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Program by

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User's Manual by

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PAUP is licensed to individual users for the sole purpose of facilitating the scientific research of the licensee. This software may be used in more than one location or by more than one person provided that there is **no possibility** that it will be used by two or more people simultaneously. Generally, this qualification means either of the following: (1) the program may be used by a **single researcher** on any number of machines in his or her possession, or (2) the program may be installed on a **single machine** and used by one or more individuals who have access to that machine.

If you cannot abide by the terms of this agreement, please return the software to the Illinois Natural History Survey immediately for a refund of the distribution fee.

Suggested Citation

PAUP 3.1 is in many ways comparable to a monographic article. In its over 70,000 lines of code, PAUP implements numerous original concepts and ideas and contains many new algorithms. For these reasons, citation of the program in a book format is recommended:

> Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1 Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Table of Contents

PRELIMINARIES

This manual is for version 3.1 of PAUP for the Apple Macintosh. It also serves as preliminary documentation for the IBM-PC and "Portable" (mainframe and workstation) releases of Version 3, which as of this writing have still not been released. The manual is not as polished as I had hoped it would be at this point. Hopefully, the weaker sections will be enhanced by the time version 3.2 is released. I apologize for not including an index. The manual is still evolving too rapidly at this point to justify the considerable amount of time that indexing would require. Please report any errors or other significant omissions.

Unfortunately, I have not yet been able to incorporate all of the many useful features that users of PAUP have requested. I will continue to plug away on the depressingly long "wish list". The most significant new feature that I did not get working in time for this release are Archie-Faith-Cranston permutation/randomization tests (PTP, T-PTP). Randomization tests will almost certainly be included in the next release. You're welcome to check the anonymous FTP server (see below) for test versions containing these and other new features, but of course I can make no promises as to when these test versions will be made available.

David L. Swofford
28 March, 1993

ACKNOWLEDGMENTS

The number of people who have had a significant impact on the development of PAUP continues to grow, and I must apologize for failing to mention everyone who has suggested features or provided assistance. I am especially grateful to Julian Humphries, an important source of useful ideas and expertise during the development of earlier versions. It was through Julian's prodding and assistance that the first interactive mainframe version of PAUP was developed. He initially convinced me to port the program to microcomputers and was instrumental in the development of the first IBM PC version.

Several of the newer features of PAUP have been heavily influenced by many hours of lively discussion with David and Wayne Maddison and by their truly outstanding program "MacClade." David served as a consultant during the early stages of development of the Macintosh version of PAUP and has patiently provided advice, assistance, and testing throughout the development of PAUP Version 3. In addition to countless ideas for interface and documentation improvements, David suggested significant improvements in tree filtering (filtering by constraints), stepwise taxon addition (random-addition-sequence enhancements), and tree-file operations (most importantly, the use of Boolean operations for combining trees in memory with trees in a file). Wayne was also heavily involved in the testing and made many additional suggestions for improvements, including introducing me to the notion of user-defined character types and sharing algorithms for dealing with multistate taxa. I have also benefited tremendously from discussions with Joe Felsenstein, particularly with respect to the branch-and-bound algorithm. The unselfishness of these individuals (who, after all, have a vested interest in the advancement of their own programs) is a constant source of inspiration to me.

Many other individuals have reported bugs, made helpful suggestions and/or provided stimulating ideas. These include Larry Abele, Vic Albert, Jim Archie, Paul Berti, David Cannatella, Jonathan Coddington, Joel Cracraft, Mike Cummings, Chris Darling, Scott Davis, Ron DeBry, Doug Eernisse, Bill Fink, George Gutman, David Hillis, Kent Holsinger, Bob Jansen, Rick Mayden, Chris Meacham, David Mindell, Mike Miyamoto, Gavin Naylor, Mark Norell, David Penny, Norman Platnick, Winston Ponder, Andrew Simons, Greg Spicer, Beth Stewart, Sherman Suiter, David Stock, Rytas Vilgalys, and Jim Woolley.

Three members of the staff of the Illinois Natural History Survey, where all previous versions of PAUP have been developed, deserve special recognition. Angie Young has cheerfully and conscientiously taken care of the day-to-day distribution matters and Mary Lou Williamson has flawlessly kept the financial matters in order. Larry Page, director of the Center for Biodiversity, provided encouragement and support throughout the development of earlier versions.

Finally, I thank my wife, Ruth Swofford, for enduring many lonely evenings while I wrote and debugged code and for willingly and unselfishly providing assistance in many phases of the development of PAUP.

ABOUT THIS MANUAL

Organization

The manual is divided into four chapters, the first three of which are common to all implementations of PAUP. The first chapter presents background material relevant to the capabilities of the program. We urge all users to become familiar with this material before using the program. Chapter 2 provides basic instruction on the use of PAUP's primary functions and other features. Chapter 3 provides a detailed reference to PAUP commands and input file organization. Chapter 4 is specific to each implementation (i.e., Macintosh, Unix, etc.), and describes details of the user interface that are specific to that implementation.

Typically, you will refer to Chapter 2 to find out what PAUP can do and get a general idea of how to do it. When you need more specific information, you will then refer to Chapters 3 and/or 4. Admittedly, this organization is not ideal. From a user's point of view, it would be much nicer if, for example, the instructions for using the menu/dialog-box interface for setting character types were presented at the same time that the equivalent command-based method was described. Unfortunately, maintaining *n* different copies of the manual for *n* different implementations would then become a nightmare from *our* point of view. By isolating the implementation-specific portion of the manual in a single chapter, we only need to maintain a single version of the remainder of the manual.

Typographical and notational conventions

The following typographical conventions are used throughout the manual:

Important term	Boldface italics are used to highlight important terms the first time they are defined.
`USER INPUT`	This font is used to represent input supplied by the user, either from a file or the command-line.
`PAUP output`	This font is used to identify output generated by PAUP.
Key	This font is used to represent a key on your keyboard.

COMMAND **Menu Command**	Command names are shown in boldface type. Commands that are used from input files or typed on the command line are indicated using all upper-case characters. Commands available for selection from menus are shown in mixed upper and lower case.
Dialog item	This font is used to refer to buttons, checkboxes, and other items contained in dialog boxes or elsewhere on the screen.

In descriptions of data-file and command formats, the following notation is used:

ITEMTEXT	Items typed entirely in uppercase are to be entered as indicated. Input of PAUP commands is case-insensitive, however, so you may enter command names, keywords, etc., in any combination of upper- and lower-case characters. In addition, PAUP allows abbreviations of command names and keywords to the shortest unambiguous truncation. Note that other NEXUS-conforming programs (MacClade, in particular; see below) may not accept these abbreviations.
[Optional-item]	Brackets around an item means that the item is optional. Square brackets may be nested as in [OptionalItem [AnotherOptionalItem]] In this case, each level of nesting depends on the specification of the item at the next higher level. You should not include these brackets when you enter the command.

{ A | <u>B</u> }	Two or more items enclosed in curly braces and separated by vertical bars indicate a set of mutually exclusive choices. The underlined item (if any) indicates the default setting. You should not include the braces or vertical bar when you enter the command.
variable	Letters, words, and symbols shown entirely in lowercase italics represent variables for which specific information must be supplied by the user when the command is entered.
item … <item1 item2>…	An ellipsis indicates that the preceding item may be repeated one or more times. If a group of items may be repeated sequentially, the group is surrounded by angle brackets.
[ ] { } … | _	These symbols are used to define the command format (see above). Unless otherwise indicated, they should not be typed when the actual command is entered.
; : . , " ' ( )	Other punctuation should be entered as shown in the command description.

Why read the manual?

Many users of microcomputer software take the position that the manual is something you refer to as a last resort. It is sometimes suggested that "well-written" software largely eliminates the need for a manual by providing an intuitive, menu-based interface that guides the user. While this idealized state-of-affairs may be strictly true with respect to the mechanics of running the program, a thorough reading of the manual is essential in order to understand many of the biological aspects of PAUP. If you do not read the manual, you will have no way of discovering many useful features and shortcuts available in PAUP. More importantly, you may be unaware of assumptions that the program is making during its calculations. We cannot emphasize strongly enough the importance of reading the User's Manual carefully, painful as this may be.

TECHNICAL SUPPORT

Assistance with the use of PAUP and interpretation of output will be provided only to licensed users. If you are unable to resolve a problem by experimentation or need information that is not available in the User's Manual, you may reach me in any of the following ways:

E-mail:	paup@onyx.si.edu	(Internet)
Standard Mail:	David L. Swofford Laboratory of Molecular Systematics MRC 534, MSC Smithsonian Institution Washington, DC 20560 USA	
Express courier:	David L. Swofford Laboratory of Molecular Systematics Smithsonian Institution Museum Support Center 410 Silver Hill Road Silver Hill Road Suitland, Maryland 20746	
FAX:	(301)238-3059 (Note that I cannot guarantee a FAXed response)	

Use of e-mail is vastly more likely to generate a quick response than the other methods.

Please provide the following information in your communication.

1. The exact wording of any error messages that you have received.

2. If a crash occurred, the sequence of events prior to the crash (e.g., commands issued, etc.).

3. The exact version number of your program. You can obtain the version number from the opening screen that is displayed when you first start the program.

4. A copy of your data file.

ANONYMOUS FTP SERVER FOR PAUP SUPPORT

We have set up an FTP server to support PAUP. We will periodically post updated programs, test versions, documentation files, and other announcements there. To use the FTP server, log in to **onyx.si.edu**

(160.111.64.54) as "anonymous" and enter your e-mail address when prompted for a password. The overall structure of the ftp directories is described in a README file in the root directory.

When minor bug-fix releases are issued, we will post an "updater" program that will convert any version of PAUP/Mac 3.1 to the new version. The updater program will only work if you already have a copy of PAUP 3.1.

Chapter 1 BACKGROUND

This chapter provides general background on the concepts underlying the methods used in PAUP. Specific information on how to use the program follows in later chapters.

GENERAL CONCEPTS

PAUP is a program for inferring phylogenies from discrete-character data under the principle of ***maximum parsimony***. Parsimony methods search for ***minimum-length trees***:[1] trees that minimize the amount of evolutionary change needed to explain the available data under a prespecified set of constraints upon permissible ***character*** changes. The best known discrete-character parsimony method, often called ***Wagner parsimony*** (Kluge and Farris, 1969; Farris, 1970) treats ***binary*** or ***ordered multistate*** characters and permits free irreversibility. Multistate characters may also be left ***unordered*** (i.e., any ***character state*** is permitted to transform directly into any other state), sometimes called ***Fitch parsimony*** after Fitch (1971). Other parsimony variants place additional restrictions on the types of character-state changes that are allowed. ***Dollo parsimony*** (Farris, 1977) permits each ***derived***, or ***apomorphic***, character state to originate only once. ***Camin-Sokal parsimony*** (Camin and Sokal, 1965) prohibits reversals from a derived state to a relatively more ***ancestral***, or ***plesiomorphic***, condition. Also introduced with version 3.0 of PAUP is a

[1]Some authors (e.g., Wiley, 1979; Nelson and Platnick, 1981) prefer to distinguish between the terms *tree*—a hierarchical statement regarding genealogical (ancestor-descendant and sister-group) relationships—and *cladogram*—a branching diagram depicting patterns of character distribution or nested sets of synapomorphies. This distinction is purely terminological and probably inappropriate (Hendy and Penny, 1984). PAUP may be used to construct to construct either trees or cladograms, the only difference being how the user interpets the output. "Tree" will be used to refer to both evolutionary trees and cladograms thoughout this manual, with apologies to those who find this usage unacceptable.

procedure based on the work of David Sankoff and his collaborators (Sankoff and Rousseau, 1975; Sankoff and Cedergren, 1983) that allows the user to specify the cost associated with a change from each character state to each other state. Each of these methods is discussed in more detail under "Character Types."

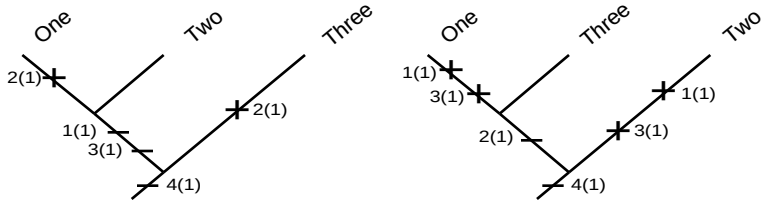
Minimization of the total tree length is equivalent to seeking trees that imply the least amount of homoplasy, or similarity not directly attributable to common ancestry. Homoplasies or "extra steps"—reversals, parallelisms, and convergences—constitute *ad hoc* assumptions required to bring observations into conformity with the "simpler" explanation that possession of the same character state in two or more taxa is due solely to inheritance from a common ancestor. A word about the relationship between computerized minimum-length-tree approaches and the manual methods used in traditional cladistics [e.g., Hennig (1966); Wiley (1981)] is in order. The latter ("Hennigian") methods place a heavy emphasis on *a priori* assessment of character ***polarity***, the specification of which of the observed character states represents the ancestral condition in the group under study. *If* character polarities could always be reliably assessed, and *if* there were no homoplasy in the data, then phylogeny reconstruction would amount to nothing more than grouping taxa according to shared derived character states (***synapomorphies***), with no relevance being attached to the sharing of ancestral states (***symplesiomorphies***). That is, all of the taxa that possess a particular derived character state could unambiguously be interpreted as descendants of the ancestor in which the state originated and taxa sharing the ancestral condition could be definitively excluded from that group. Inevitably, however, character conflicts or incompatibilities arise. For example, consider the data shown below:

Table 1. Hypothetical data for example discussed in text.

	Character			
Taxon	1	2	3	4
One	1	1	1	1
Two	1	0	1	1
Three	0	1	0	1
Four	0	0	0	0

For now, we will assume that 0 represents the ancestral state for each character. Thus, we observe that taxa *One* and *Two* share the derived state for characters 1 and 3, while taxa *One* and *Three* share the derived state for character 2. Consequently, if *One* and *Two* were, in reality, sister taxa (tree *A* below), the possession of state 1 for character 2 in *One* and *Three* would be a homoplasy, whereas if *One* and *Three* were sister taxa, the

derived characters 1 and 3 shared by *One* and *Two* would be homoplasies (tree *B* below)[2]



Tree *A* Tree *B*

Two trees for the data of Table 1.

Faced with such a conflict, a practitioner of traditional manual methods would usually decide between these two alternative hypotheses of relationship by invoking the parsimony criterion and therefore choose tree A, which requires fewer assumptions of homoplasy. But this choice is exactly the one that would have been made by the computer method as well, since the tree requiring the least homoplasy would also be the shorter tree (tree A = 5 steps; tree B = 6 steps). Clearly, there is no real difference between computerized methods and manual methods for choosing trees under the parsimony criterion. Two factors can cause the methods to differ in actual practice, however. The first lies in subjectivity introduced by the investigator, who may prefer a tree requiring two or three extra steps in a character assumed to be unreliable or "plastic" over a tree requiring a single extra step in a "better" character. The second is that the human brain is unable to deal effectively with the complexity of large and/or noisy data sets.

The above example also illustrates one reason why excessive concern with *a priori* assessment of character polarities is unwarranted. When incongruent character distributions occur as in this case, the sharing of a derived character state among two or more taxa need not indicate close relationship of those taxa. The problem is accentuated when the outgroup being used for the polarity assessment is heterogeneous. In such cases, parsimony can provide the basis for assigning character polarities, but the most parsimonious state assignment(s) for the most recent common ancestor of the ingroup depends upon the overall structure of the tree (Farris, 1982), including the relationships of the outgroup taxa (Maddison et al., 1984). Thus, the only fully satisfactory recourse is to infer the topology of the tree and the character polarities simultaneously, rather

[2]Of course, a third possibility is that neither *One* and *Two* nor *One* and *Three* represent sister taxa, with all shared derived states being homoplasies.

than going through the two-stage process of assigning polarities first and then estimating the tree.

NOTE: The concepts of character order and character polarity are often confused. The former defines the nature of permitted character-state transformations, whereas the latter refers to the *direction* of character evolution. Specifying how character states are *ordered* with respect to each other is not the same as determining which character state is ancestral.

For further amplification of these points, see the sections entitled "Character Types" and "Outgroups, Ancestors, and Roots" later in this chapter.

TREE TERMS

The most general terminology for describing the various components of trees is derived from the field of graph theory [e.g., Harary (1969); Gould (1988)]. Some of this terminology is reviewed briefly here, although formalism is minimized. A graph consists of a set of vertices and a set of edges, where each edge is a line joining a pair of vertices. Two distinct vertices are adjacent if they are joined by an edge; the edge is said to be incident to those vertices. The degree of a vertex is the number of edges with which the vertex is incident. A path is a sequence of distinct edges such that each edge shares one vertex in common with the preceding edge in the sequence and the other vertex in common with the next edge in the sequence. A path connecting a pair of vertices can also be described as a sequence of vertices, with each vertex adjacent to the preceding vertex in the sequence. A graph is connected if there is at least one path from any vertex to any other vertex. A cycle is a path that connects a vertex to itself in which no vertex is repeated. Most importantly for our purposes here, a tree is a connected acyclic graph.

Vertices and edges on trees are often called ***nodes*** and ***branches***, respectively, and this terminology will be used throughout the manual. Nodes are called ***terminal nodes*** if they have degree one and ***internal nodes*** otherwise. Unfortunately, the above terminology is not standardized, and much synonymy exists. Terminal nodes are also called ***tips*** or ***leaves***; branches (edges) are also called ***links***, ***segments***, ***intervals***, or ***internodes***. Terminal nodes corresponding to biological taxa are often called ***Operational Taxonomic Units*** (OTUs) or simply ***terminal taxa.*** Similarly, internal nodes are sometimes referred to as ***Hypothetical Taxonomic Units*** (HTUs) or ***hypothetical ancestors***.

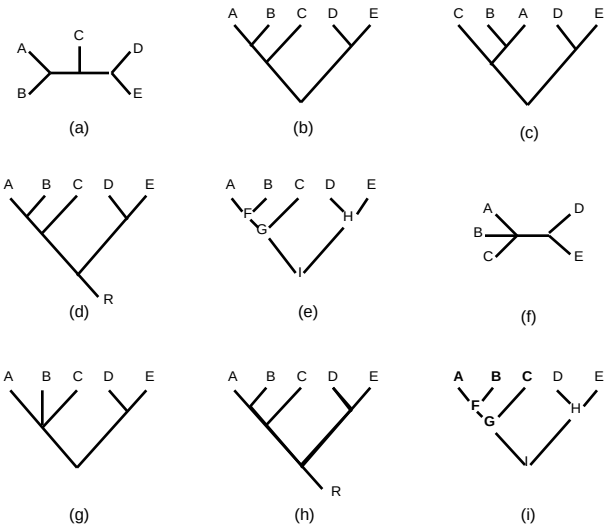
A tree is ***binary*** if none of its internal nodes has degree exceeding three. If a binary tree has at most one vertex of degree two, it is also ***full***. Full binary trees are sometimes called ***strictly bifurcating*** or ***fully dichotomous***

trees. A node having degree greater than three (e.g., one immediate ancestor and three immediate descendants; see below) corresponds to a ***polytomy*** or a ***multifurcation***, and trees containing one or more polytomies are sometimes called ***polytomous*** (nonbinary) trees.

A tree is ***rooted*** if there is a special node (the root) that imparts a direction upon the tree such that nodes lying on a path connecting the root to any other node are ***ancestors*** of (ancestral to) nodes in the path that are further from the root and ***descendants*** of (descendant to) nodes closer to the root. Typically, the root is an internal node having degree two, however in PAUP the root is usually considered to be an additional terminal node positioned at the base of the tree. A ***subtree*** is a connected subset of a tree. A subtree consisting of all of the nodes and branches that descend from a particular internal node *v* on a rooted tree is called the "subtree rooted at *v*" or simply "*v*'s subtree." Phylogeneticists often refer to the subtree rooted at an internal node as a ***clade***.

A tree is ***ordered*** if the branches incident to each node are assigned in some nonarbitrary (fixed) order. If the order in which the branches are connected is irrelevant or arbitrary (as is generally the case for phylogenetic trees), then the tree is said to be ***unordered***. (Although the trees computed by PAUP are unordered in the sense that free rotation of subtrees around internal nodes does not affect the relationships implied for terminal taxa, an order may be imposed based on other criteria purely for output purposes.) Trees are also classified according to the way in which nodes are labeled. In phylogenetic analysis, trees are usually considered to be ***terminally labeled***. That is, the terminal nodes correspond exactly to the biological taxa under study, but the labeling of the internal nodes is arbitrary. If all internal nodes are associated with actual objects (e.g., fossil taxa) and are not merely hypothetical constructs, the trees are ***completely labeled***. Note that although some programs (e.g., MacClade) may permit actual taxa to occupy ancestral positions, PAUP considers only terminally labeled trees; if a taxon is assigned to an internal node (e.g., in a user-specified tree description) it is "popped out" to a terminal position.

Examples of the types of trees discussed above are shown below.



*Examples for tree terminology. (a) Unrooted binary tree. (b) Rooted binary tree (rooted at an internal node of degree 2), (c) Another rooted binary tree. Trees **b** and **c** are equivalent as unordered trees but distinct as ordered trees. (d) Binary tree rooted at terminal taxon R. (e) Completely labeled rooted binary tree. (f) Unrooted nonbinary tree. (g) Rooted nonbinary tree. (h) Path between terminal nodes B and D (heavy lines). (h) Subtree of node G (heavy lines and boldface).*

The total number of distinct, unrooted, terminally labeled, strictly bifurcating, trees for *T* terminal taxa is given by the formula

$$B(T) = \prod_{i=3}^{T} (2i - 5) \qquad (1)$$

(Cavalli-Sforza and Edwards, 1967; Felsenstein, 1978b). Table 2 shows the value of *B*(*T*) for several values of *T*. Obviously, the number of trees quickly becomes quite large as the number of taxa increases.

Table 2. The number of unrooted, binary, terminally labeled trees, B(T)*, for* T *terminal taxa.*

T	*B*(*T*)
3	1
4	3
5	15
6	105
7	945
8	10,395
9	135,135
10	2×10^6
15	8×10^{12}

20	2×10^{20}
50	3×10^{74}

CHARACTER TYPES

PAUP implements different parsimony variants through the declaration of a ***character type*** for each character included in the data matrix. Available types are ***ordered*** (Wagner), ***unordered*** (Fitch), ***Dollo***, ***irreversible*** (Camin-Sokal), and ***user-defined***. Any combination of character types can be assigned to the characters in the data matrix. The character types (and weights) that are specified constitute the *a priori* assumptions that are in effect for a particular analysis.

Character types may be classified as either undirected or directed. An undirected character is one in which for every pair of states a and b, the "cost" in tree length is the same for the transformations $A \rightarrow B$ and $B \rightarrow A$. All of the standard character types are undirected except for irreversible. Stepmatrix characters (defined below) are undirected if and only if the stepmatrix is symmetric, i.e., $d_{ij} = d_{ji}$ for all pairs of character states i,j (see below). The "directedness" of the characters bears a close relationship to whether PAUP stores trees as rooted or unrooted trees. When directed characters are in effect, trees must be treated as rooted trees, because the position of the root may affect the length of the tree. On the other hand, if all characters are undirected, PAUP ordinarily considers the trees to be unrooted, since the length of the trees is independent of the position of the root. Unrooted trees need not be explicitly rooted before requesting subsequent output. PAUP roots trees automatically using the currently defined outgroup (or via Lundberg rooting) whenever necessary.

In addition, characters are either ***polarized*** or ***unpolarized***. Polarized characters are those for which the state ancestral to all other states is prespecified. If the ancestral state is not specified or is designated as "missing," the character is said to be unpolarized.[3]

A character type may formally be described as a ***weighted directed graph*** (***weighted digraph).*** The vertices of the graph correspond to character states, and the edges of the graph are arrows corresponding to permissible character-state changes. The weight of each edge is the "cost" associated with the transformation from one character state to another.

[3]Unfortunately, this terminology is unstandardized. Meacham (1984) and others use "directed" vs. "undirected" in the same sense that I use "polarized" vs. "unpolarized". My usage of "directed" is more consistent with graph theoretic concepts (see next paragraph).

Ordered (Wagner) Characters

"Ordered" characters are those typically associated with the "Wagner method." The character states are ordered according to their position in the "SYMBOLS list."

See "The Data Block" (Chapter 2) for information on the SYMBOLS list.

The list of symbols represents a linear transformation series. For example, if `symbols="ABCDE"` were specified in the **FORMAT** command of the DATA block, PAUP would treat ordered characters under the assumption that to get from state A to state E, the character must proceed progressively through states B, C, and D, as indicated in the character-state graph below:

No numerical or alphabetical order is assumed. If SYMBOLS="021", state 2 is assumed to be intermediate between states 0 and 1. Similarly, if SYMBOLS="ABQC", state B lies between A and Q, and state Q lies between B and C. No polarity is implied by the symbols list, however. For example, all of the following transformations are consistent with the symbols list "012"; there is no requirement that '0' be the ancestral state:

state 0 ancestral: $0 \rightarrow 1 \rightarrow 2$

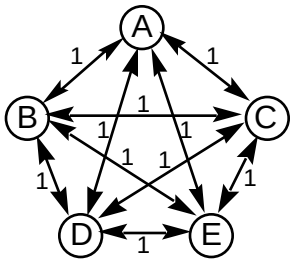
state 1 ancestral: $2 \leftarrow 1 \rightarrow 0$

state 2 ancestral: $2 \rightarrow 1 \rightarrow 0$

Note to PAUP Version 2 Users: The default character type is now unordered, rather than ordered as in earlier versions. Unless ordered characters are explicitly requested, PAUP will assume that multistate characters are unordered.

Unordered Characters

Unordered characters are defined such that any state is capable of transforming directly to any other state, with equal cost. For example, a five-state unordered character with states A through E has the character-state graph:



Character-state assignments are made to internal nodes of the tree so as to minimize the total number of character-state transformations (steps), using an algorithm based on that of Fitch (1971).

NOTE: The "ordered" vs. "unordered" concept does not pertain to binary characters. The order of a character refers to the potential pathways of character transformation, and there is only one possible path between the two states of a binary character. Consequently, it makes no difference whether freely reversible binary characters are defined as "ordered" or "unordered."

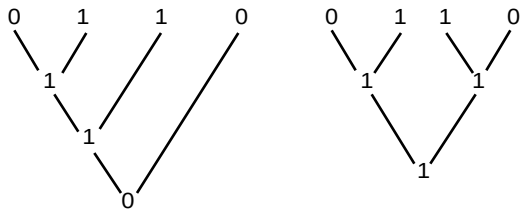
Dollo Characters

A "Dollo" character is one that is consistent with the requirement that every derived character state be *uniquely* derived. If a hypothetical ancestor is included in the analysis, this definition corresponds to the traditional Dollo model in which each character state is allowed to originate only once during evolution and all homoplasy takes the form of reversals to a more ancestral condition (i.e., parallel gains of the derived condition are not allowed).

As for ordered (Wagner) characters, character states are linearly ordered according to their position in the symbols list specified in the FORMAT command of the DATA block. However, we now define a "forward transformation" as a change from a less derived state to a more derived state, and a "backward transformation" as a change from a more derived state to a less derived state.

Rooted vs. unrooted Dollo models

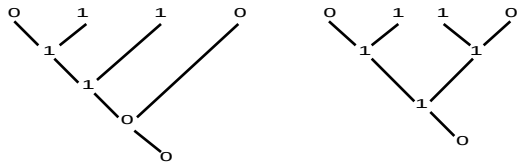
PAUP (as well as MacClade) differs from some other implementations of Dollo parsimony (e.g., Felsenstein's PHYLIP) in that it can operate as an unrooted method. The only requirement is that the reconstructed character states be consistent with the constraint that each derived state be uniquely derived. Under this definition, the position of the root affects neither the assignment of character states nor the length of the tree. For example, both of the trees below, which differ only in the placement of the root, require two steps under the unrooted Dollo model, assuming that state 1 is the derived state:



Reconstructions for a Dollo character under two different rootings of an unrooted tree. Terminal taxa are labeled according to their state for the character in question.

That is, neither tree requires more than a single origination of state 1. (In the tree on the right, the derived state (1) is assumed to be ancestral with respect to the group ABCD, but derived relative to some more inclusive group.)

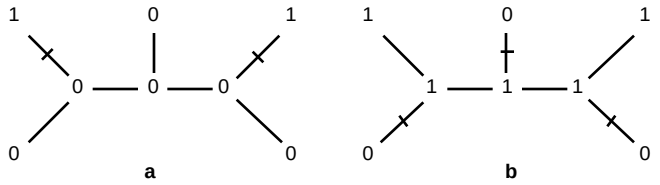
If, on the other hand, the trees are rooted by the attachment of a hypothetical ancestor possessing state 0, the left tree will be shorter (2 steps) than the right tree (3 steps):



Reconstructions for a Dollo character on the trees of the figure above under a rooted Dollo model.

The extra length in the right tree comes from the inclusion of the initial gain of state 1 in the tree length. This example makes it clear that trees computed under Dollo parsimony are intrinsically rooted *only if* we assume we know the state at the "outgroup node" [*sensu* Maddison et al. (1984)].

A more formal definition of the unrooted Dollo criterion is the following. A ***character-state reconstruction*** satisfies the Dollo constraint if it is possible to trace a path between any pair of nodes possessing the same character state without passing through a node possessing a less derived state. For example, reconstruction *A* below, while requiring fewer steps than reconstruction *B*, is not a Dollo reconstruction, as tracing the path connecting the two terminal nodes possessing state 1 requires passing through internal nodes possessing the less derived state 0. Reconstruction *B*, on the other hand, does satisfy the Dollo constraint.



*Two reconstructions for a single character on an unrooted tree. Reconstruction **a** requires 2 steps but violates the Dollo constraint. Reconstruction **b** satisfies the Dollo constraint, but requires 3 steps.*

Note that there is no possible rooting of the tree which does not require independent (parallel) gains of state 1 under reconstruction *A*, whereas any rooting of the tree is consistent with a unique origination of state 1 under reconstruction *B*, in accordance with traditional Dollo parsimony.

Unless an ancestor is included in the analysis—either explicitly or due to the presence of irreversible or asymmetric stepmatrix characters (see "Outgroups, Ancestors, and Roots")—PAUP uses the unrooted Dollo method. If, on the other hand, an ancestral taxon is included, then PAUP performs a rooted Dollo analysis. (If all characters are binary and are assigned polarity "up," as noted below, the rooted analysis corresponds to the implementation of Dollo parsimony in PHYLIP.) Since tree lengths will, in general, vary according to the position of the root, the result of the analysis is an intrinsically rooted tree—any specification of outgroups by the user is ignored. While the ability to obtain rooted trees without assuming an outgroup may seem appealing, it comes at a high price. Suppose a derived character state appears both in our ingroup and in an undisputed outgroup taxon also included in the data matrix. The analysis will place a premium on making all of the taxa possessing the derived state a monophyletic group (subject, of course, to effects from other characters in the data set), since the alternative is likely to be many independent losses. As a result, taxa that would ordinarily be assigned to the "outgroup" may spring from within the ingroup. Even when no basis exists for identifying "outgroup" vs. "ingroup" taxa, the rooting is still likely to be more artifactual than meaningful. The assumption is that the ancestor of the full tree possesses the ancestral state for the character and that the derived states must evolve somewhere on the tree from this ancestral state. Thus, the tree tends to be rooted nearest the taxa that have the fewest derived states. This may in fact be what you want, but you should at least be aware of the reasons why the program places the root where it does.

To amplify on the points made in the preceding paragraph, note that Dollo parsimony is sometimes recommended for restriction site data [e.g., DeBry and Slade (1985)] because of the asymmetry in the probabilities of losing an existing restriction site vs. gaining a new site at a particular location. (If a site is present, any substitution at any position in the recognition sequence causes a site loss, whereas even if a particular sequence is one substitution away from being a site, exactly the right substitution at exactly the right position is required to convert the "one-off site" to a site.) Thus, a rooted Dollo analysis in which the ancestral state is assumed to be "site absent" will tend to root the resulting tree(s) near the taxa that have the fewest sites. Although one could argue that this approach to rooting is reasonable, it seems a bit arbitrary to me.

There are ways of circumventing the problems of using rooted Dollo parsimony for characters like restriction site data. One approach is to infer character polarity via traditional outgroup analysis and then use a mixture of Dollo and Camin-Sokal (irreversible characters, see below) parsimony. If the site occurs only in (some) members of the ingroup, then we would designate the ancestral state as "absent" and allow a single gain of the site followed by as many losses as would be required to explain the character (i.e., traditional Dollo parsimony). But if a site occurs in the ingroup *and* in the outgroup, the assumption that the site was already present in the common ancestor of the ingroup-plus-outgroup would be reasonable. In this case, we would designate the ancestral state for this character as "present" and then treat the character as an *irreversible* (rather than Dollo) character, allowing only losses of the site in accordance with the Dollo model. A somewhat simpler but logically equivalent approach is to constrain the ingroup to be monophyletic (either through the use of a heavily weighted "dummy" synapomorphy or by using the "topological constraints" feature of PAUP) and use Dollo parsimony with an "all-absent" ("all-zero") ancestral taxon for all of the characters. Then if the site occurs in both the ingroup and the outgroup, a site gain will be forced along the basal branch of the tree (terminating at the common ancestor of the ingroup-plus-outgroup) and all subsequent character changes will be losses. If, on the other hand, the site occurs only within the ingroup, then a single origination will be assigned within the ingroup, perhaps with one or more subsequent losses.[4] Fortunately, the *unrooted* Dollo approach available in PAUP and MacClade renders these more complicated approaches unnecessary, but users should understand the logical connections between the alternative methodologies.

---

[4]An "all-missing" ancestor with polarity "up" [see "Direction of transformations (polarity)]" for all characters could also be used, with equivalent results. The same character state would be assigned to the node corresponding to the common ancestor of the ingroup-plus-outgroup in either case. The only difference is that with an all-absent

NOTE: Felsenstein (1984) has described a variation on rooted Dollo analysis that he calls the "unordered Dollo" method. (This terminology is a bit unfortunate—"unpolarized" Dollo would have been a better name.) Unlike the Dollo method implemented in PAUP, which assumes that the polarity is known, the unpolarized Dollo method evaluates each character on a given tree under all possible polarity assignments and chooses that polarity which allows the minimum number of changes. For example, in a two-state character with states 0 and 1, we would count first the number of changes required under the assumption that state 0 is ancestral, and then count the number of changes required under the assumption that state 1 is ancestral. Polarity is then assigned in accordance with the assignment requiring the fewest changes. I cannot imagine a situation in which one would be willing to assume that a character could not evolve from an ancestral state to a derived state more than once, but unwilling to postulate the character's polarity. Consequently, PAUP does not implement this method. (If you *can* imagine such a situation, let me know.) The unpolarized Dollo approach is not appropriate for restriction site data, since allowing site presence to be ancestral and site absence to be derived would, under the Dollo model, imply that sites could only be lost once and then regained as many times as necessary, clearly an unreasonable assumption.

Polarity specification

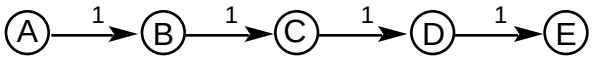
For the unrooted Dollo method, PAUP allows either the lowest or highest observed state (as defined by the SYMBOLS list) to be the ancestral state (polarity "up" vs. "down," respectively). If you do not explicitly specify "up" or "down," "up" is assumed. When an ancestor is included in the analysis (rooted Dollo), any state in the SYMBOLS list may be designated as the ancestral state.

See *Assigning Character Polarities* under "Specifying Character Types" in Chapter 2 for information on how to designate character polarities.

Irreversible Characters

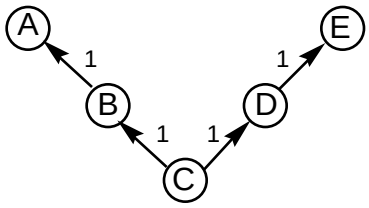
Irreversible characters are equivalent to ordered characters with the additional constraint of irreversibility being imposed (Camin and Sokal, 1965). As for ordered (Wagner) characters, character states are linearly ordered according to their position in the SYMBOLS list specified in the FORMAT command of the DATA block. However, we now prohibit transformations from a more derived state to a less derived state. For example, if SYMBOLS="ABCDE" and state A is defined as the ancestral state, the character-state graph is:

(all-zero) ancestor, one step would be added to the tree length for every character in which the site was present in both the ingroup and outgroup, corresponding to the site gain along the basal branch. With an "all-missing" (unknown) ancestor, this additional step would not be required, because the change would be from the "missing" state to present (see "MISSING DATA"). Thus, the only difference between the two methods is that a constant is added to (or subtracted from) the tree length.



Polarity specification

Although character transformations must always proceed in the direction of a more derived state, they need not proceed in a single direction with respect to the SYMBOLS list. Any state in the SYMBOLS list may be designated as the ancestral state; with states preceding and/or following this state representing more derived states. For instance, state C could instead have been chosen as the ancestral state in the example above. The character-state graph would then be:



See *Assigning Character Polarities* under "Specifying Character Types" in Chapter 2 for information on how to designate character polarities.

NOTE: Felsenstein (1984) has described a variation on irreversible-character parsimony that he calls the "unordered Camin-Sokal" method (analogous to his "unordered Dollo" method discussed above). Again, "unpolarized Camin-Sokal" would have been a more appropriate name. As in the unordered Dollo method, the unpolarized Camin-Sokal method evaluates each character on a given tree under all possible polarity assignments and chooses that polarity which allows the minimum number of changes. It seems exceedingly unlikely that one would be willing to assume that a character was irreversible, but ignorant as to its polarity, so I have not implemented unpolarized irreversibility in PAUP.

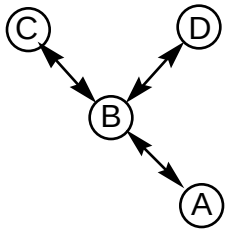
User-Defined Character Types

Two kinds of user-defined character types are available. Stepmatrices allow you to define arbitrary character types by specifying the distance from any character state to any other. Character-state trees provide an easy method for inputting branching character transformation series.

Character-state trees

Character-state trees are used to specify a non-linear, branching, relationship among character states, which are then otherwise treated as ordered characters. (Formally, a character-state tree imposes a "partial order" on the character states.) In most earlier programs, users were required to recode character-state trees using additive coding (binary or otherwise; see below). PAUP now provides this capability automatically.

As an example, suppose that you wish to assume that states C and D were derived independently from state B, which itself was derived from state A. The following character-state tree would be used to represent this relationship:



Either of the following additive codings, breaking the character into a suite of independent characters, could be used in lieu of a character-state tree:

A = 00	A = 000
B = 10	B = 100
C = 20	C = 110
D = 11	D = 101

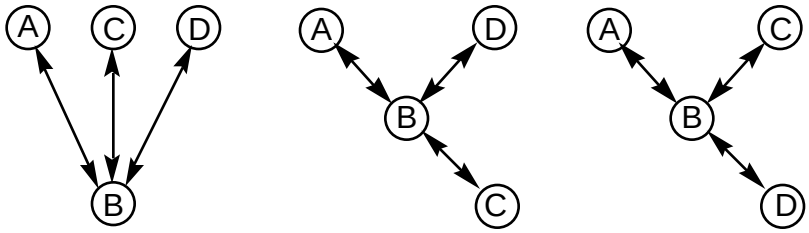
However, such a coding makes interpretation of the output more difficult because you would have to decode the new characters back to the original states of the character-state tree. In this case, the character-state tree specification

((C,D)B)A

provides an equivalent definition of the character, requires essentially identical computation time, and eliminates the coding-decoding steps.

Characters defined as character-state trees are fully reversible, however they may evolve only along the paths specified by that definition. For instance, in the above example a change from state C to state D would imply passage through state B and would require two steps.

Finally, remember that character-state trees, like linearly ordered characters, are undirected. The character-state tree only specifies available paths for character-transformation; it does not imply anything about polarity. In the example above, states B, C, or D could just as easily have been placed at the base as shown below:



(A,C,D)B ((A,D)B)C ((A,C)B)D

Three equivalent rootings of a character-state tree.

See "The ASSUMPTIONS block" for details on the specification of character-state trees in an input file.

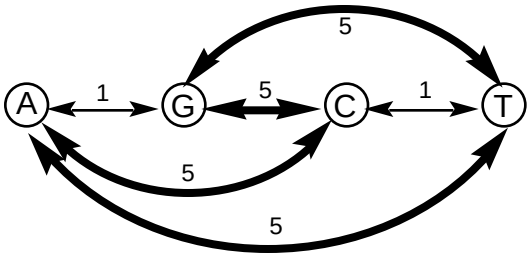
Stepmatrices

A stepmatrix is a square matrix specifying the distance from every character state to every other state. These distances represent the "cost" in tree-length units of the corresponding transformations. (Transformations may be completely forbidden by coding 'i', for infinity, as the transformation cost.) For each stepmatrix character, PAUP uses dynamic programming algorithms (Sankoff and Rousseau, 1975; Sankoff and Cedergren, 1983) to determine the minimum possible length on each tree it evaluates and to reconstruct hypothetical ancestors consistent with this length.

Stepmatrices may be either ***symmetric*** or ***asymmetric***, corresponding to the usual definition of matrix symmetry (i.e., a matrix d is symmetric if $d_{ij} = d_{ji}$ for all i,j). Symmetric stepmatrices imply free reversibility of characters, because the cost of reversing a given transformation is equal to the cost of the original transformation.

NOTE: Asymmetric stepmatrices force a rooted tree. If the stepmatrix is asymmetrical, the taxon designated "ancestor" is very important. If you want to have asymmetrical transition penalties but remain agnostic regarding the ancestral condition of the characters, you should include an ancestor with all-missing values. See the section "**Character Types**" for the relationship between character type and tree rooting.

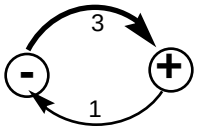
The "generalized parsimony" approach provided by stepmatrix characters is extremely powerful. Researchers with nucleotide sequence data can assign different weights to transitions vs. transversions (and even to different kinds of transitions and transversions):



	A	C	G	T
A	0	5	1	5
C	5	0	5	1
G	1	5	0	5
T	5	1	5	0

Character-state graph and corresponding stepmatrix for a character type that assigns five times more weight to transversions than to transitions

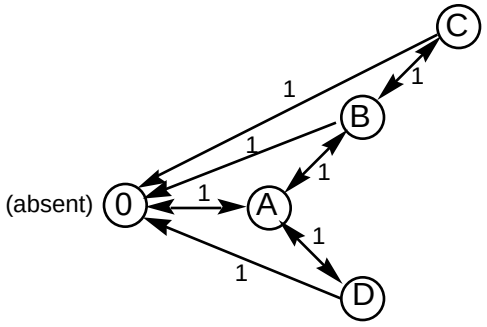
For restriction map data, site gains can be given higher weight than site losses, so that parallel loss and gain-loss events are preferred over parallel gains and loss-regains (Templeton, 1983b; Templeton, 1983a) , avoiding the perhaps overly severe strict prohibitions imposed by the Dollo and Camin-Sokal models (DeBry and Slade, 1985) :



	-	+
-	0	3
+	1	0

Character-state graph and corresponding stepmatrix for a character type that assigns three times as much weight to a gain than a loss.

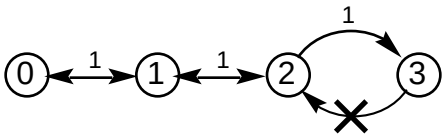
Stepmatrices may be used to define models of character transformation that cannot be expressed under any other available coding method. For instance, morphologists can define "partially unordered" characters in which some transformations are required to follow a specified order but others may occur freely. In the example below, a character-state tree for several alternative "present" states is specified, but a transformation to the "absent" condition is permitted to occur with equal cost from any state:



	0	A	B	C	D
0	0	1	2	3	2
A	1	0	1	2	1
B	1	1	0	1	2
C	1	2	1	0	3
D	1	1	2	3	0

Character-state graph and associated stepmatrix for a "partially unordered" character. Transformations between states A, B, C, and D follow a character-state tree, but immediate losses may occur from any state.

"Partial irreversibility," where reversals are permitted for some transformations but not for others, can also be implemented using stepmatrices:




```
   0 123
0  0123
1  1012
2  2101
3  i i i0
```

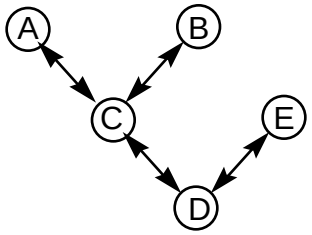
Character-state graph and associated stepmatrix for a "partially irreversible" character. State "3" cannot reverse to any other state, hence the entries of infinity ("i") in the last row of the stepmatrix.

Most of the other standard types may be defined equivalently as stepmatrices. For example, the stepmatrices

```
0 1 2     0 1 1         0 1 2
1 0 1 ,   1 0 1  , and  i 0 1
2 1 0     1 1 0         i i 0
```

correspond to the ordered, unordered, and irreversible types, respectively, for a character with 3 or fewer states. However, stepmatrix characters require significantly more computation than other character types and should be used only if it is not possible to use one of the standard types.

Stepmatrices may also be used to describe character-state trees. For example, the character-state tree



= ((A,B)C,E)D

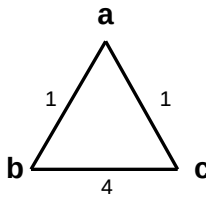
can be described using the stepmatrix:

```
   A  B  C  D  E
A  -  2  1  2  3
B  2  -  1  2  3
C  1  1  -  1  2
D  2  2  1  -  1
E  3  3  2  1  -
```

However, there are enormous computational advantages to using an explicitly defined character-state tree rather than an equivalent stepmatrix. Again, use stepmatrices only when another equivalent type is unavailable.

See "The ASSUMPTIONS block" for details on how to define a stepmatrix in an input file.

It is possible to define stepmatrix characters that violate the "triangle inequality." This inequality is a property of distances in Euclidean space, where the length of one side of a triangle is always less than the sum of the lengths of the other two sides. This means the shortest distance between two points will always be a straight line. If a stepmatrix is defined that violates this rule, one side is actually longer than the sum of the other two sides. For example, the following triplet violates the inequality, as the distance (b,c) is longer than the sum of the distances (a,b) and (a,c).



This would result from the following stepmatrix:

```
      a     b     c
a     -     1     1
b     1     -     4
c     1     1     -
```

If a stepmatrix contains a triplet that violates the "triangle inequality," PAUP will display a warning the stepmatrix is internally inconsistent, but will still allow it to be used in an analysis. In that case, you must decide if you really want to disallow shortcuts such as (bc), and what the biological meaning of that stepmatrix really is. The implications of having stepmatrices which violate the triangle inequality are discussed in more detail in Maddison and Maddison (1992).

TREE LENGTHS AND CHARACTER WEIGHTS

Parsimony analysis operates under the assumption that characters are independent of each other, so that the length L of a full tree can be computed as the (weighted) sums of the lengths l_j of the individual characters:

$$L = \sum_{j=1}^{C} w_j l_j \ ,$$

where C is the total number of characters. Consequently, for the purpose of computing tree lengths, we can treat each character in isolation from the rest. It is this property that allows the "mixing and matching" of character types. For example, nothing prevents you from assuming that one subset of the characters is ordered (Wagner), another is unordered, and yet another Dollo. For any tree, the minimum length required by each character is evaluated under the assumptions assigned to that character and the full length of the tree is obtained by summing over characters.

Typically, $w_j = 1$ for all characters ("equal weights"). However, you may choose any vector **w** of weights. For instance, if you had reason to believe that two characters were tightly coupled for reasons other than phylogenetic history, then weighting each of the characters 1/2 would be appropriate. Alternatively, you may have prior knowledge (or at least be willing to assume) that some characters are more reliable than others. These characters could be given higher weight than the rest.

Sometimes the number of states recognized for a character is highly arbitrary. This would be the case, for example, if a character varying continuously in size were broken into a set of discrete states. The character could perhaps be broken into four states (a "course-grained" approach) or ten states (a more "fine-grained" coding). Remember that under "equal weighting" the character will actually have three times as much influence on the analysis under the ten-state coding than it would under the four-state coding [(10-1)/(4-1)]. Likewise, under the ten-state coding, the character would have nine times as much influence on the analysis as an "equally weighted" binary presence-absence character. PAUP provides an option for "scaling" character weights so that the total influence of each character is the same, regardless of the number of states. Specifically, a binary character is weighted 1, a three-state character 1/2, a four-state character 1/3, and so on.

NOTE: Some workers have suggested that the problem of the above paragraph can be circumvented by breaking the multistate character into a set of additive binary characters, but this is not the case. If you break a ten-state character into nine binary characters, it is indeed true that each of the resulting binary characters has the same weight as does a truly binary (e.g., presence-absence) character, but there are now nine of them. In fact, for a linearly ordered multistate character, it makes no difference to the analysis whether you treat it as a single character or break it into a set of additive binary characters. Doing the latter serves no useful purpose and merely complicates the interpretation of the output.

Note that most implementations of PAUP 3.0 do not allow fractional or decimal character weights. Thus, to assign a weight of 1/2 to a character, you must instead assign a weight of two to all of the other characters. (The reason for this design decision is that integer arithmetic is vastly faster on microcomputers than is floating-point or "real" arithmetic.)

When scaling weights, it is desirable to choose a smallest common multiple of the desired weights in order to avoid roundoff error. For example, if your data set contains a mixture of two-, three-, four-, and five-state characters and you want to scale weights, use 60 as the "base" weight so that the weights 1/2, 1/3, 1/4, and 1/5 correspond to 30, 20, 15, and 12, respectively.

PAUP also implements the successive approximations character method (Farris, 1969) as implemented in the Hennig86 program (Farris, 1988). This method is discussed in more detail in the section "*A Posteriori* Character Weighting."

CHARACTER-STATE OPTIMIZATION

The reconstruction of character states at internal (ancestral) nodes on a given tree is called character-state optimization or character mapping. Character optimization does *not* come into play at any time during the search for optimal trees; *only* when character reconstructions are requested. Under the maximum parsimony criterion, the goal is to assign character states so as to minimize the total number of change required by a particular character on a given tree. The set of such assignments at internal nodes is called a most-parsimonious reconstruction (MPR) or simply an "optimal reconstruction" (Swofford and Maddison, 1987; Swofford and Maddison, 1992). Characters do not have to be polarized in order for these reconstructions to be made.

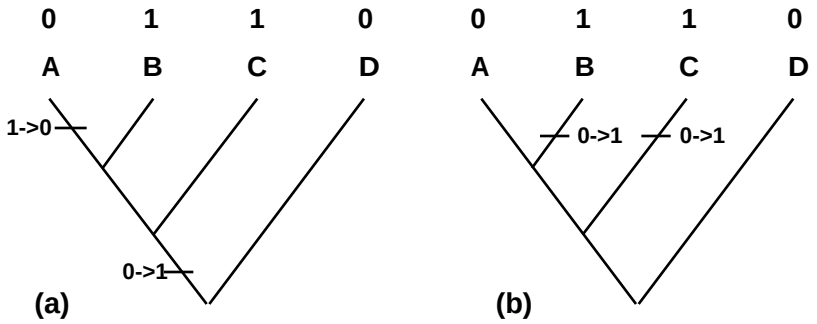
The optimal reconstruction for a given character is a function of tree topology, but also of any other assumptions about character order and/or irreversibility that have been made. Specifically, this involves the "cost" of changing from one character state to another. This is a function of the character type, whether one of the standard types or user-defined. The character optimization algorithm operates by making two passes over a tree. The first pass proceeds from the tips toward the root. At each internal node, the algorithm determines which character states are potentially allowed, and calculates the minimum possible lengths of the clade above that node for each allowed state.

The second stage proceeds from the root to the tips, and determines a set of optimally reconstructed internal states by using the information from the first pass and information from the second pass below that node. The optimal state minimizes the sum of two quantities: the length of the clade above that node given each allowed state and the cost of transforming to each allowed state given the state assigned to the ancestor of that node.

In most cases there will only be one optimal assignment at a node, but it is possible that more than one optimum may exist. In that case, a particular reconstruction may be favored on the basis of additional criteria. The most common ancillary criteria are accelerated transformation (ACCTRAN) and delayed transformation (DELTRAN) (Swofford and Maddison, 1987; Maddison and Maddison, 1992; Swofford and Maddison, 1992). Basically, these assign states at internal nodes in order to delay (DELTRAN) or accelerate (ACCTRAN) character transformation within the tree. By "pushing" transformations up or down the tree, these optimization methods lead to very different hypotheses of character change. Delaying change will lead to a preference for two origins of a character state (parallelisms), while accelerating change will lead to a preference for a single origin followed by reversal.

Both hypotheses will always involve the same number of steps on the tree. The only change will be where those steps are located. The result will be that the character change associated with a particular node will vary with the choice of optimization method. For that reason, you have to carefully examine the output that PAUP gives you (see Character Diagnostics).

In the figure below, assume that the ancestral state is 0, and that taxa A,B,C, and D have the state assigned them for that character. There are two ways that this character can be optimized: either the occurrence of state 1 in B and C is a reversal (reconstruction *a*), in which case 1 arose at the level of (ABC) and was lost in A; or the presence of 1 in B and C is due to parallelism (reconstruction *b*), or independent gain. In either case, this character requires two steps when it is optimized, so the length of the tree does not change.



*Character optimization using **ACCTRAN** and **DELTRAN**. (a) **ACCTRAN** optimization of character with distribution shown. (b) **DELTRAN** optimization of character. Both require two steps.*

This is especially important if an analysis is explicitly examining the levels of parallelism/reversal in a data set. In that instance, there are several steps that one might take. Most obviously, use both optimization methods, and see how the characters fall out—it may be that there is no

difference. Another strategy is to choose the method which best favors the null hypothesis. For example, if you are studying adaptation and want to discover instances of parallel derivations of a trait, choose ACCTRAN, which favors reversals. If a pattern of repeated parallelisms *still* appears in spite of a bias against them, the argument for adaptation is that much stronger.

A third option for character-state optimization is available: **MINF.** Under **MINF** optimization, character states are optimized so that the f-value of Farris (1972) is minimized. Two constraints are enforced: the states assigned to an HTU must be present in at least one OTU, and the tree length must be minimal (it is possible to further reduce the f-value by increasing the length of the tree). The effect of minimizing the f-value is that length is transferred from interior branches towards terminal branches whenever possible, minimizing the risk that groups will be arbitrarily resolved internally. This option will, in many instances, yield the same reconstruction as **DELTRAN**. **MINF** is not available for rooted trees.

MISSING DATA

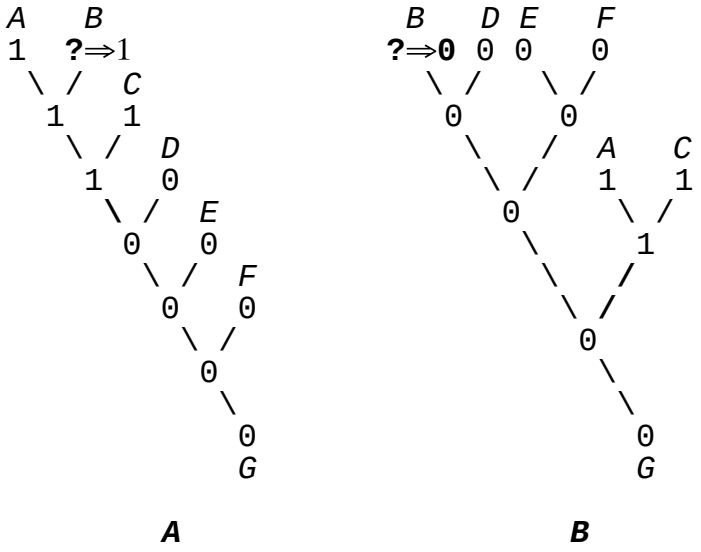
Ordinarily, you will assign a state for every character in every taxon. However, in two situations you may need to score a character state as "missing":

(1) the data for a particular character are simply unavailable in a taxon, but you want to include the taxon in the analysis anyway, or

(2) the character "does not apply" (e.g., the character represents different modifications of a structure that is absent entirely in some taxa).

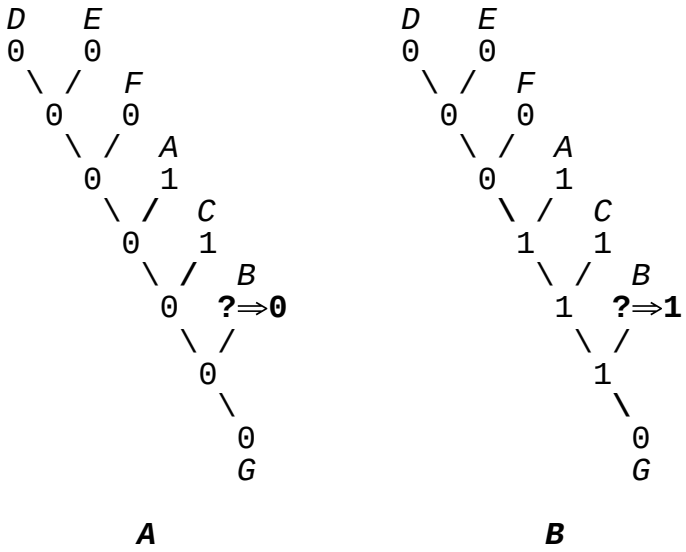
In either case, a possible solution is to assign to the taxon the character state that *would be* most parsimonious *given its placement on the tree* (but see Maddison (In press). Effectively, then, only those characters that have non-missing values will affect the location of any taxon on the tree. The example below illustrates this concept. Remember that for the purpose of computing tree lengths, each character can be treated independently; in this example, one character is considered in isolation from all of the others. For tree **A**, PAUP would assign state "1" to the taxon with missing data (=*B*). This allows a reconstruction requiring only one step over the full tree. For tree **B**, however, assignment of state "0" is required in order to minimize the length required by this character, again one step. Thus, both trees are equally parsimonious with respect to this character; if one

tree is in fact "better" than the other, evidence for this conclusion will have to come from other characters.



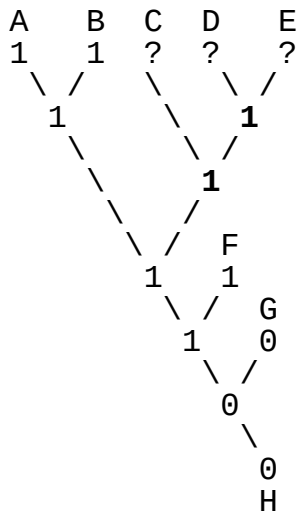
Demonstration of PAUP's handling of missing data. Reconstruction of one character on two different trees (see text).

Note that in some cases, the assignment of the "would-be" most parsimonious state is ambiguous. However, the length required is the same regardless of how the ambiguity is resolved, so that for the purposes of calculating the length of a particular tree or comparing the lengths of alternative trees, ambiguity does not present a problem. In the example below, either state "0" or "1" could be assigned to taxon *B* (reconstructions A and B, respectively). In either case, the required length of two steps is greater than that required by the trees above. Thus, although this character is not informative for placing taxon *B*, it is still useful for discriminating among tree topologies.



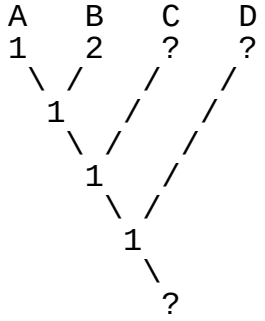
Two equally parsimonious reconstructions on the same tree, demonstrating the handling of ambiguity in the presence of missing data. Although the two reconstructions are different, the length of the reconstructions is two steps in each case.

When reconstructing characters, PAUP always assigns a nonmissing state to all internal nodes. Occasionally, users have complained about this behavior. For example, some users have argued that for the reconstruction shown below, state '?' should have been assigned to the nodes at the base of the {D,E} and {C,D,E} clades rather than state 1. This choice would imply, however, that state 0 could be assigned to these nodes in a most-parsimonious reconstruction. But if state 0 were assigned to either of these nodes, the reconstruction would require at least two steps rather than the one step implied by the indicated reconstruction. Thus, state 1 is the only permissible state for these nodes.



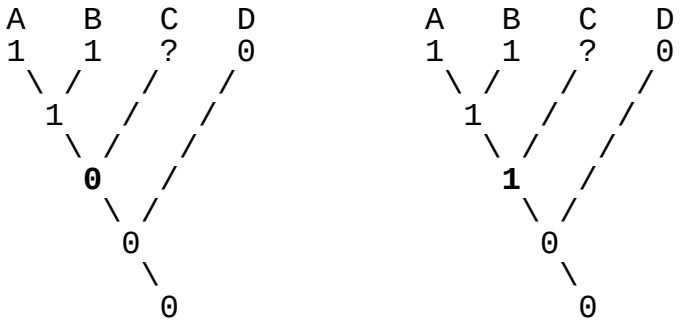
A character-state reconstruction that illustrating the inappropriateness of assigning missing values to internal nodes.

Similarly, in the example below, we must assign either state 1 or state 2 to all internal nodes, even though non-missing states are defined only for taxa A and B.



A character-state reconstruction that illustrating the inappropriateness of assigning missing values to internal nodes.

Sometimes, internal-node state assignments are truly ambiguous due to the presence of missing data. For example, either of the two reconstructions below require the minimum length of one step:



PAUP always chooses one of the potential reconstructions according to the character types and optimization options in effect. However, it also provides capabilities for examining all possible state assignments to internal nodes so that you can determine whether or not a reconstruction contains ambiguities.

> See "Character-State Optimization" for information regarding the resolution of ambiguity in character-state reconstructions.

OUTGROUPS, ANCESTORS, AND ROOTS

In most cases, trees computed by PAUP are unrooted and need be rooted for output and interpretational purposes only. The absence of a defined root is a consequence of the fact that for undirected character types, the tree length is the same regardless of the position of the root. However, rooted trees are computed when any of the following are true: (1) characters of type "irreversible" are present; (2) asymmetric stepmatrix characters are present; or (3) a hypothesized ancestral taxon is explicitly included in the analysis at the request of the user.

For irreversible and asymmetric stepmatrix characters, the length of the tree depends upon the position of the root, so the trees must be stored as rooted trees. When all characters are undirected, the inclusion of a hypothesized ancestral taxon corresponds to the traditional, but unnecessary practice (Meacham, 1984; Meacham, 1986) of *a priori* character polarization. This approach is reasonable when the polarity determination is unambiguous (i.e., there is no heterogeneity in the outgroup for characters that are variable within the ingroup). However, when the outgroup is heterogeneous, the most parsimonious assignment of an ancestral condition for the ingroup depends upon how the outgroup taxa are related to each other [see Maddison et al. (1984) for a complete analysis of this problem]. If outgroup relationships are already well resolved, you can use methods described by Maddison et al. (1984) to assess character polarities *a priori* and include an ancestral taxon in the

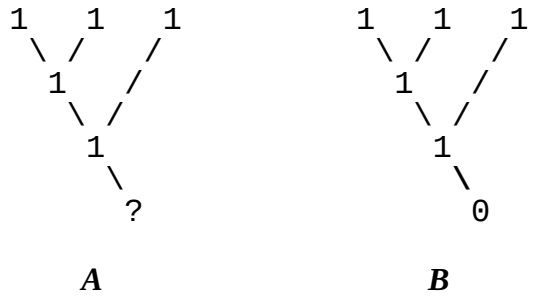
analysis [see also Donoghue and Maddison (1986)]. Otherwise, it makes much more sense either to:

(1) include several outgroup taxa in the analysis, with the root of the ingroup portion of the tree being determined by the location at which the outgroup taxa connect to the tree [i.e., Maddison et al.'s (1984) "simultaneous resolution of ingroups and outgroups taken to the extreme"; see also Farris (1972)], or

(2) compute an unrooted tree for the ingroup taxa only, making no *a priori* assumptions about polarity, and then attach an outgroup taxon (or hypothesized ancestor) to the tree *a posteriori* (Lundberg, 1972).

Although clearly better than "guessing" at the polarity, both of the above approaches have some disadvantages (Maddison et al., 1984; Meacham, 1984; Donoghue and Maddison, 1986; Meacham, 1986). In order to be fully effective, the first method requires the inclusion of characters that are informative with respect to outgroup relationships even if these characters are invariant within the ingroup (Maddison et al., 1984). On the other hand, the second method can generate "optimal" trees that are locally parsimonious within the ingroup, but not globally parsimonious.

If the trees being evaluated are rooted (either implicitly because directed characters are present or explicitly due to the user's decision to include an ancestral taxon), the character states taken by the ancestor of the full tree must be specified. By default, these ancestral states are set to "missing" (= the "standard" ancestor), however, the user may make alternative assignments using ANCSTATES definitions (see below). The default setting of all-missing values was chosen for two reasons. First, if all characters are undirected, trees may be converted from rooted to unrooted or *vice versa* with no change in tree length. Second, if directed characters are present, the program has the freedom to assign a character-state to the "first fork" of the tree (i.e., the internal node immediately descending from the root corresponding to the initial branching event) that permits the most parsimonious reconstruction subject to the constraints imposed by the character type. Consider, for example, an irreversible character with polarity "up." If, for whatever reason, the state for all terminal taxa included in the analysis is "1," then the character will contribute no length to the tree, as expected for a constant character (A, below) But if the ancestral state had defaulted to "0" rather than "missing," a step would have been assigned to the basal branch (B, below)

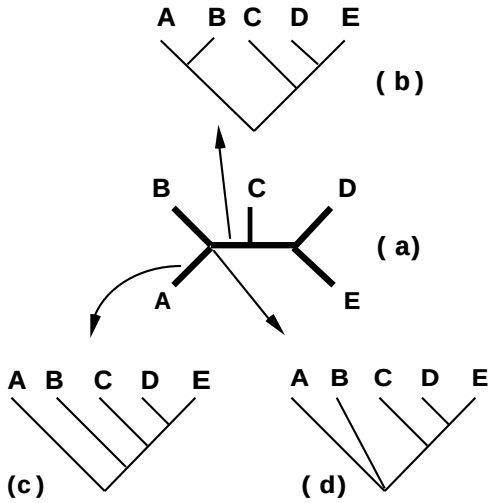


A ***B***

*Reconstruction for a hypothetical character with ancestral state "missing" (**a**) and "0" (**b**). See text.*

PAUP provides commands for converting unrooted trees stored in memory to rooted trees and *vice versa*. Note that **you do *not* have to invoke a "root trees" command** before "showing," "describing" or "printing" trees, requesting consensus trees, etc. (see command descriptions below). The only time you would ordinarily use a "root trees" command is if you want to declare one or more characters to be irreversible or an asymmetric stepmatrix type when there are already unrooted trees in memory; in this case PAUP must store the trees internally as rooted and you will have to convert them. Otherwise, the trees are stored as unrooted trees and are rooted automatically for output purposes (using either the currently defined outgroup or the Lundberg method, at your choosing).

Outgroup rooting of the unrooted tree A in the figure below can be done in three ways indicated by the three arrows leading to the rooted trees. "Pulling" the tree down at each of the different arrows leads to a different rooting: the tree can be rooted such that the outgroup is a monophyletic sister-taxon to the ingroup (tree B); it can be rooted such that the outgroup is paraphyletic with respect to the ingroup (tree C); or it can be rooted such that the outgroup taxa form a basal polytomy with the ingroup (Tree D; this is the default). The reason that taxon A and not taxon B is the basal-most taxon when the tree is rooted such that the outgroup is paraphyletic is that taxon A is the primary outgroup (the first outgroup taxon listed by the **OUTGROUP** command or chosen using the **Define Outgroup** menu command. The primary outgroup will always be the basal-most taxon when outgroup rooting and a paraphyletic outgroup is chosen.



Outgroup rooting options. (a) unrooted tree for five taxa, outgroup is A + B. (b) tree rooted with monophyletic outgroup. (c) tree rooted with paraphyletic outgroup. (d) tree rooted with basal polytomy.

You have three options when using outgroup rooting and more than one outgroup is present in the analysis. These are: rooting at an internal node with a basal polytomy; rooting such that the ingroup is monophyletic and the outgroup is paraphyletic with respect to the ingroup; or rooting such that the ingroup is monophyletic with the outgroup a monophyletic sister group. These options allow you to at least partially constrain the structure of the outgroup topology when the tree is rooted, providing the specified ingroup is monophyletic. See the section "Rooting trees for output and character reconstruction." If, however, the tree cannot be rooted such that the ingroup is monophyletic, PAUP will include as many outgroup taxa as necessary to make the ingroup plus those outgroup taxa monophyletic. The primary outgroup is never included in the ingroup, so the topology of the tree in that instance is directly affected by the choice of the primary outgroup.

SEARCHING FOR OPTIMAL TREES

If the length of a tree under a particular set of assumptions (character types and weights) is less than or equal to the length of any other possible tree for the same data, the tree is said to be *optimal* (most parsimonious). Depending on the data set, there may be a single most parsimonious tree or two or more (sometimes many) equally parsimonious trees. Ordinarily, the goal of a search is to find all of the equally parsimonious trees that exist for a particular data set under the chosen assumptions. In some cases, you may also be interested in *near-optimal* trees, i.e., trees whose length is less than or equal to some specified cutoff criterion.

PAUP provides two basic classes of methods for searching for optimal trees, exact methods and heuristics. Exact methods guarantee to find the optimal tree(s) but may require a prohibitive amount of computer time for medium- to large-sized data sets. Heuristic methods do not guarantee optimality but generally require far less computer time.

Note that the search algorithms described below consider only *binary* trees. In many cases, however, the data will not be sufficient to determine a fully resolved (i.e., binary) tree, however, so that branches of zero-length might occur. In earlier versions of PAUP, two or more trees that could be derived through different resolutions of ***polytomies*** were always considered to represent distinct binary trees containing zero-length branches, with the result that many more trees were sometimes saved than was actually necessary. For example, if a tree contained two trichotomies, nine equally parsimonious trees would be saved—three resolutions for the first trichotomy times three resolutions for the second. Version 3 of PAUP provides an option to collapse branches having zero length to yield polytomies. Because two or more distinct binary trees may collapse to the same nonbinary tree, the program then must ensure that after collapsing, a tree does not become identical to a tree already saved that was obtained by collapsing a different binary tree.

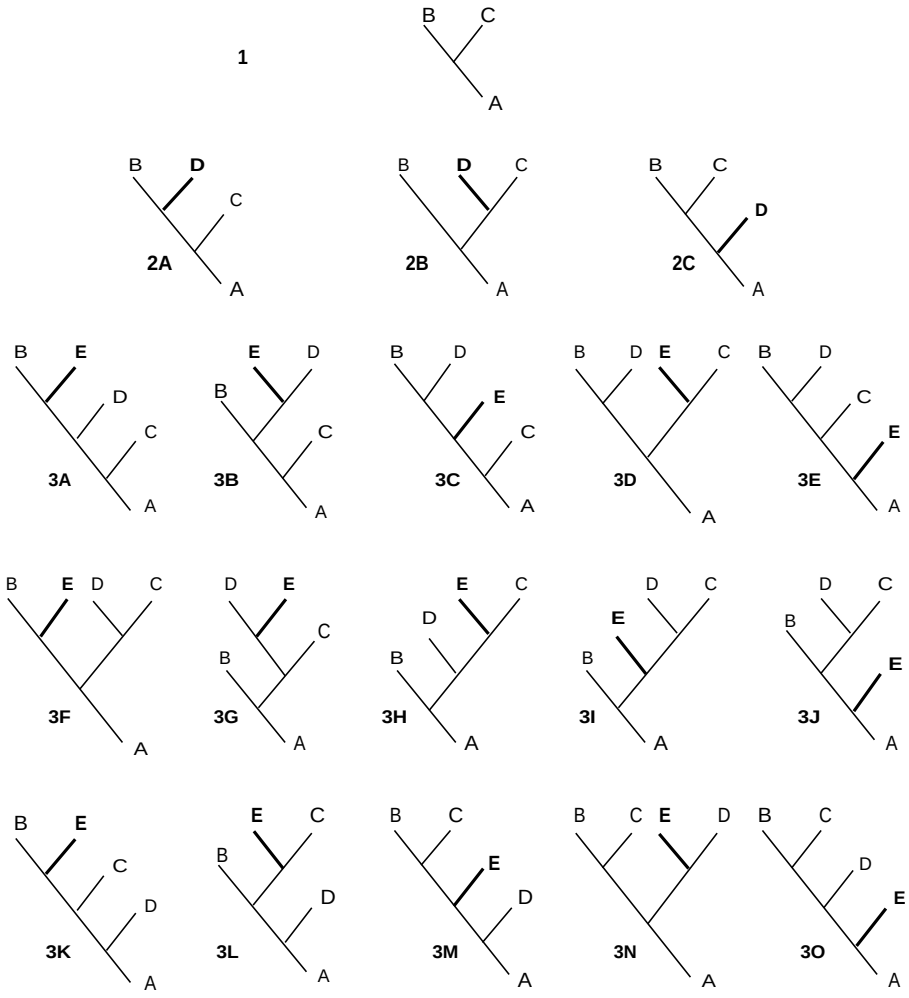
See "Zero-Length Branches" and Polytomies below for further details on this point.

Exact methods

Exhaustive search

The conceptually simplest approach to the search for optimal trees is simply to evaluate every possible tree. Assuming that exact methods exist for evaluating the length of any particular tree, an algorithm that generates all possible tree topologies and evaluates each one is guaranteed to find all of the optimal trees. The algorithm outlined in the figure below can be used for this purpose. Initially, the first three taxa in the data set are connected to form the only possible unrooted tree for these taxa (row 1). In the next step, the fourth taxon is added to each of the three branches of the three-taxon tree, thereby generating all three possible unrooted trees for the first four taxa (row 2). The process continues in a similar fashion, adding the *i*th taxon to each branch of every tree (containing *i*–1 taxa) generated during a previous step. Thus, for example, row 3 contains all 15 possible trees for the first five taxa, obtained by adding the fifth taxon to each of the five possible branches for the three trees obtained at the four-taxon stage. This process demonstrates the rationale for equation (1) for counting the number of possible unrooted bifurcating trees for T taxa: for each of the possible trees for i–1 taxa, there are $2(i–1) – 3 = 2i – 5$

branches to which the *i*th taxon can be connected. Note that the order of addition is immaterial; one could just as well have chosen a taxon at random to connect to the tree at each step.



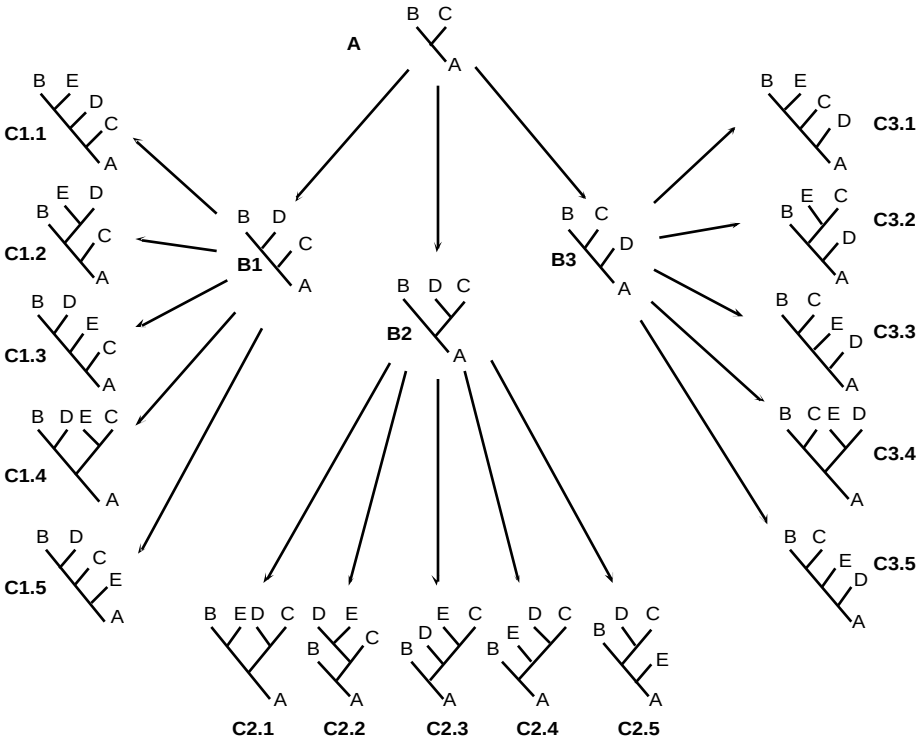
Generation of all possible binary tree topologies for five terminal taxa.

Evaluation of Equation 1 (see Table 2) quickly reveals why exhaustive search procedures are useful only for small numbers of taxa. There are over 2 million trees for 10 taxa and 34 million trees for 11 taxa, so it is doubtful that exhaustive search strategies will be useful beyond 11 taxa.

Branch-and-bound algorithm

Fortunately, an exact algorithm for identifying all optimal trees that does not require exhaustive generation is available. The ***branch-and-bound*** method, frequently used to solve problems in combinatorial optimization, was apparently first applied to evolutionary trees by Hendy and Penny (1982). This method closely resembles the exhaustive search algorithm described above. In this procedure, a ***search tree*** is traversed in a "depth-

first" sequence, as illustrated in the figure below. The root of the search tree (A) contains the only possible tree for the first three taxa. We first construct one of the three possible trees obtained by connecting taxon 4 to tree A, yielding tree B1. Then, to this tree, we connect taxon 5, yielding tree C1.1. (If the data set contained more than five terminal taxa, we would continue to join additional taxa in this manner until a tree containing all *T* taxa had been completed.) Now, we backtrack one node on the search tree (i.e., back to tree B1) and generate the second tree resulting from the addition of taxon 5 to tree B1 (= tree C1.2). When all five of the trees derivable from tree B1 (C1.1–C1.5) have been constructed, we backtrack all the way to tree A of the search tree and take the second path away from this node, leading to tree B2. As before, all five trees derivable from tree B2 (C2.1–C2.5) are constructed in turn. Then we backtrack once again to tree A and proceed down the third path toward trees C3.1–C3.5. Eventually, we will have constructed all of the possible trees culminating with tree C3.5. If the length of each tree containing all five taxa were evaluated at the time of its construction, we would have performed an exhaustive search equivalent to that described in the above section.



Search tree for branch-and-bound algorithm.

Suppose that *L* represents an upper bound on the length of the shortest tree(s). For the present, we can obtain *L*, for example, by evaluating a random tree; if we know that a tree of length *L* exists, then the length of the optimal tree(s) cannot exceed this value. If, as we are moving along a

path of the search tree toward its tips (containing all *T* taxa), we encounter a tree whose length exceeds *L*, then we need proceed no further along this path; connecting additional taxa cannot possibly decrease the length. Thus, we can dispense with the evaluation of all (phylogenetic) trees that descend from this node in the search tree and immediately backtrack and proceed down a different path. By cutting off portions of the search tree in this manner, we can greatly reduce the number of trees that must actually be evaluated.

If we reach the end of a path on the search tree and obtain a tree whose length is less than or equal to the upper bound *L*, then this tree is a candidate for optimality. If, however, this length is less than *L*, then this tree is the best one found so far, and we have improved the upper bound on the length of the optimal tree(s). This bound-improvement is important because it may enable other search paths to be terminated more quickly. When the entire search tree has been traversed, all optimal trees will have been identified.

Several factors influence the running time of the branch-and-bound algorithm. The quality of the data is perhaps the most important factor; large data sets with little homoplasy will run quickly because most paths of the search tree are terminated early. The speed with which the length of each tree can be evaluated, a function of the character types, is also important. In general, undirected character types run faster than directed types because certain algorithmic tricks for rapidly computing tree lengths can be used when the tree length does not depend on the location of the root. Ordered (Wagner) characters are much faster than unordered characters for similar reasons. User-defined stepmatrix characters, on the other hand, run very slowly due to the enormous amount of computation required to compute the length of even a single tree. Finally, of course, the speed of the available computer is critical to the run times—the branch-and-bound algorithm can be applied to larger data sets on a Cray X/MP than on a Macintosh Plus.

The above presentation of the branch-and-bound method, while correct, is an oversimplification of the algorithms actually used in PAUP, which implements algorithmic refinements that greatly speed the computations. These refinements, designed to promote earlier cut-offs in the traversal of the search tree, include: (1) using heuristic methods (see below) to obtain a near-optimal tree whose length is used as the initial upper bound; (2) designing the search tree so that divergent taxa are added early, thereby increasing the length of the initial trees in the search path; and (3) using pairwise incompatibility to improve the *lower* bound on the length that will ultimately be required by trees descending from a tree at a given node of the search tree.

Since the branch-and-bound method requires evaluation of all trees as its worst possible case, why would we ever want to perform an exhaustive search? In fact, if we were interested only in the optimal trees, the branch-and-bound algorithm would indeed be the method of choice. However, exhaustive searches can be used to generate the frequency distribution of tree lengths. We may find it useful to know, for example, whether there are few or many near-optimal trees, or where some tree of prior interest lies in the distribution of tree lengths.

Heuristic Methods

When a data set is too large to permit the use of exact methods, we must resort to heuristic approaches that sacrifice the guarantee of optimality in favor of reduced computing time. I like to apply the following analogy to the problem of searching for an optimal tree by approximate methods. Consider the plight of a myopic pilot who loses his glasses when forced to parachute from his airplane into a mountainous region. He suspects that there is a manned outpost at the top of the highest peak in the area, but he must somehow grope his way there to have any hope of rescue. Obviously, simply walking uphill from the point of landing will not necessarily lead him to his goal, since he may not have started on a slope of the highest peak. Suppose that he reaches a summit and finds no outpost. Two possibilities remain: (1) he is, in fact, at the top of the highest peak but was wrong about the existence of the outpost; or (2) he has climbed the wrong hill. Unfortunately, he will have no way of choosing between these alternatives. Although rather silly, the analogy is actually quite apropos.

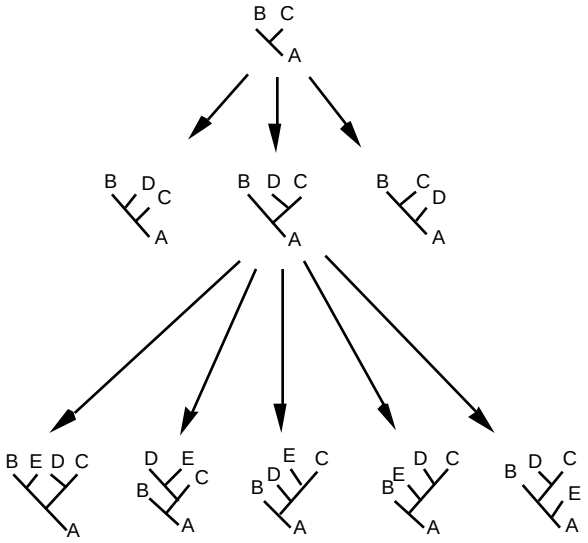
Heuristic tree searches generally operate by hill-climbing methods. An initial tree is used to start the process; we then seek to improve the tree by rearranging it in a way that reduces its length. When we can find no way to further improve the tree, we stop. Like the downed pilot, however, we generally have no way of knowing whether we ended up at the top of the highest hill. That is, we do not know whether we have arrived at a ***global optimum*** or merely a ***local optimum***.

Fortunately, the heuristic methods used in PAUP are, so far as we can tell, very effective. Two basic strategies are used. An initial tree (or set of trees) is obtained by ***stepwise addition***. Then the tree is subjected to trial rearrangements that attempt to find shorter trees. This second process is called, somewhat loosely, ***branch swapping***.

Stepwise Addition

Stepwise addition operates by connecting taxa, one at a time, to a developing tree until all taxa have been placed. First, three taxa are

chosen for the initial tree. Next, one of the remaining unplaced taxa is selected for next addition. Each of the three trees that would result from joining the unplaced taxon to the tree along one of its (three) branches is evaluated, and the one (in this example, but more can be saved at each step with the HOLD option) whose length is optimal is saved for the next round. In this next round, yet another unplaced taxon is connected to the tree, this time to one of the five possible branches on the tree saved from the previous round. Again, one of the resulting trees is saved for the next round. The process terminates when all taxa have been joined to the tree. This process is illustrated in the figure below, where five taxa are added to a tree. First, three taxa are joined (A, B, and C). Next, taxon D is attached at each of three possible places. The shortest tree resulting from the addition of taxon D is retained, and taxon E is added, resulting in five trees. Branch swapping can then begin on the shortest tree(s) from these five.



Stepwise addition of five taxa .

Of course, the above description is oversimplified. We must have some way of determining which three taxa will be joined initially and which one of the unplaced taxa will be connected to the tree at each step. PAUP provides four options for specifying the ***addition sequence***:

(1) *As is*. The taxa are simply added in the same order in which they are presented in the data matrix, starting with the first three and sequentially adding the rest. This method is usually not very effective.

(2) *Closest.* Initially, the lengths of all possible three-taxon trees—formed by joining a triplet of terminal taxa to a single internal node—are evaluated. The three taxa yielding compose the

starting tree. At each successive step, all remaining unplaced taxa are considered for connection to every branch of the tree, and the taxon-branch combination that requires the smallest increase in tree length is chosen. Obviously, the *closest* approach requires considerably more computation than does *as is*. In the latter, the number of tree lengths that must be evaluated at each stage is simply equal to the number of branches on the tree (i.e., one for each potential connection point for the already chosen taxon). In the *closest* addition sequence, however, *every* unplaced taxon must be connected to the tree at *every* possible branch. In addition, each of the C(*T*, 3) possible triplets of taxa must be evaluated at the start.

(3) *Simple*. This option corresponds to the order in which taxa are connected in the "simple algorithm" of Farris (1970). As for *as is*, an addition sequence is determined prior to beginning the stepwise addition process; however, a more elaborate criterion for determining the sequence is used. First, the distance between each taxon and a reference taxon is calculated; Farris called this distance an *advancement index*. The taxa are then added in order of increasing "advancement." That is, the reference taxon and the two taxa closest to it form the initial three-taxon tree, and the remaining taxa are added in the order given by their rank in the array of advancement indices. In Farris (1970), the reference taxon represented a "hypothetical ancestor" possessing the assumed ancestral state for each character. However, the algorithm can be used with any taxon chosen as the reference.

(4) *Random*. A pseudorandom number generator is used to obtain a permutation of the taxa to be used as the addition sequence.

When ties occur under the simple and closest addition sequences, they are broken arbitrarily.

Unfortunately, no one strategy seems to work best for all data sets; the best approach is to try as many alternatives as possible, each of which may potentially provide a different starting point for branch swapping (see below). In particular, although random addition sequences are not very effective in terms of the stepwise addition process, they are exceedingly useful in obtaining different starting points for branch swapping. For some data sets, "families" or "islands" of trees exist (Hendy et al., 1988); (Maddison, 1991). Trees from the same island are much more similar to each other than trees from different islands. Suppose we define an island as a set of trees such that for every tree in the set, there is at least one other tree in the set that is exactly one rearrangement away [Maddison (1991); see *Branch Swapping* for details on what constitutes a "rearrangement].

Generally, if we find any tree in the island, we can recursively find all of the trees in the island by a process of swapping on every new tree we find.[5] However, by definition, a tree from a different island cannot possibly be obtained. Fortunately, by initiating branch swapping repeatedly from different starting trees, we can increase the probability of "beaching" on more than one island (and hopefully landing on all of them). One way of obtaining different starting trees would be to start from randomly generated tree topologies; however, these trees are usually so far from the optimal trees that searches are slow and ineffective. A compromise is to use trees obtained from random addition sequences, which are often different enough to be from distinct islands but still not too far from optimal. Another strategy would be to effectively "lower the water level" between islands and swap on nonminimal trees. In that way you don't have to try and "drain the ocean" and swap on random trees.

Even when there is only one "island," random addition sequences can be used to circumvent the problem of entrapment in local optima. If a nonminimal tree cannot be rearranged to produce a shorter (or equal-length) tree, the rearrangement process ordinarily terminates, even though the globally optimal trees have not been found. By trying several different starting trees, however, the chances improve that at least some of these starting trees will lead to globally rather than locally optimal trees.

Finally, random addition sequences can be used to evaluate the effectiveness of the heuristics. If you do 100 different replications under the random addition sequences and get the same 15 trees in every case, you can be reasonably confident that these trees are in fact all of the optimal trees. By examining the "running status report" (available as an option), you can gain some idea of whether additional replications are likely to be effective. If, for example, the first 10 replications produce 50 trees in three different islands, and the next 20 replications terminate due to finding one of those 50 trees, the chances are reasonably good that you have found all of the islands and all of the most parsimonious trees contained in those islands. If, on the other hand, you are still finding new islands after 100 replications, a high probability exists that more trees remain to be found.

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[5]This statement is strictly true only when the "collapse zero-length branches" option is declined. "Rearrangements" are defined only in terms of binary trees (see *Branch Swapping*). Before a tree in which zero-length branches have been collapsed is input to the swapping procedure, polytomous trees are first converted to binary trees by arbitrarily resolving polytomies into dichotomies. Although precautions are taken in the algorithm to avoid consistently resolving the polytomies in exactly the same way, this arbitrary resolution process can nonetheless prevent some trees in an island of binary trees from being found (Maddison, 1991).

The biggest drawback of stepwise addition algorithms is that they are too "greedy." Like the nearsighted pilot who is unable to scan the horizon and instead must simply proceed up the nearest hill, these methods strive for optimality given the current situation rather than attempting to look more broadly into the future. Thus, one placement of a taxon may be best, given the taxa currently on the tree, but that placement may become suboptimal upon the addition of subsequent taxa. Once a decision has been made to connect a taxon to a certain point, however, we must usually accept the consequences of that decision for the remainder of the stepwise addition process, perhaps ending up in a local optimum as a result. PAUP provides one option that attempts to deal with excessive greediness. If the number of trees held at each step (HOLD) is set to *n*, the *n* shortest trees from each step are considered, in turn, during the next step. Note that *n* trees are retained even if some are longer than others. For example, we might find in a given step trees of lengths 25, 25, 25, 26, 27, 27, and 28. The 26-step tree may ultimately be the best choice for retention, but if we saved only one of the 25-step trees, then it would be discarded prematurely. If HOLD were 4 or greater, however, the 26-step tree would be retained and would eventually "rise to the top." Setting HOLD >1 is also useful for minimizing the effect of ties early in the stepwise addition process, since ties are, to some extent, "followed out" rather than being broken arbitrarily.

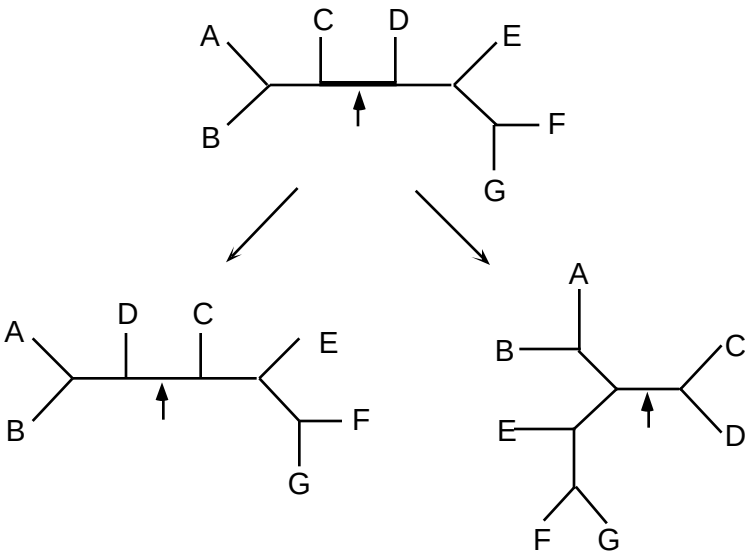
In order to determine the length of the tree that would result if a taxon were to connect to the tree at a particular point, PAUP evaluates the minimal length of the full tree, given that placement. (Some algorithmic tricks are used to minimize redundant calculations, however.) Note that this approach differs considerably from that of Farris (1970). In his method, character-state assignments made during each step are retained for all subsequent steps. These state assignments are only locally optimal given the state assignments from the previous iteration. Thus, a taxon might be connected to a branch to which it would not connect if a full optimization of the tree were performed.

Branch swapping

Because of the excessive greediness and susceptibility to local-optima problems, stepwise-addition algorithms generally do not find optimal trees unless the data are very clean. However, it may be possible to improve the initial estimate by performing sets of predefined rearrangements, a technique commonly referred to as "branch swapping." In general, any one of these rearrangements amounts to a "stab in the dark," but if a better tree exists and enough rearrangements are tried, one of them is likely to find it.

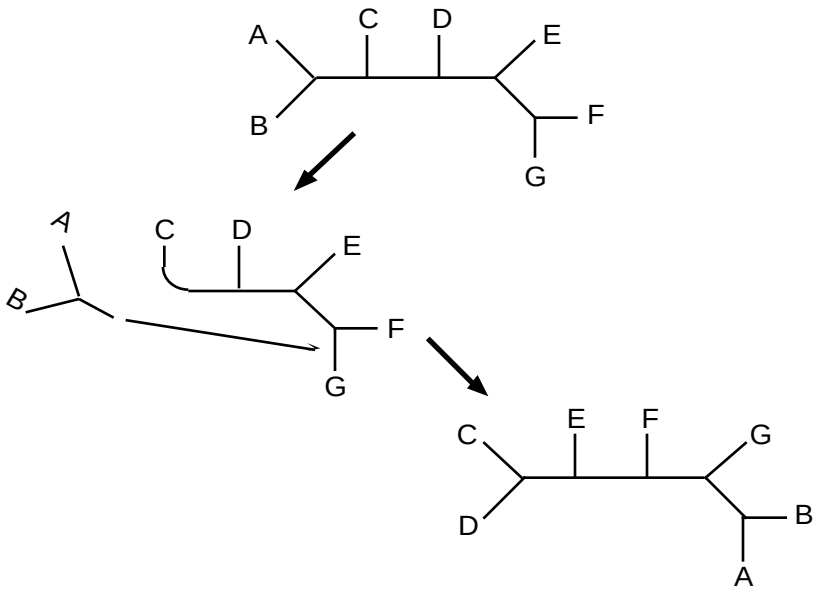
PAUP uses three branch-swapping algorithms. In order of increasing effectiveness, these are (1) nearest neighbor interchanges (NNI, equivalent to the "local" procedure used in Versions 1 and 2 of PAUP); (2) subtree pruning-regrafting (SPR, approximately, but not exactly, equivalent to the "global" procedure used in earlier versions of PAUP); and (3) tree bisection-reconnection (TBR, a new procedure).

In NNI swapping, each internal branch of the tree defines a local region of four subtrees connected by the internal branch. Interchanging a subtree on one side of the branch with one from the other constitutes an NNI. Two such rearrangements are possible for each internal branch, as shown below:



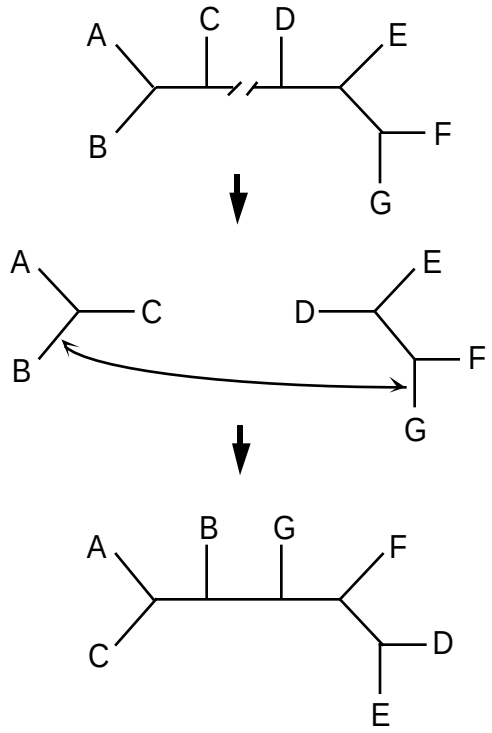
Nearest neighbor interchanges around the branch partitioning taxa {A,B,C} from {D,E,F}.

In "subtree pruning and regrafting," a subtree is pruned from the tree (e.g., the subtree containing terminal nodes A and B as indicated). The subtree is then regrafted to a different location on the tree. All possible subtree removals and reattachment points are evaluated.



An example rearrangement via "subtree pruning and regrafting." (a) the starting tree; (b) tree after pruning the subtree containing terminal taxa A and B; (c) tree after regrafting this subtree to the peripheral branch incident to terminal taxon G.

In "tree bisection and reconnection," the tree is bisected along a branch, yielding two disjoint subtrees. The subtrees are then reconnected by joining a pair of branches, one from each subtree. All possible bisections and pairwise reconnections are evaluated.



Example rearrangement via tree bisection-reconnection. Initial tree is bisected into two subtrees and then reconnected along a different pair of branches.

Of course, the globally optimal tree(s) may be several rearrangements away from the starting tree. If a rearrangement is successful in finding a better tree, a round of rearrangements is initiated on this new tree. So long as each round of rearrangements successfully finds an improved tree (according to their length under the optimality criterion), then we will eventually arrive at the global optimum. However, if the path to the optimal trees requires us to pass through intermediate trees that are inferior to the best one(s) yet obtained, we will once again find ourselves trapped in a local optimum unless an option is provided for branch-swapping on suboptimal trees (the "KEEP" option in PAUP may be used for this purpose). A related problem concerns "plateaus" on the optimality surface. It may be the case, for example, that an optimal tree lies several rearrangements away from the current tree, and that these rearrangements all correspond to trees having equal lengths under the optimality criterion. If the intermediate trees are discarded because they are "not better," then the optimal tree will not be found. The MULPARS option requests the saving of all of the equally most parsimonious trees.

Swapping on polytomous trees: The rearrangement algorithms above are defined only for dichotomous trees. However, when the COLLAPSE (zero-length branches) option is in effect, trees recovered from memory for input to branch swapping may have one or more polytomous nodes. In

this case, PAUP "dichotomizes" the tree arbitrarily in order to prepare the tree for branch-swapping. This arbitrary resolution can introduce additional complications. Most importantly, the resolution of the polytomy that PAUP chose may not lead to all minimal trees in an island. It may be that another resolution would actually have done better, but was not chosen.

There are two solutions to this problem. First, you can save all of the trees found by branch-swapping and input them into a second round of swapping. This greatly increases the chances of finding all of the trees in an island. The second solution is not to use the COLLAPSE option in the first place. With COLLAPSE off, you can save all dichotomous trees and use the **CONDENSE** command at the end of branch swapping. The problem with this strategy is that there may be too many dichotomous trees, and huge amounts of memory and long search times may be required.

The effect of using COLLAPSE has special significance when random addition-sequences are used to construct starting trees. In the normal case, if PAUP finds a tree that it has found in a previous replicate, it assumes that branch swapping in the previous round has already found all trees in the island to which that tree belongs; therefore it abandons the current round. But that assumption may not be true, since there is no guarantee that the original "dichotomization" allowed full exploration of the island. So the trees that resulted are not *necessarily* all of the minimal trees. In that case, it may be prudent not to use COLLAPSE in conjunction with random replicates. This may greatly increase computation time, but will avoid the problem of arbitrarily missing parts of islands.

Steepest descent: The first "round" of swapping begins with the trees(s) in memory. These can come from several sources: user-defined trees, an external treefile, a TREES block in a data file, or from stepwise addition. In the simplest case, PAUP will swap only on minimal trees (although this can be changed). In that case, the first round begins by discarding all nonminimal trees and swapping on the first of the minimal trees. For example, if we start with five trees in memory of length 30, 30, 30, 31, and 32, PAUP will begin by discarding the two trees longer than 30 steps. It will then begin swapping on one of the 30-step trees. If no trees shorter than 30 steps are found, it will go on to swap on another 30-step tree. If STEEPEST DESCENT is off, when a tree of less than 30 steps (say, 29 steps) is found *at any point*, all 30-step trees from that round are discarded. That 29-step tree is now the input for the next round of swapping. Subsequent rounds begin each time a shorter tree is found. The process terminates when swapping on all starting trees from a previous round does not find any shorter trees.

However, if the STEEPEST DESCENT option is on, the round is not abandoned when a shorter tree is found, but continues until *all* trees from the previous round have been examined. In the example above, this means that the round would not end as soon as a 29-step tree was found. PAUP would swap on all of the initial 30-step trees, saving all of the new shortest trees and passing them to the next round. It's called "steepest descent" because instead of discarding current trees the instant a shorter one is found, it continues looking for even shorter ones, and uses the tree(s) that give the most improvement for the next round.

Note that if STEEPEST DESCENT is off and swapping on the first 30-step tree led to a 29-step tree, PAUP would not swap on the other starting trees. Thus if you want to force swapping on *all* minimal starting trees, you must enable STEEPEST DESCENT. Steepest descent is sometimes effective in finding other islands because it may allow the search to take a different path than it would otherwise. One way to hop islands is to swap on nonminimal as well as minimal trees; steepest descent is similar in that ***more*** nonminimal trees are tried as the search continues to find shorter and shorter trees. For many data sets, my experience is that steepest descent causes the program to get bogged down finding thousands of trees that will eventually be discarded anyway, and a better strategy is to perform more replicates of the random addition sequence without steepest descent.

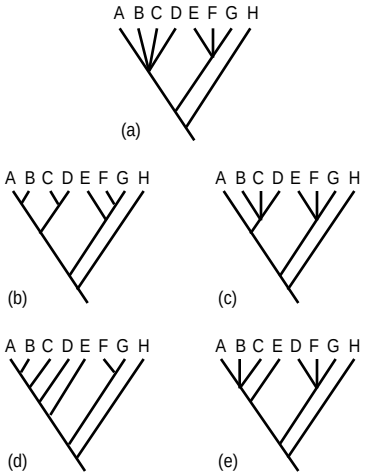
Searching under Topological Constraints

Topological constraints are a new feature in version 3 of PAUP. They are of two types: "monophyly" constraint trees, which contain all of the taxa in the data matrix, and "backbone" trees, which contain a subset of taxa. To search under constraints, you must define and select a constraint tree; this tree is used to restrict the set of trees retained by heuristic or exact searches.

"Monophyly" constraint trees

Monophyly constraint trees are usually incompletely resolved (i.e., contain one or more polytomies). These polytomies on the constraint tree indicate uncertainty with respect to relationships rather than simultaneous divergence into more than two descendant lineages. A tree being evaluated (the "trial tree") is said to be compatible with the constraint tree if and only if it is either identical to the constraint tree or it can be transformed into the constraint tree by deleting (collapsing) one or more of its branches. In more biological terms, constraint-tree compatibility means that any statement of relationship among taxa implied by the constraint tree must also be true for the trial tree. Thus, trial trees compatible with the constraint tree are (usually) "more highly resolved versions" or "refinements" of the constraint tree. For example, in the

figure below, trial trees *b* and *c* satisfy the constraints imposed by tree *a* but trial trees *d* and *e* do not.



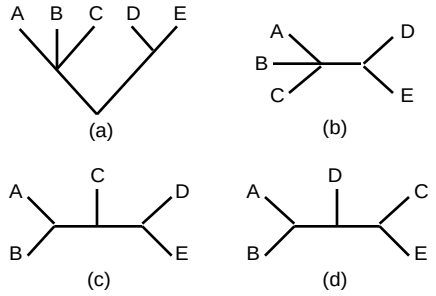
Example for constraint-tree compatibility. (a) The constraint tree. (b,c) Two trees that are compatible with the constraint tree. (d,e) Two trees that are incompatible with the constraint tree.

If a group of particular interest is not monophyletic on the minimal trees for your data set, constraining a search so that only those trees consistent with the group's monophyly are retained makes it easy to determine how much longer are the shortest trees on which the group *is* monophyletic. For example, if the most parsimonious tree for your data set does not support the monophyly of a genus according to someone else's classification (established on the basis of other data), you might be less inclined to challenge that classification if the genus were monophyletic on a tree only one step longer than if 28 additional steps were required for the group's monophyly. This notion can be extended to testing your data against an entire classification rather than only a single group. Since established classifications are usually not fully resolved, you can constrain the search so that only trees that are consistent with that classification are retained. Thus, where the classification is unambiguous, the relationships it implies must be maintained before a trial tree is accepted. However, other aspects of the classification can be resolved in the way that is most parsimonious for the data at hand.

Before the topological constraints feature was implemented in PAUP, users could force the monophyly of particular groups by including "dummy" synapomorphies in the data matrix and weighting them so heavily that any tree on which the group was not monophyletic was immediately rejected. This approach was inconvenient and sometimes tedious, and had the further drawback that the dummy characters needed to be deleted before tree lengths and consistency indices were interpretable. Furthermore, it is impossible to determine the number of steps required to "break up" a monophyletic group appearing on the most

parsimonious trees by using dummy character techniques. In addition to allowing you to force compatibility with the constraint tree, PAUP allows you to request acceptance of trees only if they are *incompatible* with the constraint tree. This information provides a crude index to the strength of support for a clade. Of course, you are then faced with the decision as to how many steps longer a tree must be before it the existence of a clade is considered to be insupportable. (One way to make this judgment is through the use of the "T-PTP" randomization tests suggested by Faith, 1991.)

Note that although constraint trees are input as rooted trees, the criterion for satisfaction of topological constraints is the same whether rooted or unrooted trees are being determined. For unrooted trees, the rooted constraint tree is first "derooted." Then each tree being evaluated is compared to the (unrooted) constraint tree; if the trial tree is equal to the constraint tree or the trial tree can be converted to the constraint tree by deleting one or more branches from the trial tree, then the constraints are satisfied. An example is shown below.



Unrooted constraints. (a) A rooted constraint tree. (b) Unrooted equivalent of the constraint tree. (c) A tree that satisfies the constraints imposed by tree "b." (d) A tree that violates the constraints imposed by tree "b."

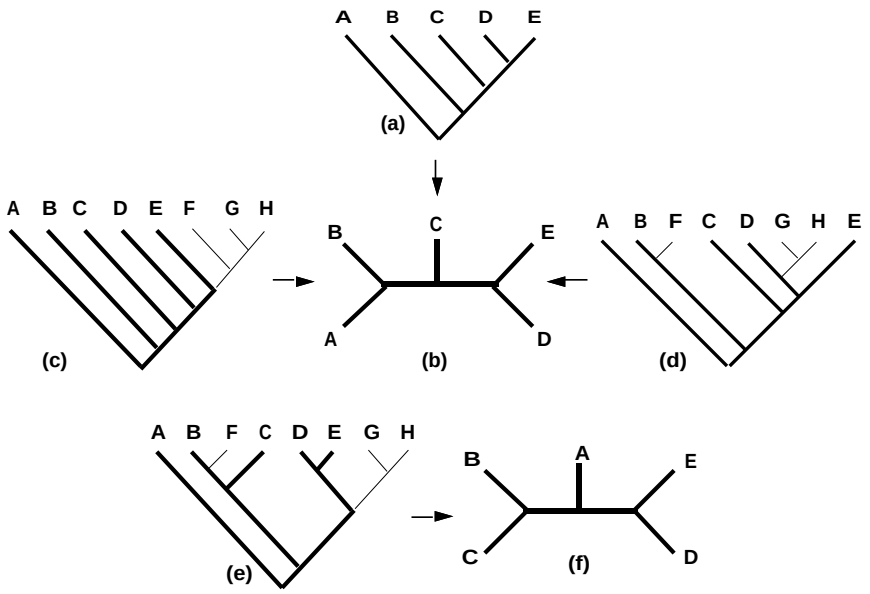
Topological constraints can also be used to restrict the "solution space" when searching using exact algorithms (Sankoff et al., 1982; Constantinescu and Sankoff, 1986). If the monophyly of particular groups is indisputable, the imposition of constraints that enforce these groupings can greatly reduce the number of possible trees that need to be examined, thereby extending the limits of usefulness of the exact algorithms. Of course, the "guarantee" of optimality is then conditional on the validity of the assumed prior groupings, but that sacrifice may be a relatively small price to pay in many situations.

Yet another use of topological constraints is to enforce ingroup monophyly or partial outgroup structure. Recall that when potential outgroup taxa vary for characters that are informative with respect to ingroup relationships, one recourse is to include several outgroup taxa in the analysis. In this procedure, the ingroup node incident to the branch

partitioning the ingroup from the outgroup becomes the root of the ingroup portion of the tree. If, however, the ingroup and outgroup taxa do not constitute a partition on the most parsimonious tree(s), you may wish to impose ingroup monophyly as a constraint. (Presumably, you would do this only if additional evidence supporting ingroup monophyly exists, but is not contained in the data set.) Simply define a constraint tree with a single group consisting of the ingroup taxa and enforce that constraint during a search. Another problem that sometimes arises when using this approach is the lack of sufficient information to adequately resolve outgroup relationships, with the result that a large number of equally parsimonious trees differing only in the relationships among outgroup taxa are found. Again, if there is support for some outgroup structure that comes from information not available in the data set, these aspects of the tree can be enforced through topological constraints. Finally, note that if you use the option to collapse zero-length branches (see "Zero-Length Branches and Polytomies," below) PAUP will not collapse a branch if this action would result in violation of the constraints for a tree that would otherwise satisfy them.

"Backbone" constraint trees

"Backbone" constraint trees differ from "monophyly" trees in that they contain only a subset of the study taxa. A trial tree is compatible with the constraint tree if pruning the taxa not present on the backbone tree from the trial tree leaves a topology identical to the backbone. Unlike the monophyly constraint trees described above, backbone trees force a *relative* topology, and other taxa may be added at any point on the backbone tree, as long as the backbone is not violated. For example, a typical backbone topology might look like tree *a* below, and the corresponding unrooted backbone topology would look like tree *b*. Trees *c* and *d* are compatible with the backbone, while tree *e* is not—when taxa F, G, and H (i.e., those not present on the backbone tree) are pruned, Tree *f* results, which differs from Tree *b*.



Backbone constraints. (a) Rooted backbone. (b) unrooted equivalent of rooted backbone. (c) and (d) Rooted trees compatible with backbone—backbone is highlighted. (e) Rooted tree incompatible with backbone. Tree connecting taxa A-E is highlighted. (f) unrooted tree for taxa A-E derived from (e).

Backbone constraints force a *relative* pattern of relationships. In the above example, the *relative* positions of A, B, C, D, and E on the tree must be the same. Using backbone constraints is less restrictive than using monophyletic constraints, in that any topology is compatible with the constraint tree as long as the relative backbone topology is preserved. This means that a backbone does not force an inclusive monophyletic group as does a monophyly constraint tree. The interesting feature of backbone trees is that they can also be unresolved (partially, as long as there are two child nodes—see above). The unresolved part of the backbone tree is even less restrictive, as even the *relative* topology within that part is not specified. In the end, whether you use a backbone or monophyletic constraint tree depends on your immediate goals—if you are interested in the lengths of trees which are compatible with a particular hypothesis of monophyly, use a monophyletic constraint tree; however if you are interested in which trees are compatible with a less restrictive topology, one that includes fewer taxa, use backbone constraints. In either case you may still be left with trees compatible with the constraint *and* those which are the shortest length, if they are different from the former group

Heuristic Searches and "converse" constraints

When "converse" constraints (i.e. trees are kept only if they are *incompatible* with the constraint tree) are used during a heuristic search,

special problems may arise. This strategy will cause trouble if a heuristic search does not find the shortest trees that do not sashays the constraint, or if a heuristic search finds some, but not all, of those shortest trees. The latter is probably more common, but the former is more serious. If for whatever reason the data set is one that does not lend itself to a branch-and-bound analysis, it may be impossible to check whether either of these events has occurred, so some degree of skepticism might be warranted.

Why might these situations occur in the first place? The heuristic search uses the specified addition sequence, finding the shortest tree *until the last step*, when it *must* place the last taxon such that the constraints are violated. This will then be the starting point for branch-swapping. Because you have asked the algorithm to deliberately narrow the solution space before branch swapping even begins, it is possible that all optimal "converse" trees will not be found by branch swapping. You have simply asked the algorithm to begin swapping on a tree from which it cannot reach the shortest trees that are incompatible with the constraint tree. In some instances, trying different swapping and addition options may find more trees, but this is not always true. Again, if your data set is small enough to allow branch-and-bound searching, this will not be a problem. What are the solutions if an exact search is not possible? In that case you must be as diligent as possible in trying different addition sequences (especially random addition) and different swapping algorithms.

Keeping "Near-Minimal" Trees

You can keep trees less than or equal to a certain length by setting the **Keep all trees ≤ length** ___ option in the search procedure dialog boxes or specifying KEEP=*length* in the **HSEARCH**, **BANDB**, and **ALLTREES** commands.

Selecting "steepest descent" forces the examination of suboptimal trees even when a shorter one has been found. This examination of suboptimal trees can be increased by selecting **Keep all trees ≤ length** ___. This may allow the hopping of islands, so that the global optimum is reached, but can also substantially increase computation time.

One of the values of setting the **Keep all trees ≤ length** ___ option is that it allows one to examine the support for particular groupings in trees that are slightly longer than the optimal tree(s). We might, for example, keep all trees 3 steps longer than the optimal trees. If a particular group is present in the optimal tree(s) and all the suboptimal trees which are kept, then we might place more confidence in the support for that grouping. If, however, a particular clade is found only in the shortest trees and becomes nonmonophyletic in trees one or two steps longer, we might have less confidence in the support for that group.

The relationship between clade monophyly and tree length can be quickly evaluated by the use of topological constraints (see "Searching under topological constraints" above), but setting the "keep" option allows evaluation of the whole topology, not just those aspects set in the constraint tree (although as an alternative, multiple constraint trees can be examined). This strategy becomes more interesting when the frequency distribution of all possible tree lengths is examined (produced as a by-product of an exhaustive search, if that search type is possible for the data set (see the section "Exhaustive searches" for a description of this option). With detailed information about the number and lengths of all trees, we can better evaluate the strength of evidence for a particular group. In the example above, we kept all trees 3 steps longer than the optimal tree(s). However, the frequency distribution might show that there are few trees 3 steps longer, but many trees 4 steps longer, which might also be profitable to examine. In this way, it can be used in concert with the "keep" option in exploring suboptimal trees. As in the case of evaluating the length of trees which do not meet constraints of monophyly, you are still faced with the decision as to how many steps longer a tree must be before the existence of a clade is considered to be insupportable. Despite this difficulty, it is satisfying to know how long a tree must be before a particular clade becomes nonmonophyletic.

ZERO-LENGTH BRANCHES AND POLYTOMIES

As discussed above, PAUP's search algorithms find only binary trees. When the data are insufficient to determine fully resolved trees, trees containing polytomies may be preferable to binary trees containing one or more branches of zero length. Dichotomies that collapse into polytomies when zero-length interior branches are deleted are said to represent ***arbitrary resolutions***, because character support for some of the groupings is absent or ambiguous. Usually (but not always), a smaller number of trees containing one or more polytomies is preferable to the full set of binary trees; all of PAUP's search algorithms therefore provide an option for collapsing interior branches of zero length.

Unfortunately, however, the criterion for collapsing zero-length branches is complicated by the fact that for some characters, more than one ***most parsimonious reconstruction*** [MPR; Swofford and Maddison (1987)] may exist. An MPR is the set of state assignments to each interior node that allow the length required by a character on a given tree to be minimized. Consequently, a branch may have zero length under one MPR but a length greater than zero under a different MPR. Swofford and Maddison (1987) described an algorithm for finding the minimum and maximum lengths of a branch over all MPRs for a single character. We

can obtain the "minimum possible" and "maximum possible" branch lengths by summing, over all characters, the minimum and maximum branch lengths for each character. Conceivably, three rules could then be used to decide whether to collapse an interior branch:

(1) Collapse an interior branch if the *minimum* possible length of the branch is zero. That is, if there exists at least one MPR for every character such that no length is assigned to the branch, the branch is collapsed.

(2) Adopt an ancillary criterion for choosing one MPR from the full set of MPRs for each character (or choose one arbitrarily). If no length is assigned to the branch for all characters, then the branch is collapsed.

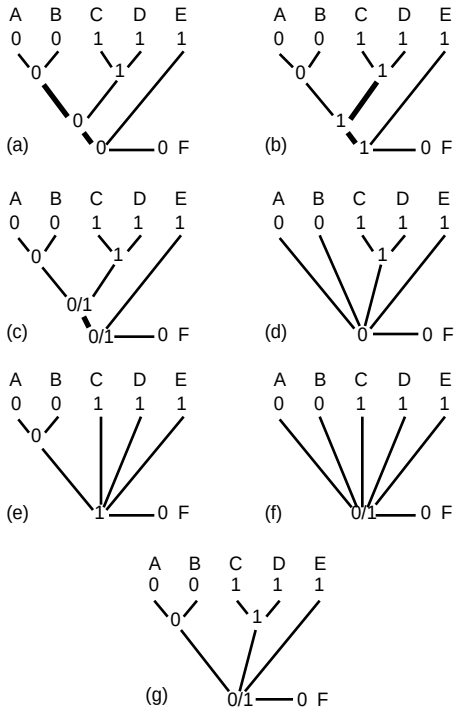
(3) Collapse an interior branch if the *maximum* possible length of the branch is zero. That is, if all MPRs assign zero length to the branch for every character, then the branch is collapsed.

I believe that only the third criterion can be justified and this rule is the one implemented in PAUP. (Comparisons of results from Farris's Hennig 86 program suggest that this rule is used by that program as well, although his documentation does not address the issue.) The rationale is simply that if potential, though perhaps ambiguous, support exists for a particular resolution of a polytomy, then the resolution should be retained. However, if no support exists for any resolution (i.e., a branch length is zero under every possible reconstruction), then a polytomy is in order. For example, if three trees found by PAUP consist of a trichotomy and two of the three resolutions of that trichotomy. This means that there is *potential* support for two of the three resolutions of the trichotomy, but for the third resolution there are no characters that provide even potential support. Note that even when a branch is not collapsed, it may still be assigned zero length under some reconstructions. Thus, if you have requested the option to collapse branches and yet zero-length branches occur in the output, you will know that there are other MPRs in which the branch has nonzero length.

The first criterion is flawed in that it may not be possible to collapse all branches whose minimum length is zero and still obtain an MPR. For instance, two branches may each potentially have zero length, but once one of the branches is reduced to zero length, the other cannot be, and vice versa. An example is shown below. Two MPRs exist (A and B) for the hypothetical discrete character on the binary tree shown, requiring two steps. It can be seen from these reconstructions that all three of the branches may potentially be assigned no changes (zero length), but only the interior branch indicated by a heavy line in tree C has zero length under both reconstructions. If we collapse the zero-length branches under

reconstructions ?a and ?b, we obtain the trees and reconstructions of trees D and E, respectively, which also require two steps. However, if all branches that are potentially of zero length (i.e., of zero length in at least one MPR) are collapsed, an MPR on the resulting tree (F) would then require three steps whether state 0 or state 1 were assigned to the single remaining internal node.

This example also illustrates why PAUP uses the third criterion listed above rather than the second. If branches having zero length under *either* reconstruction ?a or reconstruction ?b (but not both) are collapsed, then either the group (A,B) or the group (C,D) would be retained. Clearly, the decision to retain one but not both of these groups would be arbitrary. PAUP retains both groups, since there is potential support (i.e., favored by at least one reconstruction) for both. However, the group (A,B,C,D) is not retained since neither reconstruction provides even potential character support for it. Of course, some other character might provide support for the (A,B,C,D) group, and if so, the group would be retained. Otherwise, the tree would collapse as shown in tree G.



Demonstration of effect of different criteria for collapsing zero-length branches. (a,b) The two MPRs for a hypothetical character on a given tree; heavy lines indicate zero-length interior branches. (c) Possible state assignments to interior nodes consistent with at least one MPR; heavy lines indicates mandatory zero-length interior branch. (d) Reconstruction A after collapsing all zero-length interior branches. (e) Reconstruction B after collapsing all zero-length interior branches. (f) Tree after collapsing all interior branches that are of zero length under at least one MPR. (g) tree after collapsing all branches that have zero length under all MPRs, retaining groups with potential (but ambiguous, in this case) support.

TREE-TO-TREE DISTANCES

As we discussed in the context of "islands" in the section on heuristic searching above, there may be distinct families of trees that are similar with a family but very dissimilar to trees in other families (Hendy et al., 1988; Maddison, 1991). Random addition sequence searches provide one way to discover islands. Another way to examine this phenomenon is to use one of several metrics that measure the dissimilarity of pairs of trees. PAUP provides only one of these, the symmetric-difference or "partition" metric (Penny and Hendy, 1985), which is equivalent to the Robinson and Foulds's (1981) ontraction/decontraction metric. Several others are calculated by the COMPONENT program (Page, 1993). The symmetric difference metric simply counts the number of groups that are on one tree or the other but not on both. Trees that are most similar will have the

lowest distance value. The output is potentially useful in identifying "classes" of similar trees.

By default, a matrix of pairwise distances between all trees is computed, as well as a frequency distribution of the distance values. You may choose to suppress one or both of the options. You can also choose to compare all trees in memory to a reference tree rather than comparing all pairs of trees. Here is a sample of the output (generated from the 25 most parsimonious trees for the "Menidia" sample data set). The command

```
treedist;
```

with no options requests the full matrix of pairwise comparisons:

```
Symmetric-difference distances between trees

        1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
1       -
2       4  -
3       2  4  -
4       1  3  1  -
5       3  7  5  4  -
6       2  6  4  3  3  -
7       3  7  5  4  2  3  -
8       1  5  3  2  4  1  2  -
9       7  3  7  6  4  7  6  8  -
10      6  2  6  5  7  4  7  5  3  -
11      7  3  7  6  6  7  4  6  2  3  -
12      5  1  5  4  8  5  6  4  4  1  2  -
13      6  2  6  5  9  8  9  7  5  4  5  3  -
14      3  5  1  2  6  3  4  2  8  5  6  4  7  -
15      2  4  2  1  5  2  3  1  7  4  5  3  6  1  -
16      7  3  7  6 10  7  8  6  6  3  4  2  1  6  5  -
17      5  7  3  4  2  5  4  6  4  7  6  8  9  4  5 10  -
18      5  7  3  4  4  5  2  4  6  7  4  6  9  2  3  8  2  -
19      4  6  2  3  5  2  5  3  7  4  7  5  8  1  2  7  3  3  -
20      4  6  4  3  1  4  3  5  3  6  5  7  8  5  4  9  1  3  4  -
21      4  6  4  3  3  4  1  3  5  6  3  5  8  3  2  7  3  1  4  2  -
22      9  5  9  8  6  9  8 10  2  5  4  6  3 10  9  4  6  8  9  5  7  -
23      8  4  8  7  9  6  9  7  5  2  5  3  2  7  6  1  9  9  6  8  8  3  -
24      9  5  9  8  8  9  6  8  4  5  2  4  3  8  7  2  8  6  9  7  5  2  3  -
25      3  5  3  2  4  1  4  2  6  3  6  4  7  2  1  6  4  4  1  3  3  8  5  8

Symmetric-difference distances between trees (continued)

       25
25      -

Distribution of symmetric-difference distances between trees

   fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
 0 › (0)
 1 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (20)
 2 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (33)
 3 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (45)
 4 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (47)
 5 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (39)
 6 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (38)
 7 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (32)
 8 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (24)
 9 ›,,,,,,,,,,,,,,,,,,,,,,,,,, (18)
10 ›,,,,,, (4)
   fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
```

To limit the analysis to comparisons of one tree against all others, specify a tree number for the COMPARE option:

```
treedist compare=1;
```

```
Symmetric-difference distances to tree 1

Tree number    1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21
Distance       -  4  2  1  3  2  3  1  7  6  7  5  6  3  2  7  5  5  4  4  4

Tree number   22 23 24 25
Distance       9  8  9  3

Distribution of symmetric-difference distances to tree 1

  fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
0 › (0)
1 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (2)
2 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (3)
3 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (4)
4 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (4)
5 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (3)
6 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (2)
7 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (3)
8 ›,,,,,,,,,,,,,,,,, (1)
9 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (2)
  fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
```

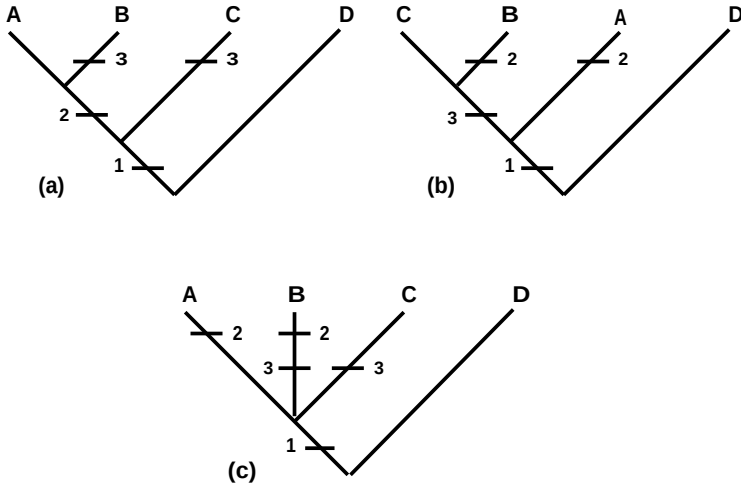
CONSENSUS TREES

Consensus trees are hierarchical summaries of the information common to a set of "rival" trees. PAUP provides the following consensus tree methods: strict, semistrict (a variation on strict), Adams, and majority-rule. These methods differ in the criteria used for retaining ***groups*** (subsets) of taxa in the consensus. Introductory descriptions of consensus methods can be found in Swofford (1991) and Wiley et al. (1991).

> **NOTE:** For all consensus methods other than that of Adams (1972), if (1) the rival trees are unrooted, (2) outgroup-rooting is in effect, and (3) there are multiple outgroup taxa, then PAUP first computes an unrooted consensus and then outgroup-roots the consensus tree. (Algorithmically, this is accomplished by rooting the tree at an arbitrarily chosen terminal taxon and applying the usual rooted consensus methods). As long as the specified ingroup taxa always comprise a monophyletic group, this approach is exactly equivalent to outgroup-rooting the rival trees first and then computing a rooted consensus. However, when the tree cannot be rooted such that the ingroup is monophyletic, the position of the root is not well specified, and the resulting consensus would depend on the method used to resolve the ambiguity in locating the root. The approach used in PAUP circumvents this problem—the uncertainty in locating the root does not come into play until after the consensus is computed. This approach can not be used for the Adams (1972) method, which is defined only for rooted trees; consequently trees are rooted prior to calculation of the consensus in this case.

In general, a consensus tree for a group of minimal trees will be longer than any of those trees (e.g., Miyamoto, 1985). This is because consensus trees are usually less-resolved than any of the rival trees, which automatically means that some characters will have to change more times on the unresolved consensus than they would have to on the more highly

resolved rival trees. For example, the figure below shows two rival trees of length four. Their strict consensus is tree C. Because this tree is unresolved, both characters two *and* three must have two steps. Its total length is then five steps, one step longer than either of the rival trees.



Tree lengths of rival trees and their consensus. (a) rival tree of length four. (b) a different rival tree of length four. (c) strict consensus of (a) and (b), length is now five.

The main point here is that *the consensus tree is not an optimal tree for a particular data set*, and should not be treated as such. It is a summary of agreement among trees, but it should not be interpreted as a phylogenetic tree. Such an interpretation assumes that polytomous nodes indicate simultaneous divergence of multiple lineages rather than uncertain resolution (i.e., "hard polytomies" vs. "soft polytomies; Maddison, 1989).

Strict Consensus

Strict consensus trees contain only those groups appearing in all of the rival trees (Sokal and Rohlf, 1981; Page, 1989). This can be considered to be the most conservative estimate of consensus, and is the simplest to interpret. In the figure below, tree *f* is the strict consensus of trees *a*, *b*, and *c*. It preserves the two groups (ABC) and (DEF), but there is no resolution within either of those groups. In the case of (EDF), two of the rival trees (*a* and *c*) agree on the resolution of (D(E,F)), while the other tree (*b*) resolves this clade as (E(D,F)). This conflict is reflected in the strict consensus by the trichotomy (E,D,F). In the case of (ABC), because only one tree resolves (c) resolves the clade and two do not, the strict consensus must then be unresolved. The liability of this technique stems from its demand for identity in each of the rival trees. For example, two trees may be identical except for the placement of a single taxon, yet may have a completely unresolved consensus. In this instance, the consensus is "too strict" (Adams, 1986; Funk and Brooks, 1990) , in that none of the

agreement between the trees is preserved in the consensus when the unstable taxon is included.

Three rival trees (a,b,c) and their strict (d), semistrict(e) and 50% majority rule (f) consensus trees.

Semistrict Consensus

This method corresponds to the "combinable-component" consensus of (Bremer, 1990) (essentially the same idea was earlier proposed by Hillis, 1987). Under this method, if all trees have either an (A,B,C) trichotomy or an ((A,B),C) dichotomy (i.e., A+B is never contradicted, just not always supported), then (A,B) is retained in the consensus. In the example above, trees *a* and *c* are unresolved, while tree *b* supports A+B. The semistrict consensus (*e*) preserves A+B, while the strict consensus (*f*) does not. When there is conflict, semistrict behaves the same as strict. E.

Majority-rule consensus

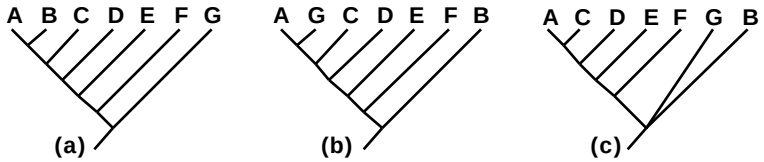
In contrast to strict consensus, it may be of interest to find groups that appear on a certain pre-specified percentage of the rival trees. Thus a group may be preserved in the consensus even if there are some trees that support conflicting groups (see Margush and McMorris, 1981 and Swofford, 1991). In the example above, the majority-rule consensus (*d*) preserves (EF) because it is found in two of the three rival trees (*a* and *c*). Likewise, (ABC) is unresolved because two of the three trees do not resolve it. Typically the majority-rule percentage is set to 50%, so that the consensus will retain all groups found in over half of the rival trees. The reason that groups must be present in over half the rival trees to be included is that two groups occurring in exactly half the trees might not be able to coexist on the same consensus tree. If only two trees are being compared, majority-rule and strict methods are equivalent.

Adams Consensus

The method of Adams (1972; 1986) (see also Swofford, 1991) was the first consensus method to be proposed. Adams trees typically preserve more structure than strict methods. The example shown below illustrates the behavior of Adams consensus. The strict consensus of trees *a* and *b* would be completely unresolved, as there are no groups that are *exactly* the same on both of the rival trees. The Adams consensus (tree *c*) shows more resolution, but it must be interpreted carefully. If this were a strict consensus, we would infer that there is a monophyletic group (ACDEF) on

all of the rival trees, and that the relationships of B and G to that group are unresolved.

The Adams tree makes no such claim. Instead of being defined on the basis of monophyletic groups, it shows "nestings" shared among the trees (one group is said to *nest* within a larger group if the most recent common ancestor of the smaller group is a descendant of the most recent common ancestor of the larger group, which need not require monophyly of either group). In this example, the most recent common ancestor of taxa A and C is a descendant of the most recent common ancestor of taxa A, C, and D on both rival trees; therefore (AC) is retained in the consensus. The Adams consensus reflects a great deal of shared structure between trees *a* and *b*, which are identical if taxa B and G are removed. It does not, however, imply that a monophyletic (ACDEF) is found on either of the rival trees. The appearance of B and G at the unresolved portion at the base of the consensus indicates that their placement relative to the other taxa is different on each of the rivals. It does *not* indicate that they both belong outside the ACDEF group, since the rival trees suggest that either B or G may be contained within that group.



Two rival trees (a, b) and their Adams consensus (c)

Note that the group (AC) appears in the Adams consensus, yet is not found on either of the two rival trees (the same is true of (ACD), (ACDE), and (ACDEF)). This is a common occurrence with the Adams method, and has been the source of much of the criticism concerning it. This criticism is valid only if Adams trees are interpreted in the same way as are strict consensus trees. On a strict consensus, the appearance of an AC clade would indicate that clade appeared on all rival trees. On an Adams consensus, the appearance of an AC clade means instead that the rival trees indicate that A and C are more closely related to each other than either is to D, E, or F.

Consensus indices

Several authors have developed indices that use the topology of the consensus tree to measure the degree of congruence of the rival trees. Many of these measures are simple functions of the "resolution" of the consensus tree: the more congruent a set of rival trees is, the more highly resolved will be the consensus of those trees. Some indices are also

affected by the symmetry of the tree. The consensus indices computed by PAUP are described in Rohlf (1982) and Swofford (1991).

GOODNESS-OF-FIT STATISTICS

PAUP outputs several indices that measure the "fit" of characters to particular trees. Three main parameters are used to define these indices (Kluge and Farris, 1969; Farris, 1989a; Farris, 1989b):

s = length (number of steps) required by the character on the tree being evaluated

m = minimum amount of change that the character may show on *any* conceivable tree

g = maximum possible amount of change that a character could possibly require on any conceivable tree i.e., the length of the character on a completely unresolved bush).

The ***consistency index*** (Kluge and Farris, 1969) for a single character, c, equals m/s. Thus, if a particular tree explains the data as well as any tree possibly could, $c = 1$. Unfortunately, the lower bound on c is not 0 but is a function of the distribution of character-states in the data matrix. For example, if two taxa have state 1 but all others have state 0, the maximum possible number of steps on any tree is 2, so that c can be no lower than 0.5. Farris (1989a; 1989b) proposed two new indices, the ***retention index*** and the ***rescaled consistency index***. For a single character, the retention index, r, is defined as $(g - s)/(g - m)$. Thus when a character fits the tree as poorly as possible, its retention index will be 0. Note that for uninformative (e.g., autapomorphic) characters, $m = g$ so that r is undefined. Farris (1989a; 1989b) recommends using r as a factor for scaling c between 0 and 1, defining the rescaled consistency index as the product of r and c ($= rc$).

An "ensemble" (overall) consistency index C (Farris, 1989b) for a suite of characters is calculated as M/S, where M and S are the sums over all characters in the suite of the individual-character m and s values, respectively. The ensemble retention index R is defined analogously to the ensemble consistency index; i.e., $(G - S)/(G - M) = (\sum g - \sum s)/(\sum g - \sum m)$. Archie (1989) independently proposed an equivalent index, calling it the "homoplasy excess ratio maximum" (=HERM). The product of R and C is referred to as the "ensemble rescaled consistency index."

In general, the homoplasy index HI is equal to 1 – C. But when multistate taxa are treated as "polymorphic," the homoplasy index has a slightly different meaning. In that instance, PAUP identifies an "ancestral" character state in the multistate taxon from which all observed states in that taxon must be derived (see "Multistate Taxa"). Thus the homoplasy index will not be the same for a character in which multistate taxa are treated as polymorphic (character change allowed within the terminal), and "uncertain" (no character change allowed within the terminal).

All of these measures are useful not only in comparing characters on a single tree, but on multiple trees as well. If several different analyses of a data set produce different trees when different assumptions are made, these indices are a quick way to estimate which characters support which hypotheses of topology. When you use the **LENFIT** command or the **Lengths and Fit Measures** menu command, you can output a summary of length and fit measures for trees *and* for characters. These can then be used to evaluate which characters fit which trees. You can choose to output information only for those characters which vary over the trees in memory—see the section "Lengths and Fit Measures" in Chapter 2.

A POSTERIORI *CHARACTER WEIGHTING*

PAUP also allows you to assign weights to characters based on their fit to trees in memory. These *a posteriori* weights are then used as input for another (successive) analysis. You can continue to reweight and reanalyze until the weights do not change for two consecutive analyses or until identical trees (or sets of trees) are found in two consecutive searches. By doing this, you may or may not converge on fewer tree topologies, depending on the data matrix. If you choose this route, however, tree lengths will no longer be comparable between successive searches as they are directly dependent on the weights which are in place. The basic idea is to penalize characters which fit the tree(s) poorly (are homoplastic) and reward characters which fit the tree well. By successively evaluating and reweighting, the goal is to arrive at some stable topology or topologies. In practice, this amounts to running a search, choosing a weighting function, reweighting, and running another search. Reweighting can be based on the consistency index (CI), retention index (RI), or rescaled consistency index (RC). Since multiple most-parsimonious trees will generate different weight sets, PAUP has the ability to weight by the best, worst, or mean fit to multiple trees.

It is up to the user to justify this approach, especially given the range of options under which it may be run. For example, the primary variable is the index chosen as the basis for reweighting, but it is not immediately

clear which index should be preferred. You must also choose whether to reweight based on best, worst, or mean fit to the trees. (The default settings of best fit according to rescaled consistency correspond to the "xsteps w" command of the Hennig86 program (Farris, 1988) . Since different index and fit choices can potentially lead to very different trees, you must be very careful about conclusions based on a search strategy with many arbitrary components built in. If you do pursue this method, it is probably a good idea to experiment with different reweighting schemes, in order to get some crude idea of how stable the resulting topologies are.

THE "BOOTSTRAP"

PAUP's implementation of bootstrap analysis follows Felsenstein (1985). The method involves sampling the original data set with replacement to construct a series of bootstrap replicates of the same size as the original data set. Each of these is analyzed, and the variation among these replicate estimates is taken be an indication of the error involved in making estimates from the original data. In Felsenstein's approach, the taxa are held constant and the characters are sampled with replacement to build a series of new data sets the same size as the original. These are then subject to a search, either heuristic or branch-and-bound. Finally, a majority-rule consensus is constructed for all of the bootstrap trees. If a group appears in X percent of the bootstrap trees, the confidence level associated with that group is taken as X percent. This method gives the investigator the ability to assign statistical confidence to hypotheses of relationship.

There are a number of assumptions underlying the bootstrap, which are discussed in Felsenstein (1985) and in more detail in Sanderson (1989). The utility of the method, like all methods, depends on the validity of these assumptions. We will not go into them in detail here, but perhaps the most important is the "i.d.d" assumption identified by Felsenstein (Felsenstein, 1985), which requires that the characters be identically and independently distributed. This is separated into two somewhat less restrictive assumptions by Sanderson (1989), namely that characters are independent, and that the observed character set is a "representative" sample of the "universe of characters." It is especially important to remember that if the sample of characters does not accurately reflect the larger underlying distribution of characters, then the bootstrap confidence intervals may be very poorly estimated. Also, the bootstrap cannot overcome systematic biases such as "long branch effects." In any event, it is wise not to take the bootstrap confidence values as absolutes—there are many factors that might lead to over- or underestimates of confidence.

LAKE'S METHOD OF INVARIANTS

PAUP provides an implementation of Lake's (1987b) method of linear phylogenetic invariants, or "evolutionary parsimony", for RNA or DNA sequence data. In brief, it is an attempt to estimate phylogeny accurately in the presence of highly unequal rates of change in different lineages. When substitution rates are high in some branches and low in others, parsimony may lead to an incorrect tree, grouping the most-substituted branches together even if they are not in reality closest relatives (also see Felsenstein, 1978). Lake's method is an attempt to circumvent the problems of unequal rates of substitution in four distantly related taxa. It examines each of the three distinct unrooted trees (labeled the E tree, F tree, and G tree) for four taxa and calculates which tree is statistically significant. We will not describe the method here; introductory presentations can be found in Holmquist et al. (1988) and Swofford and Olsen (1990).

Because the method deals with only four taxa (a "quartet") at a time, PAUP has three options for handling more than four taxa. In that case, you can choose to evaluate a specified subset of four taxa; evaluate all possible quartets; or partition the taxa into four groups, where the quartet is made up of one taxon from each of the four groups (all possible quartets of this type being evaluated) .

PSEUDORANDOM NUMBER GENERATION

A few of the capabilities provided by PAUP require generation of sequences of "random" numbers (i.e., random-addition sequence in stepwise addition, sampling of characters in bootstrap analysis, and evaluation of the lengths of random trees). PAUP uses a linear congruential method: starting with any number x_i between 1 and 2,147,483,646 (inclusive), the next number in the sequence is given by $x_{i+1} = 397{,}204{,}094 * x_i \bmod (2^{31} - 1)$ (see Fishman and Moore, 1982).

The first number represents the "seed". PAUP always uses "1" as the initial seed rather than an arbitrary value such as one obtained from the system clock. The main reason for this decision was so that runs from the same data file would always generate identical results on any kind of computer, even if the user neglected to define the initial seed explicitly. The drawback to this approach is that unless the default initial seed is explicitly overridden, exactly the same analysis will be performed in any two runs of the program with identical command sets. For example, conducting a second random-addition-sequence search on a second set of

100 replicates will produce exactly the same set of trees as an initial set of 100 replicates if the program is restarted between the two searches unless you override the default initial seed. Keep this in mind.

Chapter 2 USING PAUP

This chapter provides general information on the use and features of PAUP. For details, refer to the COMMAND REFERENCE and INTERFACE chapters. This chapter is intended to be "implementation-independent"—i.e., it pertains to all existing implementations of PAUP Version 3, including the Macintosh, IBM-PC, and mainframe/minicomputer versions. Features and capabilities available only under specific implentations are described in the INTERFACE chapter.

INPUT FILES

Input files for PAUP are standard text files containing commands and/or data. These files will usually adhere to the NEXUS format, described below. Files must be standard text files. For example, on the Macintosh, input files can be created using any editor that can output files of type 'TEXT' (i.e., word processors, spreadsheet programs, and text editors). MacClade 3.0 (and later) users can use MacClade's integrated spreadsheet editor to create files for input to either PAUP or MacClade.

The NEXUS Format

The NEXUS format was designed by David Maddison, Wayne Maddison, and David Swofford to facilitate the interchange of input files between programs used in phylogeny and classification. Data files that conform strictly to the NEXUS guidelines can be input to any program that fully supports the NEXUS standards. Currently, these programs include PAUP, MacClade (Maddison and Maddison, 1992), and COMPONENT (Page, 1993). Among other things, this means that data files created using MacClade's spreadsheet editor can be input to PAUP, and PAUP tree and data files can be input to MacClade for further analysis. The book accompanying the MacClade program has detailed instructions on the simultaneous use of PAUP and MacClade.

Blocks

The characteristic of NEXUS files that allows them to be so portable is the "block" concept. A "block" is a well-defined subsection of an input file that can either be read or ignored by any NEXUS-conforming program. The format of three blocks—DATA, ASSUMPTIONS, and TREES—is defined by the standard; any conforming program must either ignore these blocks entirely or be able to process commands in the block as defined by the standard.[6]

Other blocks are program-specific. If a program encounters a block that it either does not recognize or does not want to deal with, it simply skips over the entire block. (Of course, this feature makes it extremely important to spell block names correctly; otherwise, essential blocks might be unintentionally skipped over during processing).

Every block starts with "`begin` *`block-type`*;" directive and ends with an "`endblock;`" directive. Each block is composed of one or more commands, each terminated by a semicolon (;). If a command-name within a block is unrecognized, a warning message is issued and the rest of the command ignored by skipping forward to its terminating semicolon. (Optionally, PAUP will abort processing of a file if an unrecognized command is encountered). Blocks can also be given a name immediately after the *block-type* but before the semicolon. Currently, PAUP simply ignores these names.

Within a block, the only restriction on the ordering of commands is the following one: any command which affects the operation of a second command must precede the second command in the file. (I.e., only one pass is made through the file, and all commands are executed immediately when they are encountered; there is no "lookahead" capability).

---

[6]"Process" is defined loosely. Strictly speaking, NEXUS-conforming programs are free to ignore commands within these blocks. For instance, some commands may provide information or instructions that are irrelevant to a particular program. The only requirement is that the program should at least provide the option to continue processing the file after encountering a command that it either does not recognize or does not wish to interpret. (A program can easily skip to the end of the command by looking for its terminating semicolon, even if it is otherwise unable to interpret the command.) The precise behavior of the program upon encountering such commands is not specified by the standard. PAUP provides the options of allowing execution to continue (with a one-line warning that an unrecognized command was encountered) or terminating the processing of a file (with an error message).

NEXUS file identification

NEXUS-conforming files are identified by a #NEXUS directive at the very beginning of the file (line 1, column 1).

General format of NEXUS files

NEXUS files are entirely free-format. Blanks, tabs, and newlines may be placed anywhere in the file. Unless RESPECTCASE is requested in the **FORMAT** command, commands and data 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 character-state symbols and in names for assumption sets, ANCSTATES specifications, etc. This is only true for standard roman alphabetic characters. For example, `MÜLLERI` and `mülleri` are not considered identical if RESPECTCASE is off.

Comments may be included in the input file by enclosing them in square brackets. For example,

```
[This is a comment.]
```

Unless the first character following the '[' is an explanation point, the comment is for internal documentation of the data file, and is otherwise completely invisible during the processing of the file. If the comment begins with "[!", as in

```
[!This is a visible comment.]
```

the comment is "visible." NEXUS programs are free to treat visible comments in any appropriate manner; PAUP simply echoes them to the output destinations (display window, output file, and/or printer) exactly as they appear in the file. Although not a requirement, it is recommended that every NEXUS file begin with a visible comment that at least briefly describes the contents of the file (e.g., source of data, date entered, etc.).

Note to MacClade Users: If you use PAUP to manually create a NEXUS file for input to MacClade, note that "[!"-style comments are visible only if they are contained within a DATA, TAXA, or TREES block.

The following sections provide a brief description of the major elements of the NEXUS format. A more formal description will be published elsewhere (D. Maddison, Swofford, and W. Maddison; in prep).

The DATA Block

The DATA block contains the data matrix and other associated information. Its syntax is:

```
BEGIN DATA [block-name] ;
    DIMENSIONS NTAX=number-of-taxa NCHAR=number-of-characters;
    [ FORMAT
        [ MISSING=missing-symbol ]
        [ LABELPOS={ LEFT | RIGHT } ]
        [ SYMBOLS="symbols-list" ]
        [ INTERLEAVE ]
        [ MATCHCHAR=match-symbol ]
        [ EQUATE="<symbol = expansion >…]" ]
        [ TRANSPOSE ]
        [ RESPECTCASE ]
        [ DATATYPE = {STANDARD | DNA | RNA | PROTEIN} ]
        [ GAP=gap-symbol ] ; ]
    [ OPTIONS
        [ IGNORE={ NONE | INVAR | UNINFORM } ]
        [ MSTAXA = { UNCERTAIN | POLYMORPH } ]
        [ ZAP = "character-list" ]
        [ GAPMODE = { MISSING | NEWSTATE } ] ; ]
    [ CHARLABELS character-name …  ; ]
    [ TAXLABELS taxon-name …  ; ]
    [ STATELABELS charnum-and-state-list [ , charnum-and-state-list ] … ; ]
    MATRIX data-matrix ;
ENDBLOCK;
```

Every data block begins with a **DIMENSIONS** command specifying the size of the data matrix (number of taxa, number of characters). It is followed by one or more optional commands that specify details of the matrix format, option settings, and character and/or taxon names. The last command in the DATA block, **MATRIX**, defines the data matrix itself.

A very simple example of a DATA block follows. Note that the style of indenting shown here is not required, but merely serves to enhance readability.

```
begin data;
   dimensions ntax=4 nchar=5;
   matrix
      taxon1    00111
      taxon2    0111?
      taxon3    11001
      taxon4    10000
      ;
endblock;
```

Remember that the data matrix, like the rest of the NEXUS input file, is entirely free-format. You may use any number of physical lines to represent each row of the data matrix. We could just have well have entered the matrix above as follows:

```
matrix
   taxon1
      001
      11
   taxon2
      011
      1?
   taxon3
      110
      01
   taxon4
      100
      00
   ;
```

or even:

```
matrix taxon1 00111 taxon2 0111? taxon3 11001
   taxon4 10000;
```

NOTE: In PAUP 2.4, one of the taxa in the data matrix was designated as the hypothetical ancestor. In PAUP 3.1, the definition of the ancestor is no longer part of the matrix, and is done using the **ANCSTATES** command. This allows more flexibility in changing ancestral states for different analyses of the same data matrix (e.g. to test the effect of alternative polarity assignments).

Entering the matrix in "transposed" format

There is considerable flexibility in the form of the input matrix. By default, rows correspond to taxa and columns to characters. If you prefer to list all of the data for each character on a single row, with columns corresponding to taxa, you can use the TRANSPOSE option of the **FORMAT** command. Since the taxon names are no longer defined in the data matrix itself, you can use the **TAXLABELS** command to define the taxon names. For example, the DATA block below defines the same matrix as the one above:

```
begin data;
   dimensions ntax=4 nchar=5;
   format transpose;
   taxlabels taxon1 taxon2 taxon3 taxon4;
   matrix
      char1   0011
      char2   0110
      char3   1100
      char4   1100
      char5   1?10
      ;
endblock;
```

Placing taxon and character names

Ordinarily, taxon names precede the character-state data for each taxon. If you prefer to place your taxon names at the end of each row rather than t

he beginning, you can use the LABELPOS=RIGHT option of the **FORMAT** command (i.e., the names are to the "right" rather than the "left" of the data). For example:

```
begin data;
   dimensions ntax=4 nchar=5;
   format labelpos=right;
   matrix
      00111 taxon1
      0111? taxon2
      11001 taxon3
      10000 taxon4
      ;
endblock;
```

You can also use LABELPOS=RIGHT in conjunction with the TRANSPOSE option. In this case, the character names follow the data for each character.

Character-state symbols

You may choose any combination of digits, alphabetic characters, or other symbols to representing character states. The only symbols not allowed are whitespace characters (blank, tab, CR and LF) and the following punctuation characters:

```
" ' * ( ) { } [ ] / , ; =
```

The "SYMBOLS list" defines the set of symbols that you have chosen to represent character states in the data matrix and in other commands. For data other than molecular sequences (see below), the default SYMBOLS list is "01", which means that the only (non-missing) character-state symbols permitted are '0' and '1'. If you want to use any other symbols to designate character states, you must explicitly define an alternate SYMBOLS list in the **FORMAT** command. The format of a *symbols-list* is a sequence of single-character "symbols" enclosed within double-quotes. For example, if your data matrix contains some two-, three-, and four-state characters, you might specify

```
symbols="0123"
```

Alternatively, you could specify

```
symbols="abcd"
```

and use alphabetic characters rather than digits in the data matrix.

To specify a range of digits or alphabetic characters, you can use the '~' (tilde) character. For example,

```
symbols="0~9 A~F"
```

is equivalent to

```
symbols="0123456789ABCDEF"
```

Note that the tilde is used rather than the hyphen (minus sign) in order that '+' (present) and '-' (absent) may be used to designate character states (SYMBOLS="+-"), as in the following example:

```
format symbols="+-";
matrix
   Taxon_one    - - + -
   Taxon_two    - - + -
   Taxon_three  + + - +
   Taxon_four   + - - +
   ;
```

By default, PAUP does not distinguish between the lower- and upper-case representations of the same alphabetic characters. Thus, if you set the SYMBOLS list to "abcd", 'A' is equivalent to 'a', 'B' to 'b' and so on. If, for some reason, you want to treat the lower- and upper-case representations as different character states, you can use the RESPECTCASE option. For example, the command

```
format respectcase symbols="ABCDabcd";
```

defines a SYMBOLS list containing eight distinct character-state designations. The RESPECTCASE option pertains only to symbols used to represent character states in the data matrix. Commands, taxon and character names, etc., can still be entered using any combination of upper- and lower-case characters.

Using alphanumeric character names

PAUP automatically uses the integers 1 through NCHAR to identify characters for input and output purposes. You may also provide alphanumeric character names to supplement the numeric identifiers using the **CHARLABELS** command. For example, the command

```
charlabels amnion appendages thermoreg nostrils
    teeth;
```

assigns character names to the first five characters. You can then use these character names in any context in which a character number would be used. PAUP will also use these names to identify characters in the output.

Ordinarily, the **CHARLABELS** command will supply a name for each character in the data set. If you provide fewer than NCHAR names, a

warning will be issued (a mistake may have been made in entering the names); only the character numbers will then be available for the missing elements. You may use a single underscore (_) as a placeholder if you want to name some characters but use only numbers for others. For instance, if you wanted to name only the second and fourth characters in the above example, you could use the command

```
charlabels _ appendages _ nostrils _;
```

The remaining characters could then be identified only by number (1, 3, and 5).

You should use a **CHARLABELS** command only if the TRANSPOSE option is not in effect. If you enter the data in transposed format, the character names will be obtained from the data matrix itself.

See "Identifiers" in Chapter 3 for rules pertaining to character names.

Predefined formats for molecular sequence data

The predefined formats "RNA," "DNA," and "PROTEIN" are available for nucleotide and amino-acid sequence data. These are selected using the DATATYPE option. For example:

```
begin data;
   dimensions ntax=4 nchar=20;
   format datatype=dna gap=-;
   matrix
      One      ATGCT ATCCG TCATG ACCTA
      Two      ATCCT AGCCG T--AG ACGGA
      Three    CTGCT AGCCG TGGAG TCCTA
      Four     CTGAA ---CG ACATA AGTCA
      ;
endblock;
```

If DATATYPE is set to DNA, the SYMBOLS list is set to "ACGT" and the following "equate" macros, corresponding to the IUPAC/IUB ambiguity codes, are predefined:

```
R    =  {AG}     [ puRine ]
Y    =  {CT}     [ pYrimidine ]
M    =  {AC}     [ aMino ]
K    =  {GT}     [ Keto ]
S    =  {CG}     [ Strong ]
W    =  {AT}     [ Weak ]
H    =  {ACT}    [ not G ]
B    =  {CGT}    [ not A ]
V    =  {ACG}    [ not T ]
```

```
D    =   {AGT}     [ not C ]
N    =   {ACGT}    [ unkNown ]
```

Also, the symbol X is interpreted as "missing data".

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

```
A   =   ala         [ alanine ]
C   =   cys         [ cysteine ]
D   =   asp         [ aspartic acid ]
E   =   glu         [ glutamic acid ]
F   =   phe         [ phenylalanine ]
G   =   gly         [ glycine ]
H   =   his         [ histidine ]
I   =   ileu        [ isoleucine ]
K   =   lys         [ lysine ]
L   =   leu         [ leucine ]
M   =   met         [ methionine ]
N   =   asn         [ asparagine ]
P   =   pro         [ proline ]
Q   =   gln         [ glutamine ]
R   =   arg         [ arginine ]
S   =   ser         [serine ]
T   =   thr         [ threonine ]
V   =   val         [ valine ]
W   =   trp         [ tryptophan ]
Y   =   tyr         [ tyrosine ]
*   =   nonsense    [ chain termination ]
```

The symbol X, for "unknown", is interpreted as "missing data". In addition, two "equate" macros are predefined:

```
B   =   {DN}        [ asx = asp or asn ]
Z   =   {EQ}        [ glx = glu or gln ]
```

If you want to add additional character-state symbols to the SYMBOLS list implied by the DATATYPE in effect, you can still use the SYMBOLS= option in the format command. These additional symbols will be inserted at the beginning of the SYMBOLS list. For example, if you wanted to mix nucleotide-sequence and restriction-site characters in the same data matrix, you could use the following **FORMAT** command:

```
format datatype=dna symbols="01";
```

The symbols list would then be set to "01ACGT." See "Alignment Gaps" below for another example.

Matching the states in the first taxon

You may define character-states relative to the states of the first taxon by using the MATCHCHAR option. For example, the following **MATRIX** command is equivalent to the one above:

```
format datatype=dna gap=- matchchar=.;
matrix
   One      ATGCT ATCCG TCATG ACCTA
   Two      ..C.. .G... .--A. ..GG.
   Three    C.... .G... .GGA. T....
   Four     C..AA ---.. A...A .GTC.
   ;
```

In many cases (particularly with sequence data) it is convenient to separate the data into blocks of characters. This is accomplished using the INTERLEAVE option. For the sequence-data example above, we could first supply the data for positions (characters) 1-10 and then for positions 11-20 as follows:

```
begin data;
   dimensions ntax=4 nchar=20;
   format datatype=dna gap=- interleave;
   matrix
      One      ATGCTATCCG [1-10]
      Two      ATCCTAGCCG
      Three    CTGCTAGCCG
      Four     CTGAA---CG

      One      TCATGACCTA [11-20]
      Two      T--AGACGGA
      Three    TGGAGTCCTA
      Four     ACATAAGTCA
      ;
endblock;
```

Alignment gaps

For molecular sequence data, PAUP accepts only prealigned sequences. That is, if all of the sequences are not of the same length, "gaps" corresponding to insertions and/or deletions must be inserted into the sequences at appropriate locations.[7] If your data matrix contains gaps,

---

[7]The issue of sequence alignment is not a trivial one. Different alignments for the same set of sequences can generate markedly different results. Because current versions of

you must define the symbol used to represent them using the GAP= option of the **FORMAT** command (see the examples above).

Alignment gaps may be treated either as missing data or as an additional character-state (fifth base or 21st amino acid) using the GAPMODE option of the **OPTIONS** command. The default is GAPMODE=MISSING. If you want gaps to represent an additional character state, specify GAPMODE= NEWSTATE. You should not use GAPMODE= NEWSTATE when gaps longer than one or two bases occur, because a single evolutionary event (i.e., an insertion or deletion) will then be treated as *n* independent events, where *n* is the length of the gap. If you believe that the gaps convey important phylogenetic information that would be lost by using GAPMODE=MISSING, one strategy is to add an additional set of characters to the data matrix to signify the presence or absence of particular gaps. The INTERLEAVE option provides a handy way of adding an additional set of characters to a matrix containing sequence data. For example:

```
begin data;
   dimensions ntax=4 nchar=24;
   format datatype=dna gap=- interleave symbols="01";
   options gapmode=missing;
   matrix
      [sequence data...]
      One      ATGCT ATCCG TCATG A----
      Two      ATCCT AGCCG T--AG A----
      Three    CT--T AGCCG T--AG TCCTA
      Four     CT--A ---CG A--TA AGTCA

      [insertion/deletion data]
      One      0 0 0 1
      Two      0 0 1 1
      Three    1 0 1 0
      Four     1 1 1 0
      ;
endblock;
```

Since the two kinds of characters are in clearly defined blocks, you could easily use other PAUP commands to assign different weights to insertion/deletion vs. substitution events or to compare trees calculated with and without the information from insertion/deletion characters.

EQUATE macros

The EQUATE macro facility allows translation of particular character-state specifications in the data matrix to alternate character-state specifications. They are useful in several situations. For example, you

---

PAUP do not contain any alignment capabilities, the choice of alignment strategies is left entirely to the user.

might use two different symbols to refer to missing data, one for "unavailable" and another for "not applicable." Because PAUP allows only a single symbol to refer to missing data, you can convert one to other via by equating one symbol to the other. If you, say, issued the command:

```
format missing=? equate="- = ?";
```

then both hyphens and question-marks in the data matrix would be treated as missing data. Equate macros are also useful when more than one character-state is being assigned to some taxa (see "Multistate Taxa"). For example, if you specify

```
format equate="R={AG} Y={CT}";
```

then R's and Y's (ambiguity codes for purine = A or G and pyrimidine = C or T, respectively) in the data matrix are converted to the corresponding character-state set. In fact, use of DATATYPE=DNA (below) causes this equate macro, as well as others, to be predefined.

Using a subset of the characters

By default, PAUP uses all of the characters in the data matrix. The IGNORE and ZAP options can be used to restrict the characters actually used by the program. If you request IGNORE=INVAR, invariant or "constant" characters are ignored. Alternatively, you can specify IGNORE=UNINFORM to request that "uninformative" characters be ignored. To ignore specific characters, you can use the ZAP option. For example, the command

```
options ignore=uninf zap="1-10";
```

causes the first ten characters plus all other uninformative characters to be ignored.

"Ignoring" a character is effectively the same as physically removing it from the data matrix; the only meaningful difference is that physical removal would affect the numbering of the "downstream" characters, whereas ignoring/zapping it does not. Note that you can also "exclude" characters; "ignoring" and "excluding" are very different operations (see "Excluding Characters," later in this chapter).

An uninformative character is defined as one which contributes exactly the same length to every possible tree topology or, equivalently, one whose minimum possible length is equal to its maximum possible length. For example, an unordered character is informative only if at least two character states each occur in more than one taxon; otherwise, the singleton states can always be explained as single changes on terminal branches, regardless of the tree topology. In general, uninformative

characters simply add a constant amount to the tree length and do not otherwise affect the results. However, the above definition of an uninformative character is somewhat ambiguous when missing data are present. In this case, "uninformative" characters can nonetheless provide potential support for certain groupings, and can therefore affect whether zero-length branches are collapsed (see the sections "Missing Data" and "Zero-Length Branches and Polytomies"). Consequently, unless you decline the option to collapse zero-length branches, tree searches may find different numbers of trees depending on the setting of the IGNORE option.

Invariant characters are those in which only one non-missing state was observed. Such characters contribute zero length to any possible tree and are therefore also uninformative. When missing data are present, the same ambiguities referred to in the above paragraph with respect to the collapsing of zero-length branches with uninformative characters also apply to "invariant" characters.

The type of a character can affect its informativeness. For example, a character for which states 0, 1, and 2 were observed once, five times, and once, respectively, would be uninformative as an unordered character but informative as an ordered character.

PAUP does not attempt to evaluate the consistency index or informativeness of user-defined stepmatrix characters due to the difficulty of determining the minimum possible lengths required by these characters. In principle, this minimum length could be established by performing separate parsimony analyses on each stepmatrix character considered separately, but the amount of computation required would be horrendous. If you absolutely must determine whether a given stepmatrix character is uninformative, you could exclude all other characters and perform an exact (or heuristic) search to obtain (or estimate) the minimum possible length for that character. This minimum length could then be compared to the maximum possible length (available via the **DESCRIBE** command or the **Describe Trees** menu command).

> See Chapter 3 for the complete list of commands available in the DATA block and a description of their syntax. Additional examples can be found in the sample data files included on your distribution disk.

The ASSUMPTIONS Block

The ASSUMPTIONS block is used to declare character types and weights, assumption sets, and ancestral states (polarities). Its syntax is:

```
BEGIN ASSUMPTIONS [block-name] ;
    [ OPTIONS
```

```
        [ DEFTYPE=default-character-type ]
        [ POLYTCOUNT={ MINSTEPS | MAXSTEPS } ];
    [ USERTYPE name [ { STEPMATRIX | CSTREE } ]
        = description ; ]
    [ CHARSET character-set-name = character-list ; ]
    [ TYPESET [*] name = character-type : character-list
        [ , character-type : character-list ] … ; ]
    [ WTSET [*] weight-set-name = character-weight : character-list
        [ , character-weight : character-list ] … ; ]
    [ EXSET [*]  exclusion-set-name = character-list ; ]
    [ ANCSTATES [*] ancestor-name = character-state : character-list
        [ , character-state : character-list ] … ; ]
ENDBLOCK;
```

An example ASSUMPTIONS block follows, with examples of each of the available commands:

```
begin assumptions;
   options deftype=ord polymcount=addsteps;
   usertype myOrd = 4
      0 1 2 3
      - 1 2 3
      1 - 1 2
      2 1 - 1
      3 2 1 - ;
   usertype myTree cstree = ((0,1)a,(2,3)b)c;
   typeset * mixed = irrev: 1 3 10, unord 5-7;
   charset odd = 1-.\2;
   charset even = 2-.\2;
   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 allzero = 0:ALL;
   ancstates allone = 1:ALL;
   ancstates mixed = 0:1 3 5-8 11, 1:2 4 9-15;
endblock;
```

Any number of **USERTYPE**, **TYPESET**, **WTSET**, **EXSET**, and **ANCSTATES** commands may be provided.

The TREES Block

The TREES block is used to input user-defined trees to PAUP. A single **TREE** or **UTREE** command is used for each tree; any number of **TREE** or **UTREE** commands may be included in the block.

The syntax for the TREES block follows:

```
BEGIN TREES [block-name] ;
    [ TRANSLATE token taxon-name [ , token taxon-name ] … ; ]
    [ TREE [*] name = tree-specification; ]
    [ UTREE [*] name = tree-specification; ]
ENDBLOCK;
```

The **TREE** and **UTREE** commands are used to input rooted and unrooted trees, respectively. To input multiple trees, you may use as many **TREE** or **UTREE** commands as you need. However, you may not mix **TREE** and **UTREE** commands—all of the input trees must either be rooted or unrooted.

Tree descriptions require that taxa be referred to by the name assigned to them in the DATA block. However, the **TRANSLATE** command can be used to define a translation table that maps arbitrary tokens in the tree specification to valid taxon names. If a **TRANSLATE** command is not present, a default translation table maps the integers 1 through NTAX to the corresponding taxon names in the data matrix, so that integer values rather than taxon names may be used in the tree specifications. However, it is usually best to avoid relying on a default translation table. For example, if you rearrange the order of the taxa in the data matrix, this reordering will not affect the validity of the tree descriptions so long as you either retain the original translation table or use taxon names rather than numbers in the tree descriptions. There are two advantages to using a translation table rather than using the taxon names directly in tree descriptions. First, tree descriptions are generally much more compact when integers rather than taxon labels are used, especially if a large number of trees are being described. Second, if you decide to rename a taxon, only one element of the translation table need be changed. Without a translation table, the old taxon name would have to be changed to the new one in every tree description.

TREES-block processing is much faster if you use the consecutive integers 1, 2, …, NTAX for the arbitrary tokens in the translation table. Unless you have a good reason for not doing so, you should follow this convention, especially if a large number of trees are being defined.

A sample TREES block for four taxa named 'one', 'two', 'three', and 'four' is shown below:

```
begin trees;
   translate
      1 one,
      2 two,
      3 three,
      4 four;
   tree a = ((one,two),three,four);
   tree b = ((one,two),(three,four));
   tree c = ((one,two));
   tree d = (1,(2,(3,4))); [uses translation table]
endblock;
```

If one or more taxa are omitted from a tree specification, they are joined to the root node of the subtree described by that specification. It is recommended that taxa be excluded from tree description only for

constraints input. See the section "Defining and using topological constraints."

> See the section "Manipulating Trees" later in this chapter for more information on defining and using trees, and Chapter 3 for a description of the syntax of the **TRANSLATE**, **TREE**, and **UTREE** commands.

TAXA and CHARACTERS Blocks

An alternative to the use of the DATA block is the combination of TAXA and CHARACTERS blocks. The TAXA block contains only a **DIMENSIONS** command and a **TAXLABELS** command. Its **DIMENSIONS** command specifies only the number of taxa, as NTAX=*n*. The CHARACTERS block is essentially identical to the DATA block, except that the DIMENSIONS command specifies only NCHAR=*c* and no **TAXLABELS** command may be present. Thus, the information contained in TAXA and CHARACTERS blocks is exactly complementary.

For example, the data in the DATA block

```
begin data;
   dimensions ntax=4 nchar=5;
   matrix
      taxon1    00111
      taxon2    0111?
      taxon3    11001
      taxon4    10000
      ;
endblock;
```

could be defined equivalently as:

```
begin taxa;
   dimensions ntax=4;
   taxlabels taxon1 taxon2 taxon3 taxon4;
endblock;

begin characters;
   dimensions nchar=5;
   matrix
      taxon1    00111
      taxon2    0111?
      taxon3    11001
      taxon4    10000
      ;
endblock;
```

If you want to do analyses that only require information about taxa (e.g., user-defined tree input and consensus tree calculation), you may omit the CHARACTERS block entirely. Alternatively, you can include one TAXA block and several CHARACTERS and PAUP blocks (see below) to

analyze different data matrices for the same set of taxa. Note that taxon labels in the characters block are just placeholders; the taxon labels defined in the TAXA block are used for the remainder of the data file.

PAUP supports the TAXA block mainly for compatibility with Rod Page's COMPONENT program (Page, 1993), the latest version of which uses the NEXUS format.

"PAUP" Blocks

One or more PAUP blocks may be included in the data file in addition to the standard blocks. Any valid PAUP command may be placed in these blocks (in fact, any command outside of NEXUS blocks is assumed to be a PAUP command). A typical PAUP block would look like:

```
begin paup;
   log file=my.output replace;
endblock;
begin data;
   .....
endblock;
begin paup;
   ctype ord: all;
   bandb;
   savetrees file=my.trees;
endblock;
```

See "Commands Used in the PAUP Block or from the Command-Line" for a description of all PAUP commands.

In many cases, it may be easier to include commands in the file than to enter them from the command line or to make the corresponding requests via the menu system. An example follows:

```
#NEXUS
begin paup;
   exclude 1 5 10-12;
   wts 2:1-10, 3:11-20;
   set maxtrees=200;
   hsearch/keep=120;
   describe all/chglist apolist;
endblock;
```

The advantage of keeping separate command file is that the data matrix need only be executed a single time. When each command set is needed, simply open it and execute it. You may also use the same command file on different data sets, providing the number of characters and/or taxa do not conflict.

Batch Processing

If you wish to run (unattended) a single batch file with multiple data sets and analyses, you must use two **SET** options in the first PAUP block (NOWARNRESET and AUTOCLOSE). This is because by default PAUP will display a warning if you try and reset the data file, and will not close windows unless told to do so, thus you would never proceed beyond the first data set. Batch files allow unattended multiple analyses—useful if you have many data sets to run but do not wish to (or cannot) be present. The format of a typical file with multiple data sets would be:

```
#NEXUS
begin data;
   ......; [first data set]
endblock;
begin paup;
   set nowarnreset autoclose;
   outgroup 1 2 3;
   hsearch swap=tbr addseq=simple hold=10;
endblock;

begin data;
   ......;[second data matrix here]
endblock;
begin paup;
   ..... ;[paup commands - new search]
endblock;
```

Each analysis may have its own TREES or ASSUMPTIONS block, as these are reset when a new data set is processed.

Error Messages and Input Files

Because of the extremely free format of data-entry, it is sometimes hard for PAUP to figure out exactly where a user erred when it tries to execute an input file. This leads to "bogus" error messages, in that what PAUP reports is not the real error, but a reflection of a different error earlier in the file. This is a function of the way PAUP files are constructed. The alternative is to require more rigid formatting, which is acceptable neither to the programmer nor to users. Below are some examples of more common errors.

The number of taxa and/or characters often get changed as an analysis progresses, but if the NCHAR or NTAX settings do not match the matrix, PAUP will complain in an indirect way. For example, the following matrix has seven characters, but setting NCHAR to 6 leads PAUP to complain about the "t" in taxon B in the following way "Invalid character state 't' for taxon 2, character 1." This means that PAUP was expecting a character here, not an alphanumeric taxon name. PAUP did as it was

told—it read the first six characters of taxon 1, took the next "1" to be the label for taxon 2, the carriage return to indicate that data was coming, and the following "t" to be the first character state, about which it complained. So the error is really three lines up in the file, not where PAUP places its flag.

```
begin data;
   dimensions  ntax=5 nchar=7;
   format missing=? ;
   matrix
   taxonA    0000001
   taxonB    0100000
   taxonC    1110000
   taxonD    1111101
   taxonE    1101100
   ;
endblock;
```

If you mistakenly set NTAX to four when it should be five, PAUP will return the error "expecting semicolon at end of data matrix." This tells you immediately that PAUP had processed all the taxa you told it to, and was not expecting any more. Note that this is the same message you would get if you actually did omit the terminal semicolon from matrix.

This brings up an important point: PAUP's free format works only when the semicolons are correctly placed, so that it knows when to start and stop reading a particular part of the input file. For example, omitting the semicolon at the end of the following "hsearch" command in the PAUP block would return the error message "keyword 'set' not recognized."

```
hsearch swap=nni hold=10 addseq=asis
set tcompress;
```

PAUP thought that the **SET** command was part of the preceding **HSEARCH** command, which lacked a terminating semicolon. You should be tipped off that PAUP thought **SET** was a keyword when you know it is a command. There is nothing wrong with the **SET** command, only with the preceding **HSEARCH**. Adding the missing semicolon will cause both commands to be executed correctly.

Again, PAUP will do its best to flag errors in an input file, but if you do get a seemingly nonsensical error message, the best strategy is to work backwards in the file to see where that message might be appropriate. If you read carefully what PAUP *thought* it should find and think about what might have misled it earlier, chances are you won't have to go far to find the cause. If you use MacClade, you can largely avoid these types of errors by using MacClade's built-in spreadsheet editor, which insulates you from having to understand the details of the NEXUS format.

SPECIFYING CHARACTER TYPES

The "Standard" Character Types

The standard character types are referred to in NEXUS and PAUP commands as `ORD` (ordered), `UNORD` (unordered), `IRREV` (irreversible), and `DOLLO` (Dollo). Dollo and irreversible characters may have an optional suffix (DOLLO.UP, DOLLO.DN, IRREV.UP, IRREV.DN)

See Character Types in Chapter 1 for a description of these types.

Assigning Character Polarities

Dollo and irreversible characters require the specification of character polarities (either implicitly or explicitly). For Dollo characters, there are three options for specifying the direction of "forward" (less derived to more derived) vs. "backward" (more derived to less derived) transformations. If the ancestor currently in effect has the "missing" state, polarity can be either "up" or "down." "Up" specifies that states higher in the SYMBOLS order are derived relative to states lower in the SYMBOLS order (i.e., for `SYMBOLS="01"`, state 0 is ancestral and state 1 is derived). "Down" specifies the opposite: higher ordered states are ancestral to lower ordered states. If the state in the currently chosen ancestor is non-missing, this state defines the most ancestral state, with both lower and higher ordered states in the SYMBOLS list being relatively more derived. Note that if the 'standard' ancestor is in effect, this state will be "missing," and the "up" vs. "down" setting will apply.

Within a file or from the command line, these options are specified by optionally appending a suffix to the DOLLO keyword. If no suffix is provided ('`DOLLO`'), then "up" is assumed. '`DOLLO.UP`' and '`DOLLO.DN`' can be used to explicitly request the "up" and "down" options, respectively. In versions of PAUP that provide a menu mode, pop-up menus in the **Set Character Types** dialog box may be used to choose one of the options.

Likewise, for irreversible characters, there are three options for specifying the direction of allowed (less derived to more derived) transformations and disallowed (more derived to less derived) transformations. If the ancestral state defined by the current ancestor is not equal to "missing," then this state represents the most ancestral state, with both lower and higher ordered states in the SYMBOLS list being relatively more derived. If an ancestral state *is* "missing," then the polarity may either be "up" or "down." "Up" specifies that states higher in the symbols order are derived relative to states lower in the symbols order (i.e., for `SYMBOLS="01"`,

state 0 is ancestral and state 1 is derived). "Down" specifies the opposite–higher ordered states are ancestral to lower ordered states. Note that if the "standard" ancestor is in effect, all ancestral states will be "missing", and the "up" vs. "down" setting determines the polarity.

Within a file or from the command line, these options are specified by optionally appending a suffix to the IRREV keyword. If no suffix is provided ('`IRREV`'), then "up" is assumed. '`IRREV.UP`' and '`IRREV.DN`' can be used to explicitly request the "up" and "down" options, respectively. In versions of PAUP that provide a menu mode, pop-up menus in the **Set Character Types** dialog box may be used to choose one of the options

For further information on assigning polarities from the menus in the Macintosh and IBM-PC versions, see Chapter 4.

For further information on defining and choosing ancestors, see "Defining Ancestral States" elsewhere in this chapter.

Defining Your Own Character Types

In addition to providing the standard character types mentioned above, PAUP allows you to define your own character types via the **USERTYPE** command. Two kinds of user-defined character types are available. Character-state trees allow you to define a character-state graph that specifies a linear or branching relationship among the character states. Stepmatrices assign a cost for the transformation from every state to every other state.

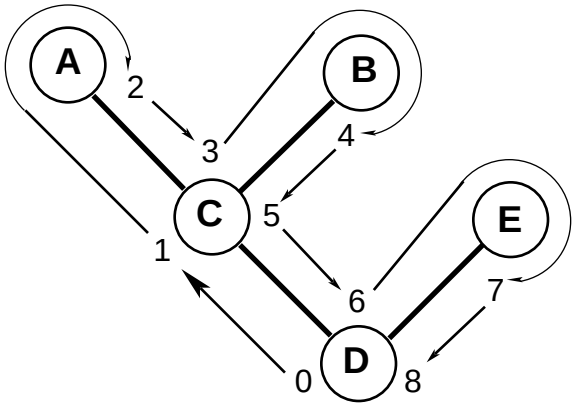
See "Character Types" in Chapter 1 for more discussion on potential uses of user-defined character types.

Character-state trees

Character-state trees are described using a parenthetical notation that defines the shape of the tree. The nodes of the character-state tree are labeled by symbols corresponding to the character-state symbols used in the data matrix. Obviously, all of the states observed in the data matrix for a particular character must be represented in any character-state tree assigned to that character. Additional states that were not observed may also be included in the character-state tree as well.

The system used to describe character-state trees is straightforward. Think of the character-state tree diagram as a set of roadways connecting the nodes of the tree (see figure below). The itinerary is to visit all of the nodes (character-states) of the character-state tree in a circuit beginning at the root node (state D), following two simple rules: (1) when you come to

an intersection or fork in the road (internal node), always bear to the left, and (2) when you come to a dead end (terminal node), turn around. The path indicated by the arrows in the figure shows the sequence in which the nodes would be visited in this example.



A character-state tree and the circuit followed in writing its description.

To write the character-state tree description, perform the following operations as you make the circuit:

- When you leave a node traveling *away from* the root toward the *leftmost* descendant, write a left parenthesis.
- When you leave a node traveling *away from* the root toward any descendant *other than the leftmost* descendant, write a comma.
- When you leave the *rightmost* descendant of an internal node traveling *toward* the root, write a right parenthesis.
- When you visit a terminal node or visit an interior node for the last time, (optionally) write the symbol for the character state. If you omit the symbol for a state corresponding to an internal node of the character-state tree, PAUP will use an asterisk to label that state in the output. For ease in interpreting the output, however, it is best to label all nodes on the character-state tree.

Applying these rules to the example above, the character-state tree description would develop as follows:

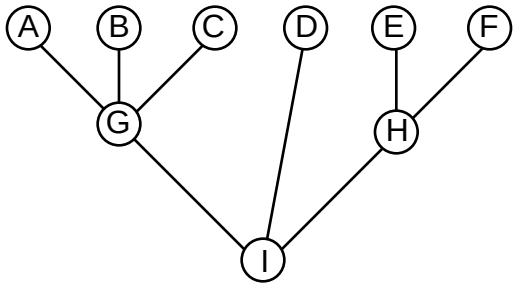
Number in sequence	Description to this point	Explanation
0	**(**	leaving for internal node D's left descendant
1	(**(**	leaving for internal node C's left descendant
2	((**A**	terminal node A visited
3	((A**,**	leaving for internal node C's right descendant
4	((A,**B)**	terminal node B visited; leaving internal node C's rightmost descendant
5	((A,B)**C**	internal node C visited for last time
6	((A,B)C**,**	leaving for internal node D's last descendant
7	((A,B)C,**E)**	terminal node E visited; leaving internal node D's rightmost descendant
8	((A,B)C,E)**D**	internal node D visited for last time

The character-state tree could therefore be defined using a **USERTYPE** command in the ASSUMPTIONS block as follows:

```
begin assumptions;
   usertype mycst cstree = ((A,B)C,E)D;
endblock;
```

The name `mycst` can be any name you choose. CSTREE is required because, by default, user-defined character types are stepmatrices (see below) rather than character-state trees.

Just for practice, here is a slightly more complicated example:



((A,B,C)G,D,(E,F)H)I

Another character-state tree and its NEXUS-format description.

In unusual situations, you may want to use character-state trees to define a linearly ordered (rather than branching) character, but with a different ordering from that implied by the SYMBOLS list. For example, if SYMBOLS="012" and you want one character to be ordered as 0-2-1 rather than 0-1-2, you could declare a user-defined type with the character-state tree specification "((1)2)0". Other ordered characters would retain the 0-1-2 ordering.

You are not required to assign a character-state symbol to all internal nodes of the character-state tree. If you do not explicitly assign a symbol

to an internal node, an asterisk (*) is used. For example, if none of the taxa in the data matrix actually possessed any of the states G, H, or I, the description of the character-state tree shown in the above tree could have been written as

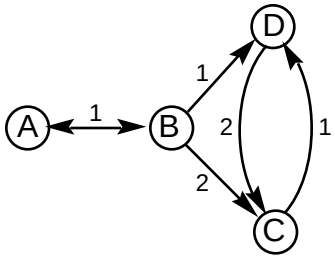
```
((A,B,C),D,(E,F));
```

The states corresponding to the internal nodes of the character-state tree would then be shown as an asterisk in the output. This practice is not recommended, however, as it then becomes impossible to distinguish between different nodes of the character-state tree that are all represented by the same symbol in the output.

User-defined character-state trees cannot include multistate taxa. In that case you must code the character-state tree using a stepmatrix (see below).

Stepmatrices

To define a stepmatrix character, first draw the character-state graph corresponding to the assumptions that you wish to impose for the character type. The character-state graph should have arrows connecting all allowed transformations (this is where you can incorporate the changes associated with a multistate taxon) with a cost (weight) associated with each transformation (see Chapter 1 for examples). Then, assign each element s_{ij} of the stepmatrix according to the sum of the costs of all the transformations required to convert state *i* (stepmatrix row) to state *j* (stepmatrix column). If there is more than one path from state *i* to state *j* (i.e., there are one or more cycles in the character-state graph), choose the path the allows the smallest total cost. Several example stepmatrices are given in Chapter 1. Here is one more example, intentionally made rather unusual in order to illustrate a few points:




```
   A BCD
A  0132
B  102 1
C  ii01
D  i i 20
```

A character-state graph and its associated stepmatrix.

First, note that state C can only transform into state D and vice versa, since the B-to-D and B-to-C arrows are unidirectional. Hence, the matrix entries for C and D to A and B are "infinity," represented by the character 'i' in the stepmatrix definition. Also observe, for example, that there are two paths from A to C: A→B→C (3 steps) and A→B→D→C (4 steps).

Therefore, we let $s_{A,C} = 3$. The stepmatrix could be defined in an assumptions block as follows:

```
begin assumptions;
   usertype weird stepmatrix = 4 ABCD
      0 1 3 2
      1 0 2 1
      i i 0 1
      i i 2 0
      ;
endblock;
```

The name `weird` can be any name you choose; `4` is the number of rows and columns in the stepmatrix; and `ABCD` is the list of states corresponding to these rows and columns. Also, the STEPMATRIX keyword is not actually required, as this is the default user-defined type class.

NOTE: The diagonal elements of a stepmatrix are always set to zero in PAUP, so you can put any one-character symbol you want there (e.g., '.' or '-') to improve readability. This is an extension to the NEXUS format, however, so other programs may not deal correctly with nonzero entries on the diagonal. MacClade, for example, allows you to substitute periods or hyphens for zeros, but other characters will generate an error.

Remember that defining a character type does *not* automatically assign that type to the characters in the data matrix. Before a user-defined character type will have any effect on subsequent analyses, you must assign the type to one or more characters using one of the methods described in the section below.

Verifying USERTYPE definitions

You can use the **SHOWUSER** command or the **Show Usertypes** menu command to verify that you have defined your character types in the way you intended. The resulting output will have one of the following two forms:

```
Character-state tree 'one':

   *--e--c--a
   |  |  |
   |  |  b
   |  |
   |  d
   |
   j--h--f
   |  |
   |  g
   |
   i

Stepmatrix '10_1':

            TO: a       c       g       t
  FROM: a       -      10       1      10
        c      10       -      10       1
        g       1      10       -      10
        t      10       1      10       -
```

Because of the limitations of lineprinter-style graphics, this ouput for character-state trees can be confusing. For example,

```
usertype a cstree=(a,b,c); showuser;
```

generates the following ouput:

```
*--a
|
+--b
|
c
```

At first glance this implies a bifurcating cstree, which is not what was specified in the original usertype command. The trick is to ignore all *apparent* nodes that do not have either a state symbol or an asterisk (*). Thus, a, b, and c all connect directly to *, because the apparent node joining the edge leading to b and c does not really exist.

Assigning Character Types

There are four ways to specify character types:

- By using the DEFTYPE option in an ASSUMPTIONS block. For example, the following ASSUMPTIONS block sets the character type for all characters to "ordered":

  ```
  begin assumptions;
     options deftype=ord;
  endblock;
  ```

  Note that the default character type must be one of the predefined character types; it can not be a user-defined type.

- By defining a type-set using one or more **TYPESET** commands in the ASSUMPTIONS block. For example, to make characters 3 and 7 unordered, characters 9 through 13 Dollo, and the remaining characters ordered, you could use the following ASSUMPTIONS block:

```
begin assumptions;
   options deftype=ord;
   typeset *mytypes = unord:3 7, dollo:9-13;
endblock;
```

  The asterisk preceding `mytypes` is important. It informs PAUP that you want the type-set to go into effect immediately. Otherwise, the type-set would not be used unless it was specifically invoked by an **ASSUME** command or a **Choose Assumption Sets** menu command.

  NOTE: Any characters not explicitly assigned a type in a TYPESET command use the character-type specified by the DEFTPYE option in the **OPTIONS** command. If you do not use an "OPTIONS DEFTYPE=___" directive, the default character type is UNORD.

- By using the **CTYPE** command (either from within a PAUP block or from the command-line). The same character types assigned in the example above could be specified using the command:

```
ctype ord:all, unord:3 7, dollo:9-13;
```

  NOTE: Later specifications override earlier specifications in a **CTYPE** command, so that characters 3, 7, and 9-13 are set to UNORD or DOLLO as intended, despite the preceding ORD ALL. This sometimes saves effort, since the alternative is to specify every character type explicitly, e.g.:

```
ctype ord:1 2 4-6 8 14-.,unord:3 7,dollo:9-13;
```

- By using the **Set Character Types** menu command.

CHARACTER WEIGHTING

By default, PAUP assigns equal weight to each character in the data matrix. You may, however, prefer to attach greater weight to some characters than to others. When you assign character weights, PAUP simply computes a weighted sum of the single-character tree lengths when calculating the total length of the tree.

Assigning Weights

You can use the **WEIGHTS** (= **WTS**) command or the **Set Character Weights** menu command to specify *a priori* character weights. For example, the command

```
weights 2:4-10 15 18-20, 3: 11 12;
```

assigns a weight of 2 to eleven characters and a weight of 3 to two characters. If a weight is not explicitly assigned to a character, the character retains the weight that was previously in effect; i.e., the effect of the **WEIGHTS** command is cumulative. The sequence of commands

```
wts 2:1-5;
[more commands]
wts 2:10;
```

results in the first five and the tenth characters having a weight of 2.

If a more than one weight is assigned to a given character, the last assignment applies. This precedence rule can be used to advantage if you want to assign a nonunit weight to most of the characters. For example, the command

```
weights 2:all, 1:2 6 11;
```

assigns weights of 2 to all characters other than 2, 6, and 11, which receive a weight of 1. Likewise, if you want to be sure that only the characters specified in a single **WEIGHTS** command receive nonunit weight, you could issue a command such as

```
weights 1:all, 2:5-15 31-45;
```

Any nonunit weights previously in effect are then reset to unity before a weight of 2 is assigned to some of the characters.

It is important to realize that the assumption of "equal weights" does not necessarily mean that each character has the same influence in discriminating among alternative tree topologies. In general, the greater the number of states observed for a character, the greater will be that character's influence—e.g., a character with five discrete states will always contribute at least four steps to the tree length, whereas a binary character may contribute as little as one step. This issue is particularly relevant if the number of states recognized is arbitrary, as for a continuous character broken into an arbitrary number of discrete states. In this case, a more "fine-grained" coding will lead to more character states and therefore more influence on the analysis than will a relatively "coarse-grained" coding. You can use the SCALE option to request that weights be assigned such

that the minimum possible length for each character is the same for all characters in a group. The simplest use of this option is as follows:

```
weights scale;
```

In this case, weights are assigned to all characters such that the minimum possible length of each character is 1000 (the default "base weight"). That is, binary characters are assigned a weight of 1000, three-state characters a weight of 500, and so on.

Because version 3.1 of PAUP uses integers to store tree lengths and character weights, fractional weights such as 0.5. 0.33, etc., can no longer be used in PAUP. You can specify a base weight other than 1000 by using the BASEWT option. For example, the command

```
wts scale/basewt=100;
```

requests a base weight of 100, provides results equivalent to using decimal weights recorded to 2 decimals. A slight problem with the use of a number like 100 or 1000 as the base weight is roundoff error. For example, if your data contains a mixture of two-, three-, and four-state characters, the scaled weights resulting from a base weight of 1000 are 1000, 500, and 333, respectively. Suppose two trees are being evaluated and that exactly one character is homoplastic (i.e., requires extra steps) on each tree. Further suppose that the first tree requires three extra steps (homoplasies) in a four-state character and that the second tree requires two extra steps in a three-state character. Even though the two trees *should* be considered equally parsimonious, the first tree is deemed shorter, since 3 x 333 = 999 is less than 2 x 500 = 1000. In this case, using a base weight of 6 solves the problem; the scaled weights then become 6, 3, and 2, with 3 x 2 = 2 x 3.

You can mix scaled and unscaled weights in the same command. For example, you might want to scale the weights for just a few characters, but use "equal weighting" for the remaining characters. In this case, the syntactically valid command

```
wts scale:3 9-11 14;
```

would probably not produce the desired results, since the specified characters would receive weights of 1000, 500, 333, etc., but the remaining characters would retain their initial weights (probably 1). Instead, you could use a command such as

```
wts 100:all, scale/basewt=100:3 9-11 14;
```

See Chapter 4 for information on the **Set Character Weights** menu command.

Excluding Characters

Excluding characters is actually a specialized version of weighting characters—excluded characters are simply assigned a weight of zero. Excluded characters therefore do not contribute to overall tree lengths. However, they are still used in other contexts; for example, it possible to examine character-changes in excluded characters with the **DESCRIBE** and **CHGPLOT** commands. An increasingly common approach is to draw inferences about the evolution of one set of characters based on a phylogeny computed from an independent second set of characters. In PAUP, this can be accomplished simply by excluding the first set of characters, searching for trees based on the second set, and then using the standard facilities for interpretation of character changes in the first set.

The **EXCLUDE** command is used to specify a list of characters to be excluded. For example, the command

```
exclude 1-25;
```

requests exclusions of the first 25 characters.

If you want to restore previously excluded characters to the analysis, you can use the **INCLUDE** command. For example:

```
include 5-15;
```

restores ten characters to their original nonexcluded status.

Ordinarily, the effect of the **EXCLUDE** command is cumulative; i.e., any characters already excluded by prior **EXCLUDE** commands remain excluded when a new **EXCLUDE** command is issued. If you want only the characters specified a single **EXCLUDE** command to be excluded, you can use the ONLY option:

```
exclude 6 8 11/only;
```

This is equivalent to the pair of commands:

```
include all;
exclude 6 8 11;
```

You can also use the **Include-Exclude Characters** menu command to exclude or re-include characters (see Chapter 4).

Successive weighting

In addition to *a priori* weighting, PAUP allows *a posteriori* weighting based on the fit of the characters to the trees currently in memory (see "*A Posteriori* Character Weighting" in Chapter 1). The simple command

```
reweight;
```

corresponds to the successive weighting method used in Hennig86 (Farris, 1988). The weight assigned to each character is proportional to the maximum rescaled consistency index over all trees in memory. The weights are scaled between 0 and the "base weight," which is initially set to 1000. The BASEWT option allows you to specify a different scaling. For example, if you issue the command

```
reweight basewt=10;
```

weights will be scaled between 0 and 10.

You can request that other fit measures be used via the INDEX option. Possible values for INDEX are RC (rescaled consistency index, the default), CI (consistency index), and RI (retention index). You can also use the FIT option to request that the minimum (worst fit) or mean fit values be used rather than the maximum (best fit). Available choices for FIT are MAXIMUM, MINIMUM, and MEAN. For example, to request reweighting using the mean (over trees) consistency index, you would use the command

```
reweight fit=maximum index=ci;
```

Ordinarily, after reweighting the characters, you will conduct another tree search using the new weights. The process of reweighting and searching continues until the weights no longer change (Farris, 1988) or until identical trees (or sets of trees) are found in two consecutive searches. You can use the **CSTATUS** command to examine the weights assigned to each character following a reweight command.

DEFINING ANCESTRAL STATES

Ancestral states are used in PAUP for three purposes. First, some character types, including irreversible and asymmetric stepmatrix characters, automatically force a hypothetical ancestor to be included in the analysis; the ancestral states assign a state to this ancestor for each character. Second, even when it is not required, you may choose to include an ancestral taxon in a search (i.e., to explicitly specify character polarities). In this case, the ancestor is treated as an additional taxon, and trees computed during the search are automatically rooted at the point where the ancestor connects to the tree. Third, if you input user-defined trees as rooted trees (i.e., you use the **TREE** rather than the **UTREE** command), character states must be assigned to the ancestor of the full tree.

Ancestral states are defined by an **ANCSTATES** command in the ASSUMPTIONS block. In addition to user-defined ANCSTATES settings, a "standard" ANCSTATES definition is defined to have the "missing" state for all characters. If no other ANCSTATES definition appears in the ASSUMPTIONS block, the default ancestor will be the "standard" (all-missing) one.

For example, to define an ancestor that assigns state 0 for all characters to the ancestor named "allzero," you would use a command such as:

```
ancstates allzero = 0:all;
```

Of course, you do not have to assign the same state for all characters. For example, the command

```
ancstates mixed = 0:1 3 6-10, 1:2 4 12;
```

assigns to the ancestor named "mixed " state 0 for the first, third, and sixth through tenth characters and state 1 for the second fourth, and twelfth characters. Any character numbers not explicitly assigned a character state (e.g., characters 5 and 11 above) retain the default "missing" state.

If two different states are assigned for the same character in an **ANCSTATES** command, the last assignment takes priority. This is convenient when you want to assign a state other than "missing" to most of the characters and a different non-missing state to a few characters. For example, the command

```
ancstates mostly_a = a:all, b:2 5;
```

assigns state 'a' to ancestor "mostly_a" for all characters other than 2 and 5, which get state 'b'.

Instead of using ranges of characters as above, you can also explicitly specify each ancestral character state by using the VECTOR format option. This is useful if not necessary if the character states for the ancestor are very heterogeneous. For example, an ancestor with five characters would be specified by the following VECTOR statement, where *name* is the name of the ANCSTATES set.

```
ANCSTATES name VECTOR = 0 1 2 0 2;
```

Any number of ancestors may be defined (via multiple **ANCSTATES** commands), but only one is in effect at any one time. You may select the current ancestor in any of three ways:

- By preceding the name in an **ANCSTATES** command with an asterisk. For example, the commands

```
ancstates allzero = 0:all;
ancstates *allone = 1:all;
ancstates mixed = 0:1-10 1:11-20;
```

define three ancestors, with ancestor "allone" being the currently chosen ancestor.

- By using the **ASSUME** command. For example, the command

```
assume ancstates = allzero;
```

would make ancestor "allzero" the current ancestor.

- By using the **Choose Assumption Sets** menu command.

ANCSTATES commands are typically issued from within an ASSUMPTIONS block, however you may also issue them from within a PAUP block or via the command line.

DEFINING AND USING OUTGROUPS

Unless you are using directed characters or you choose to include an ancestral taxon, the trees found by PAUP searches are unrooted (see "Outgroups, Ancestors, and Roots" in Chapter 1). The most commonly used method for rooting these trees is to include an assumed outgroup in the analysis. You can use the **OUTGROUP** command to define an outgroup. For example, the command

```
outgroup gar shark;
```

specifies that the taxa "gar" and "shark" are to be considered outgroup taxa relative to the remaining ingroup.

You can also assign outgroup taxa by number, e.g.:

```
outgroup 1 18-21;
```

The taxon numbers correspond to the row numbers of the taxa in the data matrix.

If you want to move a taxon previously assigned to the outgroup back to the ingroup, you can use the **INGROUP** command. For example:

```
ingroup gar;
```

returns the taxon "gar" to the ingroup.

Ordinarily, the effect of the **OUTGROUP** command is cumulative; i.e., any taxa already assigned to the outgroup by prior **OUTGROUP** commands remain as outgroups when a new **OUTGROUP** command is issued. If you want only the taxa specified in a single **OUTGROUP** command to be assigned to the outgroup, you can use the ONLY option:

```
outgroup 18/only;
```

This is equivalent to the pair of commands:

```
ingroup all;
outgroup 18;
```

You can also use the **Define Outgroup** menu command to move taxa from the ingroup to the outgroup or *vice versa* (see Chapter 4 for details).

SIMPLIFYING INPUT WITH "SETS"

"Sets" in PAUP provide a way to refer to collections of objects with single names. The use of sets can greatly reduce the amount of redundant typing needed to issue commands, and can help to avoid mistakes when typing commands into the command line.

Ordinarily, you will define sets within the ASSUMPTIONS block, although you may also define them from within a PAUP block or via the command line. Once a set has been defined, you can use it in any subsequent command that recognizes sets of that type.

Character Sets ("CHARSETs")

The use of character sets allows you to refer to a group of characters by a single name. The only restriction is that the name of the character set cannot be the same as the label assigned to an individual character, for obvious reasons. After defining a character-set using a **CHARSET** command, you can use the character-set name in any place that you would ordinarily use a character name or number. For example, you could define two character sets as follows:

```
charset larval=1-10 26-30;
charset adult=11-25 31-50;
```

and then use the CHARSET names in subsequent commands:

```
exclude larval;   [excludes larval characters]
weights 1:larval, 2:adult; [assigns double-weight to
                            adult characters]
```

The preceding two commands are exactly equivalent to the commands:

```
exclude 1-10 26-30;
weights 1:1-10 26-30, adult:11-25 31-50;
```

Note that you may freely mix character names and/or numbers with character-set names. For example, the command

```
weights 2:larval 12 14 45-49;
```

assigns a weight of 2 to all of the larval and some of the adult characters.

Character sets are especially useful when analyzing molecular sequence data. For example, you might subdivide the regions of a protein-coding gene as follows:

```
charset coding = 25-99 138-234 251-298;
charset introns = 100-137 235-250;
charset flanking = 1-24 251-276;
charset noncoding = introns flanking;
charset 1stPos = 25-97\3 138-232\3 251-296\3;
charset 2ndPos = 26-98\3 139-233\3 252-297\3;
charset 3rdPos = 27-99\3 140-234\3 253-298\3;
```

You could then easily refer to these regions as in the following examples (which are not necessarily intended to be biologically meaningful):

```
exclude flanking ; [excludes flanking regions]

wts 4:1stPos 2ndPos, 2:3rdPos, 1:noncoding;
   [assigns double-weight to 1st and 2nd positions,
    half-weight to noncoding positions]

ctype tv3:coding;[assign a user-define character type
                  to characters in the coding regions]
```

Character-sets are also available in any dialog box that contains a character-selection list. Popup menus allow you to instantly select all of the characters in a set.

Taxon Sets ("TAXSETs")

The use of taxon sets allows you to refer to a group of taxa by a single name. After defining a character-set using a **TAXSET** command, you can use the taxon-set name in any place that you would ordinarily use a taxon name or number. For example, you could define two taxon sets as follows:

```
taxset myGenus=1-15 26-40;
taxset otherSpp=16-25;
```

and then use the taxon-set names in subsequent commands:

```
delete otherspp;   [deletes taxa other than those in
                    'mygenus']
outgroup otherSpp; [assigns taxa 16-25 to the
                    outgroup]
constraints ingrp = ((myGenus)); [define an "ingroup
                                  monophyly" constraint]
```

You cannot declare a **TAXSET** name that is the same as any taxon name in the data file.

Assumption Sets

Assumption sets allow rapid assignment of character type and weight assumptions. Each assumption set specifies a character type ("type sets"), weight ("weight sets") or exclusion status ("exclusion sets") for each character. Any number of type, weight, or exclusion sets may be defined. You can then change the assumptions assigned to all characters in the data set simply by invoking a new assumption set.

Assumption sets are normally defined in the ASSUMPTIONS block, however you may also define them from within a PAUP block or via the command line.

Type sets ("TYPESETs")

Type sets assign a character type to each character. For example, if you wanted the first 10 characters to be of type UNORD, the second 10 to be of type DOLLO, and the remainder to be of type ORD, you could define a corresponding type set as follows:

```
typeset mytypes = unord:1-10, dollo:11-20, ord:21-.;
```

If you do not explicitly assign a type to one or more characters, the current default character type (specified via DEFTYPE in the **OPTIONS** command of the ASSUMPTIONS block) will apply. Note that the default DEFTYPE is UNORD, so ordinarily you will only need to assign types to those characters to which you want to assign a type other than UNORD.

Be careful to use the correct punctuation. If, for instance, you omitted the first comma in the above example, the character-type `dollo` would be interpreted as a character identifier, and would generate an error message.

Remember that you can use character sets (see above) to refer to groups of characters in the TYPESET command. Simply specify a character-set name anywhere a character name or number could be used.

Weight sets (WTSETs)

Weight sets assign a weight to each character. For example, you might define a weight set as follows:

```
wtset mywts = 2: 2 4 8-12, 3: 6 14-20;
```

Any character for which a weight is not explicitly assigned receives a weight of one. Be careful to use the correct punctuation. If, for instance, you omitted the first comma in the above example, the next "3" would be taken as a character number and assigned weight 2. However, the immediately following colon would generate a syntax error message.

An alternate format, "vector", allows you to specify the weight for each character sequentially rather than by character-lists. For example, the following command is equivalent to the one shown above:

```
wtset mywts2 vector = 1 2 1 2 1 3 1 2 2 2 2 2 1 3 3 3
   3 3 3 3;
```

Remember that you can use character sets (see above) to refer to groups of characters in the WTSET command. Simply specify a character-set name anywhere a character name or number could be used.

Exclusion sets (EXSETs)

Exclusion sets allow you to specify a set of characters that are to be "excluded" from the analysis (see "Excluding Characters" under "Character Weighting" above). For example, the command

```
exset dontwant = 4 11-20 31-34;
```

specifies a list of 15 characters that can be excluded by invoking the exclusion set "dontwant."

Remember that you can use character sets (see above) to refer to groups of characters in the EXSET command. Simply specify a character-set name anywhere a character name or number could be used.

Invoking assumption sets

The **TYPESET**, **WTSET**, and **EXSET** commands merely *define* assumption sets; they do not cause the specified types, weights, or exclusion status to go into effect. An assumption set must be *invoked* before it actually takes effect. There are three ways to invoke an assumption set:

1. By preceding the type-set, weight-set, or exclusion-set name with an asterisk. For example, if the following commands were issued in sequence:

```
typeset one = ord:5-8;
typeset *two = dollo:all;
typeset three = irrev:1-5 dollo:6-.;
```

three type sets would be defined, and all characters would currently be assigned type DOLLO.

2. By using the **ASSUME** command. This command allows you to invoke any combination of type sets, weight sets, exclusion sets, and ancestors. For example:

```
assume typeset=one wtset=mywts exset=noncoding;
```

sets the current character types, weights, and exclusion status to the settings defined in the type set "one," the weight set "mywts," and the exclusion set "noncoding," all of which must previously have been defined in **TYPESET**, **WTSET**, and **EXSET** commands, respectively.

3. By using popup menus in the dialog boxes associated with **Set Character Types**, **Set Character Weights**, and **Include-Exclude Characters** (see Chapter 4).

MULTISTATE TAXA

Ordinarily, you will assign a unique (singleton) character-state to each taxon for each character. However, two situations may necessitate the assignment of multiple character states to a taxon:

1. You may be uncertain about the state that a particular taxon possesses, but some of the potential states can be excluded as possibilities. This condition is called "partial uncertainty."

2. A terminal taxon may be an assemblage of lower-level taxa which vary in the character-state possessed. This condition is loosely referred to as "polymorphism."

When multiple states are interpreted as "uncertainty," PAUP will choose a state from the set of available states that allows minimization of the tree length. When multiple states are treated as "polymorphism", PAUP assumes that the "terminal taxon" is actually a heterogeneous group. In this case, all but one of the states in the polymorphic terminal taxon must be derived from a monomorphic ancestral taxon in the most parsimonious

way possible. In PAUP, you can choose either of the above interpretations of multistate taxa. However, it is not possible to mix the two interpretations at the same time (i.e., some multistate taxa interpreted as uncertainty, others as polymorphism). This current limitation will hopefully be eliminated in a future version.

IMPORTANT: "Polymorphism" refers only to variability within a "terminal taxon." Multistate taxa do *not* provide a mechanism for dealing with characters that are polymorphic in a population-genetic sense. In particular, there is no provision for polymorphism in hypothetical ancestral taxa (internal nodes). If a taxon is coded as having multiple states, it is assumed that this taxon represents a monophyletic collection of subtaxa, each of which are themselves monomorphic. The program then assumes that the ancestor of this monophyletic group possessed one of the observed states, from which the other states were subsequently derived. Thus, for example, if two sister taxa were both coded as being polymorphic for states 0 and 1, the ancestor of this pair of taxa would be assigned either 0 or 1 (in order to minimize the overall number of changes on the tree), and either the 0's or the 1's would be interpreted as parallelisms. While it might seem reasonable to assign both states to the ancestor with the polymorphism being retained in the two descendant taxa, this would require additional assumptions about the relative probabilities of retention of polymorphism vs. character transformation that are beyond PAUP's scope.

To assign multiple states to a taxon, enclose all of the desired states within curly braces or parentheses, as in the following example:

```
matrix
   tax1    1 1    0 0
   tax2    1 {12} 1 0
   tax3    0 2    1 (01)
   tax4    0 0    1 1
   ;
```

PAUP does *not* distinguish between the curly braces and parentheses. However, in MacClade, multiple states enclosed in curly braces imply the "uncertainty" interpretation, whereas those enclosed in parentheses imply polymorphism. Thus {12} in the above example would imply "state 1 *or* state 2," whereas the (01) would imply "*both* states 0 *and* 1." For compatibility with MacClade (and in anticipation of future versions of PAUP) you may want to adopt this convention now.

In some situations, it may be helpful to use "equate macros" to enter multistate taxa into the data matrix. The replacement of character-state specifications of the form `{abc}` by a single character allows easier alignment of columns of the data matrix. For example, the matrix above could be equivalently defined as follows:

```
format equate="a={12} b=(01)";
matrix
   tax1    1100
   tax2    1a10
   tax3    021b
   tax4    0011
   ;
```

Note to MacClade Users: Remember that if you created your PAUP input file using MacClade's spreadsheet editor, the "/" and "&" separators used to choose between the two interpretations of multistate taxa are ignored by PAUP. You will have to set the interpretation used by PAUP using one of the methods described above.

In practice the difference between designating multistate taxa "polymorphic" or "uncertain" will be manifested in some constant difference in tree length. Trees calculated using the "polymorphic" option will be longer because they force extra change within the multistate taxon (see above). When the "uncertain" option is used, PAUP chooses the state that leads to a minimal tree, and does not invoke any further change within the multistate taxon.

Complications occur when a taxon is multistate for a Dollo or irreversible character. In those cases, PAUP will select the state which minimizes overall tree length and satisfies the demands of the character type. With Dollo characters, PAUP will allow a single derivation and multiple reversals, if necessary. With an irreversible character, PAUP will allow multiple derivations, but no reversals. In the case of uncertainty, PAUP chooses the state from the multistate taxon which satisfies the character type and minimizes tree length. So PAUP ensures that the character type is maintained over the tree, and can choose the best character state from the "uncertain" multistate terminal. It is even possible that a character for which one or more taxa is multistate may not require any extra steps if that character is treated as "uncertain." However, when the multistate taxon is "polymorphic" for a Dollo or irreversible character, the *best* fit will be one or more derivations of each state internally plus whatever is required within the terminal, depending on the type of character (remember that PAUP must explain *all* of the states in a "polymorphic" multistate taxon). In the case of a Dollo character, unique derivation and reversal but *not* multiple derivation can be tolerated; in the case of an irreversible character, independent derivation but *not* reversal are allowed. Because PAUP is required to account for all the states in a "polymorphic" taxon *and* to adhere to the demands of the character type over the entire tree, the states PAUP assigns internally can be very different from those that would be assigned under a condition of "uncertainty." This of course can lead to different tree topologies, so the difference between treating multistate taxa as "polymorphic" versus "uncertain"is certainly non-trivial. Keep this in mind when you include multistate taxa in your analysis.

DELETING AND RESTORING TAXA

PAUP permits interactive deletion of taxa using the **DELETE** command, as in the following example:

```
delete salamander frog;
```

You can also delete taxa by number:

```
delete 1 10-12 21;
```

The taxon numbers correspond to the row numbers of the taxa in the *original* data matrix (i.e., before any taxa have been deleted).

If you want to restore a previously deleted taxon to the analysis, you can use the **RESTORE** command. For example:

```
restore frog;
```

restores the taxon named "frog" to the analysis.

Ordinarily, the effect of the **DELETE** command is cumulative; i.e., any taxa already deleted by prior **DELETE** commands remain deleted when a new **DELETE** command is issued. If you want only the taxa specified a single **DELETE** command to be deleted, you can use the ONLY option:

```
delete 6 8 11/only;
```

This is equivalent to the pair of commands:

```
restore all;
delete 6 8 11;
```

You can also use the **Delete-Restore Taxa** menu command to delete and/or restore taxa (see Chapter 4 for details).

DISTANCE MATRICES

A matrix of pairwise distances between taxa can be computed using the **SHOWDIST** command or the **Show Distance Matrix** menu command. Two distances are computed for each pair of taxa *i* and *j*:

1. The "absolute distance"

$$d(i,j) = \sum_{k \ S} w_k \cdot diff(x_{ik},x_{jk}) \quad ,$$

where S is the set of characters that are not "ignored" or excluded, w_k is the weight currently assigned to character k, x_{ik} and x_{jk} are the states of character k in taxa i and j, respectively, and $diff(x_{ik},x_{jk})$ is the cost of a change from state x_{ik} to state x_{jk}. The costs specified by $diff(y,z)$ are determined as follows:

If either y or z is "missing," or if both y and z are "missing," $diff(y,z) = 0$.

Otherwise, for unordered characters,

$diff(y,z) =$ 1 if $y \neq z$, 0 otherwise;

for ordered, Dollo, and irreversible characters,

$diff(y,z) =$ $|y - z|$;

for user-defined character-state trees,

$diff(y,z) =$ the number of branches lying on the path of the character-state tree connecting states y and z;

for stepmatrix characters,

$diff(y,z) =$ s_{yz}, the corresponding element of the applicable stepmatrix.

2. The "mean distance"

$$d_m(i,j) = \frac{d(i,j)}{\sum_{k \ S} w_k^*} \quad ,$$

where $w_k^* = w_k$ if both x_{ik} and x_{jk} are nonmissing and zero otherwise. That is, the absolute distance is divided by the total weight of the characters for which neither i nor j has the state "missing." Mean distances are more meaningful when some taxa have much higher proportions of missing data than others.

Distance matrix output has the following format:

```
Pairwise distances between taxa

   Below diagonal: Absolute distances
   Above diagonal: Mean distances

                  1       2       3       4       5       6       7       8
  1 taxonA        -   0.429   0.357   0.429   0.500   0.857   0.571   0.571
  2 taxonB        6       -   0.357   0.571   0.500   0.429   0.857   0.857
  3 taxonC        5       5       -   0.214   0.286   0.500   0.500   0.500
  4 taxonD        6       8       3       -   0.214   0.571   0.286   0.286
  5 taxonE        7       7       4       3       -   0.500   0.500   0.500
  6 taxonF       12       6       7       8       7       -   0.571   0.571
  7 taxonG        8      12       7       4       7       8       -   0.000
  8 taxonH        8      12       7       4       7       8       0       -
```

SEARCHING FOR TREES

PAUP provides two basic classes of methods for searching for optimal trees, exact methods and heuristics. Exact methods guarantee to find the optimal tree(s) but may require a prohibitive amount of computer time for medium- to large-sized data sets. Heuristic methods do not guarantee optimality but generally require far less computer time. Either exact or heuristic methods may be employed by the bootstrap. See Chapter 1 for a complete description of searching methods. The options available to each type of search are discussed under the appropriate heading below, but some options are available for all types of searches. These will be described first, followed by a description of each search type. All of these shared options except STATUS are available under the General window of the **Heuristic** dialog box.

Although searches are designed to find optimal trees, you can request that PAUP retain near-optimal trees as well. If you wish to do so, you can specify a tree length below which trees should be kept by using the KEEP option of the **HSEARCH**, **BANDB**, or **ALLTREES** commands or by specifying the appropriate number in the **Keep** selection of the appropriate search dialog box. Once you have done so, the optimal and near-optimal trees in memory can be saved or manipulated further. You can also choose to include an ancestor with the INCLUDEANC option or the **Include Ancestor** item in a search dialog box. The ancestor will be either the standard or one you have specified previously using the **ANCSTATES** command. If this is done, trees are rooted in memory using the designated ancestor. Next, you may choose during any search to collapse any zero-length branches. If you choose not to do so, all resolutions of optimal trees will be retained, even if there is no evidence for some of them. This is done using the COLLAPSE option or the Collapse zero-length branches selection in a search dialog box. Next, you may choose to enforce topological constraints during the search (see the section "Searching under topological constraints"), either keeping or discarding trees that are

compatible with the constraint tree. This is done using the ENFORCE option (with CONVERSE or NOCONVERSE) or the **Enforce topological constraints** selection in the appropriate search dialog box. Remember that you must have previously defined a constraint tree in order to invoke this option. Finally, you can request that PAUP output the status of the search using the STATUS option.

One option that affects only heuristic and branch-and-bound searches is MULPARS, or save all minimal trees in a **Heuristic** dialog box. Searches without MULPARS in effect can sometimes be significantly faster because less of the search tree needs to be explored. However, at the end of the search you will know the length of the shortest tree(s) but you will have no idea how many trees exist at that length.

Heuristic Searches

Heuristic searches generally provide the fastest way to find optimal trees, but the results, being approximate, may depend on the way in which the search is conducted. Unlike the exact methods, heuristic methods may find only a subset of the optimal trees for a given data set and a given set of search parameters or may fail to identify minimum length trees at all. In addition, no one combination of settings will provide the best results for all data sets. You must be prepared to spend some time exploring different options to find optimal trees. This is especially true for large data sets, for which exact methods cannot be used. See the section "Heuristic Search Strategies" below.

There are too many options to give exhaustive examples. At some point it is incumbent on the user to explore how changing options affects the analysis of her/his data set. The reason that PAUP has all these features is to make it possible for you to explore the data set in as many ways as possible. Like any analytical program, PAUP can only give you the tools - you must decide which tools to use and when to use them. Unfortunately, the bottom line is that no two data sets are the same, so no two heuristic searches will ever be the same. Searching involves changing the search options from the default values. By default zero-length branches are collapsed, all most-parsimonious trees are kept (MULPARS), and steepest descent is not invoked. You can find out the current search settings at any time by using the command

```
hsearch ?;
```

Which will list all options available during a heuristic search and their current settings. Options which are nonpersistent are marked with an asterisk.

Once you have selected the general options (listed above) that will be in effect during the search, you have other decisions to make: where to get starting trees for branch swapping; if by stepwise addition, how to add taxa; and how to branch swap. Starting trees for a search can be some or all trees in memory (using FROMTREE and TOTREE options or by selecting tree numbers in the **Heuristic** dialog box), or they can be obtained by stepwise addition (using STEPWISE or by selecting this in the **Heuristic** dialog box). Using trees in memory allows you to use the results of a previous analysis as the starting point for a new search. Any or all trees in memory may be used, so swapping can start on both optimal and suboptimal trees.

NOTE: Selecting the starting tree range cannot be done if trees in memory have been filtered. You must either start with tree(s) obtained by stepwise addition or swap on all trees in memory.

If trees in memory are not used, you must specify which addition sequence to use to obtain starting trees. You must also specify how many equal-length trees PAUP will keep during each step of stepwise addition using the HOLD option or by entering this value in the **Heuristic** dialog box. Stepwise addition is selected using the ADDSEQ option or by choosing it in the **Heuristic** dialog box. You may opt to add taxa in the sequence they are in (ASIS); to add the closest taxon at each step (CLOSEST); by following the "simple algorithm" (SIMPLE) of Farris (1970), or in random order (RANDOM). The SIMPLE option requires that you choose a "reference" taxon (default is the first taxon in the matrix—see the section "Stepwise Addition").

NOTE: The reference taxon for simple addition sequence is ignored if the tree is rooted —in that case the ancestor serves that role.

Random addition requires a few more options be set, specifically the number of replicates (NREPS or the **#reps** selection in the search dialog box); a "seed" for the random number generator (by using the RSEED option or by entering this value in the **Heuristic** dialog box) and whether or not a running status of the random addition should be displayed (the RSTATUS option or by entering this value in the **Heuristic** dialog box). HOLD values are ignored when random addition is used. The following command will begin a search with ten replicates of random addition and the initial seed set to 123321:

```
hsearch addseq=random nreps=10 rseed=123321;
```

Once the starting trees have been obtained, the only thing remaining is selection of a branch swapping algorithm. These include TBR (tree bisection-reconnection), NNI (nearest-neighbor interchange), and SPR (subtree pruning-regrafting). These algorithms are described in detail in

the section "Branch Swapping." The following command will begin a search with simple addition sequence and subtree-pruning-regrafting branch swapping:

```
hsearch addseq=simple swap=spr;
```

In addition to KEEP, heuristic searches also have the option of limiting the number of trees (NCHUCK option) *greater than or equal to* a specified length (CHUCKLEN option) which will be kept (see below). This is different from KEEP, which specifies that all trees *less than or equal* a certain length are kept. Thus

```
hsearch nchuck=25 chucklen=50;
```

will begin a heuristic search, keeping no more than twenty-five trees of fifty or more steps. If KEEP and NCHUCK are used together, once PAUP reaches the limit specified by NCHUCK and CHUCKLEN, it stops looking for shorter trees; that is, it doesn't replace longer trees satisfying the KEEP limit with shorter trees satisfying the KEEP limit. When that happens, PAUP will either stop or go on to the next step, depending on the circumstances. The command

```
hsearch nchuck=25 chucklen=50 keep=40;
```

will try to keep all trees less than forty steps, but if twenty-five trees of length greater than or equal to fifty steps are found first, PAUP will stop.

Part of the difficulty in using heuristic searches is that there are no settings that will get the best results for all data sets. Each search will be more or less unique, although different types of search will be similar. The choice of options will almost always affect the outcome of the search. This becomes critical when the data set is one which produces a very large number of trees. In that instance, there are several strategies for trying to get the best estimate of the set of shortest trees.

First, a simple illustration of how option choice can affect the number of optimal trees found. This example uses the sample *Menidia* data set distributed with the program. If this data set is analyzed with the following command

```
hsearch addseq=asis swap=nni hold=1;
```

ten trees are found. If we now choose to hold ten trees instead of 1, the search now finds 24 trees (note that the ADDSEQ=ASIS and SWAP=NNI settings are automatically retained.

```
hsearch hold=10;
```

Finally, switching to TBR swapping yields 25 trees. This is the maximum number that any other combination of settings will find in a heuristic search, as you can verify yourself by exploring the data set with other settings.

```
hsearch swap=tbr;
```

Of course, with other data sets you may reach a stage where trying further rearrangements does not yield any new trees and takes up vast amounts of computer time.

In general, the best way to identify all optimal trees is to aggressively vary the swapping and addition-sequence options to explore as much of tree space as possible. The single best strategy is use of the random addition-sequence option, which will present the rearrangement algorithms with a broad range of starting trees on which to swap. The tradeoff is that random trees can be very far from optimal, and as such are not very good places to start to find optimal trees. It may drastically increase computation time and resources to find minimal trees from a random beginning. On the other hand, random addition frequently leads to the finding of more (if not all) islands than other addition sequences. You can also hold more trees during stepwise addition, which may increase the effectiveness of the search but also greatly increases the time spent on adding taxa.

Another strategy is to invoke steepest descent, which will not abandon a round of swapping until all minimum trees from the previous round have been evaluated; it then chooses the best available tree(s) for the next round. (Without steepest descent, a round of swapping ends as soon as a shorter tree is found, and this tree is used as the starting tree for the next round). Because the rearrangements producing greater improvements are preferred over those leading to smaller ones, use of steepest descent can sometimes reduce the problem of entrapment in local optima. Unfortunately, being overly "greedy" can also produce the opposite result—in some cases, rearrangements with smaller decreases in tree length ultimately lead to the shortest trees, whereas the rearrangements preferred under steepest descent represent blind alleys (local optima).

You can also potentially increase your chances of finding minimal trees by swapping on nonminimal trees. The first way to do this is to KEEP trees above a certain length, say one or two steps above the shortest tree found so far (be aware that you may end up retaining enormous numbers of trees when you do this). Then you can begin swapping on those trees. This may have some of the same problems as using random-addition sequence, but may in fact "lower the water level" between islands enough to allow PAUP to find more of the optimal trees.

Another way to estimate optimal trees is to use the NCHUCK/ CHUCKLEN option pair (so named for historical reasons) in conjunction with random addition (but see the note about the interaction of NCHUCK and KEEP in the section above). If you set CHUCKLEN to some low value, such as one step, and set NCHUCK to ten, you will save no more than ten trees for each random replicate before the next replicate begins. If the next replicate finds ten different trees as short as the first ten, they will be added to the ten found before, and so on. In that way, you can build a set of trees in memory, say one thousand, with ten trees coming from each of one hundred random searches. This is a better estimate of the universe of optimal trees than saving one thousand trees from a single search, which probably gives a very biased estimate of the diversity of optimal trees.

For some data sets, you may want to try a huge number of random replicates but avoid wasting too much time on replicates that start with very suboptimal trees. You can specify ABORTREP along with CHUCKLEN and NCHUCK to abandon a replicate as soon as the "chucking" limits are hit. In this case, many replicates are likely to be abandoned early, but many more (perhaps thousands of) replicates can be performed.

Branch-and-Bound Search

The details of the branch-and-bound algorithm are given in Chapter 1 (see also the section above describing options that affect all searches). To begin a branch-and-bound search you must either specify the initial upper bound, for example the command

```
bandb upbound=30;
```

will set the upper bound at length 30, or you can let PAUP find it by stepwise addition.

Ordinarily, you will not need to specify an upper bound. However, the better the initial upper bound, the faster the branch-and-bound search will proceed. For large and/or messy data sets, you can sometimes reduce run times by performing a more extensive heuristic search and using the resulting tree length as the starting upper bound. The options for stepwise addition are slightly different than those available in a heuristic search, and include three choices: furthest, asis, or simple. PAUP can also output a frequency distribution of tree lengths using the FD option or the **Tree length frequency distribution** item in the **Branch and Bound** dialog box. The maximum tree length to be shown must be specified by the KEEP length. This option provides a way to examine the left tail of the distribution of tree lengths if there are too many taxa to obtain the full distribution via an exhaustive search (see below). To accomplish this, you

can perform an initial branch-and-bound search to determine the minimum tree length and then perform a second search specifying FD and a KEEP length some number of steps larger than the minimum length. In this way, you can determine the number of trees that are one step longer than the shortest, two steps longer, and so on. The output for a branch-and-bound search is nearly identical to that for a heuristic search except for when the FD is selected. For example, using the sample "Menidia" data set, the command

```
bandb fd keep=53;
```

outputs the following:

```
Frequency distribution of tree lengths:

   fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
50 › (70)
51 ›,, (959)
52 ›,,,,,,,,,,,, (7525)
53 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (40627)
   fi<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
           mean=52.803725 sd=0.453089 g1=-2.354361 g2=5.528415
```

This tells us that there are over 8,500 trees within two steps of the shortest and over 49,000 trees within three steps of the shortest. The statistics for this distribution are ordinarily not meaningful due to the fact that it has been artificially truncated, but they are output anyway.

Note that no trees are saved when this option is in effect.

Exhaustive Search

Exhaustive methods are not generally useful for more than 10 or 11 taxa, since there are over 2 million trees for 10 taxa and 34 million trees for 11 taxa. Computation of all trees for larger numbers of taxa is impractical at present. With very large data sets, heuristic methods may be the only avenue available for analysis. If however you do have a data set small enough for exhaustive analysis, there are several useful options besides the standard search options (see the section above on options affecting more all search algorithms). Obviously, addition sequence is irrelevant in an exhaustive search, since all trees are going to be evaluated anyway. The most useful option is the ability to calculate a frequency distribution of tree lengths. Normally, obtaining the frequency distribution is the only reason for doing an exhaustive rather than branch-and-bound search, so you will probably always specify this option. The frequency interval can be set using either the HINTERVAL option or the **Class Interval** selection in the **Exhaustive** (search) dialog box. By default, HINTERVAL=1, so that the number of trees at each length is output. If HINTERVAL>1, then adjacent tree length values are pooled into tree length classes of width

interval-value. An exhaustive search is begun by using the **ALLTREES** command or the **Exhaustive** menu command. for example

```
alltrees hinterval=5;
```

will begin an exhaustive search and use an interval of five steps in the tree-length histogram. If you wish to save the interval distribution to a text file for input to other programs you must use the SAVEFD option or the S**ave tree-length frequencies to file** selection in the **Exhaustive**(search) dialog box. The SAVEFD option is not persistent. You must also specify a filename to receive the output, either by using the FILE= option or by specifying a filename in the dialog box that appears when the save option is selected. For example, the following command will begin an exhaustive search, use an interval of five steps on the histogram, save the distribution to the file "test.out", replacing it without prompting if it already exists.

```
alltrees hinterval=5 savefd file=test.out replace;
```

A sample frequency distribution (for the "Xenopus" sample data file) is shown below:

```
Frequency distribution of tree lengths:

   fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
51 › (4)
52 › (1)
53 › (7)
54 ›, (14)
55 ›, (18)
56 ›,, (31)
57 ›,,,,, (65)
58 ›,,,,, (66)
59 ›,,,,,,,, (110)
60 ›,,,,,,,,, (135)
61 ›,,,,,,,,,,, (162)
62 ›,,,,,,,,,,,,,,,,, (245)
63 ›,,,,,,,,,,,,,,,,,,,, (292)
64 ›,,,,,,,,,,,,,,,,,,,,,,,, (347)
65 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (422)
66 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (552)
67 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (606)
68 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (635)
69 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (610)
70 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (746)
71 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (844)
72 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (900)
73 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (945)
74 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (913)
75 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (790)
76 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (516)
77 ›,,,,,,,,,,,,,,,,,, (261)
78 ›,,,,,,,,,, (148)
79 ›, (10)
   fi<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<
          mean=69.844156 sd=4.775776 g1=-0.664305 g2=0.029334
```

The shape of this distribution has been suggested to provide a measure of the strength of phylogenetic signal (Hillis, 1991; Huelsenbeck, 1991;

Hillis and Huelsenbeck, 1992). In general, the more left-skewed the distribution (as quantified by increasing negativity of the g_1 statistic), the greater is the amount of phylogenetic signal present in the data. However, as pointed out by Hillis and Huelsenbeck (1992) (see also Källersjö et al., 1992) the existence of signal says little about the nature of the signal; all of the "signal" may be confined to one or a few relatively uninteresting groups (e.g., mouse + rat in a study of tetrapod relationships).

Lake's Method of Linear Invariants

Lake's method is only available if DATATYPE=DNA or DATATYPE=RNA. To use the method, you must specify either 1) a quartet of taxa to be evaluated; 2) all possible quartets of taxa from the data matrix; or 3) four groups of taxa, where one taxon from each group makes up the quartet (all such quartets are evaluated). These options are selected in the **Lake's Invariants** dialog box or by using the **LAKE** command. If no options are specified, the command only calculates the invariants.

```
lake mode=choose4 taxa="1 2 3 4";
```

with the following output for the sample "Hominoid mtDNA" data. (Note that this data set is used for example purposes only; the number of positions considered informative by Lake's method is too small for meaningful application of the method to this data set).

```
Lake's method of Phylogenetic Invariants ("Evolutionary Parsimony")

E= ((human,chimp),(gorilla,orang))
F= ((human,gorilla),(chimp,orang))
G= ((human,orang),(chimp,gorilla))

Invariants:

   E tree: x = 3 + 0 - (0 + 0) = 3
             P = 0.25000 (binomial test)
   F tree: y = 0 + 0 - (0 + 1) = -1
             P = 1.00000 (binomial test)
   G tree: z = 0 + 0 - (0 + 0) = 0
```

These results would suggest that there is slightly more support for a (human,chimp) clade (the E tree) than for (human,gorilla) or (chimp,gorilla), but that the result is not statistically significant. Note that a binomial test is used rather than a chi-square (see Holmquist et al., 1988) due to the low expected frequencies.

The following command will begin a search for a single quartet and output the spectral distribution and branch lengths:

```
lake mode=choose4 taxa="1 2 3 4" spectdist brlength;
```

with the output:

```
Lake's method of Phylogenetic Invariants ("Evolutionary Parsimony")

   Output of spectral distributions requested
   Calculation of branch lengths requested

E= ((human,chimp),(gorilla,orang))
F= ((human,gorilla),(chimp,orang))
G= ((human,orang),(chimp,gorilla))

Spectral distribution:

   O (1111) = 676
   a (1222) = 21
   b (1211) = 27
   c (1121) = 30
   d (1112) = 63
   A (1333) = 1
   B (1311) = 2
   C (1131) = 3
   D (1113) = 22
   e (1122) = 14
   f (1212) = 9
   g (1221) = 13
   E (1133) = 3
   F (1313) = 0
   G (1331) = 0
   H (1233) = 0
   h (1322) = 0
   i (1344) = 0
   J (1134) = 0
   j (1123) = 4
   k (1132) = 0
   L (1323) = 0
   l (1232) = 0
   m (1343) = 0
   N (1314) = 1
   n (1213) = 2
   p (1312) = 0
   Q (1332) = 0
   q (1223) = 1
   r (1334) = 1
   S (1341) = 0
   s (1231) = 0
   t (1321) = 0
   u (1234) = 0
   v (1324) = 0
   w (1342) = 0

Total number of positions used = 893

Spectral distribution of 3-taxon components and terms for branch lengths

Pattern    wxy_        w_yz        wx_z        _xyz
-------------------------------------------------
O (111)     761         705         709         698
a (122)      35          35          30          41
b (121)      38          43          40          39
c (112)      48          72          77          77
A (133)       2           4           1           5
B (131)       3           3           2           3
C (113)       6          25          29          23
u (123)       0           5           3           6
v (132)       0           0           0           1
w (134)       0           1           2           0
-------------------------------------------------
S1            2           3          -1           5
S2            3           3           2           2
S3            6          20          26          17
R1          710         625         622         623
R2          716         641         642         619
R3          736         699         716         695
```

```
L1        2.508       4.266      -1.438       7.110
L2        3.726       4.160       2.773       2.876
L3        7.221      24.840      31.291      21.322

Branch lengths for E tree

Branch        1st estimate   2nd estimate           mean
-------------------------------------------------------
human                2.508         -1.438          0.535
chimp                3.726          2.773          3.250
gorilla              4.160          2.876          3.518
orang               24.840         21.322         23.081
central                                            4.667

Branch lengths for F tree

Branch        1st estimate   2nd estimate           mean
-------------------------------------------------------
human                2.508          4.266          3.387
chimp                2.773          7.110          4.942
gorilla              7.221          4.160          5.690
orang               31.291         21.322         26.306
central                                           -1.567

Branch lengths for G tree

Branch        1st estimate   2nd estimate           mean
-------------------------------------------------------
human                4.266         -1.438          1.414
chimp                3.726          7.110          5.418
gorilla              7.221          2.876          5.049
orang               24.840         31.291         28.066
central                                            0.000
```

If all possible quartets *or* four groups are used, you have the option of requesting only summary tables. For all possible quartets, the summary tables show the number of times significant support for particular groupings of taxa is achieved. For example, the command

```
lake mode=allquart taxa="1 2 3 4 5" sumtabonly;
```

generates the following output:

```
Number of times pairs supported/rejected at P = 0.050

   Below diagonal: Number of times pair supported
   Above diagonal: Number of times pair rejected

               1     2     3     4     5
  1 human      -     0     0     1     1
  2 chimp      1     -     0     1     1
  3 gorilla    0     0     -     0     0
  4 orang      0     0     0     -     0
  5 gibbon     0     0     0     1     -

Number of times pairs supported/rejected at P = 0.010

   Below diagonal: Number of times pair supported
   Above diagonal: Number of times pair rejected

               1     2     3     4     5
  1 human      -     0     0     1     1
  2 chimp      1     -     0     1     1
  3 gorilla    0     0     -     0     0
  4 orang      0     0     0     -     0
  5 gibbon     0     0     0     1     -

Number of times pairs supported/rejected at P = 0.005

   Below diagonal: Number of times pair supported
   Above diagonal: Number of times pair rejected

               1     2     3     4     5
  1 human      -     0     0     0     0
  2 chimp      0     -     0     0     0
  3 gorilla    0     0     -     0     0
  4 orang      0     0     0     -     0
  5 gibbon     0     0     0     0     -

Number of times pairs supported/rejected at P = 0.001

   Below diagonal: Number of times pair supported
   Above diagonal: Number of times pair rejected

               1     2     3     4     5
  1 human      -     0     0     0     0
  2 chimp      0     -     0     0     0
  3 gorilla    0     0     -     0     0
  4 orang      0     0     0     -     0
  5 gibbon     0     0     0     0     -
```

These results show that the (human,chimp,gorilla) and (human,chimp) groupings receives some support at P=0.01, but only from one quartet in each case.

The most useful approach for more than four taxa is to divide the taxa into four groups and evaluate all quartets consistent with this arrangement. For example, if we wanted to assume monophyly of (chimp,gorilla,human) but use both orang and gibbon as outgroups to resolve the trichotomy, we could use the command:

```
lake mode=fourgrps sumtabonly grpa="1" grpb="2" grpc="3" grpd="4 5";
```

which generates the following output:

```
   All output other than summary table suppressed
   Evaluating quartets with one member from each of the following groups:

      Group A:
            human

      Group B:
            chimp

      Group C:
            gorilla

      Group D:
            orang
            gibbon

2 quartets to be evaluated

"E", "F", and "G" trees:
   E = ((Group A,Group B),(Group C,Group D))
   F = ((Group A,Group C),(Group B,Group D))
   G = ((Group A,Group D),(Group B,Group C))

Summary of results from Lake's method:
--------------------------------------

                                            E      F      G
Lake's method:
   # Times tree favored                   2.0    0.0    0.0
   Total counts favoring tree              6      1     -1
Standard parsimony:
   # Times tree favored                   2.0    0.0    0.0
   Total counts favoring tree             35     20     28
Transversion parsimony:
   # Times tree favored                   2.0    0.0    0.0
   Total counts favoring tree              6      3      1
Transversion parsimony (1133,1313,1331):
   # Times tree favored                   2.0    0.0    0.0
   Total counts favoring tree              6      2      0

Cumulative statistics
---------------------

   E tree:

      means: d=3.000 s=3.000

      rho's: r1=1.000 r2=1.000 r3=1.000 r4=1.000
             r12=1.000 r13=1.000 r14=1.000 r23=1.000 r24=1.000 r34=1.000
             r123=1.000 r124=1.000 r134=1.000 r234=1.000
             r1234=1.000

      N4: c=2.000 X2=3.000 P=0.083265
      N5: c=2.000 X2=3.000 P=0.083265

   F tree:

      means: d=0.500 s=1.500

      rho's: r1=1.000 r2=1.000 r3=1.000 r4=0.000
             r12=1.000 r13=1.000 r14=0.000 r23=1.000 r24=0.000 r34=0.000
             r123=1.000 r124=0.000 r134=0.000 r234=0.000
             r1234=0.000

      N4: c=1.000 X2=0.333 P=0.563703
      N5: c=1.000 X2=0.333 P=0.563703

   G tree:

      means: d=-0.500 s=0.500
```

```
rho's: r1=1.000 r2=1.000 r3=1.000 r4=0.000
       r12=1.000 r13=1.000 r14=0.000 r23=1.000 r24=0.000 r34=0.000
       r123=1.000 r124=0.000 r134=0.000 r234=0.000
       r1234=0.000

N4: c=1.000 X2=1.000 P=0.317311
N5: c=1.000 X2=1.000 P=0.317311
```

The summary table shows that the (human,chimp) tree is favored both by Lake's method and by the three parsimony variants. Note that for a given sequence position, "transversion parsimony" supports a tree if a pair of taxa on one side of the central branch both have purines and the pair of taxa on the opposite side both have pyrimidines. Lake attaches a different meaning to transversion parsimony, requiring that the two pyrimidines be the same base and the two purines be the same base. This is indicated by "Transversion parsimony (1133,1313,1331)" where 1133, 1313, and 1331 correspond to Lake's category codes.

When there are many more than four taxa to be divided into four groups, the use of taxon-sets can greatly simplify typing the command (or selecting the groups from the lists in the dialog box). Just define the groups via TAXSET commands in the input data file and use the taxon-set names rather than actual taxon names or numbers in the GRPA, GRPB, GRPC, and GRPD option settings.

Assessing Confidence using Bootstrap Analysis

The search options for the bootstrap are the same as those for either a heuristic or branch-and-bound search. A bootstrap search is stared using the **BOOTSTRAP** command or the **Bootstrap** menu command. Because it randomly samples the data matrix with replacement, you must also specify a starting seed for the analysis. If you do not, 1 is used for the first replicate (the number is reset randomly for every subsequent replicate). For example, the following command will begin a branch-and-bound bootstrap search with the initial seed set to 12322, ten replicates, and a confidence level of 50:

```
bootstrap bseed=12322 nreps=10 method=bandb
conlevel=50;
```

If you do not specify a starting seed for subsequent runs, the seed defaults to the next number in the random number sequence initiated during the previous run. You can try different addition sequences and heuristic search options, or you can use a branch-and-bound search. You may specify that compatible trees that are below the confidence level be kept by using the KEEPALL option. For example:

```
bootstrap bseed=1 nreps=10 method=bandb conlevel=50
keepall;
```

Be aware, however, that when KEEPALL is in effect, some groups may appear in the bootstrap consensus tree that are incompatible with other groups that did not appear. For example, If two groups are different resolutions of a polytomy, they will be incompatible with one another, yet one of them may be included in the consensus and one not, although they appear at similar frequencies. The solution to this is to carefully examine the plot of partition frequencies for other incompatible groupings with similar frequencies—there may be one group found in 32% of the replicate trees that appears in the bootstrap consensus and another conflicting group found in 31% that therefore could not be included.

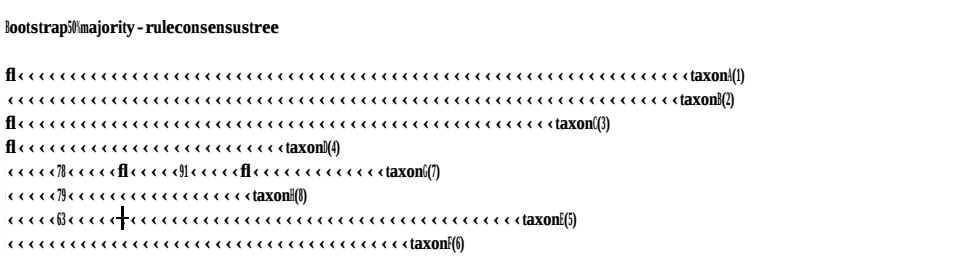
The presence of unequal character weights adds an additional complication to the use of the bootstrap. Version 3.0 of PAUP simply ignored any weights that might be in effect and weighted each character equally for the bootstrap analysis. This option is retained in Version 3.1, and two new options have been added. The first (WTS=SIMPLE) simply assigns each character an equal probability of being sampled, but then uses the weight attached to each character during the following search. The second option (WTS=REPEATCNT) treats each character weight as if it represents the number of times the character was observed. (For example, the weights might represent the number of times a particular pattern of character states was observed, with one column of the data matrix used to represent each pattern). Obviously, these two interpretations have very different implications, and you should decide which one makes the most sense for your analysis. Implied confidence will generally be higher with weights treated as repeat counts, because there will be, on average, more "characters" supporting each retained group. On the other hand, if the weights are drawn from an especially appropriate biological criterion (e.g., first and second vs. third positions in protein-coding DNA sequences), the "simple" interpretation is probably more justifiable.

The output of the bootstrap procedure consists of (1) a table showing all partitions (or groups) that were found in the bootstrap replications and their frequencies, and (2) a bootstrap majority-rule consensus tree. The numbers on the branches of the consensus tree indicate the percentage of the bootstrap replications that support the group descending from that branch.

```
Partitions found in one or more trees and frequency of occurrence:

12345678    Freq
----------------
...*..**   91.01
......**   78.66
..******   77.66
...*****   63.40
...*.***   42.83
..**.***   25.80
.**.**..    8.80
.....***    8.79
....**..    4.34
.**.....    4.07
.*..*...    3.09
..*..*..    2.39
..*.**..    0.78
.**.*...    0.40
..*.*...    0.33
.**..*..    0.30
.*..**..    0.30
..*.****    0.17
..*..***    0.12
```

The frequencies indicate the number of bootstrap replicates in which the particular partition was found. These are called "partitions" because the trees are unrooted: either the group represented by periods or the group represented by asterisks would be monophyletic, but not necessarily both (each branch "partitions" the taxa into those on one side of the branch and those on the other). Of course, if the trees were intrinsically rooted due to the inclusion of an ancestral taxon or the presence of directed character types, then the partitions would correspond directly to monophyletic groups on the tree. If *N* multiple equally parsimonious trees are found in a given replicate, each group found on each of these trees contributes 1/*N* to the total group count. This explains the presence of fractional values in the partition-frequency table.

The bootstrap consensus tree shows the relative partition frequencies (expressed as a percentage) corresponding to each branch. Note that percentages on the consensus tree are rounded.



The consensus tree that is ouput for the bootstrap will in general be longer than the minimal tree for the full data set, although this is a function of consensus trees in general and is not unique to the bootstrap. This is because in PAUP, polytomies are treated as representing simultaneous multiple splitting ("hard polytomies") (Maddison, 1989) rather than as indicating uncertain resolution ("soft polytomies").

Random Trees

If you are interested in the tree-length frequency distribution (see "Exhaustive Search" above but the number of taxa is too large to perform an exhaustive search, you can approximate the shape of the distribution by randomly sampling trees under a model in which every possible tree is equally likely. As in the case of an exhaustive search, you may save the tree-length distribution to a file for input into other programs. Random trees may be generated using the **RANDTREES** command or the **Random trees** menu command. For example, the following will evaluate the lengths of 10000 random trees with the seed set to 445667:

```
randtrees tseed=445667 nreps=10000;
```

Output consists of a tree-length frequency distribution, which may be saved to a file as in an exhaustive search. For the sample "Menidia" data set, the resulting output is as follows:

```
Evaluating 10000 trees sampled randomly from the set of all possible trees

   Trees are unrooted
   Starting seed = 445667
   Time used = 00:01:31.8

Frequency distribution of lengths of 10000 random trees:

    fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
 71 ›, (4)
 72 › (3)
 73 › (1)
 74 › (1)
 75 › (3)
 76 ›, (6)
 77 ›, (6)
 78 ›, (10)
 79 ›,, (14)
 80 ›,, (18)
 81 ›,, (18)
 82 ›,,, (26)
 83 ›,,,, (34)
 84 ›,,,,, (41)
 85 ›,,,,,,, (54)
 86 ›,,,,,,,,,, (80)
 87 ›,,,,,,,,,, (77)
 88 ›,,,,,,,,,,, (82)
 89 ›,,,,,,,,,,,,,,, (117)
 90 ›,,,,,,,,,,,,,,,, (126)
 91 ›,,,,,,,,,,,,,,,,,,, (144)
 92 ›,,,,,,,,,,,,,,,,,,,,,, (168)
 93 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (227)
 94 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (230)
 95 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (262)
 96 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (302)
 97 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (330)
 98 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (348)
 99 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (381)
100 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (382)
101 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (459)
102 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (452)
103 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (471)
104 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (477)
105 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (482)
106 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (492)
107 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (504)
108 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (487)
109 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (422)
110 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (408)
111 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (369)
112 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (354)
113 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (292)
114 ›,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, (253)
115 ›,,,,,,,,,,,,,,,,,,,,,, (174)
116 ›,,,,,,,,,,,,,,,,,,,, (155)
117 ›,,,,,,,,,,,, (95)
118 ›,,,,,,,, (61)
119 ›,,,,, (40)
120 ›,,,,, (37)
121 ›,, (19)
122 › (2)
    fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹
           mean=103.055500 sd=8.043744 g1=-0.458140 g2=0.035357
```

It is interesting to compare these results with those from the branch-and-bound search where we obtained the number of trees of length less than or equal to 53 steps for the same data set (see "Branch-and-Bound Search" above). Even though there were 49,181 trees of length less than or equal

to 53 steps (3 steps longer than the minimum-length trees), the shortest tree found by random sampling of 10,000 trees was 71 steps. Obviously, given that for this 16-taxon data set, there are over 2.134×10^{14} possible (unrooted) trees, random sampling of trees is not an effective way to search for optimal trees!

DIAGNOSING TREES

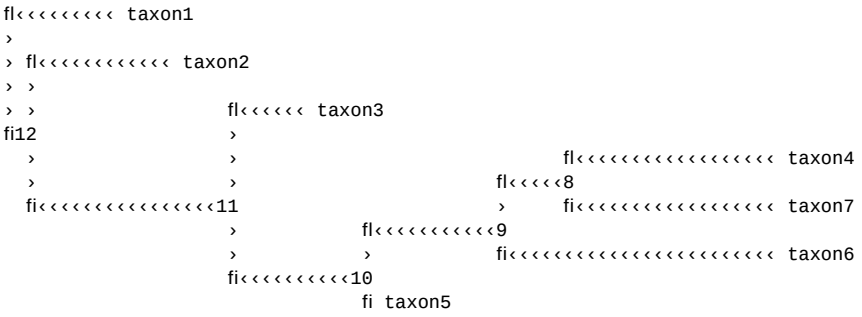
To ouput diagnostic information for a tree or a set of trees, use **DESCRIBE** option or the **Describe Trees** menu command**.** The following options are available for tree description.

"Cladograms" and "Phylograms"

Trees output by the **DESCRIBE** command may be drawn either as cladograms or phylograms. Cladograms convey only branching information; phylograms draw branch lengths proportional to number of inferred changes according to the current reconstruction. This means that cladograms will not change when a different reconstruction is chosen, but phylograms will.

Example of a phylogram, obtained by

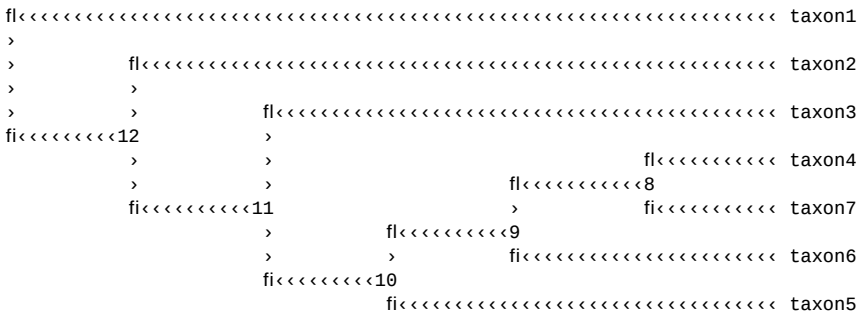
```
describe 1/ plot=phylogram
```



The command

```
describe 1/ plot=cladogram
```

gives a cladogram for the same taxa:



Consistency Indices and Goodness-of-Fit Statistics

Diagnostic information for selected trees is always displayed, even if no diagnostic character information is requested. In addition to displaying how the tree was rooted and which optimization criterion was chosen, the output lists the tree length, consistency index (CI), homoplasy index (HI), CI excluding uninformative characters, HI excluding uninformative characters, retention index (RI), and rescaled RI. See chapter 1 for a detailed discussion of these statistics. Below is sample **DESCRIBE** command with no other options, i.e..

```
describe 1;
```

```
Tree description:

   Unrooted tree(s) rooted using outgroup method
   Character-state optimization: Delayed transformation (DELTRAN)

Tree number 1:

Tree length = 23
Consistency index (CI) = 0.696
Homoplasy index (HI) = 0.304
CI excluding uninformative characters = 0.667
HI excluding uninformative characters = 0.333
Retention index (RI) = 0.562
Rescaled consistency index (RC) = 0.391
```

Table of Branch Lengths and Linkages

When a **Table of linkages** in the **Describe Trees** dialog box or the LINKS option is specified, PAUP outputs a table which shows, for each terminal taxon and each node, which node it is linked to, the assigned length of the branch under the current reconstruction, and the maximum and minimum possible lengths of that branch. over all MPRs (see Swofford and Maddison, 1987). The command format would be

```
describe 1/links;
```

Below is the format of the output. The terminal taxa are listed first, followed by the internal nodes.

```
Branch lengths and linkages for tree #5 (unrooted)

                                 Assigned       Minimum       Maximum
                  Connected        branch      possible      possible
   Node            to node        length        length        length
taxon1 (1)            12           2.000         2.000         4.000
taxon2 (2)            12           2.000         0.000         2.000
taxon3 (3)            11           1.000         0.000         1.000
taxon4 (4)             8           3.000         2.000         4.000
taxon5 (5)            10           0.000         0.000         1.000
taxon6 (6)             9           4.000         1.000         4.000
taxon7 (7)             8           3.000         2.000         4.000
     8                 9           1.000         1.000         2.000
     9                10           2.000         2.000         4.000
    10                11           2.000         2.000         3.000
    11                12           3.000         2.000         3.000
```

Change and Apomorphy Lists

There are two ways of summarizing the character changes that have taken place on a particular tree. The first is to describe the changes for each character, the second for each branch. If **List of changes** is selected in the **Describe Trees** dialog box or the command `DESCRIBE CHGLIST` is issued, the output will show for each character, where on the tree that character changed, the state it changed from, and the state it changed to. If list of apomorphies is selected in the **Describe Trees...** dialog box or DESCRIBE APOLIST is selected, the output will show *for each branch*, which characters changed, the state they changed from, and the state they changed to.

The following command will give character changes:

```
describe 1/chglist;
```

with the following output:

```
Character change lists:

 Character         CI     Steps          Changes

 1               1.000      1    node_12 0 ==> 1 node_11
 2               0.500      1    node_12 1 <=> 0 taxon1
                            1     node_8 1 ==> 0 taxon7
 3               0.500      1    node_12 0 ==> 1 node_11
                            1     node_8 1 ==> 0 taxon6
 4               1.000      1    node_11 0 ==> 1 node_10
 5               1.000      1    node_10 0 ==> 1 node_9
 6               0.500      1    node_10 0 ==> 1 taxon4
                            1     node_8 0 ==> 1 taxon7
 7               0.333      1    node_12 0 <-> 1 taxon1
                            1    node_11 0 --> 1 node_10
                            1     node_8 1 ==> 0 taxon6
 8               0.500      1    node_10 0 ==> 1 taxon4
                            1     node_8 0 ==> 1 taxon6
 9               0.667      1    node_12 0 ==> 2 taxon2
                            1     node_9 0 ==> 1 taxon4
                            1     node_8 0 ==> 2 taxon7
 10              1.000      1    node_11 0 ==> 2 node_10
                            1     node_9 2 ==> 1 node_8
```

You can see the arrows are not all the same type. This is because PAUP uses different arrows to indicate different types of change. A double-lined arrow means that the change occurs in all possible reconstructions (i.e. is unambiguous). A single-lined arrow indicates that change occurs under some reconstructions but not others. A double-headed arrow indicates that the *direction* of change is undetermined, that is the change occurs along the branch connecting the outgroup to the ingroup. A single-headed arrow indicates that the direction of change is unambiguously within the ingroup. In the example above all four types of arrow are present. Remember that these arrows are a reflection of the tree and optimization criterion. Character changes with double-lined arrows will not be affected by a different optimization scheme, while those with single-lined arrows will. This is also true of the ouput listing the apomorphies for each branch, as below. Note that multistate characters have a CI of 1.000 when each state is derived only once.

To get the list of apomorphies for each branch, type:

```
describe 1/apolist;
```

This gives the following output:

```
Apomorphy lists:

                Branch                Character     Steps    CI  Change

          taxon1 <-> node_12          2                 1  0.500 0 <=> 1
                                      7                 1  0.333 1 <-> 0
                                      9                 1  1.000 0 <=> 1
                                      12                1  1.000 0 <-> 1
         node_12 --> node_11          1                 1  1.000 0 ==> 1
                                      3                 1  0.500 0 ==> 1
                                      12                1  1.000 1 --> 2
         node_11 --> node_10          4                 1  1.000 0 ==> 1
                                      7                 1  0.333 0 --> 1
         node_10 --> taxon4           6                 1  0.500 0 ==> 1
                                      8                 1  0.500 0 ==> 1
                                      12                1  1.000 2 ==> 4
         node_10 --> node_9           5                 1  1.000 0 ==> 1
          node_9 --> node_8           11                1  1.000 0 ==> 1
                                      12                1  1.000 2 ==> 3
          node_8 --> taxon6           3                 1  0.500 1 ==> 0
                                      7                 1  0.333 1 ==> 0
                                      8                 1  0.500 0 ==> 1
          node_8 --> taxon7           2                 1  0.500 1 ==> 0
                                      6                 1  0.500 0 --> 1
                                      10                1  1.000 1 --> 0
```

Character Diagnostics

In addition to information about where the characters change on a particular tree, PAUP will display summary diagnostic information for each character. This is obtained by selecting **Character diagnostics** in the **Describe Trees** dialog box or the **DESCRIBE** command with the DIAG option. The output includes the minimum possible length for each character (i.e. the length if a minimal tree were computed for each character taken separately); the maximum possible length (i.e. the length on a completely unresolved bush); the length required on the tree being described; and four goodness-of-fit statistics: the unit consistency index (CI); homoplasy index (HI); retention index (RI); and the rescaled consistency index (RC). This output is obtained by the following command:

```
describe 1/diag;
```

and has the following format:

```
Character diagnostics:

                Minimum     Tree  Maximum
Character         Steps    Steps    Steps        CI      HI      RI      RC

 1                    1        1        2     1.000   0.000   1.000   1.000
 2                    1        2        3     0.500   0.500   0.500   0.250
 3                    1        2        3     0.500   0.500   0.500   0.250
 4                    1        1        3     1.000   0.000   1.000   1.000
 5                    1        1        4     1.000   0.000   1.000   1.000
 6                    1        2        3     0.500   0.500   0.500   0.250
 7                    1        2        4     0.500   0.500   0.667   0.333
 8                    1        2        2     0.500   0.500   0.000   0.000
 9                    1        1        1     1.000   0.000     0/0     0/0
 10                   1        1        2     1.000   0.000   1.000   1.000
 11                   1        2        3     0.500   0.500   0.500   0.250
 12                 n/a        0        0       0/0     0/0     0/0     0/0
 13                 n/a        0        0       0/0     0/0     0/0     0/0
 14                   1        2        2     0.500   0.500   0.000   0.000
 15                   1        2        2     0.500   0.500   0.000   0.000
 16                   1        2        2     0.500   0.500   0.000   0.000
```

Note that these minimum and maximum lengths are not the same as those generated by the table of branch lengths and linkages, which only consider MPRs. The retention index is undefined for uninformative (autapomorphic) characters - this is indicated in the matrix by 0/0. The consistency index is not calculated for stepmatrix characters, due to the difficulty in estimating the minimum amount of change for those types of characters.

Character-State Reconstructions

PAUP will also display reconstructed character states for the internal nodes of a tree, according to the optimization criterion currently in effect. It will also display the possible reconstructions over all MPRs. In this way you can compare the reconstructions required by the current tree and optimization with the states possible over all most parsimonious reconstructions.

There are several ways to obtain this information: assigned character changes are displayed superimposed on the current tree(s) when CHGPLOT or character changes selection in the **Describe Trees** dialog box is selected. Selected reconstructions will be output with a tree for each character. States for the terminal taxa are also displayed.

> **NOTE:** If no characters are specified for either a CHGPLOT or POSSPLOT command, the characters are taken to be those plotted in the last invocation of either of these commands. For example, CHGPLOT 1 3 5 7; POSSPLOT will cause both commands to output information for characters 1, 3, 5, and 7.

The trees below are the reconstruction of a single character using the command

```
describe 1/ chgplot;
```

The first tree optimized the character using ACCTRAN; the second used DELTRAN. You can see how different the two hypotheses are for character 1. The ACCTRAN hypothesis proposes an origin at the node leading to taxa 4,5,6, and 7, with a subsequent loss in taxon 5. The DELTRAN hypothesis prefers two independent origins of character 1: once in taxon 4 and once in taxon 7.

```
Changes in character 1 on tree 1:

fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon1
›          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon2
fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon3
           fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 1 taxon4
                      ,,,,,,,,,,,1          ,,,,,,,,,,,,,,,,,,,,,, 0 taxon5
                                 fi‹‹‹‹‹‹‹‹‹‹1          fl‹‹‹‹‹‹‹‹‹‹‹ 1 taxon6
                                            fi‹‹‹‹‹‹‹‹‹‹1‹‹‹‹‹‹‹‹‹‹‹ 1 taxon7
```

```
Changes in character 1 on tree 1:

fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon1
›          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon2
fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon3
           fi‹‹‹‹‹‹‹‹‹‹0          ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, 1 taxon4
                      fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon5
                                 fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹ 1 taxon6
                                            ,,,,,,,,,,,1‹‹‹‹‹‹‹‹‹‹‹ 1 taxon7
```

You can also display one tree summarizing the possible states for a given character. This is done using the POSSPLOT option of **DESCRIBE** or **Possible state assignments** item in the **Describe Trees** dialog box. A typical command would be:

```
describe 1/ possplot;
```

with the following output:

```
Possible state assignments for character 1 on tree 1:
fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon1
›          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon2
fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon3
           fi‹‹‹‹‹‹‹‹‹‹0          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 1 taxon4
                      fi‹‹‹‹‹‹‹‹‹‹?          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ 0 taxon5
                                 fi‹‹‹‹‹‹‹‹‹‹?          fl‹‹‹‹‹‹‹‹‹‹‹ 1 taxon6
                                            fi‹‹‹‹‹‹‹‹‹‹1‹‹‹‹‹‹‹‹‹‹‹ 1 taxon7
```

The two question marks indicate that the assignment of character 1 at those nodes is ambiguous - they do not indicate that the state is missing (default meaning of "?" in a data matrix). Reconstruction of multistate characters may be ambiguous, or two or more states may be possible at a node, as below

```
Possible state assignments for character 3 on tree 2:

fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<< 0 taxon1
›          fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<< 0 taxon2
fi<<<<<<<<<<0          fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<< 2 taxon3
           fi<<<<<<<<<02          fl<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<< 2 taxon4
                      fi<<<<<<<<<02          fl<<<<<<<<<<<<<<<<<<<<<<<<<<<< 0 taxon5
                                 fi<<<<<<<<<<?          fl<<<<<<<<<<<< 1 taxon6
                                            fi<<<<<<<<<<1<<<<<<<<<<<< 1 taxon7
```

You can also see the assigned and possible reconstructions by choosing states for interior nodes and possible state assignments selection in the **Describe Trees** dialog box or by using the XOUT option for **DESCRIBE**. The output describing the possible state assignments is a matrix of nodes by characters, as below. The possible states for each character is displayed in a row for each internal node on the tree. The output for the actual assignments for a given tree and optimization is also in matrix format. Requesting states for terminal taxa with the following command

```
describe 1/xout=both
```

results in the printing of the input data matrix as well as the matrix of assigned states, as below.

```
Data matrix and reconstructed states for internal nodes

                    1
Node        1234567890
---------------------
taxon1      0000001001
taxon2      0100000011
taxon3      1110000011
taxon4      1111100011
taxon5      1101100111
taxon6      1111011111
taxon7      1011111010
8           1111111011
9           1111100?11
10          111110?011
11          1110000011
12          0100000011
```

Once again, the question mark indicates that each available character state appears at that node in at least one reconstruction. It does not mean that any character is considered "missing" at that point.

Stepmatrix Character Reconstruction: Special Considerations

When a stepmatrix contains three or more character states, it is possible that full minimization of the tree length may require assignments of character states that were not observed in any of the terminal taxa to internal nodes. When only a few character states were observed for a stepmatrix character, but the stepmatrix is defined for a large number of character states. This will often be the case for protein-sequences analyzed in a manner similar to the PROTPARS program for analyzing

amino-acid sequences in PHYLIP. There, amino-acid sequences are analyzed using a stepmatrix specifying the cost of changing from one amino-acid to another (see the PROTPARS sample data matrix). However, not all amino-acids are always present in the terminal taxa. When this happens, execution in PAUP is slowed significantly if all character-states are allowed as candidates for assignment to internal nodes.

PAUP provides three options for dealing with this problem. First, you may choose to restrict the states for internal nodes only to those observed in terminal taxa. This is done using the STEPMATRIX=OBSONLY option of the **SET** command or selecting the appropriate box in the **Stepmatrices** dialog box**.** Second, you may choose to allow any state contained in the stepmatrix definition to be assigned to internal nodes regardless of whether it was observed in a terminal taxon. This is done using the STEPMATRIX=ALLSTATES option of the **SET** command or the **Stepmatrices** menu command. For example, the following might be issued either in a PAUP block or on the command line:

```
set stepmatrix=allstates;
```

The third option provides a compromise between these two extremes. It uses the "3+1" rule Applying the rule independently to each character, all triplets of *observed* character states are examined, in turn. For each triplet, each member of the triplet is assigned to one of the terminal nodes of a 3-branch tree. Then, every character-state contained in the stepmatrix definition is assigned, in turn, to the single internal node and the length required for the character, given these state assignments, is computed. If assignment of an unobserved character state to the internal node yields a length less than or equal to that obtained from one of the three members of the triplet, this unobserved state is added to the set of candidate states. This would be achieved by the command

```
set stepmatrix=threeplus1
```

How does the "3+1" rule work in practice? Take the following stepmatrix for amino-acids as an example (this is taken directly from the PROTPARS example included with the PAUP program - only the stepmatrix is reproduced here). Each of the symbols designates either one or more amino acids, a stop codon (*), or a deletion (-) corresponding to the standard IUB single-letter amino acid codes (see the section "Predefined formats for molecular sequence data"**).**

```
     A C D E F G H I K L M N P Q R 1 2 T V W Y * -
[A]  0 2 1 1 2 1 2 2 2 2 2 2 1 2 2 1 2 1 1 2 2 2 3
[C]  2 0 2 2 1 1 2 2 2 2 2 2 2 2 1 1 1 2 2 1 1 1 3
[D]  1 2 0 1 2 1 1 2 2 2 2 1 2 2 2 2 2 2 1 2 1 2 3
[E]  1 2 1 0 2 1 2 2 1 2 2 2 2 1 2 2 2 2 1 2 2 1 3
[F]  2 1 2 2 0 2 2 1 2 1 2 2 2 2 2 1 2 2 1 2 1 2 3
[G]  1 1 1 1 2 0 2 2 2 2 2 2 2 2 1 2 1 2 1 1 2 1 3
[H]  2 2 1 2 2 2 0 2 2 1 2 1 1 1 1 2 2 2 2 2 1 2 3
[I]  2 2 2 2 1 2 2 0 1 1 1 1 2 2 1 2 1 1 1 2 2 2 3
[K]  2 2 2 1 2 2 2 1 0 2 1 1 2 1 1 2 2 1 2 2 2 1 3
[L]  2 2 2 2 1 2 1 1 2 0 1 2 1 1 1 1 2 2 1 1 2 1 3
[M]  2 2 2 2 2 2 2 1 1 1 0 2 2 2 1 2 2 1 1 2 3 2 3
[N]  2 2 1 2 2 2 1 1 1 2 2 0 2 2 2 2 1 1 2 3 1 2 3
[P]  1 2 2 2 2 2 1 2 2 1 2 2 0 1 1 1 2 1 2 2 2 2 3
[Q]  2 2 2 1 2 2 1 2 1 1 2 2 1 0 1 2 2 2 2 2 2 1 3
[R]  2 1 2 2 2 1 1 1 1 1 1 2 1 1 0 2 1 1 2 1 2 1 3
[1]  1 1 2 2 1 2 2 2 2 1 2 2 1 2 2 0 2 1 2 1 1 1 3
[2]  2 1 2 2 2 1 2 1 2 2 2 1 2 2 1 2 0 1 2 2 2 2 3
[T]  1 2 2 2 2 2 2 1 1 2 1 1 1 2 1 1 1 0 2 2 2 2 3
[V]  1 2 1 1 1 1 2 1 2 1 1 2 2 2 2 2 2 2 0 2 2 2 3
[W]  2 1 2 2 2 1 2 2 2 1 2 3 2 2 1 1 2 2 2 0 2 1 3
[Y]  2 1 1 2 1 2 1 2 2 2 3 1 2 2 2 1 2 2 2 2 0 1 3
[*]  2 1 2 1 2 1 2 2 1 1 2 2 2 1 1 1 2 2 2 1 1 0 3
[-]  3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 0
```

Stepmatrix for amino acid substitutions.

The stepmatrix gives the minimum number of amino acid replacement substitutions needed to convert one amino acid to another, based on the genetic code used in nuclear genes of most organisms and chloroplast genes in plants. The character distribution in the five taxa is:

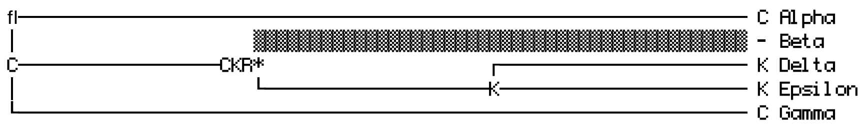
```
   matrix
Alpha   ABCDEFGHIK
Beta    AB--EFGHIK
Gamma   ?BCDSFG*??
Delta   CIKDEFGHIK
Epsilon DIKDEFGHIK
```

where "?" is a missing observation and "-" is a deletion. Reconstructing the third position on one of the unrooted trees gives the following when only the observed states are allowed at internal nodes. Both "C" (cys) and "K" (lys) are possible assignments at the internal node:

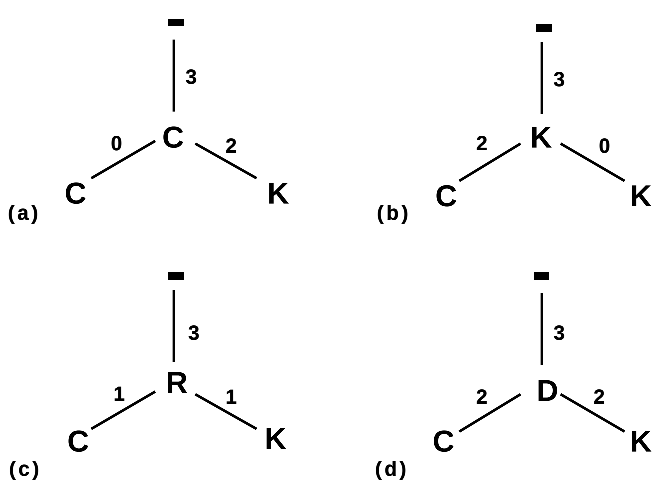
```
Possible state assignments for character 3 on tree 1:

fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ C Alpha
›                     ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, - Beta
C‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹CK                  fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ K Delta
›                     fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹K‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ K Epsilon
fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ C Gamma
```

Reconstructing internal nodes gives a different result when states that satisfy the "3+1" rule are allowed:

```
Possible state assignments for character 3 on tree 1:
```



Why are the additional states "R" (arg) and "*" (stop codon) allowed where only "C" (cys) and "K" (lys) were allowed in the previous example? The "3+1" rule takes all possible triplets of observed states and evaluates the length when every state in the stepmatrix is placed at the single internal node. Thus there is a "3" component composed of triplets of observed states, and a "1" component composed of each of the states in the stepmatrix. The figure below shows the results of the "3+1" rule when various are assigned as the "1" component. Since in this example there are only three observed character states, there is only one triplet ("C", "K", and "-") to make up the "3" component. To begin, we measure the length when the members of the triplet (the observed states) are placed at the internal node. This is shown in *a* and *b*, where the overall length is 5 (the length to any state from the "-", or missing state, is always 3). Thus for any other state to *pass* the "3+1" rule, the length of the tree must be less than or equal to 5, which is the length obtained when only *observed* states are allowed. If the calculated length from the stepmatrix is more than 5, the state in question fails the "3+1" rule, and cannot be assigned to the interior node. In *c*, a state is determined to pass the rule (length=5, equal to that obtained by using the observed states) that is *not* one of the observed states. The length of tree *d* is 7, so state "D" fails the test and cannot be a candidate for assignment to the interior node.

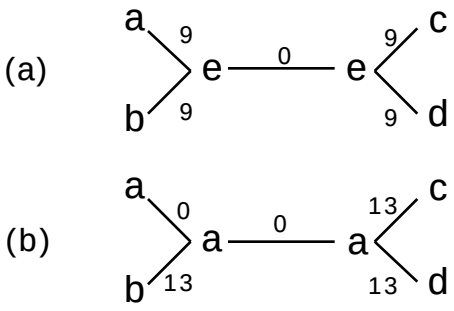


Example of the "3+1" rule. (a) and (b) three-taxon trees with observed states at node, length=5. (c) non-observed state that satisfies the rule with length=5. (d) state which does not pass the rule, length=7.

For real data sets, I have never discovered a case for which STEPMATRIX=THREEPLUS1 fails to obtain an optimal reconstruction as long as all triplets of states satisfy the triangle inequality. However, hypothetical counterexamples have been constructed (Wayne Maddison, personal communication). For example, the 3+1 rule excludes a state required to obtain an optimal reconstruction for the following stepmatrix.

```
      a  b  c  d e
a     - 13 13 13 9
b     13 - 13 13 9
c     13 13 - 13 9
d     13 13 13 - 9
e     9  9  9  9 -
```

If the only observed states are a, b, c, and d, the 3+1 rule will not allow state "e" to be reconstructed at internal nodes. The cost of assigning any of the observed states will always be 26, while the cost of assigning e will always be 27, so the 3+1 rule excludes it as a candidate for internal nodes. However, for the following four-taxon tree, state e is actually the best state for internal nodes, even though it fails the 3+1 test. Assigning e gives a tree-length of 36 (=4 x 9), while assigning any other state gives a tree-length of 39 (3 x 13 plus 1 x 0).



Example of failure of 3+1 rule. (a) optimal reconstruction with length 36 not found by 3+1 rule. (b) reconstruction of length 39 allowed by 3+1 rule.

Of course this example is derived from a fairly unusual stepmatrix. In reality the 3+1 rule will choose states that allow optimal reconstructions most of the time, and the speedup in searches can be enormous because fewer states need be considered.

The Pairwise Homoplasy and Patristic Distance Matrices

If you want to display a summary of homoplasy and patristic distances between taxa (where patristic distances (P) = sum of branch lengths on path between each pair of taxa; D = observed character difference; and the

homoplasy (H) = P - D), check the **Patristic distance matrix** and/or **Homoplasy matrix** items in the **Describe Trees** dialog box or use the HOMOPLASY or PATRISTIC option of the **DESCRIBE** command. For example, the command

```
describe 1/patristic homoplasy;
```

will output patristic distance and homoplasy matrices for the first tree:

```
Note: Multistate unordered and/or stepmatrix characters
      excluded from patristic distance calculations.

Patristic distance matrix

   Below diagonal: Adjusted character distances
   Above diagonal: Patristic distances

                  1         2         3         4         5
  1 taxon1        -         3         6         8         9
  2 taxon2        3         -         5         7         8
  3 taxon3        6         5         -         6         7
  4 taxon4        8         5         4         -         5
  5 taxon5        7         8         5         5         -

Pairwise homoplasy matrix

                  1         2         3         4         5
  1 taxon1        -
  2 taxon2        0         -
  3 taxon3        0         0         -
  4 taxon4        0         2         2         -
  5 taxon5        2         0         2         0         -
```

Stepmatrix characters and unordered multistate characters are not included in patristic distance calculations (and by extension, homoplasy matrices).

Although it is probably not a limitation in practice, you must choose costs and weights for stepmatrix characters such that the total length of the longest possible tree (a completely unresolved bush) is less than $(2^{31})-1$ = 2,147,483,647.

LENGTHS AND FIT MEASURES

Character information can be output in slightly different format by selecting **LENFIT** or the **Lengths and Fit Measures** menu command. Typically, these options are used to display the lengths and fit statistics of trees in memory, but also to display summary information about characters relative to trees in memory. You can choose to output information about all characters (SINGLE=ALL) or only those which vary over trees (SINGLE=VAR). This is a very good way to get a summary of the relative fit of various characters on different trees. You can choose to output any combination of the length, consistency index, retention index,

or rescaled consistency index of characters. The output will include the value for each character over each chosen tree, as well as an entry for the best and worst value for that character. The following command

```
lenfit 1-5/total single=all ci;
```

will, for trees one through five, output tree lengths and consistency indices for all characters (single=all), overall tree-lengths and ensemble consistency indices (total). If you wish only to output the range of minimum and maximum tree lengths (or best and worst fit measures) for each character, use the RANGE option.

For example, the command

```
lenfit/single=all total ci notl;
```

requests consistency indices (and suppression of tree lengths) for all characters as well as ensemble consistency indices:

```
   Sum of min. possible lengths = 14
   Sum of max. possible lengths = 36

Tree #        1       2       3       4       5       6
-------------------------------------------------------
CI        0.609   0.609   0.609   0.609   0.609   0.609

Consistency indices for each character:

Tree           1       2       3       4       5       6       7       8
-------------------------------------------------------------------------
   1       1.000   0.500   0.500   1.000   1.000   0.500   0.500   0.500
   2       1.000   0.500   0.500   1.000   0.500   1.000   0.333   0.500
   3       0.500   0.500   0.333   0.500   1.000   0.500   0.500   0.500
   4       1.000   0.500   0.500   1.000   0.500   1.000   0.333   0.500
   5       1.000   0.500   0.500   1.000   0.500   0.500   0.500   1.000
   6       1.000   0.500   0.500   1.000   0.500   1.000   0.333   0.500
Best       1.000   0.500   0.500   1.000   1.000   1.000   0.500   1.000
Worst      0.500   0.500   0.333   0.500   0.500   0.500   0.333   0.500

Tree           9      10      11      14      15      16
-------------------------------------------------------
   1       1.000   1.000   0.500   0.500   0.500   0.500
   2       1.000   1.000   1.000   0.500   0.500   0.500
   3       1.000   1.000   0.500   1.000   1.000   1.000
   4       1.000   1.000   1.000   0.500   0.500   0.500
   5       1.000   1.000   0.500   0.500   0.500   0.500
   6       1.000   1.000   1.000   0.500   0.500   0.500
Best       1.000   1.000   1.000   1.000   1.000   1.000
Worst      1.000   1.000   0.500   0.500   0.500   0.500
```

If there are many characters in the data matrix, the most efficient use of this option is probably to limit output only to characters which vary over trees in memory (i.e., using the SINGLE=VAR option). That way you don't waste a lot of space describing characters which do not change over trees. The output for **LENFIT** does not give the detail available from a **DESCRIBE** command, in that it does not tell you *where* on the tree a particular character changed, only the *amount* of change that had to be invoked to explain that character on each tree. The advantage of using

LENFIT is that you can quickly see which characters or sets of characters are consistent with each tree. This can be very interesting if there are both minimal and non-minimal trees in memory. It may be that some characters are consistent only with topologies that are not found on minimal trees.

NOTE: Minimum-possible tree lengths are not easily determined when multistate polymorphic taxa are present and the character type is Dollo. Consequently, character-fit measures are not evaluated in that case.

MANIPULATING TREES

Rooting Trees for Output and Character Diagnosis

The trees found by PAUP searches are unrooted (unless there is some reason to explicitly root them in memory - see "Outgroups, Ancestors, and Roots" in Chapter 1). These trees must be ouput from the tree buffer as rooted trees for character reconstructions and other analyses. There are three rooting options - outgroup; Lundberg; and midpoint. Outgroup rooting, which includes an assumed outgroup in the analysis, is the most common method.

NOTE: Remember that if you specify outgroup rooting but do not specify an outgroup, PAUP will take the first taxon in the matrix as the default outgroup.

Outgroup rooting is achieved by using the ROOT=OUTGROUP option, and perhaps specifying an OUTROOT option as well. The different outgroup rooting options are also available from the **Rooting** menu command. For example, the following command would describe a tree and root it such that the outgroup is monophyletic relative to the ingroup:

```
describe 1/ root=outgroup outroot=monophyl;
```

Other options for outgroup rooting output the outgroup in a basal polytomy (OUTROOT=POLYTOMY) or as a group paraphyletic to the ingroup (OUTROOT=PARAPHYL). Remember that if the tree cannot be rooted such the specified ingroup is monophyletic, PAUP will include as many outgroup taxa as possible with the ingroup to form a monophyletic group (though the primary outgroup is never included). Lundberg rooting involves computing an unrooted tree for the ingroup taxa only, and then attaching an outgroup taxon (or hypothesized ancestor) to the tree *a posteriori* (Lundberg, 1972). This is achieved by using either the ROOT=LUNDBERG option or the appropriate selection in the **Rooting** dialog box. The tree can be output with Lundberg rooting with a command like the following:

```
describe 1/root=lundberg;
```

Lundberg rooting forces the use of MINF optimization (the only available optimization that is independent of the location of the root), which means that it may be invoked only when ordered (Wagner) or unordered characters are in use. Other character types (Dollo, irreversible, stepmatrix) cannot be used with MINF optimization; hence are not allowed. It is possible that a particular ancestor may have multiple equal-length attachment sites, which leads to the following output:

```
Note: Ties found in Lundberg rooting.  The following root
      locations are equally parsimonious:

      9 <--> 10
      14 <--> taxonA

Tree length = 23
Consistency index (CI) = 0.609
Homoplasy index (HI) = 0.391
Retention index (RI) = 0.591
Rescaled consistency index (RC) = 0.360

                                                        fl‹‹‹‹‹‹‹‹‹‹‹ taxonA
                                              fl‹‹‹‹‹‹‹‹‹14‹‹‹‹‹‹‹‹‹‹‹ taxonB
                                    fl‹‹‹‹‹‹‹‹‹13‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonC
                          fl‹‹‹‹‹‹‹‹‹12‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonF
               fl‹‹‹‹‹‹‹‹‹11‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonE
fl‹‹‹‹‹‹‹‹‹10‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonD
›                                                       fl‹‹‹‹‹‹‹‹‹‹‹ taxonG
fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹9‹‹‹‹‹‹‹‹‹‹‹ taxonH
```

You can see that PAUP only prints *one* of the trees, but you may wish to recover all equally parsimonious trees for further analysis. To recover all the multiple roots for a optimal trees, you must include an "ancestor" taxon in the matrix with the same states as were in the original ANCSTATES definition (but do not change the ANCSTATES settings). Then, exclude the ancestor and calculate all optimal trees for the ingroup. Next, save the trees to a file. Restore the ancestor. Load the saved trees as backbone constraints. Now perform a search while enforcing each constraint tree in turn and save all trees compatible with the backbone. Output them using Lundberg rooting—since the ANCSTATES and ancestor are identical, you will be able to see each of the places the tree can be rooted. If there is only one possible rooting, a single tree will result from this process. Below are the two trees that are recovered for the example above. The first tree is identical to the tree displayed above.

```
Tree number 1:

                                                          ﬂ‹‹‹‹‹‹‹‹‹ taxonA
                                                ﬂ‹‹‹‹‹‹‹‹16‹‹‹‹‹‹‹‹‹ taxonB
                                       ﬂ‹‹‹‹‹‹‹15‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonC
                            ﬂ‹‹‹‹‹‹‹‹14‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonF
                   ﬂ‹‹‹‹‹‹‹13‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonE
         ﬂ‹‹‹‹‹‹‹‹12‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonD
ﬂ‹‹‹‹‹‹‹11                                                ﬂ‹‹‹‹‹‹‹‹‹ taxonG
›        ﬁ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹10‹‹‹‹‹‹‹‹‹ taxonH
ﬁ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ ancestor

Tree number 2:

        ﬂ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonA
        ›       ﬂ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonB
ﬂ‹‹‹‹‹‹16       ›        ﬂ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonC
›       ﬁ‹‹‹‹‹‹15        ›                        ﬂ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonD
›               ﬁ‹‹‹‹‹‹‹14               ﬂ‹‹‹‹‹‹11         ﬂ‹‹‹‹‹‹‹‹ taxonG
›                        ›       ﬂ‹‹‹‹‹‹12        ﬁ‹‹‹‹‹‹‹10‹‹‹‹‹‹‹‹ taxonH
›                        ﬁ‹‹‹‹‹‹13       ﬁ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonE
›                                ﬁ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonF
ﬁ‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ ancestor
```

These trees can then be saved or analyzed further.

The last rooting option, Midpoint rooting, is straightforward: the tree is rooted at the midpoint of the longest path connecting any pair of taxa. The following command will output a tree with midpoint rooting:

```
describe 1/root=midpoint;
```

Essentially, this is a way to output a tree without explicitly rooting it, and should only be used as a last resort. Use of this option forces the use of MINF optimization, which is the only optimization method that does not depend on the position of the root.

Saving Trees to Files

If the search required to find a given set of trees uses a significant amount of computing time, saving the descriptions of these trees to a "tree file" may be desirable for several reasons. First, you may need the trees at a subsequent time (e.g., to obtain additional output information about the trees, to plot the trees in a different way, or to use the trees as input for a subsequent search) after you have terminated the PAUP session. If you have saved the trees to a file, you can then recover the trees quickly without having to repeat the lengthy search. Second, the tree file can be input to other programs that read user-defined trees. Since the trees are written to the file as a NEXUS TREES block, any NEXUS-conforming program that accepts user-defined trees (e.g., MacClade) will be able to read the tree file. Other programs that follow the standard format for tree description described in the section Describing Trees (e.g., PHYLIP, PLOTGRAM) will be able to interpret the tree file after minor editing. \ (Note that PAUP's export facilities, described in the section "Importing

and Exporting Trees and Data" provide a more direct means of inputting trees found by PAUP to PHYLIP and Hennig86). Third, data files and tree files are standard text files that are portable across computers. For large and/or complex data sets, you may want to perform the searches on a larger, more powerful, computer, and then download the trees to a microcomputer for further analysis, graphics output, etc.

You can use the **SAVETREES** command or the **Save Trees to File** menu command to save trees to a file (the default treefile name is "*data-file-name*.trees"). You may save either all of the trees in memory or a range of trees that you specify. For example, the command

```
savetrees from=1 to=10 file=trees.out brlen ttable;
```

will save trees one through ten to the file "trees.out" along with branch-lengths and a translation table that maps positive integers to taxon names. In essence, this creates nothing more than a TREES block contained in a single file. Additionally, trees may be saved rooted (or unrooted) although this may slow down the saving process. For example

```
savetrees from=1 to=10 root file=trees.out replace;
```

will replace the contents of the file "trees.out" with rooted trees one through ten.

PAUP also includes information about the analysis that generated the trees, should you need to replicate the search. Here is a sample treefile resulting from a branch-and-bound search:

```
#NEXUS

begin trees;  [Treefile saved Tuesday, February 16, 1993  10:18 AM]
[!>Branch-and-bound search settings:
>   Initial upper bound: unknown (compute via stepwise)
>   Addition sequence: furthest
>   Initial MAXTREES setting = 100
>   Branches having maximum length zero collapsed to yield polytomies
>   Topological constraints not enforced
>   Trees are unrooted
>   Shortest tree found = 23
>   Number of trees retained = 5
]
utree PAUP_1 = (taxonA,(taxonB,(taxonC,(((taxonD,(taxonG,taxonH)),taxonE),taxonF))));
utree PAUP_2 = (taxonA,(taxonB,(taxonC,((taxonD,(taxonG,taxonH)),(taxonE,taxonF)))));
utree PAUP_3 = (taxonA,(taxonB,(taxonC,(taxonD,(taxonE,(taxonF,(taxonG,taxonH)))))));
utree PAUP_4 = (taxonA,(taxonB,(taxonC,((taxonD,(taxonF,(taxonG,taxonH))),taxonE))));
utree PAUP_5 = (taxonA,(taxonB,(taxonC,((taxonD,taxonE),(taxonF,(taxonG,taxonH))))));
endblock;
```

Recovering Trees from Files

You can also retrieve trees from a file, using the **GETTREES** command or **Get Trees from File** menu command. One or more of these imported trees may then be used as starting trees for searches, or the current data

matrix may be optimized onto imported trees. PAUP gives you the option of importing some or all of the trees in a particular treefile, or only those which meet certain conditions. This last category is available through Boolean operations.

When **GETTREES** or **Get Trees from File** is invoked, the default is to import all the trees in a particular treefile, replacing all trees in memory. For example

```
gettrees file=trees.out
```

will import all of the trees in the file "trees.out." However you don't have to import all of the trees in the file - you can also specify the tree numbers, if you know which number corresponds to which topology. For example,

```
gettrees file=trees.out from=1 to=5;
```

will replace any trees in memory with trees one through five in the file "trees.out." These trees may be the products of a previous search of the current data matrix, they may be derived from a different data matrix for the same taxa, or they may have been created by a program such as MacClade. You can then test how certain starting trees affect the search, and explore how the characters are reconstructed on different trees.

There is also a way to import trees without knowing which tree number corresponds to which topology. PAUP has the option of performing Boolean operations on tree files using the MODE option or by making the appropriate selection in the **Get Trees From File** dialog box. You can choose these operations with the following command:

```
gettrees file=trees.out mode=n;
```

where n is an integer taken from the MODE value for one of the choices below:

keep trees which are in the file, but *not* originally in memory - this means you will import only trees which are *different* from those currently in memory (MODE=1)

keep trees in the file that are also currently in memory - this will replace trees in memory with those trees in the treefile which are the *same* as those in memory (MODE=2)

replace all trees in memory by all trees in the file - this is the default (MODE=3)

keep trees in memory that are *not* also in the treefile - this excludes trees in memory that are also found in the treefile (MODE=4)

keep trees that are currently in memory or the file, but *not* in both places - this excludes trees which are shared between memory and the treefile (MODE=5)

add trees in the file to the trees in memory - all trees in the treefile are added, without duplication (MODE=7)

PAUP will echo the Boolean selection and display a summary of how many trees in memory and/or the file have been kept. For example, adding all trees in the file to the trees currently in memory gives this output:

```
Processing TREES block from file 'manual.trees':
   Keeping: trees in memory plus trees in file (without duplication)
   6 trees originally in memory
   11 trees read from file
   5 trees from file kept
   11 trees now in memory
```

A **GETTREES** command specifies either the range of trees to be imported or the boolean MODE. Setting the MODE to different values invokes the different boolean operations described above.

IMPORTANT: Getting trees using MODE=2 can be dangerous! If no trees in memory match trees in file, all trees in memory will be lost. You may want to save trees to a file before getting trees with this option.

Different tree-number options and boolean operations are selected in the **Get Trees from File** dialog box by manipulating two partially intersecting circles, one representing trees in memory, one representing trees in the file.

Manipulating trees in this way gives you the ability to compare *sets* of trees, such as those derived from different data sets, different search options, or different assumptions about characters (e.g. weight). One you have imported and kept the trees you want, you can then save them to a different treefile. This allows you to have different sets of trees in different treefiles, and to extract different subsets of trees from each of those different treefiles.

Trees may be imported either rooted or unrooted. You *cannot* have both types in memory at the same time. If you attempt to retain one type in memory and import another type, PAUP will warn you that rooted and unrooted trees cannot be mixed. Also, if characters require a rooted tree (such as Dollo and irreversible characters) and you import unrooted trees, PAUP will warn you when you attempt to reconstruct characters on those trees. In that case you must either root the trees or change character types.
.

See "Outgroups, Ancestors, and Roots" for a discussion of character types and rooted/unrooted trees.

You can also use the Import File command to get trees created by other programs, such as Hennig86 or PHYLIP. Once these have been imported into PAUP, they can either be stored as NEXUS files, or exported to the original or a different format.

Comparing Trees

Once trees are in memory, either as the result of a search or from the reading of a treefile, they can be compared using the **TREEDIST** command or the **Tree-to-Tree Distances** menu command. There are no options. This calculates the symmetric-difference or "partition" metric (Penny and Hendy, 1985) which is equivalent to the Robinson and Foulds' (1981) contraction/decontraction metric. Trees that are most similar will have the lowest distance value. The output will appear in a matrix of tree-to-tree distances. For example:

```
Symmetric-difference distances between trees

        1   2   3   4   5   6   7   8   9  10  11
1       -
2       4   -
3       4   8   -
4       4   2   8   -
5       2   4   6   4   -
6       4   2   8   2   4   -
7       4   8   2   8   6   8   -
8       8   8   6   8   8   8   6   -
9       5   7   3   7   7   7   3   3   -
10      6   6   4   8   8   8   4   4   1   -
11      4   8   2   8   6   8   2   4   1   2   -
```

Calculating Consensus Trees

If multiple trees are in memory, you can calculate the consensus for those trees in four ways: Adams, strict, semistrict, and majority-rule. The details of each of the consensus methods are discussed in the section "Consensus Trees." You may calculate one or all of the different consensus types for any given set of rival trees—you do not have to issue repeated requests for each type. Consensus trees are obtained by using the **CONTREE** command or the **Compute Consensus** menu command. Once a consensus tree is calculated, it is *not* stored in the tree buffer. If you wish to use it as a constraint or perform other tree manipulations on it, you must first save it to a treefile using the SAVE option and then load it into memory as you would any other tree. In fact, you may wish to calculate consensus trees for different subsets of trees in memory and store them in separate files for further comparison.

```
contree 1 2 3/strict save file=summary.con;
```

will calculate the strict consensus for trees 1, 2, and 3 and save it in the file "summary.con." You may also specify ADAMS for an Adams consensus, MAJRULE for a majority-rule consensus, or SEMISTRICT for a semistrict consensus. Any or all of these may be specified on the same command line or on the same line of a PAUP block.

Consensus indices will be printed following each tree if the INDICES option is included or they are selected in the **Compute Consensus** dialog box. For example:

```
contree 1 2 3/ strict adams indices file=summary.con;
```

will calculate and store the strict and Adams consensus trees and include consensus indices for both in the file "summary.con." For majority-rule consensus trees, a table of partition or group frequencies can be requested, the percentage cutoff specified, and compatible trees occurring in less than fifty percent of the trees can be included by specifying

```
contree 1 2 3/majrule cutoff=50 dotplot le50;
```

The format of the partition-frequency table is

```
Partitions found in one or more trees and frequency of occurrence:

12345678    Freq
---------------
...*****       3
...***..       3
..******       2
....**..       2
......**       2
```

This output is very useful if there is a very large number of trees in memory. If you are interested in all trees in which a particular group appears, it would be tedious to output all trees to find the ones of interest. A better strategy might be to compute a majority-rule consensus with partition-frequency table output; identify groupings of interest; construct a constraint tree that incorporates those groupings; and finally, filter the trees in memory to output only those trees on which the groupings occur.

Filtering Trees

Trees in memory can be selectively hidden by a process known as "filtering". The analogy is to a physical filter, which has certain characteristics that cause some elements to be retained, leaving a "residue" on the filter itself. PAUP's filters are of this type, with the "residue" being the trees that meet the filtering criteria. You build the filter by specifying its components, for example so that it will keep trees shorter or longer than a given length; within a certain topological distance from a reference tree; or trees compatible with a constraint tree. Unlike a physical filter, though, PAUP's filters can be inverted, that is, the "residue" will be trees that *do*

not meet the filter criteria. Filtering criteria are not cumulative—each time you filter trees the filtering criteria are applied to all undeleted trees in memory.

Filtering is invoked by the command **FILTER** or the **Filter Trees** menu command. For example, filtering trees below a certain length would be accomplished using the following command

```
filter maxlength=50 ;
```

There are several different filtering criteria you may select:

Filtering trees within three partition-metric units from a specified tree (tree 3) would be done by

```
filter sd=3 from=3;
```

Filtering with constraints would be invoked using the command

```
filter constraints=testcon
```

where the constraint-tree "testcon" will be used. This can be either a backbone or monophyly constraint tree. Constraint trees must be defined before they can be invoked, as is the case when searching under constraints. Although several constraint trees may be defined, only one may be invoked in a particular filter.

You also have the option of keeping polytomous trees *only* if there are no other compatible resolutions of them in memory. For example, two resolutions of a trichotomy might receive potential support but the third resolution might have no conceivable character changes supporting it, in which case the zero-length branch would collapse to the trichotomy. In this case, a search will retain trees containing the two resolutions of the trichotomy as well as trees containing the unresolved trichotomy resulting from the third resolution. If you check the **Do not retain a polytomous tree for which a more highly resolved compatible tree exists** item in the **Filter Trees** dialog box or specify the LESSRESOLV option of the **FILTER** command, e.g.:

```
filter lessresolv;
```

only the more highly resolved trees will be retained. Reversing this filter (see below) will keep the polytomous trees in lieu of more highly resolved versions of them.

Filters can be quite complex; several criteria can be applied at the same time, for example:

```
filter maxlength=50 constraint=testcon permdel;
```

would filter all trees below fifty steps that are compatible with the constraint tree "testcon" and permanently delete them.

If you wish to permanently remove trees from memory which do not meet the filtering criteria, use the PERMDEL option or select this in the **Filter** dialog box. The command

```
filter constraints=test1 permdel;
```

would remove all trees *not* compatible with the constraint tree "test1." The set of trees in memory available to filter will then decrease. Deleted trees cannot be recovered - you must either repeat the search that produced them or reload them from a file, if one exists.

Inverting a filter is done by using the **Filter Trees** menu command. If trees have already been filtered, you can select the **Reverse Filter** menu command. Using the NOT option with the **FILTER** command achieves the same result:

```
filter not constraints=testcon;
```

This will keep only trees that are *not* compatible with the constraint tree "testcon."

When filtering is invoked, PAUP will display the number of trees originally in memory and the number of trees retained by the filter. It doesn't show the trees—you must do that yourself to see which trees survived the filter.

```
Trees filtered according to the following criteria:
    constraint tree = 'PAUP 2'

   Number of trees originally in memory = 17
   Number of trees retained by filter = 6
```

You can also filter trees directly by tree number using the FROM and TO options of **FILTER** or by selecting the tree range in the **Filter Trees** dialog box. Thus the command

```
filter from=1 to=5
```

would retain trees one through five.

NOTE: Filtering trees does *not* reset the tree numbers - the numbers are those assigned before the invocation of the filter.

Removing the filter restores all undeleted trees. This is done by the **Remove Filter** menu command or the command

```
filter off;
```

Condensing Trees

PAUP can either keep all dichotomous trees, whether or not there is evidence to resolve all branches, or it can collapse zero-length branches. This will have the effect of collapsing all branches for which there is no character support. After collapsing, duplicate trees are eliminated from the tree buffer (if a tree is collapsed to a resolution that already resides in the buffer, only one of those trees is retained). Remember that PAUP will *not* allow trees to be condensed if this action would result in violation of the constraints for a tree that would otherwise satisfy them. Collapsing is the default, but it specified in the search dialog box or by using the COLLAPSE option during a search, such as

```
hsearch addseq=asis swap=nni hold=1 collapse;
```

Rooting and Derooting Trees

Normally, trees are stored unrooted in the tree buffer. They are automatically rooted when output is requested, based on the rooting criteria that are in effect. You need not actively request rooted trees unless you wish to define an ancestor for the full tree (see **ANCSTATES** command) or if you are using directed, for example irreversible, characters. Rooting is achieved by using the **ROOT** command or the **Root Trees** menu command. Trees are rooted according to the currently specified outgroup. There are no options. Once trees in memory are rooted, they can be derooted by the **DEROOT** command or the **Deroot Trees** menu command. Trees cannot be rooted if a filter is in effect. You must first remove the filter in order to root them.

Printing and Plotting Trees

There are two ways to output trees: plotting and printing. Plotting involves invoking the PLOT option in a **DESCRIBE** command, or with the **Describe Trees** menu command. The output can be a cladogram, phylogram, or both. The destination of the plotted trees can either be a log file (when **LOG** or **Log Output to Disk** is invoked) or a printer (when **ECHO** or the **Echo to Printer** is invoked). The trees can be output in compressed or uncompressed form (when the TCOMPRESS option is chosen or by selecting it in the appropriate dialog box). Here is an example of an uncompressed and compressed tree after plotting. Compressing can save a great deal of space, especially when there is a large number of taxa.

```
describe 1;
```

would output an uncompressed tree:

```
fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ A
›
›         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ B
›         ›
fi‹‹‹‹‹‹‹‹14          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ C
          ›          ›
          fi‹‹‹‹‹‹‹‹‹13         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ D
                     ›         ›
                     fi‹‹‹‹‹‹‹‹12          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ E
                               ›          ›
                               fi‹‹‹‹‹‹‹‹‹11         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ F
                                          ›         ›
                                          fi‹‹‹‹‹‹‹‹10          fl‹‹‹‹‹‹‹‹‹‹ G
                                                    fi‹‹‹‹‹‹‹‹‹‹9
                                                               fi‹‹‹‹‹‹‹‹‹‹ H
```

while

```
describe 1/tcompress;
```

will output a compressed tree

```
fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ A
›         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ B
fi‹‹‹‹‹‹‹‹14          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ C
          fi‹‹‹‹‹‹‹‹‹13         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ D
                     fi‹‹‹‹‹‹‹‹12          fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ E
                               fi‹‹‹‹‹‹‹‹‹11         fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ F
                                          fi‹‹‹‹‹‹‹‹10          fl‹‹‹‹‹‹‹‹‹‹ G
                                                    fi‹‹‹‹‹‹‹‹‹‹9‹‹‹‹‹‹‹‹‹‹ H
```

Trees output by plotting or printing cannot be recovered for further manipulation and analysis - for that they must be saved to a NEXUS file (see Saving Trees to Files above).

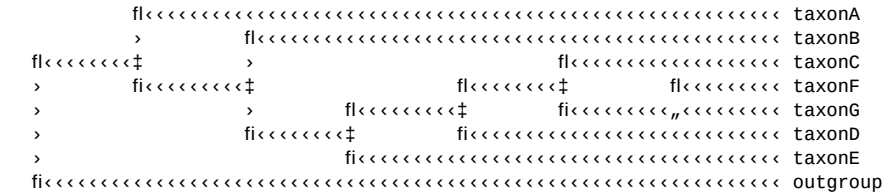
Changing the Order of Taxa on Trees

As is generally the case for phylogenetic trees, PAUP's trees are formally "unordered" (see "Tree Terms" in Chapter 1), however they may be ordered according to one of four available conventions for output purposes. You can specify which ordering to use by the TORDER option of the **SET** command or the **Tree Order** menu command. Because systematists tend to think of the root as the lowest point in the tree diagram, assume that the output has been rotated 90˚ clockwise, so that "left" is actually up, "right" is down, etc.

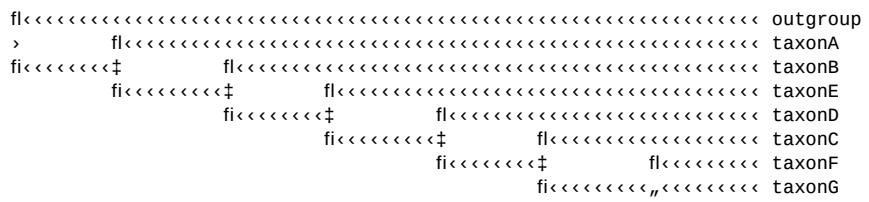
If TORDER=STANDARD, trees are ordered so that taxon names appear from left to right in, as nearly as possible, the same order as the taxa were presented in the data matrix. If TORDER= RIGHT, the tree is "ladderized" (a term used in MacClade) to the right. (Technically, this means that rotations are performed around each internal node so that the descendant node with the greatest number of ultimate descendants is placed on the right and the descendant node with the fewest descendants is

placed on the left. TORDER=LEFT is just the opposite of TORDER=RIGHT. Finally, if TORDER=ALPHABET, the tree is ordered so that the taxon names appear, as nearly as possible, in alphabetical order, regardless of the order in which the taxa were presented in the data matrix. Examples of the same tree in different orders are:

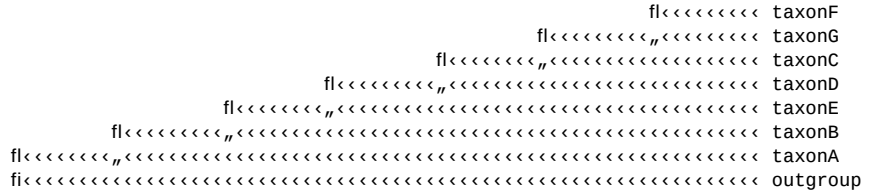
TORDER=STANDARD:



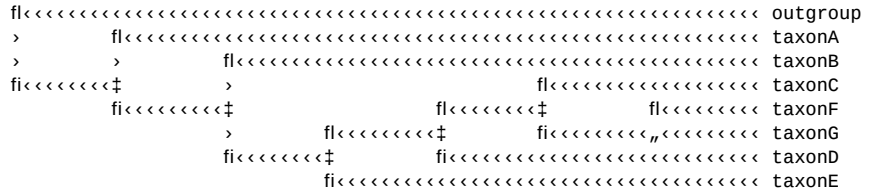
TORDER=RIGHT



TORDER=LEFT



TORDER=ALPHABET



User-Defined Trees

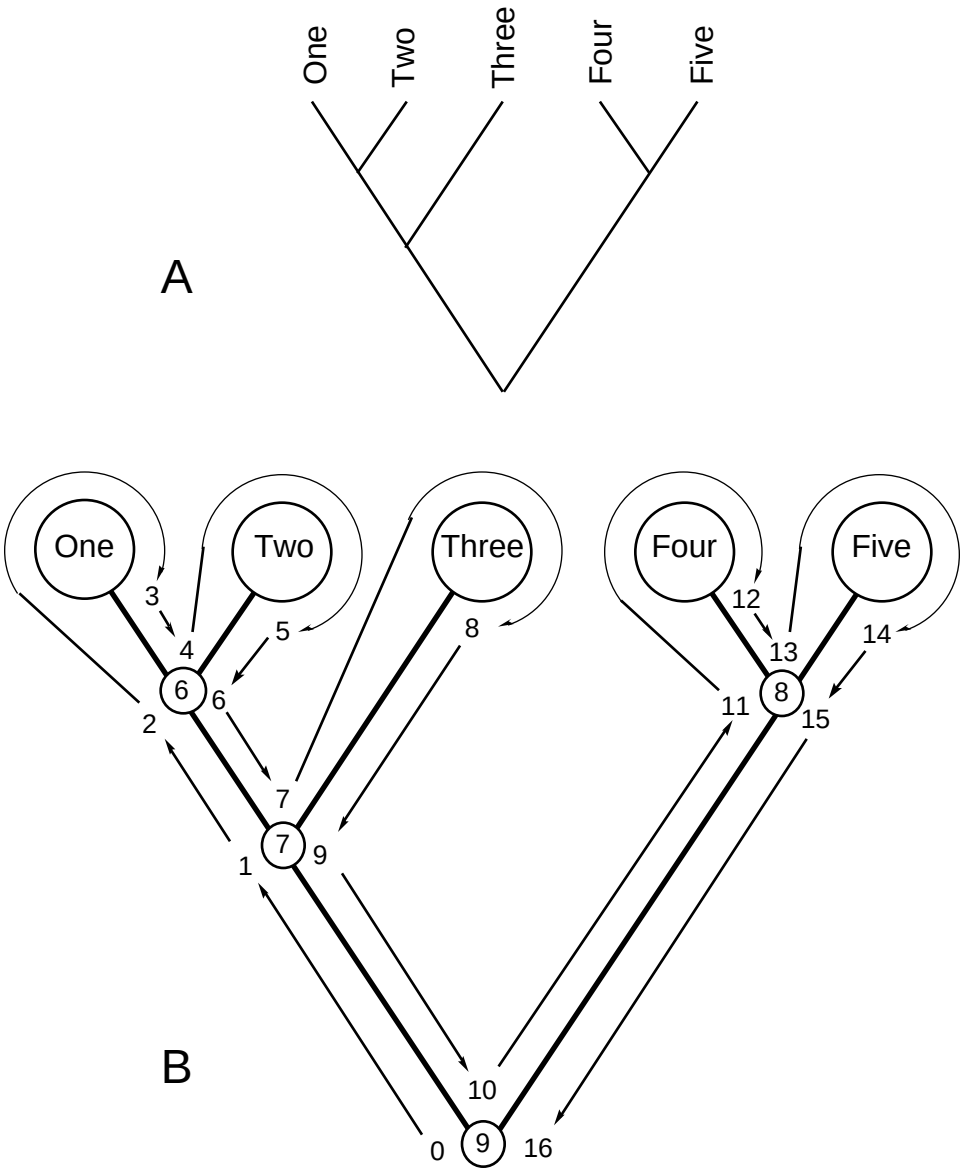
Tree descriptions follow the "Newick's 8:45" standard agreed upon by an informal committee of phylogenetic software developers[8] at the 1986

[8]The final draft was agreed upon by James Archie, William Day, Joseph Felsenstein, Wayne Maddison, Chris Meacham, F. James Rohlf, and David Swofford, meeting at Newick's, a local lobster house, on June 24, 1986.

annual meeting of the Society for the Study of Evolution in Durham, New Hampshire.

User-defined trees are described using a parenthetical notation that defines the shape of the tree. The terminal nodes of the tree correspond to the taxa included in the data matrix, and the internal nodes correspond to hypothetical ancestral taxa. Each pair of parentheses encloses all members of a monophyletic group.

Formally, the system used to describe trees is essentially the same as that for character-state trees (see "Defining Your Own Character Types," above). Once again think of the tree diagram as a set of roadways connecting nodes of the tree (see figure below). The itinerary is to visit all of the nodes of the tree (both terminal and internal) in a circuit beginning at the root node, following two simple rules: (1) when you come to an intersection or fork in the road (internal node), always bear to the left, and (2) when you come to a dead end (terminal node), turn around. The path indicated by the arrows shows the sequence in which the nodes would be visited in this example.



(a) Tree to be input as a user-defined tree; (b) Circuit followed in writing its description.

To write the tree description, perform the following operations as you make the circuit:

- When you leave a node traveling *away from* the root toward the *leftmost* descendant, write a left parenthesis.
- When you leave a node traveling *away from* the root toward any descendant *other than the leftmost* descendant, write a comma.
- When you leave the *rightmost* descendant of an internal node traveling *toward* the root, write a right parenthesis.

- When you visit a terminal node or visit an interior node for the last time, write the *node information* (if any) for the node.

NOTE: Usually, the node information will consist of a taxon identifier for terminal nodes and nothing for internal nodes. You are only entering the relative topology of the tree, therefore information about internal node labels and branch lengths is not included. In any case, branch lengths are dependent on a particular data matrix, and are not something that are fixed on a topology. When user-defined trees are read into memory, they can later be output with node labels and branch lengths, although if they are output to a treefile, only the branching topology is preserved. See the sections on **"Diagnosing Trees"** and **"Saving Trees to Files"** for descriptions of how trees are described and manipulated.

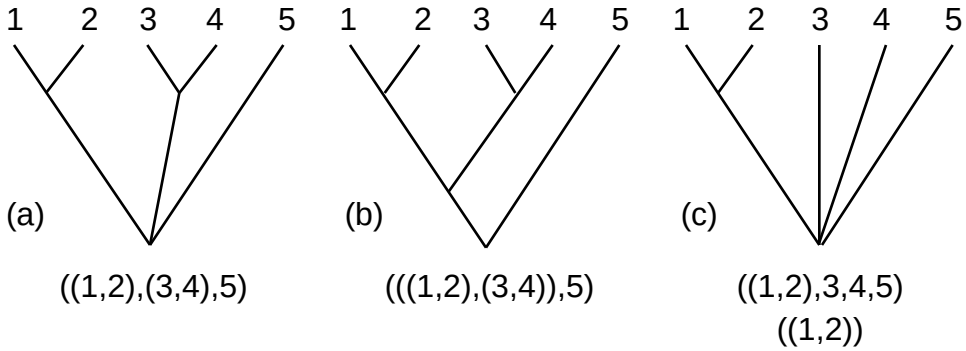
Applying these rules to the example of above, the tree description would develop as follows:

Number in Sequence	Description to this Point	Explanation
0	**(**	leaving for internal node 9's left descendant
1	(**(**	leaving for internal node 7's left descendant
2	((**(**	leaving for internal node 6's left descendant
3	(((**One**	terminal node One visited
4	(((One**,**	leaving for internal node 6's next descendant
5	(((One,**Two)**	terminal node Two visited; leaving internal node 6's rightmost descendant
6	(((One,Two)	internal node 6 visited for last time (no node information written)
7	(((One,Two)**,**	leaving for internal node 7's next descendant
8	(((One,Two),**Three)**	terminal node Three visited; leaving internal node 7's rightmost descendant
9	(((One,Two),Three)	internal node 7 visited for last time (no node information written)
10	(((One,Two),Three)**,**	leaving for internal node 9's next descendant
11	(((One,Two),Three),**(**	leaving for internal node 8's left descendant
12	(((One,Two),Three),(**Four**	terminal node Four visited
13	(((One,Two),Three),(Four**,**	leaving for internal node 8's next descendant
14	(((One,Two),Three),(Four,**Five)**	terminal node Five visited; leaving internal node 8's rightmost descendant
15	(((One,Two),Three),(Four,Five)**)**	visiting internal node 8 for last time (no node information written); leaving internal node 9's rightmost descendant
16	(((One,Two),Three),(Four,Five))	circuit completed (no node information written for root node 9)

Note that if the tree is defined as an unrooted tree (i.e., a **UTREE** command, see Chapter 3), the position of the root implied by the tree description is forgotten once the tree has been successfully stored; other methods (outgroup or Lundberg rooting) must be used to root the tree for output purposes. In this case, the tree can be rooted at any convenient point for input purposes.

All (nondeleted) taxa should be included in the tree description. If a taxon is omitted, it will be joined to the basal node of the tree described by the remaining taxa.

The examples below should clarify some of these points. In particular, observe that trees *a* and *b* specify the same *unrooted* tree, but distinct *rooted* trees. Also note that the two descriptions shown for tree *c* are equivalent, since in the second description, omitted taxa "3", "4", and "5" are joined to the basal node of the tree.



Three trees and their associated tree descriptions.

DEFINING AND USING TOPOLOGICAL CONSTRAINTS

User-defined trees may also be used to enforce constraints during a search, or as a constraint in filtering trees in memory. There are two types of constraint trees: those that include all taxa in the matrix "monophyly" constraints, and those that include only a subset of those taxa "backbone" constraints. Both of these types must be defined or loaded before they can be invoked in a search. Defining constraint trees is done by using the **CONSTRAINTS** command. The tree-description format is the same as for any other user-defined tree. See the section "User-defined trees" for details of tree description. An equivalent procedure is to use the **Load Constraints** menu command. This is quite useful—any tree in that has been previously saved may potentially be loaded as a constraint tree. These may include trees produced under different search conditions using the present data set, or trees from other studies of the same taxa. MacClade's tree manipulation interface is particularly useful in constructing and defining different constraint trees.

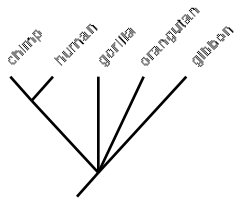
In PAUP the command (either on the command line or in PAUP block) for defining a "monophyly" constraint tree called "yourname" would look like

CONSTRAINTS yourname = *tree-specification*

where the tree-specification would follow the rules for user-defined trees above. Constraint trees are at least partially unresolved, otherwise only one tree would match the constraint. For example, the command

```
constraints
   hc=((human,chimp),gorilla,orangutan,gibbon);
```

defines an unresolved tree on which the only clade (other than the trivial all-species clade) is a group containing human + chimp.



For monophyly constraints, you do not necessarily have to enter all taxa into the tree definition. This is because omitted taxa are joined to the root node of the subtree described by the tree specification for the included taxa. For example, to force the groups ((1,2),3), specify "constraints (((1,2),3))". Note that the outer pair of parentheses are necessary, otherwise the converted specification would be ((1,2),3,4,5,6) rather than (((1,2),3),4,5,6).

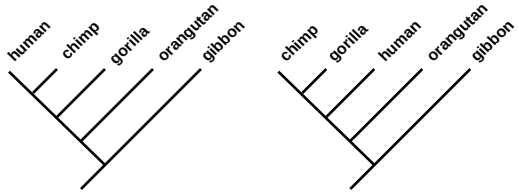
Constraints are enforced during a search by selecting this option in the search dialog box or by using the ENFORCE option as in this command:

```
hsearch enforce constraints=test1;
```

This will result in the following output when the search is begun

```
Keeping only trees compatible with constraint-tree 'other'
```

Enforcing this constraint during the search will mean that any tree on which the human+chimp group does not appear is automatically rejected, regardless of its length. For the monophyly constraint above, the tree on the left below would be accepted, while the tree on the right would not.

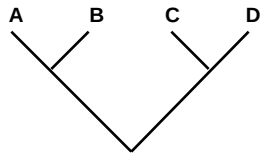


The rules for defining and enforcing backbone constraints are exactly the same as for monophyly constraint trees, with the exception that backbone trees *always* omit some of the taxa. When a backbone is specified,

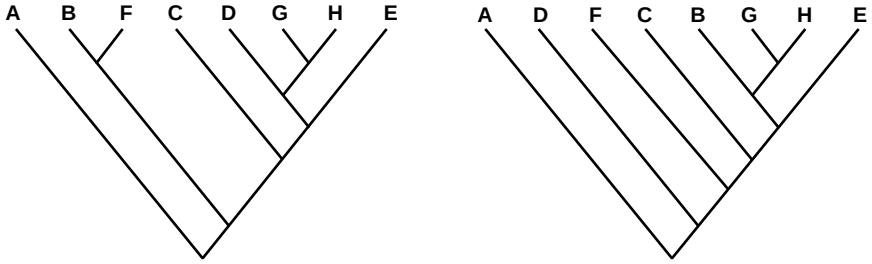
omitted taxa are left off the subtree. For example, the following command will define the constraint tree "bb1" as a backbone constraint:

```
constraints bb1
backbone=((taxonA,taxonB),(taxonC,taxonD));
```

which is the equivalent of the following rooted constraint tree:



The tree below on the left is one of the rooted trees that satisfies the backbone constraint; the tree on the right does not satisfy it. See the section "Searching under topological constraints" for a full description of "monophyly" and "backbone" constraint trees, as well as the difference between rooted and unrooted constraints.



Multiple constraint trees of either type may be defined by separate **CONSTRAINTS** commands, although only one of them may be the current constraint tree. This is conveniently done in either the data file or a command file, so that many different constraints can easily be tested on a given data matrix. Currently defined constraint trees are displayed using the **SHOWCONSTR** command or the **Show Constraints** menu command. When this is invoked, "backbone" trees are flagged, and the number of trees compatible with all monophyly constraints are displayed (this is not displayed for backbone constraint trees due to the difficulty of evaluating it).

```
Constraint-tree bb1 (backbone):

                                  fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonA
fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonB
›                                 fl‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonC
fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonD

Constraint-tree full:

                                                          fl‹‹‹‹‹‹‹‹‹‹ taxonA
                                                 fl‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹ taxonB
                                       fl‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonC
                             fl‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonD
                   fl‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonE
          fl‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonF
fl‹‹‹‹‹‹‹‹‹„‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonG
fi‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹‹ taxonH

     Of the 135,135 distinct rooted binary trees for 8 taxa, 1 is
     compatible with this constraint tree

     Of the 10,395 distinct unrooted binary trees for 8 taxa, 1 is
     compatible with this constraint tree
```

You may also choose to retain only those trees which are not compatible with the constraint tree. In the first example above, this will retain all trees in which the human+chimp group does not appear. This is achieved by using the CONVERSE and ENFORCE options during a search or by selecting this in the search dialog box. For example

```
hsearch enforce converse constraints=test1;
```

will keep trees *not* compatible with the constraint tree "test1."

Either type of constraint tree may also be used to filter trees after a search (though this is not the same as constraining during a search). In that case, the constraint trees are defined in the same way, and are invoked either by using the **FILTER** command, or by the **Filter Trees** menu command; see the section "Filtering Trees." Complications can arise when using converse constraints with a heuristic search. See the section "Heuristic Searches and 'negative' constraints."

IMPORTING AND EXPORTING TREES AND DATA

PAUP also has the capability of exporting trees and data in the format of other programs, saving you the effort of editing NEXUS files yourself. This gives you the option of importing your trees and data matrix into another program for comparative analysis, and vice versa. This is achieved using the **Import File** and **Export File** menu commands. Files can be exported in Hennig86 or PHYLIP formats, and you can choose to export the data matrix, the trees, or both. Exported files can then be read directly by the other program. You also have the option of exporting the

file as tab-delimited or plain text, should you wish to import the file into a spreadsheet or word-processing program. The only things that are preserved when a file is exported are the matrix itself and any tree descriptions contained in the data file. All other PAUP/MacClade instructions are not included. For example, the following is a sample data matrix with one user-tree and two taxa transferred to the outgroup:

```
#NEXUS
begin data;
dimensions ntax=8 nchar=16;
format missing=? ;

matrix

taxonA    0000001001012?000
taxonB    0100000011011111
taxonC    1110000011012?000
taxonD    1111101011012?000
taxonE    1101100111011000
taxonF    1111010111112?111
taxonG    1011111010112?000
taxonH    1011111010112?000
;
end;
begin trees;
   translate
      1 taxonA,
      2 taxonB,
      3 taxonC,
      4 taxonD,
      5 taxonE,
      6 taxonF,
      7 taxonG,
      8 taxonH
      ;
utree PAUP_1 = (1,(2,(3,(((4,(7,8)),5),6))));
end;
begin PAUP;
   outgroup taxonA taxonB;
end;
```

When this is converted to Hennig86 format, it appears as:

```
xread
'File "test" converted for Hennig86 by PAUP'
16 8
taxonA
0000001001012000
taxonB
0100000011011111
taxonC
1110000011012000
taxonD
1111101011012000
taxonE
1101100111011000
taxonF
1111010111112111
taxonG
1011111010112000
taxonH
1011111010112000
;

tread
'1 tree(s) from PAUP'
(taxonA taxonB (taxonC (((taxonD (taxonG taxonH)) taxonE)taxonF)));
procedure /;
```

Notice that the outgroup designation in the PAUP data file does not get exported—you must reset that yourself, either within Hennig86 or by editing the Hennig86 data file. The PHYLIP equivalent of this file looks like:

```
8 16
taxonA    0000001001012000
taxonB    0100000011011111
taxonC    1110000011012000
taxonD    1111101011012000
taxonE    1101100111011000
taxonF    1111010111112111
taxonG    1011111010112000
taxonH    1011111010112000
1
(taxonA,taxonB,(taxonC,(((taxonD,(taxonG,taxonH)),taxnE),taxonF)));
```

Excluded and ignored characters are not exported. To export all characters in the matrix, include any excluded characters and reset the "ignore" option to "ignore none".

The options for importing are more varied. Besides PHYLIP, Hennig86, and tab-delimited text, you can select two formats for molecular data: GCG MSF or NBRF-PIR. If PHYLIP is chosen, you have the option of selecting discrete data, DNA sequence, restriction site data, or protein sequence. Other data formats can be imported by selecting text in the **Import File** dialog box. You can then select from standard format, DNA, RNA, or protein. Using this option gives you great flexibility in analyzing molecular data sets in a wide variety of formats.

EXAMINING CURRENT STATUS

PAUP provides several commands for displaying the current status of data, trees, and ancestors.

Data Matrix

To display the current data matrix, use the **SHOWMATRIX** command or the **Show Data Matrix** menu command . By default, ignored/zapped characters will not be displayed. They can be displayed if SET SHOWIGNORE is used, or that option is selected in the **Character Matrix Format** dialog box. Excluded characters are always displayed. If SET CMSTATUS is used or status information is requested in the **Character Matrix Format** dialog box, excluded, uninformative and constant characters are flagged. For example, the following matrix has both ignored and deleted characters:

```
Input data matrix

                     1111111
Taxon       1234567890123456
---------------------------
 Constant:              **
   Uninf.:           *  **
 Excluded:     **
---------------------------
taxonA      0000001001012000
taxonB      0100000011011111
taxonC      1110000011012000
taxonD      1111101011012000
taxonE      1101100111011000
taxonF      1111010111112111
taxonG      1011111010112000
taxonH      1011111010112000
```

The width of each column can be controlled with the SET CMDCOLWID command or by using the **Character Matrix Format** menu command. The default is one column (CMDCOLWID=1). If "equate" macros are used in the data file (e.g. a={12}) and character-state reconstructions are requested, the "equate" symbol will be assigned to internal nodes if SET CMSHOWEQ is chosen or this is selected in the **Character Matrix Format** dialog box. **SHOWMATRIX** will always display the "equate" symbol. If NOCMSHOWEQ is selected, the possible character states will be displayed instead of the "equate" symbol.

Character Status

To display the status of characters, use the **Show Character Status** menu command. Excluded, zapped, constant, and ignored characters are

flagged. One column has a "Y" or "N" depending on whether the character is informative. Output also includes current character type (ordered etc.)., character weight, and observed states.

```
Current status of all characters:

Character        Type      Inform?  Status     Weight    States

1                Irrev.Up     Y                   1    01
2                Unord        Y                   1    012
3                Unord        Y                   1    01
4                Unord        Y                   1    01
5                Ord          Y                   1    012
6                Ord          Y                   1    012
7                Unord        Y                   1    01
8                Unord        Y                   1    01
9                Unord        N     Ignored       1    01
10               mytype       Y     Excluded     (1)   01
11               Unord        Y     Excluded     (1)   01
12               -            N     Const/Ign     1    1
13               -            N     Const/Ign     1    1
14               Unord        Y                   1    01
15               Unord        Y                   1    012

There are 1 "zapped" characters
```

User-Defined Character Types

To display the status of user-defined characters, use the **SHOWUSER** command or the **Show Usertypes** menu command. This will output all stepmatrices or character-state trees that have been defined, for example:

```
Stepmatrix 'aa':

            TO: a       b       c       d       e
  FROM: a       -       2       1       3       2
        b       1       -       3       1       3
        c       1       3       -       2       1
        d       1       1       2       -       1
        e       1       2       2       1       -


Character-state tree 'typea:

   0--2--1
```

Ancestral States (ANCSTATES)

To display the ancestral states, use the **SHOWANC** command or the **Show Ancestral States** menu command. This ouputs the name of the current ancestor and its states. For example:

```
Character states for current ancestor: "new"

                          1 1 1 1 1 1 1
          1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6
----------------------------------------
   Zapped:             *
   Uninf.:                       * *
 Excluded:       * *
----------------------------------------
new        ? ? ? ? ? ? ? 0 0 0 0 0 0 0 0 0
```

The status lines are omitted if SET NOCMSTATUS is used, or this output is suppressed in the **Character Matrix Format** dialog box.

Tree Status

To display the status of trees in memory, use the **TSTATUS** command or the **Tree Info** menu command. This will display the number of trees in memory and their source. For example:

```
3 unrooted trees in memory
No tree filter in effect
Source of trees: Heuristic search
```

If trees in a file are added to trees in memory, PAUP will identify "treefile" as the source for all trees in memory.

Chapter 3 COMMAND REFERENCE

This chapter describes the PAUP command format. Unless stated otherwise, these commands may be included in the PAUP block of a NEXUS file, or typed from the command line. The EXECUTE and EDIT commands may only be issued from the command line. All users will need to be familiar with the commands available in the DATA, ASSUMPTIONS, and TREES blocks, which are normally placed in PAUP input files. In versions of PAUP that provide a menu-based interface, only knowledge of these "block" commands is required, as other instructions to PAUP can be accomplished through the menu system. Some users, however, may prefer a command-driven interface. Consequently, except for certain machine-specific features, PAUP may be controlled entirely by a command-line system. Commands may also be placed into input files for convenience (i.e., to avoid repeated typing of complicated commands) and to provide a simple "batch" facility.

COMMAND FORMAT

The command descriptions below use the notational conventions specified in the Preface. Remember that upper-case items are to be entered as shown. Italicized items—e.g., *user-item*—represent variable items to be substituted by the user. Items inside of square brackets—e.g., [OPTIONAL_ITEM]—are optional. Items inside of curly braces and separated by vertical bars—e.g., { X | Y | Z }—are mutually exclusive options; only one of the choices indicated may be used. The default item (if any) is underlined, as in { ABC | <u>DEF</u> }. Options in commands are generally specified as "KEYWORD=VALUE" for options that have two or more potential values. For options that represent simple "on-off" ("Boolean") switches, options may be selected by simply specifying KEYWORD or deselected using NOKEYWORD. Alternatively, the forms KEYWORD=YES and KEYWORD=NO can be used to select or deselect options, respectively.

Each command begins with a command-name and ends with a semicolon. Otherwise, the commands are completely free-format. A command may span any number of lines and whitespace (tabs and blanks) may be inserted at will. Input of PAUP commands is case-insensitive, so you may enter command names, option keywords, etc., in any combination of upper- and lower-case characters (the only exception pertains to the MATRIX command). In addition, PAUP allows abbreviation of command names and keywords to the shortest unambiguous truncation. Note that other NEXUS-conforming programs may not accept these abbreviations. MacClade, in particular, does not allow abbreviations, so if you want your data file to be MacClade-compatible, then commands visible to MacClade (i.e., those not contained within a PAUP block) should not be abbreviated.

Identifiers

"Identifiers" are simply names given to taxa, characters, and other PAUP input elements such as character-sets, taxon-sets, and exclusion-sets. They may include any combination of upper- and lower-case alphabetic characters, digits, and punctuation. If the identifier contains any of the following characters:

```
* ( ) [ ] { } , ; - = : " / \ ' _
```

or a blank, the entire identifier must be enclosed in single quotes. Underscores (_) are translated to blanks, unless the identifier is enclosed in single quotes. For example, the identifiers `Homo_sapiens` and `'Homo sapiens'` are equivalent, but distinct from `'Homo_sapiens'`. To include a single-quote in the identifier, you must use two consecutive single quotes. Any trailing blanks are stripped before the identifier is stored; leading blanks are preserved.

Examples of valid identifiers:

```
subterraneus
Mus_musculus
H._sapiens
'H. sapiens #429'
'Fred''s new sp.'
'rusticus (1)'
'"shoal bass"'
AMNION
_23
x21.02
myType
```

PAUP imposes limits on the lengths of identifiers as defined below. If you use identifiers that exceed these limits, PAUP simply truncates them to the maximum acceptable length. If you use names that are longer than

the maximum lengths (e.g., for other NEXUS programs that allow longer identifiers), you should make sure that identifiers in the same class will be unique after truncation by PAUP.

MacClade will not accept all-digit character or taxon names. PAUP allows them, with a warning that taxon/character names have precedence over numbers (e.g. the if the tenth character is named "5", the command EXSET *no_5=5 causes character number five to be excluded by MacClade and character number ten to be excluded by PAUP). Do not use all-digit names in PAUP if you intend to also use the data file in MacClade (and it's not advisable to use them in PAUP in any case).

Taxon identifiers

For input, taxa may be referenced either by name or by number. The numbers are simply the row number of the taxon in the input data matrix. Note that if you delete taxa, the *original* taxon numbers are still used to refer to taxa.

Taxon names may be up to 32 characters in length, however for most output they are truncated to 16 characters. Thus, you should choose names that are unique up to at least the first 16 characters. The full 32-character names are used only for high-resolution tree plotting (currently available in the Macintosh version only).

Taxon names have priority over taxon numbers in input commands. This only becomes an issue if you use all-digit taxon names. For example, if five taxa in a data file are, for whatever reason, given the names

```
One
34
three
3
673
```

then the command `delete 3;` would cause deletion of the *fourth* taxon. Because of the potential confusion, you are strongly urged not to use all-digit taxon names.

> The name "ALL" is reserved for a taxon list containing all of the taxa in the data matrix (see below). You may not name a taxon "ALL".

Character identifiers

Ordinarily, PAUP refers to characters by consecutive integers starting with 1. These integers are used both for input commands and in the output. You may also assign alphanumeric character names if you wish. The maximum length of a character name is 32, but PAUP truncates character

names to 10 characters in its output. Thus, you should choose names that are unique up to at least the first 10 characters. If you assign alphanumeric character names, these will also be used to identify characters in PAUP output.

The rules for including punctuation, underscores, and blank characters are the same for character names as for taxon names (see above).

As for taxa, character names have priority over character numbers. For example, if you used all-digit character names (e.g., sequence positions or restriction-map locations) such as

```
2
4
11
23
89
102
```

and issued the command `exclude 4-5;` then all but the first and last characters would be excluded (i.e., *name* "4" through *number* 5). Because of the potential confusion, it is suggested that you always include at least one nondigit in alphanumeric character names.

> The name "ALL" is reserved for a character list containing all of the characters in the data matrix (see below). You may not name a character "ALL".

Other names

Identifiers are also used for TYPESET, WTSET, EXSET, CHARSET, TAXSET, and ANCSTATES definitions. These names follow the same rules as taxon and character names, and have a maximum length of 10 characters.

Common Command Elements

The following elements are used in more than one command and are defined here to minimize redundancy.

Taxon lists

A ***taxon list*** is a sequence of one or more taxon identifiers (names and/or numbers. If two taxon identifiers are separated by a hyphen, this indicates that the range of taxa between the first taxon and the second taxon (inclusive) are to be included in the list. (The second taxon must have a higher number than the first taxon).

A taxon list composed of the reserved name "ALL" specifies all of the taxa in the data matrix.

Examples of valid taxon lists (assuming that corresponding taxon identifiers have been defined in the DATA block) are as follows:

```
heteroclitus
M23-Q45 S1 T5;
1 3 5-8
all
```

Character lists

A ***character list*** is a sequence of one or more character identifiers (names and/or numbers). If two character identifiers are separated by a hyphen, this indicates that the range of characters between the first character and the second character (inclusive) are to be included in the list. (The second character must have a higher number than the first character). If the second specification in the range is followed by a backslash (\), then an integer value immediately following the backslash represents an ***increment***. For example, the list `3-24\3` consists of the characters 3, 6, 9, …, 21, 24. If a range-plus-increment is used, only those characters contained within the range are included in the list (e.g., the list `2-5\2` includes only characters 2 and 4).

The special identifier consisting only of an unquoted period refers to the last character in the data matrix (=NCHAR).

A character list composed of the reserved name "ALL" specifies all of the characters in the data matrix.

Examples of valid character lists (assuming that corresponding character identifiers have been defined in the DATA block) are as follows:

```
8
amnion appendages gizzard teeth;
1 two 5 7
11-.
1 3-7 16 31 28
LLSCALES-CPDSCALES
3-.\3
all
```

Character states

A ***character state*** is a single digit, alphabetic character, or other symbol that represents a valid character state as defined by the SYMBOLS list.

Tree lists

A ***tree list*** is simply a list of tree numbers referring to one or more trees currently stored in memory. If two tree numbers are separated by a hyphen, this indicates that the range of trees between the first tree number and the second tree number (inclusive) are to be included in the list. To include all trees currently in memory, specify "ALL" as the tree list.

COMMANDS USED IN THE DATA BLOCK

CHARLABELS

Use the **CHARLABELS** command if you want to define alphanumeric character names to supplement the default numeric identifiers. The syntax is:

CHARLABELS *character-name-list*;

The *character-name-list* is of the form

character-name$_1$ *character-name*$_2$ …

where each *character-name*$_i$ is a valid character identifier for the *i*th character (see "Identifiers" above). The underscore (_) character can be used as a placeholder if you do not want to assign names to some characters. It is permissible to supply fewer than NCHAR names; the remaining characters will then be identified by number only. The length limit on these labels is 10 characters, due to line-length constraints in PAUP output. However, on *input*, the full character label is needed. Any CHARLABELS command issued from the command line overrides labels provided in the data matrix.

DIMENSIONS

The **DIMENSIONS** command specifies the size of the data matrix. The syntax is:

DIMENSIONS NTAX= *number-of-taxa*
NCHAR= *number-of-characters*;

where *number-of-taxa* and *number-of-characters* are integer values. Technically, the limits on numbers of taxa and characters is 32767 but practical limitations on computing time and available memory will reduce the maximum numbers of taxa and characters that can be accommodated.

FORMAT

The **FORMAT** command is used to specify information pertaining to the format of the data file. The syntax is:

FORMAT *option-specification …* ;

Any or all of the following option specifications may be given:

MISSING=*missing-symbol*

The *missing-symbol* specifies a character used to represent missing data. Any alphabetic, numeric, or other character that may be used as a character-state symbol may be used as the *missing-symbol*. If MISSING is not specified, it defaults to '?'.

TRANSPOSE

If TRANSPOSE is specified, rows of the data matrix correspond to characters and columns correspond to taxa. Otherwise, rows correspond to taxa and columns to characters.

LABELPOS={ LEFT | RIGHT }

If LABELPOS=LEFT, taxon names (or character names, if the data matrix is transposed) begin each row of the data matrix (i.e., precede the character-state data). If LABELPOS=RIGHT, then these names end each row of the data matrix. The default is LABELPOS=LEFT.

SYMBOLS="*symbols-list*"

The SYMBOLS list defines a set of permissible symbols that may be used to designate character states. The default SYMBOLS list is "01" for the STANDARD data type (see below), which means that the only (non-missing) character-state symbols permitted are '0' and '1'. If you want to use any other symbols to designate character states, you must explicitly define an alternate SYMBOLS list. The standard symbols for molecular sequence types (DNA, RNA, PROTEIN) are given in the section "Predefined formats for molecular sequence data" (Chapter 2).

The format of a *symbols-list* is a sequence of single-character "symbols"; the entire list is then enclosed within double-quotes.

INTERLEAVE

If INTERLEAVE is not specified, the character-state data are entered sequentially by taxon (i.e., all character-state data for the first taxon are entered before beginning the second taxon, and so on). Specification of INTERLEAVE allows the data to be entered in "blocks" of characters. This format is often used for nucleotide and amino-acid sequence alignments, but may be useful in other contexts as well.

MATCHCHAR=*match-symbol*

If a *match-symbol* is specified, any occurrence of that symbol in the data matrix is translated to the state (or state-set) occurring in the first row of the matrix.

EQUATE= "*symbol=expansion…*"

The EQUATE option provides a simple macro facility for translating character-state specifications in the data matrix to alternate character-state specifications. The *symbol* component must be a single-character. *Expansion* is either a valid character-state or a character-state set .

Any number of EQUATE macros may be specified following the equal sign, but only one pair of double-quotes is used. For example:

```
format equate="U=T R={AG} .=- X=?";
```

EQUATE macros may not be defined recursively. That is, you cannot equate A to B and B to C, expecting A to be expanded to C. If you equate the same symbol to more than one expansion, the last definition applies.

RESPECTCASE

By default, PAUP does not distinguish between upper- and lower-case character-state symbols in the data matrix. If you want upper- and lower-case representations of the same alphabetic character to refer to different character states, specify RESPECTCASE.

DATATYPE = { STANDARD | DNA | RNA | PROTEIN }

If DATATYPE=STANDARD, the SYMBOLS list is taken from the SYMBOLS="*symbols-list*" item, above (default = "01"). If DATATYPE is set to one of the molecular sequence types, a predefined SYMBOLS list is used ("ACGT" for DNA, "ACTU"

for RNA, and the standard one-letter amino acid codes for PROTEIN). In addition, standard ambiguity codes are implementedby predefined EQUATE macros. See "Predefined formats for molecular sequence data" in Chapter 2 for more information on these types.

GAP=*gap-symbol*

The "gap symbol" refers to the symbol used to represent alignment gaps, corresponding to insertions and/or deletions. For example, `gap=-` would assign the hyphen as the gap character. The GAP setting is ignored unless DATATYPE is DNA, RNA, or PROTEIN.

Alignment gaps may be treated either as missing data or as an additional character-state (fifth base or 21st amino acid) using the GAPMODE option (see the **OPTIONS** command below).

MATRIX

The **MATRIX** command defines the data matrix. The syntax is:

MATRIX *data-matrix* ;

The form of the *data-matrix* depends on the options specified in the **FORMAT** command of the DATA block; see "The DATA Block" in Chapter 2 for details.

OPTIONS

The **OPTIONS** command of the DATA block is used to set certain options pertaining to the treatment of the data matrix. The syntax is:

OPTIONS *option-specification …* ;

Any or all of the following option specifications may be specified.

IGNORE={ NONE | INVAR | UNINFORM }

If IGNORE=NONE , all characters in the data set are used. If INVAR or UNINFORM are specified, invariant ("constant") and uninformative characters, respectively, are ignored. See the section "Using a subset of the characters" in Chapter 2 for definitions of invariant and uninformative.

MSTAXA = { UNCERTAIN | POLYMORPH }

If MSTAXA=UNCERTAIN, multistate taxa (i.e., nonsingleton character-state sets in terminal taxa) are interpreted as representing uncertainty about the state found in the taxon. If MSTAXA=POLYMORPH, multistate taxa are interpreted as polymorphism within the terminal taxon. (See "Multistate Taxa" in Chapter 2 for the differences between these two interpretations.)

Note that MacClade allows MSTAXA=VARIABLE as a third possibility. PAUP does not support this option. If you do not explicitly set MSTAXA, MacClade uses the uncertainty interpretation if the character-state set is enclosed in curly braces and the polymorphism interpretation if the set is enclosed between parentheses. If you set MSTAXA here, this setting will override the interpretation implied by the curly brace vs. parenthesis notation in MacClade.

ZAP = "*character-list*"

All characters in the *character-list* will be ignored (see "Using a subset of the characters" in Chapter 2).

GAPMODE = { MISSING | NEWSTATE }

If GAPMODE=MISSING, gap characters in sequence data are treated as "missing." If GAPMODE=NEWSTATE, gap characters are treated as a fifth base or 21st amino acid. See "Alignment gaps" in Chapter 2 for more information.

If you are satisfied with the defaults indicated above and you do not want to "zap" characters, then you do not need to include an OPTIONS command.

STATELABELS

The **STATELABELS** command is recognized but not interpreted by PAUP. In MacClade, it supplies names for the character-states of each character.

TAXLABELS

Use the **TAXLABELS** command to supply taxon names when the data matrix is entered in transposed format. The syntax is:

TAXLABELS *taxon-label*$_1$ *taxon-label*$_2$ ... *taxon-label*$_{\mathrm{NTAX}}$;

If the data matrix is transposed and you do not provide a **TAXLABELS** command, the integers 1 through NTAX will be used to identify taxa in both input and output. The length limit on these labels is 16 characters, although up to 31 characters may included in graphical tree output. This is due to line-length constraints on PAUP output. Any **TAXLABELS** command issued from the command line overrides any labels provided in the matrix. MacClade ignores **TAXLABELS** unless the data matrix is transposed.

COMMANDS USED IN THE ASSUMPTIONS BLOCK

ANCSTATES

Use the **ANCSTATES** command to define a set of ancestral states corresponding to an ancestral taxon. The syntax is:

ANCSTATES [*] *ancestor-name* = *character-state* : *character-list*
[, *character-state* : *character-list*] … ;

Any number of *character-state:character-list* pairs, separated by commas, may be specified. You may also repeat the **ANCSTATES** command to define multiple ancestors, although only one ancestor can be in effect at any given time. If you precede the *ancestor-name* with an asterisk, that ancestor becomes the "current" ancestor. You can also assign the current ancestor with the **ASSUME** command or the **Choose Assumption Sets** menu command.

ANCSTATES can also be used to explicitly specify the state for each character in the ancestor. This is done using the VECTOR format option, e.g., if NCHAR=5

```
ANCSTATES name VECTOR = 0 1 2 0 2;
```

would define an ancestor "name" with the specified states for the five characters. See "Defining Ancestral States" in Chapter 2 for information on how ancestral-state specifications are used.

CHARSET

Use the **CHARSET** command to define a "character set." Character sets are simply groups of characters that can be referred to by a single name in other commands. The syntax is:

CHARSET *character-set-name* = *character-list* ;

The *character-set-name* must not be identical to any of the original character names.

See "Simplifying Input with 'Sets'" in Chapter 2 for examples on the use of character sets.

EXSET

Use the **EXSET** command to define an "exclusion set." The syntax is:

EXSET [*] *exclusion-set-name* = *character-list* ;

If you precede the *exclusion-set-name* with an asterisk, any previously excluded characters are re-included and the characters specified by *character-list* are excluded. Otherwise, the exclusion set is simply defined for later use by the **ASSUME** command or the **Include-Exclude Characters** and **Choose Assumption Sets** menu command.

See "Simplifying Input with 'Sets'" in Chapter 2 for information on how to use exclusion sets.

OPTIONS

Use the **OPTIONS** command of the ASSUMPTIONS block to specify certain assumptions-related options recognized by PAUP and by other NEXUS-conforming programs. The syntax is:

OPTIONS *option-specification* [*option-specification*]… ;

The only option used by PAUP is the following:

DEFTYPE=*character-type*

The *character-type* specifies the character type that applies to characters not explicitly assigned a type by **CTYPE** and/or **CHARSET** commands. It must be one of the standard types (ORD, UNORD, DOLLO, DOLLO.UP, DOLLO.DN, IRREV, IRREV.UP, or IRREV.DN).

The following option is used by MacClade but ignored by PAUP:

POLYTCOUNT = { MINSTEPS | MAXSTEPS }

This option specifies whether polytomies are treated as "hard" or "soft" (see Maddison (1989)) when counting the number of steps required by a character and when reconstructing ancestral states. PAUP currently supports only POLYTCOUNT=MAXSTEPS

("hard" polytomies), and will ignore the POLYTCOUNT specification.

Future NEXUS-conforming programs may define additional options; the ERRORSTOP setting (see **SET** command) determines how PAUP will react to unrecognized option keywords.

TYPESET

Use the **TYPESET** command to define a "type set." The syntax is:

TYPESET [*] *type-set-name* = *character-type* : *character-list*
<, *character-type*> : *character-list* >…;

The *character-type* must be one of the standard character types (ORD, UNORD, DOLLO, DOLLO.UP, DOLLO.DN, IRREV, IRREV.UP, or IRREV.DN) or the name of a user-defined character type. Any number of *character-type:character-list* pairs, separated by commas, may be specified.

See "Simplifying Input with 'Sets'" in Chapter 2 for information on how to use type sets, including examples.

USERTYPE

Use the **USERTYPE** command to define a new character-type. The syntax is:

USERTYPE *character-type-name* [{ STEPMATRIX | CSTREE }]
= *character-type-description* ;]

Character-type-description must follow the rules for character-state tree or stepmatrix description outlined in the section "Defining Your Own Character Types" (Chapter 2).

WTSET

Use the **WTSET** command to define a "weight set." The syntax is:

WTSET [*] *weight-set-name* = *character-weight* : *character-list*
[, *character-weight* : *character-list*] … ;

or

WTSET [*] *weight-set-name* VECTOR = wt_1 wt_2 wt_3 ... $wt_{NCHAR;}$

Character-weight must be a nonnegative integer value. In the first form, any number of *character-weight:character-list* pairs, separated by

commas, may be specified. In the second form, a single weight is provided for every character.

See "Simplifying Input with 'Sets'" in Chapter 2 for information on how to use weight sets.

COMMANDS USED IN THE TREES BLOCK

TRANSLATE

Use the **TRANSLATE** command to define mappings of arbitrary tokens appearing in **TREE** and **UTREE** commands to valid taxon names. Ordinarily, the tokens are the integers 1 through NTAX.

TRANSLATE *token taxon-name* [, *token taxon-name*] … ;

See "The TREES Block" in Chapter 2 for more information on the use of translation tables.

TREE, UTREE

Use the **TREE** and **UTREE** commands to define user-specified rooted and unrooted tree topologies, respectively. The syntax is:

[U]TREE [*] *tree-name = tree-description*;

You will usually define *unrooted* trees. These trees are then rooted for output purposes using the outgroup or Lundberg rooting procedures. However, if you wish to define an ancestor for the full tree (see **ANCSTATES** command) or if you are using directed characters (see "Character Types" in Chapter 1), you should define rooted trees instead. In any case, you can convert rooted trees to unrooted trees and *vice versa* using the **ROOT**/**DEROOT** commands or the **Root Trees/Deroot Trees** menu commands.

Note that in order to write the tree descriptions, unrooted trees may be rooted at any convenient point (including a terminal taxon or internal node); the position of the root is simply ignored when the tree is stored.

PAUP currently ignores the tree names; trees are referred to subsequently only by number, in the order in which they are presented in the TREES block. (MacClade uses the tree names, however.) If the name is preceded by an asterisk (*), the tree becomes the "default" tree. (E.g., a **DESCRIBE** command with no tree list will result in the description of the default tree.)

If a taxon is omitted from the tree specification, it is assumed to descend from the root of the tree described by the remaining taxa.

See "Manipulating Trees: User-Defined Trees" in Chapter 2 for instructions on writing tree descriptions.

Note to PAUP Version 2 Users: The new standard requires commas in places in which versions 2.4 and earlier allowed them to be omitted. In particular, commas are now generally required between a right parenthesis and a left parenthesis or taxon number. E.g., the old description `(((1,2)(3,4))5)` must now be written as `(((1,2),(3,4)),5)`.

PAUP COMMANDS

Commands described in this section are specific to PAUP. They may be included in the PAUP block of a NEXUS file or typed from the command line.

Unless otherwise specified, options specified in these commands are "persistent;" i.e., they retain their values between successful invocations of the command. Persistence of options simplifies typing of commands because one a command requiring a large number of options has been entered once, subsequent invocations of the command need not respecify all of the options. The drawback is that you can become temporarily confused if you forget that a previously specified option remains in effect until you explicitly override it.

Options Affecting Multiple Commands

Several options apply to a number of commands. Specification of these options on one command affects all commands that use the same options. To minimize redundancy, these options are described in the following sections rather than in the description for each individual command to which the option applies.

Tree-searching options

These options pertain to the commands that request searching for trees (**ALLTREES**, **BANDB**, **HSEARCH**).

KEEP = *keep-length*

If *keep-length* is zero, only the shortest trees found will be saved. If *keep-length* ≥ zero, all trees of length ≤ *keep-length* will be

saved. By default, *keep-length* = 0, so that only the shortest trees found will be saved.

[NO]COLLAPSE

NOCOLLAPSE requests that zero-length branches not be collapsed to yield polytomies. The default setting is to collapse zero-length branches. COLLAPSE can be used to reverse a previous NOCOLLAPSE setting.

[NO]ENFORCE

ENFORCE requests that topological constraints be enforced; i.e., trees that are not compatible with the constraint tree are not evaluated. If the CONSTRAINTS option (see below) is not used to specify a constraint tree, the "current" constraint tree is used.

CONSTRAINTS=*contraint-tree-name*

The specified constraint tree, which must have been defined in a previous **CONSTRAINTS** command, becomes the current constraint tree. You must also specify ENFORCE if you want to search under constraints.

[NO]CONVERSE

If CONVERSE is specified in conjunction with ENFORCE, only trees that are *not* compatible with the constraint tree are evaluated. NOCONVERSE reverses the effect of a previous CONVERSE specification.

[NO]INCLUDEANC

If INCLUDEANC is specified, the "current" ancestor is included in the analysis, and is used to root the tree. (You can use the ANCSTATES=*ancestral-states-name* to choose a different ancestor. The ASSUME command can be used to choose the current ancestor. NOINCLUDEANC reverses the effect of a previous INCLUDEANC specification.

[NO]STATUS

Ordinarily, information on the progress of the search (number of trees examined, number of trees saved, etc.) is output to the screen or to a window. NOSTATUS suppresses this status output.

Tree-rooting options

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }

The ROOT option is used to specify how unrooted trees are to be rooted prior to output. You can choose OUTGROUP rooting, using whichever outgroup you have selected; MIDPOINT rooting, which roots the tree at its midpoint; or LUNDBERG rooting, which requires that a previous ANCSTATES command has been issued. By default, OUTGROUP rooting is in effect.

OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }

If OUTGROUP rooting is selected, there are three options for output. The outgroup can make up a polytomy next to the ingroup (POLYTOMY, the default); or it can be made to be paraphyletic relative to the ingroup (PARAPHYL); or the monophyletic sister group to the ingroup (MONOPHYL).

Tree output options

[NO]TCOMPRESS

Specify COMPRESS to output tree diagrams in a "vertically compressed" format. The resulting diagram is not as aesthetically appealing, but it allows more of a large tree to be seen on one screen (or in one window), and it takes less paper to print.

Options for character-matrix listings

[NO]SHOWIGNORE

Unless SHOWIGNORE is specified, "ignored" characters are not shown in character-matrix listings. (For information on ignoring characters, see "Using a subset of the characters" in Chapter 2.)

[NO]CMLABELS

By default, character names are used to label the columns of character-matrix listings. If you want to use numbers even when character names are available, specify NOCMLABELS.

[NO]CMCSTATUS

If CMCSTATUS is specified, characters that are constant, "zapped," uninformative, or excluded are identified by asterisks at the top of each column of a character-matrix listing.

CMDCOLWID = *column-width*

The value specified for *column-width* determines the number of columns used for each character in the data matrix. The default is CMDCOLWID=2, so that one blank column appears between each column of character state data. For sequence data, you may want to use CMDCOLWID=1 in order to fit more characters onto each line of output.

[NO]CMSHOWEQ

Unless CMSHOWEQ has been specified, if the possible state assignments to an interior node correspond to a multistate taxon code specified in an EQUATE macro, the corresponding EQUATE character is shown rather than the equivalent set of character states.

Other options

OPT = { ACCTRAN | DELTRAN | MINF }

The entry following OPT= determines how the characters are optimized on the tree(s) in memory. ACCTRAN (default) uses "accelerated transformation", DELTRAN uses "delayed transformation", while MINF optimizes so as to minimize the f-value of (Farris, 1972) . See the section on **character-state optimization** for detailed discussion of these options.

ANCSTATES=*ancestral-states-name*

Change the ancestor currently in effect to the *ancestral-states-name* defined in an earlier **ANCSTATES** command (or to STANDARD). This option affects searching and character-state reconstruction algorithms.

?

The "?" command is a synonym for "HELP". "?" with no arguments requests a list of the available commands. See help on "HELP" for further information.

!

Use the **!** command to execute a UNIX command from within PAUP. The syntax is:

! *unix-command* ;

You can type a command that contains a semicolon by enclosing the entire command within single-quotes. You can open a temporary UNIX shell by typing, for example, `!csh` or `!sh`. When you are ready to resume your PAUP session, type Ctrl-D to exit the shell and return to PAUP. (As of this writing, the UNIX version has still not been released.)

ALLTREES

Use the **ALLTREES** command to perform an exhaustive search of all possible tree topologies. The syntax is:

ALLTREES [*options* …] ;

The following options are available:

KEEP = *keep-length*
[NO]COLLAPSE
[NO]ENFORCE
CONSTRAINTS = *constraint-tree-name*
[NO]CONVERSE
[NO]INCLUDEANC
ANCSTATES=*ancestral-states-name*
[NO]STATUS

See "Tree-searching options" under "Options Affecting Multiple Commands" earlier in this chapter.

[NO]FD

Unless NOFD is specified, a frequency distribution of tree lengths is output. (Normally, obtaining the frequency distribution is the only reason for doing an exhaustive rather than branch-and-bound search, so you will probably never specify this option.)

HINTERVAL = *interval-value*

Interval-value specifies a class interval for the frequency distribution of tree lengths. By default, HINTERVAL=1, so that the number of trees at each length is output. If HINTERVAL>1, then adjacent tree length values are pooled into tree length classes of width *interval-value.*

SAVEFD

If SAVEFD is specified, the data for the frequency distribution of tree lengths are saved to a text file for input to other programs. The SAVEFD option is not persistent.

FILE = *file-specification*

File-specification specifies the name of the file to receive the frequency distribution (ignored unless SAVEFD is specified). If SAVEFD is specified and FILE is not specified, a default file name is used. If *file-specification* contains any of the characters equal-sign (=), semicolon (;), colon (:), or blank, it must be enclosed within single-quotes. Explicit specification of FILE also implies SAVEFD.

[NO]REPLACE

Ordinarily, if SAVEFD is requested and the specified file (see FILE option above) already exists, you will be prompted for confirmation that the existing file should be replaced. REPLACE suppresses this prompt; the existing file will be quietly overwritten by the new data.

ANCSTATES

The **ANCSTATES** command, used to define ancestral states, is ordinarily issued from within the ASSUMPTIONS block. You may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

ASSUME

Use the **ASSUME** command to invoke a type set, weight set, or exclusion set (see "Simplifying Input with Sets: Invoking Assumption Sets" in Chapter 2), or to select an ancestor (see "Specifying Ancestral States" in Chapter 2). The syntax is:

ASSUME [TYPESET=*type-set-name*] [WTSET=[*weight-set-name*]
[EXSET=*exclusion-set-name*] [ANCSTATES = *ancstates-name*] ;

BANDB

Use the **BANDB** command to search for trees using the branch-and-bound algorithm. The syntax is:

BANDB [*options* …] ;

The following options are available:

KEEP = *keep-length*
[NO]COLLAPSE
[NO]ENFORCE

CONSTRAINTS = *constraint-tree-name*
[NO]CONVERSE
[NO]INCLUDEANC
ANCSTATES=*ancestral-states-name*
[NO]STATUS

See "Tree-searching options" under "Options Affecting Multiple Commands" earlier in this chapter.

UPBOUND=*upper-bound*

Use this option to specify an upper bound on the length of the shortest tree(s). If you do not specify an upper bound on the length of the shortest tree (or if you specify UPPERBOUND=0), PAUP computes a starting upper bound via the stepwise addition algorithm.

ADDSEQ = { FURTHEST | ASIS | SIMPLE }

ADDSEQ specifies the way in which taxa are selected for next addition to the tree at the current node of the search tree. FURTHEST is usually the fastest, although it is not permitted unless all characters are of type ORD or UNORD (it will automatically be overridden by SIMPLE in this case).

[NO]MULPARS

Ordinarily, PAUP saves all minimal trees it finds during the branch-and-bound search. You can use NOMULPARS to save only one of the shortest trees found. If you only want to know the length of the shortest tree(s), use this option. The single tree found is guaranteed to be of minimum length and the search often runs much faster.

[NO]FD

The FD option requests output of a tree-length frequency distribution for all trees of length less than or equal to the value specified for KEEP. KEEP must be set to a value > 0. No trees are saved if FD is specified.

BOOTSTRAP

Use the **BOOTSTRAP** command to perform a bootstrap analysis using either a branch-and-bound or a heuristic search. The syntax is:

BOOTSTRAP [*bootstrap-options* …] [/ *search-options* …];

The options pertaining directly to the bootstrap analysis are:

BSEED = *starting-seed*

Starting-seed specifies an integer between 1 and 2147483646 (= 2^{31}–2) used to seed the random number generator. If you do not specify a starting seed for the first bootstrap analysis, 1 is used. If you do not specify a starting seed for subsequent runs, the seed defaults to the next number in the random number sequence initiated during the previous run.

NREPS = *number-of-replications*

Use NREPS to specify the number of bootstrap replications (resamplings) to be performed. The default is 100.

METHOD = { HEURISTIC | BANDB }

If METHOD=HEURISTIC, a heuristic search is performed for each resampling of the characters. If METHOD=BANDB, the search is performed using the branch-and-bound algorithm.

CONLEVEL = *confidence-value*

Use CONLEVEL to specify the minimum frequency of the bootstrap replicates (expressed as a percentage) in which a group is supported in order to be included in the bootstrap consensus tree (loosely, the width of the confidence interval). For example, to obtain a bootstrap consensus tree that shows only those groups which occurred on more than 80% of the trees, you would specify CONLEVEL=80.

CONLEVEL must be at least 50, which is the default.

[NO]KEEPALL

If you request KEEPALL, groups occurring at frequencies less than CONLEVEL will also be retained in the bootstrap consensus as long as they are compatible with all groups that are already included in the consensus. Effectively, this forces CONLEVEL=50, because any group occurring in 50% or more of the replicates will automatically be compatible with all more frequently occurring groups.

The *search-options* are the same as for the **HSEARCH** command (if METHOD=HEURISTIC) or the **BANDB** command (if METHOD=BANDB).

CHARSET

The **CHARSET** command, used to define character sets, is ordinarily issued from within the ASSUMPTIONS block. However, you may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

CHGPLOT

Use the **CHGPLOT** command to request output of character-state reconstructions for one or more characters on one or more trees. The reconstruction is shown by superimposing the character-states assigned to each node on a plot of the tree. The syntax is:

CHGPLOT *character-list* [/ *options* …] ;

The *character-list* specifies the character(s) for which reconstructions are shown, and consists of one or more character numbers, character names, or character-set names (see "Character lists" earlier in this chapter for details).

The following options are available:

TREES =*tree-list*

The *tree-list* specifies the tree numbers for which reconstructions are to be shown. If this is the first **CHGPLOT** command and you do not specify a tree list, reconstructions are shown for the first tree only.

OPT = { ACCTRAN | DELTRAN | MINF }
ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
[NO]TCOMPRESS

See "Options Affecting Multiple Commands" earlier in this chapter.

If no characters are specified for either a CHGPLOT or POSSPLOT command, the characters are taken to be those plotted in the last invocation of either of these commands. For example, CHGPLOT 1 3 5 7; POSSPLOT will cause both commands to output information for characters 1, 3, 5, and 7.

CONDENSE

Use the CONDENSE command to collapse zero-length branches into polytomies for all trees and then keep only those trees that are unique after the collapsing is accomplished. The syntax is:

CONDENSE [options] ;

Two options are available:

[NO]COLLAPSE

Unless NOCOLLAPSE is specified, branches whose maximum possible length is zero are collapsed to yield polytomies. COLLAPSE reverses the effect of a previous NOCOLLAPSE.

DELDUPES

Unless DELDUPES is specified, duplicate trees will be eliminated.

CONSTRAINTS

Use the **CONSTRAINTS** command to define a constraint tree. The syntax is:

CONSTRAINTS constraint-tree-name = tree-specification ;

The tree specification must follow the format described under "User-Defined Trees" in Chapter 2.

CONTREE

Use the **CONTREE** command to request computation of strict, semistrict (combinable component), Adams, and/or majority-rule consensus trees (see "Consensus Trees" in Chapter 1). The syntax is:

CONTREE [*tree-list*] [/ *options* …] ;

The tree list specifies which trees to include in the consensus; the default is "ALL." The following options are available:

[NO]STRICT

By default, a strict consensus tree is computed. Use NOSTRICT to suppress this computation.

[NO]SEMISTRICT

Specify SEMISTRICT to request computation of a semistrict (combinable component) consensus tree. NOSEMISTRICT reverses the effect of a previous SEMISTRICT specification.

[NO]MAJRULE

Specify MAJRULE to request computation of a semistrict (combinable component) consensus tree. NOMAJRULE reverses the effect of a previous MAJRULE specification.

The following options apply only if MAJRULE is in effect:

CUTOFF = *required-majority-value*

Specifies the percentage of the trees on which a group must appear in order to be retained in the majority-rule consensus. A group must appear on more than this percentage of the trees before it is retained. The default is 50.

[NO]LE50

If LE50 is specified, groups occurring on less than 50% of the trees are retained in the consensus if they are compatible with the groups already on the tree. NOLE50 reverses the effect of a previous LE50 specification.

[NO]GRPFREQ

By default, a table s output that shows all partitions (or groups) occurring on at least one tree and the frequency of each such group. NOGRPFREQ can be used to suppress this output.

[NO]ADAMS

Specify ADAMS to request computation of an Adams consensus tree. NOADAMS reverses the effect of a previous ADAMS specification.

[NO]INDICES

Specify INDICES to request calculation of a variety of consensus indices. The indices computed by PAUP are described in Rohlf (1982) and Swofford (1991) .

[NO]SAVE

If SAVE is specified, a description of all consensus trees computed is output to a file containing a NEXUS-format TREES block. This option is not persistent; you must specify it on every **CONTREE** command for which you want a tree file to be saved.

The following options apply only if SAVE is specified.

FILE = *file-specification*

Specifies the name for the tree file. If *file-specification* contains any of the characters equal-sign (=), semicolon (;), colon (:), or blank, it must be enclosed within single-quotes.

{ REPLACE | APPEND }

Ordinarily, if SAVE is requested and the specified file (see FILE option above) already exists, you will be prompted for confirmation that the existing file should be replaced. Explicit specification of REPLACE suppresses this prompt; the existing file will be quietly overwritten by the new data. Alternatively, you may specify APPEND, in which case a new TREES block will be concatenated to the end of an existing file. The APPEND option is mainly useful only for archival purposes, as current versions of PAUP and MacClade can process only the first TREES block in a file.

For obvious reasons, this option is not persistent.

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
[NO]TCOMPRESS

See "Options Affecting Multiple Commands" earlier in this chapter.

CSTATUS

Use the **CSTATUS** command to request a listing of character-status information for all characters. There are no options.

For each character, the following information is output:

1. The number and name (if any) of the character.
2. If the character is constant (invariant), excluded or uninformative, this is indicated.
3. The character's current type and weight.
4. A list of the states observed for the character.

CTYPE

Use the **CTYPE** command to assign character types to characters. The syntax is:

CTYPE *character-type* : *character-list* [, *character-type* : *character-list*] … ;

The *character-type* must be one of the standard character types (ORD, UNORD, DOLLO, DOLLO.UP, DOLLO.DN, IRREV, IRREV.UP, or IRREV.DN) or the name of a user-defined character type. Each *character-list* consists of one or more character numbers, character names, or character-set names (see "Character lists" earlier in this chapter for details). The characters specified by *character-list* are assigned the immediately preceding *character-type*. Any number of *character-type:character-list* pairs, separated by commas, may be specified.

DEFAULTS

Use the **DEFAULTS** command to specify default option settings for another command. The syntax is:

DEFAULTS *command-name options…* ;

Options for the following commands may be set:

ALLTREES
BANDB
BOOTSTRAP
CONTREE
DESCRIBE
HSEARCH
LAKE

LENFIT
RANDTREES
SAVETREES

For example,

```
DEFAULTS HSEARCH SWAP=NNI ADDSEQ=CLOSEST;
HSEARCH;
```

is equivalent to:

```
HSEARCH SWAP=NNI ADDSEQ=CLOSEST;
```

DELETE

Use the **DELETE** command to delete a taxon from subsequent analyses (see "Deleting and Restoring Taxa" in Chapter 2). The syntax is:

DELETE *taxon-list* [/ options] ;

The available options are:

Unless ONLY is specified, taxa specified in the taxon-list are simply added to the set of currently deleted taxa. If taxa have already been deleted and you want only those taxa specified in *taxon-list* to remain deleted, specify ONLY; any currently deleted taxa not explicitly specified in the list will be restored.

The following three commands pertain if there are trees in memory that will become invalidated by the deletion of taxa.

PRUNE

If PRUNE is specified, newly deleted taxa will be removed ("pruned") from the trees currently in memory, which otherwise remain unmodified.

CLEAR

If CLEAR is specified, any trees currently in memory are simply deleted.

CONDENSE

If CONDENSE is specified, any duplicate trees that result from the removal of taxa (PRUNE option) are deleted.

If you do not specify PRUNE or CLEAR, the program will prompt for your desired action. PRUNE and CLEAR are

mainly useful for batch file processing, where you do not want the program to stop and wait for a response from the user before continuing.

DEROOT

Use the **DEROOT** command to convert all trees in memory from a rooted to an unrooted representation. There are no options.

The circumstances under which you would need to use this command are rather unlikely (see "Rooting and Derooting Trees" in Chapter 2).

DESCRIBE

Use the **DESCRIBE** command to output tree diagrams and associated information. The syntax is:

DESCRIBE [*tree-list*] [/ *options* …];

The *tree-list* specifies the numbers of the trees you wish to describe. If this is the first **DESCRIBE** command and you do not specify a tree list, only the first tree is described.

The following options are available:

PLOT = { CLADOGRAM | PHYLOGRAM | BOTH }

If PLOT=CLADOGRAM, branch lengths on the tree have no meaning, and taxa are aligned at the right edge of the diagram. If PLOT=PHYLOGRAM, branch lengths are drawn proportionally to the number of changes assigned to each branch. PLOT=BOTH requests output of the tree diagram in both CLADOGRAM and PHYLOGRAM formats.

[NO]LINKS

Requests output of a table of assigned, minimum-possible, and maximum-possible branch lengths (see "Table of Branch Lengths and Linkages" in Chapter 2).

[NO]CHGLIST

Requests output of a list of changes in each character (see "Change and Apomorphy Lists" in Chapter 2).

[NO]APOLIST

List of changes along each branch (see "Change and Apomorphy Lists" in Chapter 2).

[NO]DIAG

Requests output of the minimum-possible, assigned, and maximum-possible length of each character, and goodness-of-fit measures based on these quantities (see "Consistency Indices and Goodness-of-Fit Statistics" in Chapter 2).

[NO]PATRISTIC

Requests output of the patristic distance matrix (see "Pairwise Homoplasy and Patristic Distance Matrices" in Chapter 2).

[NO]HOMOPLASY

Requests output of the pairwise homoplasy matrix (see "Pairwise Homoplasy and Patristic Distance Matrices" in Chapter 2).

[NO]LABELNODE

Ordinarily, internal nodes on the tree diagram are labeled with a node number that is referenced by other output information (change lists, apomorphy lists, etc.). NOLABELNODE can be used to suppress the labeling of internal nodes.

XOUT = { NONE | TERMINAL | INTERNAL | BOTH }

Requests output of a table of character-state assignments for each tree. XOUT=INTERNAL requests output of the character-states assigned to internal nodes for each tree. XOUT=TERMINAL requests a listing of the original data matrix. (This option is not particularly useful, as you would ordinarily use the **SHOWMATRIX** command to list the data matrix.) XOUT=BOTH requests a listing of the original data matrix plus the states assigned to internal nodes.

[NO]CSPOSS

Requests a listing of the possible character-state assignments (MPR-sets) for each tree. See "Character-State Reconstructions" in Chapter 2 for more information.

[NO]FVALUE

Requests output of the F-value and F-ratio (see "F-Value and F-Ratio" under "Goodness-of-Fit Statistics" in Chapter 1).

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
[NO]TCOMPRESS
OPT = { ACCTRAN | DELTRAN | MINF }
ANCSTATES=*ancestral-states-name*
[NO]SHOWIGNORE
[NO]CMLABELS
[NO]CMCSTATUS
CMDCOLWID = *column-width*

See "Options Affecting Multiple Commands" earlier in this chapter.

DOS

Use the **DOS** command to execute a DOS command and return immediately to PAUP or to enter a DOS shell (IBM-PC and generic DOS versions only, which do not exist at the time of this writing). The syntax is:

DOS [*dos-command*] ;

If you do not specify a command, PAUP will open a DOS shell. Type `exit` at the DOS prompt when you want to return to PAUP. If you specify a command, control is returned to PAUP immediately after the command has finished. You can type a command that contains a semicolon by enclosing the entire command within single-quotes.

EDIT

Use the **EDIT** command to edit a file using PAUP's editor. The syntax is:

EDIT file-specification ;

See "The PAUP Editor" in Chapter 4 for more information on using the editor. This command is only valid in implementations of PAUP that provide built-in editing capabilities.

EXCLUDE

Use the **EXCLUDE** command to exclude one or more characters from tree-length calculations (see "Excluding Characters" in Chapter 2). The syntax is:

EXCLUDE *character-list* [/ONLY];

Unless /ONLY is specified, characters specified in the character-list are simply added to the set of currently excluded characters. If characters have already been excluded and you want only those characters specified in *character-list* to remain excluded, specify /ONLY; any currently excluded characters not explicitly specified in the list will be re-included.

EXECUTE

Use the **EXECUTE** command to request processing of an input file. The input file should be a valid NEXUS file (see "The NEXUS Format," above). The syntax is:

EXECUTE *file-specification* ;

If *file-specification* contains any of the characters equal-sign (=), semicolon (;), colon (:), or blank, it must be enclosed within single-quotes.

The input file may contain any or all of the following: DATA blocks, ASSUMPTIONS blocks, TREES blocks, and valid PAUP commands. Although not required, you should place PAUP commands inside of a PAUP block so that other programs (e.g., MacClade) can use the same file. Blocks other than DATA, ASSUMPTIONS, TREES, and PAUP are permitted but are ignored by PAUP.

PAUP commands are processed exactly as if they had been entered from the command line, with only a few exceptions (e.g., the EDIT command cannot be issued from a file). Commands are processed until the end of the file is reached, or a QUIT command is encountered.

EXSET

The **EXSET** command, used to define "exclusion sets," is ordinarily issued from within the ASSUMPTIONS block. However, you may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

FILTER

Use the **FILTER** command to filter trees according to length, constraints, or other criteria (see "Filtering Trees" in Chapter 2). The syntax is:

FILTER [NOT] *filtering-criteria* … [PERMDEL] ;

or

FILTER OFF;

Any combination of the following filtering criteria can be specified.

MAXLENGTH = *maximum-tree-length*

If *maximum-tree-length* is nonzero, only trees of length less than or equal to the specified length are retained.

MINLENGTH = *minimum-tree-length*

If *minimum-tree-length* is nonzero, only trees of length greater than or equal to the specified length are retained.

NUMLE = highest-tree-number-to-keep

Keep only trees numbered 1 through *highest-tree-number-to-keep*.

NUMGE = lowest-tree-number-to-keep

Keep only trees numbered between *lowest-tree-number-to-keep* and the number of trees currently in memory.

CONSTRAINTS = *constraint-tree-name*

Only those trees that are compatible with the specified constraint tree are retained.

SD=*symmetric-difference-distance* FROM=*tree-number*

Only those trees within *symmetric-difference-distance* units of the specified tree number are retained.

LESSRESOLV

Keep a tree only if more highly resolved compatible trees do not exist.

If NOT is specified, trees that do *not* satisfy the specified filtering criteria are retained.

If PERMDEL is specified, trees not retained by the filter are permanently deleted from memory. Otherwise, they are only temporarily deleted, and can be recovered by issuing the command:

```
FILTER OFF;
```

GETTREES

Use the **GETTREES** command to load trees into memory from a file containing a NEXUS-format TREES block. The syntax is:

GETTREES FILE=*file-name* [/ options …] ;

The available options are:

FROM = *starting-tree-number*

If nonzero, *starting-tree-number* specifies the number of the first tree to get.

TO = *ending-tree-number*

If nonzero, *ending-tree-number* specifies the number of the last tree to get.

MODE = { 1 | 2 | 3 | 4 | 5 | 7 }

By default (MODE=3), any preexisting trees in memory are replaced by the trees read from the file. The MODE setting allows you to alter this behavior. Let *M* = the set of trees originally in memory and *T* = the set of trees from the tree file. The following mode values are then available:

1 = replace M by T – M (i.e., keep trees from the file that are *not* originally in memory)

2 = replace M by T ∩ M (keep trees from the file that are *also* originally in memory)

3 = replace M by T (i.e., replace all trees in memory by all trees from the file)

4 = replace M by M – T (i.e., keep trees in memory that are *not* also in the file)

5 = replace M by M T (i.e., keep trees that are either currently in memory or in the file, but not both places)

7 = replace M by M T (i.e., append trees from file to trees originally in memory, with elimination of duplicates)

ROOT

If directed character types are in effect for one or more characters and the trees being input are unrooted, you will ordinarily be asked if you want to convert the trees to rooted trees (otherwise, you will not be able to use the new trees unless you change the character types or root them later). If you specify ROOT explicitly, this conversion will occur automatically without a prompt.

DEROOT

If the trees being input are rooted but all character types are currently undirected, you will ordinarily be asked if you want to convert the trees to unrooted trees. If you specify DEROOT explicitly, this conversion will occur automatically without a prompt.

HELP

Use the **HELP** command to obtain help on using PAUP's command-line interface. The syntax is:

HELP [{COMMANDS | *command-name*}] ;

If invoked with no arguments, **HELP** produces a list of the available commands. If COMMANDS (or CMDS) is specified, a one-line description of each command is output. If a command-name is specified, **HELP** provides information of that command.

Examples:

```
HELP;          [requests a list of available commands]

HELP COMMANDS; [requests a list of available
                  commands, with a one- line
                  description of each]

HELP EXEC;     [requests help on the EXEC command]
```

HSEARCH

Use the **HSEARCH** command to search for optimal trees using heuristic algorithms. The syntax is:

HSEARCH [*options* …] ;

The following options are available:

KEEP = *keep-length*
[NO]COLLAPSE
[NO]ENFORCE
CONSTRAINTS = *constraint-tree-name*
[NO]CONVERSE
[NO]INCLUDEANC
ANCSTATES = *ancestral-states-name*
[NO]STATUS

See "Tree-searching options" under "Options Affecting Multiple Commands" earlier in this chapter.

[NO]STEPWISE

Ordinarily, starting trees for branch swapping are obtained via a stepwise addition procedure. Alternatively, you can request that trees already in memory be used as the starting point by specifying NOSTEPWISE. FROMTREE and/or TOTREE can be used to select a subset of the available trees as the starting point for branch swapping.

ADDSEQ={SIMPLE | CLOSEST | ASIS | RANDOM }

Use ADDSEQ to specify the addition sequence to be used in the stepwise addition procedure. These addition sequences are described in the section "Stepwise Addition".

Options for ADDSEQ = SIMPLE:

REFTAX = *reference-taxon-number*

By default, the first taxon in the data file is used as the reference taxon. Use REFTAX to specify an alternate reference taxon. This option is relevant only for unrooted-tree searches. For rooted-tree searches, the hypothetical ancestor (see ANCSTATES and ASSUME commands) is used as the reference taxon.

Options for ADDSEQ = RANDOM:

NREPS = *number-of-replications*

Use NREPS to specify the number of random-addition-sequence replications to be performed. The default is 10.

RSEED = *seed-value*

Use RSEED to initialize the seed used to generate pseudorandom numbers used in obtaining random addition sequences. The seed value must be between 1 and 2147483646, inclusive. The seed is set to 1 when PAUP first starts; it is updated every time a new random number is generated.

[NO]RSTATUS

A status report showing the results of each random-addition-sequence replication is normally output; you can use NORSTATUS to suppress this output. The status report is very useful in evaluating the effectiveness of the heuristic search (see the "Stepwise Addition" section for a description of random addition sequences).

HOLD = *n*

Specifies the number of trees to be help at each cycle of the stepwise-addition procedure (see the section "Stepwise Addition"). By default, HOLD=1, so that a single tree is held at each step. However, setting HOLD > 1 sometimes improves the length of the tree found by stepwise addition.

SWAP = { TBR | SPR | NNI | NONE }

Specifies the algorithm used by branch-swapping: TBR = tree bisection-reconnection, SPR = subtree pruning-regrafting, NNI = nearest-neighbor interchange, NONE=no branch swapping performed. NNI rearrangements are a subset of those done by SPR, and SPR rearrangements of those done by TBR. Ordinarily, you will use TBR, but SPR or NNI can be used to reduce search times.

[NO]MULPARS

Ordinarily, PAUP saves all minimal trees it finds during branch swapping. You can use NOMULPARS to save only one of the shortest trees found. Use of NOMULPARS is not recommended,

as it can drastically reduce the ability of branch swapping to find the shortest tree (see the section on "Branch Swapping").

FROMTREE = *tree-number*
TOTREE = *tree-number*

Specify FROMTREE and/or TOTREE to request use of a subset of the available trees for input to the branch-swapping procedure. Only trees with numbers ≥ FROMTREE and ≤ TOTREE are input to branch swapping. FROMTREE defaults to 1 and TOTREE defaults to the number of trees in memory; so that ordinarily all trees are swapped on. These options are meaningful only if NOSTEPWISE is specified.

[NO]USENONMIN

If NOSTEPWISE or HOLD > 1 is requested, it is possible that the trees in memory at the time branch-swapping begins are not all equal in length. Ordinarily, only the shortest available trees are input to the branch-swapping procedure. If you want to swap on nonminimal trees as well, specify USENONMIN. NOUSENONMIN reverses the effect of a previous USENONMIN specification.

[NO]STEEPEST

Specify STEEPEST to request use of the steepest-descent modification to the branch-swapping procedure (see the section on "Branch Swapping"). NOSTEEPEST reverses the effect of a previous STEEPEST specification.

NCHUCK = *maximum-number*
CHUCKLEN = *tree-length-for-chucking*

If this pair of options is used, no more than *maximum-number* of trees of length greater than or equal to *tree-length-for-chucking* will be retained in a search (or in a random-addition-sequence replicate). See "Heuristic Methods" in Chapter 2 for more information on these settings.

[NO]ABORTREP

If ABORTREP is requested and NCHUCK and CHUCKLEN values have been specified, the current random-addition-sequence replicate will be aborted if the "chucking" limits are hit.

RETAIN = *number-of-trees-to-retain*

The first *number-of-trees-to-retain* will be retained in memory throughout the search. (Ordinarily, all trees initially in memory will be replaced by trees found during the search.) The RETAIN option is not persistent; you must reset it for every HSEARCH command if you want to continue retaining the same set of initial trees.

[NO]RESETDSEED

When trees containing polytomies (e.g., due to the COLLAPSE option) are input to the swapping procedure, they must first be "dichotomized". PAUP does this by randomly resolving each polytomous node in order to obtain a binary tree. The seed for this randomization is ordinarily reset to the same initial value at the beginning of every search (or every random addition-sequence-replicate, if applicable) so that a search will generate repeatable results. However, NORESETDSEED may produce alternative dichotomizations that "get lucky" in generating better rearrangements.

INCLUDE

Use the **INCLUDE** command to re-include characters that were previously excluded (see "Excluding Characters" in Chapter 2). The syntax is:

INCLUDE *character-list* [/ONLY];

Unless /ONLY is specified, characters specified in the character list are simply removed from the set of currently excluded characters. If you want only those characters specified in the list to be included, specify /ONLY; characters not explicitly specified in the list will then be excluded.

INGROUP

Use the **INGROUP** command to return one or more taxa to the ingroup. The syntax is:

INGROUP *taxon-list* [/ONLY] ;

Unless /ONLY is specified, taxa specified in the taxon-list are simply removed from the current outgroup. If you want only those taxa specified in *taxon-list* to be included in the ingroup, specify /ONLY; any taxon that is not explicitly specified in the list will be transferred to the outgroup.

LAKE

Use the **LAKE** command to perform an analysis using Lake's (1987b) method of linear invariants. This command is available only when DATATYPE=DNA or DATATYPE=RNA. The syntax is:

LAKE [*options* …] ;

The following options are available:

MODE = { CHOOSE4 | ALLQUART | FOURGRPS }

If there are more than 4 (non-deleted) taxa in the data set, this option specifies whether you want to choose 4 taxa for analysis (MODE=CHOOSE4), analyze all possible quartets (MODE=ALLQUART), or divide the taxa into four groups and analyze all quartets containing one member from each group (MODE=FOURGRPS).

[NO]SPECTDIST

SPECTDIST requests the output of the spectral distribution (the number of positions falling into each of the 36 possible patterns considered informative by Lake's method).

[NO]BRLEN

BRLEN requests output of branch-lengths calculated by Lake's "operator metrics" (Lake, 1987a) .

[NO]SUMTABONLY

SUMTABONLY limits the output to a summary table of results rather than outputting results for each quartet.

EXACTN = *n*

An exact binomial test (rather than the chi-square approximation) will be used to test the significance of deviations of invariant scores from 0 if the number of informative positions is less than *n*.

If MODE=CHOOSE4, you must specify exactly four taxa using the following option:

TAXA = *taxon-list*

The taxon-list must contain exactly four taxa.

If MODE=ALLQUART, you may limit the number of included taxa by selecting *at least* four taxa using the following option:

TAXA = *taxon-list*

If MODE=FOURGRPS, you must assign at least one taxon to each if the four groups using the following options:

GRPA = *taxon-list-for-first-group*
GRPB = *taxon-list-for-second-group*
GRPC = *taxon-list-for-third-group*
GRPD = *taxon-list-for-fourth-group*

LEAVE

Use the **LEAVE** command to terminate processing of an input file. Ordinarily, PAUP continues processing until the end of an input file is reached. If, for whatever reasons, you do not want some of the commands in the input file to be processed, insert a **LEAVE** command into the file at the point where you want execution to stop. PAUP then continues as if the end of the file were reached at that point. **LEAVE** has no effect if issued interactively.

LENFIT

Use the LENFIT command to request a listing of tree lengths and/or fit measures for one or more trees. The syntax is:

LENFIT [*tree-list*] [/ *options ...*] ;

If no tree list is specified, ALL is assumed. By default, only tree lengths (for single characters, overall, or both) are output. The following options are available:

SINGLE = { ALL | VAR | <u>NO</u> }

The single option specifies the type of single-character output. If SINGLE=ALL, lengths (or fit measures) are output for all trees specified by *tree-list.* If SINGLE=VAR, lengths (or fit measures) are output only for those characters whose lengths vary among the trees specified by *tree-list*. If SINGLE=NO, no single-character tree lengths (or fit measures) are output.

[NO]RANGE

If RANGE is specified, only the minimum and maximum tree lengths (or best and worst fit measures) are output for each character.

[NO]TOTAL

If NOTOTAL is specified, overall tree lengths and fit measures are not output.

[NO]TL

Unless NOTL is specified, tree lengths are output. The default is to show tree lengths and no other fit measures.

[NO]CI

Use CI to request output of consistency indices.

[NO]RI

Use RI to request output of retention indices.

[NO]RC

Use RC to request output of rescaled consistency indices.

[NO]HI

Use HI to request output of homoplasy indices.

LOG

By default, output generated by PAUP goes only to the "display buffer," a region of memory set aside exclusively for this purpose. PAUP's main display window is used to view this information. The **LOG** command may be used to request direction of PAUP output to a file (e.g., for subsequent printing). The syntax is:

```
LOG
    [ FILE = file-specification ]
    [ { START | STOP } ]
    [ { APPEND | REPLACE } ]
    [ [NO]FLUSH ] ;
```

If START is specified (the default), logging is initiated to the named file. If FILE is not specified explicitly, a default name is assigned. If APPEND is specified, subsequent output is appended to the previous contents (if

any) of the file. Otherwise, subsequent output will overwrite the original contents of the file. The APPEND/REPLACE setting is retained between invocations of the **LOG** command, unless the file is changed by a FILE= directive. If REPLACE is not specified explicitly and the file already exists, you will receive a warning and will have the opportunity to cancel the command before the contents of the existing file are erased. Specification of FLUSH causes the file's buffer to be flushed after every line of output. Ordinarily, this degrades system performance and is not recommended. However, there may be situations in which immediate flushing is useful.

If the *file-specification* contains any of the characters equal-sign (=), semicolon (;), colon (:), or blank, it must be enclosed within single-quotes.

Examples:

```
LOG FILE=MyOutput;
```

(subsequent output is saved to file 'MyOutput')

```
LOG STOP;
```

(subsequent output no longer saved to file)

```
LOG START APPEND;
```

(resume logging, appending new output to file 'MyOutput')

```
LOG STOP;
```

(suspend logging once again)

```
LOG START;
```

(resume logging again, appending to 'MyOutput')

```
LOG FILE=NewOutput;
```

(begin logging to a different file ('NewOutput'), replacing it if it already exists)

MEMAVAIL

Use the **MEMAVAIL** command to find out how much memory is still available from the operating system (DOS/portable version only). There are no options.

OUTGROUP

Use the **OUTGROUP** command to assign one or more taxa to the outgroup. The syntax is:

OUTGROUP *taxon-list* [/ONLY] ;

Unless /ONLY is specified, taxa specified in the taxon-list are simply added to the current outgroup. If taxa have already been assigned to the outgroup and you want only those taxa specified in *taxon-list* to remain in the outgroup, specify /ONLY; any taxon that is not explicitly specified in the list will be transferred to the ingroup.

POSSPLOT

Use the **POSSPLOT** command to request output of possible character-state assignments (MPR-sets) for one or more characters on one or more trees, by superimposing the possible character-states for each node on a diagram of the tree. The syntax is:

POSSPLOT *character-list* [/ *options…*] ;

The *character-list* specifies the character(s) for which possible character-state assignments are shown, and consists of one or more character numbers, character names, or character-set names (see "Character lists" earlier in this chapter for details).

The following options are available:

TREES = *tree-list*

The *tree-list* specifies the tree numbers for which possible character-state assignments are to be shown. If this is the first **POSSPLOT** command and you do not specify a tree list, reconstructions are shown for the first tree only.

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
ANCSTATES = *ancestral-states-name*
[NO]TCOMPRESS

See "Options Affecting Multiple Commands" earlier in this chapter.

If no characters are specified for either a CHGPLOT or POSSPLOT command, the characters are taken to be those plotted in the last invocation of either of these commands. For example, `CHGPLOT 1 3 5 7;`

`POSSPLOT;` will cause both commands to output information for characters 1, 3, 5, and 7.

QUIT

The **QUIT** command, which has no options, causes PAUP to terminate.

RANDTREES

Use the RANDTREES command to randomly sample trees from the set of all possible trees and compute their lengths. The results are shown in the form of a frequency distribution of tree lengths. The syntax is:

RANDTREES [*options* …] ;

The following options are available:

TSEED = *starting-seed*

Starting-seed specifies an integer between 1 and 2147483646 (= 2^{31}–2) used to seed the random number generator. If you do not specify a starting seed for the first random-trees analysis, 1 is used. If you do not specify a starting seed for subsequent runs, the seed defaults to the next number in the random number sequence initiated during the previous run.

NREPS = *number-of-replications*

Number-of-replications specifies the number of random trees to be evaluated. The default is NREPS =1000.

HINTERVAL = *interval-value*
SAVEFD
FILE = *file-specification*
[NO]REPLACE
[NO]INCLUDEANC
ANCSTATES=*ancestral-states-name*

These options have the same meaning as in the ALLTREES command (see above).

RESTORE

Use the **RESTORE** command to restore currently deleted taxa for subsequent analyses (see "Deleting and Restoring Taxa" in Chapter 2). The syntax is:

RESTORE *taxon-list* [/ONLY];

Unless /ONLY is specified, taxa specified in the taxon list are simply removed from the set of currently deleted taxa. Specify /ONLY to delete, in addition, any taxon not explicitly specified in the list.

REVFILTER

Use the **REVFILTER** command to "reverse" the effect of the current filter. All trees that are currently hidden by the filter will become visible, and all trees that were previously visible will be hidden. There are no options.

REWEIGHT

Use the **REWEIGHT** command to assign weights to the characters based on their fit to the trees currently in memory (see "Successive weighting" in Chapter 2). The syntax is:

REWEIGHT [/ *options* …] ;

The following options are available:

BASEWT = *base-weight*

Base-weight specifies the maximum possible weight that a character can be assigned, corresponding to an index value (see below) of 1. Weights are scaled from 0 to this value. The default is BASEWT=1000.

INDEX = { RC | CI | RI }

The INDEX option is used to specify which fit measure to use when calculating the new character weights (RC = rescaled consistency index, CI = consistency index, RI = retention index).

FIT = { MAXIMUM | MINIMUM | MEAN }

The FIT option is used to specify whether the new weights are based on the maximum, minimum, or mean of the fit values for each character over all of the trees in memory.

[NO]TRUNCATE

Because PAUP uses integers to represent weights, they are ordinarily scaled to the nearest integer by rounding. Use the TRUNCATE option if you want to simply discard the fractional part (e.g., 8.7 goes to 8 rather than 9). I can think of no reason to use this option other than to

duplicate the weights obtained using the Hennig86 program (Farris, 1988) .

ROOT

Use the **ROOT** command to convert all trees in memory from an unrooted to a rooted representation. Trees are rooted according to the currently specified outgroup. There are no options.

The circumstances under which you would need to use this command are rather unlikely (see "Rooting and Derooting Trees" in Chapter 2).

SAVEASSUM

Use the **SAVEASSUM** command to save the current character-type, character-weight, character-exclusion, ancestral-states, taxon-deletion, and outgroup status to a file in ASSUMPTIONS and a PAUP block. You can restore the settings in effect at the time the SAVEASSUM command was issued simply by executing the commands stored in the file (use the **EXECUTE** command for this purpose). The syntax is:

SAVEASSUM FILE=*save-file-name* [REPLACE] ;

By default, if the specified file already exists, you will be asked if you want to replace it . To suppress this warning, specify REPLACE; in this case the file will be quietly overwritten.

SAVETREES

Use the **SAVETREES** command to write trees currently in memory to a file as a NEXUS-format TREES block or as a treefile accepted by another program. The syntax is:

SAVETREES [/ *options* …] ;

The following options are available:

FMT = { NEXUS | ALTNEX | FREQPARS | PHYLIP | HENNIG }

Specifies the type of treefile to be produced. NEXUS requests a file containing the standard NEXUS TREES block using a translation table (which greatly reduces the amount of disk space required to store the trees). ALTNEX also specifies a NEXUS TREES block, but no translation table is used (the full taxon names are included in each tree description). FREQPARS requests a treefile for the FREQPARS program described by Swofford and Berlocher (1987) . PHYLIP requests a treefile for input to version 3.4 of Felsenstein's (1991) PHYLIP package.

HENNIG requests a treefile for version 1.5 of Farris's (1988) Hennig86 program.

[NO]BRLENS

If BRLENS is specified, tree descriptions will include branch lengths if the program corresponding to the FMT setting supports them. NOBRLENS reverses the effect of a previous BRLENS specification.

FILE = *tree-file-name*

Specifies a name for the tree file. If you do not explicitly specify a filename, a default filename will be used.

FROM = *starting-tree-number*

If nonzero, *starting-tree-number* specifies the number of the first tree to save.

TO = *ending-tree-number*

If nonzero, *ending-tree-number* specifies the number of the last tree to save.

[NO]ROOT

If the current trees in memory are unrooted and ROOT is specified, trees are rooted (using the rooting options currently in effect) before they are saved. Note that the process of rooting the trees slows down the saving operation considerably. If you are saving the trees only with the intention of rereading them into PAUP, there is no need to root the trees. However, if you are exporting the trees to another program, rooting them may be desirable.

The ROOT option has no effect if the trees in memory are already rooted. NOROOT can be used to reverse the effect of a previous ROOT specification.

{ REPLACE | APPEND }

Ordinarily, if SAVE is requested and the specified file (see FILE option above) already exists, you will be prompted for confirmation that the existing file should be replaced. Explicit specification of REPLACE suppresses this prompt; the existing file will be quietly overwritten by the new data. Alternatively, you may specify APPEND, in which case a new TREES block will be concatenated to the end of an existing file. The APPEND option is

mainly useful only for archival purposes, as current versions of PAUP and MacClade can process only the first TREES block in a file.

For obvious reasons, this option is not persistent.

SET

The **SET** command is used to set a variety of options whose scope extends beyond single commands. The syntax is:

SET *option-specification …* ;

The available options are as follows:

[NO]DISPLAY

NODISPLAY suppresses output to the "main display" (window or terminal screen) and is useful when you want to send output to the log file and/or printer only. DISPLAY reactivates the main display.

At least one output destination must be active at all times. Consequently, if no log file is active or the "echo to printer" (ECHO) option is not set, output will be sent to the main display even if NODISPLAY has been requested.

MSTAXA = { UNCERTAIN | POLYMORPH }

The MSTAXA option specifies how PAUP treats multistate taxa (see "Multistate Taxa" in Chapter 2). This option is set at the time the data matrix is processed (see description of the **OPTIONS** command under "Commands used in the DATA Block" earlier in this chapter). You can change the interpretation of multistate taxa at any time by specifying MSTAXA in a **SET** command.

[NO]SEMIGRAPH

PAUP uses special characters in its internal font to draw trees and other items. On the IBM-PC, these characters are nonstandard "high ASCII" characters. On the Macintosh, these characters are neither in the standard 128 ASCII characters nor in the set of special characters normally included with Macintosh fonts. Thus, although the trees look nice when drawn in the main display window, they may not look right when printed on some printers. Therefore, PAUP ordinarily translates these "semigraphics" characters to standard ASCII substitutes when output is directed to

a printer, file, or document window. If you want to override this behavior, specify SEMIGRAPH. (E.g., many IBM-PC printers can print the high ASCII characters, and the Apple LaserWriter can create a "bit map" version of PAUP's internal "PAUPMonaco" font).

This option is relevant only for IBM-PC and MACINTOSH versions only.

The following three options affect the setting of the maximum number of trees that PAUP can store at any given time:

MAXTREES = *n*

The MAXTREES parameter specifies the maximum number of trees that can be saved. Setting MAXTREES to a large value will reduce the likelihood that the tree buffer will become full during a search or tree-file operation, at the expense of a larger chunk of memory being tied up and therefore unavailable for other purposes.

Ordinarily, if the number of trees found during a search reaches the value of MAXTREES, you will be given a chance to increase MAXTREES before proceeding. This behavior can be altered using the INCREASE option (see below).

MAXTREES is initially set to 100.

INCREASE = { PROMPT | AUTO | NO }

The setting of the INCREASE option determines the action taken by PAUP if the limit on the number of trees that can be stored (=MAXTREES, see above) is reached during a search or a tree-file operation. If INCREASE=PROMPT, you will be given the opportunity to increase MAXTREES. If INCREASE=AUTO, MAXTREES will automatically be increased by a number of trees equal to the current AUTOINC setting (see below). If INCREASE=NO, MAXTREES will not be increased, and no prompt will be issued. In this case, a "tree-buffer overflow" occurs which can affect the effectiveness of the search in progress. (The tree-buffer overflow condition will be documented in the output.)

AUTOINC = *n*

The AUTOINC value species the number of trees by which MAXTREES is increased when the number of trees saved

reaches MAXTREES and the INCREASE=AUTO option is in effect. AUTOINC is initially set to 100.

The following three options specify whether PAUP sounds a "beep" when various kinds of errors occur:

[NO]ERRORBEEP

Ordinarily, PAUP beeps to alert you that an error message has been issued. Specify NOERRORBEEP to suppress these beeps.

[NO]QUERYBEEP

Ordinarily, PAUP beeps to alert you when it stops for your input before it can continue a process. Specify NOQUERYBEEP to suppress these beeps.

[NO]KEYBEEP

Ordinarily, PAUP beeps when you type a key that is invalid in the current context. Specify NOKEYBEEP to suppress these beeps.

[NO]ERRORSTOP

Ordinarily, PAUP stops processing an input file when unrecognized commands, OPTIONS-command keywords, or formats are encountered. If NOERRORSTOP is specified, a warning message is issued and processing is allowed to continue.

[NO]WARNRESET

Ordinarily, PAUP issues a warning message when an input file containing a DATA block is executed and a DATA block has already been processed. Specification of NOWARNRESET suppresses this warning.

TORDER = { STANDARD | RIGHT | LEFT | ALPHABET }

Specifies the convention used to "order" the tree (see "Changing the Order of Taxa on Trees" in Chapter 2).

STEPMATRIX = OBSONLY/ALLSTATES/THREEPLUS1

When a stepmatrix contains three or more character states, it is possible that full minimization of the tree length may require assignments of character states that were not observed in any of the

terminal taxa to internal nodes. When only a few character states were observed for a stepmatrix character, but the stepmatrix is defined for a large number of character states. PAUP provides three options for dealing with this problem.

If STEPMATRIX=OBSONLY, only those character states observed in terminal taxa are candidates for assignment to internal nodes.

If STEPMATRIX=ALLSTATES, any state contained in the stepmatrix definition may be assigned to internal nodes, regardless of whether it was observed in a terminal taxon.

If STEPMATRIX= THREEPLUS1, PAUP provides a compromise between these two extremes. Character states which satisfy the "3+1" rule are considered candidates for assignments at internal nodes. See the section "Stepmatrices: Special Considerations" in Chapter 2 for a detailed explanation of the "3+1" rule. I

The following two options are specific to the Macintosh version. See the description of the **Searching…** menu command in Chapter 4 for more information.

[NO]BACKGROUND

Ordinarily, PAUP continues processing when it is moved to the background. Specify NOBACKGROUND to suppress background processing, thereby giving more time to the foreground application.

[NO]CHECKEVTS

NOCHECKEVTS disables "event-checking," causing all mouse clicks an key presses to be ignored. Speed of PAUP searches is improved somewhat, but it will not be possible to stop the search (without restarting the computer) or to switch to a different application under MultiFinder.

The following options are described in the section "Options Affecting Multiple Commands" earlier in this chapter:

[NO]COLLAPSE
[NO]ENFORCE
CONSTRAINTS = *contraint-tree-name*
[NO]CONVERSE
[NO]INCLUDEANC
[NO]STATUS

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
[NO]TCOMPRESS
OPT = { ACCTRAN | DELTRAN | MINF }
[NO]SHOWIGNORE
[NO]CMLABELS
[NO]CMCSTATUS
CMDCOLWID = *column-width*

SHOWANC

Use the **SHOWANC** command to request a listing of the ancestral character-states currently in effect (see "Defining Ancestral States" in Chapter 2). No options are available.

SHOWCONSTR

Use the **SHOWCONSTR** command to show one or more constraint-tree definitions. The syntax is:

SHOWCONSTR [{ *constraint-tree-name* | ALL }];

If you do not specify a name (or ALL), the current default constraint tree will be shown. Specify ALL to show all constraint trees that have been defined.

SHOWDIST

Use the SHOWDIST command to output a matrix of "distances" between taxa (see Chapter 2). There are no options.

SHOWMATRIX

Use the **SHOWMATRIX** command to list the current data matrix. The syntax is:

SHOWMATRIX [*options …*] ;

The options available are listed under "Options for character-matrix listings" earlier in this chapter.

SHOWTREES

Use the **SHOWTREES** command to request a diagram of one or more trees with no other information (see also the **DESCRIBE** command). The syntax is:

SHOWTREES [*tree-list*] [/ *options*];

TCOMPRESS

This option is described in the section "Options Affecting Multiple Commands" earlier in this chapter.

ROOT = { OUTGROUP | LUNDBERG | MIDPOINT }
OUTROOT = { POLYTOMY | PARAPHYL | MONOPHYL }
ANCSTATES = *ancestral-states-name*

See "Options affecting multiple commands" above. ANCSTATES is ignored unless LUNDBERG rooting is in effect.

SHOWUSER

Use the SHOWUSER command to show all user-defined character types (see "Verifying USERTYPE definitions" in Chapter 2 for examples). There are no options.

TAXSET

Use the **TAXSET** command to define a "taxon set." Taxon sets are simply groups of taxa that can be referred to by a single name in other commands. The syntax is:

TAXSET *taxon-set-name = taxon-list* ;

The *taxon-set-name* must not be identical to any of the original taxon names.

See "Simplifying Input with 'Sets'" in Chapter 2 for examples on the use of taxon sets.

TREEDIST

Use the **TREEDIST** command to request output of a matrix of tree-to-tree distances computed according to the symmetric-difference or "partition" metric (Penny and Hendy, 1985) . The syntax is:

TREEDIST [options] ;

The available options are:

COMPARE=*tree-number*

If *tree-number* equals 0, a matrix of all pairwise comparisons is calculated. If *tree-number* > 0, the specified tree is compared to all others.

[NO]FD

By default, the frequency distribution of tree-to-tree distances is output. Use NOFD to suppress this output, or FD to reverse the effect of a previous NOFD specification.

[NO}SHOWALL

By default, a matrix of the tree-to-tree distances for all comparisons performed is output. Use NOSHOWALL to suppress the output, or SHOWALL to reverse the effect of a previous NOSHOWALL specification.

TREEINFO

Use the **TREEINFO** command to obtain information on the status of trees currently in memory. No options are available.

TSTATUS

Use the TSTATUS command to obtain information on which taxa, if any, are deleted and which taxa have been assigned to the outgroup. No options are available.

TYPESET

The **TYPESET** command, used to define a type set, is ordinarily issued from within the ASSUMPTIONS block. However, you may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

USERTREE

Use the **USERTREE** command to input a single user-defined tree. The syntax is:

USERTREE *tree-specification* ;

Ordinarily, you should use a TREES block to input one or more user-defined trees. This command merely provides a mechanism for quickly specifying a user-defined tree from the command line, which may be useful in certain situations.

USERTYPE

The **USERTYPE** command, used to define a user-defined character type, is ordinarily issued from within the ASSUMPTIONS block. However, you may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

WEIGHTS

Use the WEIGHTS command to assign weights to one or more characters. The syntax is:

> WEIGHTS *character-weight* : *character-list* [, *character-weights*: *character-list*] … ;

The *character-weight* must be a valid character weight (see "Tree Lengths and Character Weights" in Chapter 1). Each *character-list* consists of one or more character numbers, character names, or character-set names (see "Character lists" earlier in this chapter for details). The characters specified by *character-list* are assigned the immediately preceding *character-weight*. Any number of *character-weight:character-list* pairs, separated by commas, may be specified.

WTS

The **WTS** command is a synonym for the **WEIGHTS** command. For some reason, I have a hard time typing "weights."

WTSET

The **WTSET** command, used to define a weight set, is ordinarily issued from within the ASSUMPTIONS block. However, you may also issue it from the command line or from within a PAUP block. See "Commands used in the ASSUMPTIONS Block" for the description of this command.

Chapter 4
THE MACINTOSH INTERFACE

This chapter describes aspects of running PAUP that are specific to the Apple® Macintosh™. PAUP has a standard Macintosh interface including pulldown and pop-up menus, dialog boxes, and scrollable lists. This chapter assumes that you are familiar with these basic interface elements. It also assumes that you already know how to use your Macintosh computer. If this is not the case, you should read or review the documentation that came with your computer. PAUP may be started by double-clicking on the PAUP application or document icon, or, under System 7, by dragging a PAUP or MacClade document icon onto the PAUP application icon.

INSTALLATION

PAUP uses no special installation procedure. Just copy the application and help files from the distribution diskette to your hard disk. (If you run PAUP from a floppy disk, always do this from a copy of the original disk). You may also want to copy the Sample Data Files folder.

Be sure the read the file READ ME FIRST! It contains information pertaining to errors in the manual or changes made to the program after the manual was printed. This is a standard text file and can be read by any text editor, word processor, or by PAUP.

Note to PAUP/Mac 3.0 users: You can delete the PAUP Defaults file (if any) in your System Folder. PAUP/Mac 3.1 stores its settings in a file named PAUP Preferences, located in the Preferences Folder (under System 7.0 and later) or the System Folder (under systems earlier than 7.0).

THE PAUP EDITOR

The PAUP editor has most of the features expected of a Macintosh text editor, including horizontal and vertical scroll bars, cut, copy, paste, clear, and undo facilities, selection of a "word" by double-clicking, etc. You may also select an entire line by triple-clicking.

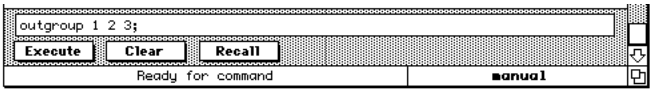
You may set the default font, size, and tab width by choosing **Editor**… from the Options menu. Although not required, use of a monospaced font like Monaco or Courier is recommended so that columns of the data matrix will line up cleanly. (The default font, PAUPMonaco, is a modification of Monaco that overcomes some of the shortcomings of Monaco. Specifically, it distinguishes between zero and the upper-case "oh," between the lower-case "el" and the upper-case "eye," and increases the size of several punctuation characters for improved readability. You can set font and tab settings for individual windows using the **Set Tabs and Fonts** dialog under the **Edit..** menu. Font/tab settings chosen there automatically override the default settings. You can change the font/tab settings for all open windows by checking the box Apply to all open windows in the **Editor...** menu command.

To edit a data file, choose the **Edit** option in the **Open** dialog box. When the file is opened, a window will appear with the data file in it. You can then make changes to the data file as you like. In order to use the file, you must then *execute* it (whether or not you saved your editing). Alternatively, if you have opened and executed the file already, it will appear in the Windows menu. Simply select the file, and the editing window will appear. If you close the window without saving your changes, PAUP will prompt you for the appropriate action.

If you wish to create a new file, choose the **New** dialog box. An untitled window will appear. You can enter a new data matrix, or paste in data from another file. When you are done, save your work and execute the file, as above.

THE COMMAND LINE

All of PAUP's capabilities may be invoked using either the menus or the command line.



The Command Line.

To use the command line, choose **Show Command Line** from the **Windows** menu and type in the desired command. For a complete list of available commands, see the section "Command Reference." When the command line is open, type in the appropriate command and press the Return or Enter keys to execute it (or click on the *Execute* button). The *Clear* button or the *Escape* key will clear the command line, while the *Recall* button will bring up the last command typed.

SELECTING ITEMS IN LISTS

Many dialog boxes contain scrollable lists from which you may select more than one item (e.g., lists containing tree numbers, character numbers/names and taxon names). To select multiple items, you must either "shift-click" or "command-click." In lists that allow multiple selections, if neither the shift key nor the command key is held down, a click causes all current selections to be deselected and the item receiving the click to be selected. If the shift key is held down, then as long as the mouse button is held down, the current selection is either expanded or contracted. If the command key is held down, then the selection status of a cell receiving a click is toggled. (I.e., if it was originally selected, it becomes deselected, and vice versa).

RUNNING UNDER MULTIFINDER® (OR SYSTEM 7.0 AND LATER)

PAUP is completely "MultiFinder-friendly." In particular, long operations such as tree searches can be run in the background under MultiFinder, allowing you to use your computer for other work during this time. Of course, search times will not be as fast as when PAUP is running in the foreground, but the benefits of not having the computer totally tied up may outweigh the extra time needed for the search. See the "Macintosh® System Software User's Guide" for details on the use of MultiFinder.

I have tried very hard to minimize the impact of PAUP background processes on the foreground application (e.g., keyboard delays, etc., that you may have seen with other programs). Unfortunately, however, in some situations PAUP may cause an unacceptable degradation in the performance of the foreground application. If this happens, you may want

to disable the **Allow background processing under Multifinder** option (**Searching…** command of the Options menu; see below). With the option disabled, PAUP will simply "sleep" while it is in the background, allowing the foreground application full control of the processor. When PAUP is brought to the foreground, its execution will resume. For example, you could start a time-consuming search at the end of the day. If it had not completed by the next morning, you could move PAUP to the background while you used your machine for other activities during the day, and then resume PAUP execution before leaving again that evening.

If a PAUP process finishes while some other application is currently in the foreground and you are running version 6.0 or later of the Macintosh operating system, you will receive notification in two forms. First, an alert box will be presented notifying you that the process has completed. Then, a small PAUP icon will alternate with the Apple logo in the menu bar until PAUP is returned to the foreground

Remember that each application running under MultiFinder reserves a certain amount of memory for its own use. Once that memory is exhausted, the application is not allowed to access other memory, even though there may still be some free memory available. Unlike some applications, the amount of memory needed by PAUP is a function of the size of the data set. Therefore, in setting PAUP's default memory size, I was forced into a compromise between requesting enough memory to handle larger data sets while not tying up more memory—which is then unavailable to other applications—than PAUP really needs for typical data sets. If you get an "out of memory" message while running under MultiFinder, you will have to increase the memory size as follows:

1. Select the PAUP icon in the Finder and choose **Get Info** from the File menu.

2. Change the value displayed in the **Application Memory Size** to a larger value.

3. Click the close box on the information window.

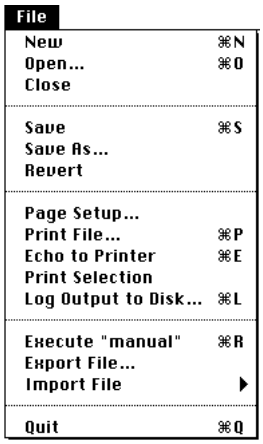
If you run out of memory and you are *not* using MultiFinder, you will either have to add additional memory to your machine or take additional steps to reduce the amount of memory used by the system (e.g., remove unneeded INITs from the System Folder, etc.).

THE APPLE (🍎) MENU

*The **Apple** (🍎) menu.*

Along with your usual set of desk accessories, the Apple menu contains choices for the "About PAUP" window (which displays, among other things, the current version number). The help system is no longer located in the Apple menu, instead it is found in the Windows menu (see below).

THE FILE MENU



The File menu is used to create, open, convert, and save input/output files, to control certain printing options and operations, and to exit the program. The options available on the File menu are described below.

*The **File** menu.*

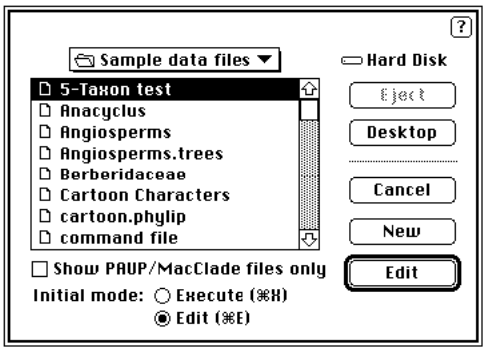
New

Use the **New** command to open a new, untitled, editor window (e.g., to create a new PAUP input file). See "The PAUP Editor" for more information on how to use the editor.

Open…

Use the **Open** command to execute or edit an existing PAUP input file. On the standard file dialog box that appears, choose either **Execute** or **Edit** as the initial mode, then select the name of the file you want to open and click **Execute/Edit** (or just double-click on the file name). Note that by default, PAUP shows all files. To restrict the display to files created by

PAUP or MacClade, click on the **Show PAUP/MacClade files only** box. A sample file dialog box is shown below:



*The **Open…** dialog box.*

Close

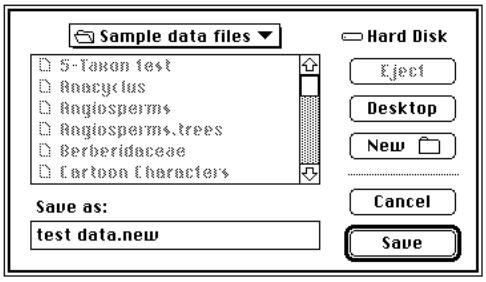
Use the **Close** command to close the active window (generally an editor window, but it may be the command-line or memory-status windows). This is equivalent to clicking the mouse in the close-box of the window. (Some windows, such as the main display and search-status windows, cannot be closed.)

Save

Use **Save** to save the file being edited in the active window to disk. The file will be saved in exactly the same place it was when you opened it. (If you want to save a copy of the file under a different name or in a different place, use **Save As** rather than **Save**.) Note that if the file has not previously been saved (i.e., if it was created via **New**), you will be requested to name it using the standard **Save As** dialog box (see below).

Save As…

Use the **Save As** command to save the file being edited in the active window to disk. Ordinarily, you will use **Save As** only when you want to save the file being edited with a different name, in a different folder, or on a different disk. Name the file using the standard file dialog box that is provided:

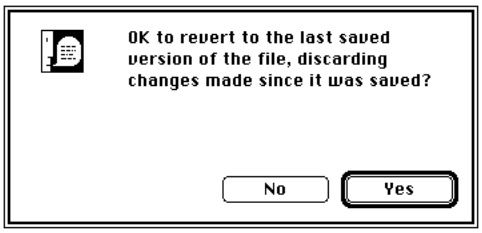


*The **Save As…** dialog box.*

By default, saving a file via the **Save As** command will also change the type of a file created using another application to a PAUP document. (The **Editor...** command in the **Options** menu can be used to request retention of the original creator setting.) After a file has been saved as a PAUP document, double-clicking on that document from within the Finder will start PAUP with the chosen file as the active file.

Revert

Use **Revert** if you want to abandon changes to a document (e.g., PAUP data file) being edited without leaving the editor. The following alert box will appear:



*The **Revert**… dialog box.*

If you answer "Yes," the most recently saved version of the file will be restored to the editor window.

Page Setup…

This command brings up the standard system "Page Setup" dialog box, which lets you define printing features such as the size and orientation of the paper on which a document is printed. The actual appearance of this dialog box will vary according to which printer has been selected via the Chooser.

> See your Macintosh documentation for further information on using the Chooser and on the available options for each printer.

Print File…

Choose **Print File** to print the document being viewed in the frontmost editor window (generally a PAUP input or output file) to the currently active printer. (You can use the Chooser desk accessory to change printers.) Note that **Print File** may only be used while in editor mode; it is disabled otherwise.

The standard system printing dialog box will appear, allowing you to specify things such as the number of copies, page range, etc. The actual appearance of this dialog box will vary according to which printer has been selected via the Chooser. See the preceding item for problems in printing PAUP output with semigraphics. Note that PAUP makes no attempt to deal intelligently with files containing lines too long to print. If for example a matrix contains very long lines, PAUP will truncate them when they are sent to the printer. One possible solution is to use the **SHOWMATRIX** command or **Show Data Matrix** dialog box and log the output to a file for subsequent printing. Alternatively, you can use those commands and then print the display buffer. See the **Log Output to Disk** dialog box for a more detailed description.

> See your Macintosh documentation for further information on using the Chooser and on the options found in the system printing dialog box.

Echo to Printer

If you choose **Echo to Printer**, all subsequent output to PAUP's main display window is also sent to the currently active printer. Each time you choose **Echo to Printer**, it will toggle the current printing status. (Printing is currently on if the item is checked in the menu, as shown below, and off otherwise.)

*Portion of **File** menu indicating the printer-echo is currently activated.*

This method of printing uses low-level printer driver calls that are not supported by some (non-Apple) printers. If you encounter problems when attempting to print using the **Echo To Printer** command, you will have to use a different method for obtaining printed output. For example, you can save output to a disk file and then print it later using PAUP or a text-editor/word-processor of your choice.

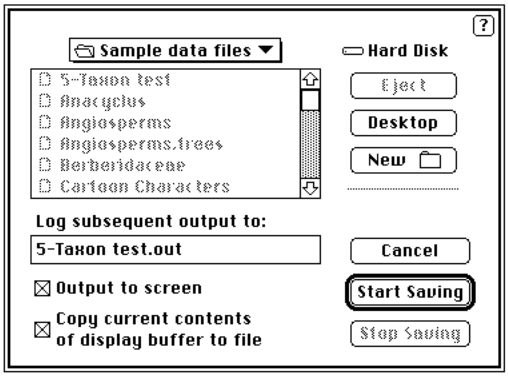
> See the **Log Output to Disk** command for how to save PAUP output to a file.

Print Selection.

This option will print any selected text, either from the display buffer or a data file. If you want to print the entire display buffer, choose **Edit Display Buffer** from the **Edit** menu, select all text, and then choose **Print Selection**. Alternatively, you can save the contents of the buffer to a file, which can be printed later (see **Log Output to Disk** below).

Log Output to Disk…

The **Log Output to Disk** command allows you to control the saving of PAUP output to disk files. The following dialog box will appear:



*The **Log Output to Disk…** dialog box.*

To begin saving output, name the output file, then press **Start saving**. All subsequent output generated by the program will be saved in this file. If you want to stop saving the output to disk, choose **Log Output to Disk** again and press the **Stop saving** button.

The **Output to screen** checkbox allows you to specify whether output is also written to PAUP's main display window. If you want to send output to the file only, without having it scroll by on the screen, uncheck this box.

The **Copy current contents of display buffer** checkbox allows you to save the buffer to a file.

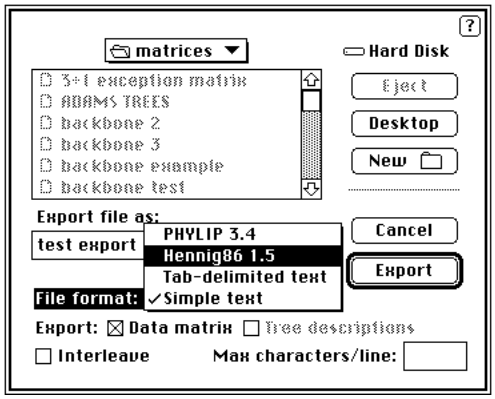
If you attempt to output to an already existing file, you will be asked whether you want to append to the file or to replace (overwrite) it.

Execute (File)

Use this command to request processing of the indicated file. If you are in editor mode, the file being edited will be executed. Otherwise, the most recently closed file will be executed. For example, you can open a file for editing, make any desired changes, save and close it, and then execute it.

Export File…

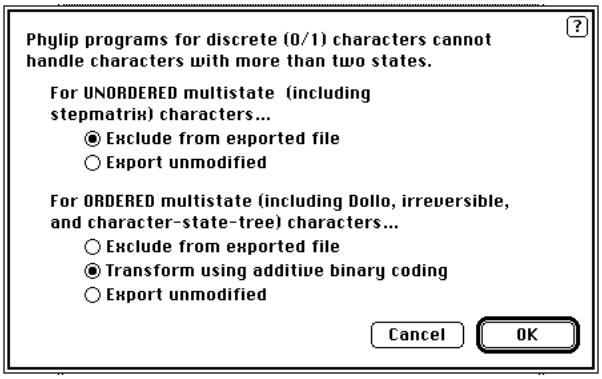
Choose **Export File** to export a data matrix and/or tree descriptions in one of three available formats: (1) PHYLIP (J. Felsenstein's Phylogeny Inference package), (2) Hennig 86 (J. S. Farris's IBM-PC program for phylogenetic analysis), and (3) tab-delimited text (a general format readable by Excel and other programs).



*The **Export File…** dialog box.*

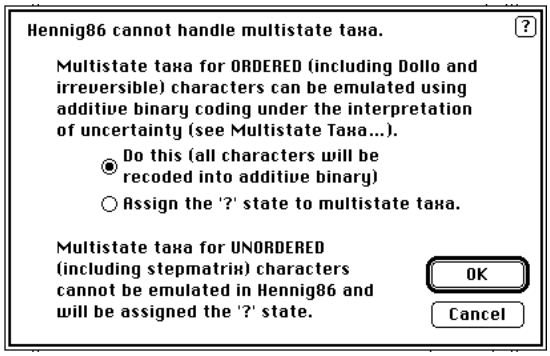
A data matrix must have been successfully input to PAUP (via a DATA block) for this command to be available. If there are trees in memory, you may export these as well; however tree export is supported only for PHYLIP and Hennig 86 formats. To export the data and/or trees, name the exported file in the field provided by the standard file dialog box, choose the desired format (pop-up menu) and export options (check boxes), and click on **Export**.

PHYLIP's "discrete (0,1)" programs are restricted to binary characters. Consequently, if the format of the current PAUP data file is "standard" (rather than DNA, RNA, or Protein) and there are multistate characters present, you will be presented with the following options:



Dialog box for specifying PHYLIP export options.

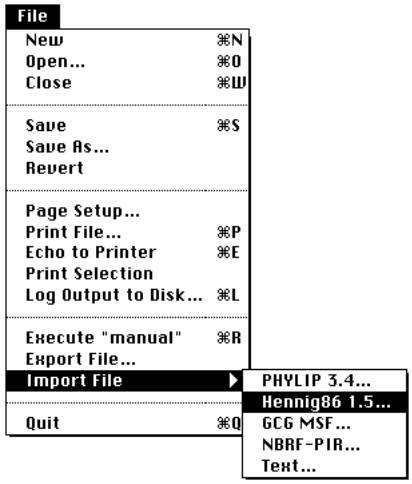
Neither Hennig86 nor PHYLIP accept multistate taxa. If the characters are binary or ordered multistate, they can be equivalently recoded (but only under the interpretation of uncertainty rather than polymorphism) using additive binary coding with missing values used to indicate the points of uncertainty. If multistate taxa are present and you request export of data to Hennig86, the following self-explanatory dialog will appear:



Dialog box for specifying Hennig86 options for exporting data containing multistate taxa.

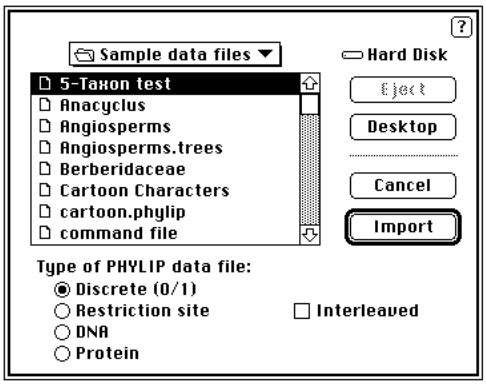
Import File…

The **Import File** command allows you to import a file in one of four primary formats: PHYLIP, Hennig 86, tab-delimited text, or simple text. A pop-up menu will appear for you to select the file format to be imported. You can select from text, PHYLIP, Hennig86, GCG MSF, or NBRF-PIR by using the pull-down menu that appears when **Import File…** is selected. Examples of GCG-MSF and NBRF-PIR file formats are included in the sample data files included with the PAUP program.



*The **Import File…** menu.*

Once that is chosen, the standard file opening dialog box will appear, unless PHYLIP is chosen, in which case you must select the PHYLIP file options as well as the file.



The PHYLIP file importing dialog box.

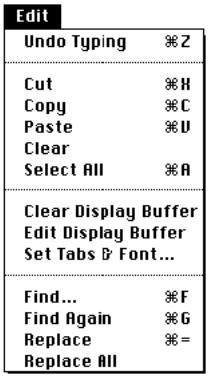
These PHYLIP file formats are described under "Importing Data and Trees from Other Programs." Remember that in the current version of PAUP, PHYLIP options that require extra lines of input (e.g., 'A', 'T', W', etc.) are not supported and will probably cause the conversion to fail. PAUP will successfully process PHYLIP user-defined trees. Although PAUP can handle the format of tree files output by Hennig86, it supports only a subset of the Hennig86 tree-description format.

If the file is successfully converted, it will be opened as a new, untitled editor document. Ordinarily, you will want to save the document to a file before proceeding.

Quit

Use the **Quit** command when you want to exit from PAUP and return to the Finder.

THE EDIT MENU



The Edit menu contains the standard Undo, Cut, Copy, Paste, and Clear items, which are available while in PAUP's editor. You may also clear PAUP's display buffer from the Edit menu.

*The **Edit** menu.*

Undo

Use this command to "undo" your last text-editing action. Generally, the word "Undo" will be followed by the undo-able action (e.g., "Undo Typing," "Undo Copy," etc.).

Cut

Cut removes the selected text and replaces it on the Clipboard. (Any previous contents of the Clipboard are replaced.) The **Cut** command is disabled if no text is selected.

Copy

Copy puts a copy of the selected text on the Clipboard. (Any previous contents of the Clipboard are replaced.) Unlike **Cut**, the selected text is not removed.

Paste

Paste inserts a copy of the contents of the Clipboard at the insertion point. (If text is selected at the time **Paste** was requested, it will be replaced.)

Clear

Clear removes the selected text without altering the contents of the Clipboard (unlike **Cut**, which deletes the selected text but places it on the clipboard).

Select All

This will select all text in the open window. You can then perform the above actions on all selected text.

Clear Display Buffer

Use this command to clear all output in PAUP's display buffer from memory, allowing you to start with a "fresh" display window.

Edit Display Buffer

This command causes the current contents of the display buffer to be placed into a new, untitled, editor window for editing or printing. For example, you might want to save only a portion of the output currently stored in the display buffer for subsequent retrieval. You can do this by choosing the **Edit Display Buffer** command, deleting the portions of the output you do not want to save, and then requesting the Save command (File menu) to save the remaining output.

The current "semigraphics" mode is respected (see the **Semigraphics…** command of the **Options** menu). If you have disabled the translation of semigraphics characters to standard ASCII characters, you will not be able to print these characters in any font other than the special Monaco font included in PAUP.

Set Tabs and Font

This allows you to choose the tab stops and font that PAUP will use while editing.

Find

This option allows you to find a specified text string within the document., and to specify other text with which to replace it (the actual replacing is done using the **Replace** command, below).

Find Again

This option repeats a previous **Find** command.

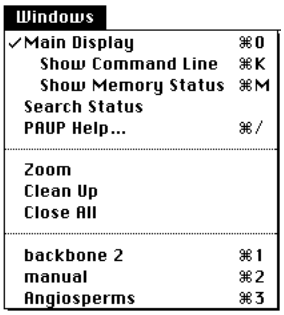
Replace

This replaces highlighted text with the text specified in the **Find** command. Use **Find Again** and **Replace** to cycle through all occurrences of the text specified in the **Find** command.

Replace All

This option finds and replaces all instances of the text specified in the **Find** command.

THE WINDOWS MENU



The Windows menu allows you to open available windows or bring them to the front if they are already open.

*The **Windows** menu.*

Main Display

Choose **Main Display** to bring PAUP's main display window to the front. (The same effect may be achieved by simply clicking on the main display window, but it may be entirely covered by other windows.) The width of the main display is the same each time it is called up. You may shrink it by holding down the "option" key while clicking and dragging on the lower right-hand corner. It cannot be expanded beyond the standard size.

Show Command Line

Choose **Command Line** to show the command line at the bottom of the main window. The command line provides an alternative way to control PAUP's actions. The available commands are identical to those found in "generic" mainframe/minicomputer versions of PAUP, and are fully documented elsewhere in this manual. If the command line is active, type in the desired command and click on the **Execute** button or press the *Return* or *Enter* key. Click **Clear** or press *Escape* to clear the command line.

You can retrieve the most recently executed command by clicking on the **Recall** button.

Some users may find the command-line to be a more efficient interface, particularly for certain operations that require a lot of "pointing and clicking" within dialog boxes.

Show Memory Status

This command adds a line at the top of the main display window that provides constant monitoring of memory availability. (If the memory-status window has already been opened, **Show Memory Status** brings the window to the front.) Two pieces of information are available: (1) the total amount of memory available (i.e., not already allocated by the System, by PAUP, or by other programs and desk accessories), and (2) the size of the largest free memory block. Knowing how much memory is available can be useful if you are working near the limits of the memory capacity of your machine.

If you no longer want to see the memory status, bring it to the front and choose **Close** from the File menu (or click in the window's close box).

Search Status

Choosing **Search Status** brings the status window for a heuristic, branch-and-bound, or exhaustive search to the front. It is useful when the status window has been completely obscured by other windows. **Search Status** is only available when a search is actively being performed; it will be disabled at other times.

PAUP Help

Choosing PAUP Help will bring up the help system. You can view the contents by topic only, or read the help entry for a particular topic. When you are done using help, choose the Exit button to return to PAUP. The help file must be in the same folder as PAUP when PAUP is started, otherwise it will not be found when Help is selected.

Zoom

This option zooms the current window to full size. Reselecting **Zoom** will restore it to its original size.

Clean Up

This option will restore all windows to their default location settings. If the "option" key is held down when this is selected, only the editor windows are affected. Editor windows will be "stacked" so that all title bars are visible.

Close All

This option will close all open editor windows.

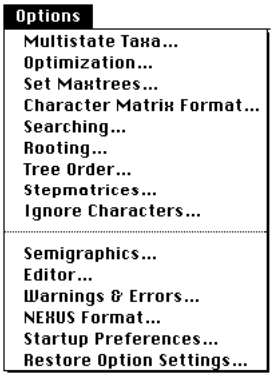
Editor windows

The Windows menu will ordinarily also contain the names of additional files that are available for editing. Choosing one of these filenames will cause the window for that file to be brought to the front, reading the file from disk if necessary.

You can also select a file for editing by clicking anywhere in an already open window. However, the window may be hidden from view by other windows, in which case the Windows menu provides a means of bringing the window to the front.

See "The PAUP Editor" for more information on PAUP's editing capabilities.

THE OPTIONS MENU

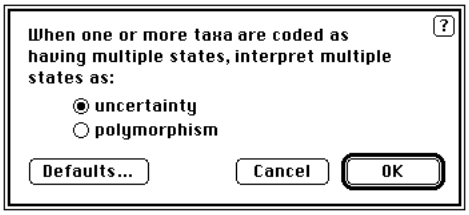


The Options menu is used to control a number of option settings that affect PAUP in various ways.

*The **Options** menu.*

Multistate Taxa…

This command allows you to specify how PAUP treats "multistate taxa" when computing tree lengths. Multistate taxa are those for which more than one character state was assigned to taxa in the data matrix for some characters; see the section "Multistate Taxa" in Chapter 2.



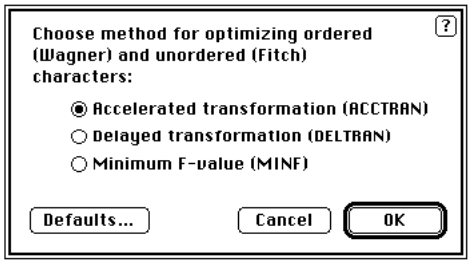
The ***Multistate Taxa…*** *dialog box.*

When multiple states are interpreted as "uncertainty," PAUP will choose a state from the set of available states that allows minimization of the tree length.

When multiple states are treated as polymorphism, PAUP assumes that the "terminal taxon" is actually a heterogeneous group. In this case, all but one of the states in the polymorphic terminal taxon must be derived from a monomorphic ancestral taxon in the most parsimonious way possible.

Optimization…

This command allows you to specify the method used to resolve ambiguity when optimizing ordered (Wagner) and unordered (Fitch) characters.



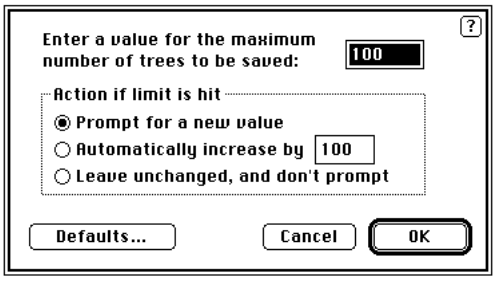
*The **Optimization…** dialog box.*

Three choices are available: (1) delayed transformation, which can be thought of as preferring parallelisms over reversals; (2) accelerated transformation, which maximizes prefers reversals to parallelisms, and (3) minimum F-value, which moves length from interior branches toward peripheral branches wherever possible.

See the section on "Character Optimization" for a discussion of the differences between ACCTRAN, DELTRAN, and MINF optimization.

Set Maxtrees…

Use **Set Maxtrees** to specify the maximum number of trees (= MAXTREES) that can be held in memory by PAUP.



*The **Set Maxtrees…** dialog box.*

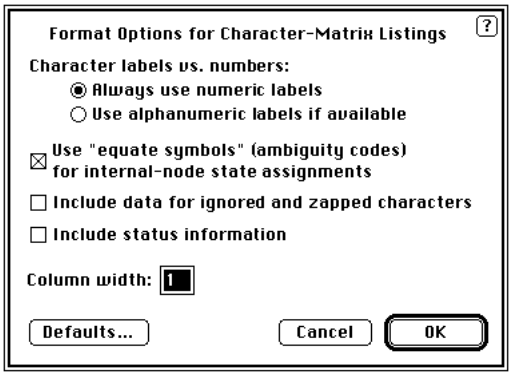
The trees are stored in a "tree buffer" which can be resized on demand if it becomes full.

Setting the MAXTREES parameter to a large value will reduce the likelihood that the tree buffer will become full during a search, at the expense of a larger chunk of memory being tied up and therefore unavailable for other purposes.

Ordinarily, if the number of trees found during a search reaches the value of MAXTREES, you will be given a chance to increase MAXTREES before proceeding. However, if you uncheck the **Prompt for a new value…** box, the program will not stop when MAXTREES is reached.

Character Matrix Format…

Use this command to change the way PAUP formats character-state matrices for output.



*The **Character Matrix Format…** dialog box.*

You may specify whether to use character numbers or alphanumeric names (if available) for the column headings. Left-to-right vs. top-to-bottom mode may be chosen for both alphanumeric names and character numbers.

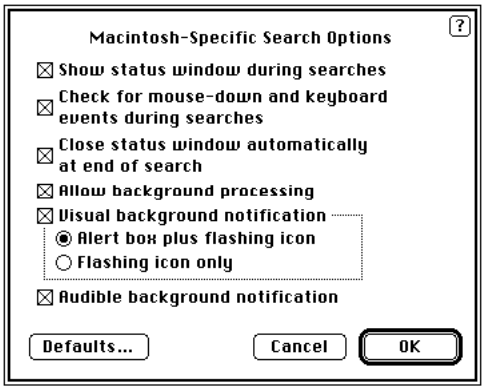
You can also control the number of columns used for each character. A column width of one character works well for nucleotide sequence data, but for other kinds of data some extra spacing may be more pleasing.

Finally, you may choose whether or not to include information on character status (i.e., deleted characters, uninformative, and/or constant characters are flagged).

The best way to learn the difference between the various formatting options is just to experiment with different settings prior to issuing a **SHOWMATRIX** or **Show Data Matrix** menu command.

Searching…

This command controls background processing, status-window display and event checking while PAUP is performing a search.



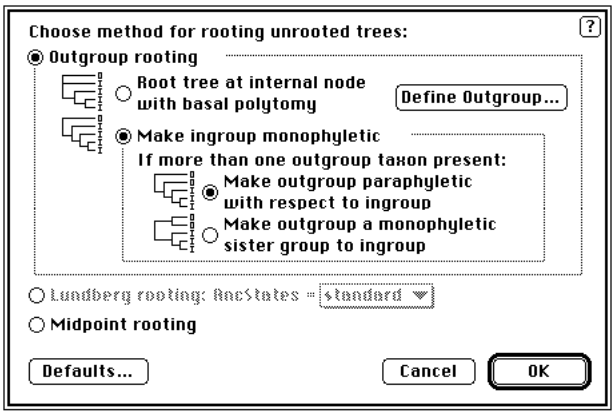
*The **Searching…** dialog box.*

Ordinarily, PAUP continues processing (e.g., tree searches) when it is switched to the background, allowing other activities (e.g., word processing) in the foreground. For large data sets, however, the responsiveness of the foreground application may be impacted. If you disable background processing, PAUP will simply "sleep" when it is switched to the background and will resume processing when it is returned to the foreground. For example, a run could conceivably continue over a period of several days by letting PAUP sleep during the day when you were using your machine for "more important" tasks but moving PAUP to the foreground when you left for the day.

If event checking is disabled, PAUP will ignore all mouse-clicks and key presses. Because event-checking steals processor time from PAUP's computations, disabling it will provide a speed improvement. However, you give up the ability to abort a search (other than by restarting the computer) or to activate a different program under MultiFinder.

Rooting…

The **Rooting…** command allows you to specify whether unrooted trees will be rooted using the outgroup or the Lundberg methods.

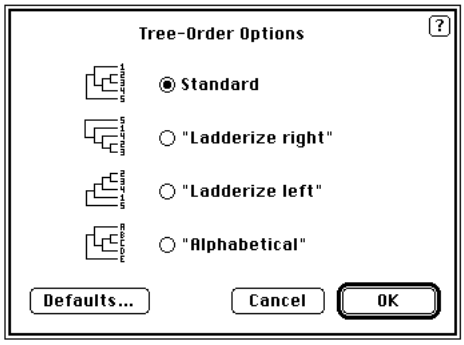


*The **Rooting…** dialog box.*

See the section in Chapter 2 for a description of the different rooting options. If outgroup rooting is selected, the outgroup may be defined from within this dialog box by clicking on the **Define Outgroup** button. This will bring up the standard **Define Outgroup…** dialog box. For Lundberg rooting, the outgroup/ancestor is set according to the current ANCSTATES setting. You can use the pop-up menu to change the outgroup/ancestor to be used.

Tree Order…

The "order" of the taxa in a tree drawn by PAUP has no physical significance; four options are available to control the ordering.



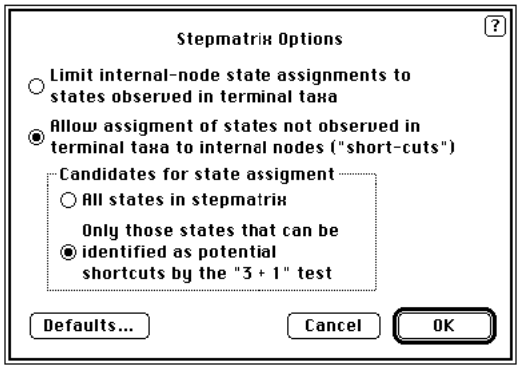
*The **Tree Order…** dialog box.*

See the section "Changing the Order of Taxa on Trees." The standard and alphabetical orders are best for visual comparison of different trees for the same set of taxa, whereas the ladderized orders sometimes provide a more

pleasing appearance (i.e., they reduce the "monkey-puzzle" like appearance that sometimes characterizes the standard and alphabetical orders).

Stepmatrices…

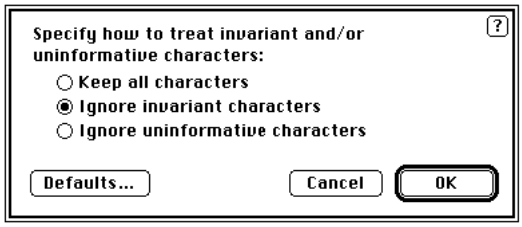
This allows you to choose which character states to allow in internal nodes.



*The **Stepmatrices…** dialog box.*

See the section "Stepmatrix Character Reconstructions" for a detailed description of these options.

Ignore Characters…



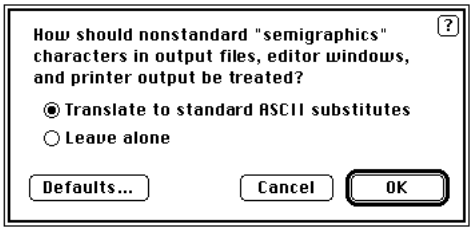
*The **Ignore Characters…** dialog box.*

Ignoring characters effectively removes them from the analysis. See the section "Using a subset of the Characters."

Semigraphics…

PAUP uses special characters in its internal font to draw trees and other items. These characters are neither in the standard 128 ASCII characters nor in the set of special characters normally included with Macintosh fonts. Thus, although the trees look nice when drawn in the display

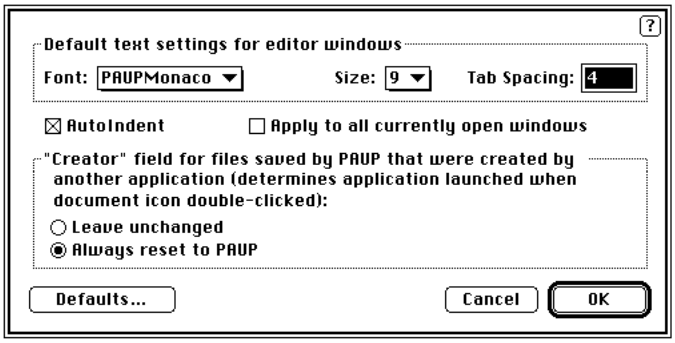
buffer, they will not look right when printed on most printers. Ordinarily, PAUP translates these "semigraphics" characters to standard ASCII substitutes when sending output to a printer, file, or document window. The **Semigraphics…** command allows you to alter this behavior via the following dialog box:



*The **Semigraphics…** dialog box.*

Editor…

The **Editor**… command allows you to change options that affect PAUP's editor.



*The **Editor…** dialog box.*

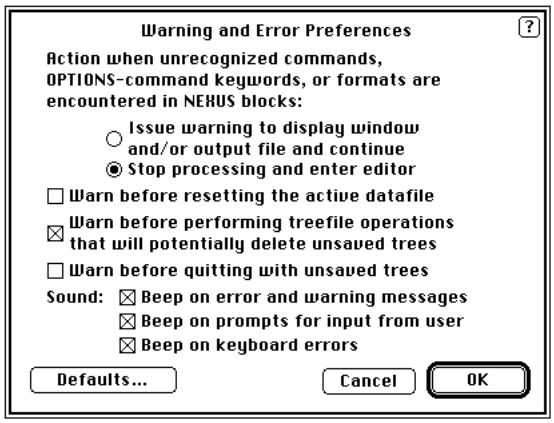
You may change the type font, size, and number of spaces that are equivalent to one tab. If you change the font, it is strongly recommended that you use a fixed-width font like Monaco or Courier so that columns will line up evenly.

In addition, you may enable or disable the "autoindent" facility. If autoindenting is on, when you hit return at the end of a line, the cursor is automatically indented to the same column position as the starting point of the previous line. You can make your changes apply to all open windows by clicking the box **Apply to all currently open windows**.

Warnings & Errors…

Use **Warnings & Errors** to specify what action PAUP will take when it encounters unrecognized commands and keywords in a file, to specify

sound options, and to enable or suppress the warnings PAUP issues before resetting the active data file.

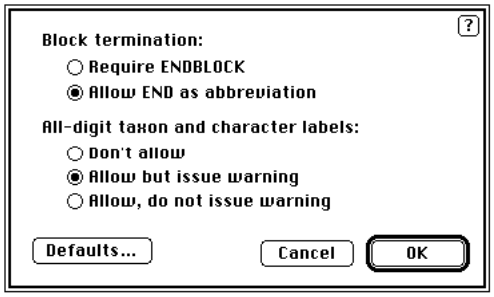


*The **Warnings & Errors…** dialog box.*

The only item requiring further explanation is **Warn before resetting the active datafile.** The "active datafile" is the file containing the most recently processed DATA block. If another 'data' block is encountered when executing a file, the previous data matrix (and any trees that may have been generated using this data matrix), will be cleared from memory. By default, PAUP warns before resetting the active data file on the chance that the 'Execute' command was issued inadvertently. If you wish to suppress this warning, uncheck the **Warn before resetting the active datafile** item.

NEXUS Format…

This option allows you to choose different options for NEXUS files.



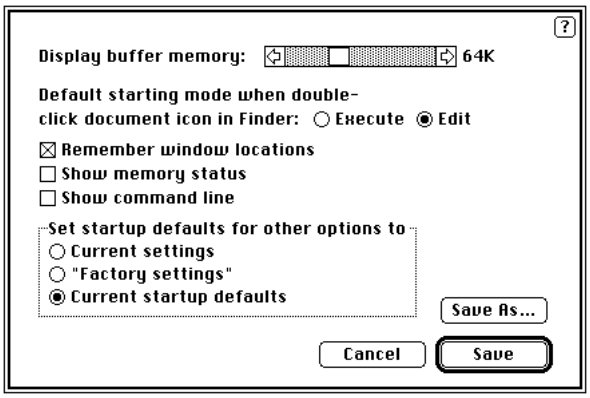
*The **NEXUS Format…** dialog box.*

In this dialog box, you can choose to have "end" be a valid block terminator in the file (as well as "endblock"). You can also choose to allow or disallow all digit taxon and character labels.

Note - MacClade uses "end" to terminate blocks. If you created your data matrix with MacClade, you *must* allow "end" to terminate a block, otherwise PAUP will not recognize end as a block terminator.

Startup Preferences…

Use this command to change the default settings that go into effect when PAUP starts.



*The **Startup Preferences…** dialog box.*

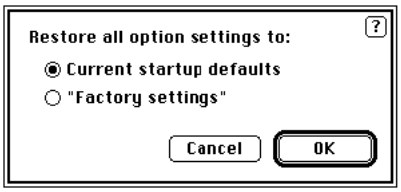
You may specify the amount of memory allocated for the display buffer in the range 32K to 128K. (The more memory that is allocated for the buffer, the greater the number of lines can be retained for subsequent recall, but the less memory that will be available for other purposes.)

You may also set the default settings for most of the options in the program to either the values that are currently in effect or to the values that are in effect at the time PAUP is originally shipped ("**Factory settings**"). If the **Save** button is pressed, a file called "PAUP Preferences" will be created or modified in the System Folder of the disk from which the computer was booted. (If a previous version of this file already exists, it will be replaced.) If you instead press the **Save As…** button, a standard file dialog box will appear that allows you to create a file with any name and folder location you choose. This new file can then be used to start PAUP (by double-clicking from the Finder) with the chosen default settings. In this way, you can have a variety of default configurations. Click on the boxes for **Show command line** and/or **Show memory status** if you wish these to be active when PAUP starts.

Finally, you can have PAUP remember the location of windows, so that the next session will start up with the windows in the same place.

Restore Option Settings…

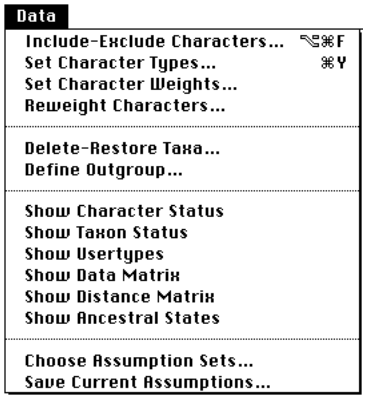
Use this command to set the current option settings to those that were in effect at an earlier time.



*The **Restore Option Settings…** dialog box.*

If you request the **Current startup defaults**, the option settings will be restored to their settings at the time the program was started. If you choose **"Factory settings,"** the option settings will be reset to the default values in effect at the time PAUP was originally shipped.

THE DATA MENU



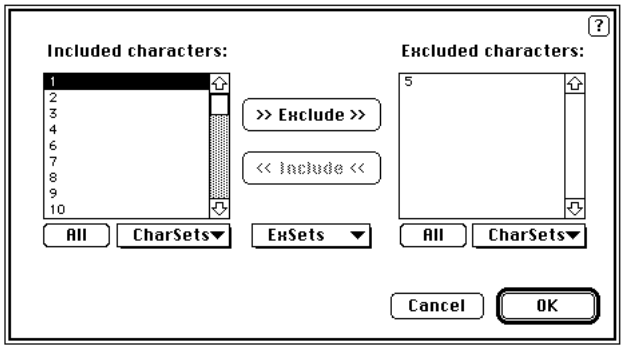
The Data menu contains commands for setting character types and weights, excluding taxa and characters from the analysis, and displaying information concerning the current data set.

*The **Data** menu.*

Include-Exclude Characters…

This command provides a facility for excluding characters from consideration when computing tree lengths. Although the excluded characters will not be allowed to influence the selection of optimal trees, they will still be optimized to these trees. For example, you can construct trees using only a subset of the characters, but still see how the remaining characters would be "mapped" onto the resulting tree(s).

A dialog box with two scrollable lists will appear.



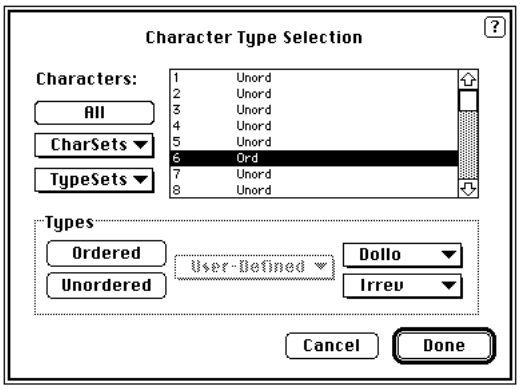
*The **Include-Exclude Characters…** dialog box.*

The left list contains the included characters, and the right list contains the characters that are to be excluded. Select the characters you want to include or exclude, and press the appropriate arrow button to move characters from one list to the other. You can also double-click on a taxon to move it from one list to the other. Click on **OK** when you are finished deleting and restoring taxa.

If you have defined character-sets, you can quickly select all of the characters contained in the set by pulling down the appropriate **Charsets** menu. If you have defined exclusion-sets, you can immediately set the exclusion status of all characters by choosing an item from the **Exsets** menu.

Set Character Types…

Use this command to change the type of one or more characters.



*The **Set Character Types…** dialog box.*

Available character types are ordered (Wagner), unordered (Fitch), Dollo, irreversible (Camin-Sokal), and user-defined.

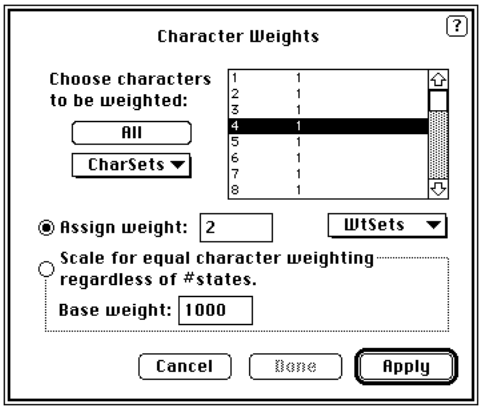
See "Character Types" for a description of these character types.

When you choose **Set Character Types…**, a dialog box containing a scrollable list of character numbers (and names, if you have provided them) will appear. Select the character(s) whose type(s) you want to change and click on one of the character-type buttons or menus at the bottom of the dialog box. (See the section "Selecting Items in Lists" for tips on making multiple selections.) The list will be updated to reflect the new character status. You may then proceed to select other characters and set their types in the same manner.

If the **Dollo** or **Irrev** buttons are pressed, a menu will pop-down allowing you to choose an option for specifying character polarity. (See the section "Character Types" for the meaning of these options.) Similarly, if the **User-Defined** button is pressed, a menu will pop-down that shows the user-defined types that were specified in the ASSUMPTIONS block. Choose the desired type from this menu.

Set Character Weights…

Use this command to change the weight assigned to each character



*The **Set Character Weights…** dialog box.*

By default, all characters are weighted equally (with a weight of unity). To change the weights, select all characters to be assigned a given weight, enter the desired weight in the box provided, and click on **Apply**. When you are finished assigning weights, click on **Done**.

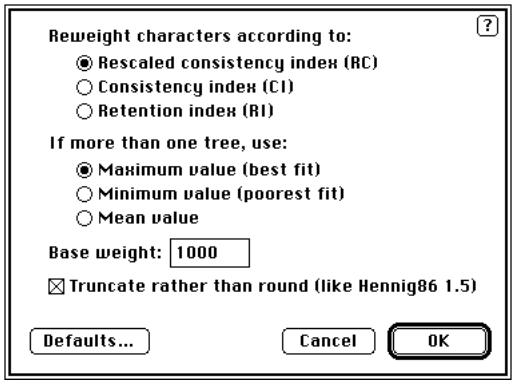
In addition to providing explicit weights, you may choose to scale character weights so that each character receives the same total weight regardless of the number of states observed. (For instance, if a data matrix contains characters with 2, 3, and 4 states, the 4-state characters could be weighted 2, the 3-state characters weighted 3, and the binary characters weighted 6. In this way, the total range of all characters is 6. (This type of weighting is especially appropriate when the number of character states

assigned to a character is essentially arbitrary, as when continuous measurements are recoded into discrete characters.)

Because Version 3.0 and later of PAUP uses integer weights and tree lengths, you can no longer use decimal weights such as 0.33, 0.5, and 1.0 for the above example. Instead, specify the "base weight." This can either be a "common denominator" or a large value like 100 or 1000. For example, using a base weight of 100 provides results equivalent to using decimal weights recorded to 2 decimals.

Reweight Characters…

This provides the option of a posteriori weighting of characters. You must first calculate some trees using equal weights. Then, reweight the characters depending on consistency index, retention index, or rescaled consistency index. If there is more than one tree, you can choose to weight by the best, worst, or average fit of the character over all trees. You can also specify whether weights are truncated or rounded. Hennig 86 truncates weights, and if you wanted to replicate a Hennig86 search, you would have the weights truncated. This means that a character with a CI of .87 with a base weight of 10 would receive a weight of 8 instead of 9, the value obtained by rounding.

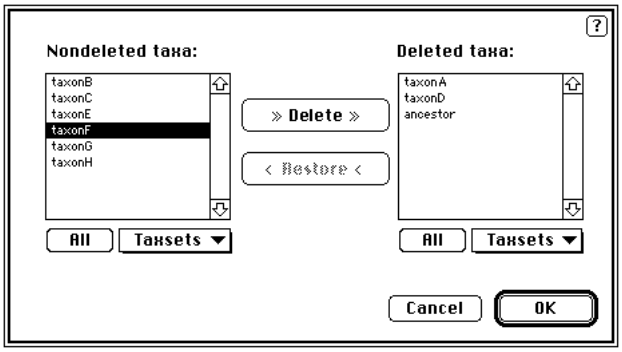


*The **Reweight Characters…** dialog box.*

You also have the option of specifying the base weight for reweighting. After assigning the new weights, a new search is run. The cycle continues until either the weights do not change over two successive runs, or the same set of trees is obtained in two successive runs.

Delete-Restore Taxa…

Use this command to temporarily delete taxa from the analysis or to restore previously deleted taxa. A dialog box with two scrollable lists will appear.



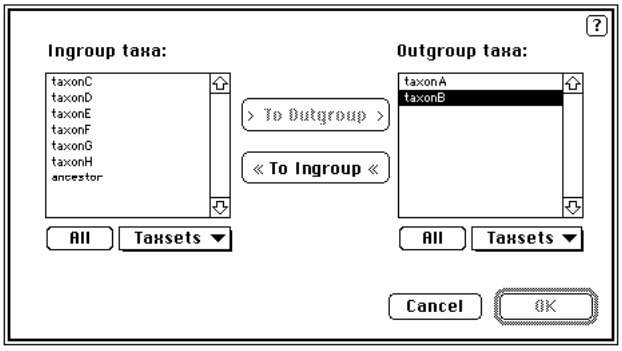
*The **Delete-Restore Taxa…** dialog box.*

The list on the left contains nondeleted taxa and the list on the right contains taxa that have previously been deleted. Select taxa in either list and click on the appropriate button to change the deletion status of the taxa.

If you have defined taxon-sets (taxsets) you can select all taxa in the set in either list by pulling down the appropriate **Taxsets** menu.

Define Outgroup…

Use this command to define the outgroup. A dialog box with two scrollable lists will appear.



*The **Define Outgroup…** dialog box.*

The list on the left contains taxa currently assigned to the ingroup, and the list on the right contains outgroup taxa. Select taxa in either list and click on the appropriate button to move taxa between the ingroup and the outgroup.

If you have defined taxon-sets ("taxsets") you can select all taxa in the set in either list by pulling down the appropriate "Taxsets" menu.

Show Character Status

This command produces a listing of the current status of all characters. Output includes the character type, states observed, exclusion status, and information on whether the character is "informative." See the command CSTATUS and the sections "Using a Subset of the Characters", "Including and Excluding Characters," "Specifying Character Types," and the DATA and ASSUMPTIONS blocks for descriptions of this output.

Show Taxon Status

This option produces output listing all deleted taxa and all taxa currently included in the outgroup.

Show Usertypes

This command generates a listing of all user-defined character types (stepmatrices and character-state trees). Character-state trees are shown in a graphical format so that you can see whether the tree you described is in fact what you intended. Input of stepmatrices is less problematical but it is nonetheless useful to include a record of the costs assigned by the stepmatrix in the output. See the section "User-Defined Character Types" for further information on interpreting this output.

Show Data Matrix

This command produces a listing of the data matrix. The same dialog box shown by the **Character-Matrix Format…** command can be used to change output options.

Show Distance Matrix

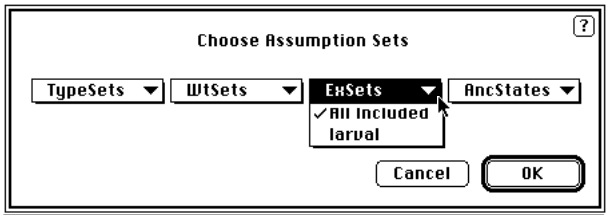
Use this command to request calculation and output of a matrix of pairwise distances between taxa. The formulas used in calculating these distances are given in the section "Distance Matrices" in chapter 2.

Show Ancestral States

This command produces a listing of the currently defined ancestral states, in a format similar to that used for showing the data matrix.

Choose Assumption Sets…

Use this command to invoke previously defined "assumption sets."



*The **Choose Assumption Sets…** dialog box.*

Four kinds of assumption sets are available. TYPESETs and WTSETs specify character types and weights, respectively, for sets of characters. (See the sample data file "5-taxon test" data set for several examples.) EXSETs define sets of characters that are to be excluded from consideration during searches. ANCSTATES specify ancestral states for each character.

Assumption sets are particularly useful when complex combinations of assumptions are being used. By declaring assumption sets, complicated sequences used to assign character types and weights, etc., can be avoided.

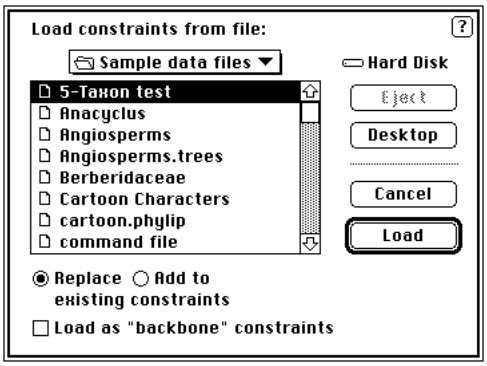
THE SEARCH MENU

Items on the Search menu allow you to search for trees using heuristic or exact algorithms, to perform "bootstrap" analyses to estimate the reproducibility of groupings, and to perform Lake's method based on linear invariants for DNA/RNA sequence data. Also, commands dealing with input and selection of constraints are included on the Search menu.

*The **Search** menu.*

Load Constraints…

Ordinarily, topological constraints are defined via one or more CONSTRAINTS commands in a PAUP block. The **Load Constraints** command provides an alternative means of defining constraints that may be simpler in some cases. When you choose **Load Constraints**, the following dialog box will appear:



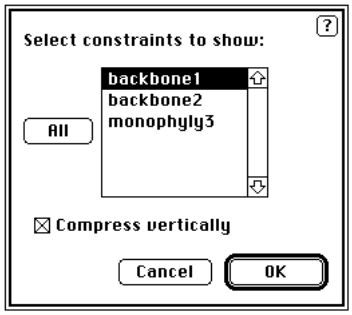
*The **Load Constraints…** dialog box.*

Select a file that is known to contain a NEXUS-format TREES block. The trees in this block will be read from the file and converted to constraints specifications. All other information in the file is ignored. If constraints have already been defined prior to issuing the **Load Constraints** command, you can choose **Replace existing constraints** to replace the existing constraint tree with the new ones loaded from the file or **Add to existing constraints** to add the new constraint trees to the ones already present. If you are loading a tree as a "backbone" constraint, check the **Load as "backbone" constraints** box.

The **Load Constraints** command is particularly useful if you are using PAUP and MacClade in conjunction with each other. It is often easier to use MacClade to graphically edit the tree descriptions that you wish to use as constraints than it is to write out the tree description in the parenthetical notation used by PAUP. MacClade can save the trees to a file from which constraints can be loaded by PAUP.

Show Constraints…

This command causes a tree representing the currently chosen constraint definition to be output. You may also choose to see other constraint definitions as well.



*The **Show Constraints…** dialog box.*

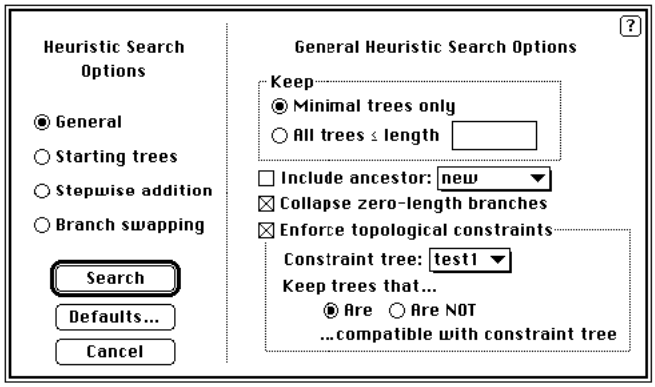
"Backbone" and "Monophyly" constraints will be flagged in the output

Heuristic…

Use **Heuristics** to request a search using a heuristic (approximate) algorithm. A heuristic search ordinarily includes two components: (1) stepwise addition of taxa to a developing tree until all have been connected, and (2) rearrangement of these trees via "branch-swapping" techniques.

There are too many heuristic search options to include them all in a single dialog box. Consequently, the controls for these options are divided into four panels. To select a different panel of options, simply click on one of the radio buttons at the left of the dialog box.

The first panel of options can be used to specify several general options:

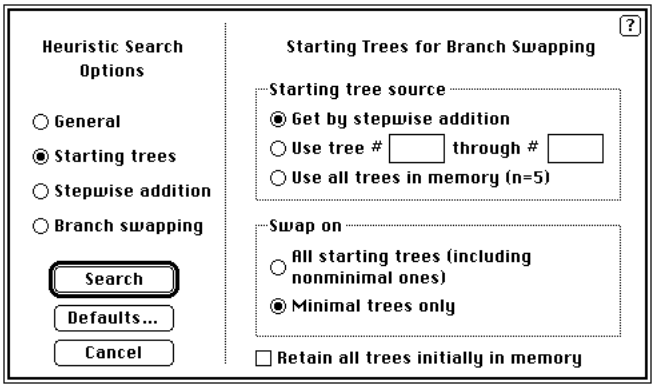


*The **General** panel of the **Heuristic…** dialog box.*

Typically, you will keep only the minimum-length trees but you may also be interested in near-minimal trees as well. You can save all trees less than or equal to a length that you specify by clicking the appropriate radio button and entering the desired length into the adjacent field. You can also choose to enforce topological constraints during the search, and keep trees that do/do not satisfy the constraint (either backbone or monophyly). When **Constraint trees** is selected, a pull-down menu will appear if there is more than one currently defined constraint tree.

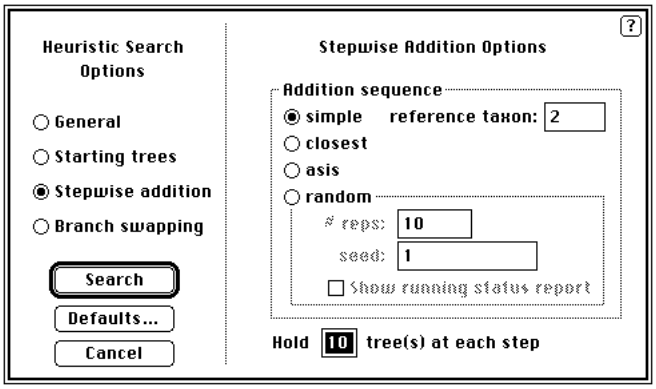
The **Include ancestral taxon** checkbox allows you to specify that the currently defined ancestral states (ANCSTATES) are to be treated as corresponding to a hypothetical ancestral taxon used to root the trees during the search. In most cases, you will leave this box unchecked (in which case trees are stored internally as unrooted trees and rooted only for output purposes only). Use it only if you have specified an ancestor different from the standard (all-missing) ancestor. If you do not, *all* rootings will be equally parsimonious and will be saved as distinct trees.

The second panel allows you to choose how the starting trees will be obtained: by stepwise addition, or by using some or all of the trees already in memory. You can also choose to swap on all the trees in memory (including nonminimal trees) or on minimal trees only by selecting the appropriate checkbox in the **Swap on** option.



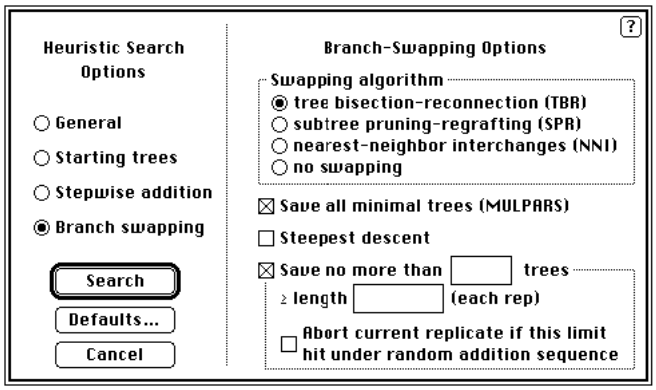
*The **Starting Trees** panel of the **Heuristic…** dialog box.*

The third panel has options for stepwise addition. the addition sequence can be simple, closest asis, or random. You can also select how many trees are held at each step. For **random** addition, you may change the number of repetitions or the seed tree. You can also select a running status report of the random search. Using the random addition sequence option is the best way to find islands of trees that may not be discovered by other addition sequence options.



*The **Stepwise Addition** panel of the **Heuristic…** dialog box.*

The final panel has options for branch swapping. You can choose the swapping option you want: tree bisection-reconnection; subtree pruning-regrafting; or nearest-neighbor interchange. If you wish, you can also select no swapping, performing the stepwise addition phase only.

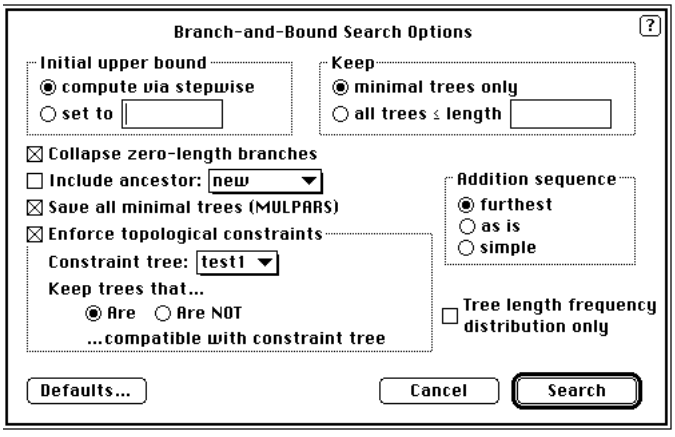


*The **Branch Swapping** panel of the **Heuristic…** dialog box.*

PAUP will save all minimal trees if **MULPARS** is selected. Selecting **Save no more than ___ trees ≥ length ____** is handy when searches get bogged down finding huge numbers of trees that are far from optimal, especially when used in conjunction with random addition sequence You can also choose to have PAUP abort the current replicate if the Save limit is hit during random addition. **Steepest descent** will not abandon a round of swapping until all input trees from the previous round have been examined by the swapping algorithm. See the section "Heuristic Searches" for more information about these options.

Branch and Bound…

Use this command to request a search using the branch-and-bound method. This algorithm is guaranteed to find all minimum-length trees.



*The **Branch-and-Bound…** dialog box.*

If you do not provide an initial upper bound, one will be calculated via stepwise addition (see **Heuristics**). However, the better the upper bound, the faster the branch-and-bound search will proceed, so for large data sets, it will generally be worth your while to perform more extensive heuristic

searches in search of a better bound prior to starting the branch-and-bound search.

The addition sequence specifies the way in which taxa are selected for next addition to the tree at the current node of the search tree. FURTHEST is usually the fastest, although it is not permitted unless all characters are of type ORD or UNORD.

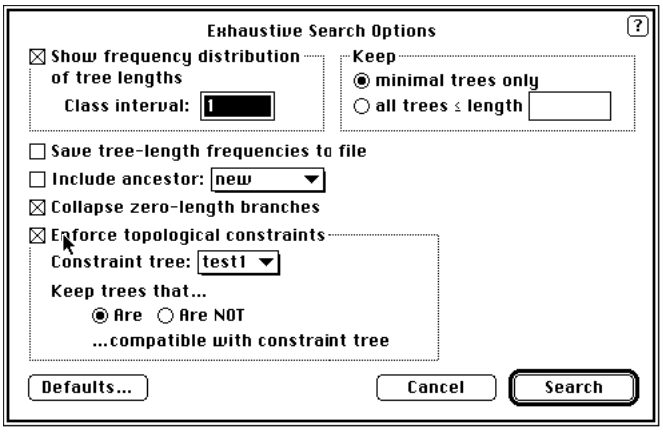
If you only want to know the length of the shortest tree(s) and do not care to obtain all trees of this length, uncheck the **Save all minimal trees (MULPARS)** option. The single tree found is guaranteed to be of minimum length and the search often runs much faster.

If you check **Tree length frequency distribution only**, PAUP will not save any trees but instead compute a frequency distribution of the number of trees of each length less than or equal to the length specified for **Keep all trees ≤ length ___.** This provides a way to obtain the number of trees that are one step from optimal, two steps from optimal, etc., for data sets that are too large to allow a full computation of the tree-length frequency distribution via exhaustive search.

The branch-and-bound run times are very data-dependent. It usually runs quickly for 12 or fewer taxa. For between 13 and 18 taxa, the run times vary considerably depending on the messiness of the data and other factors to complicated to go into here. It has been successfully run for 20 or more taxa, but for data sets this large, extremely long run times are possible. Don't even try it for data sets much beyond 20 taxa.

Exhaustive…

The **Exhaustive (Search)** command provides an alternative to the branch-and-bound algorithm for an exact search, guaranteeing to find all minimum-length trees. If all you are interested in is the most parsimonious trees, you should use the branch-and-bound method, as it will generally run much faster. However, a nice feature of the exhaustive search is that it outputs a frequency distribution of tree lengths.

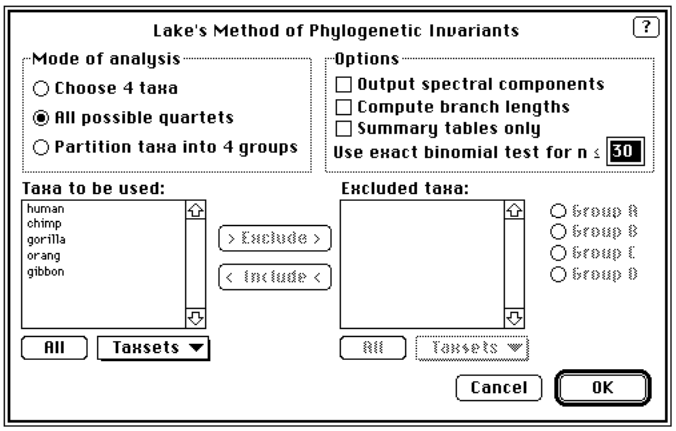


*The **Exhaustive…** dialog box.*

Although PAUP no longer enforces a limit on the maximum number of taxa that can be used in conjunction with the exhaustive search, expect long running times for 9 or 10 taxa. For more than 10 taxa, the number of trees becomes so astronomical that the exhaustive search will not generally be feasible.

Lake's Invariants…

This command requests Lake's method of invariants for nucleotide sequence data, called "evolutionary parsimony" by him. It is only available if DATATYPE=RNA or DATATYPE=DNA is specified in the FORMAT command of the DATA block.



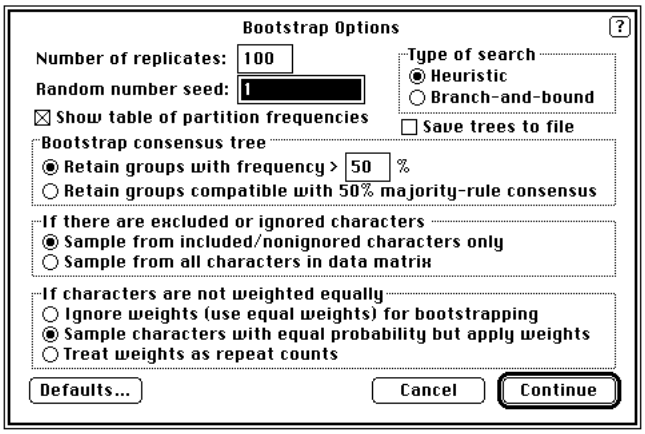
*The **Lake's Invariants…** dialog box.*

Lake's method uses quartets of taxa at a time. You have three options for searching, and in each you must select which taxa will be included or excluded. First, you can explicitly select four taxa to make up one search quartet. When you do this, you must select the taxa you want and click on the Include button. You can also double-click on a taxon to include or

exclude it. The second option is to evaluate all possible quartets. In that case you must choose which taxa will make up the group from which quartets will be taken. The third option is to partition the taxa into four groups, which will then make up the quartet. In that case, you will have to select which taxa will be included in the groups, designated Group A through Group D. Once a taxon has been assigned to a group, it is no longer available to be assigned to subsequent groups. You can also specify the output that will appear in addition to the invariants: spectral components, branch lengths, or just summary tables. See the section "Lake's Invariants"for more detail on this type of search and the output it produces.

Bootstrap…

The **Bootstrap** command implements Felsenstein's bootstrap approach to placing confidence estimates on groups contained in the most parsimonious trees.

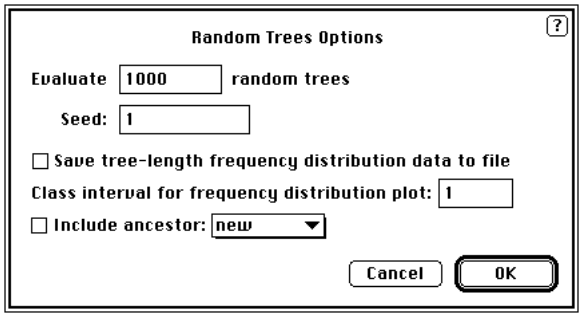


*The **Bootstrap…** dialog box.*

Specify the number of replications desired, and change the random number seed if desired. If there are excluded characters or if all characters are not weighted equally, you must tell PAUP how to treat those characters during the bootstrap replicates. Indicate whether you want to perform an exact (branch-and-bound) or heuristic search by clicking on the appropriate button; then click on **Continue.**. A second dialog box will appear allowing you to specify search options. The bootstrap procedure begins when you click the **Bootstrap** button of this second dialog box.

Random Trees…

Selecting **Random Trees** will evaluate the length of the number of random trees chosen. The output will include a frequency distribution of tree lengths.



*The **Random Trees…** dialog box.*

This distribution can also be output to a file if you select **Save tree-length frequency distribution to file**. You can choose the random seed the PAUP presents or one of your own. Usually, the box "Include ancestor" will be unchecked, unless you have defined an ancestor separate from the standard (all-missing) ancestor. If not, all rootings will equally parsimonious and will be saved as distinct trees.

THE TREES MENU

The Trees menu contains items for outputting trees and associated information, and for performing other tree-related activities.

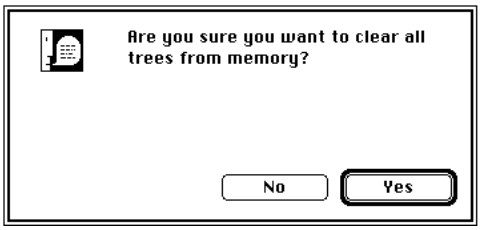
*The **Trees** menu.*

Tree Info

The **Tree Info** command provides a quick summary of the number of trees currently in memory and how they were obtained.

Clear Trees

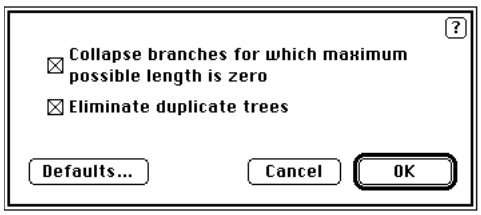
This option will clear all trees in memory. If it is selected, the following warning will appear:



*The **Clear Trees** warning box.*

Condense Trees…

This command allows you to retroactively collapse zero-length branches and keep only those trees that are unique after the collapsing is accomplished. Selecting **Condense Trees...** brings up the following dialog box.

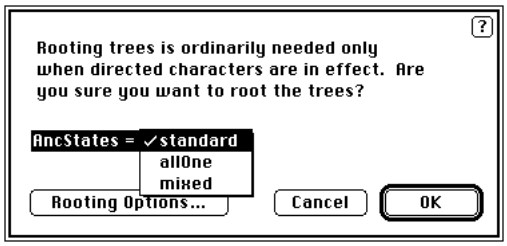


*The **Condense Trees…** dialog box.*

By default, PAUP collapses branches whose maximum length is zero, yielding polytomous trees. However, you may choose to override this default, so that only completely bifurcating trees are produced during a search by clicking on the **Collapse branches for which maximum possible length is zero** checkbox You can also have PAUP delete duplicate trees in memory by clicking on the **Eliminate duplicate trees** checkbox.

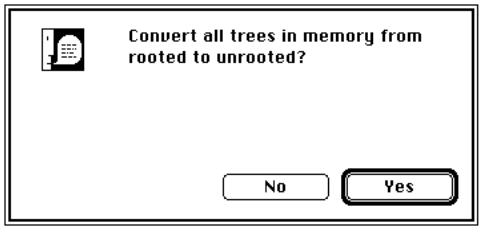
Root Trees…/Deroot Trees…

Root Trees… and **Deroot Trees…** are specialized commands that are ordinarily unnecessary. However, if you have unrooted trees stored in memory and you change one or more character types to a type that requires rooted trees (e.g., irreversible characters), the trees will have to be rooted before they can be output. Trees are rooted via outgroup rooting using the outgroup in effect at the time the command is issued. If you are using Lundberg rooting, you can select the ancestor with the pull-down menu. Selecting **Rooting Options…** will bring up the standard **Rooting…** dialog box.



*The **Root… Trees** dialog box.*

Similarly, you may want to deroot trees if current character types do not require rooted trees.

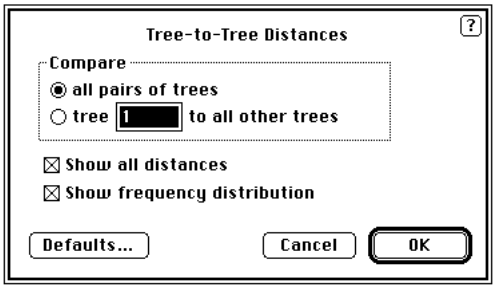


*The **Deroot Trees…** warning box.*

Note that when you convert rooted trees to unrooted trees, trees that were formerly distinct may become equivalent. You can use the **Condense Trees** command to eliminate these duplicate trees.

Tree-to-Tree Distances…

This command produces a matrix of tree-to-tree distances computed according to the symmetric-difference or "partition" metric. (Penny and Hendy, 1985)



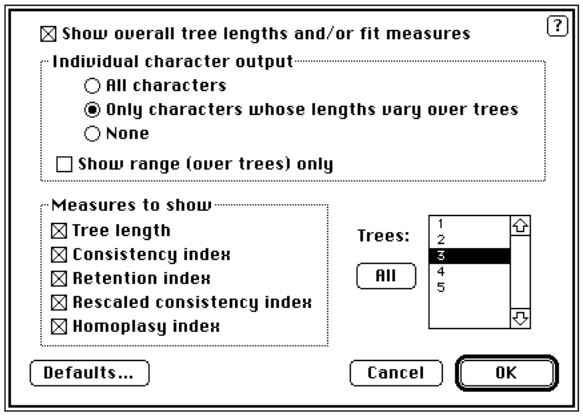
*The **Tree-to-Tree Distances…** dialog box.*

The **Compare** options allow you to output distances for all trees to all other trees, or for all trees relative to a selected tree. Clicking on the **Show all distances** checkbox will output a distance matrix for all selected trees, while clicking on the **Show frequency distribution** will only output a frequency distribution of tree distances. Trees that are most similar will

have the lowest distance value. The output is very useful in identifying "classes" of similar trees. See the section "Tree-to-Tree Distances" and Swofford (1991) .

Lengths and Fit Measures…

This outputs length and fit measures of all trees currently in memory and/or lengths and fit measures of individual characters on these trees. Tree measures include the consistency index (CI), homoplasy index (HI), retention index (RI), and rescaled consistency index (RC). See the section "Lengths and Fit Measures" for more detail on this output.

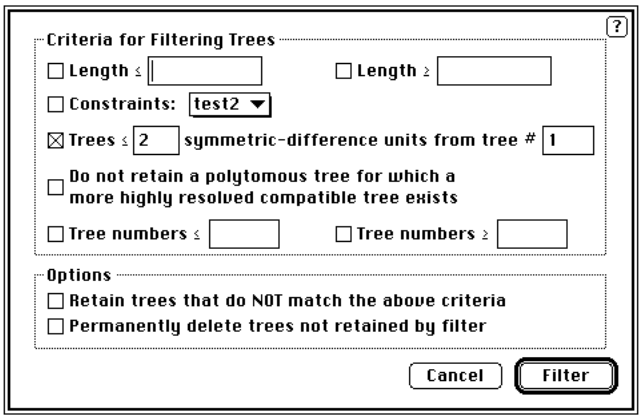


*The **Lengths and Fit Measures…** dialog box.*

For individual character output, you may choose to see the fit measures for all characters or you may restrict the output to only those characters which vary over the trees in memory. This latter information is useful in judging which characters or sets of characters provide support for which trees. If you have a number of trees in memory from different searches, this is a very good summary of character support for each rival tree, however it does not provide detailed information on specific character change such as that output by a **Describe Trees** command.

Filter Trees…

Use **Filter Trees** to temporarily limit available trees to those that satisfy certain criteria. These criteria can include (1) trees less than or equal to a certain length, (2) trees greater than or equal to a certain length, (3) trees that satisfy a particular constraint specification, (4) trees that fail to satisfy a particular constraint specification, or (5) trees that are either less than or greater than a certain number of symmetric-difference distance units from a reference tree. The filtering criteria are described in detail in the section "Filtering Trees."



*The **Filter Trees…** dialog box.*

There are many potential applications for tree filtering. For example, you may choose to save nonminimal trees during a search (via a "keep" length) and then subsequently obtain consensus trees showing the information common to only the shortest trees, trees within one step of the shortest, and so on. Also, if a search finds a great many trees, you can check (via the constraints criterion) if particular groups occur on all or some of them. Finally, you can select suites of similar trees using the symmetric-difference distance criteria (or alternatively, find trees that are very different from a particular tree). Use your imagination.

Remove Filter

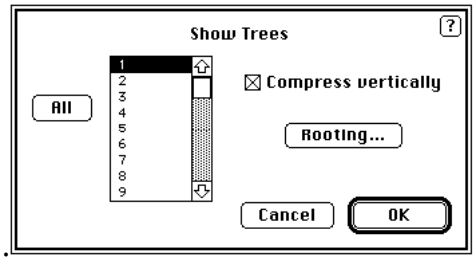
Remove Filter "undoes" the effect of a previous **Filter Trees** command, making all trees in memory available once again.

Reverse Filter

The **Reverse Filter** command simply reverses the filter status. Trees formerly hidden by the filter become available, and vice versa.

Show Trees…

Use this command to quickly output trees without associated character information.

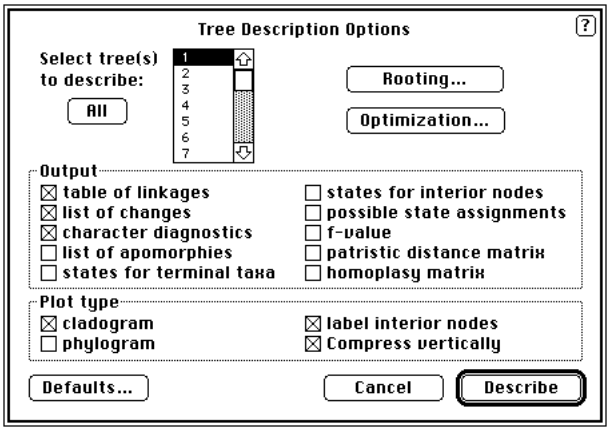


*The **Show Trees…** dialog box.*

If the trees are unrooted, the rooting method currently in effect will be used, or it can be set by clicking on the **Rooting** button.

Describe Trees…

Use this command to output trees with associated statistics and/or character information. There are a wide array of output options. See the section "Diagnosing Trees" for detail on these options and their output.

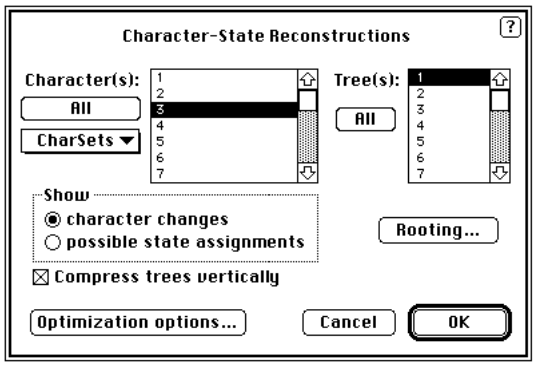


*The **Describe Trees…** dialog box.*

Available information includes a list of apomorphies (character changes by branch); list of changes (character change by character); table of linkages (assigned, minimum, and maximum branch lengths); character diagnostics (goodness-of-fit statistics for each character; states for terminal taxa; states for internal nodes; possible state assignments; the f-value of Farris for each tree chosen; a patristic distance matrix between taxa; and finally a pairwise homoplasy index. No matter what character information is selected, a tree will **always** be output, along with basic tree statistics. This is the one of the best places to explore where characters changed on a tree and which nodes are supported by which characters. Keep in mind that the output for many of these options will directly be a function of the method of optimization chosen.

Show Reconstructions…

This command produces either (a) a plot of the chosen tree(s) with the current reconstruction for each selected character "mapped" onto the tree (**Character changes**), or (b) a plot of the chosen tree(s) with the states that could be assigned to each internal node in at least one most parsimonious reconstruction (**Possible states assignments**).

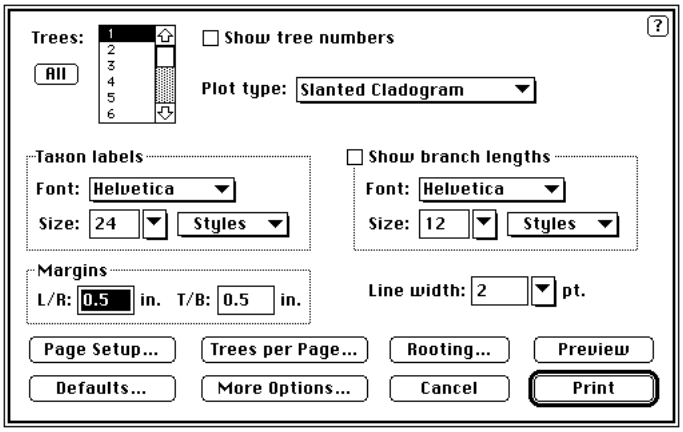


*The **Show Reconstructions…** dialog box.*

Branches are shaded at locations where character changes were assigned (**character changes** option) or where an unambiguous character changes must occur (**Possible states assignments** option). For the "possible states" plots, a '?' drawn at an internal node means that any of the observed states could be assigned to that node.

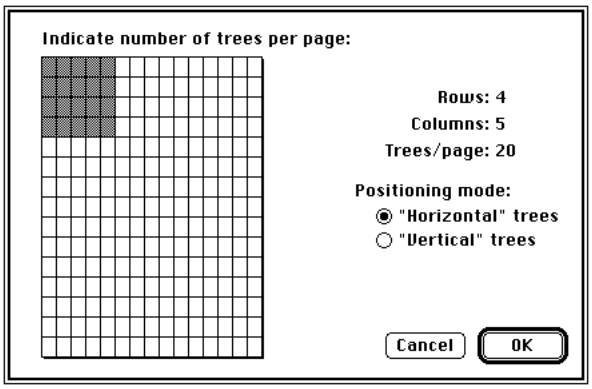
Print Trees…

This command brings up a complicated dialog box that gives you wide control over the printing of trees in memory.



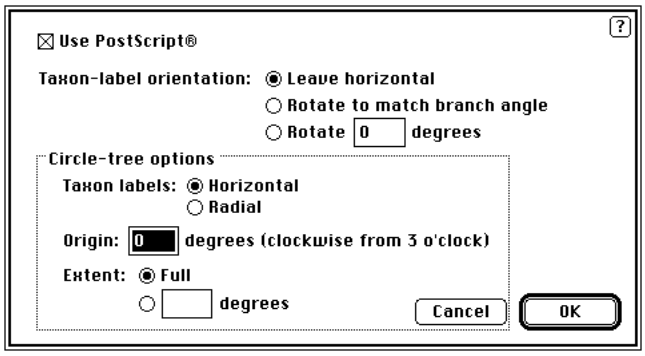
*The **Print Trees…** dialog box.*

Under the **Plot type** menu, you can select the basic form of the plot, whether a phylogram, circletree, or cladogram (rectangular or slanted). You must also choose a tree or range of trees from those in memory, and optionally include the tree number and branch lengths in the output. If there are multiple trees, you may wish to save space by printing more than one tree on a page. This is done using the **Trees per Page** option.



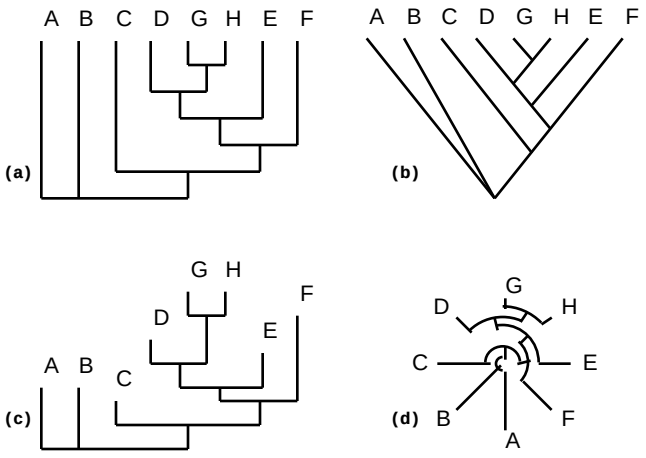
*The **Trees per Page…** dialog box.*

Select the number of rectangles that correspond to the number of trees you which displayed on each page. If you print multiple trees per page, you will probably need to reduce the font size and line width accordingly. This menu also allows you to choose whether the trees are oriented horizontally or vertically. The main **Print Trees…** menu also allows you to choose font type and size and line size for the plots. These may take some adjusting if your are printing multiple trees per page, and some experimentation is usually necessary to get the best results. The **More options...** button allows you to select taxon-label orientation, details of circleTree display, and whether or not the output will be in PostScript format.



*The **More Options…** dialog box.*

Due to the limitations of Macintosh graphics at 72 dpi, CircleTrees do not print very well unless you print directly to a PostScript printer, so use a LaserWriter for best results. The figure below illustrates the types of printed trees that PAUP can generate:



Tree printing options . (a) rectangular cladogram. (b) slanted cladogram. (c) phylogram. (d) CircleTree.

Up to 225 trees can be printed on a page, with or without tree numbers. You can also change how the trees are rooted when they are printed, using outgroup, Lundberg, or midpoint rooting. If outgroup rooting is chosen, the outgroup can be rooted at an internal node with a basal polytomy, or the ingroup can be made monophyletic relative to the outgroup. If there is more than one outgroup present, the tree can be rooted so that the outgroup is either paraphyletic relative to the ingroup, or a monophyletic sister-group to the ingroup.

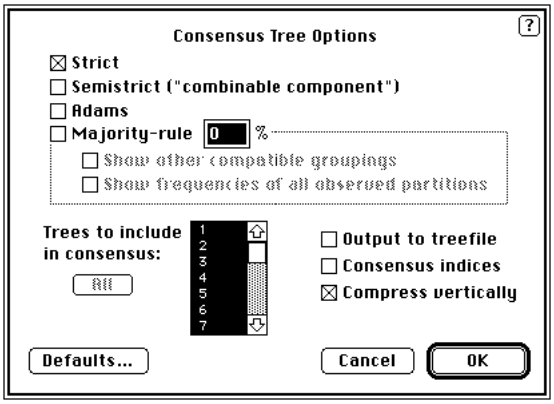
Once you have selected the display options you prefer, the **Preview** button allows you to see what the output will look like without printing. It is useful to toggle back and forth between the **Preview** menu and the main selection menu to fine-tune your selections. You also have the option in **Preview** to copy the image to a PICT file (which can be opened by any

Macintosh graphics program) or to the clipboard (from which it can be pasted into an open window of a graphics application). If you plan to import the image to an application which cannot import PICT files, use **Copy to Clipboard**. Neither **PICT file** nor **Copy to Clipboard** requires that you actually print the image.

PAUP currently makes no attempt to intelligently print files that are wider than the width of one page. If this is a problem with your file, some suggestions to reduce the size of the output are: use a smaller font; format using the interleave option or use more than one line for each taxon; print from within MacClade; or open the file in an application that automatically wraps long lines (most word processing programs).

Compute Consensus…

Use the **Compute Consensus** command to compute consensus. You will usually compute the consensus for all trees currently in memory, but you can select a subset if desired.

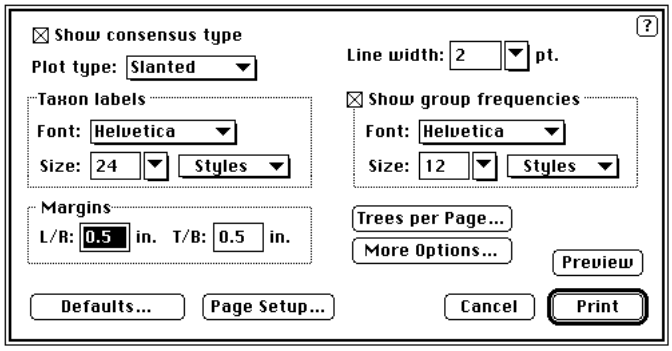


*The **Compute Consensus…** dialog box.*

Consensus indices may also be computed. These are described in Rohlf (1982) and Swofford (1991) . Available consensus techniques are semistrict, strict, Adams, and majority rule. You can include consensus indices, and output the consensus to a treefile of your choosing.

Print Consensus…

This allows you to specify the format of the consensus when it is printed.

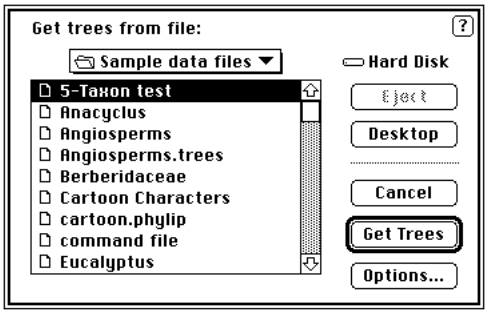


*The **Print Consensus…** dialog box.*

The options are generally the same as for the **Print Trees…** command. However, for majority-rule (including Bootstrap) consensus trees, you can request that the group frequencies be drawn on branch labels.

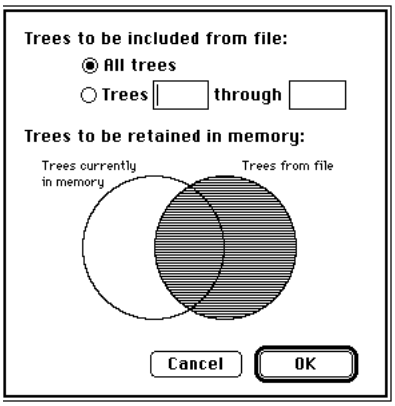
Get Trees from File…

Use this command to read trees from a file containing a NEXUS-format TREES block into memory.



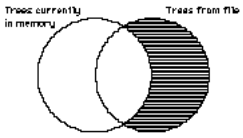
*The **Get Trees from File…** dialog box.*

By default, all trees in the file will be read in. Alternatively, by clicking the **Options…** button, you may specify a range of trees to import or you may use Boolean operations to select which trees are to be retained. The Boolean operations are controlled using the Venn diagram provided. The circle on the left represents the trees in memory; the one on the right represents the trees in the file. When a circle or a segment of it is dark, that means the trees in that region will be kept. For example, selecting all the trees in the file (the default) would be achieved by the following:

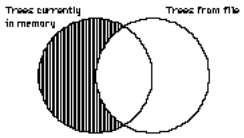


*The **Options…** dialog box.*

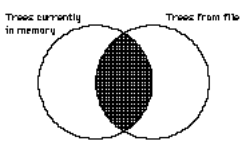
This corresponds to MODE 3 in the GETTREES command. The range of other Boolean choices are:



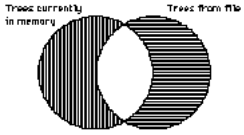
mode 1



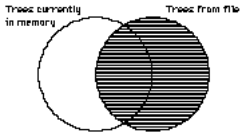
mode 4



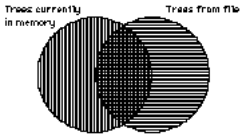
mode 2



mode 5



mode 3



mode 7

The MODE settings are as follows. Let *M* = the set of trees originally in memory and *T* = the set of trees from the tree file. The following mode values are then available:

1 = replace M by T – M (i.e., keep trees from the file that are *not* originally in memory)

2 = replace M by T ∩ M (keep trees from the file that are *also* originally in memory)

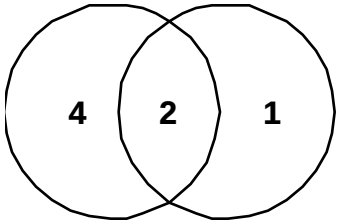
3 = replace M by T (i.e., replace all trees in memory by all trees from the file)

4 = replace M by M – T (i.e., keep trees in memory that are *not* also in the file)

5 = replace M by M T (i.e., keep trees that are either currently in memory or in the file, but not both places)

7 = replace M by M T (i.e., append trees from file to trees originally in memory, with elimination of duplicates)

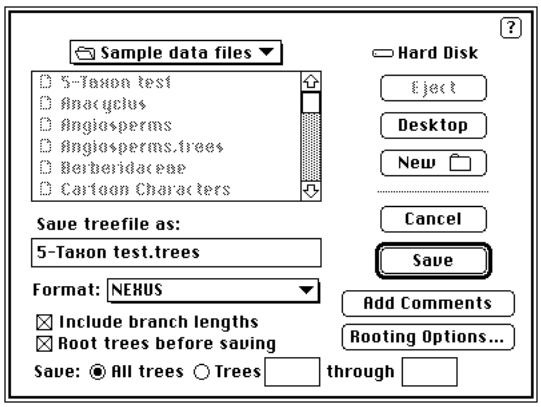
The modes are derived from the following system. Each of the three areas in the Venn diagram has a designated value: 4 for the leftmost crescent, 2 for the middle area, and 1 for the rightmost crescent.



The mode is then simply derived by selecting the regions in the Venn diagram that you wish to include and summing the values. For example, keeping all trees in the treefile encompasses the "1" region and the "2" region, hence it is mode 3. Since mode 6 amounts to reading the trees in memory back into memory, it accomplishes nothing and is not available as an option.

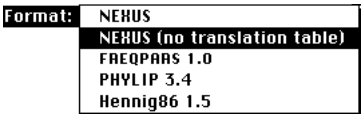
Save Trees to File…

Use this command to save some or all trees currently in memory to a file in NEXUS, PHYLIP, FREQPARS, or Hennig86 formats.



*The **Save Trees to File…** dialog box.*

The file format is chosen using the pull-down menu in the **Format…** option.



*The **Format** pull-down menu.*

You can choose to root the trees before saving by clicking on the **Root trees before saving** checkbox (not available for FREQPARS format). If

you do select the **Root trees before saving** checkbox, the **Rooting Options…** button becomes active, and selecting it will bring up the standard **Rooting…** dialog box. You can include branch length information by clicking on the **Include branch lengths** checkbox, but only if the file format is NEXUS or PHYLIP. NEXUS files can also be saved with or without a translation table. If a translation table is selected, PAUP will include a table that maps tokens in the tree specification to valid taxon names. See the section "The TREES block" for a description of the use of translation tables.

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