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# Wood ontogeny as a model for studying heterochrony, with an example of paedomorphosis in *Moringa* (Moringaceae)

**Abstract** Modern studies of heterochrony in plants have focused mostly on determinate organs or annuals, leaving long-lived plants unstudied. Wood offers remarkable access to the issue, because ontogeny during the life of a woody plant is recorded by changes in cell attributes from the inner wood to the outermost wood. An example is provided, examining vessel element ontogeny in an explicitly phylogenetic context to infer paedomorphosis in the evolution of tuberous shrubs, represented by *Moringa longituba* (Moringaceae, the drumstick tree family), from an arborescent ancestral-type ontogeny represented by sister taxon *M. ruspoliana*. Two main difficulties with the use of wood in heterochrony studies are identified and their implications are discussed: (1) Some variable must be chosen against which to plot ontogenetic data; time, either observed directly or inferred from growth rings can be used, but stem diameter may be an acceptable or more informative alternative; (2) 'Onset' and 'terminal' ontogenetic reference points are required by the prevailing paradigm for studying heterochrony. However, in most cases it will be impossible to detect differences in the onset of wood ontogeny that lead to differences in mature morphologies. Likewise, it may often be impossible to identify a terminal point (e.g. sexual maturity, the traditional zoological reference point, is usually not associated with cessation of major ontogenetic changes in woody plants). As a result, distinguishing between subcategories of heterochrony (neoteny, progenesis, etc.) is usually impossible. Nevertheless, it should usually be possible to distinguish between the major categories (paedomorphosis and peramorphosis), or to reject an hypothesis of heterochrony. Finally, issues such as the sensitivity of ancestral character state reconstructions to branch lengths and coding of terminal taxa, and opportunities for incorporating intraspecific variability into studies of wood ontogenetic evolution are shown.

**Key words** Evolution, heterochrony, *Moringa*, Moringaceae, neoteny, ontogeny, paedomorphosis, peramorphosis, phylogeny, wood anatomy

## Introduction

Plant shape and size vary over orders of magnitude, with the tiniest plants being a hundred thousand times smaller than the largest. Heterochrony – evolutionary change in the timing of developmental events – has been a major theme in explaining the evolution of this diversity at least as early as the late 1800s, with speculation on the origin of *Welwitschia* as an 'arrested sprout', (see Martens, 1977; Takhtajan, 1954, 1972). The conceptual appeal of heterochrony has resided in its power to account for even very drastic, discontinuous evolutionary changes by simple alterations in the timing of existing developmental patterns, without the need to invoke the evolution of novel structures (Gould, 1977; Müller & Wagner, 1991). Paedomorphosis – one kind of heterochrony – is a term familiar to most wood anatomists, and its use by them can be traced to Carlquist (1962). He noted that woods in many smaller life forms display features well into adulthood that are found only in the innermost xylem of more typical woody plants. Following the terminology of de Beer (1930; see also 1958), he termed such woods as showing juvenilization or paedomorphosis. The approach described here attempts to integrate this tradition of

ontogenetic thinking with explicit hypotheses of heterochrony tested in a phylogenetic context. It is suggested that wood offers features that help resolve some of the shortcomings of botanical studies of heterochrony to date. It is also argued that the special nature of wood ontogeny, which unfolds via the addition of concentric layers of cells, allows use of schemes for inferring heterochrony that build on studies of allometry (e.g. Gould, 1977; Alberch *et al*., 1979), as well as those that focus on the relative order of qualitative ontogenetic events (reviewed by Smith, 2004). Likewise, sampling schemes well-known to wood anatomists can be readily adapted to study heterochrony. One possible sampling scheme is illustrated with an example from the wood of members of the tuberous clade of *Moringa* (Moringaceae, the drumstick tree family).

The Gould–Alberch model (Alberch *et al*., 1979; 'GA model'; terminology of Guerrant, 1988) has served as a point of reference for studies of heterochrony in the last 25 years. Many studies have employed this framework, and the methods that have been proposed subsequently have largely been responses to its limitations. The GA model consists of quantifying changes in size and shape in relation to time and comparing the observed patterns in ancestors and descendants. Using four factors – the beginning of ontogeny, the end of ontogeny (indicated by sexual maturity), changes in size and shape of morphological features, and the rate of change in these attributes – the GA model distinguishes two major categories of heterochronic mechanisms that can affect parts of an organism, paedomorphosis and peramorphosis. Both have three subcategories each (based on Alberch *et al.,* 1979; Raff & Wray, 1989), outlined below.

*Heterochronic processes resulting in paedomorphosis*. A paedomorphic adult resembles the juveniles of its ancestor. Such a pattern is inferred to mean that the descendant species that manifest paedomorphosis were derived via an evolutionary 'stopping off' along an ancestral ontogeny. As a result, the ancestor's ontogeny appears to progress 'beyond' that of the descendant. Subcategories: (1) Neoteny: Sexual maturity occurs at the same time relative to an ancestor, while the somatic development of the descendant is slowed. Neotenic individuals can often be as large as the ancestral species, but of juvenile shape. In such cases, growth in size occurs at the same rate, with change in shape retarded in the descendant. (2) Progenesis: In this case, the ancestor and descendant species develop somatically at the same rate, but the descendant reaches sexual maturity earlier than the ancestor. Progenetic species are often smaller than their ancestors at maturity, and their shorter generation time may be associated with ephemeral habitats where rapid reproduction would be selectively favoured. (3) Postdisplacement: Both ancestor and descendant share the same growth rate and point of sexual maturation, but the ontogeny in the descendant species has a later time of onset.

*Heterochronic processes resulting in peramorphosis* A peramorphic ontogeny progresses 'beyond' that of an ancestor's. Haeckel's truism applies in such cases in that the descendant-type species 'recapitulates' the ontogeny of the ancestor and then exhibits additional ontogenetic stages (e.g. Gould, 1977). Subcategories: (1) Acceleration: Sexual maturity occurs at the same time relative to an ancestor, while the rate of somatic development of the descendant surpasses that of the ancestor. (2) Hypermorphosis: In this case, the ancestor and descendant species develop somatically at the same rate, but the descendant reaches sexual maturity later than the ancestor, also surpassing it in size, shape, or both. (3) Predisplacement: Both ancestor and descendant share the same growth rate and point of sexual maturation, but the ontogeny in the descendant species has an earlier time of onset.

Although the GA categorizations do map clear theoretical possibilities, the model can be too restrictive for some studies for a variety of reasons. First, it is clear that its categories do not exhaust the possibilities for heterochronic change. Likewise, the GA model reduces the definition of heterochrony to differences between species detected via comparative allometry, whereas many workers are interested in studying the change in relative occurrence of developmental events. These differences in developmental sequences between species, one of the original meanings of the term heterochrony (de Beer, 1958), are not explicitly addressed by the GA model. Additionally, for many studies, the use of absolute time as an X axis against which to plot ontogenetic data and the need for clearly identifiable ontogenetic beginning and ending points have proven difficult criteria to meet for many studies (Raff & Wray, 1989). Adding to these problems, other authors (e.g. Zelditch *et al*., 2004) have noted that the separation of 'size' and 'shape' is not always straightforward, as is implied by the GA model. As a result, some disillusion has been expressed that GA model based heterochrony may not explain as much of morphological evolutionary change as it had originally been hailed to (e.g. Raff, 1996; Zelditch, 2001). Nevertheless, even if the GA model is not able to depict some types of heterochrony, its general approach remains useful, given that changes in relative growth have certainly played a major role in the diversification of the woody plants and thus represent a fertile field for investigation.

Another approach, used in explicit phylogenetic contexts by authors such as Mabbee (1993) and Prochel *et al*. (2004), involves comparing sequences of usually qualitative developmental events, e.g. sequences of ossification defined by the order in which different bones are initiated and develop (see reviews and discussions of Smith, 2003; Schulmeister & Wheeler, 2004). Differences between species are detected via comparisons of ontogeny between pairs of species, or by optimization of ontogenetic sequences on cladograms in an effort to identify nodes associated with heterochronic changes. This method is a direct response to the GA model, which places emphasis on relative growth measured with respect to absolute time. In many instances, measures of absolute time are not available or are not informative for a given study. For example, Raff & Wray (1989) note that important differences in timing of early animal embryonic development may be a matter of hours; such differences are difficult to detect if the point of reference is sexual maturity. However, a relative sequence of developmental events can always be recovered from any ontogenetic study, e.g. vessel pitting spiral → vessel pitting opposite → vessel pitting alternate, even if no measure of time and no specific initial and terminal ontogenetic reference

points are available. Differences in the order or extent of such sequences between species can thus be used to infer at what point in a given phylogeny the developmental shift occurred. This sequence-based approach shows a strong tendency to avoid the terminology of the GA model, in part because it is often inapplicable in many situations, but more significantly because of the consensus that identifying categories is less important than documenting patterns, elucidating the mechanisms that cause them, and identifying the selective forces that have favoured evolutionary change.

The nature of its ontogeny makes wood amenable for studies of heterochrony using a variety of approaches. The methods emphasized here are largely GA-inspired because they provide an explicit method for identifying paedomorphosis, thereby complementing the concept in current use by wood anatomists (Carlquist, 1962, 2001). Because wood is accreted in concentric layers, ontogenetic changes in cell characteristics can be surveyed from cells produced when the tree was young, in the centre of the stem, to the 'adult' cells in the outermost layers. This property permits unequivocal ordering of ontogenetic events across the life of a tree, and would make wood ontogeny easily studied using sequence-based methods. Likewise, differences in size or shape of cells throughout the life of a tree can be surveyed with GA-like methods. Sampling schemes can be devised in which known ages are associated with specific ontogenetic stages, and age can even be inferred from wild trees with growth rings. In the example provided below from *Moringa*, it is argued that measures that reflect how many concentric layers of cells have accumulated may provide better measure of developmental stage than does absolute age. The methods proposed here thus incorporate elements of both the GA and sequence-based models, because ontogenetic changes are associated not with absolute time but their relative position in an ontogenetic sequence. Although the usefulness of wood as a system for recovering ontogenetic patterns (Carlquist, 2001) has been known to wood anatomists for generations, heterochrony studies in plants have yet to incorporate this tradition.

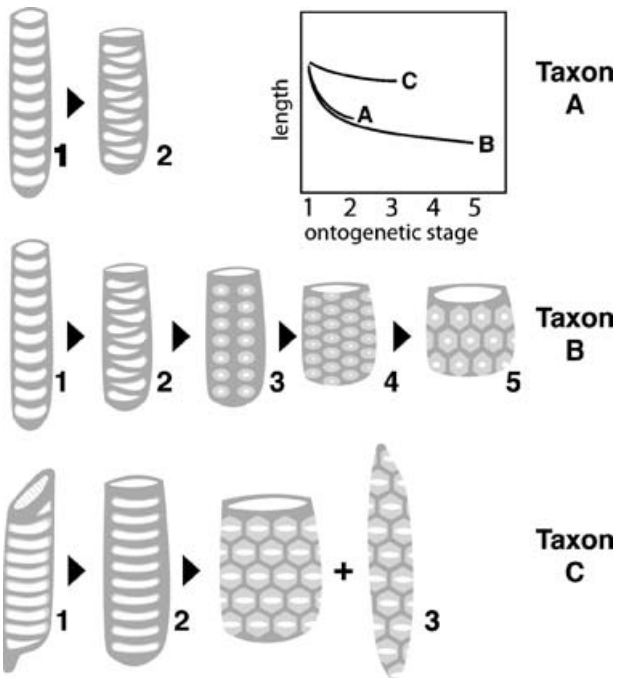


**Figure 1** Similarity in vessel element ontogenetic patterns and hypotheses of heterochrony. Ontogeny in taxon A is identical to the early stages of taxon B in cell dimensions (graph) and pit shape and arrangement, suggesting heterochrony. Which category of processes is inferred – paedomorphosis or peramorphosis – depends on the inference of which ontogenetic pattern represents the ancestral type, as determined from phylogeny reconstruction. If a taxon B-type ontogeny is inferred to be ancestral, then taxon A is inferred to have evolved via paedomorphosis. Taxon C shares no ontogenetic pattern with any other taxa, so explanations other than heterochrony must be invoked to explain these evolutionary transformations.

## Levels of plant ontogeny; phylogeny

Studying ontogeny in long-lived plants presents serious challenges. Jones (1992) noted that botanical studies can focus on 'whole plant' or 'organ level' ontogeny. 'Whole plant' ontogeny examines differences between mature metamers, for example the patterns of change in mature leaf form along a stem. Examples of such studies include Jones (1992), who studied heterochrony at the whole plant level by examining differences between mature leaves along the stems of a squash cultivar and its wild ancestor, both annuals. Likewise, Wiltshire *et al.* (1994) document mutants that cause differences at the whole plant level in peas. In addition to the whole plant level, ontogeny can be studied at the level of an individual organ such as a leaf, following change in size and shape from initiation to maturity or senescence. Most studies of heterochrony in plants have examined determinate structures such as flowers, and are thus at the organ level (see review of Li & Johnston, 2000). This tendency is in part due to the ready analogy between an individual animal and an individual plant structure (e.g. a flower) under the animal-oriented GA model. The relative lack of whole plant studies is also due to the difficulty of studying ontogeny over the life of a long-lived organism. Studies of change in the characteristics of wood cells across the life of a tree is analogous to the study of Jones (1992), and thus represent a way of gaining access to questions of whole-plant ontogeny.

Although the conclusions of a heterochrony study are based on the phylogenetic assumptions used, most have not based their findings on explicit reconstructions. Knowledge of phylogenetic relationships is necessary to evaluate a heterochrony hypothesis because conflicting evolutionary scenarios may be implied depending on the type of ontogeny that is assumed to be ancestral. For example, if taxon A in Fig. 1 is assumed to have the ancestral type ontogeny consisting of two stages, whereas species B with a five-stage ontogeny is assumed to represent the descendant type, then the conclusion reached is that species B has evolved via peramorphosis. However, if these assumptions are reversed, then species A appears to have evolved via paedomorphosis. Authors such as Guerrant (1982), Hill & Lord (1990), and Gallardo *et al.*

(1993), base their interpretations on the 'common is primitive' assumption. Guerrant (1982) found support for the evolution of the hummingbird-pollinated *Delphinium nudicaule* from insect-pollinated ancestors via neoteny. Hill & Lord (1990) and Gallardo *et al*. (1993) found paedomorphosis a plausible path of evolution of cleistogamous flowers from outcrossed ancestors. Some have solved the problem of inferring relationships by studying crops that are derived from known ancestors, e.g. Jones (1992, 1993). Similarly, Wiltshire *et al*. (1994) gauged the effect of heterochronic mutants in pea with respect to the wild type 'ancestor'.

## Comparative wood anatomical concepts of paedomorphosis

Wood anatomists have made innovative suggestions regarding the action of heterochrony, an early example being Chrysler (1937). Bailey (1925) had reported that the wood of *Zamia* was composed entirely of scalariform tracheids. Chrysler knew that most cycads produced scalariform tracheids as young plants and then acquired smaller-pitted tracheids as they grew, so he suspected that the reports were based on the examination of young material. Accordingly, he had a large plant of *Zamia floridana* sent to him. To his surprise, he found that there were only scalariform tracheids throughout the entire stem. Examinations of other populations from Florida and the West Indies revealed similar patterns. Chrysler noted that all of the individuals that had been examined were low plants with tuberous stems that never formed trunks. Upon obtaining samples of arborescent *Zamia* from Central America, he found scalariform tracheids in the central wood, but moving outward in the trunk, 'entirely normal pitted tracheids . . ., just as occurs in other trunk-forming genera such as *Dioon*' (Chrysler, 1937, p. 698). He concluded that the tuberous species remain 'persistent juveniles with respect to their growth habit and their xylem' (Chrysler, 1937, p. 709).

Carlquist (1962) used a much wider sampling of species in his formulation of a broadly applicable concept of paedomorphosis. Carlquist reasoned that certain features are nearly always characteristic of juvenile wood in dicots at large. These features include spiral and scalariformly pitted vessel elements (especially in the earliest-formed xylem), secondary xylem vessel elements that are short relative to those in the outer wood, and very tall rays composed of upright cells. Carlquist noted that plants with these features in their adult wood tend to be woody herbs, rosette trees, or otherwise more slender-stemmed than the more commonly studied arborescent plants. That is, plants that display paedomorphosis not only have adult wood features that resemble the juvenile wood of dicots at large, but tend to be smaller in habit than conventionally woody plants. This concept has been well accepted as a descriptive device by wood anatomists, who frequently report paedomorphosis using Carlquist's (1962) criteria (e.g. Jansen *et al*., 2002).

## Challenges to the study of heterochrony in wood

Remarkably, the findings of wood anatomists that suggest the action of heterochrony have never been integrated with the GA model, sequence-based schemes, or explicit phylogenetic reconstructions. At least three difficulties may be cited to explain this lack. (1) The time axis is notoriously subject to variation in plants. Unlike, say, gastrulation in mammals which involves precise timing of cell division and movements in an environment that is buffered from outside variation, plant development is affected directly by variation in temperature, and availability of light, water, and nutrients. As a result, comparing ontogenies between individuals based on absolute time is frequently meaningless because different absolute ages may correspond to different ontogenetic stages in different individuals. (2) Sexual maturity, the main reference point used in the GA model, is not associated with cessation of major ontogenetic changes in wood. (3) Whole-plant ontogeny is difficult to study in long-lived species. The few studies of whole-plant heterochrony that have been conducted reflect growth of less than a year. For example, both Jones (1992, 1992) and Li & Johnston (2000) tracked changes in leaf size and shape over the lives of annual plants. Such an approach cannot be feasibly applied to most of woody plant ontogeny, which unfolds over years or even centuries.

## Using wood to study heterochrony

Wood anatomy offers a compelling alternative to the characters that have been used to date. As a tree ages, the successive layers of cells produced differ from one another. By examining wood from the centre of the tree toward the bark, it is possible to follow the ontogeny of a tree across its whole life span (Fig. 2). The approaches proposed here for testing hypotheses of heterochrony require several key elements: a phylogenetic hypothesis for the group of interest; ontogenetic information for its members; some basis for deciding on a

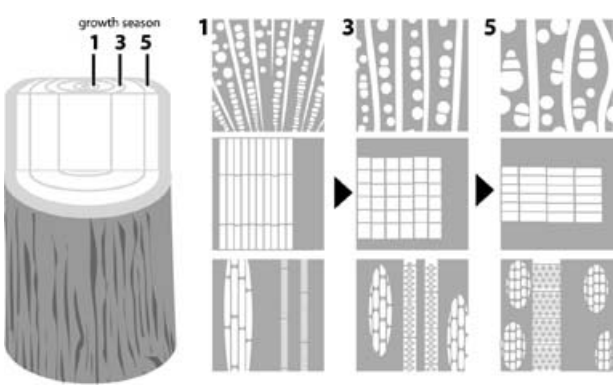


**Figure 2** Wood as an archive of development. At upper left, a sample of a stem that includes material from the pith to the bark. The numbers indicate points that correspond to ages in number of growing seasons. At right are trans, radial and tangential sections taken at these ages to show ontogenetic changes. In the transections (top row), vessel grouping drops with age as vessel diameter sharply rises and ray width increases. Ray histology changes from upright to procumbent as seen in radial sections (middle row). In tangential section (bottom row), vessel-vessel pitting can be seen to change from scalariform to polygonal alternate, while rays become much shorter.

variable against which to plot ontogenetic information; and a rationale for deciding where ontogeny begins and ends. These and other considerations are amplified below.

An example of the paedomorphic evolution of a small life form from a larger one is provided from *Moringa* (the drumstick tree family: Moringaceae). Many habits, from bottle tree to tuberous herb, are found in *Moringa*. The members of the so-called tuberous clade all have thick, water-storing roots, and are restricted to the Horn of Africa. *Moringa ruspoliana* is a 'sarcorhizal' tree with a tall, slender trunk and long, tuberous roots. Its sister species, *M. longituba*, is a diminutive single-stemmed shrublet with a large underground tuber (Olson, 2002). The hypothesis of a paedomorphic origin of the small *M. longituba* from an arborescent ancestor is tested here in two steps. First, the type of ontogeny that is ancestral to the *M. longituba* + *M. ruspoliana* clade is inferred. Then, wood ontogenies are compared between species in light of the ancestral character state reconstructions, and the type of heterochrony observed, if any, is inferred.

## Materials and methods

Basal stems of *M. ruspoliana* Engler and *M. longituba* Engler were collected from the Northeastern Province, Mandera District, of extreme northeastern Kenya near the borders with Ethiopia and Somalia. *Moringa ruspoliana* Olson 702 was collected from Rhamu-Dimtu Division, around the village of Yabicho (3°56′28′N, 41°10′00′E); *M. longituba* Olson 704 was collected nearby at a rocky plain known as Filqo (3°58′11′N, 41°45′00′E). Anatomical descriptions of these samples are given in Olson & Carlquist (2001); the voucher specimens for Olson 702 and Olson 704 are deposited at the Missouri Botanical Garden.

To infer the kind of ontogeny that is ancestral to the *M. longituba* + *M. ruspoliana* clade, the habit categories of Olson & Rosell (2006) were reconstructed on the phylogenetic hypothesis of Olson (2002), which was based on a combined parsimony analysis of three molecular data sets and one morphological one. Ancestral state reconstructions employed maximum likelihood using the StochChar module (Maddison & Maddison 2004b) of Mesquite (v. 1.05; Maddison & Maddison 2004a). Maximum likelihood ancestral character state reconstructions were preferred because they provide information regarding the likelihoods of the various possible states at each node. These methods thus provide specific information in many instances in which parsimony reconstructions would indicate only that the reconstruction is equivocal. For all characters, a Markov one-parameter model (Lewis, 2001) was used, with the varying parameter being the rate of change in state. A decision threshold of two was used, to exclude reconstructed states with very low proportional likelihoods from the pie charts shown at the nodes of the phylogeny, thus simplifying the figure. To illustrate the sensitivity of ancestral state reconstruction to coding and branch lengths, *M. arborea* was coded as tuberous rather than its actual habit, which is a sarcorhizal tree. This species was selected because it is the farthest in terms of branch lengths from *M. ruspoliana* and *M. longituba*. Likewise, ancestral character states with the 'true' coding but with all branch lengths equal to one were also reconstructed, a 'punctuational' scenario that implies that evolutionary change is associated only with speciation events. This assumption is commonly invoked in phylogenetic comparative method studies when branch lengths are not available.

For studies of wood ontogeny, slides for light microscopy were prepared using the methods of Olson & Carlquist (2001). Secondary xylem vessel diameters were measured in transections at intervals of 0.5 mm from the pith for *M. longituba* and every mm for *M. ruspoliana*. Major and minor lumen diameters were measured for 25 vessels at each sampling interval. Average vessel areas were calculated from these measurements. As for any study that uses measurements based on points that are judged to be similar between species, careful assessment of homology must be undertaken. In this case, it could be argued that vessel area might not be comparable between species. For example, a vessel that has its widest dimension oriented tangentially could have the same area as another with its widest dimension oriented radially. The comparison of area thus might not reflect similar biological characteristics. In the case of the *M. longituba* and *M. ruspoliana*, there was little difference in vessel transectional shape and thus area is very likely to represent an index that is comparable between species. Ontogenetic trajectories in vessel area were then calculated for each species, using ordinary least squares regressions of vessel area on distance from the pith were calculated using JMP 5.1 (SAS Institute, Inc., Cary, NC). The coefficients of the trajectory for both species were compared using the procedure of Sokal & Rohlf (1995, p. 495). Rationale for the choice of X axis is treated in the discussion.

## Results

Ancestral state reconstruction suggests that the small, tuberous life form in the tuberous clade of *Moringa* was derived from large ancestors (Fig. 3; Table 1). The proportional likelihood at the *M. longituba* (tuberous) + *M. ruspoliana* (sarcorhizal tree) node is 0.62 in favour of the ancestor having been a sarcorhizal tree. Similar values were recovered at other internal nodes of the tuberous clade phylogeny (Fig. 3), and suggest that the tuberous life form has evolved at least twice from sarcorhizal tree ancestors. Changing the coding of *M. arborea* from sarcorhizal to tuberous changed the proportional likelihood of the *M. longituba* + *M. ruspoliana* node to 0.82 in favour of a tuberous ancestor. Similarly, setting all branch lengths equal to one changed the reconstruction of the ancestor of the *M. longituba* + *M. ruspoliana* clade from clearly in favour of a sarcorhizal ancestor to a situation in which it is ambiguous.

Average major and minor diameters as well as average vessel areas are given for each sampling interval in Table 2. Inspection of the plot of the regressions of vessel area on distance from the pith (Fig. 4) shows that the entire ontogeny of *M. longituba* overlaps the early stages of that of *M. ruspoliana*. Indeed, regression of vessel area on distance from the centre of the stem in *M. longituba* ($n = 5$, $r^2 = 0.83$, ß = 2164,

| | 'True' observed states, original branch lengths (Fig. 3a) | | *M. arborea* coded as tuberous, original branch lengths (Fig. 3b) | | 'True' observed states, branch lengths all = 1 (Fig. 3c) | |
|---|---|---|---|---|---|---|
| **node** | **tuberous** | **sarcorhizal** | **tuberous** | **sarcorhizal** | **tuberous** | **sarcorhizal** |
| *arborea+rivae* | 0.0037 | 0.9959 | 0.8221 | 0.1750 | 0.0315 | 0.9585 |
| *borziana+pygmaea* | 0.9994 | 0.0005 | 0.9990 | 0.0001 | 0.9585 | 0.0316 |
| tuberous clade minus *longituba+ruspoliana* | 0.3565 | 0.6334 | 0.8828 | 0.1133 | 0.4645 | 0.4645 |
| *longituba+ruspoliana* | 0.3661 | 0.6209 | 0.8172 | 0.1698 | 0.4663 | 0.4663 |
| tuberous clade | 0.3582 | 0.6239 | 0.8477 | 0.1373 | 0.4150 | 0.4150 |
| rate | 0.007 | | 0.006 | | 0.068 | |
| log likelihood | 15.294 | | 13.504 | | 13.230 | |

**Table 1** Reconstructions of ancestral character states for the three scenarios in Fig. 3. Proportional likelihoods are given for the reconstructed ancestral states for the nodes of the tuberous clade. Sometimes the proportional likelihoods do not sum exactly to one because the other possible states in addition to tuberous and sarcorhizal are not included in the table.

| **Distance from pith (mm)** | **Vessel diameter ($\mu$m²)** | | **Standard deviation** | **Vessel area ($\mu$m)** |
|---|---|---|---|---|
| *M. ruspoliana* | | | | |
| 1.00 | major | 97.8 | 11.95 | 5010.19 |
| | minor | 65.2 | 7.33 | |
| 2.00 | major | 99.0 | 12.04 | 5756.15 |
| | minor | 74.0 | 10.41 | |
| 3.00 | major | 98.6 | 13.65 | 5474.35 |
| | minor | 70.7 | 10.03 | |
| 4.00 | major | 124.0 | 11.03 | 6817.26 |
| | minor | 70.0 | 8.38 | |
| 5.00 | major | 108.4 | 11.28 | 7349.04 |
| | minor | 86.3 | 13.10 | |
| 6.00 | major | 135.9 | 25.52 | 11469.36 |
| | minor | 107.4 | 16.85 | |
| 7.00 | major | 140.5 | 20.68 | 12374.92 |
| | minor | 112.2 | 18.64 | |
| 8.00 | major | 170.4 | 15.21 | 18169.01 |
| | minor | 135.8 | 13.24 | |
| 9.00 | major | 156.7 | 17.76 | 14691.74 |
| | minor | 119.4 | 18.70 | |
| *M. longituba* | | | | |
| 0.50 | major | 71.2 | 6.26 | 3221.01 |
| | minor | 57.6 | 6.18 | |
| 1.00 | major | 77.7 | 6.57 | 3479.99 |
| | minor | 57.0 | 3.95 | |
| 1.50 | major | 101.4 | 8.44 | 5997.62 |
| | minor | 75.3 | 8.35 | |
| 2.00 | major | 94.6 | 7.07 | 5216.53 |
| | minor | 70.2 | 6.62 | |
| 2.50 | major | 112.3 | 7.84 | 7763.00 |

**Table 2** Average major and minor axis diameters of vessels and vessel area per sampling interval in *Moringa ruspoliana* (Olson 702) and *Moringa longituba* (Olson 704).

$P = 0.03$) had a slope that was not significantly different ($P = 0.61$) from that of *M. ruspoliana* ($n = 9$, $r^2 = 0.84$, ß $= 1574$, $P < 0.001$). Likewise, the intercepts in both of these regressions were very similar (1889 in *M. longituba* vs. 1810 in *M. ruspoliana*).

# Discussion

In this section, important conceptual considerations for the use of wood in heterochrony studies are discussed, using the species of *Moringa* studied as an example. The chief challenges for the use of wood involve the selection of an X axis against which to plot ontogenetic data and the selection of a reference point against which to judge the 'end' of ontogeny. Likewise, some of the numerous opportunities that wood presents are discussed, including the adaptation of traditional sampling schemes examining ontogenetic changes observed radially and longitudinally in the stem, and ways that features such as growth rings might be used to infer evolutionary mechanisms.

## Paedomorphosis in *Moringa*

Wood ontogenetic information in the context of the *Moringa* phylogeny is consistent with a hypothesis of a paedomorphic derivation of *M. longituba*. Ancestral state reconstruction suggests that the small, tuberous life form is derived from an arborescent life form represented by the sarcorhizal trees *M. arborea*, *M. rivae* and *M. ruspoliana*. Therefore, for the purpose of this comparison, the ontogeny of *M. ruspoliana* can be taken to represent the type that is ancestral to that of *M. longituba*. The plot of vessel area on distance from the pith (Fig. 4) shows that the entire ontogeny of *M. longituba* overlaps the early stages of that of *M. ruspoliana*. That is, the ontogeny of *M. longituba* is identical to a truncated version of a *M. ruspoliana*-type ontogeny, with the result that adult

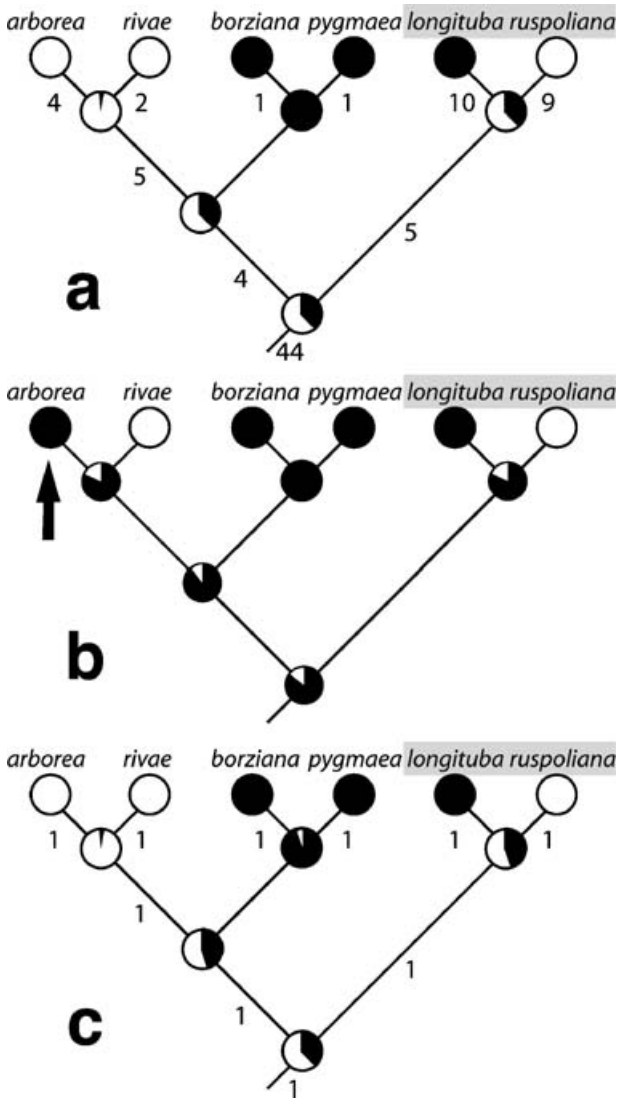


**Figure 3** Phylogeny of the tuberous clade of *Moringa*, based on Olson, 2002. Numbers are branch lengths (branch lengths are the same in Figs 3a and b, and so are shown only in 3a). The pie charts represent the known states of the terminal taxa and the proportional likelihoods of the reconstructed ancestral character states. Black = tuberous shrub, white = sarcorhizal tree. The *M. longituba* + *M. ruspoliana* clade is boxed in gray. (a) True coding, that is, in this tree, the terminal taxa are coded according to their observed life forms; (b) To illustrate the degree to which inferences of heterochrony are dependent on reconstructions of ancestral character states, which in turn are affected by factors such as tree topology, branch length and coding of terminal taxa, the coding of *M. arborea* has been changed from sarcorhizal to tuberous (arrow); (c) Same coding as in Fig. 3a, but with branch lengths all set to 1, a 'punctuational' or 'speciational' model.

*M. longituba* resembles the juvenile stages of the ancestral-type ontogeny. This scenario is congruent with the hypothesis that tuberous species are derived via paedomorphosis from larger ancestors.

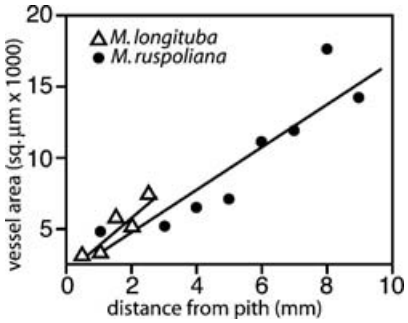


**Figure 4** Regression of average vessel area on distance from the pith for the sister taxa *Moringa longituba* and *M. ruspoliana*. The entire ontogeny of *M. longituba* is indistinguishable from the early stages of *M. ruspoliana*.

## Choosing the X axis

Because of the environmental dependence of wood production, choosing a variable against which to plot ontogenetic information is the greatest challenge to heterochrony studies of wood. The GA model plots ontogenetic data against absolute age. Estimates of age can be readily obtained in some wood studies, for example those that use plantation trees of known age. Likewise, ontogenetic changes can be readily associated with absolute ages in trees that produce annual growth rings (Fig. 1). However, especially in wild trees, or when comparing trees grown in different localities, plasticity can lead to similar absolute ages being correlated with different developmental stages. For example, it is axiomatic that with two cuttings from the same tree, one well-watered and kept warm will grow differently from one kept dry and cool. Absolute time will not correspond to ontogenetic stages between these two cuttings, even though they are from the same individual. As a result, it is to be expected that in woody plants absolute age will not provide a comparable axis for comparisons of ontogeny between individuals.

Stem size may provide the most meaningful metric for comparisons of ontogeny between individuals. Organ or body size has been found in many cases not to correlate linearly with age in animals, and thus the use of size as a proxy for age has been criticized in zoological studies (e.g. Godfrey & Sutherland, 1995; Klingenberg & Spence, 1993). However, there is a fundamental difference in the use of animal body size and measures that reflect the number of cell layers accumulated in the trunk of a woody plant. In a tree, a given wood sample is associated with the distance from the pith or from the centre of the stem from which it was collected. Because wood is produced in concentric layers, this distance situates a given sample as representing later ontogenetic stages than more interior samples and earlier stages than more external ones, providing an unambiguous ontogenetic series.

Significantly, many wood features do scale linearly with stem size (e.g. Olson & Rosell, 2006). In fact, traditional wood studies commonly plot ontogenetic information against distance from the centre of the stem or some other measure related to stem diameter (e.g. Panshin & de Zeeuw, 1980). There does appear to be some justification for expecting that, for comparisons of species with similar stem anatomy, size indices such

as stem diameter might be an acceptable or better alternative to absolute time. For a stem to remain standing, its anatomy and allometry must track its mechanical needs (e.g. Euler buckling, Vogel, 2003; or behaviour in bending, Niklas, 1992), but there is no mechanical reason to expect these aspects to reflect absolute age. Two trunks of similar length and construction would also be expected to have similar diameters though one is 10 and the other 30 years old. Because heterochrony studies examine closely related species, similar stem anatomy between species will often be observed, exactly a situation in which it is at least reasonable to expect anatomy:diameter correlations to be comparable between species. It is also clear that vessel characteristics scale with size, although the exact nature of this scaling is debated (e.g. Enquist, 2003; Meinzer, 2003; McCulloh & Sperry, 2005; see also Carlquist & Grant, 2005; Olson & Rosell, 2006). In general, in addition to requiring more water, larger trees have more extensive root systems and store more water than smaller species, thus permitting features that increase conductive efficiency; again, there does not appear to be any reason to expect these features to scale with absolute age.

Thus, although based on several assumptions regarding the interplay between stem anatomy, biomechanics, hydraulic architecture and allometry, the traditional use of stem diameter as an X axis between species seems a justifiable alternative to explore even when absolute ages are not available. When age is available, the relationship of ontogenetic data to both diameter and absolute time could be examined to test these expectations. Rosell & Olson (unpubl. data) explicitly tested the assumption that biomechanical characteristics should depend only on stem size and proportions, and should be independent of stem age in *Pittocaulon* (∼*Senecio*) *praecox*. The plants sampled represented a variety of growth rates, from individuals of slow growth in cracks on exposed boulders, to fast-growing plants from moister, sheltered locations. Despite eight-fold differences in growth rate across habitats, all stems shared the same length:diameter proportions. Mechanical behaviour, in turn, was strongly predicted by stem size. Consistent with expectations, size and mechanical parameters of a given segment of stem showed no statistical association with its age.

The degree to which anatomical differences may affect cell:stem size correlations probably differs between cell types in different clades. For example, *M. longituba* stems are made up mostly of long, slender libriform fibres, whereas *M. ruspoliana* has alternating bands of slender and wide fibres (Olson & Carlquist, 2001). Plots of dimensions of these cells would not overlap even at similar stem diameters. However, not only do the vessel element area ontogenies overlap between these two species (Fig. 4), but vessel diameters from samples of juvenile and adult wood from all 13 *Moringa* species fall along the same line when regressed against distance from the pith (Olson & Rosell, 2006). Thus, despite qualitative differences in some cell types that almost certainly are associated with mechanical and allometric differences, some cells, in this example the vessel elements, nevertheless appear to remain correlated with stem diameter in comparable ways across species. Cell types that show qualitative differences would be ideal candidates for study using sequence-based methods, or using morphometric methods that are explicitly designed to reflect shape.

## Reference points for onset and cessation of ontogeny

The GA model requires differences in onset and cessation of ontogeny to be identifiable between taxa. Because xylem production is inseparable from shoot growth, it is difficult to see how meaningful differences in onset could be found. Even if differences exist, it is likely that they will occur over a scale (days, weeks) that is too small to result in alteration of mature morphologies (cf. Raff & Wray, 1989). Identifying a terminal reference point is likewise a challenge. Sexual maturity under the GA model is assumed to fulfill three criteria: (1) it is associated with cessation of major ontogenetic changes; (2) it is independent of the heterochronic process being studied; (3) it is not environmentally triggered. A variety of reference points have been used in studies of floral ontogeny, e.g. anther dehiscence (Gallardo *et al*., 1993) and microsporogenesis (Guerrant, 1982). Flowering has been used in studies of whole-plant heterochrony in fast-growing, short-lived plants (e.g. Jones, 1992). However, none of these approaches provides a ready analogy that can be applied to studies of heterochrony in wood.

In contrast to sexual maturity in animals, in most woody plants the majority of ontogeny occurs *after* attainment of reproductive competence. However, a point at which major ontogenetic changes in the wood taper off often exists, with most changes occurring in the primary and early secondary xylem before stabilizing or even dropping off (e.g. Bailey & Tupper, 1918; Carlquist, 1975; Panshin & de Zeeuw, 1980). Such a point obviously does not meet the criterion of independence because it is identified by changes in the character of interest, but could suffice to identify points of comparison between taxa. Nevertheless, such a reference point is not universal, because in many plants of narrower diameter the ontogenetic trajectory may be too short to identify a truly level curve. In the case of the species of *Moringa* examined, no levelling off of the ontogenetic curves was detected, despite the fact that stems of maximal dimensions were collected for the populations visited. In this case, the 'end' of ontogeny thus corresponds to death of the sample imposed by collection. In species with linear ontogenies such as those given here, this artificial end point should not strongly affect inference of heterochrony, because the available points will still describe the same line even if some terminal points are missing.

## Inference of process

Clearly, the incompatibilities of wood with the GA model will have impacts on the kinds of processes that can be inferred. Without detectable differences in onset of ontogeny, post- and pre- displacement are undetectable. Likewise, if sexual maturity or some other event is not associated with cessation of ontogeny, progenesis and hypermorphosis are undetectable. Even if these subcategory processes cannot be identified, paedomorphosis and peramorphosis in the GA sense can still be distinguished. For example, knowing that species A in Fig. 1 represents the ancestral-type ontogeny, it is possible to accept

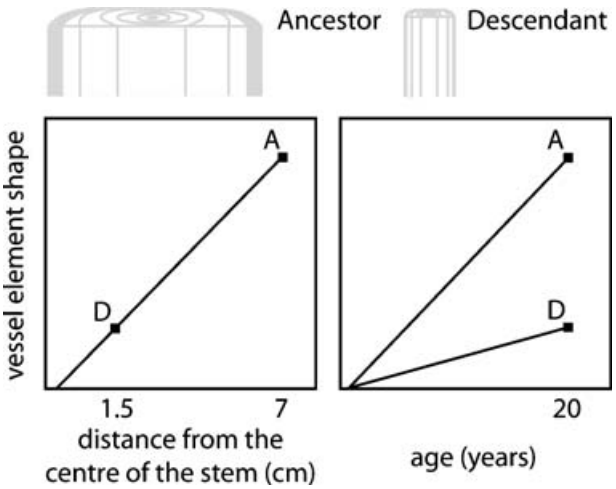


**Figure 5** Plotting ontogenetic information against absolute time or against distance from the centre of the stem. At top, two samples of the same age from different species, one with a very slender stem, and one with a very thick stem. The ontogeny of the thick-stemmed species is known to be the ancestral type. Plotting changes in vessel element shape against distance from the centre of the stem (bottom left) results in an inference of paedomorphosis. When plotted against absolute time, the graph also suggests paedomorphosis, conforming with the GA inference of neoteny.

the hypothesis that species B evolved via peramorphosis and reject the notion that heterochrony at the hierarchical level of organization studied is responsible for the changes involved in the evolution of species C. Plotting ontogenetic information against both absolute time and distance from the centre of the stem should infer the same general category of processes (Fig. 5).

## Sampling schemes

The sampling scheme used for *Moringa* is just one of many possibilities. I outline two main strategies below and discuss their merits and drawbacks. Although these sampling schemes, and many others, have long been used by wood anatomists (many are summarized by Panshin & de Zeeuw, 1980), they have never been used in a phylogenetic context to infer patterns of ontogenetic evolution.

(1) *Methods with unknown time axes*. The scheme most likely to be used for wild trees, and the only one really available for long-lived trees, requires a sample of wood that stretches from the pith to the bark. This scheme was used here to compare *M. longituba* and *M. ruspoliana*. Preparations are made that enable detection of changes in cell characteristics in the successive layers of cells. A rapid assessment can be made by examining the early secondary xylem ('juvenile wood') and of the outermost, most recently formed xylem ('mature wood'). Comparison of these preparations will identify features that undergo ontogenetic transformations. Based on these preliminary observations, a strategy for finer sampling can be devised. If the differences between juvenile and mature wood are subtle, as is likely in many cases of paedomorphosis, sampling and analysis must be particularly careful to provide adequate resolution, for example taking care to keep xylem fragments to be macerated limited in their radial and tangential extent to minimize variation. Unless annual growth rings are present, ages will usually be unknown for samples from wild trees, and so time will not be available as an X axis. However, this approach has the important advantage of using samples from natural conditions, and of permitting observations from a long ontogenetic trajectory spanning many more growth seasons than would be possible from an ontogenetic series based on cultivated plants.

(2) *Methods with a known time axis*. The following methods apply to relatively fast-growing trees (or a long study), and differ most significantly from the above in associating ontogenetic stages with known ages. The main drawback of these methods is that the xylem accumulation that can be studied is limited, significant when studying organisms that routinely span human generations. In plants that rapidly make the transition from juvenile to adult wood, this drawback is minimized. The chief advantage is that the significance of time can be tested directly. One method involves cohorts of trees of similar age, from which several are harvested each sampling period and making preparations from the outermost wood. Over a period of a few years, the series of preparations will reflect the ontogenetic changes that characterize each species. The main drawback of this method is that the sample sizes per harvest period must be small in proportion to the total population. However, it may be a useful way to recover information from a situation in which trees are harvested at regular intervals for other reasons.

A series of methods that provide much more satisfactory sample sizes involve making a small wound at the base of all trees at regular intervals, cutting through the bark and damaging the outmost layers of secondary xylem (the 'wounding' or 'pinning' method, e.g. Wolter, 1968; Yoshimura *et al*., 1981; Kuroda & Shimaji, 1984; Kuroda & Kiyono, 1997). When all the individuals are harvested at the end of the sampling period, the wounds, and thus the ontogenetic changes occurring between them, are associated with age (Fig. 6). Anatomical preparations are then made for all individuals as for sampling scheme 1. Ontogenetic series can thus be reconstructed for all individuals, all reflecting the same period. Some individuals should be occasionally sampled, especially early in the series, to ensure that the method of scoring the stems produces scars that are neither too deep nor do not reach the xylem.

## Ontogenetic features for study

Any character that varies across the ontogeny of the wood can be used, and there is no reason to limit a study to commonly examined wood ontogenetic characters such as ray height. Multivariate descriptions of shape (e.g. using characters such as those of Cannon & Manos, 2001) would likely be informative in a study of wood ontogeny, though care must be taken in interpretation due to difficulties in assessing homology (Bookstein, 1994; Olson, 2005a). By the same token, it seems likely that non-wood anatomists who would be interested in studying heterochrony are daunted by the typological nature of

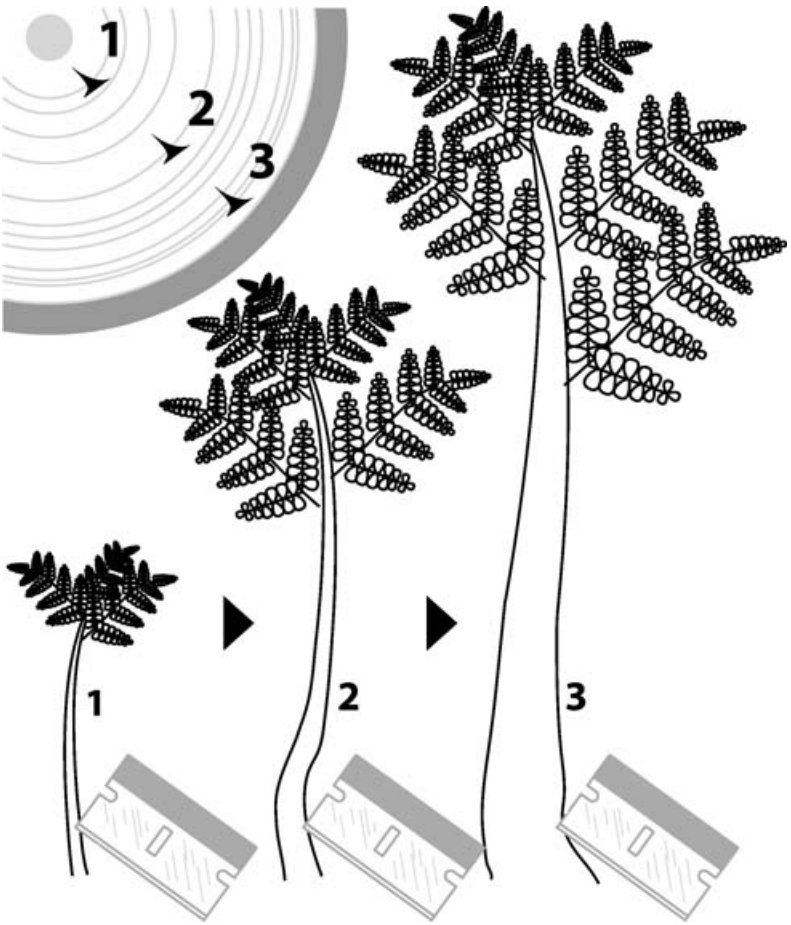


**Figure 6** Scoring the trunk to associate ontogenetic events with ages. At intervals, the bark is pierced with a blade and the outermost xylem damaged sufficiently to produce a small scar (ontogenetic series at bottom). At harvesting, the scars identify points of known ages (black wedges in the schematic stem transection at upper left).

traditional wood anatomical characters and are sceptical of the biological reality of these characters (see Olson, 2005a). Nevertheless, wood characters can and should be subjected to the same critical evaluation of homology as are applied to any other character type, thereby making the rich variation in wood available for study those interested in plant evolution generally (see also Hawkins, 2000; Rieppel & Kearney, 2002).

Numerous other features offer possibilities for studying ontogenetic evolution in woody plants. For example, in addition to changes occurring from pith to bark, woody plants also exhibit ontogenetic changes along the lengths of their trunks. Foresters have developed sampling schemes that reflect this variation as well (see Panshin & de Zeeuw, 1980). Figure 7 shows a scheme in which longitudinal changes in ontogenetic curves in cell shape are reconstructed by sampling various levels along the stem. Likewise, intra-individual variation associated with growth rings or other types of rhythmic production of differing cell types offers many opportunities for inference of heterochronic mechanisms. For example, a wood that produces alternating layers of differing cells (e.g. parenchyma and fibres) could give rise to many different stem types solely by virtue of modulations in the timing of the production of these bands. Shortening of the time of production of fibres would lead to wood that is largely parenchymatous with only narrow fibre bands; the reverse would lead to a highly fibrous wood with little parenchyma (Fig. 8a, b). Either of these scenarios can be taken to the extreme of an entirely parenchymatized or entirely fibrous wood (Fig. 8c, d).

The extent to which variation between individuals can be incorporated into heterochrony hypotheses also merits attention. Intraspecific variation in a relatively small number of species of importance to forestry has been intensively studied (e.g. Herman *et al*., 1998), but very little is known for other species. For example, authors such as Baas *et al*. (1984) suggest that dwarfed individuals with smaller stem diameters generally have shorter, narrower vessel elements than larger-stemmed individuals of the same species in more favourable locations. This observation would be expected if these features track stem diameter (although, as suggested here, cells from similar distances from the centre of the stem would represent a more valid comparison, rather than comparing anatomical features of the outer wood of stems of different sizes). However, apparently contradictory information such as that of Reyes-Santamaría *et al*. (2002) suggests that these patterns may not hold in all species. How does this variation affect mechanical behaviour, allometry, and as a consequence, inferences of heterochrony? How do the clouds of points described by ontogenetic trajectories of numerous individuals

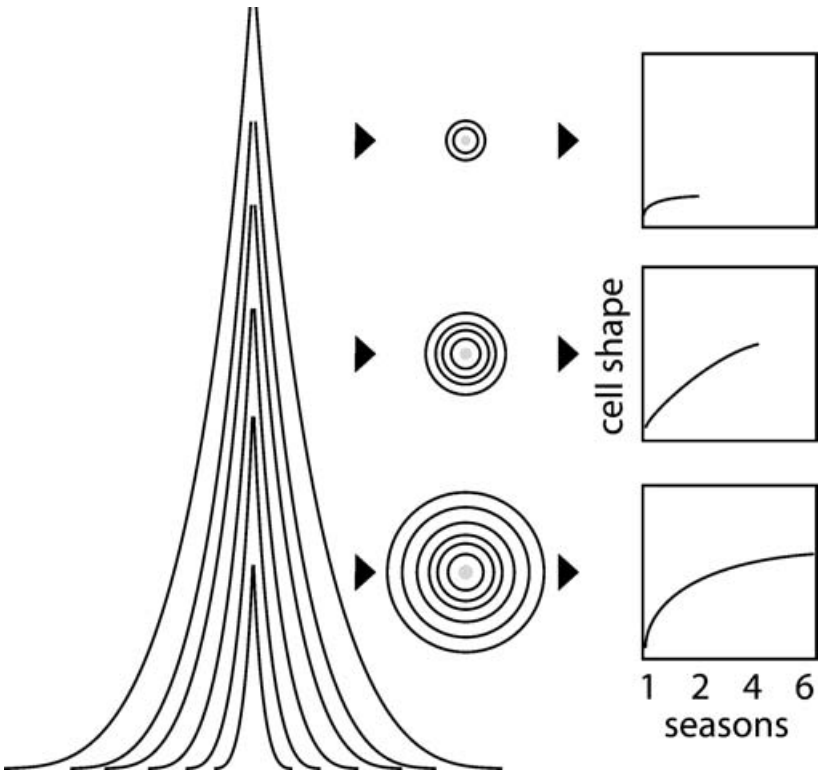


**Figure 7** A sampling scheme to reflect changes in wood ontogenetic patterns at different heights in a tree. The lines in the schematized tree trunk at left represent layers of cells deposited by the vascular cambium. The circles in the middle represent transections of the trunk drawn from three different heights. Finally, at right are the differing curves recovered from these samples, in this case 'cell shape' plotted against age as inferred from annual growth rings.

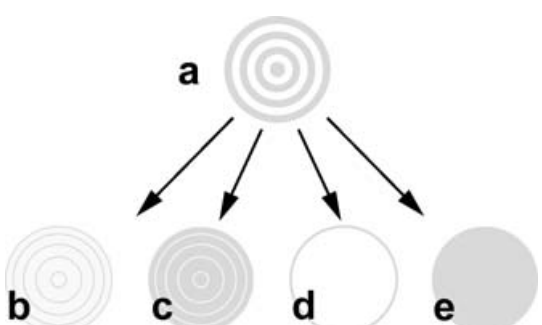


**Figure 8** An ancestor with growth rings or other types of banding (a) could give rise to descendants with a broad array of stem anatomical modes via small changes in the timing of production of different cell types, e.g. a wood that is mostly parenchyma with some fibre bands (b), a wood that is mostly fibres with parenchyma bands (c), to fully parenchymatized (d) or entirely fibrous (e) woods.

from different environments enable more detailed inference of the ways that wood ontogeny is modulated in interspecific evolution (cf. Schlichting & Pigliucci, 1998)? Such questions cannot be answered by examining only mature anatomical features, and cannot be addressed without ontogenetic information for related species in the context of a phylogenetic hypothesis.

## Inference of the ancestral-type ontogeny

Except for studies that examine crops and their wild ancestors (e.g. Jones, 1992, 1993), the descendants of a common ancestor are the subject of study and not ancestors and their descendants. The inference of the type of ontogeny that is ancestral for a given species thus rests on reconstructions of ancestral character states. Such reconstructions are strongly affected by uncertainty in tree topology, branch lengths and coding of extant species (e.g. Cunningham *et al*., 1998). For example, using the coding in Fig. 3a yields a proportional likelihood of 0.62 in favour of the ancestor to the *M. longituba* + *M. ruspoliana* clade being arborescent. Changing the coding of *M. arborea* from arborescent to tuberous tips the balance in the proportional likelihoods for the ancestor of the *M. longituba* + *M. ruspoliana* clade in favour of a tuberous ancestor (Fig. 3b, Table 1). In this scenario, *M. longituba* represents the ancestral-type ontogeny to the *M. longituba* + *M. ruspoliana* clade, and *M. ruspoliana* is inferred to have evolved from a *M. longituba*-like ancestor via peramorphosis – exactly the opposite conclusion. In contrast, using a punctuational model yields an ambiguous reconstruction for the ancestor of the *M. longituba* + *M. ruspoliana* clade (Fig. 3c). Alternative methods, such as reconstruction of quantitative ancestral character states (e.g. Webster & Purvis, 2002), would likely yield yet different results. That small changes such as the coding of the most distant member of the clade from the group of interest should reverse the heterochronic process inferred highlights the importance

of basing inferences on nodes for which reconstructions are likely to be most robust.

### Alternatives to heterochrony

Hypotheses of heterochrony are readily falsified. For example, the prediction that two species should share similar patterns of change in vessel pitting is confirmed between species A and B in Fig. 1 but would be rejected when comparing species C with the other two. Likewise, finding different slopes or Y intercepts in the regressions of the *M. longituba* + *M. ruspoliana* clade of *Moringa* would also permit rejection of the hypothesis that differences in these species are the result of simple truncation of a *M. ruspoliana* type ontogeny. Several processes other than heterochrony can lead to morphological differences between species. Some of these considerations and comments on their potential importance in wood include:

*Homoeosis* – the replacement of one structure for another. Examples of floral homoeotic mutants are readily found, e.g. roses in which all of the inner floral whorls have been replaced by petals. This process is undoubtedly of prime importance in wood evolution. All anatomists have faced cases in which the position of a cell would suggest one thing about its possible identity, and its morphology another. For example, the rays of *Misodendron* have cells that occur where ray cells 'should' be present but are indistinguishable from libriform fibres (Carlquist, 1985). Parenchymatized xylem is another potential example of homoeosis (e.g. Caricaceae, Carlquist, 1998). Distinguishing the homoeotic replacement of fibres by parenchyma from the heterochronic elimination of fibres suggested above would be difficult, and illustrates the potential overlap between these mechanisms (cf. the 'heterochronic' hypothesis of Lahaye *et al*., 2005).

*Heterotopy* is the appearance of a structure in a new location. Cauliflory is a classic example of heterotopy; another comes from *Tolmiea menziesii*, which produces plantlets at the petiole apices. Like homoeosis, heterotopy has certainly played a role in the evolution of major differences in stem structural plans, with features such as intraxylary phloem likely candidates for explanation via heterotopy.

*Novelty* – Many definitions have been offered for evolutionary novelty. Müller & Wagner (1991) suggest that a novelty is a structure that is not homologous to any other structure in an ancestor. Heterochrony, homoeosis, or heterotopy envisage morphological change occurring via the rearrangement of existing ontogenies. In contrast, novelty implies the appearance of a structure or ontogenetic pathway that cannot be explained by simple alterations of existing ontogenetic programs. Novelty observed at one level may be the result of heterochrony at a different hierarchical level of organisation.

### Carlquist's paedomorphosis concept and the GA model

The approach proposed here and the paedomorphosis concept of Carlquist (1962) partially overlap, with the most important differences being the characters used and the phylogenetic level examined. Paedomorphosis in the sense of Carlquist is identified by a specific set of features in the adult wood that are generally typical of primary xylem, e.g. long vessel elements, scalariform or scalariform-like lateral wall pitting, etc. In the approach described here, heterochrony can be diagnosed by any character that varies during ontogeny. It would thus be possible to observe GA peramorphosis in a species that would still show paedomorphosis in the sense of Carlquist. Many species that have become shrubby from less woody ancestors likely fit this scenario, e.g. the woody species of the *Chamaesyce* clade of *Euphorbia* have probably become more woody than their ancestors via peramorphosis, but nevertheless show paedomorphic wood in the sense of Carlquist (see Carlquist, 1970; Steinmann & Porter, 2002). Furthermore, Carlquist's approach applies to any phylogenetic level. Thus it is not only possible to say that the wood of a species but even of a higher taxon meets Carlquist's criteria for paedomorphosis. In contrast, the approach proposed here is designed to infer heterochrony at the interspecific level. An important overlap in the approaches would be the use of findings of Carlquistian paedomorphosis to identify taxa for study using the GA model-type or sequence-based approach.

## Conclusion

The scheme proposed here makes assumptions regarding the relationship between anatomy, biomechanics, hydraulic architecture, and allometry, and the similarity of these features between species. These assumptions can be tested readily. Characteristics worth examining in living stems include the behaviour in bending of adults and juveniles and of stems of similar diameter in different environments, wood and bark density, distribution of vessel sizes, vessel density, conductive area and hydraulics. How realistic is the assumption that anatomically similar stems of similar sizes have similar mechanical and conductive needs, regardless of age? What degree of anatomical dissimilarity is necessary before causing differences in mechanical and conductive behaviour?

In general, a strict application of the GA model to wood seems impracticable because of the impossibility of detecting significant interspecific differences in onset of ontogeny, the lack of a terminal reference point, and difficulties in using absolute time as an X-axis. Nevertheless, the methods described here can distinguish paedomorphosis and peramorphosis. However, exceptional situations can be imagined in which all GA parameters apply. Differences in onset of interfascicular cambium activity could lead to small differences in adult morphology (cf. *Pittocaulon*, Olson 2005b). Likewise, sexual maturity is associated with cessation of vegetative growth in many monocarpic monocots and some dicots such as *Cerberiopsis*. In *Melocactus*, apical growth of the vegetative body apparently ceases when the cephalium is initiated. These uncommon life forms could be profitably taken advantage of to test heterochrony hypotheses in the GA paradigm. In contrast, virtually any qualitative change, such as interspecific changes in vessel pitting type, could be examined for its heterochronic status using sequence-based methods.

Alterations to ontogenetic programs via heterochrony have certainly played a major role in the evolution of the

vast range of woody plant morphologies. Stem evolution is the result of natural selection pulling constellations of characters through morphospace, favouring different strategies for support, conduction, and storage in different species. Heterochrony and other mechanisms provide the paths by which populations move through morphospace. Understanding how natural selection has driven changes in wood and how these mechanisms have allowed or constrained change is the ultimate goal of evolutionary biology of stems. The remarkable property of wood to bear a record of its history should make it an unparalleled model system for unravelling the interaction of ontogeny and adaptive evolution.

## Acknowledgements

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