2.
Character coding

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2.1 CHARACTER TYPES

Morphological characters can be of two kinds, either discrete (qualitative) or continuous (quantitative). This chapter deals exclusively with the coding of discrete data for qualitative characters. For example, the presence of either two or four stamens in a group of plants is a qualitative character with two discrete states. By comparison, the length of corolla tubes for the same group of plants may vary between 1 cm and 10 cm, with the lengths of individual corollas overlapping within and between individuals of a population and between species. These are continuous variables.

The problem with all characters, but particularly with continuous variables, is the question of whether they are cladistically significant or not, and how they might be coded into discrete states. The latter subject is not considered here, but see Pimentel and Riggins (1987), Cranston and Humphries (1988), Chappill (1989), Felsenstein (1988*a*), and Stevens (1991) for detailed discussions.

2.2 BINARY CHARACTERS

Consider the simple problem of how to code two plant species in which species A has four stamens and species B has two stamens. This information can be coded into simple binary form (Table 2.1), in which the condition in A is represented by 0 and that of B by 1. It is important to note that there is no particular meaning regarding the assignment of 0 or 1 to a particular stamen number, as the coding could easily be reversed, i.e. 0 = 2 stamens and 1 = 4 stamens, and retain the same meaning.

Table 2.1 Binary coding for two taxa for the character stamen number (see text)

Taxa	Character	Code
A	4 stamens	0
B	2 stamens	1

2.3 MULTISTATE CHARACTERS

In a similar example, the number of stamens for five taxa A, B, C, D, and E is 1, 2, 3, 4, and 5, respectively, and this can be coded as a multistate character as shown in Table 2.2. Multistate characters have more than two character states and are coded by integers equalling the number of character states.

2.4 TRANSFORMATION BETWEEN CHARACTER STATES

Three plant species A, B, and C with 2, 3, and 4 stamens, respectively, can be coded as a multistate character (Table 2.3). Characters coded in this way imply that a character can undergo transformation, i.e. the character of stamen number has undergone modification from one state to another. For the example given in Table 2.3, which has three character states, there are nine possible ways in which the transformation of three character states may be related (Fig. 2.1a–c). In an analysis incuding a multistate character with three states it is conceivable to permit all nine transformations, as shown in Fig. 2.1a–c, or limit these nine to fewer options through choices about character order and character polarity, as discussed below.

Table 2.2 Additive coding for five taxa for the multistate character stamen number

Taxa	Character	Code
A	1 stamen	0
B	2 stamens	1
C	3 stamens	2
D	4 stamens	3
E	5 stamens	4

Table 2.3 Additive coding for three taxa for the character stamen number

Taxa	Character	Code
A	2 stamens	0
B	3 stamens	1
C	4 stamens	2

2.5 UNORDERED AND ORDERED CHARACTERS

Consider that the relationships between three character states are known, in the sense that the intermediate step of the transformation can be determined. For example, the gain or loss of stamen number may be viewed as incremental and the gain or loss of two stamens proceeds via the intermediate step of gaining or losing one stamen. In such a case this additional information can be included in the analysis by ordering the character.

If the character is treated as unordered, then any state can transform into any other state with equal cost and any of the nine transformations shown in Fig. 2.1a–c are equally possible.

If treated as an ordered character a choice is made limiting the nine possibilities to a set of three depending upon the determined order. The three possibilities if the order is for incremental stamen gain or loss are shown in Fig. 2.2. The three possible transformations shown in Fig. 2.2 can be represented in one form as shown in Fig. 2.3.

Ordering a multistate character a priori determines the relationship of each character state with every other character state but is silent about the direction of transformation.

2.6 ADDITIVE BINARY CODING

In addition to coding different states within a column, multi-column coding can be utilized for character analysis. Additive binary coding is another way of coding ordered multistate characters, although the actual coding is more tedious and the output can be difficult to interpret (Swofford 1990). The character ordered in Table 2.3 as 0-1-2 can be recoded in additive binary form as 00-01-11 (Table 2.4).
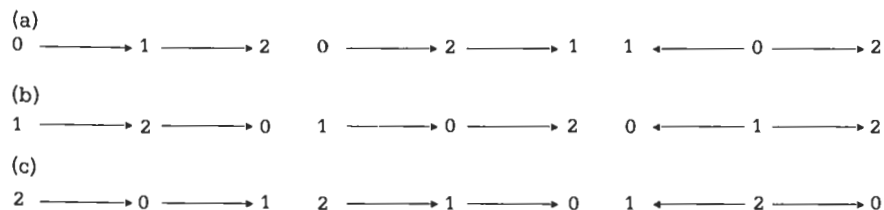
(a)

(b)

(c)

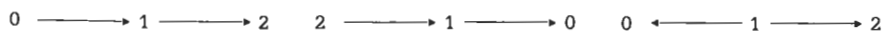**Fig. 2.1** The nine possible transformations among three character states.

Fig. 2.2 Imposition of order reduces the nine possibilities of Fig. 2.1 to a set of 3; the three possibilities if the order is for incremental gain or loss.

2.7 BRANCHED CHARACTER STATE TREES

Although only linear transformed characters have been considered thus far, it is possible to include ordered branched characters in an analysis. If the transformation between four character states was determined a priori to be that shown in Fig. 2.4, this information can be included in an analysis. PAUP v.3.0 allows direct input of 'character state trees' within the user-defined character types option. To be used in Hennig86 the branched character (Fig. 2.4) would have to be recoded in additive binary form (Table 2.5).
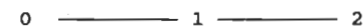
$$0 \text{——} 1 \text{——} 2$$

Fig. 2.3 The three possible transformations from Fig. 2.2 represented in one form.

Table 2.4 Coding multistate characters. For stamen number code 1 is additive, code 2 is additive binary coding

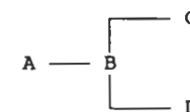| Taxa	Character	Code 1	Code 2
A	2 stamens	0	00
B	3 stamens	1	10
C	4 stamens	2	11

Fig. 2.4 Transformation between four character states determined prior to an analysis; the branched character can be coded in additive binary form (Table 2.5).

Table 2.5 Additive binary coding for the branched character state tree of Fig. 2.4. Taxa are identified as individual character states (A – D)

| Taxa	Code
A	000
B	100
C	110
D	101

2.8 OTHER USER-DEFINED MODELS OF TRANSFORMATION

Although the tendency in cladistic studies has been to minimize assumptions built into analyses, increased levels of sophistication with regard to computer software have led to many 'user-defined' options being available for analysis, especially in PAUP v.3.0. A brief mention of these 'user-defined' options will suffice to introduce two of them. As described in Chapter 1 (Fig. 1.6a,b) certain cladogram topologies could be compatible with equally parsimonious, but conflicting, character transformations. The two alternatives in which a transformation is explained either as a parallel gain or as a synapomorphy with secondary loss are reconsidered here (Fig. 2.5a,b). If, prior to analysis, it is decided that characters should take the form of secondary loss rather than parallel gain, then this hypothesis of character transformation can be included as an integral part of analysis. The Dollo parsimony option in PAUP v.3.0 prohibits parallel gain (Fig. 2.5b) and stipulates that a character must only occur once on the cladogram and therefore homoplasy always takes the form of secondary loss.

The default setting for transformations between character states of a multistate character are treated as having equal cost in terms of the number of steps. A step matrix enables a separate value to be given to each step of a linear or branched multistate character. This practice of weighting some transformations over others is used in molecular systematics for weighting transversions over transitions as the latter occur more frequently (see also Chapters 3 and 7).

2.9 CHARACTER POLARITY

Returning to the example given in Table 2.1, species which have either two or four stamens can be coded as a binary character. In its most general form this coding allows the transformation to proceed in either of two directions, from $0 \rightarrow 1$ or $1 \rightarrow 0$. If the direction of the transformation is determined, then the character is said to be polarized and one or other of the two possibilities would
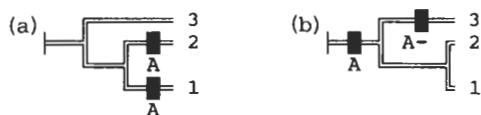
Fig. 2.5 User-defined models of character transformation. The two interpretations are equally parsimonious. (a) Character distribution interpreted as parallel gain. (b) Prohibition of parallel gain; character only occurs once on a cladogram, homoplasy explained as secondary loss.

be chosen for analysis. The determination of character polarity is particularly important for determining plesiomorphic and apomorphic characters.

The polarity of multistate characters determines the direction of transformation but not the order of transformation. Take the example in Fig. 2.3 which shows an ordered multistate character with three states. To polarize this ordered character is to determine the initial starting point of the transformation. Consequently, the ordered character sequence 0, 1, and 2 can be polarized in any of three ways, as shown in Fig. 2.6a–c. The multistate character with three states can be ordered in three ways and each of these can be polarized in three different ways, giving nine possibilities to constrain multistate characters with three states.

It is possible to polarize an unordered multistate character. (Given a binary character $0 - 1$, with a specified direction of transformation, then whether the character is said to be ordered or polarized is a moot point.) Given an unordered multistate character which has three character states $0 - 1 - 2$, it is possible to polarize the character in three ways, by choosing 0, 1, or 2 as the starting point of the transformation, and leaving the other states unordered. This then results in three possibilities for any one polarity decision as shown in Fig. 2.7a–c.
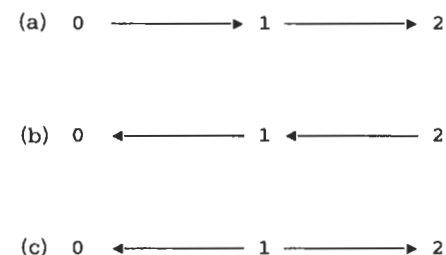
Fig. 2.6 Order and character polarity. The ordered character sequence 0, 1 and 2 may be polarized in any of the following three ways. (a) (0) most plesiomorphic. (b) (2) most plesiomorphic. (c) (1) most plesiomorphic.
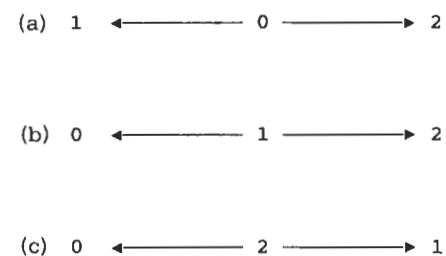
Fig. 2.7 Three possibilities for polarizing an unordered, 3 state character. (a) (0) most plesiomorphic. (b) (1) most plesiomorphic. (c) (2) most plesiomorphic.

2.10 CLADOGRAMS AND ROOTS

The results from analysing a data matrix can either be in the form of an unrooted tree (network) or a rooted tree (cladogram). Figure 2.8a–c shows all three possible unrooted solutions for four taxa. To root an unrooted tree involves imparting polarity onto at least one character transformation, although it is often the case that all or many characters will be polarized. For four taxa there are 15 possible fully resolved cladograms (Fig. 2.9).

The usual method for rooting a cladogram is outgroup comparison (see Chapters 3 and 6). This method involves choosing the sister group (or another closely related taxon) of the study group to root the cladogram. Rooting a cladogram determines the monophyletic groups, reveals paraphyly, and discovers relatively apomorphic and plesiomorphic characters.

By way of an example, Table 2.6 shows a data matrix for four taxa each with four binary characters. The study group is comprised of taxa 2, 3, and 4, with taxon 1 as the sister group. The cladogram shown in Fig. 2.10a is the most parsimonious for these taxa. The choice of root (outgroup), polarizes the characters within the ingroup and determines which states of the binary characters are apomorphic or plesiomorphic. Rooting on zeros also has the effect of grouping taxa solely on the presence of characters.

Figure 2.10a shows that characters A(1), B(1), and C(1) are synapomorphic and D(1) autapomorphic. The cladogram also determines that (3 4) and (2 3 4) are monophyletic groups. Consider that there may be some disagreement about the initial ingroup status of (2 3 4) (ingroups should be monophyletic) and that an alternative interpretation is for (1 2 3) to be monophyletic and 4 the sister group (Fig. 2.10b). This shows that A(0), B(0), and D(0) are synapomorphic and C(0) is autapomorphic. Figure 2.10b also shows that group (1 2 3) is monophyletic. This example illustrates the crucial point that even give the same data matrix the choice of root for the cladogram is of vital importance as it determines the status of cladograms and groups.
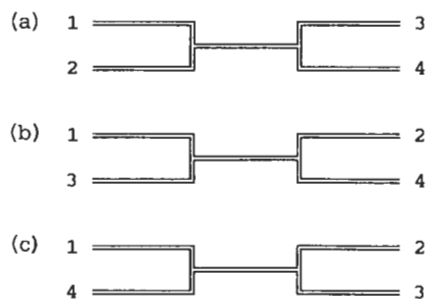

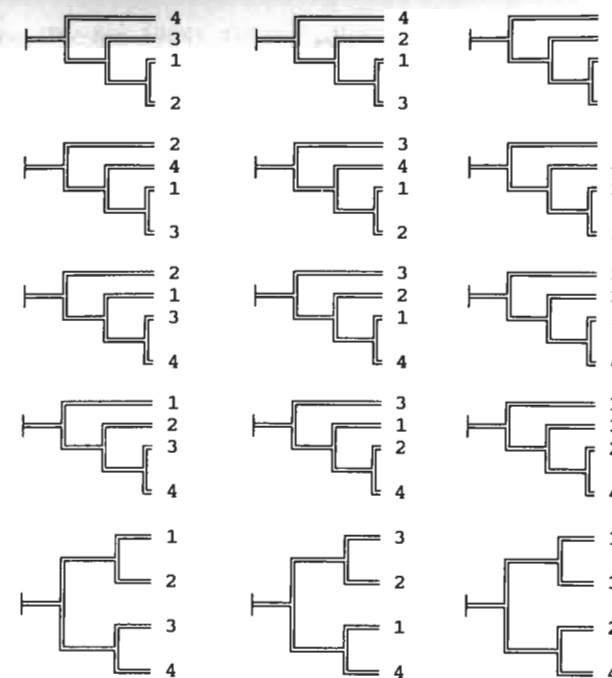Fig. 2.8 All three possible unrooted solutions for four taxa.

Fig. 2.9 All 15 possible fully resolved cladograms for four taxa.

Table 2.6 Binary coding for four taxa (1–4) and four characters (A–D); data for Fig. 2.10

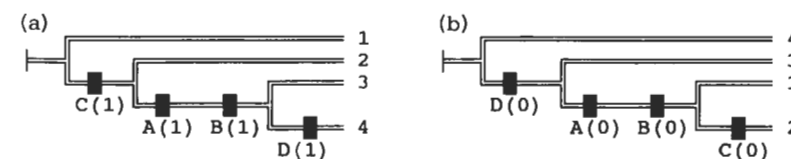| Taxa	ABCD
1	0000
2	0010
3	1110
4	1111

Fig. 2.10 Rooting, apomorphy and monophyly. (a) The most parsimonious tree taking taxon 1 as the root; characters A(1), B(1) and C(1) are synapomorphic and D(1) autapomorphic. (b) An alternative interpretation rooted on taxon 4: taxon (1 2 3) is monophyletic; and characters A(0), B(0) and D(0) are synapomorphic and C(0) is autapomorphic.

3.
The determination of character polarity

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3.1 INTRODUCTION

Swofford (1990) distinguished three properties of characters: direction, order, and polarity. Unlike the definition of Meacham (1984) in which directed characters are equivalent to polarized characters, Swofford's definition of direction refers to 'cost' in terms of tree length of a change between any two character states. An undirected character is one in which the costs are symmetrical; that is, the increase in tree length required by the transformation of state X to state Y is the same as that required by the change from Y to X. An example of a directed character is one that is optimized under the Camin–Sokal parsimony criterion, where reversals to a more plesiomorphic condition are not permitted. Step matrix characters (Swofford 1990) are directed if the step matrix is asymmetrical (see also Chapter 4). Character order specifies the type of the permitted character state transformations and has already been introduced in Chapter 2.

Polarity refers to the direction of character evolution. A character is said to be polarized if the state ancestral to all other states is prespecified. Many methods and criteria for assessing the evolutionary polarity of characters have been proposed, and have been reviewed by Crisci and Steussy (1980), de Jong (1980), Stevens (1980), and Arnold (1981). Of these, most can be reduced to variations of three main themes: the outgroup comparison (the indirect method), the ontogenetic criterion (the direct method), and criteria based on specific models.

Nelson (1973a) divided the criteria used to estimate ancestral character states into two approaches. Indirect arguments (the indirect method) involve taxa other than those of the study group and rely upon a pre-existing higher-level phylogeny to establish character polarity. Farris *et al.* (1970) demonstrated the fundamental importance of parsimony to outgroup comparison, which was therefore considered by Nelson (1978) to be the only valid indirect method. However, the higher-level phylogeny itself must be based upon yet more polarized characters, leading to an infinite regress. Eventually, a method independent of pre-existing phylogenetic hypotheses must be invoked in order to validate the outgroup

comparisons (Weston 1988). Of such direct arguments (the direct method), only ontogenetic character precedence was considered to be valid by Nelson (1973a).

3.2 OUTGROUP COMPARISON—THE INDIRECT METHOD

In its simplest form, outgroup comparison has been defined by Watrous and Wheeler (1981, p. 5) as:

For a given character with 2 or more states within a group, the state occurring in related groups is assumed to be the plesiomorphic state.

Watrous and Wheeler (1981) then gave a series of operational rules for a procedure they called the functional ingroup/functional outgroup (FIG/FOG) method. An initial hypothesis of relationships of outgroups and an unresolved ingroup is chosen. Characters are then selected and polarized using outgroup comparison, allowing partial resolution of the ingroup. Functional outgroups and functional ingroups are then established, which then permit further resolution. The procedure is repeated until either the ingroup is fully resolved or no further resolution can be achieved.

The following example, adapted from Mooi (1989), is based on the hypothetical electrophoretic allele data of Swofford and Berlocher (1987) (Table 3.1) and is optimized using the Fitch (1971) parsimony criterion (see also Chapter 4). Taxon F of the ingroup is established as the functional outgroup to the remaining ingroup taxa, A–E (Fig. 3.1a), by the presence in F and the outgroup, G, of allele 1c. Clade A–E is thus characterized by either allele 1a or 1b, but at this point in the analysis, a decision cannot be made between them.

Table 3.1 Hypothetical allelic data for seven taxa. (Adapted from Swofford and Berlocher 1987)

Taxa	Characters			
	1	2	3	4
A	b	c	a	c
B	b	c	a	c
C	b	c	b	a
D	a	b	b	a
E	a	b	b	a
F	c	d	a	a
G	c	a	c	b

Furthermore, because the alleles present in G for characters 2–4 may be autapomorphic for G or plesiomorphic for all taxa, polarity determination for these must wait.

The second iteration uses F as the FOG to A–E, ignoring the outgroup G. Alleles 4c and 3b are seen to be synapomorphic for taxa A + B and C + D + E respectively (Fig. 3.1b). Note that no decision can yet be made regarding the ambiguous status of alleles 1a and 1b.

The third iteration uses taxa A and B as the FOG to C + D + E, resulting in D + E being recognized as a clade, based upon alleles 1a and 2b. The initial ambiguity is also resolved: allele 1b is interpreted as synapomorphic for taxa A–E, with a subsequent transformation to allele 1a in taxon D + E (Fig. 3.1c). The final cladogram (Fig. 3.1d) shows one possible maximally parsimonious reconstruction (MPR; see also Chapter 4), in which alleles 1c, 2d, 3a, and 4a are interpreted as plesiomorphic for the ingroup + outgroup. Other MPRs exist; for example, alleles 2a, 3c, and 4b may be treated as plesiomorphic, whence alleles 2d, 3a, and 4a will unite taxon F with the remaining ingroup taxa as a monophyletic group.
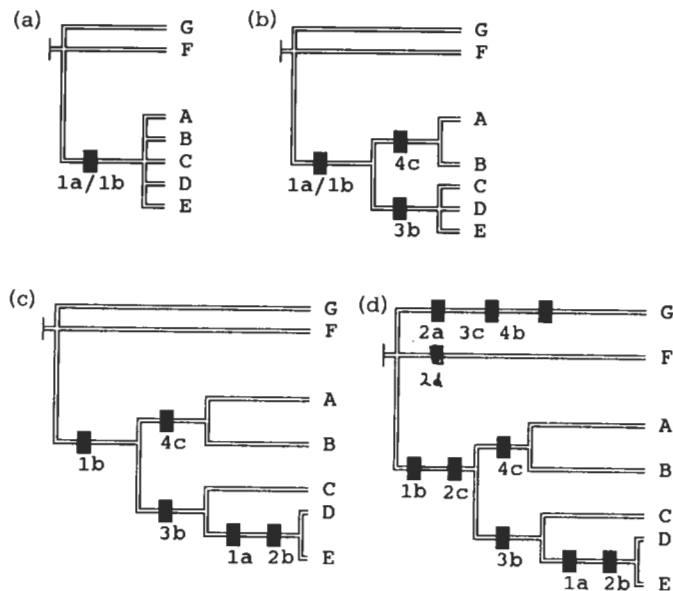

Fig. 3.1 Functional ingroup–outgroup polarization. (a) Allele 1c establishes F as the functional outgroup to the functional ingroup, A–E, which is characterized by either allele 1a or 1b. (b) With F as the functional outgroup, alleles 3b and 4c characterize (C + D + E) and (A + B) respectively. (c) Using A + B as the functional outgroup of C + D + E, D + E is recognized as a clade based on alleles 1a and 2b. Allele 1b is then seen to characterize (A–E). (d) Final resolution.

While Watrous and Wheeler's (1981) formulation is adequate when there is no variation in state within the outgroups, there are severe difficulties in applying this rule consistently when the outgroup taxa are heterogeneous. A number of specific examples of such outgroup variation have been examined by Arnold (1981) and Farris (1982a), and a comprehensive overview is provided by Maddison *et al.* (1984).

Maddison *et al.* (1984) presented a general algorithm and principles to enable the most parsimonious hypothesis of an ancestral state to be estimated given fully resolved and fixed outgroup interrelationships, and then discussed the effects of uncertainty among those relationships. Such examination of outgroups ensures that the ingroup cladograms are globally parsimonious. If the ingroup alone is studied, as in the the procedure of commonality (see below), then the chosen ingroup cladogram will only be locally parsimonious. Failure to achieve global parsimony may also result if outgroup analysis is taken to indicate the state present in the most recent common ancestor of the ingroup (for example Wiley 1981) or if the ingroup is first resolved in isolation as an unrooted tree without reference to ancestral states and the outgroup subsequently attached, as in the procedure of 'Lundberg rooting' (Lundberg 1972; Swofford 1990). Similarly, if the state occurring most commonly among the outgroups is assumed to be plesiomorphic (Arnold 1981), then non-globally parsimonious solutions may result, depending upon the precise distribution of the character state and the relationships of the outgroup taxa.

Several terms and conventions were defined by Maddison *et al.* (1984) to assist their general discussion. The most recent common ancestor of the ingroup taxa is called the ingroup node, while the next most distal node, which links the ingroup to the first outgroup is the outgroup node (Fig. 3.2). Furthermore, they assumed that the outgroup interrelationships were known and immutable.

The method of Maddison *et al.* (1984) aims to estimate the character state of the outgroup node. Such an assignment may be either 'decisive', if it can have only a single value, or 'equivocal' if it can have more than one equally parsimonious alternative state. They also noted that their results hold whether cladograms are interpreted as indicating recency of common ancestry (Nelson 1973c) or a pattern of nested sets of characters (Nelson and Platnick 1981).
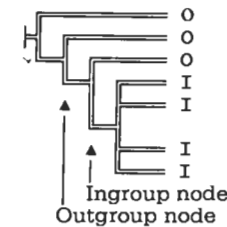
Fig. 3.2 Illustration of ingroup and outgroup nodes of Maddison *et al.* (1984). I = ingroup taxon, O = outgroup taxon.

Simple examples can be determined by visual inspection. For example, if there is only one outgroup with state x, it is more parsimonious to assign state x to the outgroup node than to assign some other state (Fig. 3.3a; but see below). Such an assignment is decisive. If the first two outgroups (Fig. 3.3b) differ in their states, then the assignment to the outgroup node is equivocal. Additional, more distal, outgroups with the same state as the second outgroup exert no influence, demonstrating that the assignment is not simply a function of the relative frequency of the states within the outgroups (but see Arnold 1981).

However, such visual inspections may fail to achieve the most parsimonious assignment in more complex cases of heterogeneous outgroups and an algorithmic approach is necessary. Maddison *et al.* (1984) adapted the methods used in Wagner and Fitch optimization to outgroup analysis. These methods are discussed in greater detail in Chapter 4 and only the outgroup comparison using binary (x/y) characters will be considered here.

A general algorithm for multistate characters was given by Maddison *et al.* (1984). First, the terminal outgroup taxa are labelled with their observed states, x or y (Fig. 3.4). Polymorphic outgroups, if any, are labelled xy. Then, beginning with pairs of terminal outgroups and proceeding towards the outgroup node, the internal nodes are labelled according to the following rules:

1. If the derivative nodes are both labelled x, or are x and xy, the ancestral node is labelled x.

Fig. 3.3 Outgroups and character state assignment at the outgroup node. (a) If there is only a single outgroup, then the assignment of the character state of the outgroup to the outgroup node is decisive. (b) If there are multiple outgroups, then if the first two outgroups disagree in their state assignments, the character state assignment at the outgroup node is equivocal.

2. If the derivative nodes are both labelled y, or are y and xy, the ancestral node is labelled y.

3. If the derivative nodes are x and y, or both xy, the ancestral node is labelled xy.

Ignoring the root and proceeding towards the outgroup node simplifies the procedure by negating the need for a preorder traversal of the tree in order to determine the MPR (see also Chapter 4).

Two simple rules follow from this procedure that allow for quick and accurate ancestral character assessment.

1. *The first doublet rule* (Fig. 3.5a,b). If the first outgroup and the first doublet (a pair of consecutive outgroups that agree in a state) share the same state, then that state is the decisive maximally parsimonious reconstruction (MPR). If they disagree, then the decision is equivocal. Furthermore, if the first two outgroups form a doublet, then their state is the MPR. A corollary to this rule is that all outgroup structure beyond the first doublet is irrelevant to the assessment.

2. *The alternating outgroup rule* (Fig. 3.6a,b). If there are no doublets, then if the first and last outgroups agree, this state is the decisive MPR; otherwise the decision is equivocal. These rules are applied with no regard for the distribution or composition of the ingroup character states. For example, if states x and y are found in the ingroup, and states x and z in the outgroup, both de Jong (1980) and Watrous and Wheeler (1981) suggested that state x is the ancestral state for the ingroup, because this is the only state to occur in both ingroup and outgroup. However, Farris (1982a) demonstrated that ignoring state z in this way may sometimes lead to a non-parsimonious result.
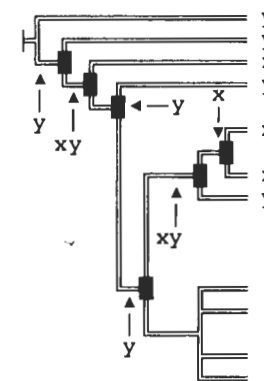

Fig. 3.4 Illustration of the algorithmic approach of assigning a character state to the outgroup node in a cladogram with heterogeneous outgroup terminals (Maddison *et al.* 1984).

Whether z, x or xz is the most parsimonious reconstruction will depend upon the outgroup relationships (Maddison *et al.* 1984).

A problem arises in the situation in which only one outgroup is used (Fig. 3.3a), for there is no outgroup node for which an MPR can be assessed (the apparent root of the tree is ignored when the general algorithm is applied). Maddison *et al.* (1984) implicitly adopted the convention that the single outgroup itself forms the outgroup node, and thus whatever state occurs in that outgroup is automatically considered to be plesiomorphic. But this convention is properly valid only if the tree is drawn as having no subtending basal branch. If the tree is so rooted (Fig. 3.7a), and the ingroup resolved (Fig. 3.7b), then
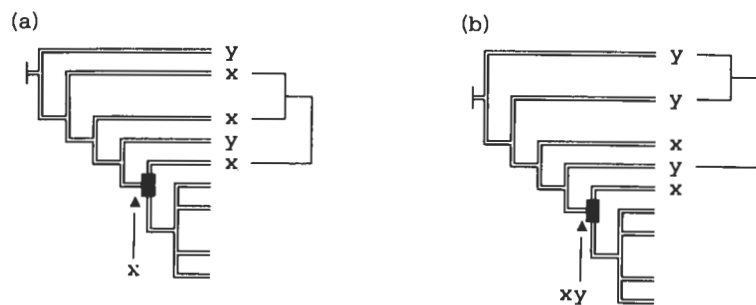

Fig. 3.5 Illustration of the first doublet rule for binary characters (see text). (a) If the character state of the first outgroup agrees with that of the first doublet, the character state assignment at the outgroup node is decisive. (b) If the character state of the first outgroup disagrees with that of the first doublet, the character state at the outgroup node is equivocal.
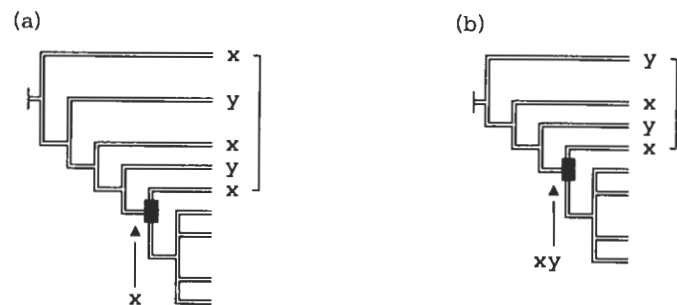

Fig. 3.6 Illustration of the alternating outgroup rule for binary characters. (a) If the character states of the first and last outgroups agree, the character state assignment at the outgroup node is decisive. (b) If the character states of the first and last outgroups disagree, the character state assignment at the outgroup node is equivocal.

a hidden assumption is involved regarding the state present at the base of the tree, which is that this state agrees with that in the sole outgroup (Fig. 3.7c). Then, by the first doublet rule, the state at the outgroup node is a decisive x, and y can be interpreted as apomorphic within the ingroup. Usually, however, no evidence is provided to support this assumption (for example Humphries and Funk 1984, fig. 5). There is an alternative assumption, which is that the base of the tree possesses state y (Fig. 3.8a). If so, then the outgroup node state is equivocal and y cannot be decisively assessed as apomorphic (Fig. 3.8b,c). Now it is true that the tree in Fig. 3.7c is more parsimonious than either of those in Figs. 3.8b and 3.8c, and could thus be chosen as the preferred interpretation on that basis alone. However, in order to avoid the hidden assumption, it is recommended that at least two outgroups be used in an analysis employing outgroup comparison.

The algorithm of Maddison *et al.* (1984) produces the globally most parsimonious ingroup cladograms, even though the ingroup character state distributions are not taken into account. The procedure is sufficient providing the sole aim is to resolve the ingroup relationships. However, Maddison *et al.* (1984)
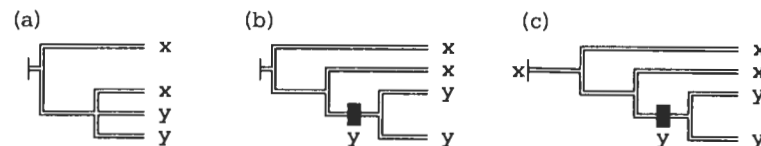
Fig. 3.7 Character state assignments for binary characters at the outgroup node for cladograms with a single outgroup. (a) Rooted cladogram with a single outgroup and character states assigned to terminals. (b) Cladogram of Fig. 3.1a with the ingroup resolved. (c) However, the resolution in Fig. 3.1b assumes that the state at the base of the cladogram is the same as that of the outgroup, hence the first doublet rule applies.
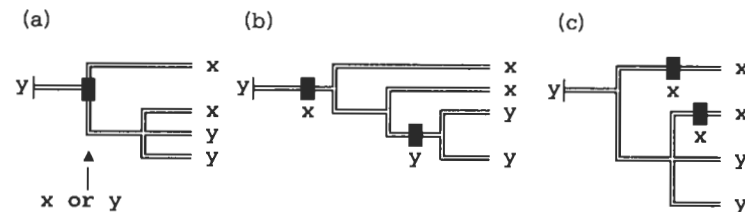
Fig. 3.8 Character state assignments for binary characters at the outgroup node for cladograms with a single outgroup. (a) Rooted cladogram with a single outgroup and assuming y is the basal state. Assignment at the outgroup node is equivocal (see text). (b) One possible MPR given the conditions in (a). (c) Alternative MPR given the conditions in (a).

noted that if the ancestral state assignments are required for other purposes, such as the evolutionary modelling of character transformation, then the ingroup should be resolved as far as possible first and then either Wagner or Fitch optimization applied to the entire tree.

Problems arise in using the algorithm when the outgroup relationships are uncertain or are not prespecified. Clearly, if all the outgroups agree in state, then their interrelationships are irrelevant. However, if they differ, then uncertainty in outgroup relationships can lead to uncertainty about the ancestral state. The algorithm could be applied to all possible outgroup resolutions, but this would be remarkably tedious for more than a very small number of outgroups. Maddison *et al.* (1984) gave six rules derived from the outgroup criterion that could be used to indicate the degree to which differing outgroup relationships may yield the same MPR. These rules describe situations in which outgroup uncertainties have no or limited effect. ('Limited' is defined as not allowing changes that will completely shift an assessment; that is (for a binary character), assessments can change from decisive to equivocal (for example x to xy, or xy to y) but not from decisive to decisive (x to y). For multistate characters, the alternative assessments must overlap, that is, they must both contain at least one state in common.)

1. Uncertainties beyond the first doublet have no effect.

2. If the root is moved within the outgroups, there is no effect on the MPR.

3. If the first outgroup or the basal node of the first subgroup of outgroups has one state, then the MPR is either decisive or equivocal for that state. The first outgroup can thus be seen to exert a considerable influence on the MPR. However, even though this taxon may be highly derived, there is no justification in appealing to more distant but supposedly more primitive outgroups.

4. The addition or deletion of one outgroup cannot completely shift an MPR; at least two additions or deletions are necessary to accomplish this. However, even the addition of a distant outgroup can affect the MPR (cf. Figs. 3.6a and 3.6b).

5. Similarly, moving one outgroup cannot completely shift an MPR. Maddison *et al.* (1984) demonstrated that this rule held for binary characters but were unable to prove or disprove it for multistate characters in general.

6. When only one of a number of outgroups possesses a particular state, the MPR cannot be decisive for that state whatever the outgroup relationships.

When there are alternative ancestral state assignments depending upon alternative outgroup resolutions, there are a number of means by which a cladistic analysis can proceed. The optimum approach would be first to conduct a higher level analysis to resolve the outgroup relationships fully. However, this rapidly leads to an infinite regression of ever higher-level analyses. In the absence of such analyses, the assessment of ancestral states could be determined under a more restrictive parsimony model (for example Dollo parsimony). Alternatively, an appeal could be made to the 'predominant states method', i.e. outgroup commonality (Arnold 1981). However, the last approach is not philosophically justified by a direct parsimony argument and may give non-globally parsimonious cladograms.

If full resolution of outgroup relationships is not feasible, then even partial resolution may reduce the number of uncertain MPRs. It may then be practicable to examine the influence of all outgroups on the MPR, both singly and in all allowable combinations (the outgroup substitution approach of Donoghue and Cantino 1984). The various ingroup resolutions could then be examined for areas of congruence using the strict consensus tree technique. Clades present in the consensus tree would be those unaffected by uncertainty in outgroup relationships. The ingroup could then be further resolved using the functional outgroup/ingroup (FIG/FOG) technique (Watrous and Wheeler 1981). Maddison *et al.* (1984) suggested resolving the ingroup cladogram using each of the possible ancestral states and selecting those cladograms that were most parsimonious according to the outgroups, ingroups or both.

The above technique can be characterized as a constrained, two-step analysis, in which the MPR of the outgroup node is first assessed, then followed by resolution of the ingroup. Clark and Curran (1986) argued that unconstrained, simultaneous analysis is superior. In this procedure, the most parsimonious cladogram for both outgroups and ingroups is estimated in one step, with no constraints placed upon the permitted resolution of either. Clark and Curran (1986) identified two a priori assumptions required by the constrained analysis of Maddison *et al.* (1984).

1. The ingroup is monophyletic, which implies that the root is basal to it.

2. Outgroup structure implies hypotheses of monophyly that are not open to testing because they are treated as immutable.

Problems can thus arise for characters that show parallel homoplasy between one or more outgroups and a subset of the ingroup. In Fig. 3.9a, where A–C are the ingroup and D–F the outgroups, character state 4 is synapomorphic for the group, A–E. A subsequent transformation to 4– characterizes the clade A + B. If two further characters are found with the same distribution as character 4, then either the outgroup formulation of Watrous and Wheeler (1981) or the general constrained algorithm of Maddison *et al.* (1984) will yield a most parsimonious tree of nine steps (Fig. 3.9b). However, if all six characters are analysed without topological constraints, then a very different, and shorter, cladogram of eight steps is found (Fig. 3.9c), in which the ingroup is not monophyletic. The lack of global parsimony in Fig. 3.9b is due to the requirement of immutability of outgroup relationships.

Clark and Curran (1986) and Farris (1982*a*) noted that a simultaneous, unconstrained analysis would never give a less parsimonious result than a two-step,

constrained analysis. The method is fully consistent with Watrous and Wheeler's (1981) 'operational rule' for the outgroup criterion. Furthermore, it can be used where the outgroup relationships are unresolved; indeed, it may help to resolve them. Therefore, because simultaneous analysis makes the fewest a priori assumptions, it is the parsimony method of choice using outgroup comparison.

3.3 ONTOGENY—THE DIRECT METHOD

Within cladistics, there are two contrasting viewpoints regarding the role of ontogenetic information (Eldredge 1979; Williams *et al*. 1990). The transformational approach, which corresponds closely to Hennig's (1966) phylogenetic systematics, is performed in three stages (Weston 1988): character transformation series are formulated, which are then polarized and used to
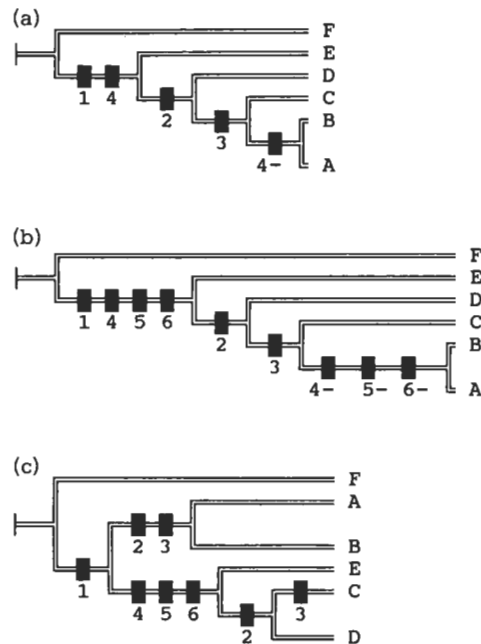
Fig. 3.9 Outgroup-constrained and outgroup-unconstrained analysis. Taxa A–C comprise the ingroup and D–F the outgroups. (a) Cladogram with characters assigned to nodes and outgroup topology predetermined and constrained. (b) Addition of two further characters (5 and 6) with the same state distribution as character 4 gives a cladogram of 9 steps with the same topology as that in Fig. 3.9a. (c) However, if the constraint on outgroup relationships is removed, a shorter cladogram of eight steps is possible in which the ingroup is not monophyletic.

construct cladograms. This contrasts with the taxic approach, considered to be embodied within pattern cladistics (Beatty 1982), which uses the distribution of homologies to hypothesize group membership. Here, character polarity is derived from the analysis rather than being an a priori assumption (Nelson and Platnick 1981; Patterson 1982*a*; Nelson 1985). A comparison of these two approaches raises the question of whether it is at all possible to determine character polarity prior to analysis.

Nelson (1978) generalized his direct argument thus:

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 character is advanced.

He explained it using the following example (Fig. 3.10a). Suppose there are two taxa, A and B, possessing characters x and y respectively. With this information alone, no decision can be made regarding which character is the more primitive. However, a study of the ontogeny of the two species reveals that embryos of both species have character x but that during the subsequent development of species B, character x transforms into character y. In other words, x is observed to be more general and y to be less general. Character x is therefore inferred to be plesiomorphic and character y apomorphic. Both Nelson and Platnick (1981) and Patterson (1982*a*) considered that this reformulation of the biogenetic law was the decisive criterion in determining character polarity.

However, Lundberg (1973) argued that if the ontogenetic transformation is treated as the character, then both cladograms are equally parsimonious (Fig. 3.10b). The essence of his argument is as follows: if the transformation of x → y
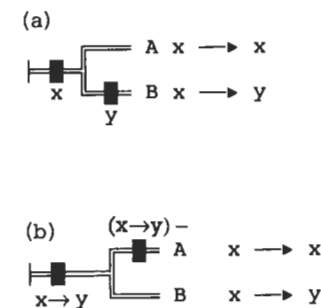
Fig. 3.10 (a) Nelson's (1973b) example of polarization using the direct ontogenetic criterion; state x, occurring in the ontogeny of both species, is the more general state and is therefore plesiomorphic. (b) Lundberg's (1973) counter-example. The gain of the transformation of x→y is regarded as a single step at the base of the cladogram, rather than two steps (gain of x, followed by gain of y). The subsequent loss of this transformation in taxon A gives a cladogram of two steps that is equally parsimonious as the cladogram in Fig. 3.10a.