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OUTGROUP ANALYSIS AND PARSIMONY

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Abstract.—Methods that use outgroups in the reconstruction of phylogeny are described and evaluated by the criterion of parsimony. By considering the character states and relationships of outgroups, one can estimate the states ancestral for a study group or ingroup, even when several character states are found among the outgroups. Algorithms and rules are presented that find the most parsimonious estimates of ancestral states for binary and multistate characters when outgroup relationships are well resolved. Other rules indicate the extent to which uncertainty about outgroup relationships leads to uncertainty about the ancestral states. The algorithms and rules are based on "simple parsimony" in that convergences and reversals are counted equally. After parsimony is measured locally among the outgroups to estimate ancestral states, parsimony is measured locally within the ingroup, given the ancestral states, to find the ingroup cladogram. This two-step procedure is shown to find the ingroup cladograms that are most parsimonious globally; that is, most parsimonious when parsimony is measured simultaneously over the ingroup and outgroups. However, the two-step procedure is guaranteed to achieve global parsimony only when: (a) outgroup relationships are sufficiently resolved beforehand; (b) outgroup analysis is taken to indicate the state not in the most recent common ancestor of the ingroup, but in a more distant ancestor; and (c) ancestral states are considered while the ingroup is being resolved, not merely added afterward to root an unrooted network. The criterion of global parsimony is then applied to evaluate procedures used when outgroup relationships are poorly resolved. The procedure that chooses as ancestral the state occurring most commonly among the outgroups can sometimes yield cladograms that are not globally parsimonious. By the criterion of global parsimony, the best procedure is one that simultaneously resolves the outgroups and ingroup with the data at hand. Finally, simple parsimony can choose among competing hypotheses, but it often fails to indicate how much confidence can be placed in that choice. [Phylogeny reconstruction; cladistic methods; outgroup analysis; character polarity; parsimony.]

This paper explores the use of outgroup analysis in phylogeny reconstruction. When reconstructing a phylogeny, a systematist asks: Given a group of organisms (the ingroup), what are the monophyletic subgroups? If the members of a subgroup share a character state that is derived within the group, the monophyly of this subgroup is corroborated (Hennig, 1966; Wiley, 1975). Hence, systematists attempting to infer phylogenies have sought methods for determining whether a given character state is derived (apomorphic) or ancestral (plesiomorphic). Many methods for assessing the evolutionary polarity of characters have been proposed, including outgroup analysis, ingroup analysis, the ontogenetic method, and the paleontological method. These approaches have been reviewed recently by Crisci and Stuessy (1980), de Jong (1980), Stevens (1980), Arnold (1981), Nelson and Platnick (1981), and others. The methods perhaps most widely accepted today are outgroup analysis and the ontogenetic method, the relative merits of which are still being debated (contrast Nelson [1978] and Patterson [1982] with Lundberg [1973], Wheeler [1981] and Voorzanger and van der Steen [1982]).

In its simplest form, outgroup analysis can be summarized by the following rule (Watrous and Wheeler, 1981): For a given character with two or more states within a group, the state occurring in related groups is assumed to be the plesiomorphic state. This rule is inadequate, however, when characters vary among the related groups (the outgroups). Arnold (1981) and Farris (1982) have dealt with some cases of

variation among the outgroups, but as yet no general treatment of the problem is available. Hence, we give general algorithms and rules which find the simplest (most parsimonious) hypothesis of ancestral state given that outgroup relationships are well enough resolved. Because outgroup relationships are often uncertain, we discuss the extent to which uncertainty about outgroups leads to uncertainty about the ancestral states.

Examination of outgroups helps to ensure that the ingroup cladograms obtained are parsimonious, not merely within the ingroup, but when considered in the context of related groups (Engelmann and Wiley, 1977; Farris, 1980, 1982). If one were to study the ingroup in isolation, one would choose ingroup cladograms that are locally parsimonious. However, some of these locally-parsimonious ingroup cladograms may force unnecessary hypotheses of convergence and reversal in the outgroups. Such ingroup cladograms should be avoided. Therefore, to ensure a more global parsimony over a larger group of organisms, one should examine the outgroups as well, choosing ingroup cladograms that are parsimonious globally, over the ingroup and outgroups together. However, not all procedures that use outgroups obtain globally parsimonious cladograms.

We examine several cladistic procedures to determine whether they achieve global parsimony. Suppose that outgroup relationships are well resolved. One common procedure estimates ancestral states using outgroup analysis, and then resolves the ingroup given these ancestral states. Although this two-step procedure examines separately parsimony in the outgroups and ingroup, we show that when properly done it finds the cladograms that are most parsimonious over the outgroups and ingroup together. However, the procedure may fail to achieve global parsimony if outgroup analysis is taken to indicate the state in the most recent common ancestor of the ingroup (as done, for instance, by Wiley, 1981) or if an unrooted network for the ingroup is first resolved without reference to the ancestral states (as done by Lundberg, 1972). We then evaluate procedures for use when outgroup relationships are uncertain. Some authors (e.g., Arnold, 1981) have suggested that the state appearing most commonly among the outgroups can be taken to be ancestral. This procedure can choose cladograms that are not globally parsimonious. The outgroup substitution approach of Donoghue and Cantino (1984) will not yield an unparsimonious cladogram, but it may leave the cladogram poorly resolved. In some cases, a more resolved cladogram results when the outgroup and ingroup relationships are resolved simultaneously with the data at hand.

Our treatment is based on what we call "simple parsimony"; that is, we prefer those hypotheses that require the fewest ad hoc hypotheses of character state change (Farris, 1983), counting convergences and reversals equally. We note in the final section that although simple parsimony can choose among competing hypotheses, it often does not specify the confidence that can be placed in the choice (Farris, 1983).

TERMS AND CONVENTIONS

Figures 1 and 2 illustrate some terms and conventions used throughout this paper. The goal of phylogeny reconstruction is to resolve cladistic structure within the *ingroup*, shown as a triangle (after Arnold, 1981). Character states ancestral for the ingroup are estimated on the basis of the states and interrelationships of the *outgroups*, shown on the left-hand portion of the cladogram. Figure 1 shows a single outgroup; Figure 2 shows four outgroups, the first outgroup (i.e., the sister group to the ingroup) having three terminal taxa. Note that we use "outgroup" to mean a clade that attaches to the stem coming down from the ingroup. The most recent common ancestor of the ingroup is represented on the cladogram by the *ingroup node*, whereas the most recent common ancestor of the ingroup and first outgroup is represented by the *outgroup node* (Fig. 1).

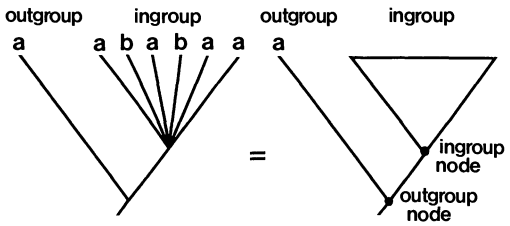

FIG. 1. Terms and symbols used throughout the paper. The unresolved ingroup is shown as a triangle. There may be more than one outgroup.

At the start of an analysis, the relationships within and among the outgroups may be well known (fully resolved), or poorly known (largely unresolved, uncertain). Figure 2 shows relationships among outgroups fully resolved (Figs. 10A and 10B show relationships among and within outgroups poorly resolved). We will deal only with cladograms that have divergent dichotomies or polychotomies representing uncertainty, not with cladograms that have reticulations or polychotomies representing multiple speciation. Whatever is assumed about outgroup relationships at the beginning of the analysis is taken as given for the subsequent analysis, and not to be challenged.

In our treatment, outgroup analysis estimates the state of a character in the most recent common ancestor of the ingroup and first outgroup (at the outgroup node). We discuss below under "Global Parsimony" our reasons for estimating at the outgroup node. Depending on the states and relationships of the outgroups, some states of the character can be more parsimoniously assigned to the outgroup node than can others; that is, some assignments to the outgroup node will require fewer hypotheses of convergence and reversal in the outgroup portion of the cladogram. Several assignments may be equally most parsimonious. The *ancestral state assessment* for a character is a statement listing all of the states that are most parsimonious assignments to the outgroup node. When only one most parsimonious assignment exists, the ancestral state assessment is said to be *decisive*. When more than one state can be assigned to the outgroup node with equal and maximum parsimony the assessment is *equivocal*.

Our results hold whether one views a cladogram as a diagram that indicates recency of common ancestry (Nelson, 1974) or as a diagram that indicates only the patterns of character distributions (Nelson and Platnick, 1981). If the cladogram is interpreted as indicating character distributions, then the assignment of states to nodes can be interpreted as merely a bookkeeping procedure to keep track of character distributions. Under this interpretation, outgroup analysis finds the assignment to the outgroup node that requires the fewest hypotheses of characters in the outgroup portion of the cladogram, and the results presented below hold *mutatis mutandis*.

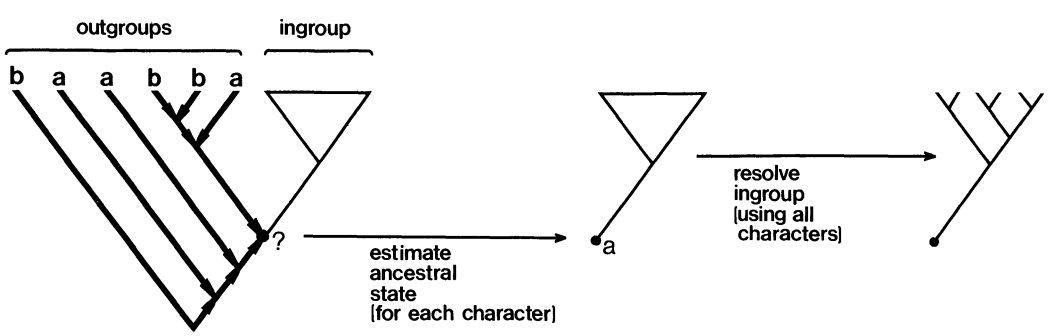

FIG. 2. Two-step cladistic analysis. First, the ancestral states (at the outgroup node) are estimated using the outgroups and parsimony. Second, the most parsimonious ingroup cladogram is sought.

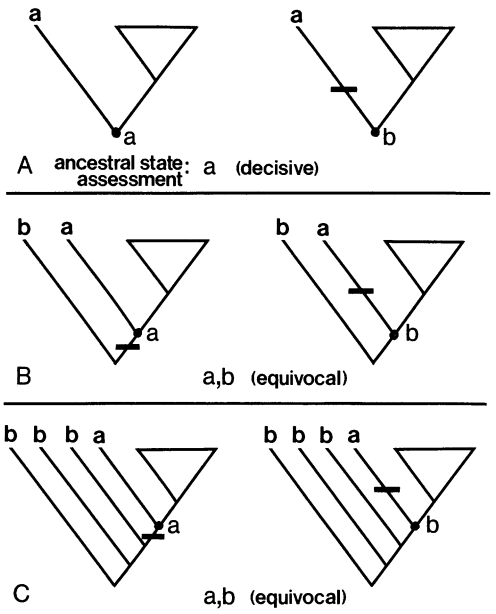

FIG. 3. The use of parsimony to assess the state at the outgroup node. Ancestral state assessment shown below each example. (A) State **a** requires no steps and, hence, is more parsimonious than state **b**. (B and C) **a** and **b** are equally parsimonious.

THE OUTGROUP ALGORITHM

In simple examples (e.g., Fig. 3), the most parsimonious estimates of the ancestral states at the outgroup node can be easily determined by inspection. If there is a single outgroup, with state **a** (Fig. 3A), it is more parsimonious to assume that **a** is ancestral, because the assumption that **b** is ancestral requires an unnecessary step between the outgroup node and the outgroup. Thus, the ancestral state assessment is a decisive "**a**." If there are two outgroups, one with **a** and one with **b**, then one step is required regardless of whether state **a** or **b** is assigned to the outgroup node (Fig. 3B). Because **a** and **b** are equally parsimonious assignments to the outgroup node, the ancestral state assessment is equivocal, written "**a,b**." When two additional outgroups with **b** are added, the assessment remains equivocal (Fig. 3C). Three more-basal **b**'s do not overrule the single **a**, which illustrates that the most parsimonious assignment to the outgroup node is not simply a function of the absolute number of outgroups with a particular state.

In more complex situations (Fig. 2), an algorithm that directly yields the most parsimonious assignments would be useful. Farris (1970) presented an algorithm for finding some of the most parsimonious assignments to all nodes of a cladogram. The algorithm applies to ordered characters and has been called "Farris optimization" (Mickevich and Mitter, 1981). Fitch (1971) presented a similar algorithm for unordered characters. These algorithms, intended for whole cladograms, can be adapted to outgroup analysis in order to find the assignments for the outgroup node that are most parsimonious according to the outgroups.

Our *outgroup algorithms* adapt the Farris and Fitch algorithms to outgroup analysis. An algorithm for binary (**a,b**) characters, consisting of steps (1) and (2) is presented immediately below. (Appendix 1 gives algorithms for multistate characters, including for the first time an algorithm for character state trees. The algorithms apply to fully resolved outgroup relationships. A sketch of the proof of these algorithms is given in Appendix 2. Immediately following the binary algorithm is an example of its application.)

(1) Label terminal taxa among the outgroups by their observed states. (When a terminal taxon has both states **a** and **b**, there are two possible procedures. One could deny that the taxon's internal cladistic structure can be resolved [because, for example, the taxon is a polymorphic species]. In this case label the taxon "**a,b**" and continue. Alternately, one could accept that the taxon's cladistic structure can be resolved. In this case the algorithm is not directly applicable, and the problem should be treated as one of uncertain outgroup relationships.)

(2) Proceed from the outgroup terminal taxa toward the outgroup node, labelling nodes according to the following rule, which is like a voting procedure (Figs. 4A–C). Label a node "**a**" if the two immedi-

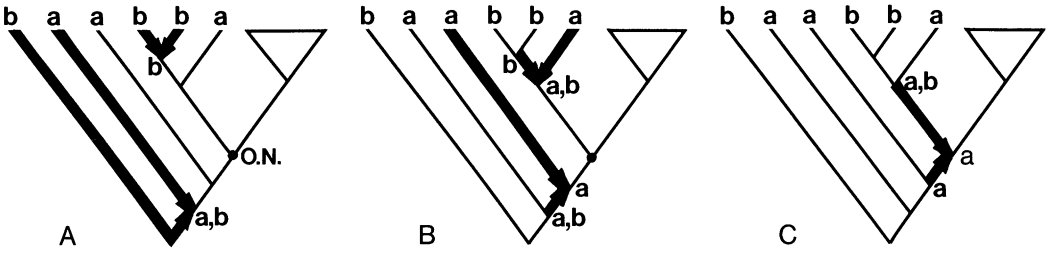

FIG. 4. An application of the outgroup algorithm for binary characters. The internal nodes are labelled, starting at the outgroup terminal taxa and proceeding toward the outgroup node (O.N.).

ately-adjacent nodes that are farther from the outgroup node are labelled "**a**" and "**a**," or "**a**" and "**a**,**b**." Label a node "**b**" if the two immediately-adjacent nodes that are farther from the outgroup node are labelled "**b**" and "**b**," or "**b**" and "**a**,**b**." Label a node "**a**,**b**" if they are labelled "**a**" and "**b**," or "**a**,**b**" and "**a**,**b**." Count the terminal taxa as nodes, but not the root of the cladogram (the algorithm would have difficulty around the root if it were counted as a node). Labelling proceeds toward the outgroup node, until finally a label is applied to the outgroup node (Fig. 4C). This label indicates the assignment(s) to the outgroup node that is (are) most parsimonious according to the outgroups. If the outgroup node is labelled "**a**,**b**," the assessment is equivocal.

To clarify the algorithm, we will describe its application to the example in Figure 4. Within the first outgroup, one proceeds down toward its basal node (Figs. 4A and 4B). Among outgroups, one proceeds up toward the outgroup node. Since the root is not counted as a node, the two nodes immediately adjacent to the node labelled "**a**,**b**" in Figure 4A, but farther from the outgroup node, are the last and second last outgroups. Thus, the labels from the last and second last outgroups are combined to give this label "**a**,**b**" (Fig. 4A). Then, this label "**a**,**b**" is combined with the label "**a**" from the third last outgroup to yield "**a**" (Fig. 4B). Then, this label "**a**" is combined with "**a**,**b**" from the first outgroup's basal node to yield "**a**" at the outgroup node (Fig. 4C). Thus, one considers votes of successively less and less distant outgroups, until at the final step the label from the first outgroup is combined with the label derived from all more distant outgroups.

Labelling toward the outgroup node simplifies the outgroup algorithm. Both Farris's (1970) and Fitch's (1971) algorithms have a first pass (Farris's R-1 and R-2) or preliminary phase which proceeds from the terminal taxa toward the root of a cladogram. The first pass or preliminary phase alone is sufficient to find all most parsimonious assignments to the root (Appendix 2). Subsequent procedures assign states to nodes other than the root. Our outgroup algorithm is equivalent to the first pass or preliminary phase, modified to proceed toward the outgroup node instead of the root (the first pass or preliminary phase can be used unmodified if the outgroup node is first made into the root by rerooting). Because the outgroup algorithm proceeds toward the outgroup node as if it were the root, it succeeds in finding all most parsimonious assignments to the outgroup node. Thus, we waive the subsequent procedures, and avoid the occasional failure of Farris's algorithm to find all most parsimonious assignments to nonroot nodes (Swofford and Maddison, unpubl. manuscript).

The outgroup algorithm gives rise to two simple rules that allow quick and accurate assessment of the ancestral state for binary characters. Suppose each outgroup has only a single state. Then there are two possibilities. First, as one looks outward

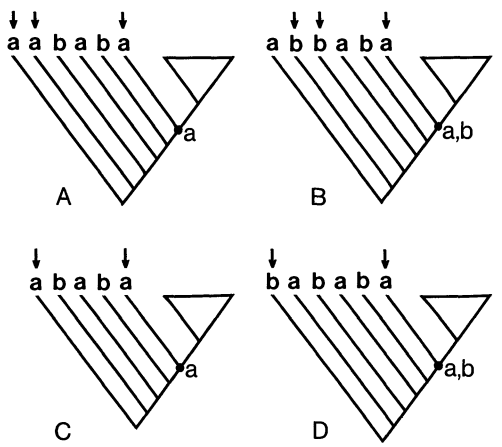

FIG. 5. Simple rules derived from the algorithm. (A and B) First doublet rule. (C and D) Alternating outgroup rule.

from the ingroup, one may encounter a doublet (i.e., a pair of consecutive outgroups that agree in state). In this case the *first doublet rule* applies: If the first outgroup and the first doublet have the same state, then that state is the most parsimonious assignment (Fig. 5A); if they differ, then the decision is equivocal (Fig. 5B). A special case of this rule is particularly important: When the first two outgroups have the same state, then this state is the most parsimonious assignment, no matter what states are shown by outgroups farther from the ingroup. However, there may be no doublets. In this case, the *alternating outgroup rule* applies: If the first and last outgroup have the same state, that state is the most parsimonious assignment (Fig. 5C); if they differ, the decision is equivocal (Fig. 5D). These two rules apply even when there is a heterogeneous outgroup, as long as enough is known of its internal cladistic structure. Any such outgroup can be represented by a single labelled node by applying the outgroup algorithm within the outgroup to yield a label for the outgroup's basal node. This procedure can be thought of as estimating the ancestral state for that outgroup. For example, in Figure 2 the first outgroup could be replaced by a node labelled "**a,b**." Any outgroups represented by "**a,b**" can be eliminated, and the rules applied.

The outgroup algorithm should be applied to the outgroups without regard to the states that appear in the ingroup. Suppose there is a multistate character with states **a** and **b** in the ingroup and states **a** and **c** among the outgroups. De Jong (1980: 12) and Watrous and Wheeler (1981) suggested that, in such a case, **a** should be the estimate of the ancestral state, since it is the only state that occurs in both the outgroups and ingroup. As Farris (1982:331) showed by example, this procedure which ignores state **c** is sometimes not parsimonious. One should instead apply the outgroup algorithm to states **a** and **c** (if outgroup relationships are sufficiently resolved). Depending on the outgroup relationships, the resulting ancestral state assessment could be "**a**," "**a,c**" or "**c**." When the ancestral state assessment at the outgroup node is a decisive "**a**," the outgroups will prefer (at least with respect to this character) those ingroup cladograms that place **a** at the adjacent ingroup node. When the assessment is a decisive "**c**," the outgroups show no preference between those ingroup cladograms that place **a** and those that place **b** at the ingroup node, because one step is required in either case between the outgroup and ingroup nodes. Similarly, with an "**a,b**" assessment, the outgroups would show no preference between those ingroup cladograms that place **a** and those that place **b** at the ingroup node. Thus, with respect to ingroup resolution, an assessment of "**c**" is equivalent to "**a,b**." An assessment of "**a,c**" is equivalent to an assessment of "**a**" with respect to ingroup resolution.

Is information from outgroups adequate to estimate the ancestral states at the outgroup node, or should information from the ingroup also be used? The outgroup algorithm determines the set of estimates of the ancestral state that are most parsimonious according to the outgroups only. Once the ingroup is resolved, it is possible that some of these estimates will no longer be among the most parsimonious or that there will be additional states that are

equally parsimonious, when parsimony is then measured considering the ingroup as well as the outgroups. Should one therefore make new estimates of ancestral states based on the outgroups and ingroup, and redo the cladistic analysis? No. The set of old estimates based only on the outgroups is adequate to find the globally most parsimonious ingroup cladograms (discussed under "Global Parsimony"). Hence, one could do no better with the new estimates, *if* one only needs to resolve the ingroup cladogram. However, if one needs to obtain ancestral state estimates for other purposes, such as evolutionary modelling of character transformation, it is advisable to resolve the ingroup cladogram as much as possible, and then apply the algorithms of Fitch (1971) or Farris (1970, as modified by Swofford and Maddison, unpubl. manuscript) to the outgroups together with the ingroup, in order to determine the state at the outgroup node (or any node). Thus, ancestral state estimates based only on the outgroups are adequate for finding the globally most parsimonious cladograms, but sometimes they are not adequate for general discussions of character transformation.

UNCERTAIN OUTGROUP RELATIONSHIPS

Will an uncertainty about outgroup relationships lead to uncertainty about the ancestral state? If so, how does one proceed with cladistic analysis? In this section we consider these two questions in turn.

Uncertain resolution of outgroup relationships may or may not lead to uncertainty about the ancestral state. In the trivial case, if all outgroups have the same state, then that state will be the ancestral state estimate regardless of outgroup relationships. When the character varies, one can determine whether the ancestral state assessment differs under alternative outgroup resolutions by applying the outgroup algorithm to each resolution. Because it can be tedious to apply the algorithm to every one, we have derived some rules from the outgroup algorithm which give an indication of the extent to which different resolutions yield the same ancestral state assessments.

Most of the uncertainties addressed by the rules, and indeed throughout the paper, involve uncertain resolution of the included outgroups. There can also be uncertainty about whether all and only relevant outgroups have been included in the analysis. As more specimens are examined or higher level relationships are better understood, outgroups could be added or subtracted. Rule 4 below describes the effects of adding or subtracting outgroups.

The rules describe situations in which the uncertainties in the outgroup relationships will have no (Rules 1 and 2) or only limited (Rules 3 to 6) effects on the ancestral state assessment. The effects are limited in that the assessment will not *completely shift* if the outgroup relationships are found to be one of the alternatives instead of another. That is, for binary characters, the assessment can change from decisive (say, "**a**") to equivocal ("**a**,**b**"), or from equivocal to decisive, but it cannot completely shift from decisive "**a**" to decisive "**b**" (or vice versa). For the more general case of multistate characters, the new and old assessments must overlap—at least one of the states judged to be a most parsimonious assignment to the outgroup node by one alternative resolution will also be judged most parsimonious by the other. There are circumstances in which such limited changes will not affect the ingroup cladogram, but in other circumstances they will. Although a binary character with an equivocal ancestral state assessment can still help resolve the unrooted form of the ingroup cladogram, the character has lost all power to choose the root of the cladogram. Thus, if an assessment changes from decisive to equivocal, the balance among conflicting characters may change, resulting in a different ingroup cladogram.

Rules 1 to 4 and 6 are valid for binary and the three sorts of multistate characters of Appendix 1. Rule 5 is valid for binary characters.

Rule 1.—If two consecutive outgroups (as one looks outward from the ingroup, Fig.

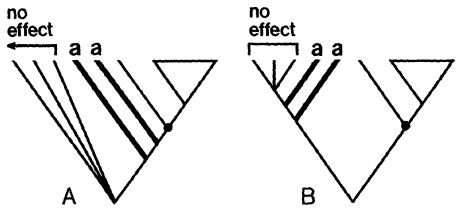

FIG. 6. The buffering effect of doublets. Groups beyond the doublet have no effect on the polarity assessment (Rule 1 of "Uncertain Outgroup Relationships").

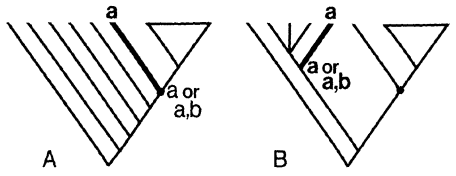

FIG. 8. The importance of (A) the first outgroup and (B) the basalmost member of a heterogeneous outgroup (Rule 3).

6A) have the same single state then outgroups farther out will have no effect on the ancestral state assessment. Thus, uncertainties about outgroup relationships or states beyond a doublet are irrelevant. Likewise, within an outgroup, if two consecutive terminal taxa (as one looks upward from the base of the outgroup, Fig. 6B) have the same state then terminal taxa farther up have no effect.

Rule 2.—The position of the root within the outgroup portion of the cladogram can be changed without affecting the ancestral state assessment (Fig. 7). The outgroup algorithm proceeds from the terminal taxa toward the outgroup node (step 2) and ignores the "true" root of the outgroup portion. Thus, for instance, when there are only two outgroups, the same ancestral state assessment results regardless of which group is placed as the sister group to the ingroup (see Fig. 3B).

Rule 3.—If the first outgroup has a single state (or if its basal node receives a label containing a single state), the assessment for the outgroup node must be either decisive for that state, or equivocal (Fig. 8A). More distant outgroups cannot completely shift the assessment away from the state in the first outgroup. In general, at least one of the states in the label of the first outgroup's basal node must be a most parsimonious assignment to the outgroup node. The first outgroup has the most influence on the most parsimonious assignment even if that outgroup is considered highly derived. An appeal to a supposedly primitive but distant outgroup, and avoidance of a highly derived sister group, are not justified by our parsimony framework. Within an outgroup, a basalmost terminal taxon (i.e., one that is the sister group to the rest of the outgroup) has the most influence on the label given to that outgroup's basal node by the algorithm (Fig. 8B). According to our parsimony framework, the basal members of a heterogeneous outgroup have the most influence on the ancestral state assessment not because they are primitive in most characters and thus should have primitive states of the characters of interest, but because they will have the most influence on the most parsimonious assignment to the outgroup node.

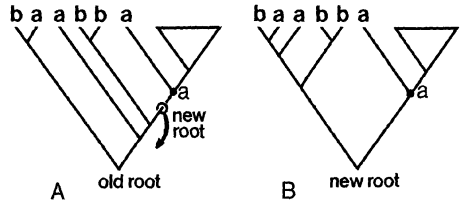

FIG. 7. If the position of the root moves from one point to another among the outgroups, the polarity assessment is not affected (Rule 2).

Rule 4.—A single addition or deletion of an outgroup (or of a single terminal taxon in an outgroup) cannot completely shift an assessment, no matter where the outgroup is added or subtracted (Fig. 9A). It takes at least two additions or deletions to shift an assessment. Note, however, that even a distant addition can affect the assessment (compare Figs. 5C and 5D).

Rule 5.—Moving a single outgroup (or a

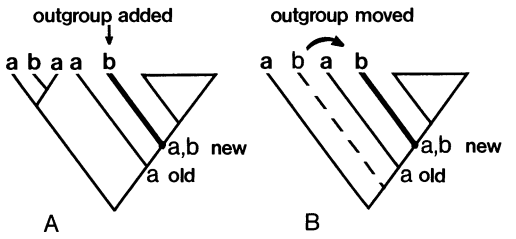

FIG. 9. The effect of adding or moving a single outgroup. New and old assessments shown. The assessment can change from decisive to equivocal or vice versa, but not decisive "**a**" to decisive "**b**" (Rules 4 and 5).

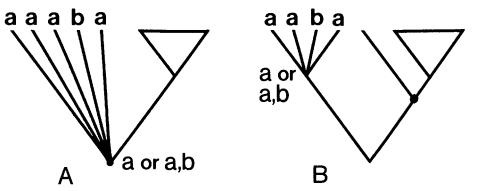

FIG. 10. (A) If one state is present in only one of several outgroups, the ancestral state assessment cannot be decisive for that state (Rule 6). (B) The same is true for the assessment of the ancestral state within an outgroup.

single terminal taxon) from one position to another cannot completely shift an assessment (Fig. 9B). It takes at least two moves to shift an assessment. This rule holds for binary characters. We have been unable to prove or disprove the rule for multistate characters in general.

Rule 6.—When only one of several outgroups has state **b** (for example), then the ancestral state assessment cannot be decisive for state **b**, regardless of how the outgroups are arranged (Fig. 10A). Thus, for binary characters, the assessment must be either "**a**" or "**a,b**." Likewise, within an outgroup, the basal node cannot receive the label "**b**" when only one of several terminal taxa has state **b** (Fig. 10B).

We should clarify the distinction between equivocal assessments and the uncertainty in assessments described above. An equivocal assessment arises when, according to a single outgroup resolution, there are equally parsimonious assignments to the outgroup node. Uncertainty in assessments arises when there are several plausible outgroup resolutions which yield different ancestral state assessments (some of which may be equivocal, others decisive). This uncertainty might be overcome by resolving the outgroups to a single arrangement, but even then the resulting assessment could be equivocal. Equivocal assessments can only be overcome by adding or subtracting outgroups or terminal taxa within outgroups.

When the ancestral state assessment differs according to the different outgroup resolutions, how does one proceed with cladistic analysis? We will not discuss the case where uncertainty exists about whether all and only the relevant outgroups have been included in the analysis (see instead Donoghue and Cantino, 1984). Since uncertainty about inclusion arises from uncertainty about resolution at a higher level, in some cases it may be better to include all plausibly related groups that have been investigated and treat the uncertainty as uncertainty about the resolution of their interrelationships. We describe below some procedures (evaluated in the next section) one might follow when uncertainty exists about the relationships of the included outgroups.

In the best of worlds one would perform a higher level analysis to resolve outgroup relationships with more and new data. This procedure is attractive, but it transfers the problem to a higher level where the same uncertainties may be present. Eventually, practical limitations will force a systematist to stop backtracking, and to analyze the data at hand.

Failing a higher level analysis, one might appeal to a criterion other than simple parsimony to make an ancestral state assessment despite uncertain outgroup relationships. One could assume irreversibility or progress towards presumed optimality. Alternately, one could use the predominant-states method, in which the state appearing most commonly among the outgroups is taken to be ancestral for the ingroup. This method has been implied or at least allowed by previous formulations of outgroup analysis. Kluge and Farris

(1969) used the criterion: "The primitive state of a character for a particular group is likely to be present in many of the representatives of related groups." Arnold (1981:10) explicitly accepted the predominant states method even though he rejected common-is-primitive ingroup analysis. Predictably, an appeal to criteria other than simple parsimony to make an ancestral state assessment can lead to cladograms that are not globally parsimonious.

Another option when faced with uncertain outgroup relationships is to stay within the bounds of simple parsimony, possibly reducing some of the uncertainty with the data at hand. For each alternative outgroup resolution, ancestral states can be estimated. All of the alternative ancestral state estimates in all of the characters can be summarized by a set of possible hypothetical ancestors, each describing the ancestral state in all characters. For each hypothetical ancestor, a candidate ingroup cladogram can be resolved. If there are many alternative outgroup resolutions, there may be many candidate ingroup cladograms among which to choose. Some candidates can be selected as more parsimonious with respect to the ingroup, outgroups, or both. Thus, despite uncertainty about ancestral states it may be possible to select a single ingroup cladogram. We discuss such a selection procedure in more detail below.

GLOBAL PARSIMONY

A globally most parsimonious ingroup cladogram is one which requires the fewest hypotheses of convergence and reversal (in all characters examined) within the ingroup and among the outgroups. Thus, such a cladogram is not merely most parsimonious locally, but is most parsimonious in the context of related groups (Engelmann and Wiley, 1977; Farris, 1980:511; Wiley, 1981:112, 128). An ingroup cladogram can be more or less parsimonious with respect to the outgroups because it can force unnecessary hypotheses of convergence and reversal in the outgroups or below the ingroup. For instance, the ingroup cladograms in Figures 11A and 11B

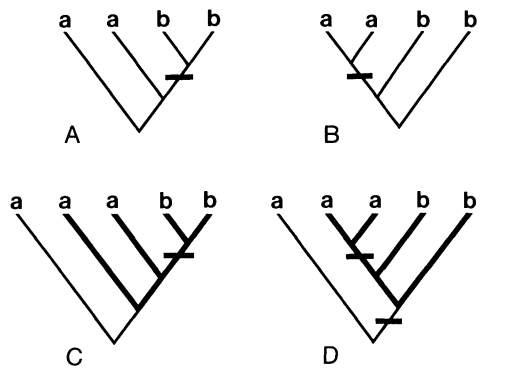

FIG. 11. Global parsimony. Cladogram A is more parsimonious than B when an outgroup is considered (C and D).

each require one step in isolation, but when they are placed in the context of an outgroup (as in Figs. 11C and 11D, respectively), cladogram 11B forces an unnecessary step beneath the ingroup or within the outgroup (Fig. 11D). Thus, cladograms 11A and 11B are equally parsimonious locally, but cladogram 11A is more parsimonious globally. Although outgroups must be examined to ensure that cladograms are globally parsimonious, not every procedure that uses outgroups will yield globally most parsimonious ingroup cladograms. We evaluate several procedures below.

Outgroup relationships well resolved beforehand.—When outgroup relationships are well resolved beforehand, a two-step procedure can be followed (Fig. 2). First, ancestral states are estimated. Second, the ingroup cladogram is sought. In practice, cladistic analysis often follows this model. Without aid of a computer, one estimates ancestral states using outgroups, then attempts to find the most parsimonious ingroup cladogram (remembering that the ingroup's ancestor started out with the estimated ancestral states). By computer, one includes a hypothetical ancestral taxon (synthesized by outgroup analysis) with the ingroup terminal taxa in an analysis searching for Wagner trees (Farris, 1970), then afterward roots the network at the hypothetical ancestor (Kluge and Farris, 1969).

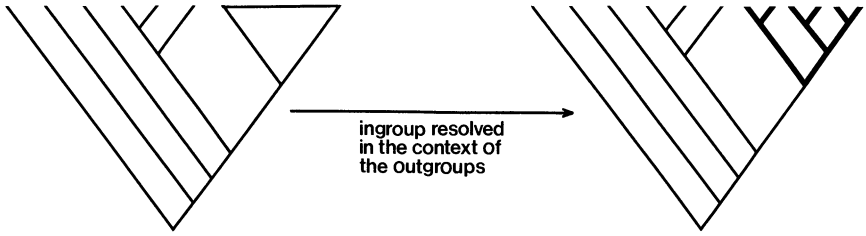

FIG. 12. One-step cladistic analysis. The parsimony of each possible ingroup cladogram is measured globally, over the ingroup and outgroups together, using all characters. Thus, the ingroup is resolved with the outgroups attached.

This two-step procedure can be compared with a one-step procedure which maintains the outgroups attached to the ingroup throughout the analysis (Fig. 12) and chooses ingroup cladograms by measuring their parsimony over the ingroup and outgroups together. This one-step procedure directly measures the global parsimony of an ingroup cladogram, and therefore it is guaranteed to find the globally most parsimonious ingroup cladograms, providing it exhaustively examines all possible ingroup cladograms. In contrast, the two-step procedure evaluates parsimony in two disjoint phases. First, parsimony is considered locally among the outgroups to estimate the ancestral states. Second, parsimony is considered locally within the ingroup (given the estimated states are ancestral) to choose the ingroup cladogram.

Does the two-step procedure find the ingroup cladograms that are most parsimonious over the outgroups and ingroup together? In a sense, the globally most parsimonious ingroup cladogram is a compromise, resulting from the conflict between the states that the outgroups "want" to have at the outgroup node, and the states suggested by the resolution that the ingroup "wants" to have on its own. The first step of the two-step procedure, estimating the ancestral states, makes a commitment to the states at the outgroup node. Hence, the outgroups make their final offer of ancestral states at the beginning of the analysis, after which the ingroup must suffer with the ancestral state assessments—bargaining back and forth is prevented. It seemed possible to us that the cladogram obtained would not represent the best mutual compromise. Fortunately, however, the two-step procedure does in fact give the ingroup cladograms that are most parsimonious over the ingroup and outgroups together (proof in Appendix 3). More precisely, the following two procedures yield all and only the same cladograms. (1) Estimate ancestral states by the outgroup algorithm, then find the most parsimonious ingroup cladograms given these ancestral states (two-step procedure, Fig. 2). (2) Maintain the outgroups throughout the analysis and find the most parsimonious ingroup cladograms when parsimony is measured over both ingroup and outgroups together (one-step procedure, Fig. 12). This global parsimony result holds for data sets consisting of binary characters, any of the three sorts of multistate characters described in Appendix 1, or combinations thereof. It holds whether these characters are equally or differentially weighted.

An initial commitment to the states in the most recent common ancestor of the ingroup (the ingroup node) might prevent the discovery of the globally most parsimonious cladograms. For this reason, we treat the estimate of the ancestral states as referring to an ancestor more distant than the ingroup node. For example, suppose state **a** occurs in the outgroups, and states **a** and **b** in the ingroup. Wiley's (1981) formulation of outgroup analysis and the second part of the operational rule of Watrous and Wheeler (1981) would assume that **b** is apomorphic. If **b** is pre-

sumed apomorphic within the ingroup, then state **a** was present in the most recent common ancestor of the ingroup (the basal dichotomy of the ingroup) and state **b** was derived one or more times within the ingroup. Wiley (1981:141), in his discussion of his figure 5.11f, assumes as much, for he assumes his state **2'** was at the basal dichotomy of the ingroup, and ignores the equally parsimonious possibility that **2** was there (see also Farris, 1982:331). The assumption that state **a** was at the basal dichotomy of the ingroup might later force one to view as independent derivations two instances of state **b** that appear on opposite sides of the dichotomy. On the other hand, treating outgroup analysis as a procedure for estimating the state in an ancestor below the point of the dichotomy allows the state to change to **b** before the dichotomy. This can save steps and lead to more parsimonious cladograms. For example, in Figure 13A characters 1–30 partially resolve the cladogram as shown. When an initial commitment is made to the states at the ingroup node (Fig. 13B), (DEF) is resolved to ((DE)F) by character 32. When the initial commitment is to the states at the outgroup node, the globally most parsimonious resolution (D(EF)) is obtained (Fig. 13C). An initial commitment to the state in the most recent common ancestor places too strong a constraint on the resolution of the ingroup cladogram (Lundberg, 1972:410); an initial commitment about the more distant outgroup node does not. One *can* treat outgroup analysis as estimating the state in the most recent common ancestor of the ingroup as long as it is only a provisional estimate and not a commitment. In cases of character conflict within the ingroup, one should be willing to reassess the state at the ingroup node, perhaps putting **b** there and having **a** apomorphic within the ingroup. Such a provisional estimate at the ingroup node, if done properly, is equivalent to an initial commitment at the outgroup node.

Because the outgroup node is below the basal dichotomy of the ingroup and because it is a convenient landmark, we have

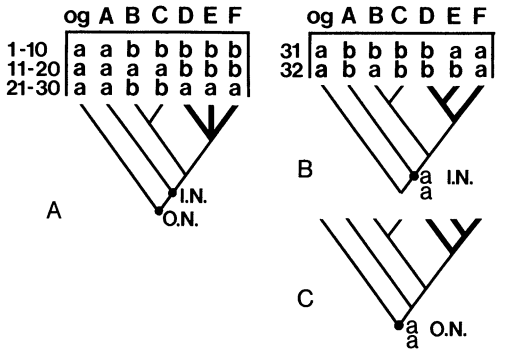

FIG. 13. Hypothetical data set of 32 characters with states **a** and **b** distributed as shown. (A) Characters 1–30 partially resolve ingroup. (B and C) Characters 31 and 32 resolve ingroup further. Treating the estimate of ancestral state as referring to the outgroup node (O.N.) gives more parsimonious cladogram (C) than treating it as referring to the ingroup node (I.N.; B). Definitions: OG, outgroup; A–F, ingroup terminal taxa.

used the outgroup node for our ancestral state assessments. Any point between the ingroup node and the outgroup node could have served as well.

The ancestral state estimates must be included in the analysis while the ingroup is being resolved (Fig. 2). If an unrooted network is found for the ingroup alone without reference to the ancestral states, and this network is afterward rooted by attaching the "hypothetical ancestor" representing the estimated ancestral states (Lundberg, 1972), a cladogram that is not globally parsimonious might be chosen. Imagine that T were the only terminal taxon in the outgroup in the example of Figure 14. If T were added to A–E after an unrooted network had been found for A–E, the cladogram obtained would be (AB)(C(DE)) (Fig. 14B). This resolution, chosen when the ingroup is resolved in isolation from the outgroup, is most parsimonious locally. However, when T is considered throughout the analysis, (AB)((CD)E) is obtained (Fig. 14C), which is more parsimonious globally.

In order for the two-step procedure to find the globally most parsimonious cladograms, the second step must find the ingroup cladograms most parsimonious

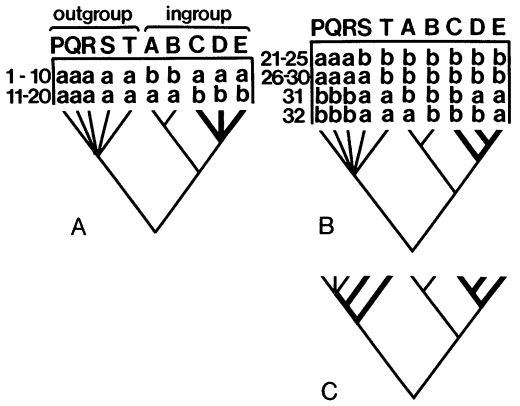

FIG. 14. Another data set of 32 characters with states **a** and **b** distributed as shown. (A) Characters 1–20 partially resolve the ingroup. (B and C) Characters 21–32 resolve the ingroup further. Simultaneously resolving the outgroup and the ingroup gives more parsimonious cladogram (C) than assuming the most common state within the outgroup to be ancestral (B). If T were the only outgroup terminal taxon, finding the unrooted network for the ingroup alone then attaching T would give a less than most parsimonious cladogram.

locally given the ancestral states. If the second step fails to do this, the two-step procedure may fail to find the globally most parsimonious cladograms. Given the difficulty of judging parsimony by eye and of finding the most parsimonious cladograms by computer (Felsenstein, 1982), the second step may often fail.

Outgroup relationships poorly resolved.— Two procedures were introduced above for dealing with uncertain ancestral state assessments arising from uncertain outgroup relationships. The first is the predominant-states method. The second resolves various candidate ingroup cladograms and possibly selects among them. We discuss these procedures below and evaluate them by the criterion of global parsimony. Our main result is that the ingroup and outgroups can sometimes be resolved simultaneously with the data at hand to yield a well resolved and globally most parsimonious ingroup cladogram.

The predominant-states method steps outside of simple parsimony to make a decision about the ancestral state. When interrelationships among outgroups are unresolved (Fig. 10A), the predominant-states method assumes that the ancestral state for the ingroup is the state most common among the outgroups. When the relationships of terminal taxa within an outgroup are unresolved (Fig. 10B), the state most common among the terminal taxa is assumed to be ancestral for that outgroup (thus, the basal node of the outgroup in Fig. 10B would be labelled "**a**").

The predominant-states method has yet to be justified by a direct parsimony argument, and in fact it can yield cladograms that are not globally parsimonious. One might attempt to justify the method with a probability argument (Arnold, 1981). For example, if 8 of 10 outgroups have state **a**, most of the possible outgroup resolutions will have state **a** as the most parsimonious assignment to the outgroup node. Thus one could suppose that **a** is most probably most parsimonious. However, in Figure 14, the predominant-states method uses character 31 to resolve (CDE) into (C(DE)) which is not globally most parsimonious (this example is discussed further below). Our Rule 6 appears to make similar claims, but in fact it makes much weaker claims and is based strictly on parsimony.

Instead of stepping outside of simple parsimony, one could resolve a candidate ingroup cladogram using each of the possible ancestral states and select those candidates that are most parsimonious according to the outgroups, ingroup, or both. For example, suppose that for one character some of the alternative outgroup resolutions yield the ancestral state assessment "**a**," whereas others yield "**b**," and for another character some resolutions yield "**x**" and others yield "**y**." There are four combinations that might be placed at the ancestral outgroup node: **a** and **x**, or **a** and **y**, or **b** and **x**, or **b** and **y**. Each of these combinations represents a hypothetical ancestor. Each of these four could be used to resolve a candidate ingroup cladogram. Their strict consensus cladogram, consisting of just those clades common to all the candidates (Sokal and Rohlf, 1981), could

be used as the final ingroup cladogram. However, because the consensus cladogram must agree with all four candidates, it would probably be poorly resolved. Such ambivalence is not necessary. Some hypothetical ancestors may be preferred by (i.e., may be more parsimonious according to) the outgroups or ingroup. Hence, candidate ingroup cladograms yielded by these hypothetical ancestors can be selected, and a more resolved consensus might result.

First, some of the hypothetical ancestors may be preferred by the ingroup. For instance, when **a** and **x** are placed at the outgroup node, the ingroup may be able to arrange itself into a cladogram with fewer steps than it can when **b** and **y** are placed at the outgroup node.

Second, not all of these hypothetical ancestors are actually supported by an outgroup resolution. Each outgroup resolution yields a combination of ancestral states. For example, suppose there are only two plausible resolutions, under one of which the assessments are "**a**" and "**x**," under the other "**b**" and "**y**." Some combinations, like "**a**" and "**y**," are not obtained under either of the outgroup resolutions. Thus, although each of "**a**," "**b**," "**x**," and "**y**" are individually supported as ancestral states by an outgroup resolution, some of their combinations (i.e., some of the hypothetical ancestors) are not supported by any of the resolutions. Ignoring this preference for combinations could cause one to choose a cladogram that is not globally parsimonious. In Figure 14, the hypothetical ancestor having "**b**" for each of the characters 21–32 is allowed if the outgroup's preference for combinations is ignored. Because the ingroup prefers this combination of **b**'s, this hypothetical ancestor could be chosen and (CDE) resolved to (C(DE)). But in fact, the outgroup dislikes this combination of **b**'s, because there is no outgroup resolution under which **b** is a parsimonious estimate of the ancestral state for each of the characters 21–32. (C(DE)) is not globally most parsimonious, because it was resolved using a hypothetical ancestor for which the outgroup had a strong dislike. The outgroups may be uninformative about each character taken separately, but they can be informative about preferred character combinations.

Third, even among the ancestral state combinations yielded by the various outgroup resolutions, some may be more parsimonious than others according to the outgroups. The alternative outgroup resolutions may have been proposed on the basis of previous data but, on the basis of the data at hand, some of these resolutions of the outgroups may have poor parsimony. Hence, the hypothetical ancestors that they yield are not parsimonious according to the outgroups.

Thus, among the many hypothetical ancestors that may arise from the alternative outgroup resolutions, one might select those preferred by the outgroups, ingroup, or both. In selecting these ancestors, one is also selecting the candidate ingroup cladograms that they yield.

The outgroup substitution approach of Donoghue and Cantino (1984) uses all and only those ancestral state combinations yielded by the alternative outgroup resolutions (and thus it takes into account the second preference, above). Under each alternative outgroup resolution an ancestral state combination (hypothetical ancestor) is estimated, which is then used to resolve a candidate ingroup cladogram. The strict consensus cladogram of all the resulting candidate cladograms is used as the ingroup cladogram, the rationale being that the clades of this cladogram would be valid regardless of how the outgroups were eventually resolved. It is a conservative estimate in the face of uncertainty.

The consensus cladogram from this approach will not contradict the globally most parsimonious cladograms. Because all and only hypothetical ancestors actually yielded by outgroup resolutions are used, the globally most parsimonious ingroup cladogram(s) must be among the candidates resolved. Therefore, the consensus of the candidates cannot contradict these most parsimonious ingroup cladograms. However, the consensus cladogram may

be poorly resolved. In the example of Figure 14, the outgroup resolution (P(Q(RST))) yields (C(DE)), whereas (((PQR)S)T) yields ((CD)E). Therefore, (CDE) is unresolved in the consensus cladogram. The outgroup substitution approach does not consider the first and third preferences of the ingroup and outgroups, respectively.

If one fully considers the ingroup and outgroup preferences, one can discard more of the hypothetical ancestors, and so possibly obtain a consensus cladogram more resolved than obtained by the outgroup substitution approach. Indeed, in some cases a single hypothetical ancestor and thus a single fully resolved ingroup cladogram can be chosen. For example, in Figure 14 the hypothetical ancestor consisting of **b** in characters 21–30 and **a** in 31 and 32, obtained from the outgroup resolution (((PQR)S)T), is much preferred by the outgroup and only a little disliked by the ingroup. This hypothetical ancestor best balances the preferences of the ingroup and the outgroup. In selecting this hypothetical ancestor, one also selects the outgroup resolution that gave rise to it (((PQR)S)T) and the ingroup cladogram that it yields (AB)((CD)E). Together, they are the most parsimonious simultaneous resolution of the outgroup and ingroup.

The most parsimonious simultaneous resolution is the combination of an ingroup resolution and an outgroup resolution which requires the fewest hypotheses of convergence and reversal over the ingroup and outgroups together. This simultaneous resolution could be sought by various methods. The technique described above tried each alternative outgroup resolution, resolved the ingroup for each hypothetical ancestor, and chose the hypothetical ancestor (and hence the resolutions of the outgroups and ingroup) that best balanced parsimony in the outgroups and ingroup. If an exhaustive examination of hypothetical ancestors cannot be done, one could attempt to find the most parsimonious simultaneous resolution without the aid of a computer, using reciprocal illumination between the resolution of the outgroups and the resolution of the ingroup. A resolution of the outgroups could be attempted, ancestral states for the ingroup estimated, a resolution of the ingroup attempted, ancestral states for the outgroups re-estimated based on the ingroup, and so on. For instance, in Figure 14 characters 1–20 support (AB)(CDE) strongly enough not to be outvoted by the other characters (Fig. 14A). Given this arrangement, the ingroup has a strong preference for having **b** at the outgroup node for characters 21–30. This partially resolves the outgroup to (((PQR)S)T). In turn, this polarizes characters 31 and 32 so as to yield ((CD)E). The ingroup and outgroups can bargain back and forth about the states at the ingroup and outgroup nodes, until the best mutual compromise in the resolutions of the outgroups and ingroup is reached. In cases of character conflict the bargaining could be complex and, if not done thoroughly, the resulting resolutions might not be globally parsimonious. To seek the most parsimonious simultaneous resolution by computer, one could include the outgroups and ingroup in a computer analysis searching for unrooted Wagner trees. The analysis could be constrained to maintain the ingroup monophyletic and to maintain any prior assumed structure among the outgroups, perhaps by loading the data set with dummy variables which support this structure (J. S. Farris, pers. comm.). Thus, the program would resolve the ingroup, and resolve uncertainties among the outgroups, but leave any assumed structure intact.

A two-step procedure that makes an initial commitment about the ancestral states, then resolves the ingroup cladogram, is sufficient when outgroups are well resolved, but not when they are poorly resolved. The initial commitment to ancestral states prevents bargaining back and forth between the ingroup and outgroups. When both the outgroups and ingroup have freedom to be rearranged, they can affect each other's resolution. Hence, to find the best mutual compromise, it is important not to make an initial ancestral state commitment based on outgroups alone. Instead, one should choose the out-

group and ingroup resolution by measuring parsimony over the outgroups and ingroup together, or by allowing the outgroups and ingroup to bargain back and forth.

In practical terms, is a procedure that deals simultaneously with the outgroups and ingroup feasible? The characters whose ancestral state assessments are uncertain initially are those that vary among the outgroups, and thus are characters that may indicate a resolution of outgroup relationships. This suggests that simultaneous resolution could be performed when needed. However, for performing simultaneous resolution it would be advisable to use more extensive data sets than are presently used. Suddenly, the ingroup and outgroups have the same status and should be treated with equal care. Characters that vary among the outgroups should be used even if they are invariant within the ingroup. Parallel observations on all characters may be needed for each terminal taxon within heterogeneous outgroups, instead of scattered observations for each character. Thus, to be thorough, one could no longer be a specialist on just the "ingroup." Given limited time and resources, one might think that the simultaneous resolution procedure would simply be impractical. But while it may be infeasible to perform simultaneous resolution in a thorough way, it is nevertheless best in terms of parsimony to resolve the outgroups as much as one can with the data at hand, however scanty these data may be. That is not to say that the simultaneous resolution procedure will necessarily give decisive or convincing results. If the data for the outgroups are incomplete or full of homoplasy, the simultaneous resolution procedure may have little power with which to resolve the outgroups, and the ingroup cladogram may be fraught with uncertainty. In such a circumstance one may prefer the more conservative outgroup substitution approach (Donoghue and Cantino, 1984). Otherwise, one could gather more data and hope.

It is instructive to reconsider some of the procedures described in this section as variants of a constrained computer analysis searching for Wagner trees (Farris, 1970) in which the ingroup and outgroups are included together. When the analysis is constrained to hold the ingroup monophyletic, but there is complete freedom for rearrangement among the outgroups and within the ingroup, we have a simultaneous resolution procedure with completely unresolved outgroup relationships. When some but not all of the outgroup relationships are constrained to remain fixed, we have a simultaneous resolution procedure with partially resolved outgroup relationships. When the analysis is constrained to maintain fixed a fully specified set of outgroup relationships, then we have the one-step procedure for fully resolved outgroup relationships (Fig. 12). This one-step procedure is equivalent to the two-step procedure of Figure 2 (Appendix 3). The two-step procedure is more efficient in that, after estimating the ancestral states by outgroup analysis, it dispenses with the outgroups and searches for the restricted Wagner tree for the ingroup and the hypothetical ancestor.

At the beginning of a cladistic analysis one is faced with a choice about how much to constrain the analysis. Does one assume a particular resolution for the outgroup relationships, or does one treat some or all of the outgroup relationships as initially unresolved?

The monophyly of the ingroup and the outgroup structure were discovered using characters whose ancestral states were presumably estimated by even more distant outgroups. Thus, all outgroup analysis is a functional ingroup, functional outgroup procedure (Arnold, 1981; Watrous and Wheeler, 1981). One could imagine starting with all life, resolving a few major groups, then proceeding stepwise to finer and finer resolution. At each stage the monophyly of the functional ingroup and the interrelationships among the outgroups are assumed to have been previously well demonstrated. Because characters are used sequentially instead of simultaneously (Farris, 1982), errors may result. The assumptions about outgroup

structure made along the way are strong assumptions, and early missteps can prevent finding the ingroup cladogram that is universally most parsimonious—over all life (and nonlife). When we say that the two-step cladistic analysis of Figure 2 yields "global" parsimony, we do not mean parsimony "over all life"—in fact it only gives parsimony over a larger local region (outgroups plus ingroup) and even then relies on assumed outgroup structure.

The use of the ontogenetic method to determine ancestral states does not necessarily avoid this stepwise approach. Nelson (1978) has suggested that the ontogenetic method is a foundation upon which outgroup analysis rests, since it avoids these assumptions of higher-level phylogeny. As we suggest in the next section, the use of ontogeny alone may be best thought of (from our parsimony perspective) as an appeal to inanimate nature as an outgroup, and thus one is simply starting anew at the highest level of the stepwise resolution.

J. S. Farris (pers. comm.) and D. L. Swofford (pers. comm.) have both suggested a procedure wherein the ingroup monophyly and the outgroup structure are not simply assumed. In this procedure, an attempt is made to include the data that were thought to have supported ingroup monophyly and outgroup structure, and then to give the groups complete freedom in the analysis. If the analysis results in a cladogram which holds the ingroup together, so be it, but if characters such as those in Figures 2 and 5 break up the ingroup, take what the data suggest. This is a simultaneous resolution procedure taken to the extreme, with most or all restrictions on monophyly loosened.

SIMPLE PARSIMONY

Most of this paper is based on "simple parsimony"; that is, the estimation of ancestral states and the choice of cladograms has depended only on the number of character state changes required, counting reversals and convergences equally. There are two things that simple parsimony does *not* do: (1) although it can choose among competing hypotheses, sometimes it fails to specify the confidence with which the choice can be made; and (2) it does not assume that loss of a character state is easier than gain, or gain easier than loss.

Parsimony works on the available information, however little. Even when information is available only from a seemingly distant outgroup (e.g., dandelions used as the outgroup for the Felidae), parsimony can still yield a decision. Nelson (1973) suggested that it is more parsimonious to assume that a transforming ontogeny in which state **x** transforms to **y** is advanced and a nontransforming ontogeny in which **x** remains **x** is primitive. This argument seems to be based on the supposition that the world began without transforming ontogenies and that they had to be derived during the course of evolution. Hence, the argument appears to use inanimate nature as an outgroup. Inanimate nature is a very distant outgroup. Nevertheless, when one has only this much information, and until one looks to closer outgroups, the argument is valid in terms of parsimony.

However, even when parsimony supports a particular ancestral state assessment, the support may not be convincing (Farris, 1983:14). More work is needed to explore how much confidence can be placed in the hypotheses that parsimony prefers. One might, for example, have little confidence in a hypothesis because only scanty information supports it, or because the characters seem unreliable.

Why should one have more confidence in a hypothesis based on more information? Perhaps the answer concerns robustness. Distant outgroups can provide a decision based on parsimony, but outgroups closer to the ingroup have the power to overrule them (see Rules 1 and 3 above). Thus, the ancestral state assessment is more robust the closer and more comprehensive the outgroups, for the estimated ancestral states are more likely to remain most parsimonious even as more knowledge of outgroups is gathered. A similar argument might apply to attempts to simultaneously resolve the ingroup and outgroups when data on the outgroups are scanty. Al-

though parsimony can supply a decision whether data are plentiful or not, the more data available for the outgroups and ingroup, the less the simultaneous resolution might be expected to change as more data are added. When information is available from only scattered outgroups, some of which may be distantly related to the outgroup, the simultaneous resolution could be unstable. Donoghue and Cantino (1984) preferred the more conservative ingroup substitution approach in such a situation. However, our arguments do not directly answer the question of confidence, since robustness measures resistance to further change as opposed to closeness to truth. We are left uncertain about how to measure the amount of confidence that can be placed in hypotheses based on different amounts of information.

Some systematists have said to us that they would hesitate to use characters that show as much homoplasy as those in Figures 2 and 5. This response suggests a belief in constancy of rate: If a character shows many changes in the outgroup portion of the cladogram, it will be equally labile in the ingroup portion and, hence, the character cannot be used with confidence to resolve the ingroup. This belief may be valid, but it goes beyond simple parsimony. Simple parsimony does not indicate whether such homoplasious characters should be used with less confidence. Simple parsimony merely finds the most parsimonious ancestral state assignment, whether the character has much homoplasy or not.

Our algorithms, rules, and global parsimony results hold when characters are given different weights, but not when assumptions such as irreversibility are made. If some characters are considered of greater overall weight than others, the outgroup algorithm and rules are not affected because they treat each character separately. The results discussed under "Global Parsimony" are also unaffected (Appendix 3). However, if some character state changes within a character are given greater weight than others, our results may not hold. The multistate ordered characters and character state trees weight some character state changes more than others (for example, a change from state **1** to state **2** is one step, whereas from **1** to **4** is three steps), but our results accommodate this. Assumptions that loss is easier than gain or vice versa, or that evolution is irreversible, can render our results invalid. Some of these assumptions may be justifiable by broader parsimony considerations. The use of these assumptions may ensure that cladistic hypotheses remain parsimonious when we consider not only the data at hand, but also theories about the evolutionary process corroborated by studies with other character systems or organisms (see Wiley, 1975; Farris, 1983).

One might say the simple parsimony approach has a purity of logic uncluttered by assumptions about evolutionary process. Alternately, one could say it is just a beginning, a framework upon which a more organic approach could be built. It is not our intent to argue for or against the exclusive use of simple parsimony—we are only exploring the consequences of such a view. Regardless of whether this view is generally adopted, application of simple parsimony can expose assumptions of irreversibility, weighting, and so on, so that if these assumptions are employed, they will be open to discussion.

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APPENDIX 1

Outgroup Algorithms for Multistate Characters

The following algorithms find all and only the state assignments to the outgroup node that minimize convergence and reversal in the outgroup portion of the cladogram. The binary algorithm presented in the text is a special case of each of the multistate algorithms.

Step 1 is the same as in the binary algorithm. We assume that outgroup relationships are fully resolved. When a terminal taxon has more than one state, there are two possible procedures (see discussion in text under step 1 of the binary algorithm). First, one could treat the terminal taxon's internal cladistic structure as unresolvable, in which case label the terminal taxon by the set (for unordered characters), by the smallest range (for ordered characters), or by the smallest subtree (for character state trees) containing the states of the taxon. Second, one could treat the taxon's cladistic structure as resolvable, in which case treat the problem as one of unresolved outgroup relationships.

Multistate unordered characters.—The character states are unordered; that is, one step is counted between any two states. One may prefer to treat characters as unordered if no particular assumption about order is well supported before the cladistic analysis (Gaffney, 1979). For example, changes in the DNA nucleotides from A to C, C to U, G to A, and so on, could each be counted as one step (Fitch, 1971).

This algorithm is a modification of the preliminary phase of Fitch's (1971) algorithm. The labels to the nodes are sets containing one or more character states. Step 2 of the outgroup algorithm for multistate unordered characters is: Label a node by the *intersection* of the label sets of the two nodes immediately farther from the outgroup node *if* the sets intersect. Otherwise label the node by the *union* of the two label sets.

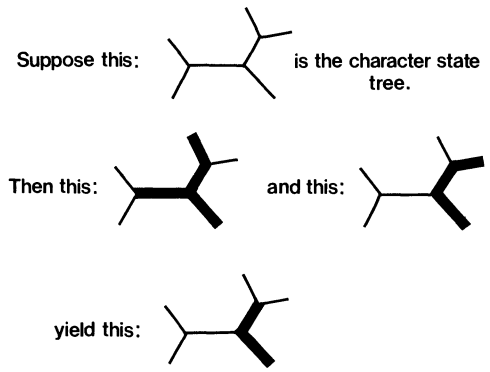

FIG. 15. Application of step 2 of the outgroup algorithm for character state trees. Upper row, the character state tree. The two label subtrees (heavy lines) in the middle row yield the subtree in the bottom row.

(Thus, {**a**,**b**,**d**} and {**b**,**c**,**d**} yield {**b**,**d**}; {**a**,**b**} and {**e**} yield {**a**,**b**,**e**}.)

Multistate ordered characters.—The character states are ordered in a linear sequence; that is, the number of steps between **ci** and **cj** plus the number of steps between **cj** and **ck** is equal to the number of steps between **ci** and **ck** (for any states **ci**, **cj**, and **ck**, $i < j < k$). The states can be discrete or continuous. If the outgroup node assessment is **[3,6]**, for example, then states **3**, **6**, and any states between them are equally parsimonious assignments to the outgroup node.

This algorithm is a modification of the first pass (steps R-1 and R-2) of Farris's (1970) algorithm. The labels to the nodes are ranges of character states. Step 2 of the outgroup algorithm for multistate ordered characters is: Label a node by the intersection of the label ranges of the two nodes immediately farther from the outgroup node *if* the ranges intersect (Farris's R-1). Otherwise label a node by the range between the ranges of the two nodes, including the end points (Farris's R-2). (Thus, **[3,8]** and **[4,9]** yield **[4,8]**; **[2,5]** and **[7,8]** yield **[5,7]**.)

Character state trees.—The character states are arranged into a character state tree. The number of steps between any two states is the distance between them along this tree. The tree is unrooted (and thus perhaps more appropriately called a network); reversals are allowed.

This is a generalization of the algorithm for multistate ordered characters. The labels to the nodes are convex pieces or "subtrees" (heavy lines in Fig. 15) of the character state tree. The outgroup node assessment will be the label subtree given to the outgroup node; any of the states in this subtree are equally parsimonious assignments to the outgroup node. Step 2 of the outgroup algorithm for character state trees is: Label a node by the intersection of the label subtrees of the two nodes immediately farther from the outgroup node *if* the subtrees intersect. If they do not intersect, label the node by the shortest (i.e., the linear) path between the two label subtrees. For instance, in Figure 15, the label subtree on the bottom row results from the intersection of the two label subtrees on the middle row.

APPENDIX 2

An Outline of a Proof of the Algorithms

A sketch of the proof of the algorithms is given here. It follows the same basic pattern for the binary and the three multistate cases. Hartigan (1973) presented proofs which justify the algorithm for multistate unordered characters. Farris (1970) presented no proof, but Swofford and Maddison (unpubl. manuscript) have developed proofs which justify the algorithm for multistate ordered characters. A more complete proof of the algorithm for character state trees can be obtained from the senior author (WPM).

To make the proof easier we presume that the ingroup has been removed and the outgroup portion rerooted at the outgroup node. Also, we will treat this rerooted outgroup portion mathematically as a cladogram even though it is not necessarily meant to represent phylogeny (having been rerooted). Once it is proved that the outgroup algorithm finds the assignments to the outgroup node in its new position as root that are most parsimonious according to the outgroups, it follows that these are also the most parsimonious when it is in its original position (see Farris, 1970:85).

Define the *subcladogram* of an internal node n to be the clade which has n at its base. The internal nodes and hence the cladistic relationships within the clade are included in the subcladogram. Define a *decoration* of a subcladogram to be a set of assignments to nodes, one state to each of the subcladogram's nodes. A most parsimonious decoration of a subcladogram is a decoration which has a minimal number of steps as measured locally, within the subcladogram only.

The following proposition (compare Hartigan's Theorem 2, 1973) can be proved by mathematical induction, proceeding down the cladogram from the terminal taxa towards the root: (a) If a node n is assigned any state in the label set given it by the outgroup algorithm, its subcladogram can be most parsimoniously decorated (i.e., there is a most parsimonious decoration of its subcladogram with n assigned this state). (b) If a node n is assigned any state outside of its label set, then its subcladogram cannot be most parsimoniously decorated *and* any decoration of its subcladogram will be longer than minimal by at least the distance between the state chosen and the nearest state in n's label set (for unordered characters this distance will be one step; for ordered and tree characters this distance may be different from one). When n is the root, the proposition guarantees that the cladogram (or, in the case of outgroup analysis, the outgroup portion) can be most parsimoniously decorated if and only if its root is assigned one of the states in its label set.

APPENDIX 3

Proof of the Global Parsimony Result

One needs to show that the following two procedures—the two-step and one-step procedures—choose

all and only the same ingroup cladograms when outgroup relationships are well resolved beforehand. For the two-step procedure (Fig. 2), parsimony is initially measured locally among the outgroups to estimate the state at the outgroup node for each character, then the most parsimonious ingroup cladograms are chosen, where parsimony is measured (over all characters) locally within the ingroup but given the estimated ancestral states at the outgroup node. For the one-step procedure (Fig. 12), the most parsimonious ingroup cladograms are chosen where parsimony is measured (over all characters) globally, over the ingroup plus outgroups together. By showing the equivalence of these two procedures we show that the two-step procedure achieves global parsimony, because the one-step procedure is guaranteed to find the globally most parsimonious cladograms, by definition.

One has a candidate K for an ingroup cladogram. The following definitions apply: *whole cladogram*, the candidate ingroup cladogram K with the outgroups attached; *outgroup portion*, the outgroup node and the outgroups; *ingroup portion*, the candidate K, the outgroup node, and the outgroup node–ingroup node internode. We assume for the moment that one is considering only one character **C** with states **c1**, **c2**, and so on (**C** can be binary or one of the three sorts of multistate characters in Appendix 1).

In the one-step procedure, in order to evaluate K's parsimony one finds any most parsimonious decoration (Appendix 2) of the whole cladogram for character **C**, and counts how many steps the decoration has. One of these most parsimonious decorations of the whole cladogram has at the outgroup node one of the states that would have been an ancestral state estimate from outgroup analysis, had the two-step procedure been followed. A most parsimonious decoration of the whole cladogram can be found by applying the algorithm of Farris (1970) or Fitch (1971) after the whole cladogram has been rerooted on the outgroup node–ingroup node internode (call the new root R). (The rerooting affects neither number of steps nor the most parsimonious decorations.) On the first pass of these algorithms down the cladogram (R-1 and R-2 of Farris's; the preliminary phase of Fitch's), the outgroup node will be labelled just as it would be by outgroup analysis, because the outgroup algorithms and the first pass are equivalent in form. (When we refer to labels in this proof, we mean the labels applied to the nodes on this first pass.) At least one of the states in the outgroup node's label (call this state **cp**) will be included in the label given to R (see step 2 of the algorithms). Because R is the root, each of the states in R's label (**cp** included) is a most parsimonious assignment to R according to the whole cladogram (Appendix 2). Because **cp** is both in the outgroup node's label and a most parsimonious assignment to R, there exists a most parsimonious decoration to the whole cladogram which has **cp** at the outgroup node. Call this decoration "W(K,**C**)." The number of steps in the outgroup portion of W(K,**C**) will be minimal, because **cp** is part of the outgroup node's label set (see proposition, Appendix 2).

In the two-step approach, the parsimony of K is measured locally within the ingroup portion given the estimated ancestral states. When more than one ancestral state was allowed by outgroup analysis (i.e., the assessment was equivocal), one uses the ancestral state that allows the ingroup to be most parsimoniously decorated. Thus, one finds a most parsimonious decoration of the ingroup portion using each estimated ancestral state, counts the number of steps, and chooses a decoration with the smallest number of steps. Suppose that the ingroup portion decoration chosen (call it I(K,**C**)) has **cq** (which may or may not be the same as **cp**) at the outgroup node.

I(K,**C**) must have the same number of steps as are in the ingroup portion of W(K,**C**). The number of steps in I(K,**C**) cannot be more than the number of steps in the ingroup portion of W(K,**C**), because **cp** is an available choice from outgroup analysis and would have been chosen instead of **cq** to make the ingroup portion decoration. The number of steps in I(K,**C**) cannot be less than the number in the ingroup portion of W(K,**C**), since this would imply there is a decoration to the whole cladogram (with **cq** at the outgroup node) which has fewer steps than W(K,**C**).

Similarly, for any other candidate ingroup cladogram L there exists a most parsimonious decoration (call it W(L,**C**)) of the whole cladogram which places an ancestral state estimate from outgroup analysis at the outgroup node. The outgroup portion of W(L,**C**) therefore has a minimal number of steps. Because W(K,**C**) also has a minimal number of steps in the outgroup portion, W(L,**C**) and W(K,**C**) differ in number of steps only in the ingroup portion. I(L,**C**) has the same number of steps as in the ingroup portion of W(L,**C**), just as I(K,**C**) has the same number as in the ingroup portion of W(K,**C**). Hence,

$$\#W(K,\mathbf{C}) - \#W(L,\mathbf{C}) = \#I(K,\mathbf{C}) - \#I(L,\mathbf{C}) \quad (1)$$

(where # is read "the number of steps in"). This holds for any character **C**, whether binary or one of the three sorts of multistate characters described in Appendix 1, and whether **C** is weighted differently from the other characters. The one-step procedure measures the parsimony of candidate K by the sum over all characters **C** of #W(K,**C**), whereas the two-step procedure measures the parsimony of K by the sum over all characters **C** of #I(K,**C**). Therefore,

$$\begin{aligned}\sum_{\mathbf{C}} \#W(K,\mathbf{C}) - \sum_{\mathbf{C}} \#W(L,\mathbf{C}) &= \sum_{\mathbf{C}} [\#W(K,\mathbf{C}) - \#W(L,\mathbf{C})] \quad (2)\\ &= \sum_{\mathbf{C}} [\#I(K,\mathbf{C}) - \#I(L,\mathbf{C})]\\ &= \sum_{\mathbf{C}} \#I(K,\mathbf{C}) - \sum_{\mathbf{C}} \#I(L,\mathbf{C}).\end{aligned}$$

Therefore, the one-step and two-step procedures will always agree about whether K or L is more parsimonious. Hence, they will choose the same ingroup cladograms.