

THE EFFECT OF ORDERED CHARACTERS ON PHYLOGENETIC RECONSTRUCTION

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Abstract Morphological structures are likely to undergo more than a single change during the course of evolution. As a result, multistate characters are common in systematic studies and must be dealt with. Particularly interesting is the question of whether or not multistate characters should be treated as ordered (additive) or unordered (non-additive). In accepting a particular hypothesis of order, numerous others are necessarily rejected. We review some of the criteria often used to order character states and the underlying assumptions inherent in these criteria.

The effects that ordered multistate characters can have on phylogenetic reconstruction are examined using 27 data sets. It has been suggested that hypotheses of character state order are more informative than hypotheses of unordered and may restrict the number of equally parsimonious trees as well as increase tree resolution. Our results indicate that ordered characters can produce more, equal or less equally parsimonious trees and can increase, decrease or have no effect on tree resolution. The effect on tree resolution can be a simple gain in resolution or a dramatic change in sister-taxa relationships. In cases where several outgroups are included in the data matrix, hypotheses of order can change character polarities by altering outgroup topology. Ordered characters result in a different topology from unordered characters only when the hierarchy of the cladogram disagrees with the investigator's *a priori* hypothesis of order. If the best criterion for assessing character evolution is congruence with other characters, the practice of ordering multistate characters is inappropriate.

Introduction

Two fundamental prerequisites for a robust cladistic analysis are good data and a proper treatment of such data. The search for proper analytical methods has fostered many of the classic debates in phylogenetic systematics such as methods of character polarization (Maddison et al., 1984; Meacham, 1984, 1986; Donoghue and Maddison, 1986), the validity of character weighting (Neff, 1986; Wheeler, 1986), the use of the principle of parsimony (Farris, 1970) and methods of cladogram construction. Another significant debate centers on the treatment of multistate characters. Specifically, the controversy centers on whether multistate characters should be ordered or unordered. Because of the commonality of multistate characters, the decision of how to treat such characters is far from trivial. Some of the theoretical aspects of this question, and its underlying assumptions, have been examined by previous authors (Mickevich, 1982; Mabey, 1989a; Mickevich and Weller, 1990). In this paper, we explore the meaning of an ordered character, review criteria often used to order multistate characters and examine the effects that ordered characters can have on phylogenetic reconstruction through the examination of 27 data sets.

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Ordered vs. Unordered Characters

Because a given structure can undergo more than a single evolutionary change, multistate characters will be encountered in most phylogenetic studies and must be dealt with by systematists. Current phylogenetic computer programs allow multistate characters to be treated as ordered, partially ordered (by means of a step matrix or various other coding methods) or unordered. Mabee (1989a) lists three types of ordered character state hypotheses (Fig. 1): reticulate, branched, and the most common form, linear. The branched and reticulate forms impose only a partial ordering; a linear form imposes total ordering.

In its various forms, an ordered multistate character, optimized by Wagner parsimony (Kluge and Farris, 1969; Farris, 1970) can be referred to as a character phylogeny (Zimmermann, 1937, 1940, 1953), character state tree (Farris et al., 1970), morphocline (Maslin, 1952) or transformation series (Hennig, 1966). An ordered character represents a specific hypothesis regarding the evolutionary relationships among the character states. It is a restrictive statement that excludes all other possible hypotheses of character state order [Fig. 2(a)]. The number of alternative hypotheses of order rejected increases with the number of states in a multistate character. When a multistate character is ordered, the distance between non-neighboring states is always more than one step in a linear transformation series and is generally more than one step in the reticulate and branched forms. An unordered multistate character, optimized by Fitch parsimony and based on the Fitch minimum mutation model (Fitch, 1971), is also a specific statement about the evolutionary relationships between the individual character states as well as the distances between those states. It is, however, a less restrictive statement because all possible character state trees are initially seen as equally probable. As such, the distance between any state, whether it is a neighboring state or not, is a single step [Fig. 2(b)]. Both hypotheses of order and unordered make certain assumptions about character evolution: hypotheses of order assume that a particular character state sequence is more probable than any alternative sequence while hypotheses of unordered assume all possible character state trees are equally likely.

It is important to recognize that ordered multistate characters are not directly equivalent to a set of non-additive binary characters. To illustrate this point, consider the following hypothetical set of binary characters: (1) presence or absence of structure

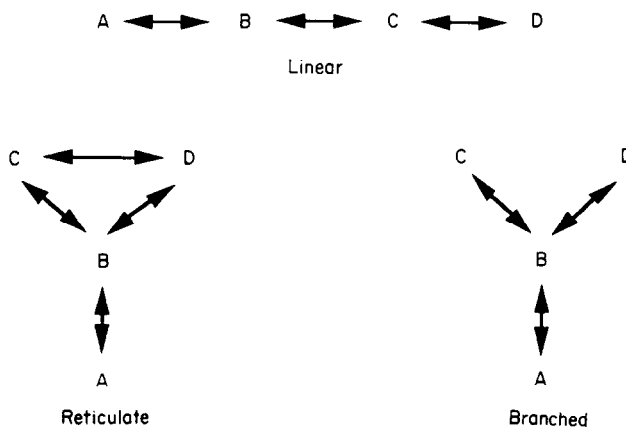


Fig. 1. Three types of ordered characters (from Mabee, 1989a).

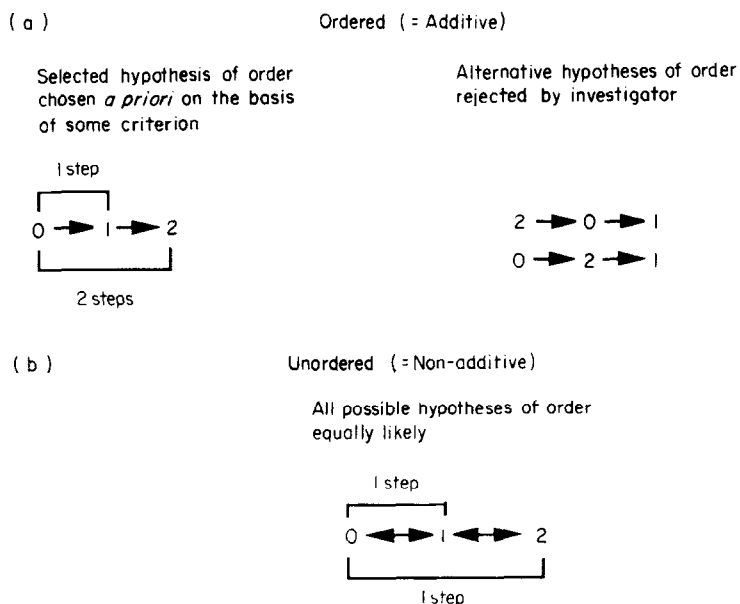


Fig. 2. Ordered and unordered characters differ in how the distances between states are defined and the degree of restrictiveness. Ordered characters (a) are more restrictive than unordered (b), and require more than a single step between non-neighboring character states.

A; and (2) structure A large or small. One way these two characters can be depicted in multistate form is “structure A absent \leftrightarrow small \leftrightarrow large”. Operating under the assumption that structure A is primitively absent, it can be demonstrated that ordering the character states forces an assumption not present in the non-additive binary or unordered forms. In Fig. 3(a), the character state changes of the non-additive binary form are depicted on a hypothetical cladogram. Note that only two steps are required regardless of whether the initial change in structure A is “large” or “small”. The same number of steps results when the binary characters are recoded as an unordered multistate character. The nature of both the non-additive binary and unordered codings make no restrictions on the sequence of structure A’s size; an absent \leftrightarrow large transformation is equally parsimonious as an absent \leftrightarrow small one. If the multistate form is ordered linearly (0 \leftrightarrow 1 \leftrightarrow 2), however, a specific assumption is made regarding the sequence of the derived states that results in an additional character state change [Fig. 3(b)]. Treating the character in additive binary form would result in the same number of steps as the ordered form. Thus, a non-additive binary character is equivalent to an unordered multistate character and an additive binary character is equivalent to an ordered multistate character.

There has been some confusion regarding the meaning of the term “unordered” (Mickevich and Weller, 1990: 146):

"Further, 'unordered' is a misnomer, because the states [of a multistate character] must be ordered to be used in a phylogenetic analysis, and this method does postulate an order of transformation".

This explanation fails to recognize the fundamental difference between an ordered and unordered character. Ordering defines the distances between states such that, in most cases, only neighboring states are one step apart; in unordered characters, the distance between all states is a single step. Second, the statement that multistate

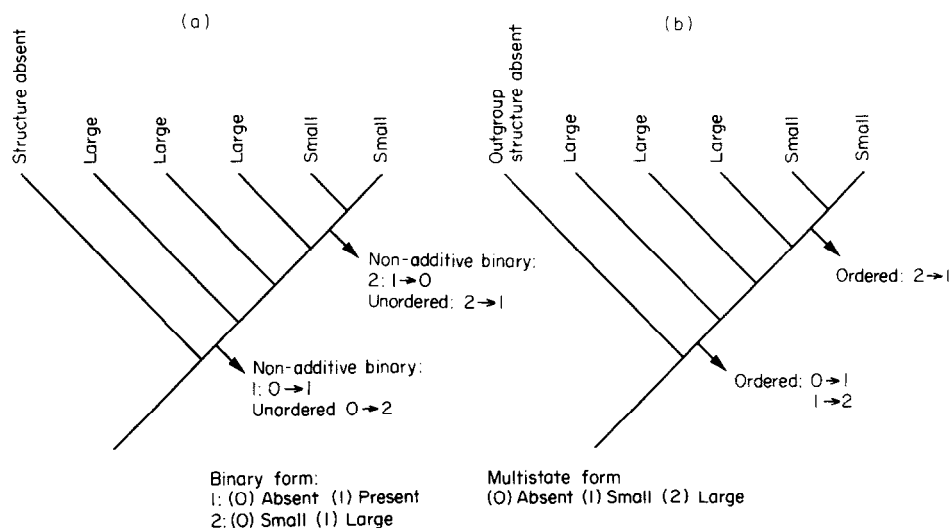


Fig. 3. Example illustrating the difference between ordered multistate and non-additive binary characters. Treating the above transformation series as non-additive or unordered (a) results in two character state changes. An ordered (or additive binary) treatment results in an additional character state transformation (b).

characters must be ordered to be processed by current phylogenetic programs implies that the algorithms require that the distance between non-neighboring states be more than one step. The evidence against this is *prima facie*, as the programs run perfectly well when all characters are treated as unordered. The idea that the programs postulate an order of transformation reduces to saying that an unordered character has a particular distribution on the resulting cladogram(s). This statement can be made about any character, be it binary or multistate, ordered or unordered. It does not alter the fact that hypotheses of order and unordered define the distance between character states in different ways and often make different assumptions about evolutionary processes.

As has been pointed out by others (Mickevich, 1982; Swofford, 1985; Mabee, 1989a), order and polarity are distinctly different concepts. Whereas hypotheses of order pertain to the relationships among all the states, polarity is simply the recognition of which state is plesiomorphic—no specific statement is made with regard to the relationships of the derived states. The distinction between ordered and unordered only has meaning when applied to multistate characters because all binary characters, by definition, have only a single transformation series. Hypotheses of polarity and order also have different effects on phylogenetic reconstruction. When modern phylogenetic programs analyse character state distributions, the initial result is an undirected tree. When an undirected tree is rooted, usually at the ancestor, direction (or polarity) is forced and a particular tree is produced. Hypotheses of polarity only affect the topology of directed trees. Hypotheses of order, however, affect the topology of the undirected trees (Mabee, 1989a).

HYPOTHESES OF ORDER AND PHYLOGENETIC RECONSTRUCTION

There is not a large literature on the effect of hypotheses of order (or unordered) on phylogenetic reconstruction. Much of the recent work focuses on various methods of coding multistate characters (O'Grady and Deets, 1987; Goldman, 1988, 1989; Farris,

1990). Only a few studies have addressed the relative merits of ordered characters (Meacham, 1984, 1986; Mickevich, 1982) and the question of how such characters affect phylogenetic reconstruction (Mabee, 1989a; Mickevich and Weller, 1990). Mickevich (1982: 467) made the following statement regarding the relative merits of the two character types:

"Additive features distinguish groups of taxa and their interrelationships without the aid of the cladogram, or information offered by other features. . . . The non-additive character . . . where all states are equally related, suggests that each pair of taxa can be equally closely related. Compared to possible alternative characters, this statement of taxonomic relationship is the equivalent of indifference".

More recently, Mickevich and Weller (1990: 164) made a similar remark:

"Using the Fitch minimum mutations model [i.e. unordered] for all characters may lead to other problems. First, the tree may have polytomies because the information available for tree construction is simply that the taxa possessing these states are different . . . The second concern is that the tree will be unstable. The addition of new taxa or characters can and probably will result in large rearrangements of clades. For simple, small data sets with low homoplasy, admittedly, rearrangement will rarely be a problem. However, for large numbers of taxa, characters, and character states with some homoplasy, this is a serious consideration against using this approach".

The implication that unordered characters may lead to unresolved polytomies and ordered characters should result in an increase in resolution seems to stem from the analysis of examples that deal with a single character. For instance, in Fig. 4, a single multistate character is treated as both unordered [Fig. 4(a)] and ordered [Fig. 4(b)].

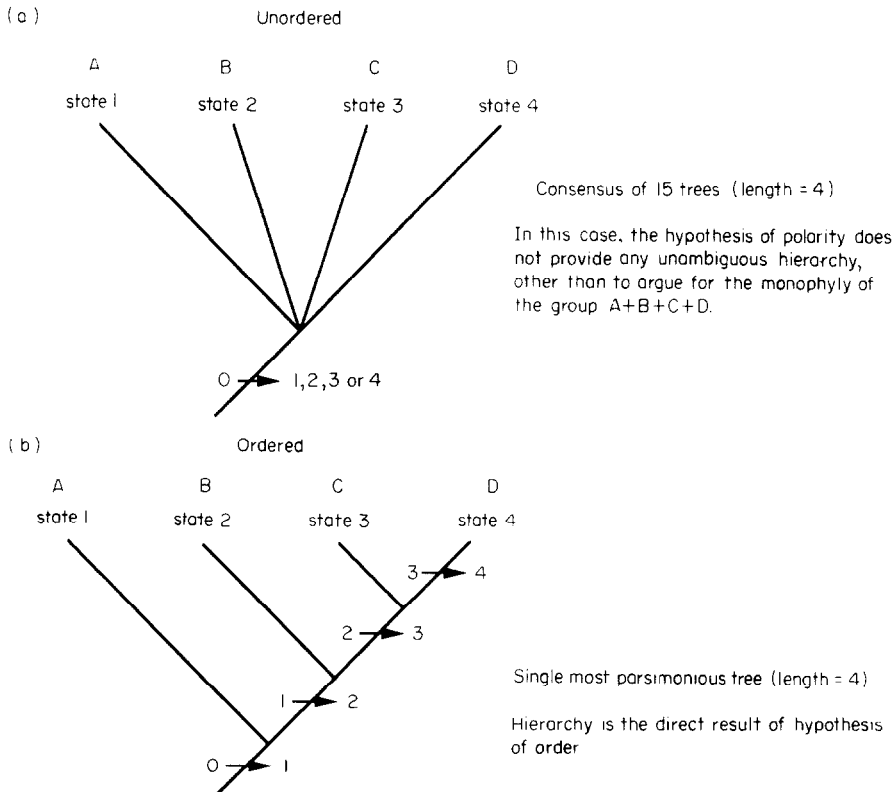


Fig. 4. Example, based on a single character, in which an ordered character (b) results in more resolution than unordered character (a).

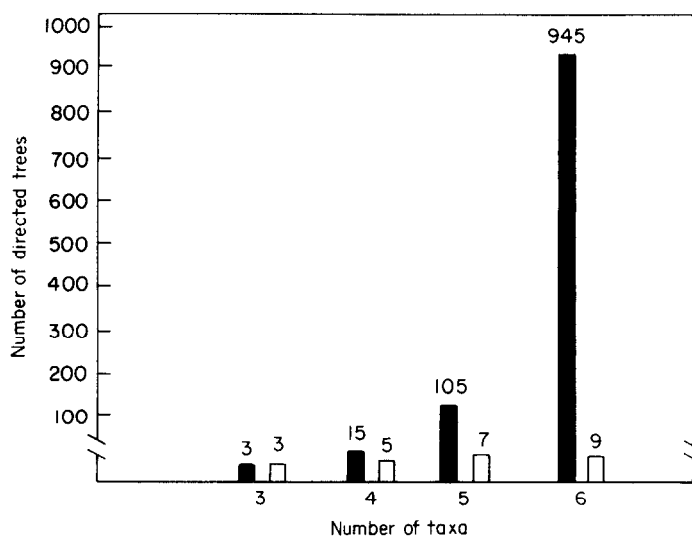


Fig. 5. Histogram demonstrating the effect of hypothesized character state order on phylogenetic reconstruction. As the number of states and taxa increase, the relative constraint on phylogenetic reconstruction increases (from Mabee, 1989a). ■, total number of directed trees. □, number consistent with hypothesis of linear order.

When treated unordered, the only unambiguous statement of relationship, the monophyly of ABCD, is based on polarity alone. Fifteen equally parsimonious trees are possible and the consensus tree is completely unresolved. Treating the character as ordered, however, results in a single, completely resolved tree. The resolution, of course, is solely the result of the hypothesis of order. A different hypothesis of order would result in a different tree.

Mabee (1989a) examined the effect of ordered characters on the number of equally parsimonious trees. Her conclusion was based on an analysis of a single, linearly-ordered multistate character. In her study, the number of character states of the multistate character was equal to the number of taxa and each state was uniquely represented. Data sets with three, four, five and six character states and taxa were examined. The number of possible directed trees was compared to the number of trees consistent with the hypothesis of linear order (Fig. 5). As the number of taxa and states increased, the proportion of directed trees consistent with the hypothesis of order decreased dramatically. Based on this, Mabee concluded:

"Ordered character state hypotheses polarized or unpolarized restrict the number of equally parsimonious undirected trees, and consequently, the number of directed trees, because it is information on order and not polarity that is used in undirected tree construction". (Mabee, 1989a: 153)

Because of the frequency in which multistate characters are encountered, and their possible effect on phylogenetic studies, it is important to examine quantitatively the effects that hypotheses of order and unordered can have on tree topology and tree resolution.

Methods

We examined the validity of the above claims regarding the effect of ordered characters on phylogenetic reconstruction. While random data sets are a useful tool in

Table 1
A list of the 27 data sets examined in this study.

Citation	Organisms studied	Type of data
Sundberg (1989)	Nermerteans	Morphology
Meylan and Gaffney (1989)	Turtles	Morphology
Frost and Etheridge (1989)	Lizards	Morphology
Estes et al. (1988)	Lizards	Morphology
Bolick (1981)	Plants	Morphology
Bolick (1983)	Plants	Morphology
Green (1986)	Frogs	Karyological
Pregill (In press)	Lizards	Morphology
Caunatella and de Queiroz (1989)	Lizards	Morphology
Ladiges et al. (1989)	Plants	Morphology
Crother et al. (1986)	Lizards	Morphology
Kluge (1989)	Snakes	Morphology and biochemical
Kraus (1988)	Salamanders	Morphology
de Queiroz (1987a)	Lizards	Morphology
Sokal (1983)	Carnivalcules	N.A. (Imaginary data set)
de Queiroz (1987b)	Lizards	Morphology
Gilmartin et al. (1989)	Plants	Morphology
Young (1981)	Plants	Morphology
Humphries (1981)	Plants	Morphology
Etheridge and de Queiroz (1988)	Lizards	Morphology
Drews (1984)	Frogs	Morphology
Arnold (1989)	Lizards	Morphology
Presch (1988)	Lizards	Morphology
Duellman and Trueb (1986)	Salamanders	Morphology
Presch (1980)	Lizards	Morphology
Les and Sheridan (1990)	Plants	Morphology
Farris (1968)	Fish	Morphology

examining many problems in cladistics, we have chosen 27 data sets from the literature upon which to base our analysis. Most of the data sets utilized morphological characters and dealt with vertebrates, primarily lizards, although some dealt with invertebrates and plants (Table 1). The proportion of multistate characters in a given data set ranged from 8% to 95%.

The majority of the data sets were run on Farris's Hennig86 (version 1.5) and Swofford's PAUP (Macintosh version 3.0d). The heuristic and the Branch and Bound algorithms were used in PAUP, depending on the size of the data set. Some of the data sets, however, were run only on Hennig86 or PAUP because of differences in program performance. For instance, some of the large data sets (over 15 taxa) took 10–15 hours to run on PAUP; Hennig86, however, could analyse the same data set in about 1–3 hours. Thus, some of the data sets with 15 or more taxa were run on Hennig86 alone. On the other hand, some of our runs on Hennig86 that dealt with smaller data sets often ran into memory problems in that the number of equally parsimonious trees often surpassed the computer's storage capacity. In these cases, the data sets were analysed by PAUP, which did not suffer from memory limitations to the same degree as Hennig86.¹

Following Presch (1989), we provide the options used in the phylogenetic programs so as to provide a basis for others to test our results: PAUP (Macintosh version 3.0d) heuristic method: Addition sequence = random; number of replications = 10; Mulpars

¹ This limitation of memory is not an attribute of Hennig86 *per se* but rather a function of the particular computer and the amount of memory available to the program.

Table 2

Tabulated results of changes in tree measures and tree resolution resulting from treating the data sets from Table 1 as ordered and unordered. See text for further explanation.

	Characters		Tree length		Number of trees		Consistency index		Resolution (number nodes)	
	Total no.	No. multi	Uno	Ord	Uno	Ord	Uno	Ord	Uno	Ord
Presch (1988)	88	11	101	103	1	1	0.750	0.750	6	6
Pregill (In press)	38	12	114	121	12	9	0.430	0.410	11	13
Sundberg (1989)	38	7	63	68	5	10	0.577	0.557	10	8
Green (1986)	13	3	17	18	1	2	1.000	0.917	5	3
de Queiroz (1987a) ¹	75	6	96	107	3	4	0.850	0.830	4	4
de Queiroz (1987b) ²	21	3	27	27	72	12	0.867	0.882	6	5
Etheridge and de Queiroz (1988)	49	26	305	386	1091+	1090+	0.280	0.220	35	23
Frost and Etheridge (1989)	67	11	204	214	288	2044	0.392	0.374	26	20
Drewes (1984)	24	14	146	184	1005+	1007+	0.375	0.300	25	23
Duellman and Trueb (1986)	30	8	66	76	37	3	0.603	0.519	6	11
Presch (1980)	24	2	58	59	750	194	0.460	0.450	5	7
Estes et al. (1988)	148	20	378	396	8	1	0.440	0.420	14	17
Cannatella and de Queiroz (1989)	13	8	38	42	870	48	0.630	0.561	9	10
Gilmartin et al. (1989)	20	6	45	55	48	5	0.643	0.519	3	5
Arnold (1989)	84	21	284	337	1391+	1392+	0.360	0.310	25	29
Young (1981) ³	41	19	234	284	1072	37	0.240	0.198	6	13
Humphries (1981) ⁴	47	5	72	73	30	2	0.646	0.633	6	8
Kraus (1988)	32	8	46	98	25	6	0.833	0.800	8	8
Crother et al. (1986)	30	6	41	45	3	3	0.688	0.679	2	2
Ladiges et al. (1989)	26	12	72	82	8	11	0.620	0.543	11	10
Kluge (1989)	77	12	133	136	2	4	0.662	0.652	8	7
Sokal (1983) ⁵	106	101	302	345	800+	800+	0.640	0.560	53	42
Bolick (1981)	24	5	37	40	4	3	0.759	0.706	7	7
Bolick (1983)	17	4	33	38	18	10	0.636	0.553	7	6
Les and Sheridan (1990)	29	19	139	189	34	1	0.489	0.358	3	24
Meylan and Gaffney (1989)	48	11	88	92	1	9	0.663	0.641	14	7
Farris (1968)	52	10	165	176	25	66	0.400	0.390	7	9

¹ Original data set had 95 characters. Autapomorphies were removed to produce 75 characters.

² Outgroup recoded: Galapagos iguanas, *Iguana* and *Cyclura* were condensed into a single O.T.U. as was *Disposaurus* and *Brachylophus*.

³ Data set taken for sample data files in PAUP (Macintosh version 3.0a).

⁴ Data set taken from sample data files in PAUP (Macintosh version 3.0a).

⁵ Data set recoded by Presch.

in effect; TBR branch swapping; branches with zero length collapsed to form polytomies. Hennig86: Mhennig* and BB (heuristic branch swapping).

For each data set, all multistate characters were treated as linearly ordered or unordered. Because the particular sequence of character state order is rarely provided, we had no choice but to assume a simple numerical sequence (i.e. $0 \rightarrow 1 \rightarrow 2 \rightarrow 3$, etc.). This assumption is not as precarious as it first seems because the default mode in PAUP (Macintosh version) and Hennig86 assumes such a linear sequence when a character is treated as ordered. In spite of this, because our goal is to assess the *potential* effects of particular hypotheses of order on phylogenetic reconstruction and not to evaluate an author's systematic conclusions, it is not necessary to make our hypotheses of character state order consistent with those of a published study. Whether or not we used an

author's hypotheses of order, our results are still valid. Certain alterations were made to a few data sets for the sake of simplicity and these are mentioned in Table 2.

The tree length, number of equally parsimonious trees, amount of resolution, number of multistate characters and consistency index were recorded for each data set (Table 2). We did not determine the number of trees that were common to both the ordered and unordered runs. Because we compared the results of unordered runs to those that assumed particular hypotheses of linear order rather than all possible hypotheses of order, we are actually examining effects of specific, linear hypotheses of order on phylogenetic reconstruction.

Results

EFFECT ON RESOLUTION

In order to examine the claim by Mickevich (1982) and Mickevich and Weller (1990) that the use of unordered characters may result in unresolved polytomies, a measure of tree resolution must be utilized. We follow Kraus (1988) and measure the degree of resolution by counting the number of resolved nodes present in the resultant tree. When only a single equally parsimonious tree is discovered in both ordered and unordered runs, determining the amount of resolution is straightforward. In almost every data set we examined, however, at least one of the runs (ordered or unordered) had more than one final tree. In those cases, resolution was determined by counting the number of nodes present in the strict consensus tree. We recognize that a consensus tree is not a phylogeny (Miyamoto, 1985), but rather a statement regarding the topologies common in all the discovered trees. There were instances in which the amount of resolution in the individual trees in the ordered and unordered runs was identical, yet the amount of resolution in the strict consensus trees was different. While the number of nodes in a consensus tree is, at best, only a rough estimate of tree resolution, we chose this method to evaluate resolution for two reasons: (1) several of the data sets resulted in a large number of equally parsimonious trees (over 1000 trees) in both ordered and unordered runs. Counting the number of nodes in each individual tree would have required the examination of at least 14 000 trees (see Table 1). While such a method would have been more accurate, it seemed to us impractical. (2) The consensus tree allows one to see which nodes were consistently resolved in all discovered trees. When a particular data set results in more than one equally parsimonious tree, a consensus tree is usually published because it represents resolution that is common to all the discovered trees. Given this, the use of the consensus tree allowed us to measure the amount of unambiguous resolution present in each data set. It is possible that our measure of resolution may be affected by problems inherent to the way the algorithm formulates the consensus tree, such that the amount of resolution common to the individual trees may not be accurately captured by the consensus tree.

Our results do not support the notion that unordered characters tend to produce unresolved polytomies while ordered ones are likely to increase resolution. Both ordered and unordered data sets each produced a greater degree of resolution in 11 cases; in five cases, there was no difference between treatments (Table 2). This distribution suggests that the amount of resolution in a cladogram is not dependent on hypotheses of order and unordered alone, but the interaction of such hypotheses with all the characters in the

data set. Whether more or less resolution will be achieved cannot necessarily be determined prior to analysis.

As mentioned earlier, the reasoning behind the idea that unordered characters have a greater tendency to produce polytomies stems from examining the behavior of a single multistate character. The problem with examples of this type is that they are not necessarily applicable to phylogenetic studies because most, if not all, published studies deal with more than one character. When data sets with more than a single character are examined, it is clear that hypotheses of order can result in unresolved polytomies as well. In Fig. 6, a simple data set of four characters, one of which is multistate, is treated as ordered and unordered. The multistate character, number three, is depicted as having two states (0 and 2). The "1" state has been excluded from the figure for the sake of simplification; the example, however, suffices to illustrate our point. In the unordered run, an unambiguous hypothesis of relationship is present, with the monophyly of taxa B and C being supported by characters two and four. In the ordered run, however, this unambiguous hypothesis is lost because the $0 \rightarrow 2$ transformation is interpreted as requiring two individual transformations ($0 \rightarrow 1$ and $1 \rightarrow 2$). Thus, the character has the effect of two binary characters. As a result, there is equal support for the monophyly of taxa A + B and B + C, and a polytomy results in the consensus tree. Whether or not ordered characters will increase or decrease resolution does not depend solely on the nature of such characters but also on their interaction with other characters in the data set.

We did not test Mickevich and Weller's (1990) claim that unordered characters will result in unstable trees. The fact that minor changes in a data matrix can substantially alter cladogram topology is not new (Coombs et al., 1981). It seems to us, however, that the effect on tree topology of adding characters or taxa has more to do with the amount of character conflict in a given data set than with hypotheses of order and unordered. Before any truly valid statements can be made on the notion of tree stability, experimental addition of taxa and characters in ordered and unordered data sets must be performed. Even if unordered characters did result in consistently unstable trees, this instability would not, by itself, be an argument against the use of unordered characters. A stable cladogram is not necessarily more correct than one that is unstable. The essential factor is not the stability of an ordered tree, but rather the validity of the criteria used to order the characters that produce the stability.

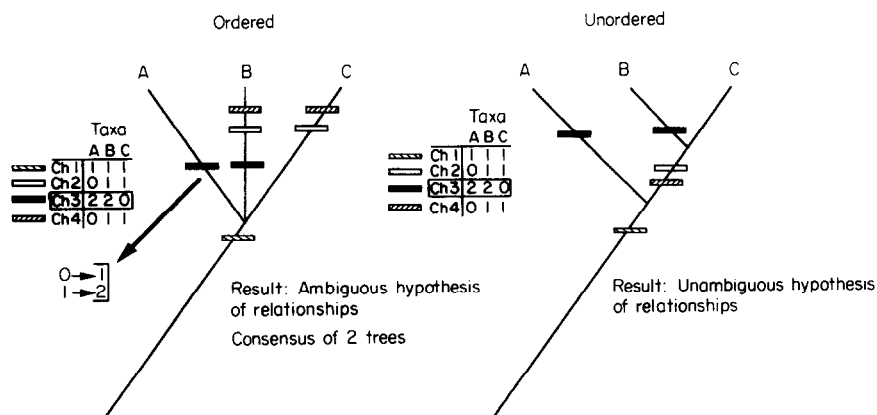


Fig. 6. Example in which an ordered character results in an unresolved polytomy.

EFFECT ON TREE NUMBER

We do not argue with the logic of Mabee's (1989a) example concerning the effect of ordered characters on phylogenetic reconstruction (see Fig. 4). We point out, however, that her example illustrates the effect ordered characters have on a data set composed of a *single* character. Again, conclusions derived from such a limited data set are not necessarily applicable to phylogenetic studies that utilize more than one character.

In our study, ordered characters resulted in fewer equally parsimonious trees in 13 data sets, while the unordered characters resulted in fewer trees in only eight (Table 2). In two cases, the number of equally parsimonious trees discovered did not vary. In four data sets, the total number of trees could not be determined because of memory limitations on Hennig86 and extremely long run times on PAUP. While the ordered data sets resulted in fewer equally parsimonious trees in the majority of runs, we do not feel this difference is sufficient to support the idea that ordered characters are more likely to result in fewer equally parsimonious trees simply by virtue of the hypotheses of order. As with tree resolution, the effect of ordered characters on tree number is, in part, based on their interaction with other characters in the data matrix. Given this, the effect of hypotheses of order and unordered on tree number is difficult to determine in an exclusively *a priori* fashion.

In addition to testing effects on tree number and resolution, additional aspects of how hypothesis of order can affect phylogenetic reconstruction were examined, particularly those dealing with the nature of the changes in tree topology. Specifically, we examined whether ordering multistate characters could produce changes in sister-taxon relationships.

In Fig. 7, cladograms derived from treating a data set (Meylan and Gaffney, 1989) both ordered and unordered illustrate a case in which sister-taxon relationships were substantially altered. In the ordered run, *Hoplochelys* is the sister-taxon to a clade composed of *Staurotypus* + *Kinosternon*. In the unordered run, however, the sister-taxon to *Hoplochelys* is *Emarginachelys*, while the *Staurotypus* + *Kinosternon* clade is nested in a more inclusive clade. Changes of this nature, of which we found several examples, are of

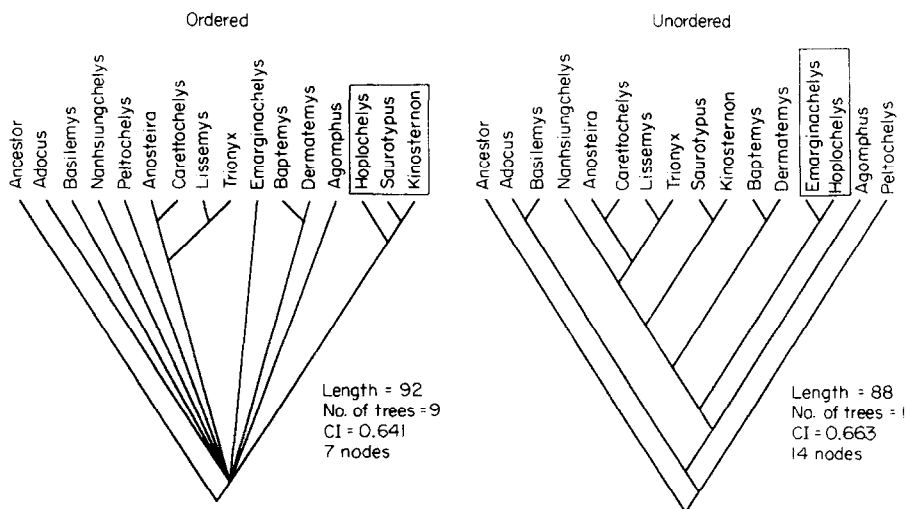


Fig. 7. Consensus tree derived from Meylan and Gaffney (1989) that demonstrates the effect of hypotheses of order on sister-taxon relationship. Boxes highlight clades in which sister-taxon relationships are different between the ordered and unordered trees.

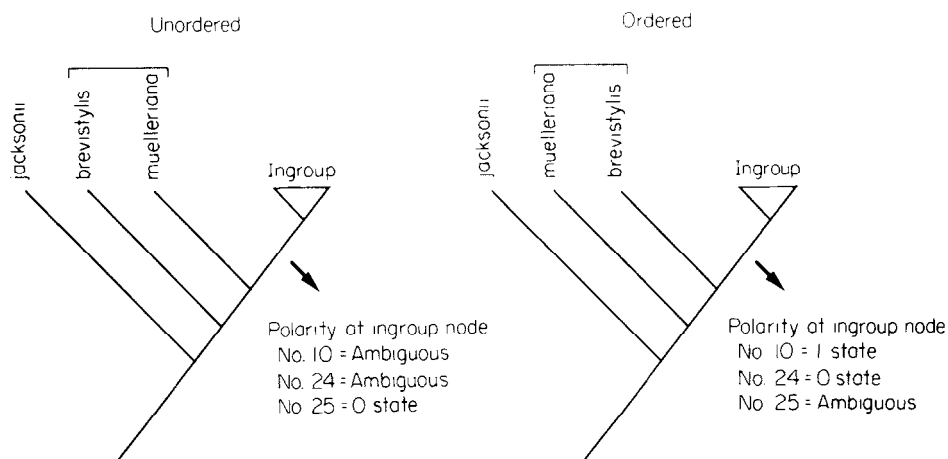


Fig. 8. Example illustrating the effect of ordered characters on polarity. Changes in outgroup topology in the unordered and ordered trees result in changes in polarity decisions. Character numbers are those used in the original study. Data from Ladiges et al. (1989).

particular interest to systematists because they can significantly affect the result of evolutionary studies that rely heavily on sister-taxa relationships (i.e. biogeography, co-evolution, etc.).

Changes in sister-taxon relationships as a result of hypotheses of order can also affect character polarities when several outgroups are included in the data matrix. This method is often employed when relationships among the outgroups is unknown because it allows for the most globally parsimonious arrangement of the outgroups with respect to the ingroup (Maddison et al., 1984). Changes in character polarity can occur when hypotheses of order result in changes in topology between the outgroups, as was seen in our analysis of the data from Ladiges et al. (1989). Treating all multistate characters as unordered [Fig. 8(a)] results in a different outgroup topology than when all multistate characters are treated as ordered [Fig. 8(b)]. As a result, the polarity of two characters (Nos 10 and 24) changed from ambiguous to decisive while another (No. 25) changed from decisive to ambiguous. While no character underwent a reversal in polarity in this particular data set, one can easily imagine situations involving three or more outgroups in which character polarities could be reversed depending on how the multistate characters were treated.

Up to now, we have examined the effects of hypotheses of order on phylogenetic reconstruction when all multistate characters in a data matrix were treated as ordered or unordered. However, treating only a portion of the multistate characters in any given matrix as ordered or unordered can result in changes in tree topology and tree measures that are more or less dramatic than those seen when all characters are treated in a singular manner. In one data set (Frost and Etheridge, 1989), the number of equally parsimonious trees ranged from 36 to over 4000 depending on which multistate characters were ordered. Dramatic changes in tree topology resulted as well, including the loss of monophyly for some groups.

Discussion

A variety of criteria can be used to determine the evolutionary relationships between the states of a multistate character. Our attempt to categorize these criteria was

hampered by the singular fact that, in the vast majority of studies examined, no explicit discussion was provided regarding the criteria used to determine character state order. In addition, only a few studies explicitly mentioned what the specific hypothesis of character state order was (i.e. $0 \leftrightarrow 1 \leftrightarrow 2$ or $1 \leftrightarrow 2 \leftrightarrow 0$, etc.), the remaining studies indicating only that a character was ordered. Despite this, we briefly discuss some of the criteria that have been advocated to determine character state order. Our list, however, is by no means exhaustive. Mickevich and Weller (1990) examine some criteria not dealt with in this paper and the reader is referred to their study for additional discussion.

TRENDS

Patterns of evolutionary change, such as patterns of digit loss, change in body size (e.g. Cope's Rule), etc., seen in other taxa can be used to determine character state relationships in the group under study. Ideally, the relevant trend would be based on a rigorous cladistic study of closely related groups as this would offer some assurance that the trend is phylogenetically valid. The fundamental problem with this approach is that it assumes some homogeneity in pattern production: patterns found in one group are likely to be found in a closely related group. This seems a rather dubious claim to make prior to a cladistic analysis.

ONTOGENY

Most of the recent discussions involving ontogeny and systematics have focused on the use of ontogeny to determine character polarity. Hailed as a "direct" method of polarity inference, its advocates have claimed that it does not suffer from the central problem that plagues the outgroup criterion, namely, the need for some *a priori* knowledge of relationships. While the use of the ontogenetic method is supported by some (Nelson, 1978, 1985; Nelson and Platnick, 1981; Rosen, 1982; Kraus, 1988; Wheeler, 1990), others have pointed out the theoretical problems associated with this method (Alberch, 1985; Brooks and Wiley, 1985; de Queiroz, 1985; Kluge, 1985; Kluge and Strauss, 1985; Mabee, 1989a,b; Wake, 1989). Although others have discussed the problems associated with the use of ontogeny to order character states (Mabee, 1989a), it is useful to discuss briefly the arguments pertinent to this issue.

Any use of ontogeny in phylogenetic reconstruction, and thus hypotheses of character state order, rests upon certain assumptions regarding the nature of ontogeny (see Alberch, 1985). In general, the use of ontogeny to polarize or order character states assumes that ontogenetic transformations are somehow reflective of phylogeny to the degree that the transformations can provide phylogenetic information in isolation of other forms of evidence. To this end, acceptance of some degree of recapitulation (Haeckelian or von Baerian) is necessary (Wake and Roth, 1989; Mabee, 1989b).

There is not a single "ontogenetic method" utilized by systematists. Some utilize a strictly traditional Haeckelian approach and assume that the ontogeny of a given character is altered at the terminal end; thus, the ontogenetic transformation and the phylogenetic history of the character are identical (i.e. ontogeny recapitulates phylogeny). Perhaps more common is the use of Nelson's Law, which states (Nelson, 1978: 327):

"given an ontogenetic character transformation, from a character observed to be more general to a character observed to be less general, the more general character is primitive and the less general advanced".

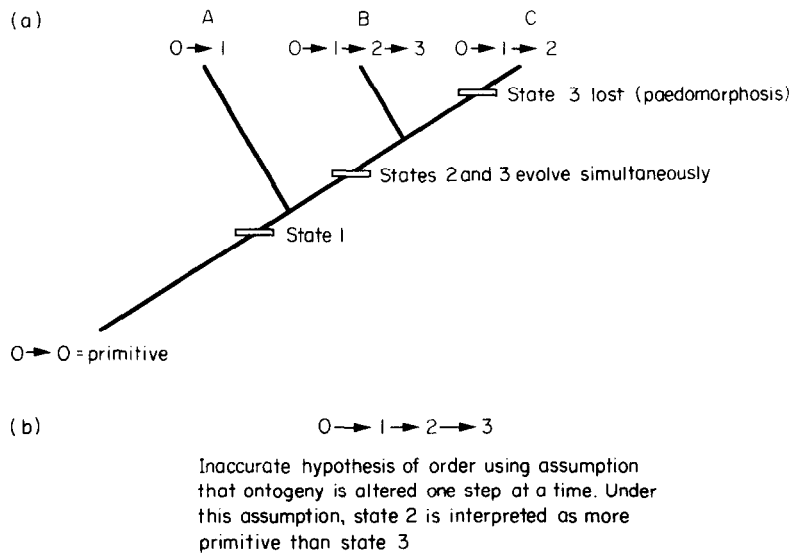


Fig. 9. Example in which the simultaneous evolution of two ontogenetic states leads to a misleading hypothesis of character state order. The transformation series (b) is not an accurate representation of the actual evolutionary history of the character states (a).

Nelson's version is restricted to those cases in which the ontogeny proceeds from more general to less general and is essentially von Baerian in nature. De Queiroz (1985) introduced a slightly different version of the ontogenetic method that differs from both the Haeckelian and Nelson version in that the ontogenetic sequence is considered irrelevant (de Queiroz, 1985:289):

"given that ancestral characters are retained in descendent ontogenies, ancestral characters are more general than derived characters"

The central problem with all of the above mentioned criteria is that, because it appears that ontogeny can be altered at almost any point, certain alterations can confound any attempt to use ontogeny to order character states. For instance, de Queiroz (1985) and Mabee (1989a) pointed out that any change in an ontogenetic sequence that involves the simultaneous addition or deletion of more than one state would result in an inaccurate hypothesis of order. In Fig. 9(a), an ontogenetic sequence is arranged in its proper evolutionary sequence (let us assume we know for certain what the evolutionary sequence was). In taxon B, states 2 and 3 originated simultaneously; in taxon C, state 3 is lost by paedomorphosis. In Fig. 9(b), the sequence is arranged in accordance with the assumptions inherent in all the ontogenetic methods discussed above. The result is an hypothesis of order that fails to reflect the simultaneous occurrence of states 2 and 3, instead forcing the latter state to evolve from the former.

If one accepts the notion that ontogeny can be divided into discrete states that are hierarchical in nature, the problem of determining whether a given ontogeny was altered terminally, non-terminally, or whether two or more states within a sequence were modified simultaneously still remains. We feel the most appropriate way to determine the answer to these questions is to interpret the evolution of ontogenetic transformations from a cladogram (O'Grady, 1985). In this way, all the character information in the data matrix can be brought to bear on the problem. Treating ontogenetic sequences in this way is consistent with the notion that these sequences are

best viewed as characters themselves whose evolutionary history is determined by a cladogram (de Queiroz, 1985; Wake, 1989; Mabee, 1989a,b; Wake and Roth, 1989).

SIMILARITY

The most common criterion used to order the states of a transformation series appears to be similarity, based on gross morphology, between the derived states. This is also known as morphocline analysis. This criterion is particularly amenable to meristic and morphometric characters (see Farris, 1990 for discussion on the use of morphometric characters in phylogenetic analyses). It is common practice for character states to be arranged in a manner suggested by Maslin (1952), in which the amount of morphological change between states is minimized. For example, if a multistate character characterized various lengths of a bone, the states would be arranged small ↔ intermediate ↔ large. In other words, it is assumed that the states that are most similar morphologically share a closer evolutionary relationship to each other than to some less similar state (i.e. phenotypic intermediacy equals phylogenetic intermediacy). While this concept makes some intuitive sense, it operates under the assumption that the process responsible for the character state change is phyletic gradualism (Mabee, 1989a) – a punctuated change in character state evolution is ruled out. The validity of assuming a purely gradual mode of evolution, however, has been questioned (Eldredge and Gould, 1972; Stanley, 1979; Wiley and Brooks, 1982; Brooks and Wiley, 1986). While both gradual and punctuated modes of evolutionary change have undoubtedly occurred, it seems unwarranted to restrict the interpretation of data to one or the other processes in the absence of phylogenetic information. It has been argued that ordering characters by overall similarity is more parsimonious in that it requires the fewest evolutionary steps (Kemp, 1988). As Mabee (1989a) correctly points out, however, parsimony provides no justification for such a position in the absence of phylogenetic information. The *a priori* ordering of a transformation series requires reference to a particular evolutionary process.

Non-phylogenetic information also has bearing on this issue. In situations where the states of a multistate character could be produced via heterochrony, such as in the bone example above, current knowledge of development does not support the ordering of the states on the basis of similarity. Bone length can be altered via a differential change in the timing of bone growth onset or offset, as well as by altering the rate at which the bone grows. A bone can stop growing at almost any time after a certain critical point, and can grow at a variety of rates. There is no reason to assume, in the absence of phylogenetic information, that the rate or duration of bone growth is altered in a manner that produces successively longer or shorter states. It seems apparent that the proper interpretation of any heterochronic change requires phylogenetic information (Fink, 1982, 1988).

TRANSFORMATION SERIES ANALYSIS

TSA is described as an iterative procedure that seeks to determine the best corroborated character state tree by means of reciprocal illumination with the cladogram (Mickevich, 1982). As such, it does not make assumptions regarding any particular process of evolutionary change. Recently, several authors have argued that this method is the most appropriate for determining character state order (Mickevich and Weller, 1990;

Lipscomb, 1990; Pogue and Mickevich, 1990). While we agree with TSA's basic premise, that transformation series should reflect the hierarchy of the cladogram, we make the following observations. First, the topology of the initial tree can affect the results of TSA (Mickevich and Weller, 1990). Therefore, if the data set of the initial tree contains multistate characters, the decision of how to treat these characters must still be made. Second, and most important, it is impossible to perform TSA on an unordered tree. The iterative aspect of TSA is the result of initial hypotheses of order being falsified by the resulting cladogram. When the individual transformation series derived from the resulting cladogram (i.e. cladogram characters) differ from those used initially to construct the tree, the initial transformation series are replaced with the cladogram characters. This process continues until all transformation series used in the construction of the tree are identical to the cladogram characters. However, when multistate characters are treated as unordered, there is no initial hypothesis of transformation to be falsified because the hierarchy of the tree determines the transformation series. Given this, one would assume that TSA would converge on the same cladogram(s) produced by an unordered data set. We analysed the data set from Lipscomb (1990) and found that the unordered tree is, in fact, identical to the TSA tree. Pogue and Mickevich (1990), however, report significantly different results between TSA and what they refer to as the "unordered outgroup method". That TSA and unordering should produce different trees is puzzling. A thorough comparison of trees derived from TSA and unordering would shed light on this problem.

The fact that hypotheses of order and unordered can have dramatic effects on tree topology and/or tree measures prompts some important questions: (1) how should multistate characters be treated; and (2) how does one choose between the ordered and unordered trees? Below, we discuss some measures that are, in our opinion, not reliable criteria for choosing between ordered and unordered trees. Subsequently, we will discuss the validity of ordering multistate characters.

CONSISTENCY INDEX

Mickevich (1982) measured the 'goodness' of the TSA method by comparing the CI of both the resultant tree and the multistate characters of the TSA tree and the Wagner tree. Because TSA resulted in a higher CI for both of the tree itself and the individual multistate characters, it was judged to be a superior method.

If one uses this criterion to choose between ordered and unordered trees, the decision appears to be straightforward: in every data set but one in which the hypotheses of order and unordered resulted in different tree topologies, the unordered trees as well as those multistate characters that exhibit a non-sequential character state change, had a higher consistency index. Part of the reason for this lies in fact that ordered multistate characters that undergo a non-sequential character state change exhibit homoplasy in the intermediate states, which results in a lower CI for the whole tree. Changes in tree topology, however, can also affect the distribution, and thus the CI, of other characters in the data set as well. It is therefore possible for an ordered tree to have a higher overall CI if the distribution of several other characters is altered in such a way that the overall amount of homoplasy is decreased. Only one of our data sets exhibited this condition (de Queiroz, 1987b).

We do not advocate the use of the consistency index in choosing between an ordered and unordered tree simply because the different assumptions inherent in hypotheses of

order and unordered render the data sets themselves fundamentally different. In addition, the use of the consistency index to determine confidence in a particular phylogenetic hypothesis has been criticized (Archie, 1989; Sanderson and Donoghue, 1989). Above all, the variation seen in the CI, or any other homoplasy index, is not the pertinent issue --what must be addressed is the validity of the hypotheses that produced the variation.

TREE LENGTH

If the length is argued to be a viable criterion to choose between unordered and ordered trees, the choice in most cases would be straightforward: the unordered tree was always shortest in every data set but one examined in this study, even when no change in tree topology occurred. Due to the manner in which the distance between character states is defined in current phylogenetic computer programs, an ordered tree can be of equal or greater length than an unordered tree, but never shorter. However, we agree with Mickevich and Weller (1990) that tree length is not a reliable measure for determining which tree is a more accurate portrayal of phylogenetic history. One reason, mentioned earlier, is that hypothesis of order and unordered render the data sets intrinsically different. More significantly, tree length is not necessarily altered solely by non-sequential changes in character state transformation. Tree length could also be affected by altering patterns of homoplasy in other characters as a result of a change in tree topology. As such, characters other than those ordered may be forced to undergo more or less character state changes than in the unordered tree.

RESOLUTION

It is tempting to choose the set of trees with the most resolution, perhaps because publishing a final cladogram that is essentially unresolved is somewhat anticlimactic. When choosing between trees on the basis of resolution, however, it is imperative that the source of the resolution be identified and discussed. If an ordered tree is chosen because it exhibits a greater degree of resolution in comparison to an unordered tree, the validity of the added resolution is directly proportional to the validity of the criteria used to determine character state order. As was pointed out earlier, it is often impossible to assess this validity because no explicit mention is usually made regarding the criteria utilized.

SHOULD MULTISTATE CHARACTERS BE ORDERED?

When the preponderance of the evidence (i.e. the distribution of other, presumably independent, characters) argues for a tree topology that is consistent with all the *a priori* hypotheses of character state order, there is no change in tree topology, consistency index, tree number or tree length. In such cases, there is no reason to choose between the ordered and unordered runs. Changes in tree topology and other tree measures results only when some or all of the investigators *a priori* hypotheses of character state order are consistent with the tree topology as determined by the entire data set. In such a case, a non-sequential character state transformation is required (e.g. $0 \leftrightarrow 2$) somewhere in the cladogram. When a multistate character is forced to undergo a non-sequential transformation, the hypotheses of order results in the differential weighting of that character, a practice that is very controversial (see Wheeler, 1986; Neff, 1986; Sharkey, 1989; Bryant, 1989 for discussions). This can be demonstrated with a simple example

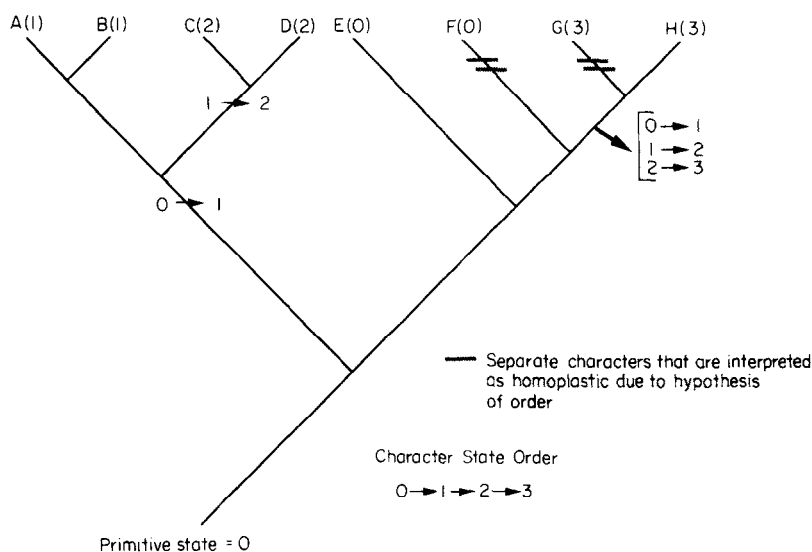


Fig. 10. Example of differential weighting caused by non-sequential transformation in an ordered character. Because of hypothesis of order, a change from 0 → 3 requires three steps. Thus, G and H are sister-taxa despite the presence of two characters that argue for the monophyly of F and G. Numbers in parentheses correspond to character states present in the taxa.

(Fig. 10). In the cladogram, the monophyly of the group G + H is supported by state three of character one. However, two binary characters support a sister-group relationship of F + G. Because character one is ordered, it is assumed that the actual evolutionary transformation is 0 → 1, 1 → 2, and finally 2 → 3. Thus, there are three character transformations supporting the monophyly of the group G + H. The two binary characters that argue for the monophyly of F + G are seen as homoplastic. If one unorders the multistate character, the transformation 0 ↔ 3 is seen as a single step and would be interpreted as homoplastic and the monophyly of F + G would be supported. Depending on the number of intermediate steps involved, a multistate character can be equivalent to a single-state change (a change between neighboring states) or several state changes. In some cases, the differential weighting will not be sufficient to change tree topology because of the presence of other characters. In this event, only tree measures (tree length, consistency index and possibly tree number) will be affected.

In cases where the investigator's hypothesis of character state order is not supported by the cladogram, a common argument is that there exists as yet undiscovered taxa (extinct or extant) that possess the intermediate state(s) and thus support the hypothesis of order. The invention of non-existent taxa, however, is not a convincing argument. Until such taxa are discovered, if they ever are, it is best to argue from known data.

If the consistency index, tree length, tree number and amount of resolution do not provide a basis to choose between trees derived from ordered and unordered data sets, how then does one choose between the competing hypotheses of phylogeny? Our first observation on this question is that we find it difficult to accept the validity of any criterion that assumes specific knowledge of character state evolution in the absence of any phylogenetic information. Because both hypotheses of order and unordered make strong assumptions about the evolutionary process, an argument could be made that both hypotheses have equal merit. The fundamental question, however, is not whether assumptions are involved in a given hypothesis but how those assumptions are to be falsified. Once an answer to this question is formulated, a more appropriate method of

determining character evolution can be developed. We believe the most legitimate criterion that can be used to test the validity of, and thus potentially falsify, a particular hypothesis of order is the cladogram itself. Because hypotheses of order and unordered are phylogenetic hypotheses, a legitimate, and perhaps the best, falsifier of a given hypothesis of character phylogeny is a cladogram formed from presumably independent characters (i.e. character congruence). This principle, to various extents, has been suggested by others (Lauder, 1981, 1990; Mickevich and Weller, 1990; Pogue and Mickevich, 1990). At first, our reasoning may appear circular: cladograms are formed from character transformations, which are, in turn, determined by the cladogram. As we have pointed out, however, cladograms formed from ordered characters do not always support the hypotheses of order. The use of a phylogeny to re-examine character evolution is not necessarily circular, it is iterative. For example, homoplasy is seen as a real evolutionary phenomena on the basis that there is no reason that reversal, convergence and parallelism cannot occur given our current concept of evolution. The reality of a homoplastic event is not made *a priori* in the absence of phylogenetic information, but rather is determined by corroboration of other independent characters and is manifested in the final tree(s). If: (1) hypotheses of character state order result in a different tree topology (compared to hypotheses of unordered) only when the cladogram's hierarchy argues against the *a priori* hypotheses of order; and (2) an appeal to a cladogram based on all the characters in a data set is the most robust way to determine character state order, then one comes to the following conclusion: *The ordering of character states in an a priori fashion is inappropriate in phylogenetic systematics*. This conclusion is applicable within the context of the methods discussed earlier that are used to construct a transformation series. It does not constitute a blanket statement concerning other methods that might be advocated, the validity of which must be determined in a case-specific manner. In general, however, we feel that any method that assumes a particular evolutionary process should not be used to order character states—the evolutionary relationships between character states, and the evolutionary processes that are compatible with those relationships, are not assumptions to be made but rather questions to be asked.

If, despite our argument, an investigator decides to order a given multistate character, it is imperative that an explicit discussion is provided explaining how the character state order was determined, and what the character state order is. It is not sufficient simply to state that a character is ordered. In the case of a five-state character, there are numerous possible hypotheses of character state order; failure to indicate which hypothesis was chosen makes it difficult for others to replicate the final tree. The cladogram in which all characters are treated as unordered should be presented as well so that it can be clearly seen where the tree resolution has been gained or lost and thus which sister-taxa relationships, and the evolutionary scenarios (biogeography, co-evolution, etc.) based on them, are the sole result of the hypotheses of order.

Conclusion

In recent years, interest in character evolution has increased. Understanding the evolutionary history of a particular character, or a subset of characters, is often necessary for examining and testing hypotheses of co-evolution (Brooks and Bandoni, 1988), adaptation (Ridley, 1983; Coddington, 1988; Donoghue, 1989), functional morphology (Lauder, 1981, 1990; Schaefer and Lauder, 1986; Gatesy, 1990), behavior (Dobson, 1985) and many others. Clearly, a proper understanding of character evolution is a

powerful tool in many types of evolutionary studies. The question of how to treat multistate characters is not a trivial one—such alterations can dramatically affect our concept of character evolution.

Hypotheses of order and unordered are specific statements, usually made in the absence of phylogenetic information, about the evolutionary relationships between the states of a multistate character. Hypotheses of order are more restrictive than those of unordered and generally define the distance between non-neighboring states as more than a single step. Numerous criteria have been used to determine hypotheses of order, such as evolutionary trends, ontogenetic transformations, similarity and TSA. Most of these criteria assume a particular evolutionary process and then interpret character evolution in that light. Ordered characters are often perceived as being more informative and likely to produce greater resolution and fewer equally parsimonious trees. Unordered characters have been perceived as “phylogenetic indifference” and likely to produce poor resolution and more equally parsimonious trees.

Our analysis of 27 data sets indicates that the manner in which ordered or unordered characters alter tree number and resolution is determined by the nature of the entire data set. Both ordered and unordered characters can potentially lead to: (1) more or less equally parsimonious trees; (2) an increase or decrease in tree resolution; (3) altered polarity decisions; and (4) changes in sister-taxa relationships.

If one accepts that the most reliable criterion for determining the evolutionary sequence of a multistate character is the cladogram itself (i.e. congruence with other, presumably independent characters), then hypotheses of order are unnecessary. If an investigator insists on ordering a multistate character, an explicit justification should be provided explaining why a particular hypothesis of character state order was chosen. This allows others to assess the validity of the hypothesis of order and the resulting change in tree topology and/or tree measures. In addition, a comparison should be made between the unordered and ordered tree with the explicit aim of isolating the changes in tree topology and/or tree measures that are the sole result of the hypotheses of order.

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REFERENCES

- ALBERCH, P. 1985. Problems with the interpretation of developmental sequences. *Syst. Zool.* 34: 46–58.
- ARCHIE, J. W. 1989. Homoplasy excess ratios: New indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. *Syst. Zool.* 38: 253–269.

- ARNOLD, E. 1989. Toward a phylogeny and biogeography of the Lacertidae: Relationships within an Old-World family of lizards derived from morphology. *Bull. Br. Mus. Nat. Hist. (Zool.)* 55: 209–251.
- BOLICK, M. R. 1981. A cladistic analysis of *Salmea* D.C. (Compositae-Heliantheae). *In*: V. A. Funk and D. R. Brooks (eds). *Advances in Cladistics I, Proceedings of the Willi Hennig Society*, New York Botanical Garden, pp. 115–126.
- BOLICK, M. R. 1983. A cladistic analysis of the Ambrosiinae Less and Engelmanniinae Stuessy. *In*: N. I. Platnick and V. A. Funk (eds). *Advances in Cladistics, Vol. 2. Proceedings of the Willi Hennig Society*. Colombia University Press, pp. 125–142.
- BROOKS, D. R. AND S. M. BANDONI. 1988. Coevolution and relics. *Syst. Zool.*, 37: 19–33.
- BROOKS, D. R. AND E. O. WILEY. 1985. Theories and methods in different approaches to phylogenetic systematics. *Cladistics* 1: 1–11.
- BROOKS, D. R. AND E. O. WILEY. 1986. *Evolution as Entropy: Toward a unified theory of Biology*. University of Chicago Press.
- BRYANT, H. N. 1989. An evaluation of cladistic and character analyses as hypothetico-deductive procedures, and the consequences for character weighting. *Syst. Zool.*, 38: 214–227.
- CANNATELLA, D. C. AND K. DE QUEIROZ. 1989. Phylogenetic systematics of the Anoles: Is a new taxonomy warranted? *Syt. Zool.* 38: 57–68.
- CODDINGTON, J. 1988. Cladistic tests of adaptational hypotheses. *Cladistics* 4: 3–22.
- COOMBS, E. A. K., M. J. DONOGHUE AND R. J. MCGINLEY. 1981. Characters, computers and cladograms: A review of the Berkeley cladistic workshop. *Syst. Bot.* 6: 359–372.
- CROTHER, B., M. MIYAMOTO AND W. PRESCH. 1986. Phylogeny and biogeography of the lizard family Xantusiidae. *Syst. Zool.* 33: 37–45.
- DE QUEIROZ, K. 1985. The ontogenetic method for determining character state polarity and its relevance to phylogenetic systematics. *Syst. Zool.* 34: 28–299.
- DE QUEIROZ, K. 1987a. Phylogenetic systematics of the iguanine lizards. A comparative osteological study. *Zoology Vol. 118*. University of California Press, Berkeley, California.
- DE QUEIROZ, K. 1987b. A new spiny-tailed iguana from Honduras, with comments on relationships within *Ctenosaura* (Squamata: Iguania). *Copeia* 1987: 892–902.
- DOBSON, F. S. 1985. The use of phylogeny in behavior and ecology. *Evolution* 39: 1384–1388.
- DONOGHUE, M. J. 1989. Phylogenies and the analysis of evolutionary sequences with examples from seed plants. *Evolution* 43: 1137–1156.
- DONOGHUE, M. J. AND W. P. MADDISON. 1986. Polarity assessment in phylogenetic systematics: A response to Meacham. *Taxon* 35: 534–538.
- DREWES, R. C. 1984. A phylogenetic analysis of the Hyperoliidae (Anura): Treefrogs of Africa, Madagascar and the Seychelles Island. *Occ. Pap. Calif. Acad. Sci.*, No. 139.
- DUELLMAN, W. AND L. TRUEB. 1986. *Biology of Amphibians*. McGraw-Hill, New York.
- ELDRIDGE, N. AND S. J. GOULD. 1972. Punctuated equilibria: An alternative to phyletic gradualism. *In*: T. J. M. Schopf (ed.). *Models in Paleobiology*. W. H. Freeman, San Francisco, pp. 82–115.
- ESTES, R., K. DE QUEIROZ AND J. GAUTHIER. 1988. Relationships within Squamata. *In*: R. Estes and G. Pregill (eds). *Phylogenetics Relationships of the Lizard Families. Essays Commemorating Charles L. Camp*. Stanford University Press, pp. 119–281.
- ETHERIDGE, R. AND K. DE QUEIROZ. 1988. A phylogeny of the Iguanidae. *In*: R. Estes and G. Pregill (eds). *Phylogenetic relationships of the lizard families. Essays commemorating Charles L. Camp*. Stanford University Press, pp. 283–368.
- FARRIS, J. S. 1968. The evolutionary relationships between the species of killifish genera *Fundulus* and *Profundulus* (Teleostei: Cyprinodontidae). Ph.D. Thesis, Univ. Michigan.
- FARRIS, J. S. 1970. Methods for computing Wagner trees. *Syst. Zool.* 19: 83–92.
- FARRIS, J. S. 1990. Phenetics in camouflage. *Cladistics* 6: 91–100.
- FARRIS, J. S., A. G. KLUGE AND M. J. ECKARDT. 1970. A numerical approach to phylogenetic systematics. *Syst. Zool.* 34: 280–299.
- FINK, W. L. 1982. The conceptual relationships between ontogeny and phylogeny. *Paleobiology* 8: 254–264.
- FINK, W. L. 1988. Phylogenetic analysis and the detection of ontogenetic patterns. *In*: M. L. McKinney (ed.). *Heterochrony in Evolution: a Multidisciplinary Approach*. Plenum Press, New York.

- FITCH, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.
- FROST, D. R. AND R. ETHERIDGE. 1989. A phylogenetic analysis and taxonomy of iguanian lizards (Reptilia: Squamata). Misc. Publ. University of Kansas No. 81: 1–65.
- GATESY, S. M. 1990. Caudofemoral musculature and the evolution of theropod locomotion. *Paleobiology* 16: 170–186.
- GILMARTIN, A. J., G. K. BROWN, G. S. VARADARAJAN AND M. NEIGHBOURS. 1989. Status of *Glomeropilcairnia* within evolutionary history of Bromeliaceae. *Syst. Bot.* 14: 339–348.
- GOLDMAN, N. 1988. Methods for discrete coding of morphological characters for numerical analysis. *Cladistics* 4: 59–71.
- GOLDMAN, N. 1989. Fewest variable coding method for multistate characters. *Syst. Zool.* 38: 79–85.
- GREEN, D. M. 1986. Systematics and evolution of Western North American frogs allied to *Rana aurora* and *Rana boylei*: Karyological evidence. *Syst. Zool.* 35: 273–282.
- HENNIG, W. 1966. *Phylogenetic Systematics*. University of Illinois Press, Urbana.
- HUMPHRIES, C. J. 1981. Cytogenetic studies in *Anacyclus* (Compositae: Anthemidae). *Nord. J. Bot.* 1: 83–96.
- KEMP, T. S. 1988. Haemothermia or Archosauria? The interrelationships of mammals, birds and crocodiles. *Zool. Jour. Linn. Soc.* 92: 67–104.
- KLUGE, A. G. 1985. Ontogeny and phylogenetic systematics. *Cladistics* 1: 13–28.
- KLUGE, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* 38: 7–25.
- KLUGE, A. G. AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18: 1–32.
- KLUGE, A. G. AND R. E. STRAUSS. 1985. Ontogeny and systematics. *Ann. Rev. Ecol. Syst.* 16: 247–268.
- KRAUS, F. 1988. An empirical evaluation of the use of the ontogenic polarity polarization criterion in phylogenetic inference. *Syst. Zool.* 37: 106–141.
- LADIGES, P. Y., M. R. NEWNHAM AND C. J. HUMPHRIES. 1989. Systematics and biogeography of the Australian "Green Ash" *Eucalypts* (Monoclyptus). *Cladistics* 5: 345–364.
- LAUDER, G. V. 1981. Form and function: Structural analysis in evolutionary morphology. *Paleobiology* 7: 530–442.
- LAUDER, G. V. 1990. Functional morphology and systematics: Studying functional patterns in an historical context. *Annu. Rev. Ecol. Syst.* 21: 317–340.
- LES, D. H. AND D. J. SHERIDAN. 1990. Hagstrom's concept of phylogenetic relationships in *Potamogeton*. *Taxon* 39: 41–58.
- LIPSCOMB, D. L. 1990. Two methods for calculating cladogram characters: Transformation series analysis and the iterative FIG/FOG method. *Syst. Zool.* 39: 277–287.
- MABEE, P. M. 1989a. Assumptions underlying the use of ontogenetic sequences for determining character state order. *Trans. Amer. Fish. Soc.* 118: 151–158.
- MABEE, P. M. 1989b. An empirical rejection of the onto genetic polarity criterion. *Cladistic* 5: 409–416.
- MADDISON, W. P., M. J. DONOGHUE AND D. R. MADDISON. 1984. Outgroup analysis and parsimony. *Syst. Zool.* 33: 83–103.
- MASLIN, T. P. 1952. Morphological criteria of phyletic relationships. *Syst. Zool.* 1: 49–70.
- MEACHAM, C. A. 1984. The role of hypothesized direction of characters in estimation of evolutionary history. *Taxon* 33: 26–38.
- MEACHAM, C. A. 1986. More about directed characters: A reply to Donoghue and Maddison. *Taxon* 35: 535–540.
- MEYLAN, P. A. AND E. S. GAFFNEY. 1989. The skeletal morphology of the Cretaceous cryptodiran turtle, *Adocus*, and the relationships of the Trionychoidea. *Am. Mus. Novitates* 2941: 60 pp.
- MICKEVICH, M. F. 1982. Transformation series analysis. *Syst. Zool.* 31: 461–478.
- MICKEVICH, M. F. AND S. J. WELLER. 1990. Evolutionary character analysis: tracing character change on a cladogram. *Cladistics* 6: 137–170.
- MIYAMOTO, M. M. 1985. Consensus cladograms and general classifications. *Cladistics* 1: 186–189.
- NEFF, N. A. 1986. A rational basis for a prior character weighting. *Syst. Zool.* 35: 110–123.

- NELSON, G. 1978. Ontogeny, phylogeny, paleontology, and the biogenetic law. *Syst. Zool.* 27: 324–345.
- NELSON, G. 1985. Outgroups and ontogeny. *Cladistics* 1: 29–45.
- NELSON, G. AND N. PLATNICK, 1981. *Systematics and Biogeography*. Columbia University Press, New York.
- O'GRADY, R. T. 1985. Ontogenetic sequences and the phylogenetics of parasitic flatworm life cycles. *Cladistics* 1: 159–170.
- O'GRADY, R. T. AND G. B. DEETS. 1987. Coding multistate characters, with special reference to the use of parasites as characters of their hosts. *Syst. Zool.* 36: 268–279.
- POGUE, M. G. AND M. F. MICEVICH. 1990. Character definitions and character state delineation: the bête noire of phylogenetic inference. *Cladistics* 6: 319–361.
- PREGILL, G. Systematics of the West Indian lizard *Leiocephalus* (Squamata: Iguania: Tropoduridae). *Miscel. Pub. Mus. Nat. Hist. Univ. Kansas*. (In Press.)
- PRESCH, W. 1980. Evolutionary history of the South American microteiid lizards (Teiidae: Gymnophthalminae). *Copeia* 1980: 36–56.
- PRESCH, W. 1988. Phylogenetic relationships of the Scincomorpha. In: R. E. Estes and G. Pregill (eds). *Phylogenetic Relationships of the Lizard Families: Essays Commemorating Charles L. Camp*. Stanford University Press, pp. 471–492.
- RIDLEY, M. 1983. *The Explanations of Organic Diversity*. Oxford University Press, 272 pp.
- ROSEN, D. E. 1982. Do current theories of evolution satisfy the basic requirements of explanation? *Syst. Zool.* 31: 76–85.
- SANDERSON, M. J. AND M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. *Evolution* 43: 1781–1795.
- SCHAEFER, S. A. AND G. V. LAUDER. 1986. Historical transformation of functional design: Evolutionary morphology of feeding mechanisms in loricarioid catfishes. *Syst. Zool.* 35: 489–508.
- SHARKEY, M. J. 1989. A hypothesis independent method of character weighting for cladistic analysis. *Cladistic* 5: 63–68.
- SOKAL, R. 1983. A phylogenetic analysis of the caminalcules. I. The data base. *Syst. Zool.* 32: 159–184.
- SUNDBERG, P. 1989. Phylogeny and cladistic classification of the paramonostiliferous family Plectonemertidae (Phylum Nemertea). *Cladistics* 5: 87–100.
- STANLEY, S. M. 1979. *Macroevolution: Patterns and Process*. Freeman, San Francisco.
- SWOFFORD, D. L. 1985. Documentation for PAUP, version 2.4.1. Illinois Natural History Survey, Urbana.
- WAKE, D. B. 1989. Phylogenetic implications of ontogenetic data. *Geobios, Memoire special No.* 12: 369–375.
- WAKE, D. B. AND G. ROTH. 1989. The linkage between ontogeny and phylogeny in the evolution of complex systems. In: D. B. Wake and G. Roth (eds). *Complex Organismal Functions: Integration and Evolution in Vertebrates*. John Wiley and Sons Ltd, pp. 361–377.
- WATROUS, L. E. AND Q. D. WHEELER. 1981. The outgroup comparison method of character analysis. *Syst. Zool.* 30: 1–11.
- WHEELER, Q. D. 1986. Character weighting and cladistic analysis. *Syst. Zool.* 35: 102–109.
- WHEELER, Q. D. 1990. Ontogeny and character phylogeny. *Cladistics* 6: 225–268.
- WILEY, E. O. AND D. R. BROOKS. 1982. Victims of history—a nonequilibrium approach to evolution. *Syst. Zool.* 31: 1–24.
- YOUNG, D. A. 1981. Are the angiosperms primitively vesselless? *Syst. Bot.* 6: 313–330.
- ZIMMERMANN, W. 1937. Arbeitsweise der botanischen Phylogenetik und anderer Gruppierungswissenschaften. In: Abderhalden (ed.). *Handbuch der Biologischen Arbeitsmethoden*, Abteilung 3.2, Teil 9, pp. 941–1053 (Not seen; cited in Hennig 1966).
- ZIMMERMANN, W. 1940. Die Methoden der Phylogenetik. In: G. Heberer (ed.). *Die Evolution der Organismen*. Jena, pp. 20–56. (Not seen; cited in Hennig, 1966).
- ZIMMERMANN, W. 1953. *Geschichte ihrer Probleme und Erkenntnisse*. Freiburg und Munchen. (Not seen; cited in Hennig, 1966).