(3,4));
```

is a rooted tree, whereas

```
TREE mytree=[&U] ((1,2),(3,4));
```

is an unrooted tree. The NEXUS standard does not specify whether rooted or unrooted is default.

An example tree with branch lengths is:

TREE tree4=((beetle:4.3,fly:1.1):1.8,spider:2.5);

If a file (and its data matrix) has four defined taxa, Crocodile, Bluebird, *Archaeopteryx*, and Rattlesnake, the following tree:

```
TREE tree4=(((Bluebird)Archaeopteryx,Crocodile)Archosauria,Rattle-
snake);
```

would indicate that the taxon *Archaeopteryx* is ancestral to Bluebird, and that Crocodile is their sister. Archosauria, because it does not refer to a taxon that has been defined in a TAXA or DATA block, is interpreted as the name of the clade including Archaeopteryx, Bluebird and Crocodile.

Any additional information about a clade, its ancestral node, or the branch below it, is to be placed in NEXUS comment commands associated with the node. Although different programs may choose their own conventions for how to embed information in comments, the comments that begin with &N are reserved for future NEXUS comment commands.

The NEXUS standard places no restrictions on the number of taxa contained in each tree.

The observance of the Newick tree standard for the TREE command causes some elements of this command to differ from that of more standard NEXUS commands. For example, the use of [&U] and [&R] to designate unrooted and rooted trees is not the natural way of specifying this information in a NEXUS command, as this critical information should not be relegated to a command comment. However, in the interests of compatibility with other computer programs that read Newick format trees, but do not support other aspects of the NEXUS format, the NEXUS standard follows the Newick format rather than creating a new tree format. In future versions of NEXUS, a more flexible GRAPH command will be present, allowing not only clearer designation of root position, but also of other aspects not currently available in Newick trees (including reticulations).

NOTES block

The NOTES block stores notes about various objects in a NEXUS file, including taxa, char-

acters, states, and trees.

The format is as follows:

```
BEGIN NOTES;
TEXT [TAXON=taxon-set] [CHARACTER=character-set]
[STATE=state-set] [TREE=tree-set]
SOURCE={Inline|File|Resource}
TEXT=text or source descriptor;
PICTURE [TAXON=taxon-set] [CHARACTER=character-set]
[STATE=state-set] [TREE=tree-set]
[FORMAT={PICT|TIFF|EPS|JPEG|GIF}]
[ENCODE={NONE|UUENCODE|BINHEX}]
SOURCE={INLINE|FILE|RESOURCE}
PICTURE=picture-or-source-descriptor;
END;
```

There are no restrictions on the order of commands.

If the written description of the taxon-set, character-set, state-set, or tree-set contains more than one token, it must be enclosed in parentheses, as in the following example:

TEXT TAXON=(1-3) TEXT='these taxa from the far north';

If both a taxon-set and a character-set are specified, then the text or picture applies to those characters for those particular taxa. If both a character-set and a state-set are specified, then the text or picture applies to those states for those particular characters.

<u>TEXT</u>.--- This command allows text to be attached to various objects.

The SOURCE subcommand indicates the location of the text. The INLINE option indicates that the text is present at the end of the TEXT command; the FILE option indicates that it is in a separate file (the name of which is then specified in the TEXT subcommand); the RESOURCE option indicates that it is in the resource fork of the current file, in a resource of type TEXT (the numerical ID of which is then specified in the TEXT subcommand).

For example, in the following

```
TEXT TAXON=5 CHARACTER=2 TEXT='4 specimens observed';
TEXT TAXON=Pan TEXT='This genus lives in Africa';
TEXT CHARACTER=2 TEXT='Perhaps this character should be deleted';
TEXT CHARACTER=1 STATE=0 TEXT='This state is hard to detect';
```

the first command assigns the note "4 specimens observed" to the data entry for taxon 5, character 2, the second command assigns the note "Perhaps this character should be deleted" to character 2, the third command assigns the note "This genus lives in Africa" to the taxon "Pan," and

the last command assigns the note "This state is hard to detect" to state 0 of character 1.

The text or source descriptor must be a single NEXUS word. If the text contains NEXUS whitespace or punctuation, it needs to be surrounded by single quotes, with any contained single quotes converted to a pair of single quotes.

<u>PICTURE</u>.--- This command allows a picture to be attached to an object.

The FORMAT subcommand allows one to specify the graphics format of the image.

The SOURCE subcommand indicates the location of the picture. The INLINE option indicates that the picture is present at the end of the PICTURE command; the FILE option indicates that it is in a separate file (the name of which is then specified in the PICTURE subcommand); the RESOURCE option indicates that it is in the resource fork of the current file, in a resource of type PICT (the numerical ID of which is then specified in the PICTURE command). The RESOURCE option is designed for Apple Macintosh[™] text files.

For example, the following command

```
PICTURE TAXON=5 CHARACTER=2 FORMAT=GIF SOURCE=FILE
PICTURE=wide.thorax.gif;
```

assigns the image in the GIF-formatted file wide.thorax.gif to the data entry for taxon 5, character 2.

The picture or source descriptor must be a single NEXUS word. If the picture contains NEXUS whitespace or punctuation, it needs to be surrounded by single quotes, with any contained single quotes converted to a pair of single quotes.

Most graphics formats do not describe pictures using standard text characters. For this reason many images cannot be included INLINE in a NEXUS command unless they are converted into text characters. The ENCODEd subcommand specifies the conversion mechanism used for inline images. The following command shows a UUencoded Compuserve® Graphics Interchange Format (GIF) file:

E>2!''249#;VYV97)T97(@,BXS+C(@;V8@36%R(#8L(#\$Y.3,`.R`@ end';

(Although it may not appear so, all of the text from the "begin" to the "end" is a single NEXUS word.)

FUTURE OF THE NEXUS FORMAT

The NEXUS format will evolve as required by the evolution of computer programs. A number of new elements to the format are being developed. For example, the USERTYPE command in the assumptions block, currently containing assumptions relevant for parsimony analyses, will likely be replaced by a more general command that will include cost matrices, character state trees, probabilistic rate matrices, and so on, useful for parsimony, maximum likelihood, and distance methods. There will be new set operators added, a GRAPH format for describing reticulate phylogenies, and the ability to name and refer to separate blocks of characters, among other features. The most recent version of the file format, including additions and proposed additions to the format, is available on the World Wide Web at http://phylogeny.arizona.edu/nexus/nexus.html. This site should be checked by those who are considering adopting or extending the NEXUS format.

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APPENDIX 1

GLOSSARY

Block.—A block is a sequence of NEXUS commands, starting with a BEGIN command, and ending with the next END command. (In MacClade, PAUP, and COMPONENT the ENDBLOCK command has been used as a synonym of the END command.)

Character number.— This is the number of a character, as defined by its position in a CHAR-LABELS command or MATRIX in a CHARACTERS or DATA block. For example, the third character listed in a CHARACTERS block is character number 3.

Character-set.— This is a set of characters, described in a *list* (see below). A character-set can be given a formal name using the CHARSET command (see SETS block description).

Character transformation type.—A character type is a specification of the costs and rules imposed on specific state-to-state changes in a parsimony analysis.

There are several predefined types: UNORD (unordered), ORD (linearly ordered reversible), IRREV, IRREV.UP, IRREV.DN (all linearly ordered irreversible), and DOLLO, DOLLO.UP, and DOLLO.DN (variants of Dollo; Swofford, 1989; Maddison and Maddison, 1992). In addition, Mac-Clade has the predefined type STRAT (Fisher's stratigraphic type [Fisher, 1992; Maddison and Maddison, 1992]). UNORD specifies a cost matrix in which all state to state changes cost 1 step; this is the assumption embodied by Fitch parsimony (Fitch, 1971, and Hartigan, 1973). ORD specifies a cost of change between two states to be the absolute value of the difference in their state numbers (e.g., a change from state 3 to state 7 costs 4 steps); this is the assumption in Wagner parsimony (Farris, 1970; Swofford and Maddison, 1987). IRREV and IRREV.UP are equivalent, and specify the same cost of change as ORD except that all changes that would involve a decrease in state number are disallowed. IRREV.DN is identical to IRREV except that the changes that are disallowed are those that involve an increase in state number. DOLLO and DOLLO.UP are equivalent, and specify the same cost of change as ORD except that each increase in state number can occur only once. DOLLO.DN is identical to DOLLO except that the changes that can only occur once are decreases in state number.

Command.—A command is a collection of tokens, terminated by a semicolon. Commands cannot contain semicolons, except as terminators, unless the semicolons are contained within a comment, or within a quoted token consisting of more than one text character. If a delimiter between command sections is needed, it should be a comma (for examples, see STATELABELS in the CHARACTERS block, WTSET in the ASSUMPTIONS block).

Command comment.—A comment containing a command is a command comment. Comments containing commands are indicated by a special symbol (\setminus or &) as the first text character within the comment. Comments whose first text character is \setminus house commands relating to display of tokens (see Display of Text, above). Comments whose first text character is & are of the form [&*word* ...], where "*word*" is an unquoted NEXUS word. The word contains the command (e.g., [&U] indicating unrooted trees in the TREE command).

If the word begins with & (so that the comment itself is of the form "[&&...]", then the rest of the word indicates to which processing programs the command applies (e.g., [&&PAUP *command*]). "&&MC" and "&&PAUP" are reserved for MacClade and PAUP, respectively.

In general, the command contained in such a command comment should not affect the nature or interpretation of the NEXUS file elements contained outside of comments. For example, one should not add a command comment to a WTSET that will tell some program to set the weights to be the reciprocal of the weights listed, as this information would be ignored by most programs, leading to different interpretations of the same file by different programs. Examples of the information that could be housed in these command comments are the status of a tree search, or the positions of windows on screen.

There are two exceptions to this rule, in the TREE and CSTREE commands. For these two commands, comments are allowed that can actually change the interpretation of the tree and character state tree. The [&U] and [&R] commands are of this nature (see the TREES block), and similar commands might be invented in the future. These exceptions are allowed in the TREE and CSTREE command because adding information to a tree description outside of comments would cause the file to be non-compliant with the Newick tree standard, and support of this broader standard is more desirable than strict adherence to the NEXUS standard.

Comment.— Any comments in the data file are delimited by brackets:

[please ignore me]

Comments are ignored by all programs, with few exceptions. These exceptions can occur if the first text character inside the comment is any of !, &, %, /, \, or @. Comments that begin with an exclamation point are output comments (see below); those beginning with any of the rest are command comments (see below).

Comments may contain any combination of dark character and whitespace (which need not be organized into NEXUS tokens). They can extend over multiple lines (that is, they can contain newline characters). Quotes are ignored within these comments. All text characters are treated equally within such comments, with the exception of open and close brackets. An close bracket will terminate a comment. Comments can be nested:

[this is a comment [nested inside]]

If a program encounters an open bracket within a comment, it will expect two close brackets to close both the main comment and the nested comment.

Comments do not break tokens. Thus, ASSUMP[comment]TIONS is processed as ASSUMP-TIONS.

Left and right brackets are not treated as symbols that delimit a comment if they are found within a quoted token.

Dark characters.—All text characters that are not whitespace are darkspace.

Data block.—A data block is any of the following blocks: CHARACTERS, UNALIGNED, DIS-TANCES, DATA.

Entry.— An element in a data matrix, consisting of the information for one character for one taxon or, for a distance matrix, the information for one taxon by taxon comparison, is a matrix entry. It may consist of a single symbol, e.g. "A", for instance if the data are discrete and the taxon shows a single character state. On the other hand, it may include a long list of information, such as the average value from the taxon, the variance, and sample size for continuous data.

List.—The description of a set of objects (taxa, characters, trees) is a list. Elements in the list are separated by whitespace. If a series of contiguous elements are to be listed, a hyphen can be used in place of the intermediate values. If all elements are to be listed, then the word "ALL" can be used in place of listing the numbers. The word "REMAINDER" designates all elements not previously referred to in a command. On each of the following lines is a legal list:

A period represents the number of the last element; thus, if there are 320 characters in the matrix, the character-set described by 56-. means characters 56 through 320 inclusive.

If, immediately following a contiguous range of numbers, one places a backslash, followed by an integer *n*, this indicates that only every *n*th element, beginning at the first element in the range, is to be included in the set. For example, the character-set 2-12\3 is equivalent to 2 5 8 11, and ALL\2 means every second character, beginning with character 1. This is especially useful for protein-coding nucleotide sequence data for which one might wish to specify, say, every third position.

Names of elements can also be used in the list. For example, if character 3 has been given the name eye color, and character 5 leg length, then

```
'eye color'-'leg length'
```

specifies characters 3, 4, and 5.

For unaligned data, the only valid list of characters is "ALL".

If sets of elements have been formally defined by a CHARSET, TAXSET, or TREESET command in the SETS block, then the names of those sets can also be used in a subsequent list. For example, if the CHARSET larval has been defined in the SETS block, a subsequent character-set could be "2-5 larval" which would mean characters 2– 5 plus all those contained within the CHAR-SET larval.

Newline character.— A newline character consists of any of the following: (1) single text character for carriage return (ASCII 13), (2) linefeed (ASCII 10), (3) carriage return followed immediately by a linefeed, or (4) other text characters used on the current operating system to designate a new line in a text file. Options 1– 3 are valid for all programs that are reading ASCII text files, no matter what operating system they are running under.

Object.— An option is any of the many entities defined in a NEXUS file that are or could be given a name. The only type of objects that need not be given a name are characters and states; for all others, names are given when they are defined. Objects include taxa, characters, trees, genetic codes, taxon-sets, etc. The NEXUS standard allows for creation of new kinds of objects.

Object definition command.— A number of commands consist of definitions of objects; these definitions follow a standard format:

category [*] object-name [(format)]=description;

where the kind of object is given by *category*, the name of the object is given by the token *object name*, optionally followed by tokens, enclosed in parentheses, describing the format of the following description, an equal sign, and then the description. The standard defines no limit to the length of the name, although individual programs might impose restrictions. If the object is to be considered the default object of its category, then this can be indicated by an asterisk between the category and object name. (This is not used for categories in which there are predefined objects, e.g., USERTYPE and GENETICCODE.) If more than one object is so designated, then the last one encountered is taken to be the default.

Some examples are as follows:

```
WTSET * myweights=2:1 3 5;
```

This is equivalent to

WTSET * myweights (VECTOR)=2 1 2 1 2;

A number of object definitions can be written in standard or vector formats. STANDARD format is a listing of values (weights, types, and so on), each followed by a character or taxon-set including those characters or taxa that are to be of that value, or, if no such value is needed (e.g., EXSETS), then the format is just a list of taxa or characters.

In the following

WTSET myweights (STANDARD)=2:1 3 5;

the weight value 2 is assigned to characters 1, 3, and 5. Different value assignments are separated by commas:

WTSET myweights (STANDARD)=2:1 3 5, 0:4;

This assigns to the first 5 characters weights of 2, 1, 2, 0, and 2.

VECTOR definitions consist of a string of values:

WTSET myweights (VECTOR)=2 1 2 0 2;

The first value in a vector format is assigned to the first object (character, taxon, etc.), as appropriate, the second value to the second object, and so on. Unless specific exceptions are made (i.e., for the commands EXSET, CHARSET, STATESET, TAXSET, TREESET), or unless the NOTOKENS formatting option is used, a VECTORED object definition requires whitespace between the values.

Output comments.— If the first character within the brackets is an exclamation mark (!), then these are considered output comments. These may optionally be displayed, in some fashion, to the user of the program. Consider the following example:

[!this is an output comment]

PAUP deals with these by rewriting them to the output; MacClade displays them in the About *datafile* or About Trees windows if they are within the TAXA, CHARACTERS, DATA, or TREES block, respectively (if they are not in these blocks, MacClade ignores them).

Punctuation.— Any of the following text characters are considered punctuation at some times: () [] {} / \, ; := * ' "` + - <> The following punctuation have special properties: [] do not break a word; + and - are allowed as state symbols, but none of the rest are allowed; - is considered as punctuation, except were it is the minus sign in a negative number.

State range.— A description of the range of values for a continuously valued character is the state range. If there is a single state, the state range is a single real value, e.g., 0.86. If there are multiple values, the state range is indicated by placing a tilde between the boundary values, e.g., 0.86~1.12.

State-set.— A description of the set of states of a discrete character is the state-set. If there is a single state in the set, the state-set is the state symbol, e.g., 0.

In some areas of the file, if there is more than one state in the set, then the state-set consists of the state symbols inside of parentheses or braces, e.g., (012) or (0~3) or $\{013\}$. Parentheses indi-

cate that the taxon is polymorphic, whereas braces indicate that the states of the taxon are uncertain. For example, (012) indicates 0 and 1 and 2, whereas {012} indicates 0 or 1 or 2.

Tilde indicates all intermediate states are included, according to the ordering of the states as determined in the list of state symbols (see *state symbol*).

For molecular sequence data, there are some symbols that are defined to represent statesets with multiple states. For example, R is the state-set that is equivalent to the state-set {AG} (see *state symbol*).

DATATYPE=STANDARD sets the default acceptable symbols to 0 1 (these symbols may be altered using the SYMBOLS subcommand, as described in the specification of the FORMAT command of the CHARACTERS block, above).

If DATATYPE is set to DNA, the SYMBOLS list is set to ACGT and the following equate macros (see EQUATES subcommand), corresponding to the IUPAC (IUB) ambiguity codes, are defined:

R	= {AG}	[puRine]	H =	{ACT}	[not G]
Y	= {CT}	[pYrimidine]	B =	{CGT}	[not A]
Μ	= {AC}	[aMino]	V =	{ACG}	[not T]
K	= {GT}	[Keto]	D =	{AGT}	[not C]
S	= {CG}	[Strong]	N =	{ACGT}	[unkNown]
W	= {AT}	[Weak]	X =	{ACGT}	[unknown]

Lowercase symbols have the same meaning as their uppercase equivalents.

If DATATYPE=RNA, the SYMBOLS list is set to ACGU and the same set of equate macros are defined, except that U is substituted for T.

If DATATYPE=NUCLEOTIDE, the SYMBOLS list is set to ACGT, with U treated as synonymous to T.

If DATATYPE=PROTEIN, the symbols list is set to ACDEFGHIKLMNPQRSTVWY*, corresponding to the standard IUB single-letter amino acid codes:

A=	ala	[alanine]	Ν	=	asr	ı [asparagine	2]
С	=	cys	[cysteine]	Р	=	pro	[proline]
D=	asp	aspartic a	icid]Q	=	gln	[glutamine]	

E=	glu [glutamic acid]R	=	arg [arginine]
F=	phe [phenylalanine]S	=	ser [serine]
G=	gly [glycine] T	=	thr [threonine]
H=	his [histidine] V	=	val [valine]
I =	ile [isoleucine]W	=	trp [tryptophan]
K=	lys [lysine] Y	=	tyr [tyrosine]
L=	leu [leucine] *	=	stop[chain termination]
M=	met [methionine]		

In addition, two equate macros are defined:

 $B = \{DN\}[asx=asp \text{ or } asn]$

 $Z = {EQ} [glx=glu \text{ or } gln]$

Lowercase symbols have the same meaning as their uppercase equivalents.

Symbol.—A single dark text character is a symbol.

Taxon number.— The taxon number is the number of a taxon, as defined by its position in a TAXLABELS command, or in a MATRIX in a CHARACTERS, UNALIGNED, or DISTANCE block (in which NEWTAXA is invoked in the DIMENSIONS command), or in a MATRIX in a DATA block. For example, the third taxon listed in TAXLABELS is taxon number 3.

Taxon-set.— A set of taxa, described in a *list*, is a taxon-set. A taxon-set can be given a formal name using the TAXSET command (see SETS block description).

Token.— A token is a NEXUS word or punctuation.

Tree number.— The number of a tree, as defined by its position in a TREES block, is a tree number.

Tree-set.— A tree-set is a set of TREEs, described in a *list*. A tree-set can be given a formal name using the TREESET command (see SETS block description).

Whitespace.—Whitespace consists of any of the following text characters: tab (ASCII 9), newline character (as defined in this glossary), blank (ASCII 32), as well as ASCII 0–6.

Word.—Any string of text characters bounded by whitespace or punctuation is a NEXUS word. Unless the word is delimited by single quotes, the string cannot contain whitespace or punctuation. If the first character of a word is a single quote, then the word ends with the next single quote (unless that single quote is in a pair of consecutive single quotes; if so, then the word ends at the first unpaired single quote). Any character, including punctuation and whitespace,

may be contained within a quoted word. A word cannot consist of only whitespace and punctuation.

On each of the following lines is a single legal word:

Bembidion
B._zephyrum
'John''s sparrow (eastern)'

Underscores are considered equivalent to blank spaces, except that underscores are dark characters, and blank spaces are whitespace. Thus, a program encountering B._zephyrum and 'B. zephyrum' should judge them to be identical.

Any doubled single quotes within a quoted word should be converted to single quotes within the program. Thus, 'John''s' would be treated internally within a program as John's. Of course, if the program writes out such a word to a NEXUS file, the quote should be redoubled, and quotes should be written around the word: 'John''s'.

A string of dark characters is broken into several words by whitespace and any punctuation other than brackets, unless the string is surrounded by quotes. Thus, [\i]Bembidion_velox[\p]_Linné consists of one word, as does '- - A single word', whereas two()words consists of four tokens: the word two, two punctuation characters, and the word words. FIGURE 1. Two character state trees and the NEXUS commands that define them. The first tree has an unnamed state.