Cladistics (1993) 9:413–426

ON OUTGROUPS

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Received for publication 26 April 1993; accepted 24 Auust 1993

Abstract—The relations among polarity, outgroups and rooting are clarified. The "outgroup algorithm" and "outgroup substitution method" are irrelevant forms of relaxed parsimony. They should be discarded in favor of unconstrained, simultaneous analysis of all terminals. A revised outgroup method is described both in text and with a *computer-generated* flowchart. Lundberg rooting is consistent with cladistic parsimony only under specific circumstances involving hypothetical ancestors.

> "Both sides seemed convinced that the 'real enemy' is a vicious conspiracy of some kind."
> Hunter S. Thompson (1979: 145).

Introduction

While methods and computer implementations of cladistic analysis have evolved and improved over the last few decades, certain topics have received uneven attention in the literature. One such topic is outgroup analysis. Interminably entwined with notions of outgroups is the concept of character polarity. In some fuzzy way, rooting is also involved. While these topics are mundane and obvious to many workers, it is clear that widespread confusion persists, especially among students. This paper provides an overview of modern discussions of outgroups, as well as a working methodology for selection and use of outgroups in cladistic analysis. We hope that in the process of completing this task, we can dispel myths about the intractability of outgroup selection and use that are pervasive and serve only to inhibit application of sound cladistic principles.

Historical Perspective

Much of the discussion of outgroup analysis by cladistics is given in the context of debate about whether the "outgroup criterion" is superior to other criteria for determining character polarity, such as ontogenetic precedence. Arguments about the relative efficacy of these particular criteria persist to the present (e.g. Brooks and Wiley, 1985; de Queiroz, 1985; Kluge, 1985; Kluge and Strauss, 1985; Nelson, 1985; and papers in Humphries, 1988). We refer not to that debate as such, because we regard all the criteria as specific applications of cladistic parsimony, whose relative success is to be judged in that general framework (e.g. Miyazaki and Mickevich, 1982; Kraus, 1988; Mabee, 1989; Q. Wheeler, 1990).

Hennig (1966: 96ff.) discussed precladistic concepts of outgroups, in the context of his discussion of the "Criterion of the correlation of series of transformations" as a means of inferring "character phylogeny". For example, he pointed out that Maslin's (1952) criterion, "If one extreme of a morphocline resembles a condition

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found in the less modified members of related groups of the same rank, this extreme is primitive", is what Naef (1931) termed the "principle of systematic character precedence". Hennig characterized this notion as a special case of correlation between transformation series. (See Rieppel, 1988: 130, for discussion of even earlier ideas.) Hennig did not use the term "outgroup". That word came into use gradually for a concept that previously went under various names. Numerous authors, even critics of cladistics, used the term "related group". For example, Colless (1967: 292) criticized division of character states ("attributes" in then-current phenetic parlance) into plesiomorphic and apomorphic on the basis of "inferences drawn from 'related' taxa, outside the group under study". Throckmorton (1968), although advocating phenetic clustering for phylogenetic inference, referred repeatedly to "outside groups" that might be "exgroup" (p. 367), and stated (p. 369): "Outside groups provide a basis for making strong inferences regarding the ancestral, or old, states. States common both to species inside and outside our group must be ancestral states, or they must be of convergent origin". Schlee's (1969: 131) rebuttal to Colless stressed that use of a "pre-existing system" by cladistics is open to revision: "Even when they turn to the relationships of partial groups, they try to solve the problem of whether the total group is founded correctly". Kluge and Farris (1969:5) explicitly tied the following criterion to cladistic parsimony: "The primitive state of a character for a particular group is likely to be present in the representatives of closely related groups". Crowson (1970: 106) stated that "a condition which occurs in related forms outside the group is probably primitive within the group". Nelson (1973: 87) described the outgroup concept as "indirect arguments, involving consideration of species, or groups, other than those of immediate concern". Ross (1974) called it "ex-group comparison". Wiley (1975: 239) showed how adding taxa to "*raise the level of universality* of the phylogenetic hypothesis" achieved cladistic parsimony in a hypothetico-deductive framework. Kluge (1976: 22) referred to "the taxonomic assemblage chosen for study as the primary group and the related groups as secondary".

The term outgroup came into use at the American Museum of Natural History in the early 1970s, and became common there. The earliest mention of "out-group comparison" that we have found in the cladistic literature is Platnick and Gertsch (1976: 2). As Nelson (1978: 329) stated: "As regards Haeckel's words on comparative anatomy, I understand them to refer to an indirect technique of character phylogeny, which has been widely used, in the evolutionary interpretation both of fossil and of recent organisms, to order data in relation to a prior phyletic hypothesis (the technique sometimes called 'outgroup comparison')". Gaffney (1979: 94) pointed out that outgroup comparison had "no logical dependence on 'higher-level phylogenies,' because, in principle, each hypothesis is falsifiable independently of all other phylogenetic hypotheses by comparison with all other organisms".

For some time usage of the term was inconsistent. For example, Hecht (1976) and Crisci and Stuessy (1980) characterized outgroup analysis in terms of the concept that the character state most widely distributed in the study group is plesiomorphic, a concept respectively termed the "commonality principle" or "common is primitive". Fallacies of that concept have been pointed out by numerous authors (e. g. Stevens, 1980, 1981; Watrous and Wheeler, 1981; Wheeler, 1981), and the criterion has been discarded (but not universally; see Stuessy, 1990).

The identity of outgroup selection with rooting was clearly delineated by Farris (1972: 657), who also pointed out that the result of non-monophyly of the ingroup after analysis indicates simply that the data analyzed challenge the presumption of ingroup monophyly. Subsequent reviews have either overlooked this point or downplayed its importance (e.g. de Jong, 1980; Stevens, 1980; Arnold, 1981; Watrous and Wheeler, 1981; Wiley, 1981; Maddison et al., 1984; Schoch, 1986). Lundberg (1972) made the first attempt to codify outgroup comparison, but was hampered by contemporary concepts of tree construction and the capabilities of available computer programs. Various later authors (Watrous and Wheeler, 1981; Donoghue and Cantino, 1984; Maddison et al., 1984) proposed formalizations of the "outgroup method" to establish character polarity, generally predicated on holding the composition of the outgroup and ingroup constant, and treating the two groups separately. These practices continued even after Farris (1982: 329) had explicitly characterized polarity in terms of rooting: "As parsimony analysis attributes character states to the hypothetical stem species of the tree, fixing the position of the root determines the direction of character transformations, and so the relative plesiomorphy of features". Farris pointed out that the "outgroup method" was simply an application of cladistic parsimony.

OUTGROUPS AND "GLOBAL PARSIMONY"

Maddison et al. (1984) provided the paper that seems to be the most widely cited for outgroup analysis in recent literature (Hillis, 1990, recorded 15 citations in 1986, more than any other paper published in *Systematic Zoology* in 1984). The Maddison et al. "outgroup algorithm" is a series of rules that require fixed relationships of outgroup taxa (e.g. from a previous analysis) in relation to each other as well as the ingroup, or alternatively, "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" from (Maddison et al., 1984: 89). In all cases, outgroup and ingroup are maintained as separate; that is, ingroup monophyly is assumed. Confusingly, they referred to this as achieving "global parsimony", and conflated under the term "simultaneous analysis" both their constrained procedure and the suggestion of Farris and Swofford (cited as pers. comm. in Maddison et al., 1984: 99) that the identity of the outgroup not remain fixed, i.e. that ingroup monophyly not be simply assumed, but established with the data at hand by a simultaneous analysis of the outgroup and ingroup without constraints. So-called "global parsimony" is actually a hybrid of parsimonious optimization of characters on an arbitrary cladogram (the constrained relationship between the ingroup and outgroup) and the criterion of minimum length applied during cladogram construction to choose topology (the parsimony criterion defined by Farris, 1970). "Global parsimony" in the sense of Maddison et al. does not necessarily achieve true parsimony over a given set of taxa. It is a form of relaxed parsimony, not cladistic parsimony.

Delving further, it can been seen that the Maddison et al. method is equivalent to treating the ingroup as a single unresolved terminal taxon that is polymorphic for characters that vary among the component ingroup taxa. Character states of the first "outgroup node" are then calculated by adapting standard optimization methods, viz. Farris (1970) or Fitch (1971). Maddison et al. were careful to point out that the "first outgroup" node is not equivalent to the common ancestor of the ingroup; indeed, states assigned to the first outgroup node may or may not be equivalent to those of the common ancestral node of the ingroup (see example below). Maddison et al. were also careful not to claim that this method provides "ancestral state assessments" *within* the ingroup, but unfortunately the method is *now often presented under discussions of character "polarity", such as when it was* the sole topic treated by Wiley et al. (1991: 25) in their section entitled "Polarity Decisions". Indeed, Wiley et al. (1991: 27) equated polarity assessment with the method: "Maddison et al. (1984) treat the problem of polarity as one in which the investigator attempts to determine the character to be assigned to the OG node". Likewise, Brooks and McLennan (1991:48) stated: "Maddison, Donoghue, and Maddison (1984) began the quest for character polarities by attempting to determine the character state at the outgroup node. They reasoned that because this precedes the ingroup node, it will give us information about the character state in the common ancestor of the ingroup". Wiley et al. and Brooks and McLennan have overstated the case. The following example data set illustrates how states of the *"OG" node, calculated by Farris or Fitch optimization as suggested by* Maddison et al., may have no predictive value for a particular character in determining the "ancestral state" for an ingroup that is evaluated separately:

OG1	000000
OG2	100000
OG3	110000
IG1	111001
IG2	111101
IG3	111111
IG4	111110

"OG" denotes the outgroup taxa, and "IG" those of the ingroup. The single most parsimonious cladogram for this matrix is completely resolved (Fig. 1; length=7, consistency index=0.85, retention index = 0.87, found with the "ie" command of

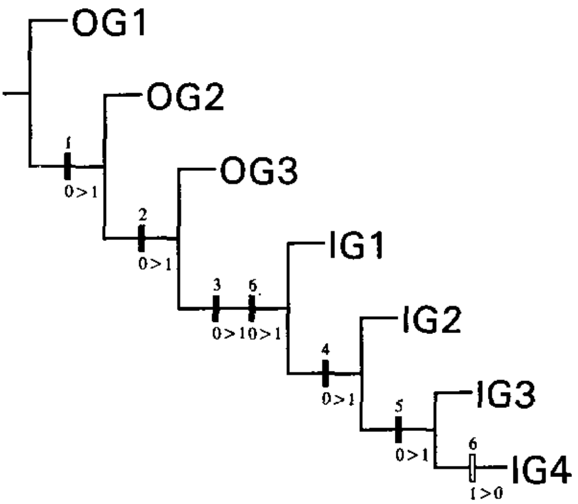

Fig. 1. Cladogram resulting from analysis of the hypothetical data set (see text).

Hennig86; Farris, 1988). Variable 6 is a synapomorphy for the ingroup under any parsimonious optimization (verified with Clados; Nixon, 1992), with a reversal in IG4. This variable character is assigned a value of 0 at the "outgroup node" (Maddison et al., 1984), and if states within the ingroup are ignored (say, by treating these taxa as missing), the ingroup node is also assigned a value of 0. Thus, the *impression*, often voiced by students, that the Maddison et al. method provides a means of assessing ingroup polarity (assignment of ingroup ancestral states) is incorrect.

If the Maddison et al. (1984) "doublet" rule is not a means of determining character polarity, what is it and what purpose does it serve? Perseverance in searching the literature reveals that in general their paper has been used as an all-purpose citation for "outgroup methods" in materials and methods sections, even when the method itself has not been used, as can be verified by scanning recent issues of primarily non-systematic journals. When actually used as Maddison et al. proposed, their method has usually been a way of performing compartmentalization on data sets, such as the within-angiosperm analyses of Donoghue and Doyle (1989) and Donoghue (1989), which relied on the "fixed" outgroup of seed plants from their earlier analysis (Doyle and Donoghue, 1986a, b, 1987). Because the "doublet rule" itself is only a special case of standard character optimization methods published in the 1970s, it is unnecessary to use for purposes of compartmentalization. Instead, it is only necessary to read the OG node from computer output of currently available programs.

Even as a form of compartmentalization of data, the Maddison et al. method suffers because it relaxes cladistic parsimony, in that the parsimony criterion is applied independently to the outgroup and ingroup. The resulting problems are identical to those discussed by Nixon and Davis (1991) when polymorphic terminals are included in analyses, which indeed is exactly how the method has been implemented (e.g. Donoghue, 1989; Donoghue and Doyle, 1989). As pointed out by Nixon and Davis (1991), when a polymorphic terminal (the ingroup) is actually recorded with its component subterminals, it is possible that the outgroup relationships, if this character were included in a simultaneous analysis, would be different than those hypothesized. Any character that has a distribution such as those provided by Maddison et al. in their "equivocal" examples could potentially affect relationships of the outgroup to the ingroup, thus potentially nullifying their assumptions about outgroup topology. In other words, any character that varies both within the ingroup and outgroup may not be treated truly parsimoniously when the two independent networks are spliced together.

Perhaps the most striking aspect of this subject is that the logically inconsistent treatment of ingroup and outgroup data proposed in Maddison et al. (1984) has largely escaped attention for 10 years. Meacham (1984) argued that character polarity need not be determined before cladistic analysis, citing Farris (1970) regarding parsimony of undirected networks. Donoghue and Maddison (1986) took issue with Meacham's suggestion that an undirected analysis be performed first and then rooted, arguing that (constrained) "simultaneous analysis" was superior in terms of their "global parsimony". In his rejoinder, Meacham (1986: 539) stated that such constraint:

> "is a restriction on the outcome of the analysis. It would seem more convincing to me if the possibility that the outgroup and ingroup were convex [monophyletic or outgroup paraphyly, in

> clique terminology] were a *result* of the analysis rather than a prior restriction on the analysis. The restriction can be easily removed by performing an undirected analysis on the outgroup plus ingroup. If the undirected parsimony analysis produces a tree on which the ingroup and outgroup are both convex, then the contention that both groups might be monophyletic or that the ingroup is monophyletic with respect to a paraphyletic outgroup is supported by global parsimony of ingroup plus outgroup. If the ingroup and outgroup are both convex, rooting the tree at the internode between the two groups will produce a tree on which both the ingroup and outgroup are monophyletic. Rooting the tree within the outgroup will produce a tree on which the ingroup is monophyletic and the outgroup is paraphyletic. These rootings, in turn, will suggest the ancestral states of the characters".

Colless (1985) concurred with Meacham (1984), and cited Farris (1972) to the effect that simultaneous analysis of the ingroup and outgroup together would properly implement outgroup analysis and rooting. Perhaps because Meacham advocated clique analysis and Colless advocated phenetics, their papers are seldom cited. Clark and Curran (1986) amplified the comments of Farris and Swofford (in Maddison et al., 1984: 99) on (unconstrained) simultaneous analysis, but misapprehended the connection between that point and the arguments advanced by Meacham and Colless. However, the points these authors argued have been accepted by numerous subsequent workers (e.g. Swofford and Olsen, 1990: 453; Kitching, 1992: 32; Maddison and Maddison, 1992: 53; Mayden and Wiley, 1992: 169, 176), although citations have often been lacking or misleading.

OUTGROUP SUBSTITUTION

Donoghue and Cantino (1984) provided a method called the "outgroup substitution method", which is similar to that proposed by Maddison et al. (1984), in using fixed outgroup topologies. Outgroup substitution uses *alternative* outgroup topologies, involving "plausible" sister-group arrangements based on "previous cladistic analysis" or arrangements that have been "suggested by phylogenists" (p. 193). Alternative outgroup topologies were mentioned also by Maddison et al.; the outgroup substitution approach differs in that points of congruence in the various resultant ingroup topologies are used to generate hypotheses of relationship within the ingroup. However, Donoghue and Cantino explicitly stated that the goal and final step of their method is to "assess character polarities" (abstract, p. 192). The method formalized an approach taken in earlier papers (Cantino, 1982; Donoghue, 1983a; see also de Queiroz, 1987, who used a modified outgroup substitution method).

One logical inconsistency in the Donoghue and Cantino paper is the confusion of the "outgroup node" (which logically occurs when more than one outgroup is used), with an unnecessary node (a root) placed between a single outgroup terminal and the ingroup. In their figures 3 and 4 (p. 194), the example of a single outgroup with state "a" is given, and the "outgroup node" in this case is mapped as "a" in figure 3 and shown to be one step longer if assigned "b" as in figure 4.

This logical inconsistency (confusing the root with the "outgroup node"—also in Maddison et al., 1984) seems to be the cause of the myth that at least two outgroups are necessary to "polarize" characters in an analysis (e. g. Wiley et al., 1991: 5). It can easily be seen that the correct assignment of the "ancestral node" for an ingroup results from parsimony (via optimization) applied over the entire tree, and that the "outgroup node" as illustrated in Donoghue and Cantino (1984) does not exist when a single outgroup is used, and so *has no effect* on the assignment of the ancestral node for the ingroup. Thus, a single outgroup is no better or worse than multiple outgroups for "polarizing" the ancestral ingroup node. Whether the "ingroup ancestral node" is eventually equivocal or assigned a fixed state is merely a function of whether the single outgroup *itself* is fixed or equivocal, under rules of optimization applied over the *whole* ingroup–outgroup topology. In the case of a single outgroup, that outgroup is logically *equivalent* to the "outgroup node" in an example with more than one outgroup. In fact, what seems to have been overlooked by Maddison et al. and Donoghue and Cantino is that a single outgroup, if it has few ambiguous characters, is likely to "polarize" *more* characters in the ingroup than the "outgroup node" generated by optimization of characters over multiple outgroups. The idea that at least two outgroups are necessary to polarize characters unambiguously is simply wrong.

POLARITY MYTHS

The most pervasive misunderstanding of outgroups and their use in cladistics is thus tied to the concept of "character polarization" and centers on the belief that we must know "primitive" and "derived" states before we attempt analysis. This belief contrasts with the procedure described by Farris (1972), implicit in Farris' (1970) description of networks, in which character polarity is determined by the resultant topology after analyzing outgroup and ingroup taxa simultaneously.

Another pervasive misunderstanding is that the sister group is necessary, or all-important as an outgroup (e.g. Wiley, 1980; Donoghue, 1983b; Donoghue and Cantino, 1984; Stuessy, 1990; Mayden and Wiley, 1992; and many earlier citations). The undue emphasis placed on the "outgroup node" (the common ancestor of the sister group and ingroup) in the Maddison et al. method doubtless helped considerably in propagation of this myth, as did perhaps their "Rule 3", on the importance of the "first outgroup". To be sure those authors did not themselves originate the myth, but the viewpoint has been taken up wholeheartedly by various authors who cite Maddison et al. (1984), including some who write introductory materials for young minds (e.g. Wiley et al., 1991, and repeated almost verbatim in Brooks and McLennan, 1991:52):

> "The most important outgroup is the sister group, and considerable phylogenetic research may be needed to find the sister group. Usually more than one outgroup is needed in an analysis" (Wiley et al., 1991: 5), and: "(1) Whatever the resolution of the outgroup relationships, the sister group is always dominant in her influence on the decision. If the sister group is decisive for a particular state, e.g. 'a', no topology of outgroups farther down the tree can result in a decisive 'b'. (2) If you are faced with no sister group but only an unresolved polytomy below the group you are working on, the frequency of a particular character among the outgroups in the polytomy has no effect on the decision for the OG node" (Wiley et al., 1991: 31).

The second sentence in the first quotation perpetuates a third pervasive myth, the notion that more than one outgroup is somehow required for correct polarity determination. An early expression of this idea is found in Donoghue (1983b: 153):

> "Determining polarity by the outgroup method requires some initial understanding of the cladistic relationships of a group. Specifically, to assess whether a character state possessed by the members of a group is ancestral or derived, its sister group and at least one group cladistically basal to these two groups must be known or hypothesized".

Outgroups and polarity are the same problem; that is, the problem of rooting. Polarity in fact need not be decided before analyses, contrary to common misconception; this notion is a holdover from earlier methods and programs which required construction of prerooted trees. The following section illustrates the connections among outgroups, polarity and rooting.

A Revised Outgroup Method

We propose the following formalization of the outgroup method, as outlined in the flowchart in Fig. 2.

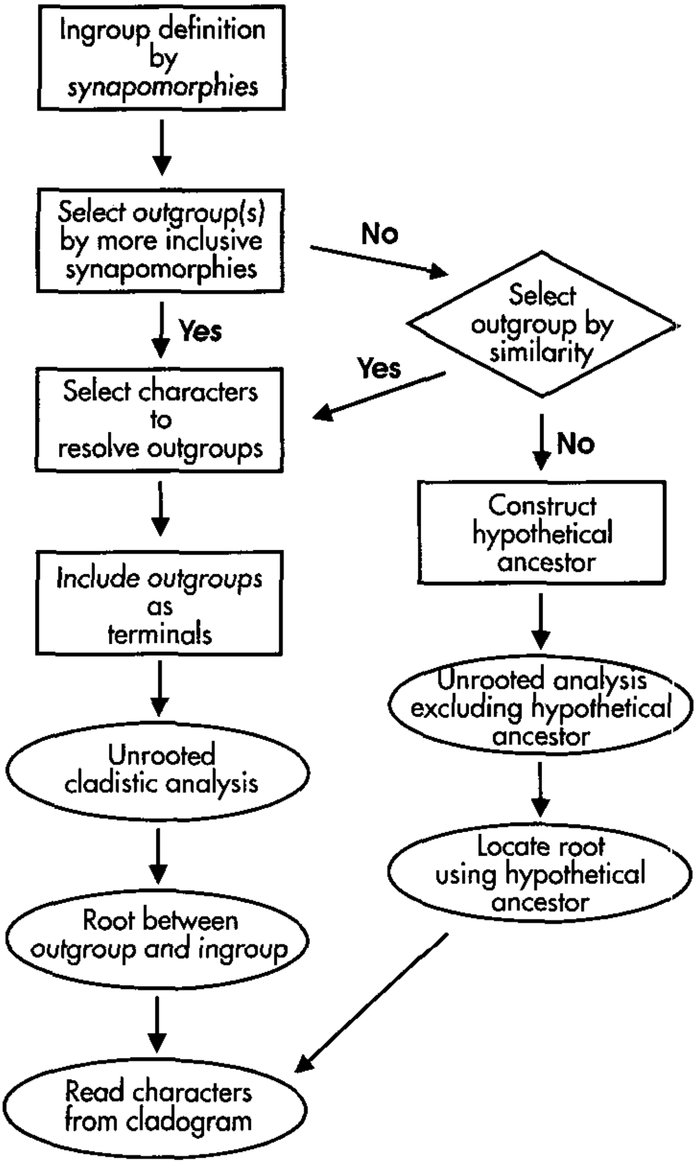

Fig. 2. Flowchart of general method for simultaneous outgroup analysis.

1. *Ingroup definition*

Ingroup definition (or diagnosis, if the reader prefers) is accomplished by identifying synapomorphies of the ingroup relative to other taxa. While this has been characterized as a daunting task that requires extensive knowledge of the phylogeny of the group (e.g. citations in Donoghue and Cantino, 1984), in practice it requires only hypotheses that one or more characters of the ingroup are apomorphic. As de Jong (1980: 12) put it: "Generally speaking out-group comparison of character states needs the presence of the relevant character outside the group. That is the only prerequisite". This concept is explicit in Hennig (1966) as a basic tenet of phylogenetic systematics.

2. *Outgroup selection*

One or more outgroups are selected on the basis of possession of more inclusive synapomorphies shared with the ingroup. If the outgroup is the sister group, the cladistic inferences might be better founded—but might not. Likewise, the more outgroups, the better founded might be the inference. Basic sampling theory predicts that denser samples are better, and this pragmatic conclusion has been echoed by many authors. There are no guarantees, however, that any particular outgroup or number of outgroups will ensure a "correct" inference. Claims for requirements in this regard are simply confusion about the nature of scientific inference, or, at best, guesses about the results of studies that have never been done.

If it is not possible to identify more inclusive synapomorphies, previous classifications or even "similarity" can be used in outgroup selection. Such similarity need not necessarily be specified very precisely, but again is a pragmatic choice: "If we are, e.g., interested in the evolution of fleas, why would it *a priori* be impossible to compare fleas with whales? Indeed, the results would be very meagre, as characters common to fleas and whales are mainly at the physiological level. That is the reason why it is more sensible to compare fleas with other insects" (de Jong, 1980: 12). As Ax (1987: 117) put it: "this out-group may be seen as a very coarse preliminary arrangement and may remain so. It need not necessarily represent the adelphotaxon of the 'in-group' nor itself be established as a monophylum. It is enough, if the out-group consists of organisms that possess one of the alternative features of the in-group". We agree with Eldredge and Cracraft's (1980: 26) suggestion that: "Intuitively, it may seem that organisms most like those being investigated would be most appropriate; after all, morphological features within the group must be compared to similar structures in outside organisms. A reasonable first approximation, then, is to compare those organisms sharing one or more similarities and see how far the within-group comparison can proceed, i.e. see if a cladogram can be constructed".

On occasion, it may be neither feasible nor convenient to select a specific outgroup. For example, in the case of highly divergent molecular sequence data the root may be essentially random (Miyamoto and Boyle, 1989; W. Wheeler, 1990). In such cases, as well as when one wishes to apply criteria for rooting other than outgroup analysis, it may be necessary to estimate a "hypothetical ancestor". Such a hypothetical ancestor is better not included as a terminal in a simultaneous analysis, as it may affect the ingroup topology in ways other than would be found if real outgroups were included. Therefore, in such cases, an unrooted ingroup analysis should be performed, and the root for the ingroup should then be determined as occurring along the internode that provides the overall most parsimonious tree length for the hypothetical ancestor + unrooted ingroup cladogram. As in the simultaneous analysis method, polarity of characters can then be read from the resulting rooted cladogram. It should be noted that this procedure is exactly that proposed by Lundberg (1972). Maddison et al. (1984: 94) observed that a real outgroup, if included in the analysis, might lead to different results than Lundberg rooting. This is trivially obvious, but is simply an argument for including outgroups if available. After all, adding or deleting *any* taxon may affect the outcome of an analysis (Farris, 1970; Donoghue et al., 1989).

3. Select outgroup characters

The resolution among multiple outgroup terminals, both in relation to each other and to the ingroup taxa, may affect both the position of the ingroup relative to outgroups and the topology of relationships within the ingroup. Therefore, characters that resolve outgroup relations are just as important as characters that resolve ingroup relationships, and a serious effort to find such characters should be undertaken. Inclusion of "dummy" characters to specify outgroup relationships *a priori*, as in Kraus (1988), misses the point of cladistic analysis.

4. Analyze the full matrix including unconstrained ingroup and outgroup terminals

In the words of Eldredge and Cracraft (1980: 63): "This procedure, then, can be viewed as a search for levels of character distribution in the observed similarities".

5. Root the cladogram between outgroup and ingroup

If this is not possible due to "mixing" of outgroup terminals with the ingroup, the conclusion is that the ingroup is non-monophyletic. That conclusion, after all, is that supported by the data just analyzed under the parsimony criterion. The phenomenon may simply be a case of overlooked characters that are constant in the ingroup but are synapomorphies that provide structure in the outgroup.

6. Read character polarity directly from the cladogram on which characters have been optimized

The above method is merely a codification of that proposed 20 years ago by Farris (1972: 657):

> "For example, if we believe that all carnivores had a common ancestor which was not the ancestor of any monkey, then to construct a rooted evolutionary tree for a set of carnivores, we construct a rooted evolutionary tree for several carnivores and at least one monkey. The location on the undirected tree of the common ancestor of all the carnivores in the study is then estimated to be a node, *U*, connected to another node, *V*, say, such that the path from any carnivore OTU [sic] to any noncarnivore OTU [sic] includes both *U* and *V*, and the path from *V* to any carnivore OTU includes *U*.
>
> For arbitrary [sic] data, a tree including both carnivore and noncarnivore OTUs [sic] need not have any node with the properties prescribed for *U* above. If this occurs, then it may be concluded that the data utilized do not support the hypothesis that the carnivores form a holophyletic [sic] group".

This characterization itself follows directly from Farris' (1970: 83) original description of the Wagner Tree method:

> "We can think of a tree as being specified in two components, the first being the relative position of the nodes in the branching pattern, and the second being the location of the root. A network with a certain set of nodes may correspond to a wide class of trees with the same nodes, each tree differing from the others in the class only in the position of its root".

While the above quotations clearly identify Farris as the earliest proponent of simultaneous analysis, some authors attribute the origin of this concept to others, in later publications. For instance, Kraus (1988) credited the procedure to Clark and Curran (1986)! Maddison and Maddison (1992: 53) explained simultaneous analysis as follows:

> "Two methods have been proposed for rooting trees that avoid specification of character polarity. First, if one incorporates into a tree inference not only the study group but also any outgroups, then the root of the study group tree will be the branch that connects to the outgroups (Farris, 1972). Such an analysis will require assuming some phylogenetic structure, namely what groups might be considered outgroups and what groups comprise the study group. At no point in the analysis would one actually make an explicit assumption about ancestral states. In some circumstances including outgroups as extra taxa is equivalent to use of assumptions about character polarity, if the outgroup data are condensed into a claim about basal polarity (Maddison et al., 1984). However, in other circumstances, particularly when outgroup relationships are uncertain, it is better to avoid specifying character polarity, and instead include all outgroup taxa along with the study group during tree inference (Maddison et al., 1984)".

In fact, the 1984 paper cited in the quotation did not mention Farris (1972), and presented (unconstrained) simultaneous analysis as an afterthought that was received as a personal communication from both Farris and Swofford. We find no discussion of "other circumstances" favoring simultaneous analysis in Maddison et al. (1984). Instead, one is left with the impression that they considered it an extremist approach, relative to the "outgroup algorithm" to which most of the paper was devoted. Maddison et al. (1984: 99) stated only:

> "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.... This is a simultaneous resolution procedure taken to the extreme, with most or all restrictions to monophyly loosened".

We prefer to view true simultaneous analysis as retaining restrictions of cladistic parsimony, not "loosening" restrictions of monophyly, because, outside of divination, it is hard to imagine any means other than the application of the parsimony criterion to characters to determine monophyly, or to test an original hypothesis of the same.

Conceptual Conclusions

The following conclusions need to be reiterated concisely, considering the massive confusion in the recent literature. We apologize that this will be tiresome for some but present them in the hope that they will not need to be repeated except in future revisions of various currently available texts and workbooks:

Outgroup(s) need not be (or include) the sister group of the ingroup, although the more complete the sampling of related taxa, the greater our expectation of stability in future studies.

Outgroup(s) need not be a monophyletic group relative to the ingroup. The outgroup(s) may be paraphyletic relative to the ingroup. In fact, it is not necessary to have more than one outgroup.

Outgroup(s) need not be "primitive" relative to the ingroup. Cladistic parsimony will deal with the problem of polarity and "primitiveness" if the user provides the root.

Outgroups/ingroups should not be constrained in the analyses. Instead, outgroups should be treated as all other terminals, and thus may test monophyly of the ingroup.

Acknowledgements

We're not claiming originality, and we thank Joel Cracraft, Darrel Frost, David Maddison, Gary Nelson and Norman Platnick for comments on the manuscript.

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