

## INTEGRATING MOLECULAR PHYLOGENETIC AND PALEOBOTANICAL EVIDENCE ON ORIGIN OF THE FLOWER

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Inferences on the origin of the angiosperm flower require consideration of other seed plants, especially fossils. Molecular data favor a relationship of Gnetales to conifers rather than to angiosperms, and both alternatives are equally parsimonious in terms of the morphological data set presented here. However, if molecular relationships among extant taxa are accepted, morphology still associates glossopterids, *Pentoxylon*, Bennettitales, and *Caytonia* with angiosperms. Bennettitales had flowerlike structures, but if *Caytonia* is sister to angiosperms, aggregation of fertile parts probably occurred independently in Bennettitales and angiosperms. These results and developmental genetic data are consistent with homology of the angiosperm bitegmic ovule with the cupule of glossopterids and *Caytonia*, while the carpel could represent a leaf and a cupule-bearing axillary branch. Origin of an adaxial cross zone could produce a uniovulate, ascidiate carpel, as in living basal angiosperms. Stamens may represent similar units bearing two microsynangia. However, ovulate structures of *Pentoxylon* and Bennettitales are more difficult to interpret, and any homologue of the carpel wall in *Caytonia* is unclear. Further progress may require better understanding of homologies in known fossils and/or recognition of closer stem relatives of angiosperms. A proposed Cretaceous stem relative, *Archaeofructus*, is more likely a crown-group angiosperm related to Hydatellaceae (Nymphaeales).

**Keywords:** angiosperms, seed plants, paleobotany, phylogeny, flower, ovule.

**Online enhancement:** appendix.

### Introduction

Reconstruction of the first flower and its origin—what the angiosperm flower and its component parts came from—are two separate but related questions. In this article, I approach the second question with phylogenetic methods, integrating results of both morphological and molecular analyses.

From a phylogenetic point of view, the original morphology of the angiosperm flower can be addressed by asking where the phylogenetic tree of angiosperms is rooted. Even in the absence of morphological information on outgroups, if the angiosperm tree can be rooted with molecular data from other living plants, it is possible to optimize (plot) morphological characters on the tree using parsimony (or more probabilistic likelihood methods) and thereby estimate ancestral states. This “top-down” approach (Bateman et al. 2006) is easiest if there are several lines with similar character states that branch off sequentially at the base of the tree before the main radiation of the group, as appears to be the case in angiosperms. This situation may help overcome the valid objection that single low-diversity “basal” groups are not necessarily “primitive” in all their characters (Crisp and Cook 2005).

Molecular analyses have provided highly consistent and statistically robust results concerning the rooting of the angiosperms and the identity of the first branches—*Amborella*, Nymphaeales, and Austrobaileyales—that are basal to the vast majority of angiosperms, often called “core angiosperms”

and formally designated Mesangiospermae by Cantino et al. (2007). The main uncertainty is whether *Amborella* and Nymphaeales form two successive lines or a clade, with more data favoring the former arrangement (Zanis et al. 2002; Soltis et al. 2005; Moore et al. 2007). Using parsimony to optimize characters on a tree based on sequences of three genes and morphology, Doyle and Endress (2000) presented a list of estimated ancestral states for 108 characters, such as vesselless wood; simple, ovate leaves with pinnate venation and chloranthoid teeth; unilacunar two-trace nodes; undifferentiated perianth; stamens with adaxial microsporangia; several ascidiate carpels; and fleshy, indehiscent fruits. Pollen characters were further refined and evaluated by Doyle (2005), floral phyllotaxis by Endress and Doyle (2007), and leaf architecture by Doyle (2007). Ronse De Craene et al. (2003) presented a similar analysis of floral characters. It can be expected that such results will be improved by integration of the increasing numbers of Early Cretaceous floral fossils, many described by Friis et al. (2006).

The second question—what sort of structures in earlier plants the flower came from, how they were transformed into the perianth, stamens, and carpels, and how they were assembled into the flower—can be approached phylogenetically by asking what the closest outgroups of angiosperms are. Examination of the seed- and pollen-bearing organs in these plants and optimization of the relevant characters on a cladogram may provide an improved picture of the precursor structures for the flower at the closest outgroup node. This “bottom-up” approach (Bateman et al. 2006) is therefore a question of seed plant phylogeny and the place of angiosperms in it, and because

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of the highly relict and divergent nature of living gymnosperm taxa, it requires consideration of fossil as well as living plants.

Unfortunately, relationships among seed plant lines are far more uncertain and fraught with controversy than relationships within angiosperms. Much of what we thought we knew 10 years ago about seed plant phylogeny, based on morphology, has been thrown into doubt by molecular analyses. Resolution of these problems requires integration of molecular, morphological, and fossil data in a phylogenetic framework. Fortunately, the better information on rooting and relationships within angiosperms helps to improve the situation by clarifying ancestral states in angiosperms and thereby counteracting the long-standing problem of comparing outgroups with angiosperm taxa that were incorrectly assumed to be primitive, such as comparisons by von Wettstein (1907) and others between Gnetales and “Amentiferae” (mostly Fagales sensu APG 1998, 2003).

#### *Hypotheses on Seed Plant and Angiosperm Relationships*

Before the advent of phylogenetic (cladistic) methods, botanists had proposed a plethora of hypotheses on seed plant relationships and the origin of the flower. Often these attempted to identify what group was “ancestral” to the angiosperms, a cladistically invalid concept: only a species in the geologic past can be ancestral to a clade, and this is seldom if ever distinguishable from a plesiomorphic sister group; ancestral groups are paraphyletic and would be broken up into monophyletic units in a cladistic classification. A more appropriate question is which other taxa are the sister group and the next-closest outgroups of the angiosperms. Many precladistic hypotheses have been eliminated by both morphological and molecular phylogenetic analyses (e.g., those postulating that angiosperms are polyphyletic), whereas others have reappeared in various cladistic studies. Here I concentrate on results of phylogenetic analyses and restrict comments on precladistic hypotheses to those that have been supported by phylogenetic analyses.

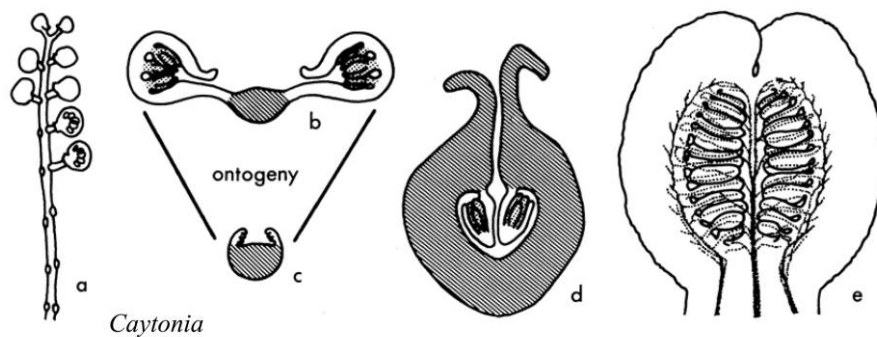
The first phylogenetic analysis of both living and fossil seed plants was that of Crane (1985a), who concluded that the closest relatives of angiosperms were living Gnetales and Mesozoic Bennettitales and *Pentoxylon* (both with more or less “cycadlike” leaves). Crane pointed out that all these plants have fertile parts grouped into flowerlike structures. His results implied that the flower originated long before the origin of angiosperms, in their common ancestor with Gnetales, Bennettitales, and *Pentoxylon*, which led to proposal of the name “anthophytes” (previously used as a synonym of angiosperms) for the whole clade (Crane 1985b; Doyle and Donoghue 1986). This is essentially an updated version of the hypothesis of Arber and Parkin (1907), who postulated that angiosperms were related to Gnetales and Bennettitales (*Pentoxylon* was unknown at the time) and derived from a common ancestor with a “proanthostrobilus” consisting of an axis bearing perianth-like sterile appendages, microsporophylls, and megasporophylls, in that order.

Equally important for homologies of floral parts, however, are the taxa that Crane’s (1985a) analysis identified as outgroups of the anthophytes—three groups of so-called Mesozoic seed ferns, namely, glossopterids (actually Permian), *Caytonia*, and crustosperms. This result appeared to support a hy-

pothesis proposed by Gaussen (1946) and adopted by Stebbins (1974) and Doyle (1978; fig. 1A), based on *Caytonia*. The female structures of *Caytonia* (to which the name was first applied; Thomas 1925) consisted of an axis bearing fleshy multiovulate structures termed cupules, which Harris (1940, 1951) and Reymanówna (1973) interpreted as a rachis bearing two rows of leaflets with ovules on their adaxial surface. The cupules are folded tip to base in an anatropeous fashion, like the bitegmic ovules of angiosperms. They could be transformed into a structure like a bitegmic ovule by reduction from several ovules to one. In other words, what is conventionally called an ovule in angiosperms is actually a cupule containing one ovule. The most serious problem is explaining the carpel: the structure bearing the cupules in *Caytonia* is narrow and rachislike, not a flat, leaflike structure with marginal or adaxial cupules that could be folded to enclose the cupules. In Doyle (1978), I suggested that the carpel was derived from the rachis, which was presumably larger relative to the cupules early in development, by paedomorphic retention of its juvenile proportions followed by folding to enclose the cupules. This general scenario was adopted by Crane (1985a).

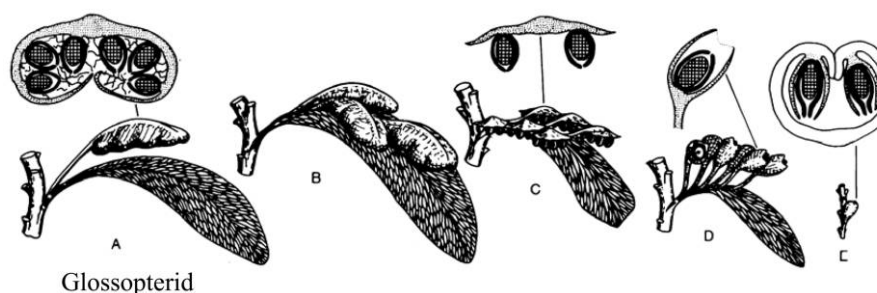
Stebbins (1974) actually favored glossopterids over *Caytonia* as the closest relatives of angiosperms. This concept was anticipated by Melville (1963), on the basis of subsequently refuted reconstructions of glossopterids, and elaborated by Retallack and Dilcher (1981; fig. 1B). Glossopterids had one or several cupules (also called sporophylls) attached to the midrib of a more or less unmodified leaf, together called a fertiler, bract-sporophyll complex, or leaf-cupule complex (Schopf 1976; Pigg and Trivett 1994; Doyle 1996, 2006). Here I use the term “leaf-cupule complex” (as in Doyle 2006). The cupules could be transformed into bitegmic ovules by reduction in ovule number, as in the *Caytonia* hypothesis, but the leaf/bract would provide a better precursor for the carpel: as Stebbins (1974) emphasized, it would not have to be enlarged, just folded around the cupule. This hypothesis would predict that angiosperm bitegmic ovules were originally attached to the midrib or adaxial base of the carpel rather than on or near its margins, the position that most earlier authors assumed was ancestral.

Subsequent morphological cladistic analyses differed in many respects, but they all associated Gnetales and Bennettitales with angiosperms. Doyle and Donoghue (1986) obtained trees like those of Crane (1985a) but with Gnetales closer to Bennettitales than to angiosperms. In the analysis of Nixon et al. (1994), angiosperms were nested within Gnetales as the sister group of *Welwitschia* and *Gnetum*, with *Chloranthus* and *Ceratophyllum* basal in some most parsimonious trees and *Casuarina* and other Fagales basal in others. Anthophytes as a whole were associated with coniferophytes rather than with Mesozoic seed ferns, a result also obtained by Rothwell and Serbet (1994). Trees with angiosperms nested in Gnetales were also obtained by Hickey and Taylor (1996). As discussed by Doyle (1994) and Hickey and Taylor (1996), these results would imply that angiosperm flowers were originally simple and derived from simple flowers like those of Gnetales and that more complex flowers of other angiosperms were derived either by multiplication and elaboration of parts or by grouping of simple flowers into pseudanthia, as proposed by von



*Caytonia*

A (Doyle 1978)



Glossopterid

B (Retallack & Dilcher 1981)

**Fig. 1** A, Proposed homologies between ovulate structures of *Caytonia* and angiosperms, from Doyle (1978), following Gausson (1946) and Stebbins (1974). B, Proposed transformation of the ovulate leaf-cupule complex of glossopterids into an angiosperm carpel, from Retallack and Dilcher (1981). See text for discussion.

Wettstein (1907). In the analysis of Doyle (1996), Gnetales were the closest living relatives of angiosperms, but *Caytonia* was their sister group rather than being situated lower as an outgroup of anthophytes. This would support the homology of the *Caytonia* cupule and the bitegmic ovule, but it would challenge the hypothesis that flowers are homologous in angiosperms, Bennettitales, and Gnetales, since the large sporophylls of *Caytonia* were probably not grouped into anything resembling a flower.

All these hypotheses have been thrown into doubt by molecular analyses. Molecular studies say nothing directly about the relationships of fossil taxa, but they do address the hypothesis that Gnetales are the closest living relatives of angiosperms. All analyses have indicated that Gnetales are monophyletic, thus refuting the hypothesis that angiosperms are nested in Gnetales (Nixon et al. 1994; Hickey and Taylor 1996). A few analyses of ribosomal DNA have supported a relationship of angiosperms and Gnetales, but only weakly (Hamby and Zimmer 1992; Rydin et al. 2002), and analyses of other genes and sequences of several genes combined, some from all three genomes, give different trees (see reviews in Donoghue and Doyle 2000; Magallón and Sanderson 2002; Burleigh and Mathews 2004; Qiu et al. 2007). In some molecular trees, Gnetales are the sister group of all other seed plants, and angiosperms are linked with cycads, *Ginkgo*, and conifers. This arrangement is implausible from a stratigraphic point of view, considering that cycads, ginkgophytes, and conifers extend

back to the Late Carboniferous or Permian but Gnetales and angiosperms are not known until the Mesozoic–Late Triassic for probable stem relatives of Gnetales and the Early Cretaceous for crown-group Gnetales and angiosperms (Crane 1988, 1996; Doyle 1996). However, in an increasing number of multigene analyses, Gnetales are related to conifers, as often proposed before cladistics (Bailey 1949; Eames 1952; Bierhorst 1971; Doyle 1978), and angiosperms are the sister group of all living gymnosperms. In most such trees, as first shown by Bowe et al. (2000), Gnetales are nested within conifers, linked with Pinaceae—the so-called gnepine hypothesis.

The paucity of molecular support for a relationship between Gnetales and angiosperms casts strong doubt on the anthophyte hypothesis, but the variety of molecular trees might be taken as a caution against molecular triumphalism. However, more sophisticated methods are showing progress toward sorting out which alternatives are most likely. First, the branches leading to and within Gnetales are unusually long (as measured by numbers of nucleotide substitutions), suggesting that rates of molecular evolution accelerated in this line. This suggests that trees in which Gnetales are basal might be a result of long-branch attraction, specifically between Gnetales and the stem lineage of seed plants, due to reversals of seed plant synapomorphies on the stem lineage of Gnetales. Second, the signal favoring the basal position of Gnetales is primarily in third-codon positions, which evolve faster because they usually do not affect amino acid composition.

These conjectures have been supported by maximum likelihood analyses (Sanderson et al. 2000; Magallón and Sanderson 2002; Soltis et al. 2002; Burleigh and Mathews 2004), which should theoretically be less sensitive than parsimony analysis to long-branch effects. Specifically, most data sets that yield Gnetales-basal trees when analyzed with parsimony give gnetpine trees when analyzed with likelihood methods. Many molecular systematists think that this issue is now resolved; this may be premature, but morphologists and paleobotanists should consider seriously what the molecular results imply if they are correct. It is in this spirit that I undertook the analyses presented in this article.

First, some general remarks about what gnetpine trees do not imply may be useful. They do not mean that angiosperms and gymnosperms were separately derived from Devonian “progymnosperms” or that angiosperms extend back to the Carboniferous. There could be any number of extinct seed plant lines attached to the stem lineage leading to living seed plants and to the lineage leading to angiosperms. Such plants would be called “gymnosperms” under the traditional typological definition “plants with naked seeds,” which led Cantino et al. (2007) to propose the more restrictive name *Acrogymnospermae* for the molecular-based crown clade that includes all living gymnosperms. In the phylogenetic nomenclature of Cantino et al. (2007), the “gymnospermous” stem relatives of angiosperms would be members of the stem-based clade *Pan-Angiospermae* but not of *Angiospermae*, a name restricted to the crown clade. Identification of angiosperm stem relatives is essential for understanding the origin of features such as the carpel and the flower; living acrogymnosperms are so divergent from angiosperms (and each other) in so many characters that they can say very little about the origin of characteristic angiosperm structures.

The only way to identify fossil stem relatives of angiosperms is by consideration of their morphological characters, preferably analyzed with cladistic methods. This is a daunting task in light of the apparent failure of morphological cladistics to infer the correct relationships of Gnetales. Scotland et al. (2003) argued that such cases mean that morphological cladistics is obsolete for phylogeny reconstruction (which would effectively preclude integration of fossils into phylogenies; Wiens 2004). However, this viewpoint is overly pessimistic. The fact that morphological cladistics was wrong about angiosperms and Gnetales does not mean that it was wrong about everything or that it was no improvement over precladistic reasoning. In fact, morphological cladistics provided answers to many previously controversial questions that have been confirmed by molecular data. Before cladistics, it was often suggested that coniferophytes and other seed plants (cycadophytes) were separately derived from progymnosperms, but both morphological and molecular analyses indicate that coniferophytes are nested within other seed plants. It was widely claimed that angiosperms, Gnetales, and conifers were polyphyletic (with Taxaceae not related to other conifers), but both kinds of analysis agree that these taxa are monophyletic (if conifers are defined so as to include Gnetales if the latter are nested within them, as in the definition of Cantino et al. 2007). The morphological analysis of Doyle and Endress (2000) failed to recover some relationships inferred from molecular data and morphological and molecular data combined

(e.g., placement of Nymphaeales in the basal “ANITA” grade), but it did find several groups that had not been recognized (Winteraceae with Canellaceae, Aristolochiaceae and *Lactoris* with Piperales) or had been differently constituted (Magnoliales and Laurales) before cladistic studies. The recent discovery that Hydatellaceae are related to Nymphaeales rather than to monocots was supported by both molecular and morphological cladistic analyses (Saarela et al. 2007). Examples could be multiplied from other taxa.

### *Objectives and Approach*

Most of this article is based on my recent phylogenetic analysis of seed plants (Doyle 2006), concentrating on its implications for the origin of the flower and its parts and discussing some aspects not previously treated. However, I also incorporate more recent advances in understanding of basal angiosperms and Gnetales, bring up to date the discussion of developmental genetic evidence on homologies of the angiosperm ovule, and evaluate the suggestion that the Early Cretaceous fossil *Archaeofructus* (Sun et al. 1998, 2002) is a stem relative of living angiosperms. Problems concerning the origin of other angiosperm features, such as leaf morphology, pollen, and siphonogamy, are discussed in Doyle (2006).

One of my goals in Doyle (2006) was to evaluate whether the conflict between molecular and morphological evidence on the relationships of angiosperms and Gnetales was as strong as it seems. I began with my previous seed plant data set (Doyle 1996), itself an update of that of Doyle and Donoghue (1986), and modified it in several ways. Some modifications were based on doubts concerning homologies of characters that seemed to link angiosperms and Gnetales (Donoghue and Doyle 2000). For example, I had coded both taxa as having a tunica layer in the apical meristem, but in angiosperms the tunica is two cells thick, whereas in Gnetales it is only one cell thick. This could be evidence that the two conditions are not homologous. I therefore redefined this character as having three unordered states (tunica absent, one cell thick, and two cells thick), so it is equally parsimonious for the two tunica types to arise independently or one from the other. In addition, new similarities had been recognized between Gnetales and conifers. Carlquist (1996) showed that Gnetales are like conifers and *Ginkgo* in having a torus in the pits on their vessel walls. Conifers have a distinctly tiered stage in embryo development, which I had scored as absent in Gnetales, but actually, embryos of Gnetales are more or less tiered at a comparable stage (Martens 1971; Singh 1978). Reassessments of characters that are more directly related to the flower problem, especially potential homologies of different types of cupules and the angiosperm bitegmic ovule, are considered in the “Discussion.”

Other changes in Doyle (2006) were related to molecular results concerning the rooting of the angiosperms. The taxon sampling of angiosperms in Doyle (1996) included two Magnoliales (now placed well above the base of the angiosperms—seven nodes higher in the combined analysis of Doyle and Endress [2000]) but only two members of the basal ANITA grade, not including *Amborella*. In Doyle (2006), I replaced these with a more up-to-date sampling of ANITA groups and mesangiosperms.

Here I also add Hydatellaceae, formerly thought to be poalian monocots (placed in Centrolepidaceae until studies by Hamann [1975, 1976]) but now linked with Nymphaeales (Saarela et al. 2007). The reproductive structures of these minute aquatics appear to be inflorescences of unisexual flowers consisting of one stamen or one carpel. Except for their linear leaves, most morphological features of Hydatellaceae resemble those of Nymphaeales rather than those of Poales: the stomata are anomocytic rather than paracytic, the pollen is monolacinate rather than porate, and the seed has a minute embryo, starchy perisperm, a palisade exotesta, and an operculum formed by enlargement of cells of the inner integument, as in Nymphaeales. The carpels are ascidiate, and the embryo sac is four-nucleate (Hamann 1975; Friedman 2008), as in most other members of the ANITA grade (Williams and Friedman 2004). Because Stevens (2007) has enlarged Nymphaeales to include Hydatellaceae, I henceforth refer to Nymphaeales in the older sense of Cabombaceae and Nymphaeaceae as “core Nymphaeales.”

Consideration of *Archaeoфраuctus* is an outgrowth of a collaboration with Peter Endress (Doyle and Endress 2007) on expansion and revision of the morphological data set of Doyle and Endress (2000) and its use to evaluate the position of Early Cretaceous fossils in the phylogeny of living angiosperms. Preliminary results indicate that the most parsimonious position for *Archaeoфраuctus* within angiosperms is in Nymphaeales, linked with Hydatellaceae (Doyle and Endress 2007; Endress and Doyle, forthcoming), but because this analysis includes only angiosperms, it does not address the hypothesis that *Archaeoфраuctus* is a stem relative of angiosperms. Inclusion of *Archaeoфраuctus* in the present study is intended to test this hypothesis. Because the morphology of the fertile shoot of *Archaeoфраuctus* is controversial, I have analyzed the position of *Archaeoфраuctus* using two alternative sets of character scorings, one assuming that the shoot is a bisexual flower or preflower (Sun et al. 2002) and the other assuming that it is an inflorescence of reduced unisexual flowers (Friis et al. 2003).

### Material and Methods

The data set is presented in the appendix in the online edition, with a list of characters and their states and changes from Doyle (2006). For documentation and reasoning behind character definitions and taxon scorings that have not been modified, readers are referred to Doyle (2006). Some modifications and decisions on scoring of Hydatellaceae and *Archaeoфраuctus* are treated in the appendix, but those requiring more explanation or argumentation are discussed in this section. As in Doyle (2006), most characters concerning inflorescence and floral organization and the morphology of stamens, carpels, and whole bitegmic ovules and seeds derived from them have been scored as unknown outside the angiosperms because they cannot be applied without making questionable assumptions on homology.

Several characters have been added in order to express previously uninformative features of core Nymphaeales, Hydatellaceae, and *Archaeoфраuctus* or as a result of ongoing reevaluation of characters by J. A. Doyle and P. K. Endress (unpublished data). Some are characters from Doyle and Endress (2000) that would

have been autapomorphies of core Nymphaeales in Doyle (2006) but also occur in Hydatellaceae: protoxylem lacunae, absence of vascular cambium, boat-shaped pollen, and operculum in the seed. A new character is presence or absence of floral bracts, which are absent in *Archaeoфраuctus* (as stressed by Sun et al. [2002]) and Hydatellaceae but present in all other angiosperms in this data set.

Other changes are reorganizations of characters and states based on unpublished work of J. A. Doyle and P. K. Endress. Lack of fibers or sclerenchyma in the pericyclic region was treated as a state of the pericycle character by Doyle and Endress (2000) but eliminated in Doyle (2006) because it was an autapomorphy of core Nymphaeales. Because this condition also occurs in Hydatellaceae, I have reinstated it but as a separate character, on the presumption that loss of sclerenchyma deserves recognition as a major change independent of transitions between the two patterns of fiber and sclerenchyma distribution. I split leaf phyllotaxis, formerly a single three-state character, into two binary characters, one for alternate versus opposite and the other for presence of at least some axes with distichous phyllotaxis, on the presumption that the distinction between opposite and alternate phyllotaxis is more fundamental than variation between spiral and partly or wholly distichous. I also split the stigma surface character, previously defined as papillae (0) unicellular only (or stigma smooth), (1) some or all uniseriate pluricellular, or (2) some or all pluriseriate pluricellular (including multicellular protuberances). Structures described as pluriseriate papillae intergrade with protuberances that are not obviously papillar, and uniseriate papillae and protuberances occur together in *Amborella* and *Trimenia* (Endress and Igersheim 1997), suggesting that they represent independent transformations. I have also redefined the uni- and pluricellular papilla types to refer to the emergent portion of the papilla, which appears to be a more consistent distinction. Other changes represent adjustments to the reduced taxon sampling in angiosperms, such as simplification of the carpel sealing and fruit wall characters.

I made several changes in floral characters to reduce problems and bring definitions into line with analyses of Eklund et al. (2004), Endress and Doyle (2007), and J. A. Doyle and P. K. Endress (unpublished data). In Doyle (2006), I recognized four perianth characters: phyllotaxis, number of whorls, merism (=merosity), and differentiation of the outer cycle. These are all informative with a larger taxon sample but are less so with the present reduced number of angiosperms and not entirely independent of each other. Thus, irregular merism overlaps with spiral phyllotaxis. If irregular is eliminated as a state of the merism character and spiral taxa are scored as unknown (for inapplicable), merism becomes uninformative: among whorled taxa in the data set, the only departures from trimery are dimery in Winteraceae and polymery in the *Barclaya*-Nymphaeoidae clade nested in core Nymphaeales. Similarly, number of whorls is problematic in spiral taxa, and among whorled taxa the only deviations from having more than two whorls and no perianth occur in some but not all members of taxa (two whorls in Cabombaceae but more in Nymphaeaceae, one in *Hedyosmum* but none in other Chloranthaceae, and one or two in Asaroideae). I therefore replaced these three characters with one character for presence or absence of perianth and one for spiral or whorled phyllotaxis. The perianth dif-

ferentiation character of Doyle (2006), which distinguished outer cycle not differentiated or forming a continuum with inner parts from sepaloïd, was too closely tied to phyllotaxis, since spiral perianths tend to show a continuity of forms and whorled ones more abrupt transitions (Endress and Doyle 2007; Endress 2008). I therefore modified this character to distinguish all perianth parts sepaloïd from outer sepaloïd and inner petaloïd. Because this distinction can be applied only to flowers with more than one perianth cycle, I scored Chloranthaceae (one cycle or none) and Asaroideae (one cycle or two) as 0/1.

Similarly, I modified androecium characters by adding a character for one stamen per flower, as in Chloranthaceae (reconstructed as ancestral in the family by Eklund et al. [2004]) and Hydatellaceae, and deleting androecium merism. If irregular merism is eliminated as redundant with spiral phyllotaxis, the only deviation from the trimerous state is polymery in Winteraceae.

Outside angiosperms, the only changes in scoring are based on the reinterpretation of reproductive structures in Gnetales by Mundry and Stützel (2004). In a developmental study, these authors concluded that the cup bearing six microsyngangia in the male “flower” of *Welwitschia*, previously interpreted as either six simple sporophylls or two pinnate sporophylls, actually consists of two lateral strobili, each with an apical meristem and three simple sporophylls. They interpreted the male “flower” of *Ephedra* as consisting of two similar units with four simple sporophylls, and they proposed plausible homologies with parts in the female “flowers” of both genera. I have therefore rescored microsporophyll morphology as simple (1) rather than either pinnate/paddle shaped or simple (0/1). Mundry and Stützel argued that their interpretation may also apply to *Gnetum*, but because this has not been confirmed, I have scored this character in *Gnetum* as unknown. This reinterpretation also affects the microsporophyll fusion character, previously scored as fused. The two lateral strobili are fused basally into a cup, but because it is unclear whether the individual microsporophylls are fused to each other or borne separately around the apex, I have rescored this character as unknown in all Gnetales. Under the Mundry and Stützel interpretation, the number of sporangia per microsporophyll in *Ephedra* is usually two, as in Pinaceae and Podocarpaceae, not more than two, as scored in Doyle (2006). However, because some species have more than two microsporangia (Martens 1971; Hufford 1996) and the ancestral number is not established (S. Ickert-Bond, personal communication), I have rescored *Ephedra* as uncertain (0/1) for this character.

Although I did not score presence or absence of a perianth outside angiosperms, I attempted to address the problem of its origin by defining a related character applicable across seed plants, which I did not use in the actual phylogenetic analyses because of problems in homology of the fertile organs (e.g., sporophylls in some taxa may correspond to cupules in others). If specialized, determinate, fertile shoots in other seed plants are considered potentially homologous with angiosperm flowers, some taxa have shoots bearing only sporophylls that might be compared with flowers that lack a perianth, namely, Ginkgoales and probably peltasperms (*Autumia* and *Peltaspermum*), which had paddle-shaped or peltate spo-

rophylls borne on axes with no other appendages (Townrow 1960; Kerp 1988). Other taxa have fertile shoots with basal cataphylls or other sterile appendages that might be compared with a perianth, such as fertile branches of corystosperms (Axsmith et al. 2000; Klavins et al. 2002), axillary fertile short shoots of cordaites and Paleozoic conifers such as *Lebachia* and *Emporia* (Florin 1951; Mapes and Rothwell 1984), male and possibly female “flowers” of *Pentoxylon* (Bose et al. 1985), and “flowers” of most but not all Bennettitales (Crane 1985a, 1988). However, there are other fossil seed plants that lack strobili or flowers, to which this distinction is difficult or impossible to apply. In some, the seeds were borne on normal leaves (medullosans and *Callistophyton*; Rothwell 1981); in others, they were borne on appendages that differed from vegetative leaves but were probably not grouped into special strobili or flowers (glossopterids and *Caytonia*). Cycads are a special case. Female plants of the basal genus *Cycas* produce regular leaves, cataphylls, and megasporophylls in alternating zones along the stem, but male *Cycas* plants and all plants of the other genera produce strobili, with sterile cataphylls on the peduncle in *Cycas*, *Dioon*, *Encephalartos*, *Lepidozamia*, and *Macrozamia* (Hermsen et al. 2006; D. W. Stevenson, personal communication), which are relatively basal, according to molecular analyses (Rai et al. 2003; Chaw et al. 2005).

I expressed these distinctions with three unordered states: (0) fertile appendages not borne on special shoots, (1) special fertile shoots without sterile appendages (no perianth), and (2) special fertile shoots with sterile appendages (perianth). I scored extant conifers as unknown, on the assumption that their cone scales are derived from whole fertile short shoots, and Bennettitales as 1/2. To test implications of the assumption that glossopterids and *Caytonia* did not have flowers or strobili, I scored them as 0. I considered two alternative scorings of cycads, to take into account the different conditions in male and female plants of *Cycas*. One (0/2) assumes that it would be most appropriate to score *Cycas* as having the nonstrobilar condition of the female plants, and the other (2) assumes the strobilar condition of the male plants. Although the interpretation of *Ephedra* and *Welwitschia* by Mundry and Stützel (2004) implies that the male strobili lack sterile appendages, I scored Gnetales as having special fertile shoots with sterile appendages, on the basis of the presence of an outer envelope around the ovule in the female “flowers.”

Scoring of Hydatellaceae generally follows that by Saarela et al. (2007), who added Hydatellaceae and two poalian monocots to the morphological data set of Doyle and Endress (2000), along with characters for porate pollen (not represented in the present data set) and four- versus eight-nucleate embryo sac (already used by Doyle [2006]; confirmed by Friedman [2008]). Data on general morphological characters were from Hamann (1975, 1976); those on vegetative anatomy were from Cutler (1969), Cheadle and Kosakai (1975), and Behnke (2000); those on pollen were from Linder and Ferguson (1985); and those on seedlings were from Cooke (1983). Unless otherwise indicated, scorings of Saarela et al. (2007) are followed for those characters that occur in the present data set. Scoring of seed plant characters that were not included by Saarela et al. is based on the same references. Rudall et al. (2007) provided valuable new data on several characters, for example, showing that the inflorescences are reduced thyrses (indeterminate

axes bearing lateral cymes). Many characters are scored as unknown in Hydatellaceae because the plant body is so reduced that state distinctions are inapplicable or questionable (vegetative buds, short shoots, nodal anatomy, leaf teeth, laminar venation, and characters of secondary xylem and phloem).

Scoring of *Archaeofructus* is based on descriptions of *Archaeofructus liaoningensis* and *Archaeofructus sinensis* by Sun et al. (1998, 2002), with confirmation of paired stamens and carpels by Friis et al. (2003). One of the two alternative scorings (*Archaeofructus* inf), following Friis et al. (2003), assumes that the fertile shoot was a raceme of male and female flowers consisting of usually two stamens and one or two carpels. The other (*Archaeofructus* flo), following Sun et al. (2002), assumes that the shoot was a bisexual flower or pre-flower with paired stamens below and carpels above. Sun et al. (2002) speculated that the apparent paired stamens may actually be single, branched sporophylls, simplified from the pinnately branched sporophylls of many fossil seed plants but less so than stamens of living angiosperms. To acknowledge and test this intriguing hypothesis, I have scored microsporophylls of *Archaeofructus* as pinnate under the flower interpretation. Morphology of *Archaeofructus eoiflora* (Ji et al. 2004), which may represent either a smaller species or a younger stage of *A. sinensis*, is generally consistent with that of *A. liaoningensis* and *A. sinensis*. Ji et al. (2004) interpreted seeds of *A. eoiflora* as orthotropous, but the orientation is not clear in their illustrations. Figure 2C of Sun et al. (1998), showing the end of a seed of *A. liaoningensis*, is more suggestive of an anatropous ovule.

In their cladistic analysis, Sun et al. (2002) scored the finely dissected leaves of *Archaeofructus* as having one order of dichotomous laminar venation, as in cycads (except *Bowenia*) and *Ginkgo*, which, along with *Ephedra* and *Pinus*, were the outgroups in their data set. Friis et al. (2003) compared the ternate organization with that of Ranunculales and the Early Cretaceous fossil *Vitiphyllum*, but in the present data set it is most similar to the pinnately compound condition of Paleozoic seed ferns. To preserve the spirit of the comparisons of Sun et al. (2002) as well as possible in terms of the present data set and to test their implications, I have scored the leaves as pinnately compound with one order of open dichotomous laminar venation.

Judging from the figures of Sun et al. (2002), the stamens of *Archaeofructus* may have a typical angiosperm morphology, but this is not well enough demonstrated to score microsporangial position, number, fusion, and dehiscence. Although Friis et al. (2003) questioned whether the bodies that Sun et al. (2002) described as pollen were in fact pollen grains, because of their irregular size and shape, I have provisionally assumed that they are pollen and scored them, on the basis of the most convincing figure (fig. 2F) in Sun et al. (2002), as boat shaped and monosulcate, with a continuous tectum and unknown infratectal structure. It is unclear whether the ovules were anatropous or orthotropous, so I have scored this and related characters as unknown. Sun et al. (1998, 2002) described the carpels as conduplicate (=plicate), but in extant carpels of similar appearance this cannot be determined without developmental or anatomical evidence (Friis et al. 2003; Endress 2005). Sun et al. (1998, 2002) described the fruits as

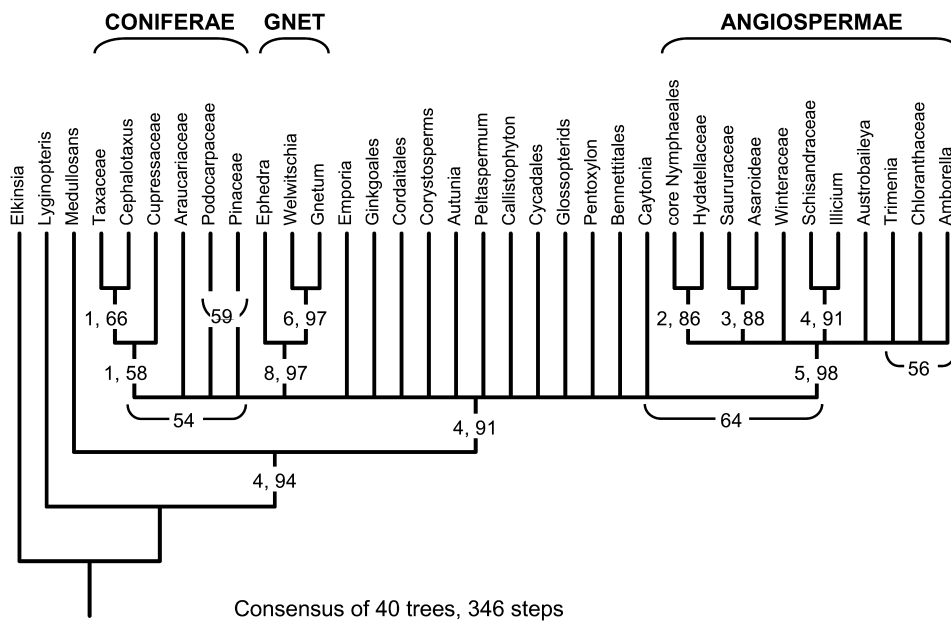
follicles, but they did not actually report dehiscence. The seeds appear to have a palisade exotesta; Sun et al. (1998, 2002) described the surface as consisting of epidermal cells with cutinized anticlinal and periclinal walls.

Searches for most parsimonious trees used PAUP (Swofford 1990), with 100 heuristic search replicates, stepwise random addition of taxa, and tree bisection reconnection branch swapping. In all cases, the Late Devonian seed fern *Elkinsia* (Serbet and Rothwell 1992) was specified as outgroup. The relative parsimony of alternative arrangements was evaluated by searching for trees consistent or inconsistent with appropriate constraint trees or by moving taxa manually with MacClade (Maddison and Maddison 2003). Many of these analyses involved “backbone constraint” trees of extant taxa and *Elkinsia* only; fossil taxa attach to this backbone at the most parsimonious location in terms of their morphology. Bootstrap analyses were performed with PAUP, using 1000 bootstrap replicates, with each replicate consisting of one heuristic search with closest taxon addition sequence, holding five trees at each step, and retaining 100 trees per replicate but allowing branch swapping to continue beyond this limit in order to increase the probability of finding shorter trees. Decay analyses (Bremer support; Bremer 1988; Donoghue et al. 1992) were conducted by searching for trees that were one, two, three, or (when memory was sufficient) four steps longer than the shortest trees and observing which clades disappeared in the resulting strict consensus. Decay values for the few groups that remained after these searches were determined by searching for shortest trees not consistent with a constraint tree in which the group formed a clade but other relationships were unresolved.

I performed three main sets of analyses. The first were unconstrained parsimony analyses of the data set without *Archaeofructus*. Second, to test the implications of molecular trees for the angiosperm question, were constrained analyses with living taxa fixed in the arrangement seen in trees of the gnepine type (Bowe et al. 2000). Third were analyses including *Archaeofructus*, with extant taxa constrained to the molecular arrangement. The relative parsimony of other positions of *Archaeofructus* was evaluated by searching for trees that were one to three or four steps less parsimonious, filtering the resulting trees with constraint trees to determine different sorts of relationships obtained, or by moving *Archaeofructus* manually to other positions with MacClade (Maddison and Maddison 2003). I repeated most analyses without Hydatellaceae and with appropriately modified constraints to evaluate the effects of addition of this group. I used MacClade (Maddison and Maddison 2003) to reconstruct character evolution by parsimony optimization of characters on trees and to identify characters that support various clades and alternative arrangements of taxa.

## Results

The unconstrained analysis of the new data set without *Archaeofructus* yielded 40 most parsimonious trees of 346 steps (strict consensus in fig. 2, with bootstrap and decay values). These form two islands, of 24 and 16 trees, that differ most significantly in the relationships of angiosperms and Gnetales.



**Fig. 2** Strict consensus of 40 most parsimonious trees of 346 steps obtained from the unconstrained analysis, with decay and bootstrap support values for nodes. Numbered brackets indicate clades with bootstrap values of >50% that did not occur in the consensus of most parsimonious trees (e.g., *Caytonia* plus angiosperms). GNET = Gnetales.

Trees of the first island (consensus in fig. 3A) are of the anthophyte type, with *Pentoxylon*, Gnetales, Bennettiales, and angiosperms linked with *Caytonia*, glossopterids, and the whole combined clade nested in coniferophytes. *Pentoxylon* and Gnetales form either two successive branches or a clade. There are two different rootings of the angiosperms. In all trees, Winteraceae are linked with a “paleoherb” clade consisting of core Nymphaeales plus Hydatellaceae (Nymphaeales) and Saururaceae plus Asaroideae (Piperales). In 16 trees, the Winteraceae-paleoherb clade is sister to the remaining angiosperms, with *Illicium* plus Schisandraceae, *Austrobaileya*, *Trimenia*, Chloranthaceae, and *Amborella* branching pectinately in that order. In the other eight trees, *Amborella* is basal, followed pectinately by Chloranthaceae, *Trimenia*, *Austrobaileya*, *Illicium* plus Schisandraceae, Winteraceae, and the paleoherb clade (fig. 3B).

In trees of the second island (consensus in fig. 3C), Gnetales are nested within conifers, as in many molecular analyses, but are linked either with Araucariaceae or with Araucariaceae, Cupressaceae (including Taxodiaceae), *Cephalotaxus*, and Taxaceae rather than with Pinaceae. The closest outgroups of angiosperms are glossopterids, *Pentoxylon*, Bennettiales, and *Caytonia*, with *Caytonia* the sister group of angiosperms. Nymphaeales (including Hydatellaceae) and Piperales are either two successive basal branches or a basal clade, and Winteraceae are sister to the remaining taxa.

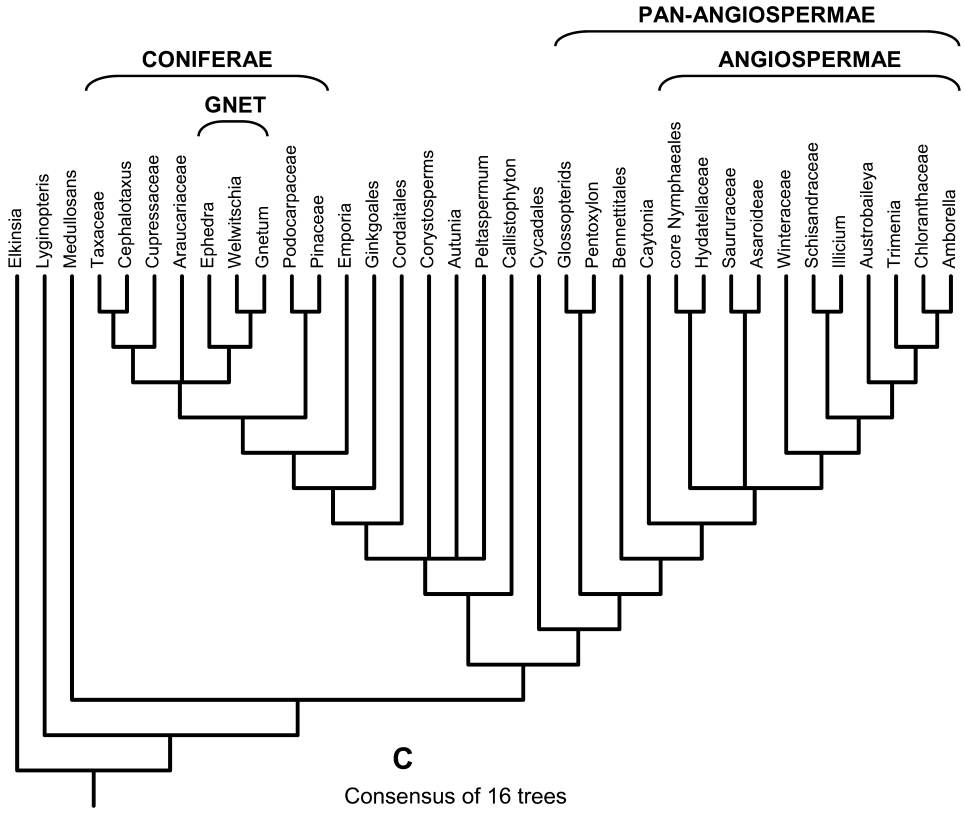
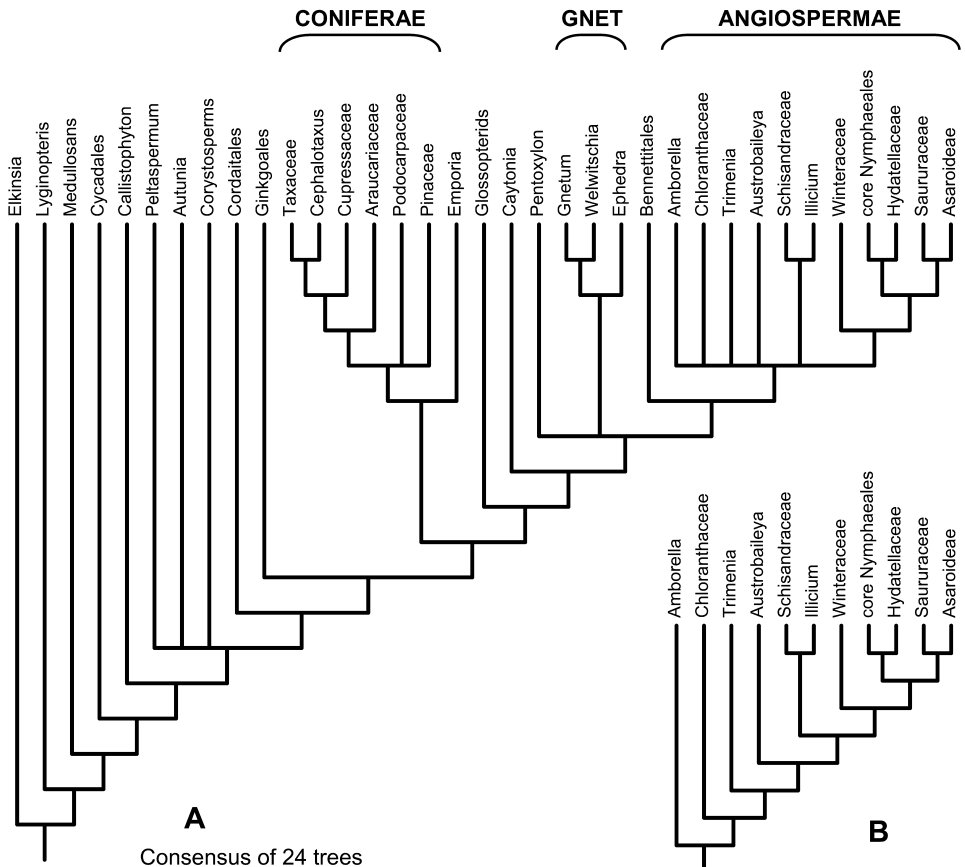
An unconstrained analysis with Hydatellaceae removed yielded 56 trees of 330 steps, which represent two islands of 40 and 16 trees. Outgroup relationships in the two islands are the same as those found when Hydatellaceae were included (fig. 3A, 3C). In the first island, core Nymphaeales and Piperales form a paleoherb clade, but Winteraceae are not always linked with this clade; again, *Amborella* is basal in eight trees.

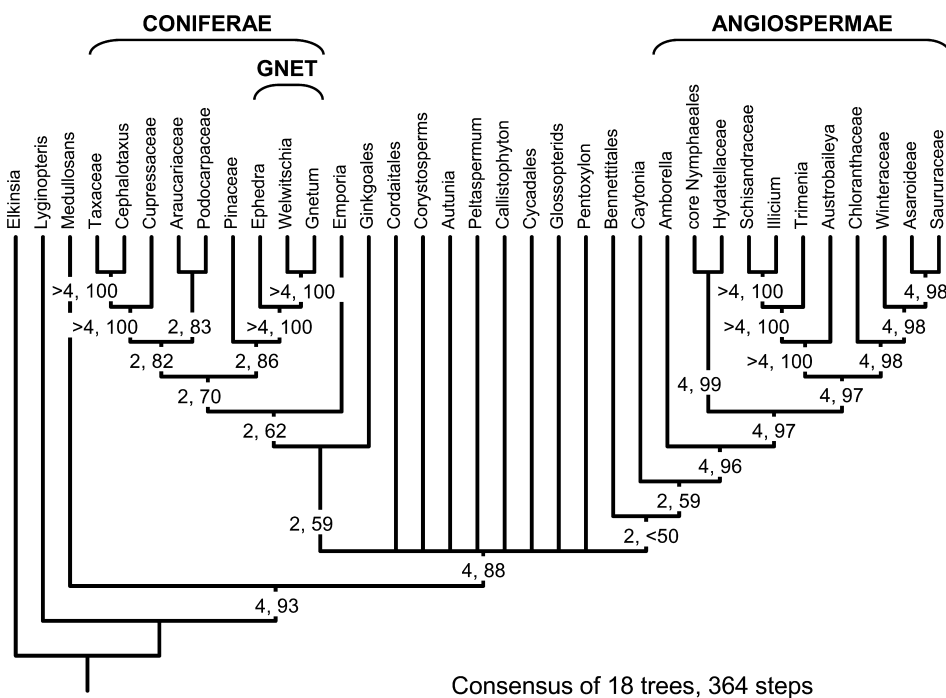
In the second island, relationships among angiosperms in the taxon set are the same as those found when Hydatellaceae were included (fig. 3C).

The analysis with extant taxa constrained to the molecular (gnepine) arrangement resulted in 18 trees of 364 steps. In the strict consensus (fig. 4), relationships at the base of the crown clade that includes all living seed plants are poorly resolved. This is a function of the existence of two islands, of six and 12 trees, and the “jumping” of *Callistophyton* between very different positions in trees of the second island. In the first island (representative tree in fig. 5A, with leaf organization optimized on the branches), the outgroups of angiosperms are the same as in the unconstrained trees where Gnetales were nested in conifers (fig. 3C): *Caytonia* is the sister group of the angiosperms, Bennettiales are the second outgroup, and a clade consisting of glossopterids and *Pentoxylon* is basal in the pan-angiosperms. All variation among trees concerns the arrangement of cordaites and the platyspermic seed fern taxa *Callistophyton*, conyosperms, *Autunia*, and *Peltaspernum* within the acrogymnosperm clade. In the second island (fig. 5B), relationships among the platyspermic seed ferns are still more unstable, with *Callistophyton* attached below the common ancestor of all living seed plants in five trees and nested among the other taxa in the other seven. However, all trees in this island have the same arrangement at the base of the acrogymnosperms, with cycads, *Pentoxylon*, and glossopterids diverging pectinately in that order. The comparable constrained analysis without Hydatellaceae yielded 18 trees of 349 steps, with the same outgroup relationships found when Hydatellaceae were included (figs. 4, 5).

The analyses including *Archaeofructus*, with relationships among living taxa constrained to the molecular arrangement, support the view that *Archaeofructus* is a crown-group angiosperm







**Fig. 4** Strict consensus of 18 most parsimonious trees of 364 steps obtained from the analysis with the arrangement of living taxa fixed to a molecular backbone constraint tree, with decay and bootstrap support values. GNET = Gnetales.

related to Nymphaeales. However, the relative parsimony of alternative relationships varies with the two interpretations of the reproductive structures. Characters supporting these results are most conveniently presented in the “Discussion.”

When *Archaeoфраuctus* was scored as having an inflorescence of unisexual flowers, the analysis yielded 18 trees of 371 steps, in which *Archaeoфраuctus* is the sister group of Hydatellaceae (fig. 6). Otherwise, these trees are identical to those found without *Archaeoфраuctus* (figs. 4, 5). The next-best positions for *Archaeoфраuctus* are as sister to core Nymphaeales, which is one step less parsimonious (372 steps), and as sister to both Hydatellaceae and core Nymphaeales, which is two steps less parsimonious. A position as sister to all living angiosperms is five steps less parsimonious (376 steps). Several positions near the base of crown-group seed plants (which would imply that the seed-containing structures are convergent with angiosperm carpels) have the same score (fig. 6). When Hydatellaceae are removed from the analysis, it is most parsimonious to link *Archaeoфраuctus* with core Nymphaeales, but a position as the sister group of living angiosperms is only two steps less parsimonious.

The analysis with *Archaeoфраuctus* scored as having a bisexual flower or preflower yielded 36 trees of 371 steps, in which *Archaeoфраuctus* is the sister group of either Hydatellaceae or core Nymphaeales (fig. 7). Again, relationships outside the angiosperms are not affected. The shortest trees in which

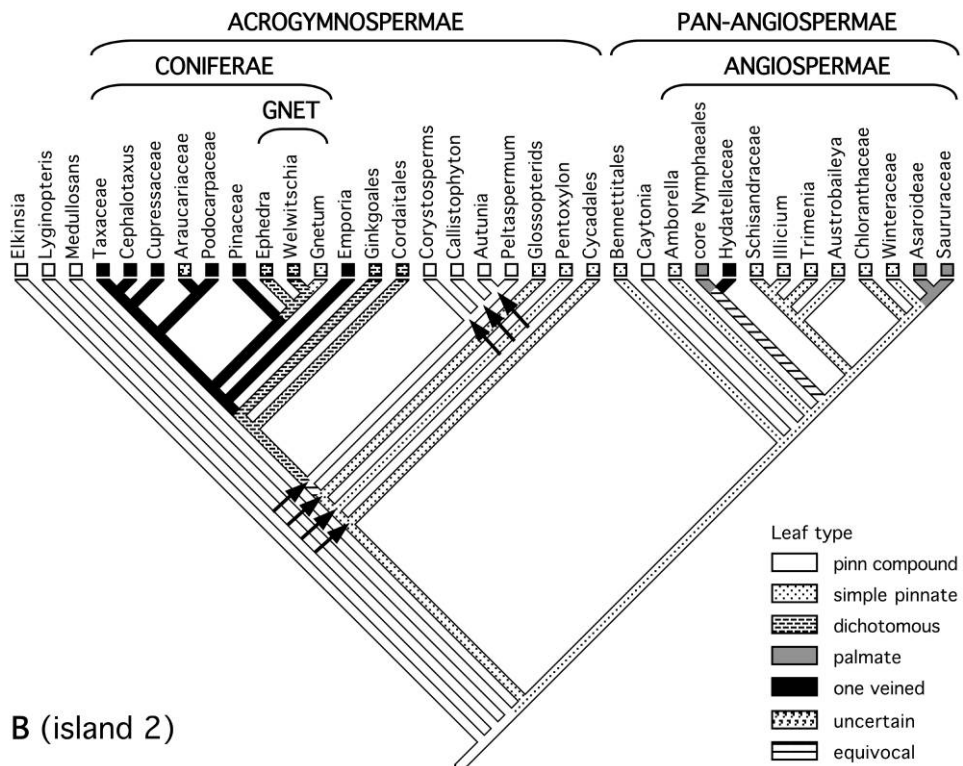
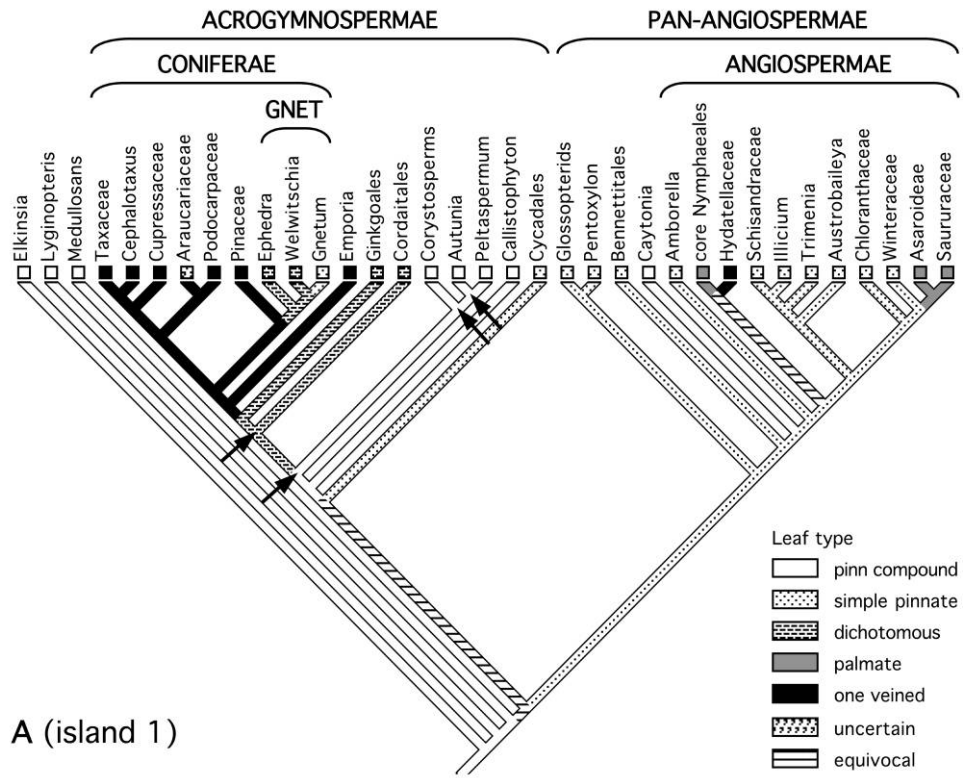
*Archaeoфраuctus* is sister to living angiosperms are three steps longer than the most parsimonious trees, but several positions lower in the tree are only two steps longer. When Hydatellaceae are removed, *Archaeoфраuctus* is associated with core Nymphaeales in the shortest trees, but trees in which it is sister to living angiosperms are only two steps longer.

## Discussion

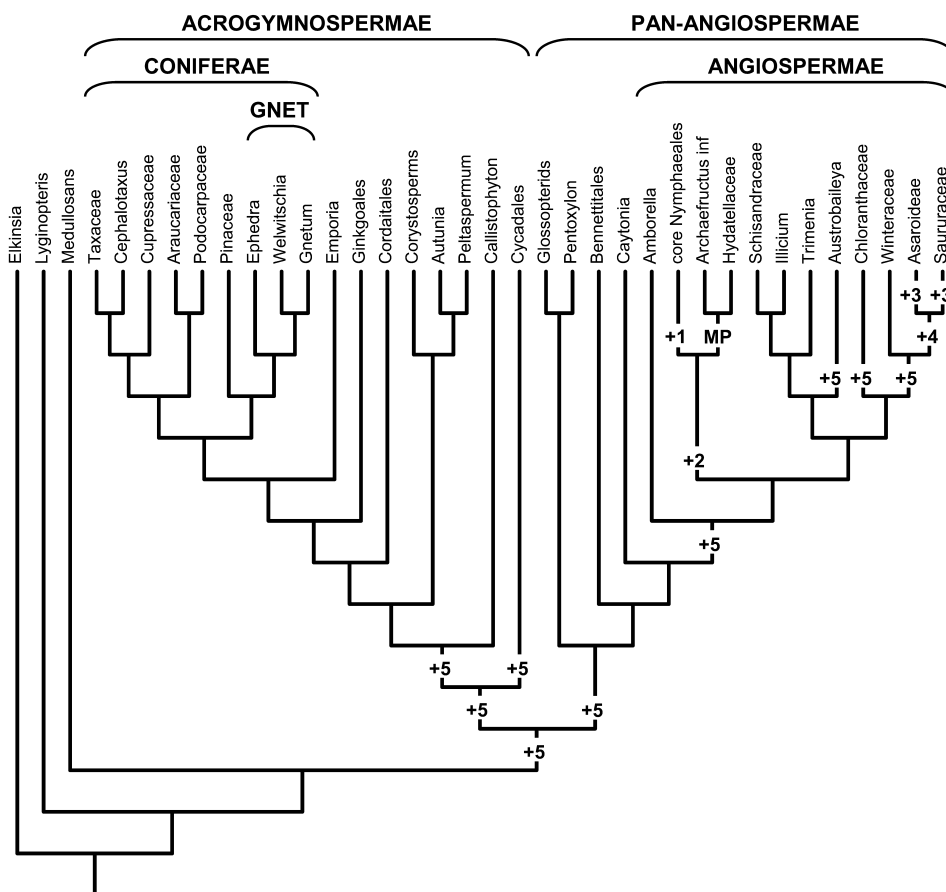
### General Phylogenetic Implications

These results differ most significantly from those of Doyle (2006) in that trees with Gnetales nested in “anthophytes” and in conifers are equally parsimonious, whereas previously, trees of the former type were favored by one step. This change is primarily a result of scoring *Ephedra* and *Welwitschia* as having simple microsporophylls rather than either pinnate to paddle shaped or simple, following the interpretation of Mundry and Stützel (2004). As a result, nesting Gnetales among anthophytes (which have more complex microsporophylls) adds a step that did not occur when Gnetales were scored as uncertain. Other changes in character definition and scoring had no evident effect on inferred outgroup relationships, except for the appearance, in the first island, of trees in which *Pentoxylon* and Gnetales form a clade rather

**Fig. 3** Strict consensus trees for the two islands of most parsimonious trees found in the unconstrained analysis (fig. 2). A, Consensus of the 24 trees in the first island, with Gnetales (GNET) in the “anthophyte” clade. B, One of two most parsimonious topologies of angiosperms in the first island, with *Amborella* basal. C, Consensus of the 16 trees in the second island, with Gnetales in conifers.



**Fig. 5** A, Representative tree of island 1 from the analysis with the molecular backbone (fig. 4), with nodes not found in all trees of the island indicated by arrows and with shading of branches showing the inferred evolution of leaf organization (character 31; see appendix in the online edition). B, Representative tree of island 2 from the same analysis. GNET = Gnetales.



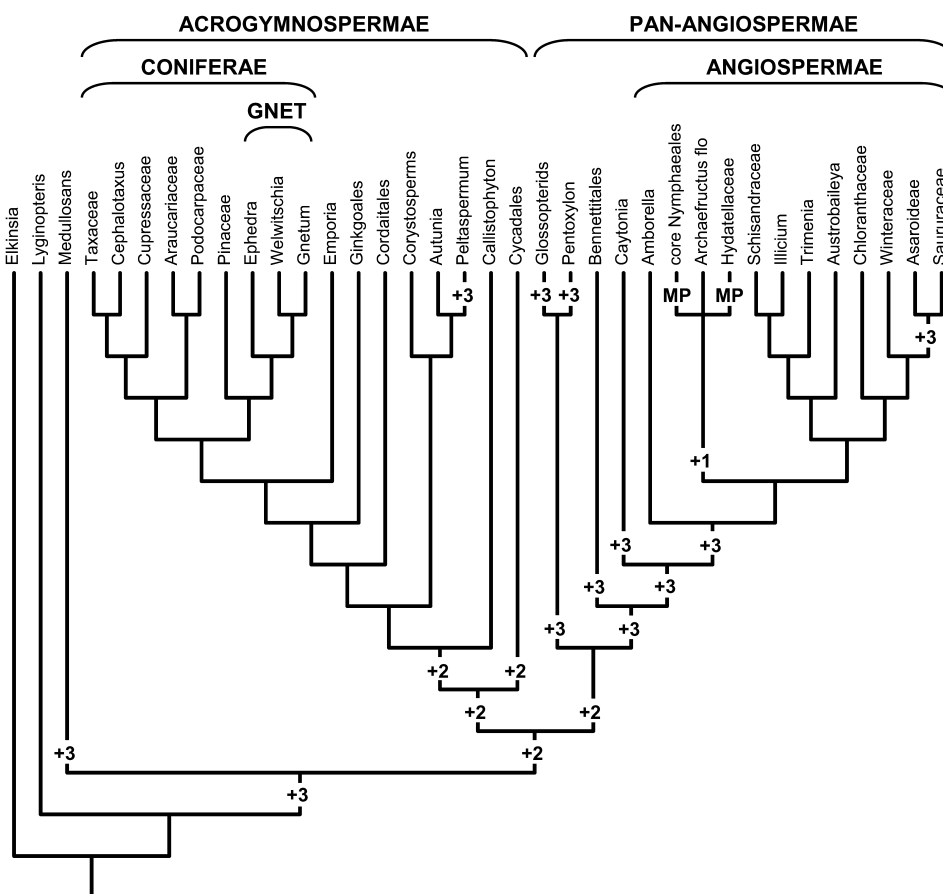
**Fig. 6** Representative most parsimonious tree from the analysis with *Archaeofructus* added, with its fertile shoot interpreted as an inflorescence of unisexual flowers and with the arrangement of living taxa fixed to the molecular backbone. Positions of *Archaeofructus* up to five steps less parsimonious than the best position (assuming the arrangement of other taxa shown) are indicated with MP = most parsimonious, +1 = one step longer than most parsimonious, +2 = two steps longer, etc. GNET = Gnetales.

than two successive branches. Addition of Hydatellaceae had no effect on outgroup relationships.

These results strengthen the view expressed in Doyle (2006) that morphology does not strongly contradict molecular data on the position of Gnetales, as previously believed, but rather is deeply ambiguous: Gnetales share morphological advances both with Bennettiales and angiosperms and with conifers, half of which are homoplastic. The nearly complete lack of resolution among crown-group seed plants in the consensus (fig. 2) does not mean that morphology is ambiguous for all aspects of seed plant phylogeny. Inspection of the consensus trees of the two islands (fig. 3A, 3C) shows that most of the lack of resolution is due to jumping of Gnetales between their two most parsimonious positions; many other relationships are consistent in the two kinds of trees. Other reflections on the similar but less complete shift in relative parsimony of the two positions of Gnetales between Doyle (1996) and Doyle (2006) were presented in the latter article. In an analysis that also began with the Doyle (1996) data set and improved the sampling of taxa and characters among basal seed plants, Hilton and Bateman (2006) found a greater parsimony difference between trees of the two kinds, but they made fewer changes

relevant to the relationships of angiosperms and Gnetales and did not modify the obsolete taxon sampling of angiosperms.

As in Doyle (2006), another major result is that trees with Gnetales nested in conifers, found both with and without backbone constraints (figs. 3C, 4, 5), identify the same previously implicated fossil taxa as the most probable relatives of angiosperms (=other Pan-Angiospermae of Cantino et al. 2007): glossopterids, *Pentoxylon*, Bennettiales, and *Caytonia*. The resulting pan-angiosperm clade corresponds to the “glossophytes” of Doyle (1996), except for the elimination of Gnetales. This result is surprisingly insensitive to relationships within angiosperms, at least with the several rootings inferred from this data set or specified by the molecular backbone tree. In theory, correct identification of outgroups might require correct rooting of the angiosperms, as well as vice versa (as argued by Doyle et al. [1994] and Bateman et al. [2006]), but this problem may be less severe in practice. However, the low statistical support values for the relationships obtained (a common situation with fossils, because of the large proportion of missing data) indicate that the inferences made here on outgroups should be viewed as only a marginally favored hypothesis.



**Fig. 7** Same as fig. 6, but with the fertile shoot of *Archaeofructus* interpreted as a bisexual flower or preflower. Positions of *Archaeofructus* up to three steps less parsimonious than the best position are indicated as in fig. 6. GNET=Gnetales.

Another intriguing change from Doyle (2006) occurred in angiosperms, in the first island from the unconstrained analysis, where the number of most parsimonious trees increased from eight to 24 (fig. 3A). Trees in which Nymphaeales are sister to all other angiosperms (as in all unconstrained trees of Doyle 2006) disappear (Nymphaeales s. l. are always linked with Piperales and Winteraceae), but eight trees appear in which *Amborella* is basal, as in molecular analyses (fig. 3B). This rooting has not been seen in previous purely morphological analyses of seed plants. Plesiomorphic states of *Amborella* that support this position are lack of vessels, alternate phyllotaxis, lack of oil cells, and continuous tectum.

Several other aspects of the unconstrained trees with *Amborella* basal conflict with molecular results. As in the morphological analyses of Doyle and Endress (2000) and Eklund et al. (2004), in which *Amborella* was specified a priori as outgroup, Chloranthaceae are basal in the remaining angiosperms, followed by *Trimenia*, and Nymphaeales are linked with Piperales. In contrast, molecular analyses exclude Chloranthaceae from the basal ANITA grade and place them in the strongly supported mesangiosperm clade (Cantino et al. 2007), which contains all other angiosperms, and place Nymphaeales above or with *Amborella* in the ANITA grade. In both cases, the combined morphological and three-gene

analysis of Doyle and Endress (2000) strongly supported the molecular arrangement, indicating that the contrasting morphological results are due to homoplasy: similarities between *Amborella* and Chloranthaceae (such as a single pendent, orthotropous ovule) and between Nymphaeales and Piperales (palmately veined leaves and other features that may be functionally related to herbaceous habit).

In those unconstrained trees in which Gnetales are nested in conifers (fig. 3C), relationships within angiosperms changed only slightly from those found in the most comparable analysis of Doyle (2006), where conifers and Gnetales were constrained to form a clade but other relationships were left unspecified. In that analysis, (core) Nymphaeales were basal, followed by Piperales, and Winteraceae were either basal in the remaining taxa or linked with Piperales, but in the present analysis, Nymphaeales and Piperales form either two successive branches or a basal clade, and Winteraceae are sister to the remaining taxa.

Bateman et al. (2006) cited Doyle (2006) as finding that constraining other seed plants into a molecular arrangement resulted in a shift toward angiosperm relationships found in molecular studies, but this perception was apparently due to confusion with the analysis in which relationships in angiosperms were constrained to a molecular tree. In fact, the oppo-

site is true. With the present data set, the most parsimonious trees with *Amborella* basal in angiosperms (as in molecular analyses) are of the anthophyte type (fig. 3B), which conflicts with molecular data, whereas trees constrained to agree with molecular studies in having *Amborella* basal and Gnetales in conifers (not shown) are three steps longer (349 steps).

In the following discussion, I concentrate on the constrained analyses (figs. 4, 5). Trees from the unconstrained analyses have many interesting features (some discussed in Doyle 2006), but, as already noted, combined analyses show that the conflicting morphological relationships in angiosperms are strongly overruled by molecular data (Doyle and Endress 2000). Molecular relationships within conifers (not considering Gnetales) also have strong statistical support (Quinn et al. 2002), including the relationship of Podocarpaceae with Araucariaceae rather than Pinaceae, the only difference from the morphological result. Bateman et al. (2006) described this procedure as accepting an unparsimonious tree, but although the constrained tree is less parsimonious in terms of morphology, the Doyle and Endress (2000) study indicates that the angiosperm portion is more parsimonious in terms of morphology and molecular data combined. The weakest aspect of the molecular constraint tree, the position of cycads at the base of the acrogymnosperms, has only minor effects on the patterns of character evolution discussed here (Doyle 2006). A true “total-evidence” analysis of both morphological and molecular data might be theoretically preferable, with fossils scored as unknown for molecular characters. However, the present procedure may be a valid approximation of such an analysis, and it may be of interest as a heuristic exercise that asks what the implications of molecular analyses are if they are correct.

Of the constrained trees, I have chosen one from the first island (fig. 5A). First, trees of this island are more consistent with stratigraphic evidence, since they entail a shorter gap (ghost lineage; Norell 1992) in the record of the pan-angiosperm clade. All the constrained trees imply that the pan-angiosperm line diverged before the Late Carboniferous, the age of the oldest known acrogymnosperms (cordaites and conifers). However, in trees of the second island (fig. 5B), no pan-angiosperms are known until the Late Triassic (Bennettitales, *Caytonia*), whereas in trees of the first island, the pan-angiosperm clade includes the glossopterids, which are abundant in the Permian.

Second, trees of the second island (fig. 5B) imply that the leaf type in the common ancestor of living seed plants was simple pinnate, a term used here to include both simple, pinnately veined leaves of the *Taeniopteris* type and the compound leaves of living cycads, in which the leaflets have parallel or dichotomous venation (for discussion of the distinction between the simple pinnate and pinnately compound states, see Doyle 1996). The fernlike, pinnately compound leaves of *Callistophyton*, corystosperms, and peltasperms (*Autunia* and *Peltaspermum*) must be derived from this type rather than being homologous (symplesiomorphic) with the similar leaves in basal seed ferns. In those trees in which *Callistophyton* is basal to all living seed plants, the close similarities between its leaves and those of corystosperms and peltasperms (which differ mainly in having rachial pinnules) must also be homoplastic. Both scenarios would require a remarkable reversal to the ancestral leaf type. In contrast, in

trees of the first island (fig. 5A), the fernlike, pinnately compound leaves of *Callistophyton*, corystosperms, and peltasperms can be either homoplastic or homologous with those of earlier seed ferns. In the latter case, simple pinnate leaves would be derived twice, in cycads and the pan-angiosperm line. This scenario is favored if one assumes that simplification more often produces similar forms than does secondary elaboration.

### General Floral Organization

The distinction between strobili and flowers is rather arbitrary (see review in Bateman et al. 2006); both are special, determinate short shoots bearing closely spaced fertile appendages, with or without associated sterile appendages. Both can be distinguished from cases in which fertile appendages are borne on shoots that are not strongly differentiated from vegetative shoots. I use “strobilus” for specialized fertile shoots in which the axis is relatively elongate at the time of pollination and “flower” for shoots in which the axis is much shorter relative to the whole structure (Doyle 1994). However, some have used the term “flower” for both types of structure, for example, Florin (1951) in describing the fertile short shoots of cordaites and early conifers. Some have defined flowers as restricted to angiosperms (Gifford and Foster 1989; Loconte and Stevenson 1990), but this would leave no term to express the similar appearance of the fertile structures of Bennettitales (and Gnetales under older interpretations, but not if the “flowers” are compound strobili; Mundry and Stützel 2004).

Retallack and Dilcher (1988) reconstructed the leaf-cupule complexes of glossopterids as borne on fertile short shoots, but Pant and Singh (1974) illustrated two leaf-cupule complexes attached to a normal stem, and in any case vegetative leaves were borne on both long and short shoots (Pant and Singh 1974). Given the large size of the pinnate sporophylls of *Caytonia*, it seems unlikely that they were grouped into flowerlike structures. This would be supported by a specimen, figured by Thomas (1925) and reexamined by Retallack and Dilcher (1988), of a megasporophyll attached to a slender stem, but more specimens in organic connection would be desirable for confirmation. Microsporophylls of many Bennettitales were quite large, but they often remained attached to the floral axis. If the present analysis is correct in identifying *Caytonia* as the sister group of the angiosperms, it implies that aggregation of fertile parts into flowers occurred independently in Bennettitales and angiosperms or that aggregation occurred in the common ancestor of these groups and *Caytonia* reverted to a nonfloral state.

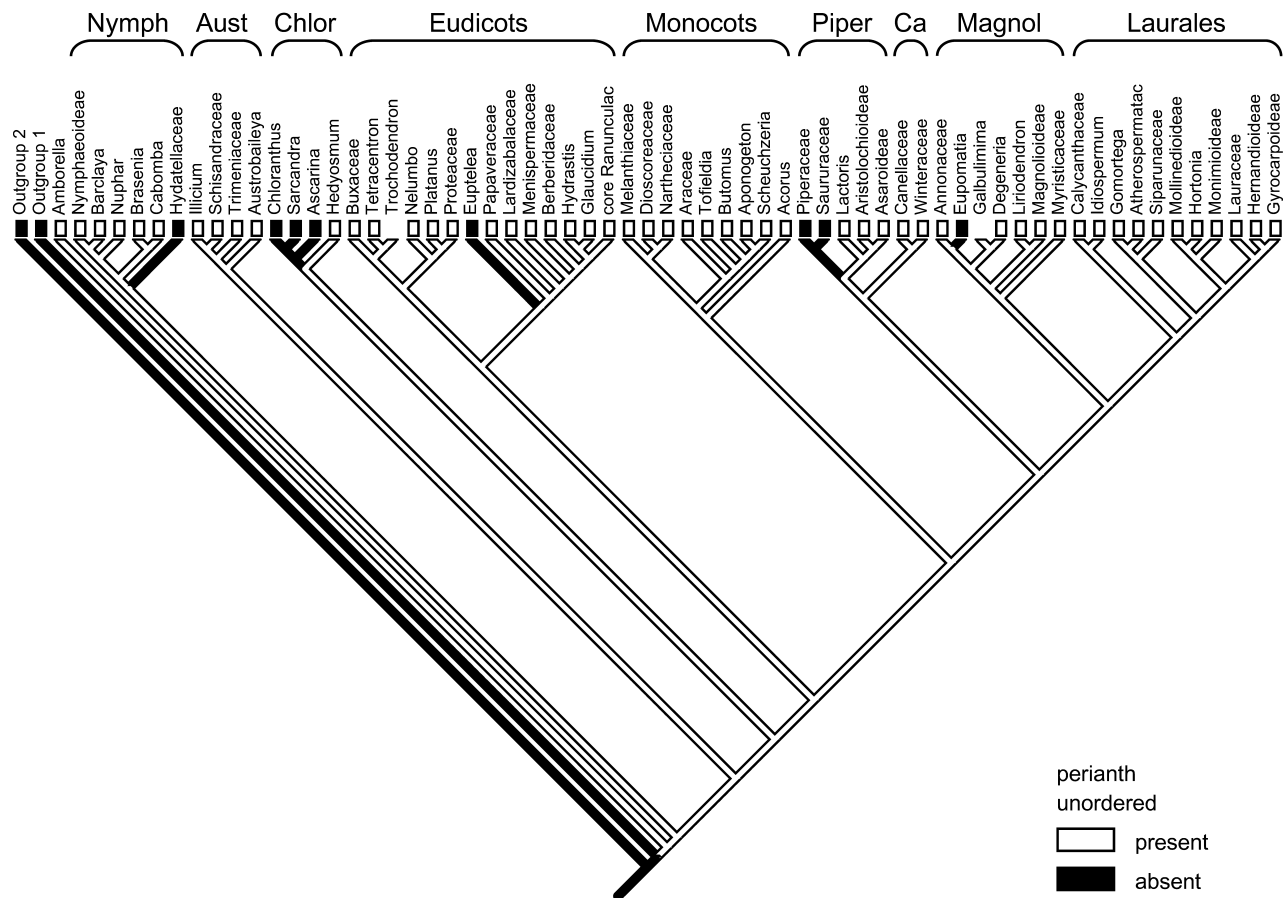
Whether the initial aggregation of fertile parts into a flower occurred before or after the divergence of *Caytonia*, an important question is whether it also involved sterile appendages that became the perianth. Optimization of characters on current angiosperm phylogenies, without considering outgroups, implies that the most recent common ancestor of all living angiosperms had a perianth (Doyle and Endress 2000; Ronse De Craene et al. 2003). This conclusion still holds with the addition of Hydatellaceae, which lack a perianth, because this group is located two nodes above the base of the angiosperms. In another near-basal group, Chloranthaceae, the genera *As-*

*carina*, *Sarcandra*, and *Chloranthus* have no perianth, but *Hedyosmum*, their sister group, has three small tepals (Endress 1987; Eklund et al. 2004). A perianth is also reconstructed as ancestral if one assumes that the closest outgroups of angiosperms lacked a perianth; it is most parsimonious to assume that the perianth arose in the common ancestor of living angiosperms and was lost in Hydatellaceae and other taxa with no perianth. This is illustrated by a tree from the combined analysis of Doyle and Endress (2000), with modifications based on more recent molecular data (Endress and Doyle 2007) and with two hypothetical outgroups that lack a perianth added at the base (fig. 8).

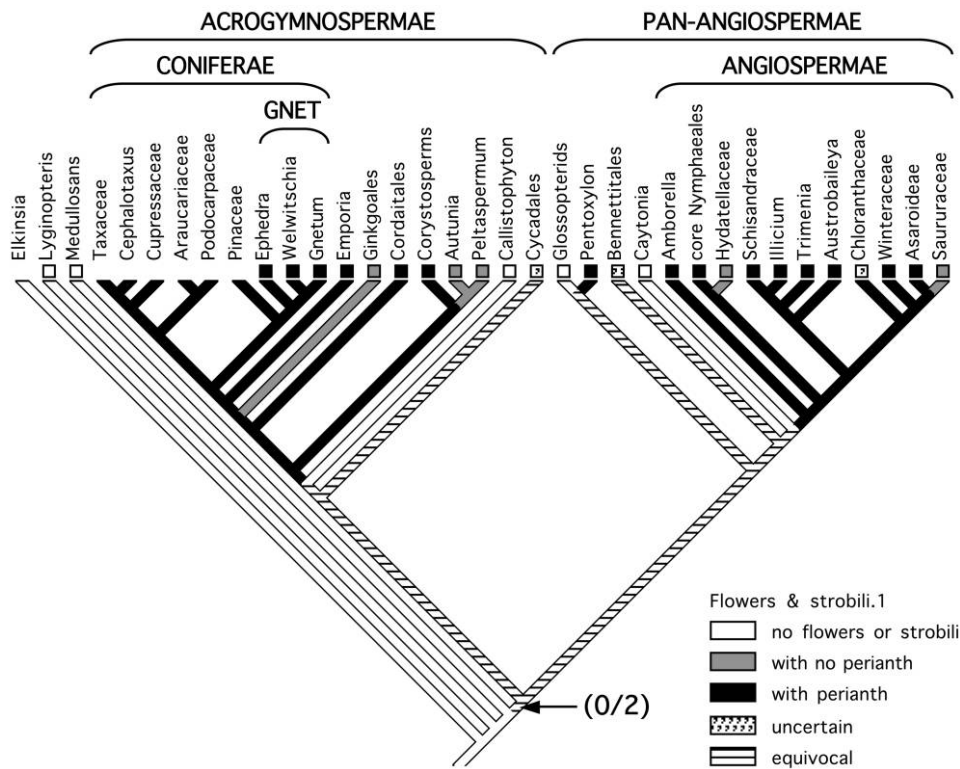
Although these arguments imply that both fertile and sterile appendages became aggregated on the line leading to angiosperms, they do not say whether the two kinds of appendages were aggregated at the same time or the fertile ones first; i.e., whether the first flower had a perianth. This question can be addressed only by considering outgroups, but, as already noted, this poses problems concerning homologies of the fertile structures and the fact that the sporophylls of some fossils (and female plants of *Cycas*) are not aggregated into flowers or strobili. This raises the possibility that aggregation of sporophylls occurred more than once and followed more than

one pathway. For simplicity, in the following discussion I use “strobili” for both flowers and strobili and “cataphylls” for all associated sterile appendages.

Mapping the three-state character devised to address this question on the preferred tree (fig. 9) underlines the ambiguity of existing data on this question. Under all trees and with both scorings of cycads, the ancestral state for seed plants as a whole is no strobili, but the situation in crown-group seed plants is complex. When cycads are scored as either lacking strobili or having strobili with cataphylls (0/2), the state at the crown-group node is equivocal: either no strobili or strobili with cataphylls (fig. 9). There are five equally parsimonious scenarios for the evolution of this character. In four scenarios, the common ancestor of the crown group lacked strobili, and strobili with cataphylls originated within cycads and in the common ancestor of corystosperms, peltasperms, and coniferophytes; cataphylls were then lost independently in Ginkgoales and peltasperms. In the pan-angiosperm line, strobili originated three times, in *Pentoxylon*, Bennettitales, and angiosperms, with cataphylls in *Pentoxylon* and angiosperms but either (1) without or (2) with cataphylls in Bennettitales; (3) twice, with cataphylls, in *Pentoxylon* and the common ancestor of Bennettitales and angiosperms, with a reversal to no



**Fig. 8** Angiosperm tree from Endress and Doyle (2007), with addition of Hydatellaceae, showing the inferred evolution of presence or absence of perianth. Two hypothetical outgroups without perianth added at the base. Nymph = Nymphaeales, Aust = Austrobaileyales, Chlor = Chloranthaceae, Piper = Piperales, Ca = Canellales, Magnol = Magnoliales.



**Fig. 9** Representative tree from the analysis with the molecular backbone (fig. 5A), showing the inferred evolution of a character (134 [see appendix in the online edition], not used in phylogenetic analyses) that distinguishes fertile appendages not borne on differentiated shoots from strobili or flowers with and without perianth or comparable sterile appendages. GNET = Gnetales.

strobili in *Caytonia*; or (4) once, with cataphylls, and with separate reversals to no strobili in glossopterids and *Caytonia*. Under the scenario 5, strobili with cataphylls originated in the common ancestor of living seed plants, and there were independent reversals to no strobili in female *Cycas*, *Callistophyton*, glossopterids, and *Caytonia*. Alternative 5 is the only most parsimonious scenario when cycads are scored as having strobili with cataphylls. Under both scorings of cycads, if *Callistophyton* is placed in the corystosperm-peltasperm clade or below crown-group seed plants, it is most parsimonious to assume that the common ancestor of living seed plants had strobili with cataphylls.

This exercise underlines the slippery nature of the distinctions among flowers, strobili, and less differentiated fertile shoots. However, it does suggest that the common ancestor of *Caytonia* and angiosperms had either fertile appendages borne on unspecialized shoots or flowerlike shoots with both fertile organs and sterile appendages that could be homologous with a perianth. It provides no support for a scenario in which flowers were derived from special fertile shoots with no associated sterile appendages. Further clarification may require recognition of closer angiosperm outgroups, as discussed further below.

#### Homologies of the Bitegmic Ovule and the Carpel

The origin of the carpel is often considered the most important aspect of the origin of the flower. However, as discussed in the "Introduction," the origin of the peculiar bitegmic ovule

of angiosperms, especially its outer integument, is an equally serious problem, and the two questions are closely linked.

Several of the advances incorporated in Doyle (2006) concern morphology and potential homologies of the angiosperm bitegmic ovule and the ovule-bearing structures of fossil seed plants, particularly those called cupules. The term "cupule" has been used in many taxa, but it is widely recognized that cupules of the first seed plants, which were dichotomously organized structures borne at the tips of special fronds or parts of fronds (Kidston 1924; Galtier 1988; Retallack and Dilcher 1988; Serbet and Rothwell 1992), were probably not homologous with cupules of groups such as *Caytonia* and corystosperms, which appear to be laminar, with seeds on one surface, and are therefore either leaves (sporophylls) or leaflets. This has been confirmed by phylogenetic analyses of seed plants (cf. figs. 2–5), which place several lines with no cupules and seeds on more or less unmodified leaves (such as medullosans, *Callistophyton*, and cycads) between basal seed plants with dichotomous cupules (such as *Elkinsia* and *Lyginopteris*) and later groups with cupules of the laminar type. Recently, it has become clearer that laminar cupules were of two kinds: in some the seeds were abaxial on the cupule lamina, and in others they were adaxial.

In Permian and Triassic peltasperms (*Autunia* and *Peltaspermum*), the seeds were borne on the abaxial side of spoon-shaped or peltate structures. Some authors interpreted these as leaflets of a pinnate leaf (Townrow 1960; Doyle and Donoghue 1986; Retallack and Dilcher 1988), but it has been

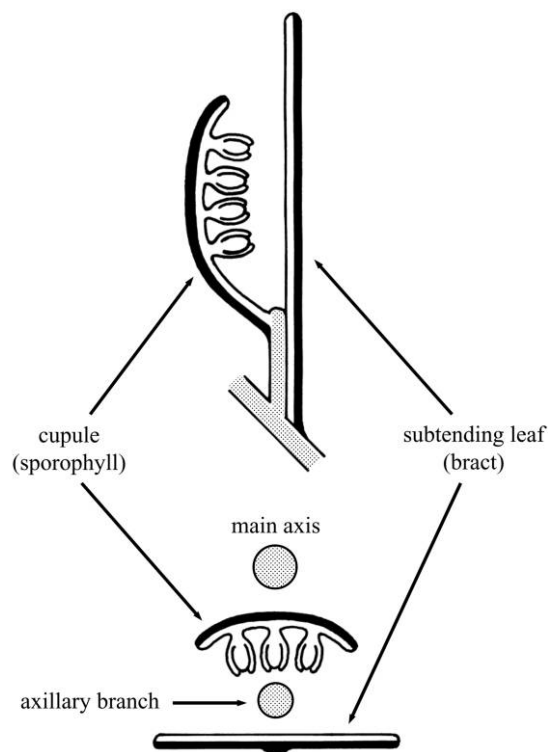


shown that they were attached helically to an axis and were therefore presumably sporophylls (Meyen 1987; Kerp 1988; Nixon et al. 1994). In Triassic corystosperms, it has also been uncertain whether the systems bearing the helmet-shaped cupules were branches with simple sporophylls modified into cupules (Thomas 1933) or pinnately compound sporophylls with ovule-bearing leaflets (Harris 1951; Doyle and Donoghue 1986; Retallack and Dilcher 1988). However, studies on compressed and silicified material from Antarctica (Axsmith et al. 2000; Klavins et al. 2002) have shown that they were branches with spirally arranged cupules, plus a few scalelike appendages below. Furthermore, in the vascular bundles in the cupule, the xylem was located toward the outside of the cupule, identifying that side as adaxial, and the phloem was toward the inside, implying that the seeds were borne on the abaxial surface, as in peltasperms.

In *Caytonia*, Harris (1940, 1951) and Reymanówna (1973) argued that the cupules were adaxially folded leaflets, based especially on their relation to the strongly dorsiventral rachis. The cupules were attached to the flatter and presumably adaxial side of the rachis and enrolled toward its midline in circinate fashion, implying that the enclosed seeds were adaxial (fig. 1A). In contrast, Retallack and Dilcher (1988) reconstructed the cupules as abaxially folded, on the basis of Thomas's (1925) specimen, mentioned above, of a sporophyll attached to a stem. However, after examination of this specimen, I was not convinced that the orientation of the sporophyll could be determined. Whether or not Harris was correct about the orientation of *Caytonia* (a question discussed further below), adaxial seeds are known in at least one Triassic cupule-bearing structure, *Petriellaea* (Taylor et al. 1994), from the position of xylem and phloem in vascular bundles of the cupule wall.

Adaxial ovule position has also been demonstrated in glossopterids. The cupules (or sporophylls) of glossopterids, which are attached to the midrib of a more or less unmodified leaf, have been reconstructed as having seeds on the surface facing the leaf (Schopf 1976; Retallack and Dilcher 1981; fig. 1B), but this has become controversial. Work by Taylor and Taylor (1992) on isolated silicified cupules with preserved vascular bundles showed that the seeds were attached to the xylem side of the cupule and therefore adaxial. They took this to mean that the seed-bearing surface faced away from the subtending leaf. Taylor (1996) also argued that this orientation would be necessary for pollination of the ovules, a questionable argument in light of the fact that pollen reaches the ovules of conifers even though they are borne on cone scales that are barely separated at the time of pollination. However, careful studies of impressions split in various planes reaffirm that the side of the cupule bearing the ovules faced the leaf (McLoughlin 1990; Adendorff 2005).

These observations can be most simply reconciled by interpreting the cupule as a sporophyll borne on an axillary branch (fig. 10) on the opposite side from the subtending leaf, like the adaxial prophyll of monocots and many magnoliids, such that the adaxial surface faced the leaf (Retallack and Dilcher 1981, fig. 3B). Another view, that the cupule is a modified branch (cladode; Schopf 1976; Retallack and Dilcher 1981, fig. 3C) has become more difficult to maintain in light of its typically foliar anatomy (Gould and Delevoryas 1977; Taylor and Taylor 1992). A more exotic possibility, suggested by Kato (1990),



**Fig. 10** Interpretation of positional relationships in the leaf-cupule complex of glossopterids, with abaxial surfaces indicated in black.

is that the cupule is the adaxial fertile segment of a three-dimensional leaf, as seen in the eusporangiate fern order Ophioglossales. Kato's suggestion that the adaxial fertile segment of the Ophioglossales, the glossopterid cupule, and the angiosperm bitegmic ovule were homologous is ruled out by molecular evidence that Ophioglossales are not related to seed plants (Pryer et al. 2001; Rothwell and Nixon 2006), but these structures might be morphologically similar.

In angiosperms, if the bitegmic ovule was derived from a cupule, by reduction of the number of ovules to one, this cupule was apparently of the type with adaxial ovules. In other words, the nucellus plus the inner integument (=original seed plant ovule) is borne on the adaxial side of the outer integument (=cupule wall). This has nothing to do with the adaxial position of the bitegmic ovule on the carpel. As noted by Frohlich (2003), when there are vascular bundles in the outer integument, the phloem is to the outside (Svoma 1997), identifying the outer surface as abaxial and the inner as adaxial. As Frohlich (2003) recognized, the finding that ovules are abaxial in the cupules of corystosperms (Axsmith et al. 2000; Klavins et al. 2002) contradicts the homology between corystosperm cupules and angiosperm bitegmic ovules postulated in the original version of his "mostly male" hypothesis (Frohlich and Parker 2000).

Nixon et al. (1994) questioned the homology of the *Caytonia* cupule and the angiosperm bitegmic ovule because of their observations on *Caytonia*, which showed that the lip of the cupule extends only to either side of the stalk rather than forming a complete ring. However, as discussed in Doyle

(1996), this condition is similar to that in many anatropous angiosperm ovules, in which the outer integument is interrupted by the funicle, a condition called “synbi” (Taylor 1991) or “semiannular” (Umeda et al. 1994; Imaichi et al. 1995; Endress and Igersheim 1997; Igersheim and Endress 1997; Yamada et al. 2001a). From the analysis of Doyle and Endress (2000), the semiannular condition can be reconstructed as ancestral in angiosperms, assuming that bitegmic ovules were originally anatropous rather than orthotropous.

Additional evidence comes from developmental genetic studies in *Arabidopsis* based on phenotypes of developmental mutants and localization of gene expression. Genes of the YABBY family are required for laminar growth and differentiation of abaxial tissues in leaves and other lateral organs (Bowman 2000; Floyd and Bowman 2007). One of these, *INO* (=INNER NO OUTER because mutants have an inner but no outer integument), is expressed in the outer epidermis of the outer integument (Villanueva et al. 1999; Balasubramanian and Schneitz 2000; Meister et al. 2002; Skinner et al. 2004) but not in the inner epidermis or the inner integument. This identifies the outside of the outer integument as abaxial, consistent with its derivation from a laminar structure with an ovule on its adaxial surface (as noted by Meister et al. [2002]; Frohlich [2003]; Yamada et al. [2003]; Skinner et al. [2004]). However, Sieber et al. (2004) questioned this interpretation because the class III HD-Zip (homeodomain-leucine zipper) gene *PHB*, which is involved in determining adaxial identity in leaves, is expressed on the inside of the inner integument but not on the inside of the outer integument. They suggested that this may indicate an origin of the two integuments from one by splitting. This hypothesis would greatly widen the field of comparison of angiosperms with outgroups to include taxa with unitegmic ovules not borne in cupulelike structures.

Since my earlier review of this topic (Doyle 2006), understanding of the genetic control of ovule polarity has improved, and new data strengthen the interpretation of the outer integument as a separate laminar structure. Genes of the KANADI family, which along with YABBY genes specify abaxial development in leaves (Eshed et al. 2001, 2004), are required for normal growth of both the inner (*ATS*) and the outer (*KAN1* and *KAN2*) integuments, and *ATS* is known to be expressed on the outside of the inner integument (McAbee et al. 2006). McAbee et al. (2006) suggested that class III HD-Zip genes are adaxial determinants in both integuments, as well as in leaves, but they stressed that this has not yet been demonstrated. However, D. Kelley (personal communication) has found that not only *PHB* but also the class III HD-Zip gene *PHV* is expressed on the inside of the inner integument. The fact that both YABBY and KANADI (*KAN1* and *KAN2*) genes are involved in development of the outer integument but that only a KANADI gene (*ATS*) is involved in development of the inner integument has been taken as further evidence that the inner and outer integuments have different origins (McAbee et al. 2006).

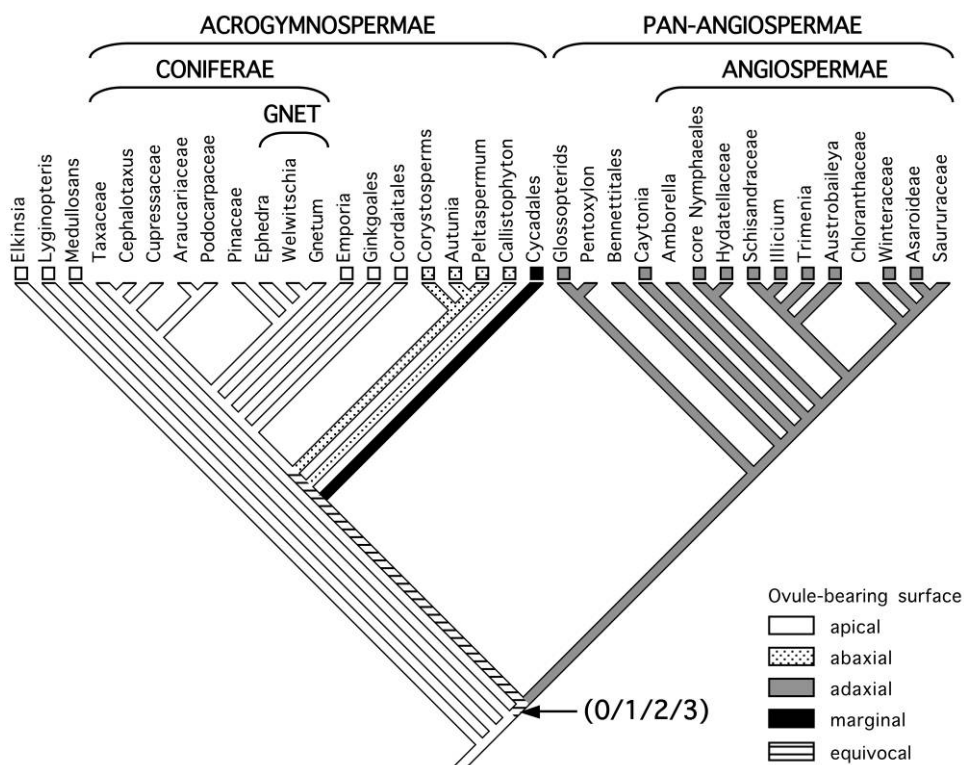
Floyd and Bowman (2007) noted that YABBY genes have been found in all investigated seed plants but not in lycophytes or ferns, and they speculated that this reflects the independent origin of leaves in seed plants and other lines. These observations can be related to the hypothesis that origin of the ancestral fernlike leaf of seed plants was a two-

step process. The first step, beginning with the dichotomous sporophyte body of the first vascular plants, resulted in main stems with simple dichotomous, overtopped lateral appendages, as in “trimerophytes,” or basal euphyllophytes (Kenrick and Crane 1997; Euphyllophyta [Cantino et al. 2007]), such as the Early Devonian genus *Psilophyton*. This presumably occurred in the common ancestor of the euphyllophyte clade, which includes ferns, sphenophytes, “progymnosperms,” and seed plants. The second step involved planation of whole branch systems of the progymnosperm type, which bore numerous dichotomous appendages, into pinnately compound fronds (Beck 1970; Doyle and Donoghue 1986; Kenrick and Crane 1997; Doyle 1998). The systematic distribution of YABBY genes suggests that they were involved in the second step but not the first. This would be consistent with the hypothesis presented here that the outer integument is a cupule derived from an ovule-bearing leaf, itself borne on an axillary branch, which was earlier derived from a compound leaf by simplification, like the vegetative leaves of glossopterids.

These observations can be related to the present phylogenetic results by plotting the position of the original ovule relative to the laminar structure on which it is borne, whether this is called a sporophyll or a cupule (fig. 11). This implies that ovules in the first seed plants (seed ferns) were apical on frondlike leaves, first in cupules at the tips of branched fronds or segments of fronds (Kidston 1924; Galtier 1988; Retallack and Dilcher 1988; Serbet and Rothwell 1992) and later without cupules at the tips of pinnae. With the topology under discussion (fig. 11), because of the variation in ovule position in acrogymnosperms, ovule position at the base of both crown seed plants and acrogymnosperms is equivocal (any of the four states). However, ovules became marginal in cycads and abaxial in *Callistophyton*, corystosperms, and peltasperms, either once or twice; in corystosperms and peltasperms the sporophylls were modified into what are called cupules. It is equivocal whether the apical position of ovules in coniferophytes (evident in cordaites and Ginkgoales but obscured in extant conifers by transformation of the fertile short shoots into cone scales) is ancestral or derived from an abaxial position. In other most parsimonious arrangements where *Callistophyton*, corystosperms, and peltasperms form a clade, abaxial position originates once from apical; with other topologies, it originates once and reverses to apical in coniferophytes.

In contrast, the two fossil taxa with adaxial ovules—glossopterids and *Caytonia*—are in the pan-angiosperm clade, associated with angiosperms, where the presumed equivalent of the original seed plant ovule is adaxial relative to the outer integument (and with *Pentoxylon* and Bennettitales, where ovule position is problematic and was scored as unknown). The inferred relationships confirm that the cupules of these groups are not homologous with those of corystosperms and peltasperms. These relationships are not based solely on this character; they are also supported by such angiosperm-like features as scalariform pitting in the secondary xylem, siphonogamy, and reduction of the megaspore membrane in Bennettitales and reticulate venation and “flat” stomata without raised guard cell poles (Harris 1932; Doyle and Donoghue 1986; Barbacka and Bóka 2000) in *Caytonia*.

Setting aside Bennettitales and *Pentoxylon*, in which reproductive morphology is more obscure, possible homologies of



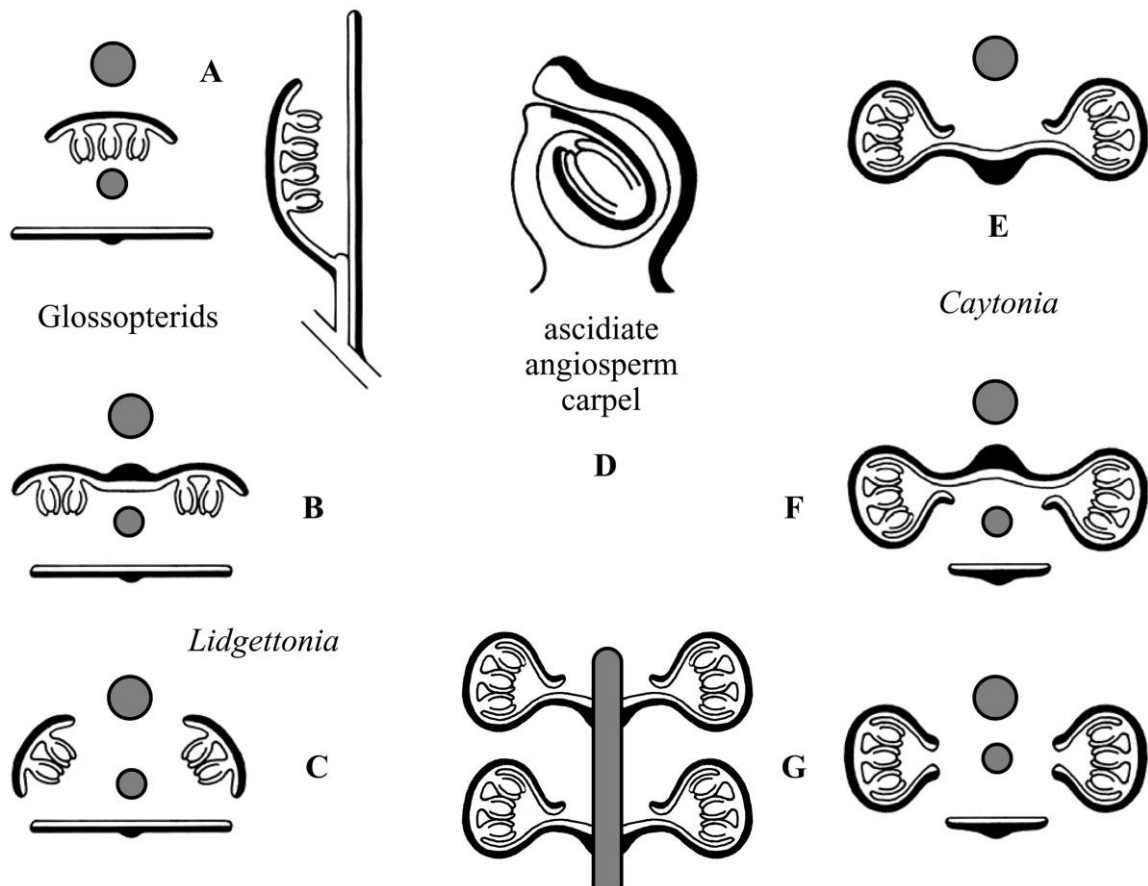
**Fig. 11** Representative tree from the analysis with the molecular backbone (fig. 5A), showing the inferred evolution of ovule position on the supporting laminar structure (character 85; see appendix in the online edition). GNET = Gnetales.

the ovulate structures in glossopterids, *Caytonia*, and angiosperms can be discussed with reference to several diagrams in which abaxial surfaces of leaflike structures are indicated in black (fig. 12). For angiosperms (fig. 12D), I have shown an ascidiate carpel with one apical ovule attached to the adaxial cross zone, as in many living basal angiosperms (Endress and Igersheim 2000; Buzgo et al. 2004). The positional correspondences between this type of carpel and the glossopterid leaf-cupule complex (fig. 12A) are particularly close: the carpel wall corresponds to the subtending leaf, the outer integument to the cupule with its adaxial ovules, and the adaxial cross zone to the area of fusion of the leaf and the axillary cupule-bearing branch. This is consistent with the view of many developmental geneticists (Skinner et al. 2004) that the carpel wall and the placenta are separate structures. At the time when Stebbins (1974) and Retallack and Dilcher (1981) proposed homologies of this sort, the correspondences with angiosperms seemed less close, because most authors assumed that the ancestral carpel had two rows of laminar or marginal bitegmic ovules. However, the analysis of Doyle and Endress (2000) reconstructed the most parsimonious ancestral condition as either more than one ovule (as in core Nymphaeales, *Austrobaileya*, and Schisandraceae) or one (as in *Amborella*, *Trimenia*, *Illicium*, and Chloranthaceae), and with the addition of Hydatellaceae, which have one apical ovule, it becomes most parsimonious to reconstruct the uniovulate condition as ancestral (fig. 13, right).

The present results imply that the next step in the modification of the cupule was its circinate incurvation into an anatropous

structure (fig. 13), as seen in *Caytonia* (figs. 1A, 12E–12G), followed by reduction of the number of ovules per cupule to one in angiosperms. *Amborella* and Chloranthaceae have orthotropous rather than anatropous bitegmic ovules, and if outgroups are not considered, it is equivocal whether the ancestral state in angiosperms was anatropous or orthotropous (Doyle and Endress 2000). In the present analysis, it is most parsimonious to interpret orthotropous ovules as derived from anatropous ovules (fig. 13), because the only outgroups reported to have an orthotropous cupule or bitegmic ovule are some Bennettitales (Harris 1954; Crane 1985a; Pedersen et al. 1989; Friis et al. 2007). As discussed further below, Rothwell and Stockey (2002) and Stockey and Rothwell (2003) have interpreted Bennettitales as having no outer envelope, and I therefore scored them as uncertain (1/3). If I had scored Bennettitales as having an orthotropous cupule, the ancestral state in angiosperms would be equivocal: either orthotropous or anatropous. Consistent with the view that the ovule orientation of *Amborella* is derived, it is not completely orthotropous at maturity, and in both *Amborella* and *Chloranthus*, the integument is semiannular and dorsiventral early in development (Yamada et al. 2001b).

Even if a uniovulate carpel is assumed to be ancestral in angiosperms, the fact that ovules are arranged in two rows in several near-basal groups (and many more derived taxa) may seem difficult to reconcile with the hypothesis that their precursor structure was borne on an axillary branch. However, the laminar to median (“dorsal”) placentation of the near-basal Cabombaceae and Nymphaeaceae (Taylor 1991; Iger-



**Fig. 12** Alternative interpretations of the homologies of ovulate structures in glossopterids (A–C), an ascidiate angiosperm carpel (D), and *Caytonia* (E–G), with abaxial surfaces indicated in black. See text for discussion.

sheim and Endress 1998) might be more consistent with an original axillary position. Under any scenario, ovule number and position must have been highly labile since the beginning of the angiosperm radiation. This lability was cited by Frohlich and Parker (2000; also Frohlich 2003) as support for their “mostly male” hypothesis that ovules (=cupules) were transferred ectopically to organs that had previously been microsporophylls, but it might simply indicate an early breakdown in positional relationships within angiosperms.

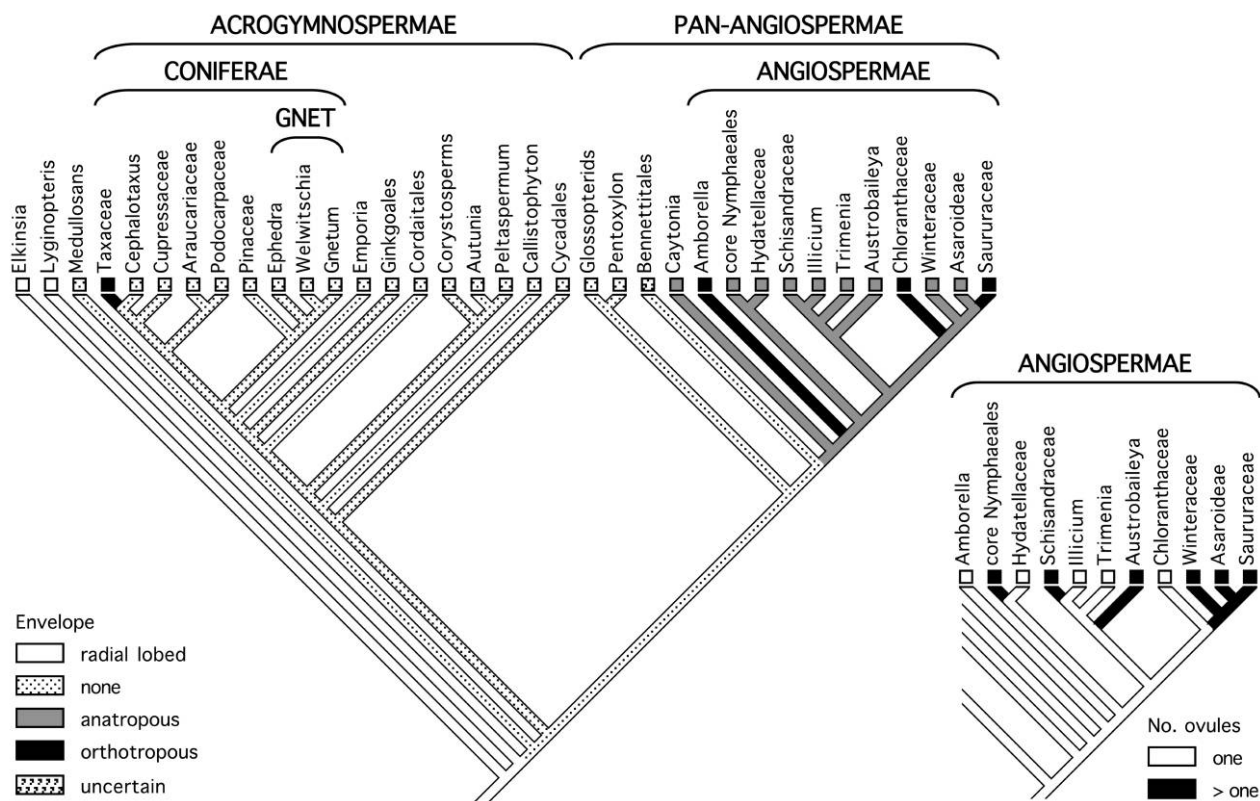
Other uncertainties concern the diversity of ovulate structures in glossopterids (cf. Pigg and Trivett 1994; Taylor 1996). Some glossopterids (e.g., *Lidgettonia*; Thomas 1958; Surange and Chandra 1975; Schopf 1976; Retallack and Dilcher 1981; fig. 1B) had more than one cupule attached to the midrib of a leaf. As discussed in Doyle (2006), there are several possible interpretations of the morphology of these structures (fig. 12B, 12C). In addition to the possibility that the cupules were part of an adaxial fertile leaf segment analogous to that of Ophioglossales (Kato 1990), one alternative is that the cupules were leaflets of a pinnate sporophyll borne on a branch (fig. 12B), and another is that they were several sporophylls borne on an axillary branch, twisted to face the subtending leaf (fig. 12C).

All attempts to homologize parts in glossopterids and angiosperms must contend with the long stratigraphic gap be-

tween Permian glossopterids and the first known crown-group angiosperms of the Early Cretaceous. This requires that plants with one or more cupules apparently borne in the axil or on the midrib of a leaf or bract persisted through the Triassic and Jurassic without being recognized in the fossil record. However, given the remarkable but poorly understood diversity of Late Triassic seed plants, most graphically seen in the Molteno Formation of South Africa (Anderson and Anderson 2003), the idea that such forms exist but have escaped recognition may not be so implausible.

Compared with glossopterid cupules, the cupules of *Caytonia* are more similar to the bitegmic ovules of angiosperms in their anatropous form, and the ovules inside are more angiosperm-like in having no pollen chamber, a thick nucellar cuticle, and no megaspore membrane. However, the structure that bore the cupules is hard to explain in terms of the homologies proposed here between glossopterids and angiosperms.

Harris (1940, 1951) and Reymanówna (1973) interpreted the cupule-bearing structure of *Caytonia* as a pinnate sporophyll and the cupules as adaxially enrolled leaflets. However, this is uncertain because there is little evidence on how the structure was attached to the plant, except for the specimen of Thomas (1925) cited by Retallack and Dilcher (1988) and discussed above. If it was a sporophyll borne on a normal stem (fig. 12E), the situation would be different from that in



**Fig. 13** Representative tree from the analysis with the molecular backbone (fig. 5A). *Left*, inferred evolution of cupules and comparable envelopes (character 87; see appendix in the online edition); *right*, angiosperm portion of the same tree, showing the inferred evolution of number of ovules per cupel (character 100). GNET = Gnetales.

glossopterids, where cupules were apparently borne on an axillary branch. Existence of such a structure in the common ancestor of *Caytonia* and angiosperms might be more consistent with the rachis expansion hypothesis for origin of the carpel (Gausson 1946; Doyle 1978; fig. 1A). Glossopterids and *Caytonia* might be more comparable if the *Caytonia* structure was a pinnate sporophyll borne on an axillary branch (fig. 12F); it could then correspond to the pinnate sporophyll model for multicupulate glossopterids (fig. 12B). Perhaps the two groups could be most easily compared if the *Caytonia* structure was not a sporophyll but rather an axillary branch with cupules derived from simple sporophylls (fig. 12G), as in the second model for multicupulate glossopterids (fig. 12C). This hypothesis would conflict with the dorsiventrality of the presumed rachis emphasized by Harris (1940, 1951; fig. 1A). It would also remove Harris's main argument that *Caytonia* ovules were borne on the adaxial side of the cupule, although it would not disprove that they were. Deciding among these alternatives may require discovery of more specimens that show attachment of the cupule-bearing structures to stems. However, even if such specimens are found, it may not be easy to determine the exact morphological relationships at the point of attachment in the absence of developmental data.

It is even less clear whether and how Bennettitales might fit into this scheme. They had numerous stalked ovules attached to an ovuliferous receptacle, mixed with interseminal scales

that are usually interpreted as sterilized ovules. Crane (1985a) proposed that each stalked ovule was a reduced cupule-bearing sporophyll, following the interpretation of Harris (1954) that some Late Triassic Bennettitales had a cupule, e.g., *Bennetticarpus crossospermus* and *Vardekloeftia*, which was restudied by Pedersen et al. (1989). However, in studies of petrified material, Rothwell and Stockey (2002) and Stockey and Rothwell (2003) argued that the layer in *Williamsonia* and *Cycadeoidea* that some authors had interpreted as a cupule is the sacrotesta of a single integument, not a morphologically distinct organ, and they questioned whether a cupule existed in any Bennettitales. Because Pedersen et al. (1989) provided seemingly convincing evidence for a discrete envelope around the ovule in *Vardekloeftia*, especially the fact that the tubular micropyle clearly protrudes through a hole in the cuticle of the structure in question, I scored Bennettitales as uncertain (1/3) for the cupule character.

A more exotic suggestion is that the ovuliferous receptacle was a single radialized sporophyll bearing many ovules (Doyle and Donoghue 1986; Crane 1988; Doyle 1994). The finding of Rothwell and Serbet (1994) that the supposed ovuliferous shoots of *Pentoxylon* had leaflike bilateral anatomy illustrates that fertile stems and sporophylls with seeds on both surfaces cannot always be easily distinguished. Because of these problems, I scored Bennettitales as unknown for sporophyll morphology and ovule position. As a result, these characters had no direct effect on inferred relationships, but

if they were better understood, they might either support or undermine the homologies proposed here between angiosperms and glossopterids.

A new element in this discussion is the description by Friis et al. (2007) of Early Cretaceous dispersed seeds with a micropylar tube, as in both Bennettitales and Gnetales, and a four-part outer envelope, which they compared with the bipartite envelope in Gnetales and the tripartite envelope in the less completely reconstructed fossil order Erdtmanithecales (producers of *Eucommiidites* pollen; Friis and Pedersen 1996; Mendes et al. 2008). In a cladistic analysis building on the seed plant data set of Hilton and Bateman (2006), in which Friis et al. (2007) rescored Bennettitales as having a “partite (valvate) outer envelope,” the dispersed seeds were sister to Gnetales in a clade including Bennettitales, Erdtmanithecales, and Gnetales, whereas previously Bennettitales and Gnetales were separated.

Friis et al. (2007) noted that their results may suggest a radical reinterpretation of the bennettitalian ovuliferous receptacle as an axis bearing many simple “flowers” of the gnetalian type. A problem for this hypothesis is the fact that the stalked ovules of Bennettitales are borne among numerous interseminal scales rather than in the axils of obvious subtending bracts, as in Gnetales. Taylor and Kirchner (1996) did attempt to interpret the ovules and interseminal scales of Bennettitales in terms of gnetalian units, but Rothwell and Stockey (2002) found that the vascular strands supplying both kinds of appendages depart from the stele in the same phyllotaxis and have similar anatomy. Finally, it is difficult to explain the large, apparently pinnate microsporophylls of Bennettitales in terms of Gnetales, especially with evidence confirming that the microsporophylls of Gnetales are simple structures (Mundry and Stützel 2004). Because of these uncertainties, I did not modify the scoring of Bennettitales in the present analysis.

Even if Gnetales are related to Bennettitales, this would not rule out homologies among angiosperms, glossopterids, and *Caytonia*, since it would place two taxa with similarly divergent morphology on the same side branch from the line leading to angiosperms. If Bennettitales are rescored like Gnetales and placed (with or without Gnetales) below angiosperms and *Caytonia*, as found here and by Friis et al. (2007; fig. 3B), it is still most parsimonious to homologize the anatropous envelope in *Caytonia* with that in angiosperms. The bitegmic structure that Friis et al. described in their fossil seeds and Gnetales is not easy to compare with that in angiosperms, considering the one-sided development of the angiosperm outer integument (Umeda et al. 1994; Imaichi et al. 1995; Yamada et al. 2001a), even when the ovule is orthotropous (Yamada et al. 2001b). Their comparisons also provide no obvious homologue for the angiosperm carpel.

It is also difficult to explain the reproductive structures of *Pentoxylon* in terms of a glossopterid prototype (leaf morphology and wood anatomy would be more compatible with a relationship of the two groups). Crane (1985a) interpreted the headlike seed-bearing structures, several of which were produced near the apex of a short shoot (Bose et al. 1985), as axes covered with numerous reduced sporophylls, which he compared with the ovuliferous receptacle of Bennettitales. However, based on the U-shaped configuration of the vascular tissue, Rothwell and Serbet (1994) interpreted the “head”

as a sporophyll with ovules borne on both surfaces. Such a sporophyll might be compared with the cupule of glossopterids, but this would require a shift of the cupule from its presumed position on an axillary branch, loss or fusion of the subtending leaf, or some equally major change in morphological relationships.

### *Origin of the Stamen*

As in most discussions of the flower problem, I have emphasized the origin of the carpel, but the morphology of the angiosperm stamen also has to be explained, and it poses as many problems. Perhaps because the stamen looks simpler to the human eye, it is easy to overlook the fact it is as unique in a seed plant context as the carpel. Because stamens have four microsporangia, authors beginning with Thomas (1925) have compared them with the microsynangia of *Caytonia*, which consisted of four sporangia. However, as noted by Harris (1937), stamens have a distinctly bilateral symmetry, with the four sporangia in two lateral pairs (thecae) separated by the connective, whereas in *Caytonia*, the sporangia formed radial groups not subdivided by sterile tissue, which were borne at the tips of a branched structure most commonly interpreted as a pinnate sporophyll. Angiosperm thecae are distinctive in other respects, such as the presence of a subepidermal endothelial layer, but this is an autapomorphy that has no direct bearing on outgroup relationships. In order to explain the positional relationships, it might be better to homologize the stamen with a whole *Caytonia* sporophyll, drastically reduced to only one synangium of two sporangia on either side. Following this interpretation, Doyle and Donoghue (1986) scored angiosperm stamens as pinnate sporophylls, but in Doyle (1996), I replaced this with a separate, less informative but more theory-neutral state.

It is also noteworthy that the stamens of most basal angiosperms have adaxial sporangia, and it is most parsimonious to reconstruct this state as ancestral (Doyle and Endress 2000; this study). In this respect they are like microsporophylls of Bennettitales, in which microsynangia were borne on the adaxial side of the sporophyll. Glossopterids had a branched unit bearing clusters of microsporangia that was attached to the adaxial side of a leaf or bract, reminiscent of the female structures (Surange and Maheshwari 1970; Surange and Chandra 1975; Schopf 1976; Gould and Delevoryas 1977). This suggests the possibility that the angiosperm stamen was derived from a leaf plus an adnate axillary branch, as hypothesized for the carpel. The male structures of *Caytonia* are less readily comparable if they were pinnate sporophylls, but in the absence of organic connection, it is hard to rule out the possibility that they were actually branches in the axil of a leaf or bract.

### *Closer Stem Relatives: The Question of Archaeofructus*

Even if glossopterids, *Pentoxylon*, Bennettitales, and *Caytonia* are related to angiosperms, there is a great morphological gap between them and angiosperms. The preferred tree (fig. 5A) implies that at least seven synapomorphies arose on the line between *Caytonia* and angiosperms (more than one order of laminar venation, stamens with two pairs of microsporangia, endothelial anther dehiscence, columellar exine,

sculptured sulcus membrane, reduced endexine, and carpel), and angiosperms have additional synapomorphies that cannot be localized on the tree because the relevant characters are not preserved in fossils (the characteristic morphology of the male and female gametophytes, double fertilization, etc.). This highlights the need for closer stem relatives of the angiosperms.

Some supposed pre-Cretaceous angiosperms might be such plants, but their morphology and relationships are uncertain. Cornet (1986, 1989b) associated Late Triassic pleated leaves of *Sanmiguelia* with structures that he interpreted as flowers and carpels. However, details in the highly compressed fossils are problematic (Crane 1987), and the male structures look more like ginkgophyte strobili, raising the possibility that the female structures are homologous with the ovuliferous “capsules” of the ginkgophyte group *Czekanowskiales*. Wang et al. (2007) described female structures of the Jurassic genus *Schmeissneria* as bicarpellate gynoecia, but this is based on uncertain interpretations of difficult compression material and the fact the ovules are enclosed rather than on a convincing morphological analysis of the supposed gynoecium. Otherwise, the plant appears to be typically ginkgophytic in its short shoots and leaf architecture (Kirchner and van Konijnenburg-van Cittert 1994), contrary to the discussion of Wang et al. (2007).

Cornet (1989a) also described angiosperm-like monosulcate pollen from the Late Triassic, with a tectal reticulum supported by columellae, and similar pollen has been found in Triassic rocks elsewhere (Doyle and Hotton 1991; Hochuli and Feist-Burkhardt 2004). However, well-preserved specimens have a uniformly thick, laminated endexine, as in non-angiospermous seed plants (Cornet 1989a; Doyle and Hotton 1991). This could mean that these grains were not related to angiosperms, but it could also mean that they were angiosperm stem relatives (Doyle and Hotton 1991; Doyle 2001, 2005). However, until these fossils are associated with other plant parts, there is little way to evaluate what, if anything, they say about the origin of angiosperms and the flower.

A better preserved fossil that has been explicitly proposed as an angiosperm stem relative is *Archaeofructus*, from the Barremian-Aptian of China, which had multiovulate carpels borne along an elongate axis (Sun et al. 1998), finely dissected leaves, and pairs of stamens below the carpels (Sun et al. 2002). The cladistic analysis of Sun et al. (2002), who used other living seed plants as outgroups, indicated that *Archaeofructus* was sister to all living angiosperms, on the basis of leaf characters (one vein order, dichotomous laminar vein form, nonanastomosing veins) and the absence of a perianth. Stuessy (2004) took this result as evidence that the carpel evolved before double fertilization and condensation of parts into a typical flower.

The interpretation of Sun et al. (2002) was questioned by Friis et al. (2003), who argued that the fertile shoot was more likely an inflorescence of unisexual flowers, reduced as an adaptation to aquatic conditions; this argument was based particularly on the fact that the stamens and often the carpels occur in pairs. Friis et al. argued that two of the features supporting the stem position of *Archaeofructus* are equivalent, dichotomous venation and lack of anastomoses, and that some potential fossil outgroups have the derived reticulate state

(glossopterids and *Caytonia* in the present data set). They questioned whether the perianth character can be polarized using modern outgroups, since it is not readily applicable in taxa that lack structures comparable with flowers (except Gnetales, which Sun et al. [2002] scored as polymorphic), and they noted that perianth-like appendages do occur in at least one potential fossil outgroup, Bennettitales. They also argued that there are aquatic crown-group angiosperms with leaves like those of *Archaeofructus*, such as *Cabomba* in the Nymphaeales, and that loss of perianth and reduction in number of floral parts are common trends in aquatics. When they added *Cabomba* to the Sun et al. (2002) data set and scored outgroups as unknown for perianth, they found that it was equally parsimonious to place *Archaeofructus* either on the angiosperm stem lineage or with *Cabomba*.

Crepet et al. (2004) rejected these criticisms on several grounds. Obviously, the flowers of core Nymphaeales, which have a well-developed perianth and are almost always bisexual, are very different from those of *Archaeofructus*. Crepet et al. noted that *Cabomba* has peltate as well as dissected leaves and argued that it should have been scored as polymorphic for this character. They called the scoring of the leaves of *Cabomba* as dissected an “empirically verifiable character miscoding” (Crepet et al. 2004, p. 1673). However, this coding is valid if the character states are defined as most or all leaves on the plant dissected versus none at all. Surely origin of leaf dissection can provide evidence of relationship in descendant species, even if it does not affect all leaves on the plant, when contrasted with the complete lack of dissection in vast majority of basal angiosperms. Crepet et al. correctly noted that plausibility arguments based on analogies with cases of floral reduction in other aquatics provide no specific phylogenetic evidence on the position of *Archaeofructus*.

The point of Friis et al. (2003) was not that *Archaeofructus* belonged to Nymphaeales but rather that its outgroup position was weakly supported. However, the discovery that Hydatellaceae are related to Nymphaeales shows that there is more floral diversity in this line than previously imagined, including flowers even more reduced than those of *Archaeofructus*—only one stamen or one carpel, with no perianth or subtending bract. Saarela et al. (2007) therefore made a speculative suggestion that Hydatellaceae might be more derived relatives of *Archaeofructus*.

This suggestion is supported by analyses of Doyle and Endress (2007; Endress and Doyle, forthcoming), which link *Archaeofructus* with Hydatellaceae in a data set of angiosperms only, and by the present analysis (fig. 6). Assuming that the fertile shoot of *Archaeofructus* is an inflorescence of unisexual flowers, its most parsimonious position is with Hydatellaceae. A position as the sister group of living angiosperms is five steps less parsimonious, and several positions much lower on the tree have the same score, because of similarities between the leaves of *Archaeofructus* and seed ferns. Unequivocal synapomorphies of *Archaeofructus* and Hydatellaceae (i.e., character state changes unambiguously located at this node) are loss of floral bracts and loss of perianth. Sun et al. (2002) cited the absence of bracts below the carpels and stamen pairs as evidence that the fertile shoot of *Archaeofructus* was a flower rather than an inflorescence, but this feature is shared with Hydatellaceae (Hamann 1975; Rudall et al. 2007), as well as

with other taxa, such as *Acorus* and Araceae (Buzgo and Endress 2000; Remizova and Sokoloff 2003). Synapomorphies of *Archaeofructus* and Nymphaeales as a whole (not counting those of Hydatellaceae and core Nymphaeales that are not preserved in fossils) are (semi)herbaceous habit, short or long and narrow stamen base (rather than long and wide), boat-shaped pollen, and palisade exotesta. The single order of laminar venation in *Archaeofructus* would be more consistent with a stem position than with a position in the crown group (it would undergo two changes across the tree rather than three), but its open venation would not, because venation in the next outgroup, *Caytonia*, is reticulate. This illustrates the dangers of relying on only living outgroups to polarize characters in divergent living groups.

Lack of a perianth in *Archaeofructus* also contributes to its inferred position in the crown group. Even though outgroups were scored as unknown for this character (for inapplicable), if *Archaeofructus* is separated from Hydatellaceae, this character undergoes three steps on the tree rather than two (the other being in Saururaceae). But even if outgroups had been scored as lacking a perianth, as in Sun et al. (2002), a relationship of *Archaeofructus* with Hydatellaceae would be four steps more parsimonious than a position on the stem lineage.

The discovery that Hydatellaceae are related to Nymphaeales increases support for the hypothesis that *Archaeofructus* is a crown-group angiosperm. When Hydatellaceae are removed from the analysis, it is still most parsimonious to group *Archaeofructus* with the remaining Nymphaeales, but the relative parsimony of a position as sister to living angiosperms improves from five steps less parsimonious to only two.

These results might be questioned because they assume that the fertile shoot of *Archaeofructus* was an inflorescence. As Sun et al. (2002) argued, perhaps it was a preflower more primitive than anything today; for example, they speculated that the supposed paired stamens could be remnants of the branched male structures of other seed plants. With *Archaeofructus* scored along these lines (fig. 7), one of its most parsimonious positions is still with Hydatellaceae, supported by absence of perianth, but it is equally parsimonious to link it with core Nymphaeales, and a stem position becomes less unparsimonious: it is three steps worse than a position in Nymphaeales rather than five. Features that would support a stem position are one order of laminar venation and pinnate microsporophylls, whereas a link with Hydatellaceae is no longer supported by absence of floral bracts. It may therefore be premature to rule out the possibility that *Archaeofructus* is an angiosperm stem relative. Even though we have whole plants of *Archaeofructus*, we need to know more about its morphology before we can be sure what it is.

Even if *Archaeofructus* is a crown-group angiosperm and does not affect inferred outgroup relationships, it could affect reconstruction of the ancestral flower. In a commentary on Saarela et al. (2007), Friis and Crane (2007) suggested that the increasing number of taxa with simple flowers near the base of the angiosperm tree, including not only Hydatellaceae and *Archaeofructus* but also Chloranthaceae and *Ceratophyllum*, raises the possibility that these plants represent a prefloral state. An alternative (Rudall 2007) is that the flower was still poorly integrated and could easily lose its distinction from an inflorescence. The present analysis is more consistent

with this “floral disintegration” hypothesis. For example, it implies that the ancestral flower had a perianth (fig. 8), more than one stamen, and more than one carpel. However, because of the number of unisexual lines (including *Amborella*) near the base of the tree, it is equivocal whether the ancestral flower was bisexual or unisexual. Addition of *Archaeofructus*, which has several ovules per carpel, also weakens the inference based on living taxa that one apical ovule was ancestral in angiosperms; the reconstructed ancestral ovule number becomes equivocal. These issues are more appropriately explored in the context of a broader analysis of angiosperms (Endress and Doyle, forthcoming).

Whether or not *Archaeofructus* affects ideas about the first flower, it reveals important early trends in floral evolution. It is also significant for the ecological radiation of angiosperms. Especially if it is related to the Albian genera *Vitiphycium* and *Caspiocarpus* (Friis et al. 2003), which had similar but less finely dissected leaves, it represents an important trend for invasion of Early Cretaceous aquatic ecosystems, represented today only by Hydatellaceae and core Nymphaeales.

Stuessy (2004) suggested that his hypothesis that the carpel evolved before double fertilization and the flower, which he argued was supported by *Archaeofructus*, could reconcile the apparent conflict between fossil evidence that angiosperms began to radiate in the Early Cretaceous and older dates based on molecular data. He proposed that molecular dates mark the origin of the first angiosperm feature (the carpel), as seen in *Archaeofructus*, whereas the Cretaceous radiation began after all three advances had evolved. If the present analysis is correct in placing *Archaeofructus* in the crown group, *Archaeofructus* does not support this or any other scenario for the order of origin of angiosperm features, but even if it did, Stuessy’s proposal for reconciliation of fossil and molecular dates would be invalid. Molecular dates are based on living taxa only, so by definition they provide ages for crown groups, not earlier events that occurred on the stem lineage, such as origin of the carpel in Stuessy’s scheme. There is every reason to assume that some angiosperm apomorphies evolved before others rather than all at once and that the angiosperm radiation did not begin until a suite of apomorphies had accumulated (Doyle and Donoghue 1993), but until closer stem relatives are confidently recognized, the order in which these apomorphies evolved cannot be determined.

## Conclusions

These considerations underline the point that the present analysis should be regarded as a heuristic exercise that may suggest avenues for future research, not as a definitive account of the origin of the flower. They also emphasize how much we do not know about the morphology of critical fossils. However, they also suggest that better information on these fossils could result in major progress toward understanding of the origin of the flower and its parts. There are also even less well understood fossils that may be relevant, such as the gigantopterids of the Permian of China, which had leaf venation approaching that of angiosperms and shared the triterpenoid oleanane (Taylor et al. 2006) but have not been associated with reproductive structures, and the diverse seed plants of the Late Triassic Molteno flora of South Africa (Anderson and Anderson



2003). Furthermore, continued progress in understanding the diversity and relationships of living and fossil basal angiosperms and the morphology of other fossil seed plants has brought the nature of the remaining problems into far clearer focus than was the case 25 years ago.

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